



INSTITUTE OF ANIMAL SCIENCE
FACULTY OF AGRICULTURE
UNIVERZITETA U BEOGRADU - SRBIJA

INSTITUT ZA ZOOTEHNIKU
POLJOPRIVREDNI FAKULTET
UNIVERZITET U BEOGRADU - SRBIJA

V INTERNATIONAL CONFERENCE “AQUACULTURE & FISHERY

CONFERENCE PROCEEDINGS

June, 1 - 3. 2011.

V MEĐUNARODNA KONFERENCIJA ”AKVAKULTURA I RIBARSTVO”

ZBORNIK PREDAVANJA

1. - 3. jun, 2011. godine

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GOVERNANCE IN PROFESSIONAL EUROPEAN AQUACULTURE

COURTNEY HOUGH
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UPRAVLJANJE U PROFESIONALNOJ EVROPSKOJ AKVAKULTURI

The word 'governance' relates to how policies and decisions are put into place and achieved through measurable actions. Governance has also been defined as the rules of the political system to solve conflicts between actors (within the scope of action) and adopt decision (legality). The term has also been used to describe the "proper functioning of institutions and their acceptance by the public" (legitimacy). Governance is also a reflection of the efficacy of government and the achievement of consensus by democratic means (participation). In all terms, good governance reflects the legality, the legitimacy and the participation of the appropriate stakeholders, be they from government, business or civil society representatives.

In the case of the professional European aquaculture sector, governance also refers to consistent operational and corporate management, the application of cohesive policies, guidance, processes and decision-rights for a given area of responsibility of the business in question.

Professional European aquaculture is composed of family firms, SMEs and large Enterprises, each of which will have its own governance 'model', which become more visible with the increasing size and responsibilities of the enterprise. Such models will be directed towards operating within legislative conditions – such as water use and effluent control – or be adapted to best practice and/or certification guidelines, for production that is geared towards – as examples - specific quality conditions or organic production.

While governance in an aquaculture business will reflect operational actions, such as following best practice, working conditions and declared corporate policies, the different representative structures of European aquaculture are increasingly involved in governance actions at National and European levels as well. These would target improving the legislative or operating framework for the sector as opposed to an individual enterprise.

The complexity of operating a business that provides food to the consumer, that uses natural resources, that rears livestock and provides employment means that aquaculture covers a large legislative framework while having to demonstrate clarity and transparency in order to establish its position in the market and in society.

Aquaculture is done throughout Europe and most European States have a national aquaculture association that acts as the interlocutor for the profession with National and Regional governments. At the European level, the Federation of European Aquaculture Producers, established in 1969, groups these interests within a federal structure. This body sits on the European Commission's Advisory Committee on Fisheries and Aquaculture, which is a consultative structure for issues and legislation affecting the sector within the European Union. This represents 'participatory governance' where sectoral representation is expected to play a higher role in political issues and decision-making, thus imposing more than just a critical viewpoint.

The Federation is also linked, as an observer, to the Food and Agriculture Organization of the United Nations – principally to the sub-Committee on Aquaculture of the Committee on Fisheries, the European Inland Fisheries Advisory Committee and the Committee on Aquaculture of the General Fisheries Council of the Mediterranean.

Finally, the FEAP is also a founder member of the European Aquaculture Technology and Innovation Platform, which is looking to improve governance within the RTDi interests within Europe, so as to promote effective innovation and competitiveness for European aquaculture.

Assuring constructive and effective participation of European aquaculture representation is a challenge, given the need to be knowledgeable on the details not only of the legislation itself but also of the reasons, be they political or technical, for this. This has imposed change on how the representative sector works, how it is structured and the skills and experience required for its participation in modern governance procedures.

Currently, the reform of Europe's Common Fisheries Policy and its related instruments and the influence of Europe 2020, the Union's guideline policy for coming years, provide the greatest challenges and opportunities for European aquaculture. It is the effective practice of good governance that gives new prospects for Europe to realise the objectives of both the sector and the Strategy for the Sustainable Development of European Aquaculture within the next decade.

UPRAVLJANJE U PROFESIONALNOJ EVROPSKOJ AKVAKULTURI

Reč „upravljanje“ se odnosi na to kako se politika i odluke stavljaju na svoje mesto i dostižu putem odgovarajućih mera. Upravljanje je takođe definisano kao skup pravila političkog sistema za rešavanje konflikta između učesnika (u okviru date mere) i usvojene odluke (zakonitost). Ovaj termin se takođe koristi da opiše „pravilan rad institucija i to koliko ih je javnost prihvatila“ (zakonitost). Upravljanje je takođe odraz efikasnosti vlade i dostizanja koncenzusa na demokratski način (sudelovanjem). Sve u svemu, kvalitetno upravljenje znači zakonitost, legitimnost i sudelovanje odgovarajućih interesnih strana, bilo da su oni predstavnici vlade, iz poslovnog sveta ili društva.

Što se tiče sektora profesionalne Evropske akvakulture, upravljanje se takođe odnosi na dosledan operacioni i korporativni menadžment, primenu kohezivnih politika, smernica, procesa i odluka- prava za određenu oblast odgovornosti datog posla.

Profesionalnu Evropsku akvakulturu čine porodične firme, mala i srednja preduzeća i velike kompanije, od kojih svaka ima svoj „model“ upravljanja, koji postaje vidljiviji sa porastom veličine i odgovornosti kompanije. Takvi modeli će biti usmereni ka radu u okviru zakonodavnih uslova- kao što su korišćenje vode i kontrola efluenata- ili će biti adaptirani za najbolju praksu i/ili smernice za sertifikaciju, za proizvodnju koja je usmerena ka – kao u primerima- specijalnim uslovima kvaliteta ili organske proizvodnje.

Dok će upravljanje u poslovima akvakulture predstavljati operativne mere, kao što je ugledanje na najbolji primer, uslove rada i proglašene korporativne politike, različite reprezentativne strukture Evropske akvakulture sve više imaju veze sa upravljačkim merama na nacionalnim i Evropskim nivoima. Ovo ima za cilj unapređenje zakonodavnog i operativnog okvira za sektor nasuprot individulanom delovanju.

Kompleksnost upravljanja delatnošću koja obezbeđuje hranu potrošačima, koja koristi prirodne resurse, koji uzgaja stoku i pruža mogućnost za zapošljavanjem znači da akvakultura pokriva veliki zakonodavni okvir dok istovremeno mora da pokaže bistrinu i transparentnost da bi ustanovila svoje mesto na tržištu i u društvu.

Akvakultura se praktikuje širom Evrope i većina Evropskih država ima nacionalna udruženja za akvakulturu koja imaju funkciju profesionalnih sagovornika sa Nacionalnim i Regionalnim vladama. Na Evropskom nivou, Federacija Evropskih Proizvođača u Akvakulturi, osnovana 1969 godine, grupiše ove interese u okviru federalne strukture. Ovo telo spada u okvir Savetodavnog Odbora za Ribarstvo i Akvakulturu Evropske Komisije, koji ima funkciju konsultanta za pitanja i zakone koja imaju uticaja na ovaj sektor u okviru Evropske Unije. Ovo predstavlja „participativno upravljanje" gde se očekuje da sektorsko predstavljanje odigra bitnu ulogu u političkim pitanjima i donošenjima odluka, i na taj način nametne više nego samo kritički pogled.

Federacija je takođe povezana, kao posmatrač, sa Organizacijom za hranu i poljoprivredu FAO Ujedinjenih Nacija - naročito za pod-Komitet za akvakulturu Komiteta za ribarstvo, za Evropski Savetodavni Komitet za kopneno ribarstvo, kao i za Komiteta za akvakulturu Generalnog Saveta za ribarstvo mediterana.

Na kraju, Federacija Evropskih Proizvođača u Akvakulturi (FEAP) je takođe osnivač Platforme za Evropske Tehnologije i Inovacije u Akvakulturi, čiji je cilj da unapredi upravljanje u okviru Istraživanja, Razvoja Tehnologije i Inovacija (RTDi) u Evropi, radi promovisanja efektivnih inovacija i konkurentnosti za Evropsku akvakulturu.

Osiguravanje konstruktivnog i efektivnog sudelovanja u predstavljanju Evropske akvakulture je izazov, s obzirom na potrebu da se znaju detalji ne samo samog zakona već i razloga, bilo oni političke ili tehničke prirode. Sve ovo je nametnulo promenu u načinu rada reprezentativnog sektora, u tome kako je on struktuiran i koje su neophodne veštine i iskustvo za njegovo učešće u modernim procedurama upravljanja.

Trenutno, reforma Evropske Politike o Zajedničkom Ribarstvu (Europe's Common Fisheries Policy) i s njom povezani instrumenati i uticaji Evrope 2020, Unijine smernice za dolazeće godine, predstavljaju najveće izazove i mogućnosti za Evropsku akvakulturu. To je efektivan primer prakse dobrog upravljanja koji pruža nove mogućnosti za Evropu da shvati koji su ciljevi i samog sektora i Strategije za održivi razvoj u Evropskoj akvakulturi za sledeću deceniju.

CHALLENGES AND FUTURE OPPORTUNITIES OF EUROPEAN AQUACULTURE

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IZAZOVI I MOGUĆNOSTI EVROPSKE AKVAKULTURE

Prošireni abstrakt

Evropski udeo u globalnoj akvakulturi je bio samo 4.5 % u 2008; ipak, Evropa je vodeći region za proizvodnju vrsta kao što su losos, pastrmka, brancin, orada, riba list i dagnje. Između 2000. i 2009. godine, obim proizvodnje Evropske akvakulture je porastao za 20,8 %, sa 2 056 000 tone na 2 484 000 tone, dok se proizvodna vrednost skoro udvostručila, sa 4 638 miliona američkih dolara na 8 799 miliona američkih dolara. Ovaj se porast najviše može pripisati porastu akvakulture morskih riba, dok je proizvodnja slatkovodnih riba opadala. Takođe, došlo je do znatne promene u trendovima proizvodnje po regijama. Dok proizvodnja u akvakulturi stagnira u zemljama Evropske Unije, postoji porast u proizvodnji u severnim, istočnim i južnim Evropskim zemljama koje nisu članice Evropske Unije, naročito u Norveškoj, Turskoj i Rusiji. Oko 75% Evropske proizvodnje u akvakulturi potiče iz morskih sredina, gde su najčešće proizvedene vrste losos, brancin i orada. Sistemi i tehnologije gajenja takođe pokazuju veliku raznolikost u Evropi i uključuju ekstenzivno gajenje u jezerima, lagunama i priobalnim oblastima; poluintenzivno gajenje u jezerima i lagunama; intenzivno gajenje u protočnim sistemima, recirkulacionim sistemima i morskim kavezima, u zaklonjenim i izloženijim zonama. Školjke se tradicionalno gaje na dnu ili na stubovima i splavovima. Obalska i marikultura otvorenog mora predstavljaju rastući udeo u ukupnoj proizvodnji.

Povećano takmičenje za resursima sa drugim privrednim aktivnostima (urbanizacija, poljoprivreda, industrija, turizam, zaštita životne sredine, itd.) je verovatno glavni izazov za dalji razvoj Evropske akvakulture. Takođe, pristup pogodnim lokacijama za proizvodnju u akvakulturi je jedno od najbitnijih pitanja. Zakonodavstvo takođe predstavlja prepreku za razvoj Evropske akvakulture. Akvakultura je najviše kontrolisan sektor za proizvodnju hrane u Evropi. Što se samih usluga tiče, glavni izazovi su sledeći: Ograničen pristup početnom kapitalu i/ili zajmovima za inovacije, naročito u centralnim i istočnim Evropskim zemljama; dostupnost specijalnih usluga koje zahteva moderna industrija;

dostupnost dozvoljenih veterinarsko medicinskih proizvoda. Zamena ribljeg brašna i ribljeg ulja, fazno hranjenje i druge inovacije doprinose održivosti resursa koji se koriste u proizvodnji hrane; ipak, trenutna zabrana za hraniva u Evropskoj Uniji za prerađenje životinske proteine takodje predstavlja veliku prepreku za uvođenje više održivih sistema ishrane. Glavni problemi koji se tiču interakcije akvakulture i životne sredine su: stroge uredbe, kvalitet vode za vodosnabdevanje, uticaj klimatskih promena (ekstremni vremenski uslovi, poplave, itd.); uticaj životne sredine na akvakulturu (korišćenje terapeutskih sredstava i lekova, nedostatak hranljivih materija, bekstvo); EIA (Environment Impact Assessment, Procena uticaja na životnu sredinu); korišćnje egzotičnih vrsta; konflikti sa drugim korisnicima istih resursa; negativan stav javnosti. Zajedno sa proizvodnjom u akvakulturi koja stagnira, Evropsko tržište veoma zavisi od uvoza. Evropska unija je najveći svetski uvoznik morske hrane (više od 65% konzumirane morske hrane je uvezeno). Proizvođači iz Evropske Unije ne mogu da se takmiče sa jevtinim uvezenim morskim proizvodima iz drugih zemalja gde je zakon mnogo manje strog nego u Evropskoj Uniji.

Eksterni faktori koji utiču na akvakulturu mogu se sažeti na osnovu sledećeg: faktori životne sredine (npr. Klimatske promene, zagađenje vode); variranje uloga (npr. kapital, riblje brašno i ulje, energija, radna snaga); regulativa (zakonodavni okvir); trgovina (promene u politici i cenama); finansijski faktori (ulaganja, kamatna/devizna stopa, oporezivanje, osiguranje); faktori konkurencije (nove vrste, proizvodi, proizvođači); ekonomska kriza (promene kupovne moći potrošača).

Buduće mogućnosti i način napretka i razvoja akvakulture u Evropi mogu se ukratko sumirati na sledeći način: (1) odgovorno korišćenje resursa i zaštita životne sredine će ostati glavni izazovi (izdavanje dozvole, EIA); (2) Pravni okvir treba da bude pojednostavljen i konsolidovan. Ovom sektoru je potreban iznivelisan teren; (3) Postoji potreba za posebnim prikupljanjem podataka za proizvode u akvakulturi i ribarstvu radi podržavanja regulative; (4) Interesne strane treba da procene posledice globalnih klimatskih promena i moguće scenarije; (5) Uspeh modernog, profesionalnog sektora akvakulture zavisi od dostupnosti usluga visokog kvaliteta; (6) Nove tehnologije za akvakulturu pružaju mogućnost razvoja; (7) Eliminacija negativnog javnog stava potrošača i zakonodavaca; (8) Razvoj "ekološke etikete" koja će garantovati praktikovanje akvakulture u Evropi na nacin koji štiti životnu sredinu; (9) Bolja komunikacija kako u okviru lanca vrednosti, tako i sa potrošačima.

Ključne reči: *Evropski, akvakultura, izazovi, trendovi, mogućnosti.*

Beleška: *Ovaj rad predstavlja rezime FAO Regionalnog Pregleda o Akvakulturi Evropskog kontinenta (FAO European Regional Review on Aquaculture) koji je za Globalnu Konferenciju o Akvakulturi (Global Aquaculture Conference) održanoj 22-25 Septembra, Phuket, Thailand), pripremio tim: L. Varadi, A. Lane, Y. Harrache, E. Bekefi, G. Gyalog i P. Lengyel pod rukovodstvom U. Barg, FIRA, FAO.*

CURRENT STATUS AND TRENDS

The European share from global aquaculture production was only 4.5% in 2008; however, Europe is a leading region in the production of species such as salmon, trout, seabass, seabream, turbot and mussels. Between 2000 and 2009, the production volume of European aquaculture increased by 20.8 percent, from 2 056 000 tonnes to 2 484 000 tonnes, while the production value almost doubled, from US$ 4 638 million to

US$ 8 799 million (Figure 1.). The increase was mainly attributable to the growth of marine finfish aquaculture, while freshwater aquaculture production declined. European finfish culture is dominated by salmonids, sea bass, sea bream and common carp, but significant growth has come from higher-value fish species, particularly turbot and tuna. Cyprinids are the dominant species in the Central and Eastern European region (75 percent of the total freshwater aquaculture production), while trout dominates Western European aquaculture (68 percent of the freshwater aquaculture production). The share of the major species of European aquaculture in 2008 is shown in Figure 2.

Farming systems and technologies also show a great diversity in Europe and include extensive culture in ponds, lagoons and coastal areas; semi-intensive farming in ponds and lagoons; intensive culture in flow-through systems, recirculating aquaculture systems and sea cages, both in sheltered and more exposed zones. Shellfish is traditionally bottom-cultured or grown on stakes or rafts. Coastal and offshore long-line culture represents a growing percentage of the total production and a recent trend is the increasing supply of juvenile oyster from hatcheries in the main producing countries. Significant research and development has focused on further improvement in the efficiency of production systems and the quality of the fish produced therein, while mitigating environmental impact. Examples include the development of underwater surveillance to manage feeding and biomass; upscaling of recirculating systems; the development of cages and nets that can be used in higher-energy locations and the development of integrated multi-trophic production systems.

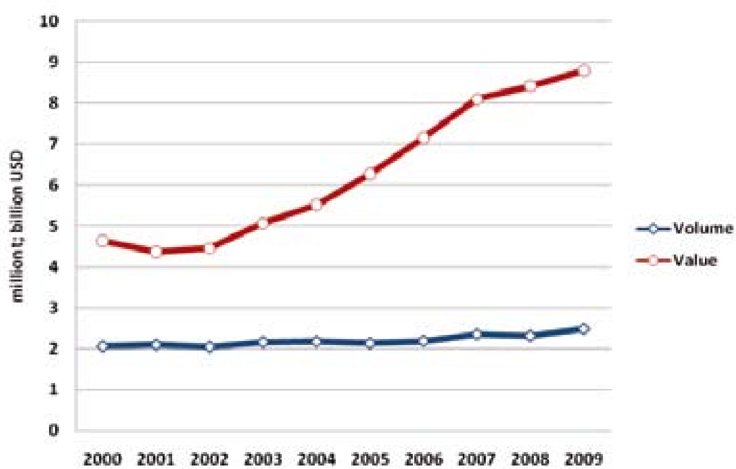

Figure 1. European aquaculture production 2000-2009 (Source: FAO, 2011)

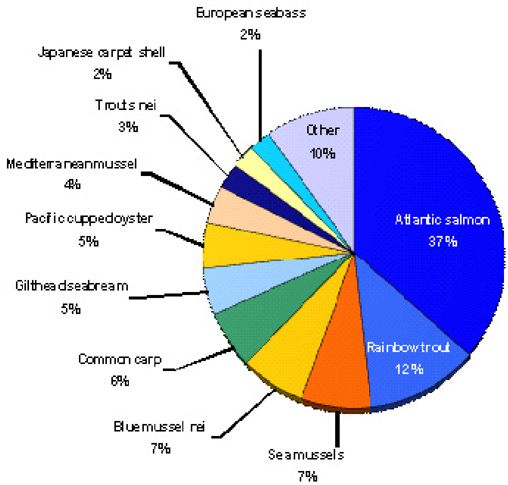

Figure 2. Major species of European aquaculture production in 2008 (Source: FAO, 2010).

CHALLENGES

The increasing competition for resources with other economic activities (urbanization, agriculture, industry, tourism, nature protection etc.) is probably the main challenge for the further development of European aquaculture and access to suitable sites for aquaculture production is a critical issue. Legislation is also seen as a burden on European aquaculture development. Aquaculture is the most heavily regulated food production sector in Europe. Some specific aspects of regulatory and legal constraints are the following: there is no common approach of licensing at all levels; aquaculture is not recognised as an equal user of suitable sites; the Water Framework Directive (WFD) in the EU constrains aquaculture development in various areas (e.g. in shellfish waters); the predation of protected species on stocks is not compensated; there is no common approach in the interpretation and application of environment impact assessment (EIA) rules. As far as services are concerned, the main challenges are: the limited access to seed capital and/or loans for innovation, especially in Central and Eastern European countries; availability of special services required by a modern industry; availability of authorised veterinary medical products. The sustainability of the use of feed resources is improving through the replacement of fish oil and fish meal, phase feeding and other innovations; however, the current EU feed ban for processed animal proteins also presents a major obstacle for the introduction of more sustainable feeding systems. The main issues regarding aquaculture and environment interaction are: heavy regulations; water quality of supply water; effects of climate change (extreme weather conditions, floods, etc.); environmental impact of aquaculture (use of therapeutics and drugs, nutrient discharge, escapees); EIA; use of exotic species; conflicts with other resource users; negative public perception. With stagnating aquaculture production, the European market is increasingly dependent upon imports. Some 1.65 million tonnes (live weight equivalent) of farmed seafood products were imported into Europe in 2008. The European Union is the largest seafood importer of the world (more than 65% of the consumed seafood is from import). EU producers cannot compete with cheap imported seafood products that come from countries, where regulations are far less strict than in the EU. Although the contribution of aquaculture to food security, social and economical development is relatively low in terms of the share from the GDP, aquaculture has a significant role in some coastal and rural regions of Europe. The external factors that affect aquaculture can be summarised according to the following: environmental factors (e.g. climate change, water pollution); variations in inputs (e.g. seed, fish meal and oil, energy, labour); government policy (regulatory framework); trade (changes in policy and tariffs); financial factors (investment, interest/exchange rates, taxation, insurance); competitive factors (new species, products, producers); economic crises (changing consumer preferences and purchasing power). While Europe as a whole enjoys a rich aquaculture research environment, it is very diversified and fragmented between public and private institutes, universities and other higher education establishments and private companies. There are, however, important initiatives and programs that assist the share of information, and collaboration such as the European Research Area (ERA) since 2000, the EU Framework Programmes; the establishment of research networks (EFARO and NACEE) and the AQUA-TNET educational network. The European Aquaculture Technology and Innovation Platform (EATIP) is an important pan-European initiative aiming to assure that European aquaculture as a sustainable industry and to consolidate the role of aquaculture in the society. Europe is a heterogeneous region regarding socio-economic conditions, and thus, aquaculture governance and management systems also vary greatly from country to country. Aquaculture in the EU region is covered by the Common Fisheries Policy but is also closely dependent on developments in other policy areas – environment, maritime spatial planning, animal welfare, animal health, food safety, research, etc. The Commission has brought together all these policies in its 2009 communication. This is the basis of a new European aquaculture strategy "*Building a sustainable future for aquaculture. A new impetus for the Strategy for the Sustainable Development of European Aquaculture*", which aims to make European aquaculture more competitive, to ensure sustainable growth and to improve the sector's image and governance. In European Union Member States, the European Fisheries Fund (EFF) 2007–2013 is the principal financial tool for fisheries and aquaculture developments. The EU aquaculture governance system also includes stakeholder interactions (e.g. ACFA, CONSENSUS Project) and sector self-governance (e.g. the FEAP Code of Conduct).

FUTURE OPPORTUNITIES

The future opportunities and the way forward of European aquaculture development are briefly summarised as follows: (1) The responsible use of resources and the protection of the environment will remain key challenges (licensing, EIA); (2) The legislative framework should be simplified and consolidated. The sector needs a level playing field; (3) There is a need for specific data collection for aquaculture and fishery products to support policy; (4) The consequences of global climate change and potential scenarios have to be assessed by all stakeholders; (5) The success of a modern, professional aquaculture sector is dependent on the availability of high-quality services; (6) New aquaculture technologies provide opportunities for development; (7) Eliminating the negative public perception of consumers and policy makers; (8) Development of an „ecolabel" that can certify environmentally friendly aquaculture practices in Europe; (9) Better communication within the value chain and towards consumers.

The challenge for European aquaculture is to achieve innovative and economic growth. European aquaculture must be perceived as an environmentally, economically and socially sustainable activity, based on scientific evidence and consumer confidence. However, European aquaculture needs strong political will and a "level playing field" to be competitive.

Note*: The paper is a brief summary of the FAO European Regional Review on Aquaculture that was prepared for the Global Aquaculture Conference (22-25 September, Phuket, Thailand), by a team consisting of L. Varadi, A. Lane, Y. Harrache, E. Bekefi, G. Gyalog and P. Lengyel under the supervision of U. Barg, FIRA, FAO.*

AQUACULTURE IN ISRAEL: AN OVERVIEW

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PREGLED AKVAKULTURE U IZRAELU

Abstract

Due to the severe water constrictions in Israel (60% of the country is a desert with less than 80 mm rainfall per year), the aquaculture practices in the country have had to rely on innovative methods designed to overcome the water shortage. These include: the extended use of saline water not suitable for other agricultural crops yet very suitable for fish culture; dual use of irrigation reservoirs for fish culture and intensive production ponds in which the fish yields per cubic meter of water are extremely high. Pelleted fish feeds of high quality, especially designed for the different cultured fish species are used in all the ponds. The main edible fish cultured in freshwater are tilapia and carp. In the marine water sea bream and European sea bass are the main cultured species. In addition there is a growing sector of ornamental fish these include cold water fish such as koi carp and warm water tropical species such as guppies. Bird predation of fish is major problem and a number of methods are utilized in order to overcome the problem. These include various scaring devices such as: noise makers, scarecrows of different shapes and colors, self propelled small airplanes in the shape of predatory birds and nets.

Key words: *desert aquaculture; irrigation/fish culture reservoirs; saline water*

Background

Due to the severe water constrictions in Israel (60% of the country is a desert with less than 80 mm rainfall per year), the aquaculture practices in the country have had to rely on innovative methods designed to overcome the water shortage. In the course of this presentation, the different innovations and rearing systems used will be discussed.

Pond types utilized

- Earthen ponds

The average depth of these ponds is typically around 1-1.5 meters. The average size of the ponds ranges from one tenth of a hectare to a few hectares. These sizes are preferred since they are easier to handle. Production levels are on average between 5-10 tons per hectare per annum.

- Dual - purpose reservoirs utilized for both irrigation and fish farming.

These are usually large size reservoirs ranging in size from 10 to 50 hectares and the average depth ranges from 3 to 12 meters. In some cases, to prevent water losses, thick plastic is used to line the bottom of the reservoirs. Production levels range from 10 to 20 tons per hectare per annum.

- Intensive fishponds in which the bottom is either plastic lined or made of concrete to prevent seepage.

Often these ponds are conical shaped with a drainage opening in the center. The water is circulated with the aid of paddle wheel oxygenators causing all the particular organic matter, excess food and feces as well as dead fish to concentrate in the center. Periodic draining of the pond through the central drainage can eliminate the build-up of pollutants and enable higher stocking levels. Production levels range from 150-250 tons per hectare per annum.

- Highly intensive production ponds built of concrete or thick plastic. These ponds are similar in their design to the intensive ponds. Higher production levels are achieved using liquid oxygen in order to meet the high oxygen demand of the densely stocked fish. These ponds are usually small in size with a volume of about 200-500 cubic meters on the average. Production levels range from 75-150 kg per cubic meter of water corresponding to 750-1500 tons per hectare. At present these ponds are very expensive to use and highly risky, requiring additional monitoring and alarm systems. Nonetheless, these ponds can provide better marketing possibilities as fish of larger sizes can be supplied on any given day during the year.

Due to the severe shortage of freshwater in the country, the aquaculture sector has utilized saline water, which cannot be used for other agricultural crops (water salinity of 1000mg chlorine and above). The main area used for fish culture in Israel is the Bet Shean valley close to the Jordan River, an area which has many saline springs.

Fish species

The two main species reared in Israel are tilapia hybrids (*Oreochromis niloticus x O. aureus*) accounting for 40% of the yield and common carp (*Cyprinus carpio*) accounting for 38%. Other fish reared are mullet (*Mugil cephalus*), trout (*Oncorhynchus mykiss*), hybrid striped bass (*Morone saxatilis x Morone chrysops*) as well as various carps.

A genetic selection program has been employed, selecting strains more suitable for the culture conditions in the country.

In mariculture the two main species reared are sea bream (*Sparus aurata*) and the European sea bass (*Dicentrarchus labrax*).

A growing industry in Israel is the production of ornamental fish. These include the cold water fish – mainly koi carp reared in outdoor ponds. In addition a variety of different warm water tropical ornamental fish are reared in indoor facilities where the conditions are kept at optimal levels for these fish. The main fish reared are live bearers such as the guppy (Poecilia reticulata) of which dozens of different varieties are reared. Most of the ornamental fish are exported – the main market is Europe.

Feeds

The rearing of the fish does not rely on natural food and all cultured fish are fed artificial diets. Pelleted feeds especially designed for the different fish species are used. Most of the pellets are produced using extruders enabling the preparation of floating pellets. The pellet size of the feeds is carefully selected to fit the mouth orifice of the reared fish. The feeds are manufactured in Israel by a number of feed mills and only one of them exclusively specializes in fish feeds. Since the climate in Israel is relatively hot, special care is taken to reduce the level of fats in the diets. This is especially important during the hot summer months.

Bird predation

Israel is on the path of migrating birds from Europe to Africa and back. Large flocks of cormorants pass through the region during the spring and autumn and this creates a serious problem for the aquaculture industry. On average, an adult cormorant can consume ½ kg of fish per day. The size of some of the flocks can reach 2000 birds and therefore the damage caused by these birds can be devastating. The major problem is in the spring when the small fish are consumed by the birds. In addition, the birds are vectors of parasites and diseases. The following methods have been used over the years with limited success: scarecrows of different sizes and colors, exploding/noise making devices, balloons of different designs; and providing the birds with feeding ponds stocked with small or undesirable fish. More successful methods include the use of a small self-propelled airplane in the shape of a large predatory bird, or covering the ponds with nets.

PREGLED AKVAKULTURE U IZRAELU

Abstrakt

Usled ozbiljnog nedostatka vode u Izraelu (60 % zemlje je pustinja sa manje od 80 mm padavina godišnje), akvakultura je morala da se osloni na inovativne metode koje prevazilaze nedostatak vode. Ove metode su: upotreba slane vode koja inače ne odgovara ratarskim kulturama ali je pogodna za gajenje riba, dvostruka upotreba rezervoara za navodnjavanje za gajenje riba i intenzivna proizvodnja u ribnjacima sa ekstremno visokim prinosima po kubnom metru vode. U ribnjacima se koristi visokokvalitetna peletirana hrana, posebno pripremljena za različite vrste. Najvažnije jestive vrste gajenih riba su tilapija i šaran. U morskoj vodi su najznačajnije orada i brancin. Postoji i rastući sector ukrasnih, ornamentalnih riba koje obuhvataju hladnovodne ribe poput koi šarana i toplovodne vrste kao što je gupi. Piscivorne ptice su veliki problem koji se rešava brojnim metodama: naprave koje prave buku, razna, strašila, aviončići na propeler koji liče na ptice predatore i mreže.

Ključne reči*: pustinjska akvakultura, navodnjavanje/gajenje riba, slana voda.*

AQUACULTURE AND FISHERY IN SERBIA – STATUS AND POTENTIALS

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AKVAKULTURA I RIBARSTVO U SRBIJI – STANJE I POTENCIJAL

Apstrakt
Akvakultura u Srbiji se najvećim delom realizuje u objektima akvakulture: pastrmskim i šaranskim ribnjacima. Ukupna površina pod šaranskim ribnjacima je oko 14 000 hektara, od čega je oko 20% van funkcije. Pod pastrmskim ribnjacima je oko 14 hektara od čega je oko 15 % van funkcije. U šaranskim ribnjacima se proizvodi šaran, beli i sivi tolstolobik, beli amur i grabljivice: som i smuđ. U pastrmskim ribnjacima se proizvodi kalifornijska (dužičasta) pastrmka. Na malom broju ribnjaka se proizvodi mlađ toplovodnih (deverika, linjak, zlatni karaš, šaran divljak) i hladnovodnih vrsta riba (potočna pastrmka, mladica, lipljan) za poribljavanje tekućih i stajaćih vodenih ekosistema. Ukupna proizvodnja u poslednjih dve godine se kreće u količini od oko 15 000 tona, od čega oko 2 000 tona kalifornijske pastrmke (od čega konzumne pastrmke oko 75%), i oko 13 000 tona toplovodnih vrsta riba (oko 11 000 tona šarana i oko 2 000 tona pratećih vrsta riba), od čega 70 do 75% konzumne ribe.

Svi vodeni ekosistemi u Srbiji, sem objekata akvakulture (pastrmskih i šaranskih ribnjaka) i hidroakumulacija čije su prioritetne namene vodosnabdevanja se svrstavaju u ribolovne vode. Ukupna dužina svih potoka i reka je 65 980 km. Broj vrsta riba koje naseljavaju vodene ekosisteme u Srbiji je 89, svrstanih u 57 rodova i 20 familija. Od košljoriba koje naseljavaju vode u Srbiji 52 vrste se love rekreativnim i privrednim ribolovom. Privrednim ribolovom se bavi oko 260 privrednih ribara. Prosečan privredni ribar obavi oko 200 ribolovnih izlazaka na vodu u toku godine. Broj rekreativnih ribolovaca se u poslednjih desetak godina kreće od 58 000 (2001) do preko 100 000 (2002. godine). Prosečan rekreativni ribolovac godišnje obavi oko 50 ribolovnih izlazaka na vodu. Prema podacima RZS izlov iz reka u Srbiji je poslednjih godina između 2 000 i 3 000 tona.

Potencijal razvoja akvakulture je baziran na mogućnostima unapređenja proizvodnje na postojećim ribnjacima, povećanju proizvodnih ribnjačkih površina, proizvodnji hra-

ne za ribe, prerađivačkim kapacitetima za koje Srbija poseduje sirovine i potencijal za plasman preradjevina od ribe na tržište zemalja EU i drugih država.

Potencial ribolova se može podeliti na onaj koji pružaju brdsko planinske tekućice i potencijal koji pružaju ravničarske reke. Potencijal brdsko planinskih tekućica baziran je na rekreativnom ribolovu, odnosno ribolovnom turizmu koji bi se razvio kao posledica želje da se dođe baš na izabrano područje. U cilju stvaranja atraktivnih ponuda, stvoren je osnov da se u narednom periodu poribljavanje obavlja sa mlađi čiji su roditelji iz istog toka, čime je stvoren preduslov za očuvanje autohtonih genotipova. Potencijal ravničarskih reka je znatno veći. Baziran je na rekreativnom i privrednom ribolovu. Potencijal koji pruža rekreativni ribolov je zasnovan na činjenici da je grad sa najvećim brojem stanovnika u Srbiji – Beograd, upravo lociran na obalama dve velike reke: Dunavu i Savi, ali i na činjenici da su gradovi Novi Sad, Šabac, Smederevo... locirani na obalama velikih reka, što svakako ukazuje na činjenicu da bi broj rekreativnih ribolovaca mogao biti višestruko veći.

Veoma značajan potencijal je i u privrednom ribolovu koji bi uz unapređenje politike poribljavanja, gazdovanja i izlova ribe, ali i statusa privrednih ribara mogao predstavljati egzistenciju znatno većeg broja ljudi od sadašnjih.

Ključne reči: *akvakultura, ribarstvo, Srbija, stanje, potenciali*

Aquaculture in Serbia

Aquaculture in Serbia is performed primarily in aquaculture objects: trout and carp farms (Marković and Poleksić, 2009). The total area under carp farms is about 14 000 ha, 20% being out of function. Area under trout farms is about 14 ha, of which 15 % out of function. On carp farms following species are reared: carp, white bighead, gray bighead, white grasscarp, and predators: wells and pikeperch. Rainbow trout is produced on trout farms. On some farms fry of warmwater fish: bream (*Abramis brama*), tench (*Tinca tinca*), crucian carp *(Carassius carassius)*, "wild" carp *(Cyprinus carpio*) and coldwater fish species: brown trout (*Salmo trutta*), Danubian salmon (*Hucho hucho*), and grayling (*Thymallus thymallus*) are produced for stocking of running and stagnant aquatic ecosystems. Total production in the last two years varies from 15 000 tons: 2000 tons of rainbow trout (of that number market size trout is about 75%) and about 13 000 tons of warmwater species (approx. 11 000 tons of carp and 2000 tons accompanying species). 70 to 75 % of the warmwater species quantity is market size fish.

Fishery in Serbia

All the aquatic ecosystems in Serbia, besides aquaculture objects (trout and carp fish farms) and reservoirs (hydro accumulations) that primarily are for urban water supply are classified as fishery waters. Total length of all rivers and water currents is 65 980 km. Rivers belong to following catchment areas: Black sea (92,46%), Adriatic sea (5,36%) and Aegean (2,18%) (Gavrilović, 2002). Fishery waters are divided in 6 fishery areas: Serbia Vojvodina, Serbia West, Serbia – South West, Serbia South, Serbia East, and Serbia Center. According to the Law on protection and sustainable use of fishery resources (Official Gazette No 36/09) fish in fishery waters are a natural resource and property of common interest. There are 89 fish species classified in 57 genera and 20 families, inhabiting aquatic ecosystems (Simonović, 2001). Among Teleost fish inhabiting Serbian waters 52 species are caught using recreational and commercial fishery. From that number economically important are: sterlet (*Acipenser ruthenus*), carp (*Cyprinus* *carpio*), wells (*Silurus glanis*), pikeperch (*Sander lucioperca*), northern pike (*Esox lucius*), bream (*Abramis brama*), Prussian carp (*Carassius gibelio*), gray bighead (*Arystichthys nobilis*), white bighead (*Hypophthalmichthys molitrix*), barbel (*Barbus barbus*), Volga pikeperch (*Stizostedion volgensis*), white grasscarp (*Ctenopharyngodon idella*), asp (*Aspius aspius*), orfe (*Leuciscus idus*), nase (*Chondrostoma nasus*) and burbot (*Lota lota*).

Fishery in Serbia can be divided, depending on fishing purpose, on commercial and recreational (this includes sport fishery). Commercial fishery is an occupation of 260 professional fishermen. Average professional fisherman is going out on water about 200 times per year. Number of fishermen in the past ten years varied from 58 000 (in 2001) till over 100 000 (in 2002). Average recreational fisherman is going out on water about 50 times. According to the data from the Statistical Office of the Republic of Serbia catch of fish from rivers in Serbia is between 2 000 and 3 000 tons (in 2008 – 2 128 827, in 2009 – 2 171 317 kg).

Serbia's potential in aquaculture and fishery

Improvement of production on fish farms could be carried out through programs for production improvement on carp and trout farms.

Improvement of production on carp farms takes place through carp feeding programs and provision of quality stocking material for carp farms. Feeding program comprises creation of several formulations, as a result of research on formulations adapted to: different age categories, pond water quality, and growing season. Additional improvement of feeding is based on feed processing in the extrusion process of feed preparation. Extrusion improves physical properties (it increases pellets durability in water, enables choice between floating and sinking pellets), chemical properties (starch gelatinization), and microbiological properties of the feed. This creates a precondition for better weight gain and better quality fish meat. Particularly important part of the program is creation of fish feed with improved fatty acid composition: with increased omega 3 content and better ratio of omega 3 and omega 6 fatty acids in carp flesh.

Provision of quality stocking material for warmwater fish farms is carried out through a program of selective breeding (family selection). It will be the basis for future carp fry production in Serbia. By the use of selected fry inbreeding is prevented, as well as restocking of ponds with fry obtained from spawning in the wild.

Improvement of production on existing carp farms could result in production increase for 50 to 70 %. Thus, production in Serbia will reach 2 tons per hectare on average. Duration of the production period to obtain the market size carp will be shortened from 3 to 2 years.

Improvement of production on trout farms takes place through recently established selective breeding program, based on crossings of broodstock obtained in Serbia. By monitoring fry growth families with best growth rate and diseases resistance will be selected. That way production will increase and fish health protected against diseases that can be imported with imported fertilized eggs.

In order to increase fish farm area in trout aquaculture sufficient water quantity and first class quality is essential, as well as space for farm establishment. Compared to existing area, it can be increased 3 to 5 times.

Contrary to relatively small potential for increase of surface area under trout farms, available surface for carp farms is ten, even several tenths times greater. Thus, a rationale use of surfaces with low fertility located near rivers and canals will be provided. New surfaces under fish farms will significantly increase carp production in Serbia.

Potential for **fish feed production** is based primarily on raw material produced in Serbia, in Vojvodina (Northern part of Serbia). In the extruded feed for semiintensive carp production, a relatively small quantity (3 – 6 %) of fish meal is added but main components are soy, corn, wheat, barley, crops that are mainly exported from Serbia. It is much better to export fish feed or carp. Available resources for fish feed production in Serbia lie in 3 production companies producing carp feed. Their total capacity exceed 5 to 6 times actual need for fish feed in Serbia.

Concerning **fish processing,** capacities existing in Serbia are primarily related to carp. Since trade of carp in Europe is mainly connected with live fish. This largely increases the price of transport and stocking, thus a change in placement method is needed. These changes are related to the need to gradually include in carp trade chilled vacuum packed long lasting (20 to 40 days) packages. Since consumers easily accept smoked products, smocked carp vacuum packed in family (150 to 600 g) packages could be a challenging option for placement of this fish. In order to increase a range of products, a smoked carp could be combined with dry plums (traditional in Serbia), mushrooms, spices vegetables and herbs (Marković et al., 2011). Such processed carp could become much more attractive compared to live carp that looses battle against semi prepared other fish species.

EU is one of the biggest importers in the world. Annual deficit EU for the year 2008 is 1 550 000 tons of fish (Varadi, 2010). It represents a chance and a need to offer a smoked carp with added plums, mushrooms, herbs to European consumers. Such a product could find a market in countries that traditionally consumes carp (Germany, France, Poland, and Czech Republic). Additional improvement of the fatty acid content in carp flesh by the use of extruded feed will contribute to better selling of carp.

Potential of fishery in natural waters

Fishing potential could be divided on hilly mountain water currents potential and potential of the lowland rivers.

Potential of the hilly mountain water currents is based on recreational fishing, fishery tourism that will be developed for particular places. In order to create attractive offers, in the future restocking with fry obtained from parents from the same water body, thus preserving autochthonous genotypes. This will be possible by keeping record and master data on broodstock from the mountain water currents and by establishment of the data base on every broodstock. This is planned to be realized this year. In order to additionally improve the activity it is necessary to increase the number of new hatcheries based on spawning of autochthonous fish species.

Potential of lowland rivers is higher than the potential of mountain water currents. It is primarily based on rivers: the Danube, 588 km long in Serbia, with average flow of 2 413 m^3/s; the Sava 206 km in Serbia and average flow of 1 640 m^3/s; and the Tisza 164 km long in Serbia and average flow of 870 m^3/s. It is based on recreational and commercial fishery. Potential offered by recreational fishery is based on the fact that the city with the largest population, the capital Belgrade, is located on the banks of two largest rivers: Danube and Sava, as well as other big cities such as Novi Sad, Šabac, Smederevo… located on big river banks, pointing out that the number of recreational fishermen could be multiplied. Very important potential is in commercial fishery. By improving stocking policy, management and fish catch, and also the status of commercial fishermen, commercial fishery could represent better existence to a higher number of people. Particularly important segment and precondition for improvement of fish catch is organized buying up of the catch, as well as increased diversification of species caught. Part of fish caught could be further processed, or offered on fish markets. Thus, a number of persons and families involved in businesses connected with fishery will be increased.

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FISHERIES OF BOSNIA AND HERZEGOVINA

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RIBARSTVO BOSNE I HERCEGOVINE

Abstrakt

Bosna i Hercegovina ima stoljetnu tradiciju proizvodnje ribe, čiji početak datira još iz rane Austrougarske vladavine. Iako je zemlja sa dugom tradicijom proizvodnje ribe, još uvijek proizvodnja i potrošnja su na vrlo niskoj razini. U ovom radu prikazana je proizvodnja ribe u B&H, proizvodnja (morske ribe i vodeni organizama, proizvodnja slatkovodne ribe, šarana i pastrve). Također u radu su obrađeni podaci o B&H uvozu i izvozu ribe, te je procjenjena potrošnja ribe u 2009. god., predstavljena je riblja bilanca.

Prelazak na tržišno gospodarstvo u Bosanskohercegovačkoj poljoprivredi (uključujući i ribarsku-akvakulturnu proizvodnju) utjecalo je na pad proizvodnje, smanjenje zaposlenosti, tehnološka zaostalost, zaduženost, pogoršanje bilance trgovinske razmjene i nelikvidnost. Ulaskom B&H u članstvo WTO dodatno je povećana i onako jaka međunarodna konkurencija. Strateški prioriteti se nužno moraju odrediti, te da se redefiniraju prioriteti u svrhu reorganizacije i prenamjene postojećih resursa voda prilagođavajući akvakulturu i ribarstvo načelima održive i ekonomsko opravdane iskorištenosti. Sustavom poticaja i stimulacija treba obuhvatiti sve osobito obiteljska ribarska gospodarstva, a preradu ribe treba podići na veću razinu i iskoristiti instalirane kapacitete te proširiti na nove subjekte i proizvode. U svrhu zadovoljenja osnovnih pretpostavki navedenih u ovom radu nužno je educirati postojeću strukturu uposlenih u akvakulturi, športskom ribolovu te izobrazbu i zapošljavanje visokostručnih kadrova iz slatkovodnog ribarstva. B&H je zemlja sa snažnim potencijalom voda za višestruko korištenje, osobito za proizvodnju kvalitetne slatkovodne ribe. Cjelovit režim iskorištavanja i gospodarenja podrazumijeva očuvanje i razvoj samoodrživog ribarstva, čija ponuda može zadovoljiti B&H potrebe, a realno može se i izvozno orjentirati, poštojući tržište i ekološke zakonitosti koristeći se njima za marketinšku promidžbu kao atraktivnog izvoznog proizvoda. B&H raspolaže sa 24 kilometra morske obale, odnosno 1.400 hektara morske površine. Svakako da to nije veliki prostorni potencijal za razvitak marikulture, ali i kao takav on je još uvijek neznatno iskorišten.

Vodni potencijali B&H još uvijek nisu iskorišteni iako mogu predstavljati snažan izvor egzistencije stanovništva. U B&H je registrirano 46 poslovnih subjekata za proizvodnju konzumne ribe, od kojih 11 ima značajne objekte za proizvodnju mlađi. Tri subjekta su otpočela s preradom ribe. Pored registriranih subjekata, postoje i male obiteljske riblje farme, oko 100 ovih farmi imaju prosječnu proizvodnju 500-1.500 kg ribe. Od svih proizvodnih subjekata dva su na moru, dok su ostali na slatkim vodam, tekućim vodama i akumulacijama. Riba je prvi proizvod animalnog porijekla koja ima odobrenje za izvoz u EU.

Ključne reči: *B&H , ribarstvo, proizvodnja, uvoz, izvoz.*

INTRODUCTION

The records about organized fishing in Bosnia, dating from the early Austro-Hungarian rule are stating that the goverment`s first income from fishing came in 1882, and that the organized water quality protection and the legislature were introduced in 1886 (*Hamzić, 2003*). The beginnings of the economic fishing in Bosnia-Herzegovina are bound to fish farm "Vrelo Bosne", which was established in 1894. Development of carp fishing started in 1902, when a Polish citizen purchased land near Prijedor and Bosanska Gradiska and started building ponds.

There were 13 trout ponds in Bosnia in 1964, with a total area of 38 000 m^2. Intensive development of fisheries in B&H happened in 60- and 70-ies of the last century, when the largemouth fish farms were built with the capacity of 100-300 tons of fish/year. In the 1987-1989 period B&H has produced 2 997 tonnes of freshwater fish which represented 13% of the then Yugoslav production. The carp ponds produced 2 155 tons of fish for the market or 11%, and 842 tons of trout (19%) of then Yugoslav production (*Kosorić et al. 1991*). The development of cage fish farming in B&H started in 1987. There were already 607 tonnes of rainbow trout produced in 1990. (*Mikavica et al. 2001*).

MATERIALS AND METHODS

The standard *Desk research* method was applied (Research at the table, *Bazala*, 1973) using secondary data, as well as information obtained from the companies engaged in the fish production in B&H. We used historical and normative methods that are common in the agroeconomic research. The historical method was used in order to study the chronology of events in fish production. The normative method was used for collection and processing of many secondary data and information quoted or reported in the literature. The relationship between total production and fish market, was studied using the balance-sheet method and the assessement of further development of domestic fisheries was made using forecasting method.

RESULTS AND DISCUSSION

The Adriatic Sea is a relatively poor in quantity but rich in fish species that inhabit it. Catch of marine fish in B&H is small; it is disorganized, not yet registered; and is performed by fishermen selling their catch to the local market, mainly restaurants. There are 2 fish farms and shellfish in B&H sea area, each of 2500 m^2, with a total capacity of 300 tons and their production parameters are shown in Table 1.

Table 1*. The total production of marine fish and shellfish (2005-2009) in tons*

Production	**Years**				
	2005	**2006**	**2007**	**2008**	**2009**
1/ Fish					
European seabass (*Dicentrarchus labrax* L.)	70	75	75	60	80
Gilthead seabream (*Sparus aurata* L.)	85	85	80	80	95
Catch	15	15	15	15	15
Total fish	**170**	**175**	**170**	**155**	**180**
2/ Shellfish	65	65	66	70	70
Total (fish and shellfish)	**235**	**240**	**236**	**225**	**250**

Source: *Documentation of the company ''Ancora ''and '' ''Karaka and Herzegovina - Neretva County*

Production has increased significantly since 2005, so the total aquaculture production in 2009 reached 250 tons, and the catch was estimated to be 15 tons.

The cyprinid ponds are located in the northern plains of B&H in the Republic of Srpska. The production areas of the carp ponds are about 2800 ha (organized in 6 ponds), and the estimated size of the small ponds is about 500 ha. The production of carp (*Cyprinus carpio* L.) for the period 2005-2009 is presented in Table 2.

Table 2. *Production of carp in B&H from 2005 to 2009. in tonnes*

Production	**Years**				
	2005	**2006**	**2007**	**2008**	**2009**
Republic of Srpska					
Organized ponds	2.785	2.925	2.894	2.815	2.699
Small ponds	270	290	290	290	300
Federation of B&H					
Small ponds	100	120	120	130	130
TOTAL	**3.155**	**3.335**	**3.304**	**3.232**	**3.129**

Source*: Statistical Office of the Republic of Srpska, 2010. - Organized ponds; for small ponds - estimates.*

Production areas of carp ponds in the period 2003-2009 increased by 554 ha.

In salmonid fish farms is predominantly grown rainbow trout (*Oncorhynchys mykiss* Wal.), as well as some spawn and brown trout (*Salmo trutta* m. *fario* L.). A significant portion of production is realized in classical fish farms and in the cage fish farming system in hydro accumulations (HA) Bocac, Bileca, Rama, Grabovica, Salakovac and Mostar, with the evident growth trend (Table 3).

Table 3. *Production of salmonid fish in running waters and HA in B&H (2005-2009) in tons*

Type of fish	**Year**				
	2005	**2006**	**2007**	**2008**	**2009**
*Federation of B&H**	1.560	1.611	1.768	2.046	2.257
*Republic of Srpska***	1.070	1.151	1.276	1.609	1.594
Total	**2.630**	**2.762**	**3.044**	**3.655**	**3.851**

Source: **From the manufacturer's documentation and documentation HNC (2010), **Department of Statistics RS (2010).*

Production of salmonid fish (mostly rainbow trout) in running waters and the HA has reached 3851 tons in 2009 (42 % in the Republic of Srpska and 58 % in FB&H). It was estimated that there are about 100 small ponds in B&H, with capacity of 500-1500 kg.

There is no commercial fishing in B&H. According to data from SRS B&H there are 13200 fishermen registered in Federation, and 14020 in RS, with estimated annual catch of 433 tons of fish or 15,67 kg/fisherman - average of 2003-2009 period (*B&H Sports Fishing Association, 2009*).

The total fish production in B&H is presented in Table 4, showing that salmonid fish species (mostly rainbow trout) account for about 41 % of total production, cyprinids (carp and others) account for about 50%, marine fish (sea bass and sea bream) and other sea organisms account for 3,2%, and 5,5% accounts for fishermen catch. Total production in 2005-2009 period increased by 21%, and the increasing production trend is continued.

Table 4. *The total fish production in B&H (2005.-2009) in tonnes*

Type	**2005**	**2006**	**2007**	**2008**	**2009**
Freshwater fish - cyprinids	3.155	3.335	3.304	3.232	3.129
Freshwater fish - salmonids	2.630	2.762	3.044	3.655	3.851
The catch of sport fishermen	-	-	428	444	426
Saltwater fish	170	175	170	155	180
Other marine organisms	65	65	66	70	70
Total	**6.020**	**6.337**	**7.012**	**7.556**	**7.656**

Source: *From the manufacturer's documentation and documentation HNC (2010), Department of Statistics RS (2010).*

The total fish production could certainly be higher if the hatcheries could provide enough quality spawn, and if smaller producers could find an easier way to supply fish food which is nowadays entirely imported at much higher prices.

B&H in its official economic policy is proclaiming an open and competitive market of products and services. The demand unmet by domestic production, produced a high and diverse range of imported food products and fish.

Table 5. *The quantities of imported fish in B&H*

Year	Quantity (tons)	The value of BAM
2005	8.582,18	31.841.685,00
2006	14.885.28	52.134.381,85
2007	14.821,60	56.420.852,51
2008	16.808,62	67.033.450,26
2009	15.697,94	62.867.912,10
2010	13.963.50	57.858.798,92

Source: *Indirect Taxation B&H, 2010.*

B&H represents a small consumer power in the European and world relations, but that does not affect presence of many countries offering a wide range of fish and fish products, among which are especially prominent Croatia, Germany and Slovenia. Countries from which we import over 100 tons of fish are Spain, Thailand and Austria. The value of imported fish in 2008 amounted to 67 million BAM (Table 5), which was the largest amount in last 10 years, and the import of fish almost tripled compared to 1999. As for the amount of imported fish to B&H, Croatia is leading with 47,4%, then Slovenia with 18% and Germany with 10,7%. Import of marine fish and other marine organisms seems to be almost 100%, of which about 89% in sea fish, 5% in marine fish fillets. The remainder consists of mollusks, crustaceans, molluscs and other marine fish products. B&H is a significant importer but also a small exporter of fish (Table 6).

Table 6. *Quantities of fish exported from B&H*

Year	Quantity (tons)	The value of BAM
2005	1.739,67	6.294.547,00
2006	4.964,81	18.918.150,55
2007	4.086,09	18.054.514,53
2008	5.423,26	24.466.891,37
2009	4.975,69	22.945.066,47
2010	3.820,45	16.981.051,33

Source: *Indirect Taxation B&H , 2010.*

The export of fish increased in quantity and value since 2006, and the main reason for this is the capacity-building for fish processing and packing. The main importers of fish from Bosnia are Serbia, Montenegro, Slovenia and Hungary.

In recent history, B&H is a permanent net importer of food and fish constantly being among the imported products. The negative trade balance of fish only mirrors the overall trade balance of food. On the other hand, the export of food, after an initial good growth, with some variations, is showing signs of encouragement, but on the whole it still remained small with strong barriers to development (*Selak et al.2003*). The import of fish does not exceed 1% of the total food imports in B&H.

***Table** 7. Values and quantities of imports and exports of fish in B&H*

Description	***2007***	***2008***	***2009***	***Index***
A/ Value in millions BAM				*2009/2007*
Import	56.420.852,51	67.033.450,26	62.867.912,10	*111*
Export	18.054.514,53	24.466.891,37	22.945.066,47	*127*
Balance	**- 38.366.337,98**	**- 42.566.558,89**	**- 39.922.845,63**	***104***
B/ Quantity in tonnes				
Import	14.821,60	16.808,62	15.697,94	*106*
Export	4.086,09	5.423,26	4.975,69	*122*
Balance	**- 10.735 ,51**	**- 11.385,36**	**- 10.722,25**	***100***

According to FAO estimates, the average fish consumption per capita for the period 1997-1999 was 1,6 kg in B&H (*FAO, 2000*); it was 2,8 kg for the period 1999-2001 (*FAO, 2003, 2005*), and 3,88 kg in 2007 (*Pavlicevic, 2007*). The evaluation presented in this paper was done using model applied in Croatia, where the consumption is automatically increased by 20-30%. Table 8 shows the total realized and by us estimated (increased by 25%) per capita fish consumption.

***Table 8**. Fish consumption in B&H*

Elements	**Year**		
	2007	**2008**	**2009**
Available at tons;			
a / Calculated	17.747	18.741	16.645
b / increased by 25 %	22.184	23.426	20.806
Present population in thousands	3.922	3.961	4.001
Consumption kg/capita			
a / calculated	4,52	4.73	4,16
b / increased by 25 %	5,66	5,91	5,20

***Source:** Authors' calculations*

Fish consumption per capita in B&H, even with an increase of 25%, thus providing the higher consumption of 5 kg/capita (Table 8), still remained rather low.

CONCLUSION

Bosnia and Herzegovina with its current production, meets only about 31% of its already modest consumer needs, and imports 69%, the import - export balance being negative in 2009 (-10 721 tonnes, or about 40 million). Fish consumption per capita is still quite small (5-6 kg/capita 2007-2009), although since 2003 significantly increasing.

Water resources, which relate only to the lake accumulations, provide opportunities for a strong development of aquatic production in B&H. Only with an increase in production in already built carp and trout fish farms (better use) and with fish from stagnant water, a significant market surplus of fish and products can be created.

Fishing, with full economic, technological, environmental, social and other arguments can stand out as an important branch in the general development of the domestic food production. There are significant obsticles in the fishery development, the main problems being lack of favorable credit lines and insufficient incentives. Therefore, the scientific and professional approach, accompanied by the appropriate development strategies and legislations in B&H, would certainly be desirable, and it would put this area on the list of activities that should not wait for implementation.

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IMPORTANCE AND PRINCIPLES OF GENETIC IMPROVEMENT IN AQUACULTURE PRODUCTION

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ZNAČAJ I PRINCIPI GENETSKOG UNAPREĐENJA U AKVAKULTURI

Need for genetic improvement programs High yields obtained in agriculture rely heavily on the use of domesticated and genetically improved breeds and varieties. Until quite recently this has in general not been the case for most farmed aquaculture species that, in the genetic sense, are still much closer to the wild state than are the major terrestrial animals and food crops. Less than 10 % of the total world aquaculture production is based on improved strains. Due to a growing human population and a decline in production from capture fisheries, there is therefore a great disparity between the need for increased aquaculture production and the genetic quality of the strains available to meet that need. Moreover, full benefits of investments in management improvements (feed and feeding practices, rearing water quality, control of diseases, etc.) can only be obtained through the use of genetically improved animals.

Potential for genetic improvement Prospects of genetic improvement of economically important traits are well documented in several fish species (Gjedrem, 1997). In general, estimates of heritability for traits of economic importance in fish species are within the range of those observed for terrestrial species while the magnitude of non-additive genetic effects has been shown to be more important than previously assumed. For growth rate, genetic gains of about 10% of the mean per generation are frequently reported, implying that growth rate can be doubled over a period of seven to eight generations. Such changes will significantly increase both turnover of production and resource efficiency (feed, land, water) and hence reduce cost of production. Another benefit of genetic improvement programs is development of strains with better product quality for the benefit of both producers and consumers.

Research and education Most of the genetic research activities in aquaculture species in many countries have until quite recently been on the development of inter- and intraspecies hybrids and studies in cytogenetics, biochemical genetics and biotechnology including cell, chromosome and gene engineering. Very little research has been on quantitative genetics and genetic improvement through selection. The main reason is the shortage of courses in quantitative genetics and selection theory at fisheries colleges and universities. Consequently, the scientists have had little knowledge about the great potential for genetic improvement of aquaculture species through selective breeding.

Special characteristics of fish The very high fecundity and the possibility of collecting eggs and semen separately in many aquaculture species facilitates a wide range of mating designs, and allows very high short-term genetic gains through intense and accurate selection. On the other hand, the use of few breeding animals can lead to a rapid accumulation of inbreeding and therefore implementation of measures for restricting inbreeding is essential in fish nucleus selective breeding programs. In order to control inbreeding and to use information on relatives in selection decisions (and thus to increase accuracy of selection) genetic relatedness among the breeding candidates needs to be monitored. However, a specific problem in fish breeding programs is the difficulty of uniquely identifying individuals at hatching due to their small size. In most large scale programs this was solved by keeping fullsib families in separate units until the fish are large enough to be individually tagged, but to a relatively high cost. The separate rearing may also induce environmental effects common to the members of the same family which reduces the genetic gain. The application of DNA-markers (e.g. microsatellites) for parental assignment of individuals in mixed family groups can solve this problem, but the technology is still relatively expensive.

Opportunities and challenges The first large scale selective breeding program for farmed fish was set up for Atlantic salmon (and rainbow trout) in Norway in the nineteen seventies (Gjøen and Bentsen, 1997). Its design was based on basic knowledge in quantitative genetics, experiences from livestock breeding programs and available technologies. Full-sib families were reared separately until family identification was obtained by cold-branding and fin-clipping. With the exception of some improvements (e.g. individual tagging at a smaller size using PIT-tags), rather few changes have been taken place since then. Other family based programs for salmonids and also other species, developed mainly in the late nineteen eighties and nineties, have followed, to a large extent, the same breeding design. Presently, about 30 improvement programs that use sib information in the selection decisions are in operation in the word today involving nine different species.

The opportunities and challenges in designing sustainable fish breeding programs have been discussed in general terms (e.g. Gjerde *et al*., 2002), but studies on optimum designs are few and limited to programs that apply individual (mass) selection. The design of current programs is thus only partially based on well-defined scientific grounds and research is needed to adapt and develop new theory and tools to account for the special reproductive characteristics of fish species and their small size at hatching that make physical tagging not possible. In particular, research is required for determining how best 1) define optimum mating and selection decisions for maximising gain while restricting rate of inbreeding; 2) create sufficient connectedness between generations, sub-populations and cohorts in order to measure and monitor genetic levels and gain; 3) estimate and exploit non-additive genetic variance; 4) use DNA markers for genome wide selection; and 6) to efficiently disseminate the improved genetic material to the industry.

Interaction between cultured and wild fish Repeated cycles of reproduction and selection in captivity will inevitably result in genetic changes in the nucleus breeding population. This raises the concern of possible adverse impact of genetically improved escapees on wild strains. It is in all interest to prevent cultured fish from escaping, but no large-scale aquaculture operation can be regarded as completely escape proof. A limited gene flow from genetically well managed aquaculture strains into wild strains of the same genetic origin should be tolerated if the alternative is to ban selective breeding programs for aquaculture. As long as genetically improved strains are available somewhere, they will most likely find their way, legally or illegally, into aquaculture farms in the absence of improved local strains (e.g. Bentsen et. al., 1992).

Conclusions Genetic improvement programs are important to make farmers more competitive in future food markets. Highest priority should be given to the development of breeding programs for species with high present or future production. The breeding programs should be based on purebreeding (to capitalise on additive genetic effects) and utilise both individual and/or family information in selection of breeders. For less important economic species, simpler programs utilising only individual information primarily for growth should be initiated and later extended to more advanced programs if production increases. Crossbreeding (to capitalise on non-additive genetic effects) and sex and ploidy manipulation (Hulata, 2002) may be applied to further improve the productivity of the grow-out animals. Recent and future developments in molecular genetics present the possibility of using genotype selection for performance traits (either through marker assisted or genome wide selection. Methodology for efficiently integration of these technologies into classical selective breeding programs needs further investigation.

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ZNAČAJ I PRINCIPI GENETSKOG UNAPREĐENJA U AKVAKULTURI

Potreba za programima genetskog unapređenja. Visoki prinosi koji se dobijaju u akvakulturi oslanjaju se u ogromnoj meri na domestifikovane i genetski unapređene rase i podvrste. Do skora ovo nije bio slučaj za većinu gajenih vrsta u akvakulturi, koje su, u genetskom smislu, još uvek mnogo bliže divljim vrstama nego što je većina suvozemnih životinja i ratarskih kultura. Manje od 10 % ukupne svetske proizvodnje u akvakulturi se zasniva na unapređenim sojevima. Rast humane populacije i opadanje ulova ribe dovelo je do velike razlike između potrebe za povećanjem proizvodnje u akvakulturi i genetskog kvaliteta sojeva koji su na raspolaganju za povećanje proizvodnje. Povrh toga, puna korist investicije u unapređenje upravljanja u akvakulturi (hrana i načini ishrane, kvalitet vode, kontrola bolesti itd.) se jedino može dostići upotrebom genetski unapređenih životinja.

Potencijal za genetičko unapređenje. Mogućnosti genetskog unapređenja ekonomski važnih osobina dobro su dokumentovane za nekoliko vrsta riba (Gjedrem, 1997). Uopšteno, procene heritabilnosti za ekonomski važne osobine ribljih vrsta nalaze se u okviru vrednosti određenih za terestrične vrste, dok se pokazalo da je magnituda neaditivnih genskih efekata veća nego što se prethodno pretpostavljalo. Za stopu rasta, genetska dobit od oko 10 % od srednje vrednosti po generaciji je često prijavljivana ukazujući da se stopa rasta može udvostručiti u toku perioda od 7 do 8 generacija. Ovakve promene će značajno uvećati prihod od proizvodnje i efikasnost korišćenje resursa (hrana, zemljište, voda) te tako umanjiti troškove proizvodnje. Druga korist od genetskog unapređenja je razvijanje rasa sa boljim proizvodnim osobinama u korist i proizvođača i potrošača.

Istraživanje i obrazovanje. Većina genetskih istraživanja vrsta koje se gaje u akvakulturi u mnogim zemljama zasnivala su se do skora na razvijanju inter i intra specijskih hibrida, kao i studijama iz citogenetike, biohemijske genetike i biotehnologije uključujući ćelijski, hromozomski i genetski inženjering. Malo je bilo istraživanja iz kvantitativne genetike i genetskog unapređenja putem selekcije. Osnovni razlog je nedostatak kurseva iz kvantitativne genetike i teorija selekcije na visokoškolskim institucijama gde se izučava ribarstvo. Kao posledica, naučnici nemaju dovoljno znanja o ogromnom potencijalu za genetsko unapređenje vrsta koje se gaje u akvakulturi putem selekcije (selektivnog uzgoja).

Osobenosti riba. Veoma visoka plodnost i mogućnost odvojenog prikupljanja jaja i mleča od vrsta koje se gaje u akvakulturi omogućava širok raspon šema ukrštanja i dozvoljava veliku genetsku dobit u kratkom vremenskom roku kroz intenzivnu i preciznu selekciju. Sa druge strane, upotreba malog broja matica može brzo da dovede do aakumulacije efekata inbridinga (ukrštanja u srodstvu), tako da je implementacija mera za prevazilaženje inbridinga u središtu programa selektivnog uzgoja. U cilju kontrole inbridinga, ali i iskorišćavanja srodničkih odnosa u selekciji (a tako i u cilju povećanja preciznosti selekcije) genetička srodnost između kandidata za ukrštanje mora da se prati. Poseban problem u selekciji riba je poteškoća u identifikaciji individua prilikom izleganja, usled njihovih malih dimenzija. U većini velikih programa selekcije ovo se rešava čuvanjem sestrinskih familija u odvojenim jedinicama dok ribe dovoljno ne odrastu da budu tagovane (obeležene), i to se radi po relativno visokoj ceni. Posebno gajenje može da dovede do ispoljavanja sredinskog efekta kod članova iste familije što umanjuje genetsku dobit. Upotreba genetskih markera (na primer mikrosatelita) za određivanje roditeljskog porekla jedinki iz grupa izmešanih familija može da reši ovaj problem, ali je tehnologija još uvek relativno skupa.

Mogućnosti i izazovi. Prvi veliki program selektivnog uzgoja za gajenu ribu uspostavljen je za Atlanskog lososa i kalifornijsku pastrmiku) u Norveškoj sedamdesetih godina 20 veka (Gjøen and Bentsen, 1997). Dizajn ovog programa zasnivao se na osnovnom znanju kvantitativne genetike, iskustvu iz uzgoja stoke i tehnologiji koja je bila na raspolaganju. Sestrinske familije su gajene odvojeno do trenutka označavanja familija hladnim žigosanjem i obeležavanjem (zasecanjem) peraja (cold-branding and fin-clipping). Sa izuzetkom nekih poboljšanja kao što je individualno obeležavanje PIT čipovima, malo je izmena u metodi od tada učinjeno. Drugi programi zasnovani na selekciji familija kod salmonida i drugih vrsta, razvijeni krajem osamdesetih i devedesetih sledili su u velikoj meri isti dizajnuzgajanja . Trenutno u svetu postoji oko 30 programa unapređenja osobina koji koriste informacije o sestrinskim familijama u procesu selekcije za 9 različitih vrsta u akvakulturi. Mogućnosti i izazovi dizajniranja održivih programa selekcije riba razmatrani su načelno (e.g. Gjerde *et al*., 2002), ali i dalje nedostaju istraživanja u vezi optimalnog dizajna ukrštanja koja su ograničena na individualnu (masovnu) selekciju. Dizajn trenutnih programa je tako samo delimično zasnovan na dobro definisanoj naučnoj osnovi i neophodno je dalje istraživanje za prilagođavanje i razvijanje novih teorija i tehnika. Nove metode su neophodne za prevazilaženje specifičnih reproduktivnih karakteristika ribljih vrsta i nemogućnosti obeležavanja usled malih dimenzija jedinki. Neophodna su istraživanje radi određivanja kako najbolje: 1) definisati optimalno ukrštanje i selekcione odluke koje će uvećati genetsku dobit i umanjiti stopu inbridinga; 2) stvoriti dovoljnu povezanost između generacija, subpopulacija i kohorti da bi se izmerili genetski parametri i dobit; 3) proceniti i iskoristiti ne aditivnu genetsku varijansu; 4) upotrebiti DNK markere za selekciju genoma; 6) efikasno umnožiti i proslediti unapređen genetski materijal proizvođačima.

Interakcije između gajenih i divljih vrsta. Ponovljeni reproduktivni ciklusi i selekcija u uzgoju će neminovno rezultirati genetičkom promenom u nukleusu uzgojne populacije. Ovo izaziva zabrinutost o mogućem nepovoljnom uticaju genetiski unapređenih odbeglih jedinki na divlje sojeve. U interesu je svih strana je sprečavanje bežanja gajene ribe, ali nema velikih operacija u tom smislu. Ograničen genetski upliv u divlju iz genetski unapređenegajene sorte istog genetskog porekla može se tolerisati ukoliko je alternativa zabranaprograma selekcije u akvakulturi. Dokle god genetski unapređene sorte negde postoje, naći će svoj put do farmi riba, u nedostatku lokalnih sorti, legalno ili nelegalno (e.g. Bentsen et. al., 1992).

ZAKLJUČCI

Programi genetskog unapređenja su značajni jer povećavaju kompetitivnost farmera na budućem tržištu. Potrebno je dati veći prioritet razvoju uzgojnih programa selekcije za vrste sa viskom sadašnjom ili budućom proizvodnjom. Programi selekcije bi trebalo da budu zasnovani na iskorišćavanju aditivnog genetičkog efekta i korišćenju i individualnih i/ili informacija o familijama prilikom izbora matica. Za ekonomski manje važne vrste jednostavniji programi koji koriste samo informacije o individuama, uglavnom prirast, trebalo bi da ubuduće prerastu u naprednije programme, ukoliko dođe do povećanja proizvodnje. Ukrštanje radi iskorišćavanja neaditivnog genetskog efekta i pola i manipulacija ploidijom (Hulata, 2002) mogu se primeniti za dalje unapređenje produktivnosti životinja u uzgoju. Skorašnji i budući razvoj molekularne genetike predstavlja mogućnost upotrebe selekcije genotipova za proizvodne osobine, ili kroz upotrebu markera ili selekcije celog genoma. Metodologija efikasne integracije ovih tehnologija u klasične programe selekcije zahteva dalja istraživanja.

IMMUNOPREVENTION IN INTENSIVE FISH CULTURE

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IMUNOPREVENCIJA U INTEZIVNOM SISTEMU GAJENJA RIBA

Hemoterapeutici, posebno antibiotici, su dosta korišćeni u intentzivnom sistemu gajenja riba, ali se danas smatra da njihovu potrošnju treba svesti na minimum. Intenzivno korišćenje antibiotika je indukovalo rezistenciju mnogih ne patogenih, ali i patogenih bakterija. Ovakva situacija se smatraju velikom pretnjom za ljudsko i životinjsko zdravlje. Sa razvojem novih imunopreventivnih metoda u akvakulturi, upotreba antibiotika i drugih hemoterapeutika u gajenju riba je značajno redukovana tokom proteklih godina.

Imunoprevencija je novi koncept kontrole zdravlja i bolesti riba koji obuhvata klasičnu specifičnu imunoprofilaksu (vakcine) i nespecifične imunomodulacije ili imunostimulacije. Takođe, aplikacija pro- i prebiotika u ishrani su veoma važan deo efektivne nespecifične imunoprevencije u intezivnom sistemu gajenja riba.

Specifična imunoprofilaksa (vakcine)

Zaštita riba od bolesti primenom imunizacije je godinama predstavljao važan koncept u akvakulturi. Međutim, odbrambeni mehanizmi nižih vertebrata su u neku ruku drugačiji od onih kod sisara, i neke tehnike vakcinacije, kada se primene u uslovima mrestilišta, nisu tako efikasne kao što se očekuje. Jedna od najčešćih nedoumica koje se odnose na upotrebu vakcina je koliko će zaštita da traje. Stoga, istraživanje je usmereno u pravcu poboljšavanja potencije i efikasnosti vakcina kao i kako optimalno aktivirati nespecifične odbrambene mehanizme, ćelijski imnitet kao i specifični imuni odgovor. Vakcinacija riba ima dugu istoriju, i komercijalne vakcine su dostupne za više od polovine glavnih bakterijskih i viralnih oboljenja u akvakuturi. Vakcine za ribe koje se apliciraju injekcijama, imerzijom ili se daju kroz hranu, su postale rutina u specifičnoj zaštiti protiv bakterijskih i virusnih infekcija. Međutim, do sada nisu napravljene komercijalne vakcine protiv parazitskih invazija. Uglavnom su to antibakterijske vakcije (protiv *yersinioze*, *Furunculoze* ili *vibrioze*), bazirane na inaktiviranim bakterijskim suspenzijama. Takođe su značajno razvijene auto-vakcine bazirane na lokalnim patogenim bakterijama. Ove auto-vakcine pružaju dobru ili adekvatnu zaštitu i mogu da se koriste kao imerzione vakcine, korisne za mlade ribe. Žive ili atenuirane bakterije ili virusi su takođe bili uspešni u pripremanju vakcina, na primer, protiv virusa koi herpesa (KHV) kod šarana. Povezani rizici od reverzije na virulentne forme će limitirati opštu upotrebu takvih vakcina u akvakulturi. Vakcine za ribe bazirane na molekularnoj tehnologiji, uključujući i DNK vakcine i upotreba rekombinantnih proteinskih antigena daju dobre rezultate. Međutim, praktični problemi su do sada ometali opštu upotrebu molekularne tehnologije u proizvodnji anti-viralnih vakcina u akvakulturi. Ova vrsta vakcina koristi rekombinantne proteine, uglavnom površinske viralne glikoproteine produkovane u bakterijama ili intramuskularne injekcije plazmida DNK koji kodiraju odgovarajući antigen. Mnoge od ovih ekperimentalnih DNK vakcina su relativno brzo dale visokospecifičnu i dugotrajnu zaštitu u veštačkim infekcijama (challenge tests). Negativna strana je više etičko pitanje koje se odnosi na sudbinu bakterijskih plazmida DNK u organizmu riba. Eksperimentalne studije su pokazale da se oni nepotpuno razgrađuju na mestu injektiranja i mogu dospeti u druga tkiva ili čak biti izlučena iz tela. Ovo dovodi do opasnosti od introdukcije plazmida u druge slučajne domaćine sa nesagledivim posledicama po životnu sredinu.

Uticaj vakcina na zaštitu od bolesti, odnosno efikasnost vakcine, zavisi od vrste ribe, starosti, kondicije i kvaliteta vode, naročito temperature u vreme vakcinacije. Specifična antitela su primarni imuni odgovor, i jedinstvena u fiziologiji riba, jer ovi molekuli imunoglobulina su specifično usmereni protiv individualnih antigena. Uobičajeni Ig riba je tetramerna forma antitela uglavnom označena kao IgM. Suprotno tome, dimerna forma je česta (IgG) i efikasna kod sisara, a pentamerna forma (IgM) je molekul koji se produkuje rano tokom infekcije ili imunizacije vakcinom. Specifično antitelo predstavlja molekul koji ima sledeće funkcije:

- može da stvara komplekse sa antigenima i da inicira druge serumske proteina da započnu komplementarnu kaskadu u liziranju invazivnih patogena.,
- može biti stacionaran ili pričvršćen za ćeliju ili može da predstavlja kominikator koji upravlja cirkuisanjem citotoksičih ćelija u prepoznavanju ili napadu na patogeni agens.

Iniciranje specifičnog imunog odgovora je kontrolisano antigenima koji predstavljaju ćelije (APC, uglavnom makrofagi) koje učestvuju u preuzimanju čestica ili antigena. Informacija o tome kako da se napravi antitelo protiv antigena se prenosi na B ćelije. Kompleksni receptori na ćelijama, kao što su veliki histokompatibilni kompleksi, koji nadgledaju selekciju antitela, uz pomoće informativnih hemijskih molekula, citokinina i interleukina, su uključeni u ekspresiju i kontrolu sekrecije antitela. Specifičan imuni odgovor, može da pamti, i kad se ribe susretnu sa patogenima po drugi put, specifična antitela se mnogo brže i u većeoj količini proizvode.

Starost riba i temperatura okruženja su kritični faktori za uspeh vakcina u akvakulturi. Potrebno je da larve i mlađ riba imaju potpuno razvijene adaptivne sisteme pre nego što se vakcinišu. Mnoge studije su pokazale da je vakcinaciona kupka kalifornijske pastrmke protiv enterične bolesti crvenih usta jedno uspešna na 15 °C ali ne i na temperaturama ispod ili iznad ove. Takođe, naši eksperimenti su pokazali da optimalna temperatura za različite vrste riba determiniše efikasnost vakcina protiv furunkuloze ili jersinioze. Variranje temeprature vode takođe utiče na stepen i dužinu adaptivnog imunog odgovora koji sledi nakon vakcinacije.

Zapravo, ustraživanja su usmerena ka potenciju i efikasnosti antigena kao i kako optimalno aktivirati ne samo specifične humoralne imune odgovore, već i nespecifične odbrambene mehanizme sa celularnim i humoranim imunim odgovorom. Kada se riba inicijalno susretne sa patogenom, nespecifični odbrambeni mehanizam je važniji nego specifični imuni odgovor, jer ovaj zahteva puno vremena za stvaranje antitela i specifičnu celularnu aktivaciju. Generalno, ribe imaju kratak životni vek i većina živi u hladnom okruženju koje usporava razvoj specifičnog imunog odgovora.

Nespecifična imunostimulacija i imunomodulacija

Barijere nespecifične odbrane koje su već prisutne indukuju epitelijalni štit od krljušti, kože i mukusa. Ukoliko je patogeni agent zarobljen mukopolisaharidnim kompleksima mukusa, može biti odstranjen sa ribe ili razgrađen litičkim enzimima. U većini slučajeva, patogeni mikroorganizmi bivaju uništeni digestivnim ili litičkim enzimima. Infekcije se mogu desiti na mestu oštećenog tkiva, što rezultuje u migraciji leukocita u oblast rane i porasta koncentracije serumskih komponenata, uključujući lizozome, transferin, C-reaktivni proteina, ceruoloplazmin i komplementarne komponente.

Fagocitoza i fagociti su glavno oruđe nespecifičnog imunog odgovora na patogene mikroorganizme kao što su virusi, bakterije, gljive i paraziti. Funkcije fagocita efektora su značajne, ali je najvažnije njihova sposobnost da ubijaju patogene. Viralni agensi mogu indukovati produkciju interferona pomoću viralno inficiranih ćelija, koje stimulišu druge ćelije da aktiviraju intracelularne endonukleaze koje će uništiti viralne DNK i RNK i zatvoriti fiziološke puteve koje virusi koriste za replikaciju. Ovaj fenomen se dešava posredstvom jedne populacije nespecifičnih citotoksičnih ćelija (NC). Slično natural kiler (NK) ćelijama, NC ćelije su ne-T, ne-B ćelije koje nemaju sposobnost fagocitiranja. Ove ćelije produkuju vrlo visok nivo citotoksičnih substanci protiv velikog broja ciljanih ćelija.

Upotreba imunomodulatora ili imunostimulatora u intenzivnom sistemu gajenju riba pruža veliki izbor atraktivnih metoda za indukovanje i povećavanje zaštite protiv infektivnih oboljenja. Uopšteno, imunostimulanti i/ili imunomodulatori čine grupu sintetičkih i bioloških jedinjenja koja pojačavaju ćelijski i humoralni imunitet u ljudima, životinjama kao i u ribama. Neki od njih stimulišu samo odbrambene mehanizme, drugi su sposobni da oporave ili moduliraju imunitet nakon supresije indukovane ksenobioticima. Stimulacija odbrambenog mehanizma može biti naročito važna za ribe koje su gajene u ili puštene u okruženja gde su vrste ili serotipovi patogena nepoznati pa imunizacija specifičnim vakcinama može biti beskorisna. Levamisole je bio jedan od prvih imunostimulanata koji je korišćena na ribama radi podizanja nespecifičnih celularnih i humoralnih odbrambenih mehanizama. Drugi imunostimulanti i modifikatori biološkog odgovora koji su korišćeni u istraživanjima na ribama uključuju dimerizovane lizozime (KLP-602), hitozan, bakterijski lipopolisaharid, HMB, metisoprinol i glukani. Upotreba probiotika značajno prisutna u gajenju životinja a od skoro je počela da se koristi u gajenju riba i dala generalno dobre rezultate. Kako funkcionišu probiotske bakterije u različitim životinjama još uvek nije poznato. Smatra se da žive probiotske bakterije utiču na intestinalnu floru svojom antagonističkom aktivnosti i kompeticijom za nutrijente i prostor, redukujući tako broj patogenih bakterija. Probiotici takođe, smatra se, poboljšavaju apetit i rast. Osim toga, stimulišu prirodnu odbranu putem TLR-a, drugim celularnim receptorima, ali da bi do ovog došlo, probiotske bakterije ili njihovi produkti moraju da prođu kroz mukoznu i epitelijalnu barijeru alimentarnog trakta i pripreme normani odbrambeni mehanizam riba. Blagotvoran uticaj može biti dugotrajan, minimalno mesec dana nakon aplikacije u hranu.

Uticaj imunomodulatora i/ ili imunostimulatora na celularni mehanizam riba se može utvrditi uzimanjem uzoraka neletalne krvi i hematopoetskih organa i praćenjem promena u brojnosti leukocita, aktivnosti i funkcije. Makrofage, neutrofili i druge fagocitarne ćelije povećavaju mobilizaciju enzima, oksidativnu radikalnu produkciju i fagocitarnu aktivnost. Subpopulacija T limfocita povećava proliferacioni odgovor i citotoksičnu aktivnost, a povećava i proliferativni odgovor B limfocita kao i nivo antitela sekretornih ćelija. Uticaj imunostimulatora na humoralni odbrambeni mehanizam riba se može pratiti u uzorcima krvi, analizom promena Ig u serumu ili plazmi i nivoa specifičnih antitela, lizozima ili ceruplazminske aktivnost i nivoa citokinina.

Imunostimulanti se mogu koristiti na sličan način kao hemoterapeutici ili hemijske materije ili u kombinaciji sa vakcinama. Ribe se mogu pripremiti za ovakve buduće tretmane sezonskim izlaganjem patogenima ili stresom rukovanja. Mnogi parametri okruženja i fiziološki parametri utiču na ekperimente i protokole za upotrebu imnostimulanata za ribe, kao što je vreme, oprema za doziranje, temperatura okruženja, stabilnost svake komponente, karakteristika vakcine i vrsta ribe. Prvo razmatranje je da li će se substanca koristiti samostalno, kao jedan tretman ili će biti davana zajedno sa programom za imnuizaciju.

Nekoliko proizvođača riblje hrane sada nude obroke sa dodatnim prirodnim i sintetičkim imunomodulatorima za upotrebu u akvakuturi. Trenutno je preporučeni način davanja imunostimulanata u hrani svaki dan tokom jedne do dve nedelje. Mnoga pitanja ostaju otvorena koja se odnose na potrebe i korišćenje imunomodulatora, posebno da bi se odredio načina davanja, odgovarajuća veličina doze, vreme i dužina apliciranja, kao i uticaj na okruženje.

IMMUNOPREVENTION IN INTENSIVE FISH CULTURE

Chemotherapeutics, but especially antibiotics have been used extensively in intensive fish culture in may years but the present consensus is that should be kept at minimum. Excessive used of antibiotics induced resistance of many non-pathogenic but also pathogenic bacteria. These situations are considered a major threat to human and animal health. With the development of new immunoprevention methods in aquaculture, the use of antibiotics and other chemotherapeutics in fish culture has been greatly reduced in the past years.

Immunoprevention is a new concept of control fish health and diseases that involves classical specific immunoprophylactic (vaccine) and nonspecific immunomodulation or immunostimulation. Also the application of pro- and prebiotic in diets are very important part of effective nonspecific immunoprevention in intensive fish culture.

Specific immunoprophylactic (vaccines)

The protection of fish against diseases by immunization has been an important concept for many years in aquaculture. However, defence mechanisms in the lower vertebrates are somewhat different from those in mammals, and some vaccination techniques when actually applied to hatchery conditions are not as effective as they should be. One of the most frequent uncertainties regarding the use of vaccines is how long the protection will last. Therefore, research is concentrating on how to improve the potency and efficacy of the vaccines and how to optimally activate the nonspecific defence mechanisms, cell-mediated immunity and the specific immune response. Fish vaccination has a long history but commercial vaccines are available for more than half of the major bacterial and viral diseases in aquaculture. Fish vaccines administrated by injection, immersion or in the feed are now start to by routinely used in specific protection against bacterial or viral diseases. However, no commercial vaccines have so far been produced against parasitic invasions. Generally anti-bacterial vaccines (against *Yersiniosis*, *Furunculosis* or *Vibriosis*) are based on inactivated bacterial suspension. Also, auto-vaccines based on the local pathogenic bacteria are very intensively are developed. These auto-vaccines have given good or adequate protection and can be used as immersion vaccines, useful for younger fish. Also live attenuated bacteria or virus have also been used successfully in vaccine preparation, for example, against koi herpes virus (KHV) infection in common carp. However, associated risks of reversion to virulent forms will limit the general use of such vaccines in aquaculture. Fish vaccines based on molecular technology, including DNA vaccines and the use of recombinant protein antigens given promising results. But practical considerations have so far hindered the general use of molecular technology for production anti-viral vaccines in aquaculture. This type of vaccine, make use of recombinant proteins, mainly surface viral glycoproteins, produced in bacteria or of intra muscular injection of plasmid DNA encoding the antigen of interest. Many of these experimental DNA vaccines have given relatively fast, high specific and long time protection in challenge test. But the negative side is the ethical question of what happens to the bacterial plasmid DNA in the fish organism. Experimental study showed that this is not completely degraded at the injection side and may reach other tissues or even be secreted. This introduces the danger of the plasmid being introduced to another unrelated host with unforeseeable consequences for the environment.

The influence of vaccine on the protection against disease – vaccine effectiveness is depending on the fish species, the age of fish, their condition and quality of water, especially the temperature in vaccination time. Specific antibody is the primary result of the specific immune response, and unique in the fish physiology because these immunoglobulin molecules are specifically directed against individual antigens. The common Ig in fish is a tetrameric form of antibody and is usually designated as IgM. By contrast, the dimeric form is common (IgG) and efficient in mammals with the pentameric form (IgM) being a molecule produced early in infection or immunisation by vaccine. The specific antibody is a recognition molecule and as such has several function:

- it can complex with antigens and initiate other serum proteins to begin a complement cascade in lysing invasive pathogen,
- it can be stationary or attached to cells or act as a communicator to direct circulating cytotoxic cells to recognise and attack pathogenic agents.

The initiation of the specific immune response is controlled by antigen presenting cells (APC, mostly macrophages) involved in particle or antigen uptake. The information concerning how to make antibody against is transferred to the B cells. Complex receptors on the cells, such as the major histocompatability complexes, that guide the selection of the antibody, assisted by messenger chemical molecules, cytokines and interleukins, that are involved in the expression and control antibody secretion. The specific immune response can hold memory, and when the fish meet the pathogen for a second time, specific antibody is more rapidly produced and in a greater quantity.

The age of the fish and environmental temperature are a critical factors for successful of vaccination in aquaculture. Fish larvae and fry need to have fully developed adaptive system before vaccination is attempted. Many studies showed that bath vaccination of rainbow trout against enteric red mouth disease is only successful at 15°C but not at temperature below or above this. Also our experimental study showed that optimal temperature for fish species determined the effectiveness of vaccines against *Furunculosis* or *Yersiniosis*. Variations in water temperature can also affect the magnitude and duration of adaptive immune response following vaccination.

Actually research is concerned on the potency and efficacy of the antigens and how to optimally activate not only specific humoral immune response but also nonspecific defence mechanisms with cellular and humoral immune responses. When a fish initially encounters a pathogen, the nonspecific defence mechanisms are more important than the specific immune response, as the latter requires a long time for antibody build-up and specific cellular activation. In general, fish have short life spans and most live in cool water environments which slow development of the specific immune response.

Nonspecific immunostimulation or immunomodulation

The nonspecific defence barriers already in place induce physical epithelial shield of the scales, skin and the mucus. If an pathogenic agent is entrapped by the mucopolysaccharide complexes of the mucus, it may be scuffed from the fish or held to be digested by the mucus lytic enzymes. In most cases, the pathogenic microorganisms are destroyed by digestive and lytic enzymes. Inflammation may happen at a tissue-damaged site, resulting in the migration of leukocytes to the wound areas and the elevation of serum component concentrations, including lysozyme, transferrin, C-reactive protein, ceruloplasmine and complement components.

Phagocytosis and phagocytes are the main tools of the nonspecific immune response to pathogenic microorganisms such as viruses, bacteria, fungi and parasites. The effectors functions of phagocytes are considerable, but most important is their ability to kill pathogens. Viral agents may induce the production of interferon's by viral-infected cells, stimulate other cells to activate intracellular endonucleases that will destroy viral DNA or RNA and will shut down physiological pathways that viruses use for replication. This phenomenon is mediated by a population of nonspecific cytotoxic (NC) cells. Similar to natural killer (NK) cells, NC cells are non-T, non-B cells which are not phagocytic. These cells produce very high levels of cytotoxicity substance against a wide variety of target cells.

The use of immunomodulators or immunostimulators in intensive fish culture offers a wide range of attractive methods for inducing or increasing protection against infectious diseases. In general, immunostimulants or/and immunomodulators comprise a group of synthetic and biological compounds that enhance the cell-mediated and humoral mediated immunity in human, animals and also in fish. Some of them only stimulate the defence mechanisms, others are able to restore or modulate immunity after suppression induced by xenobiotics. The stimulation of the defence mechanism may be particularly important for fish that are raised in or released to environments where the species or serotypes of pathogens are unknown and immunization by specific vaccines may be futile. Levamisole was one of the first immunostimulants used in fish to elevate the nonspecific cellular and humoral defence mechanisms. Other immunostimulants and biological response modifiers that have been used in fish research include dimerized lysozyme (KLP-602), chitosan, bacterial lipopolisaccharides, HMB, methisoprinol and glucans. The use of probiotics is well established in animals farming and has in recent years also been used in fish culture with generally good results. How probiotic bacteria work is not always fully understood in different animals. Live probiotic bacteria are believed to affect the intestinal flora by their antagonistic activity and competition for nutrients and space reducing the number of pathogenic bacteria. Probiotics also thought to improve appetite and hence increase growth. They may also stimulate innate defence through TLRs, other cellular receptors but for this to take place the probiotic bacteria or its products would have to pass the mucosal and epithelial barrier of the alimentary tract and prime the normal defence mechanism of the fish. The beneficial influence can be long lasting, minimum after one month of application in feed.

The influence of immunomodulators or/and immunostimulators on the fish cellular mechanisms can be followed by taking samples of nonlethal blood and haematopoietic organs and observing changes in leukocytes numbers, activity and functions. Macrophages, neutrophils and other phagocytic cells increase the enzyme mobilisation, oxidative radical production and phagocytic activities. The subpopulation of T lymphocytes increase the proliferative responses and cytotoxic activity, as well as increase the proliferative response of B lymphocytes and level of antibody secreting cells. The influence of immunostimulators on fish humoral defence mechanisms can be followed in blood samples analysed for changes serum or plasma total Ig and specific antibody levels, lysozyme and ceruloplasmine activity and cytokines levels.

Immunostimulants may be used in patterns similar to those of chemotherapeutics or chemicals and in combination with vaccines. The fish could be prepared for a predicted event, such as seasonal exposure to pathogens or handling stress, by a treatment prior to the event. Many environmental and physiological variables will influence experiments and protocol for the use of immunostimulants in fish, including timing, dosage requirement, environmental temperature, stability of each component, the characteristic of the vaccine and species of fish. The first consideration is whether the substance is to be used alone as a single treatment or whether it will be used in conjugation with an immunization program.

Several fish food manufactures now offer diets supplemented with natural and synthetic immunomodulators for use in aquaculture. Currently recommended schedules for feeding some immunostimulators all for administration every day for 1 to 2 weeks. Many question remain concerning the regiments and use of immunomodulators, particularly as to routes of administration, appropriate dosages, time and length of application, and influence on the environment.

COMPARISON OF MEAT QUALITY OF TENCH AND CARP

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POREĐENJE KVALITETA MESA LINJAKA I ŠARANA

Abstrakt
U poslednje dve godine postoje veliki problemi u plasmanu slatkovodnih riba proizvedenih na našim ribnjacima. Zahvaljujući nekontrolisanom uvozu, došlo je do toga da se na našem tržištu nađe velika količina jeftinih vrsta riba, pre svega Pangasius pangasius iz Vijetnama , čiji je uvoz zabranjen u mnogim razvijenim zemljama, pre svega SAD, Kanadi i većem broju zemalja Evropske Unije. Velprodajna cena šarana je veoma niska, pa je doveden u pitanje opstanak naše ribarske privrede, u čijoj strukturi šaran učestvuje najvećim delom. Stoga je potrebno razmišljati o uvođenju drugih slatkovodnih vrsta koje bi se mogle plasirati na tržište Evropske Unije, što se pre svega odnosi na linjaka. Takođe, treba razmišljati i o organskoj proizvodnji riba i o kreatnju ka ekstenzivnijim metodama gajenja. Neophodno je voditi računa i o nutritivnom kvalitetu mesa jer se od ribarske privrede očekuje proizvod sa niskim sadržajem masti i povoljnim odnosom nezasićenih masnih kiselina, pre svega ω-3 i ω-6 masnih kiselina. Poznat je povoljan uticaj ω-3 polinezasićenih masnih kiselina (PNMK) iz mesa ribe na zdravlje čoveka, kao i da povećana potrošnja ribe utiče na sprečavanje nastanka oboljenja kardiovaskularnog sistema, kao i u prevenciji inflamatornih, autoimunih i malignih oboljenja, dijabetesa i drugih bolesti. Literalni podaci o randmanu dvogodišnjih riba linjaka i šarana su veoma oskudni, dok su dostupne informacije o konzumnim ribama koje su starosti tri i više godina. Kako kvalitet mesa zavisi od starosti jedinke, neophodna su istraživanja i o kvalitetu mesa mlađih kategorija riba. Osim randmana analizirani su i hemijski sastav mesa dvogodišnjeg linjaka i šarana sa akcentom na zastupljenost masnih kiselina i poređenje odnosa ovih vrednosti kod linjaka i šarana. Uzorci dvogodišnjeg linjaka i šarana uzeti su na oglednom ribnjaku "Mošorin". Proizvodnja ovih riba odvijala se u ekstenzivnom sistemu, gde je povećanje organske produkcije vršeno pregorelim goveđim i ovčijim

stajnjakom. Ishrana dodatnim hranivima nije vršena. U pripremi objekta i tokom vegetacionog perioda korišćen je hidratni kreč. Analize hemijskog sastava i masnokiselinskog sastava lipida ribe su izvršene u Institutu za higijenu i tehnologiju mesa, Beograd. Analizom morfometrijskih karakteristika ribe utvrđena je dobra kondicija. Randman mesa linjaka povoljniji je od istoga kod šarana što se može objasniti manjom masom digestivnog trakta ove vrste. Razlog za niži procenat proteina kod linjaka i kod šarana nego što je to prikazivano u klasičnoj literaturi je što se radi o mesu dvogodišnjih riba dok se pomenuti literaturni podaci odnose na starije kategorije koje imaju manji sadržaj vode. Veći sadržaj vode kod dvogodišnjih riba doprinosi boljem gastronomskom kvalitetu mesa. Po sadržaju masti meso linjaka je približno vrednostima tolstolobika i amura, iz čega se može zaključiti da je veoma pogodno kao dijetalna hrana za potrebe posebnih zdravstvenih kategorija ljudi. I dvogodišnji šaran ima nizak procenat masti iz čega se može zaključiti da šaran ne spada u masne ribe jer ima niži procenat sadržaja masti od pastrmke. Tehnologija gajenja odnosno vrsta dodatne ishrane je najodgovornija za procenat masti. Odnos nezasićenih masnih kiselina kod linjaka povoljniji je nego kod šarana i pastrmke i u sličnom nivou je sa istim kod morskih vrsta riba. Meso dvogodišnjih riba po svom hemijskom sastavu ima prednost u odnosu na meso riba gajenih u trogodišnjem i višegodišnjem sistemu gajenja. Ekstenzivni sistem ima prednost u kvalitetu mesa ali treba analizirati njegovu ekonomsku opravdanost. Kvalitet mesa linjaka je izuzetnih nutritivnih vrednosti što je razlog za njegovu reintrodukciju i repopulaciju. Meso riba visoke nutritivne vrednosti ima perspektivu kao izvozni artikal u zemlje Evropske Unije i druge razvijene zemlje.

Ključne reči: *linjak, šaran, randman, nezasićene masne kiseline, ekstenzivna proizvodnja*

INTRODUCTION

The placement of freshwater fish produced in our ponds has become a problem in the past two years. The import lobby takes care that in the Serbian market a large amount of cheap fish species, especially *Pangasius pangasius* from Vietnam, forbidden in the U.S. market, in Canada and in a lot of EU countries, can be found (Ćirković et al., 2010). The cost of production is higher than carp sale prices that raises the question of the survival of our fish industry in which carp is the main cultured fish. It is therefore necessary to think about the introduction of other freshwater species that could be exported to the EU market, primarily tench whose production is practically closed in Serbia. Due to its palatable meat and high attractiveness for anglers, the tench is likely to have a great potential in the future, either as a supplementary species for pond aquaculture or for stocking into open waters (Ćirković et al., 2009). Also, it is necessary to think about organic production and extensive methods that was recommended at a meeting of Western European and Eastern European countries 1996 in Budapest (Ćirković and Ćirković., 1996). It is necessary to take into account the nutritional quality of meat because fish is one of the best sources of animal protein (Ozogul et al. 2006) and has been widely accepted as a good source of ω-3 polyunsaturated fatty acids (PUFA), such as eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids. These fatty acids appear to play a key role in neural development, functioning of the cardiovascular and immune systems (Lauritzen et al., 2001), besides the prevention of some types of cancer, including colon, breast and prostate cancer (Connor., 2000), brain aging and Alzheimer disease (Kyle, 1999). This paper analyzed the meat quality of two years-old tench and carp grown in the extensive system using well water (Ćirković et al., 2009).

MATERIALS AND METHODS

Samples of two-years old tench and carp were taken at the experimental pond »Mošorin«. Production of these fish were in extensive systems where the increase of organic production was done by adding burned-out beef and sheep manure. There was no supplemental feeding. In the facility preparations and during the growing season hydrated lime was used (Ćirković et al., 2000). During the meat yield determination, the edible part of each fish was filleted separately. Analysis of meat was carried out from three pooled samples. The chemical composition of fish was determined using standard BAS ISO methods: protein content (N x 6.25) was determined by Kjeldahl, on a Kjeltec Auto 1030 Analyzer (Manual book, Tecator, Sweden), the water content was determined by drying at 103±2°C to constant mass, total fat determined by fat extraction with petroleum ether, using the Soxhlet apparatus, after acid hydrolysis of the sample (EN ISO methods), the ash content was determined by measuring the mass of residue after annealing at 550±25°C (HRN ISO method). The fatty acids composition of the samples was determined after methyl esterification. Analyses were done at the Institute of Hygiene and Meat Technology, Belgrade.

RESULTS AND DISCUSION

Results of the morphometric measurements of analyzed tench and carp are given in tables 1 and 2, and dressing percentage in table 3. Results of chemical composition and content of unsaturated fatty acids in the meat of two-old carp and tench are presented in tables 4 and 5.

Table 1. Morphometric measurements of analyzed tench

L(overall length) (cm)	19.5	18	21.2	17	18	17	17.5	17.5	16.7	17.8
l (body length)	12	11.5	13.5	10.5	11.5	11	11.5	11.2	11	11.8
lc (head length)	4.5	4.5	5	4	4.3	4	4.5	4.2	3.6	4
Width	5.5	4.2	5	4.3	4	3.8	3.8	3.8	4	4.1
m(mass)(g)	116	88	129	66	78	71	70	64	67	77

Table 2. Morphometric measurements of analyzed carp

L (overall length) (cm)	30	28	29.5	26	26
l (body length)	19.2	17	18	15.5	15.5
lc (head length)	8	8.5	8.5	8.2	7.5
Width	10.5	10.7	12	11.2	9.5
m (mass)(g)	577	531	647	490	371

Table 3. Dressing percentage of tench and carp meat

	Tench	Carp
The total weight after evisceration (g)	810,72	2308,32
Fillets (g)	496,71	1318, 83
Dressing percentage (%)	60	50,38

Table 4. The chemical composition of tench and carp meat

Tench	Protein content(%)	Water content (%)	The total lipids (%)	Ash content (%)
1.tench	14,56	82,31	1,06	1,99
2.tench	14,55	82,15	0,92	2,03
3.tench	14,48	82,10	1,20	1,89
1.carp	16,15	80,31	2,82	1,02
2.carp	15,97	80,12	2,47	1,05
3.carp	16,25	80,14	2,62	1,03

Table 5. Fatty acid composition (% of total fatty acids) of tench and carp

Fatty acid	Tench 1	Tench 2	Tench 3	Carp 1	Carp 2	Carp 3
Myristic acid, C14:0	1.22	1.30	1.18	1.16	1.16	1.17
Pentadecylic acid, C15:0	0.91	0.95	0.99	0.55	0.53	0.53
Palmitic acid, C16:0	24.34	24.40	24.18	21.09	20.78	20.89
Palmitoleic acid, C16:1	5.45	5.71	5.81	5.00	5.14	5.00
Margaric acid, C17:0	1.10	1.12	0.95	0.72	0.70	0.72
Stearic acid, C18:0	8.31	8.02	7.94	5.61	5.14	5.61
Oleic acid, C18:1cis-9	17.85	18.43	17.64	32.03	32.92	32.03
Vaccenic acid,C18:1cis-11	4.50	4.64	4.07	4.15	4.27	4.15
Linoleic acid, C18:2, ω-6	8.98	8.82	8.83	13.34	13.84	13.34
Linolenic(GLA)C18:3,ω-6	0	0	0	0.17	0.21	0.17
α-Linolenic, C18:3, ω-3	3.77	4.06	4.11	4.61	4.71	4.61
Arachidic acid, C20:0	0.46	0.44	0.38	0.28	0.23	0.20
Eicosenoic acid, C20:1	0.61	0.66	0.58	1.56	1.51	1.55
Behenic acid, C20:2	0.85	0.86	0.82	0.77	0.75	0.77
Dihomo-gamma-linolenic acid, C20:3, ω-6	0.82	0.86	0.90	0.74	0.66	0.90
Eicosatrienoic acid, C20:3, ω-3	1.63	1.49	1.56	0.87	0.91	0.81
Erucic acid + Arachidonic acid, C22:1+20:4	6.81	6.43	6.51	2.44	2.82	2.23
Eicosapentaenoic acid, C20:5, ω-3	2.62	2.63	3.15	1.11	1.21	1.06
Docosapentaenoic acid, C22:5, ω-3	2.13	2.17	2.64	0.97	1.09	0.90
Docosahexaenoic acid, C22:6, ω-3	7.64	7.01	7.76	2.27	2.71	2.05
SFA	36.34	36.23	35.62	29.43	29.03	28.63
MUFA	28.41	29.44	28.10	43.08	42.69	43.88
PUFA	35.25	34.33	36.28	27.48	28.27	27.48
n-6	17.46	16.97	17.06	17.63	17.74	17.95
n-3	17.79	17.36	19.22	9.85	10.53	9.53
n-3/n-6	1.02	1.02	1.13	0.56	0.59	0.53

Analyzing morphometric characteristics of fish, good body form was found. Dressing percentage of tench was better than the same in carp that can be explained by the smaller mass of the digestive tract of this species (Ćirković et al., 2002). Tench is one of the cyprinid species that grow relatively slowly even when fed with live food and at the optimal temperatures (Wolnicki et all., 2003). The reason for the lower percentage of protein in the tench and carp meat than descried in literature (Marošević, 1982; Ćirković, 2000) is that we used two-year old fish, while the in mentioned literature data were refering to older fish categories that have a lower water content. Higher water content in two-year fish contributes to the higher culinary quality of meat. The fat content of tench meat is similar to that of big head carp and grass carp, and has a bit more fat than frog leg meat (Ćirković, 2000), from which it can be concluded that it is very suitable as dietary food for special health categories of people. Two-year old carp has also a low percentage of fat from which it can be concluded that carp are not concerned to be fatty fish because it has a lower percentage of fat content than trout (Spirić et al., 2009). Production technology and type of additional feeding is most responsible for fat percentage (Steffens and Wirth, 2007). The ratio of unsaturated fatty acids in tench is better than that of carp and trout and is at a similar level as in marine fish species (Kris-Ehterton et al., 2002).

CONCLUSION

The chemical composition of two years-old fish meat has an advantage over the flesh of older (three and four year old). Extensive systems have the advantage regarding meat quality, but it is necessary to analyze its economic feasibility. Meat quality of tench has an exceptional nutritional value being the reason for its reintroduction and repopulation. Fish meat with high nutritive value and low residual activity has a perspective as an export article to the European Union and other developed countries.

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ROLE AND SIGNIFICANCE OF ZOOPLANKTON IN SEMI-INTENSIVE CARP PRODUCTION

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ULOGA I ZNAČAJ ZOOPLANKTONA U POLUINTENZIVNOM SISTEMU GAJENJA ŠARANA

Apstrakt

Jedan od svetski najzastupljenijih sistema za gajenje riba je poluintenzivni sistem koji čini više od 80% (Tacon and De Silva, 1997). U najtradicionalnijem obliku, prisutnom uglavnom u Aziji, veći deo nutritivnih potreba riba se zadovoljava iz prirodne hrane, prisutne u jezeru, dok se manji deo zadovoljava kroz dodatnu hranu. Osnovna ideja ovog sistema je da se potrebe riba u proteinima zadovoljavaju konzumiranjem zooplanktona, zoobentosa i druge prirodne hrane, dok se energetske potrebe zadovoljavaju žitaricama i drugim lokalnim proizvodima u obliku dodatne hrane. Poslednjih godina u zemljama jugoistočne Evrope (Srbiji, BiH, Bugarskoj, Rumuniji) sve se više koristi peletirana i ekstrudirana hrana, umesto žitarica ili u kombinaciji sa žitaricama uz iskorišćavanje prirodne hrane iz samog ribnjaka.

Zooplankton predstavlja važanu prirodnu hranu za larve i odrasle stadijume mnogih vrsta riba koje se gaje u akvakulturi. On je i glavna prirodna hrana za larve i mesečnjake šarana, a zajedno sa faunom dna, predstavlja hranu za mlađ i gajenog konzumnog šarana. Zooplankton je značajan izvor proteina, amino kiselina, lipida, masnih kisenina, minerala i enzima (Kibria et al., 1997). U novim ribnjacima zooplankton je uglavnom poreklom iz vode za vodosnabdevanje, dok u starijim objektima, deo ovih organizama potiče iz banke trajnih jaja koja se nalaze u sedimentu, najčešće do dubine od 2 do 10 cm, odakle se jedan deo svake sezone razvija i prelazi u vodu. Razvitak zooplanktonskih organizama (Rotatoria, Cladocera, Copepoda), ima izraženu sezonalnost, pa je maksimum produkcije karakterističan za maj i jun (Paterson, 1993). Na žalost, tokom letnjih meseci (jul i avgust), kada postoje optimalni temperaturni uslovi za rast šarana, najčešće se javlja značajan pad uglavnom svih grupa zooplanktona a naročito kladocera i kopepoda. Ovaj pad u produktivnosti je pre svega rezultat sezonalnosti zooplanktonskih organizama a može biti i posledica velikog pritiska riba na zooplankton u uslo-

vim gustog nasada riba. Do izvesne mere se produkcija prirodne hrane može podstaći upotrebom veštačkih đubriva ili stajnjaka, koji zapravo dovode do porasta produkcije fitoplanktona koji je osnov ishrane za zooplanktonske organizme.

Larve šarana mogu da konzumiraju manje oblike zooplanktona kao što su rotatorije i nauplius larve kopepoda. Sa porastom, šaran počinje da konzumira krupnije oblike kladocera i kopepoda, a potom i organizme faune dna, hironomide i oligohete. Za šarana i druge vrste Teleostea, krupniji oblici zooplanktona, naročito krupne kladocere (*Daphni magna* i *D. pulex*) predstavljaju lako uočljiv plen, ne samo zbog dimenzija tela, već i zbog dobro pigmentisanog i krupnog oka. Osim toga, starije uzrasne kategorije šarana imaju tako razvijeni usni aparat koji je prilagođen za sakupljanje hrane koja je veća od 250 μm (Sibbing et al., 1986). Usled ovoga, na početku proizvodne sezone, na većini ribnjaka nasađenih sa jedno- i dvogodišnjom mlađi, vrlo brzo dolazi do istrebljenja krupnih zooplanktonskih organizama, što dovodi do dominacije rotatorija, larvi kopepoda kao i malih kladocera, kao što je *Bosmina longirostris*, jer više nema kompeticije za hranom.

U slučajevima kada postoji niska produkcija prirodne hrane, a ne postoji mogućnost đubrenja, usled npr. visoke temperature vode, dodatni objekti kao što su neupotrebljiva ribnjačka jezera, jame ili pak veliki tankovi, mogu poslužiti za dodatnu produkciju prirodne hrane – zooplanktonskih organizama. Masovna proizvodnja prirodne hrane na ovaj način može biti relativno jeftina posebno kada se kao đubrivo koristi stajnjak. Za manje količine zooplanktona, sakupljanje se može obaviti pomoću velikih planktonskih mreža zakačenih za čamac ili se mogu vući sa obale. Ukoliko su u pitanju veći objekti u kojima se gaji zooplankton, za sakupljanje se može koristiti adaptiran čamac po ugledu na "Baleen sistem" koji je poseduje deo koji zahvata vodu i mrežu za procеđivanje, nakon čega se organizmi propuštaju kroz seriju mrežica čime se raspoređuju po veličinskim klasama.

Razmatrajući svetski problem nedostatka vode i potrebe za održivom upotrebom vode i nutrijenata u akvakulturi neophodno je unapređenje dosadašnje prakse poluintenzivnog gajenja šarana. Prirodna hrana predstavlja obnovljiv izvor proteina, amino kiselina, masti i masnih kiselina, za gajene ribe i zato usavršavanje poluinteinzivnog sistema između ostalih mera unapređenja treba da ide i u pravcu njihovog većeg korišćenja bilo iz samih ribnjačkih objekata za gajenje šarana ili iz planktonskih jama, jezera ili tankova za njihovo gajenje. Na ovaj način će se omogućiti ekonomičnije iskorišćavanje prirodne hrane, a u kombinaciji sa optimalnom dodatnom hranom će nutritivne potrebe riba biti u potpunosti zadovoljene.

Ključne reči: *zooplankton, prirodna hrana, poluintenzivan sistem*

INTRODUCTION

Semi-intensive is one of the most practiced system for fish production in world, making over 80 % (Tacon and De Silva, 1997). There are different modes of intensification, but in the most traditional type of semi-intensive system, typically practiced in Asia, bigger share of the nutritional requirements of fish are met through the consumption of natural food, available in the pond, and to a lesser extent through consumption of supplementary feed of different quality. The basic idea is that the proteins requirements are mainly fulfilled from zooplankton, zoobenthos and other natural food and energy requirements are met though carbohydrates provided through row cereals and other locally available sources in the form of supplementary feed.

In recent years, in Southeast Europe (Serbia, BiH, Bulgaria and Romania), a shift towards a higher utilization pelleted and extruded feed instead of cereals or the combination of cereals and natural food from the fish pond has been in the scene.

Ecological patterns of zooplankton assemblages in fish ponds

Zooplankton has been recognized as an important source of natural food for both larvae and adults of many aquaculture species. Most fish and prawn species rely on zooplankton at some stage of their life span, and some are exclusively zooplankton feeders throughout their life. Zooplankton is the main natural food for larvae and fry carp (*Cyprinus carpio*), and together with zoobenthos are food for marketable size fish.

Zooplankton that inhabits earthen carp ponds in newly formed fish ponds usually originate from the water bodies used as water supply. In older fish ponds, a part of the zooplankton community comes from the egg bank deposits that form in the sediments, usually up to a few centimeters (2 – 10 cm) thick depending on the age of the pond.

Nutrients in carp ponds usually originate from various sources of water supply coming from adjacent rivers, lakes, canals, or other water resources as groundwater pumped from wells, from pond sediments, due to resuspension, or from applied agrotechnical measures. It has been shown that deep tube well water of certain characteristics, as high conductivity, low hardness and high concentration of ammonia nitrogen, can have a negative effect on zooplankton production, especially on Cladocerans, in aquaculture ponds (Dulić et al., 2011).

Zooplankton important as natural food for carp in natural and artificial water bodies has a pronounced seasonality, given that there is a maximum of development usually in May and June (Paterson, 1993). Unfortunately, during midsummer, when environmental conditions, as water temperature, are optimal for carp growth in fish ponds, there is usually a sharp depression in zooplankton production.

Feeding habits of carp

After the partial absorption of the egg yolk, 3 to 5 days after hutching, carp larvae start feeding exogenously on Rotifers and naupliar larvae of Copepods. After a short period of consuming these smallest zooplankters, carps move on to bigger pray, as Cladocera that are apparently a strong dietary preference particularly for carp up to one month old (Billard, 1999). Except cladocerans and copepods, juveniles and adult carp consume zoobentos, mainly Chironomids and Oligochaetes, but also young sprouts of aquatic plants.

Predation on zooplankton for adult carp is highly size selectable, with preferences for larger individuals, mostly big cladocerans, such as certain species of daphnids (*Daphnia magna* and *D. pulex*) as they are pigmented, so visible for carp. Additionally, Jha et al. (2006) reported a relative preference of carp and other cyprinides, towards Cladocerans, due to their slower locomotion compared to rather faster swimming Copepods. Due to this, at the beginning of the production season, in most ponds stocked with yearlings or adult carp, there is a rapid depletion of large zooplankton species, leading to a domination of Rotifers, copepod larvae and small cladocerans as *Daphnia longispina* and *Bosmina longirostris* since they are have no competitors for food resources.

Significance of zooplankton as nutritional food for carp

Zooplankton is a valuable source of proteins, amino acids, lipids, fatty acids, minerals and enzymes (Kibria et al., 1997). Carp larvae are rather incomplete organisms since organs, specially the digestive system, are still under development after hutching. However, fish larvae start with exogenous feeding before the yolk sac is totally depleted. This undeveloped type of digestive tract can digest only very simple food items as zooplankton organisms, and therefore poorly digest artificial diets (Hofer, 1995). It is assumed that enzymes of zooplankton can support the digestive process in larval gut (Kolkovski, 2001).

Dimension of feed particle that fish larvae can consume depend on the size of the mouth gape. Apparently the size of the mouth gape is in correlation with body size, and hence, this parameter depends on the egg diameter and the period of endogenous feeding (Dabrowski, and Bardega 1984). Feeding of juvenile and adult common carp, as it is mentioned earlier, consist of larger natural food organisms. Older carp are generally concerned to be benthvorous and facultative zooplanktivorous fish and their feeding apparatus is adjusted for collecting food items bigger than 250 μm (Sibbing et al., 1986). Large zooplankters as *Daphnia magna* and *D. pulex* are a very good source of proteins presenting up to 50% of their dry body mass, for older carp (Kibria *et al*., 1997). Apart from their size, for carps that are visual predators these zooplankton species present a noticeable prey even more due to the pigmented and large compound eye characteristic for genus Daphnia. On a whole fish pond experiment, surface ranging from 112 to 200 ha, it has been shown that when *D. magna* and *D. pulex* when comprising over 54% of the total content of the dominant Cladocera group in the pond, there has been a statistically significant ($p<0.01$) effect on growth rate of one and two year old carp (Dulić, 2007). However, in a research on the impact of fishery management on Cladoceran population, that used a sampling net of different mesh size as a preliminary selection method of cladoceran size, it has been hypothesized that carp of bigger biomass predate more intensively on larger daphnids (Prazakova, 1991).

In circumstances when there is a low production of natural food in the fish pond, and no possibilities for fertilization due e.g. high water temperature, adjacent unusable ponds or big tanks can be used for additional production of natural food organisms. Mass production of live food organisms can be rather cheap when maintained on organic manures. There is a lot of data on exploitation of live or dried zooplankton, as food for fish in aquaculture. For smaller amounts of zooplankton, harvest can be done with a large plankton net towed behind a boat, or pulled from shore. If bigger ponds are used for culturing zooplankton, a Baleen harvesting system (Zoothech, Australia) can be applied. It consists of a boat equipped with a dewatering screen after which organisms are graded through a series of sieves. Selective harvest of needed zooplankton species can be obtained by choosing appropriate mesh size (Lavens and Sorgeloos, 2006). Additionally, zooplankton, especially daphnids, can be grown abundantly in sewage and wastewater, and possibly can be grow on effluents from fish ponds. Further on, this can be a way to decrease the amount of water pollution coming from semi-intensive fish production.

CONCLUSIONS

Semi-intensive production is the main type of fish in the world. In the view of worldwide concern for sustainable usage of water in aquaculture, semi-intensive fish production is also under reconsideration. In this production system the dietary requirements of the cultured species are obtained by feeding on natural food organisms available from the pond, and through direct consumption of supplementary feed (Tacon and De Silva, 1997). Natural food is a renewable resource and a very valuable source of proteins, lipids, amino and fatty acids, minerals and enzymes for cultured fish (Kibria et al., 1997). Improvements should be made toward a more comprehensive usage of natural food, either in the fish ponds or in additional ponds and tanks, as a cheap way to provide proteins of high quality. Still, a good balance between natural food and good quality supplementary feed should be made in order to fulfill all the nutrient requirement of cultured fish on one hand and to preserve the environment from pollution due to overload by uneaten additional feed.

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RESPONSE OF COMMON CARP AND TILAPIA TO DIETS BASED ON PLANT PROTEIN SUPPLEMENTED WITH ESSENTIAL AMINO ACIDS

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ODGOVOR ŠARANA I TILAPIJE NA ISHRANU ZASNOVANU NA PROTEINIMA BILJNOG POREKLA DOPUNJENIM ESENCIJALNIM AMINO KISELINAMA

Prošireni abstrakt
Usled svog odličnog sastava mikro i makro nutrijenata i velike palatabilnosti riblje brašno je bilo osnovni sastojak hrane za vodene životinje. Danas se više od 65% svetske proizvodnje ribljeg brašna koristi za hranu za vodene organizme, mada su rastuće cene na tržištu dovele do značajnog povećanja upotrebe proteina biljnog porekla. Biljno brašno, koncentrati i izolati biljnog porekla deficitarni su bar u jednoj ili više od 10 za ribe esencijalnih amino kiselina (essential amino acids (EAA), pa je i integrisanje u hranu ograničeno. Čak i kada se nedostaci nadoknade kristalnim amino kiselinama javlja se problem nesposobnosti nekih riba da iskorišćavaju biljne proteine. Na primer, pastrmke mogu dobro da iskorišćavaju biljne proteine obogaćene kristalnim amino kiselinama dok je kod šarana različito. Jedan razlog može biti nedostatak želuca kod šarana a tako i nedostatak pepsina i kisele faze varenja. Kristalne amino kiseline mogu da dovedu do visokog sadržaja jedne amino kiseline u krvnoj plazmi šarana koja tada ne može da se iskoristi za nastanak novog tkiva, na primer mišića, već se koristi kao izvor energije.

Da bi se ispitale moguće razlike u iskorišćavanju biljnih proteina kod šarana i tilapije, izvedena su dva odvojena randomizovana hranidbena ogleda sa šaranom (*Cyprinus carpio*, starosti 12 nedelja) i tilapijom (*Oreochromis niloticus*, starosti 9 nedelja). Eksperimenti su izvedeni u postrojenju za akvakulturu na vTI-Institutu, Institut za ribarstvenu ekologiju u Ahrensburgu, Nemačka. Za oba eksperimenta 12 akvarijuma su nasađeni sa 7 šarana ili 10 tilapija. Pre početka eksperimenta pripremljena su 3 izo-azotna, izo-lipidna i izo-energetska test obroka sastavljena na osnovu potreba šarana: obrok FM sastojao se od 100% protein iz ribljeg brašna, obrok WG od 100% protein pšeničnog glutena i obrok WG+AA od proteina pšeničnog glutena + EAA. Da bi se ispitali efekti

dodavanja EAA testirane ribe su dobijale umanjenu količinu hrane u odnosu na njihovu metaboličku telesnu masu.

Uprkos sličnim nutricionim potrebama šarana i tilapije, utvrđene su jasne razlike u rastu i parametrima iskoristljivosti hrane, kao i u telesnom sastavu. Dok je najveće povećanje telesne mase dobijeno sa obrokom WG+AA kod šarana, tilapija je imala najveće povećanje telesne mase sa obrokom FM. I šaran i tilapija su mogli da koriste dodate EAA. Kod šarana je dodavanje EAA dovelo do povećanog sadržaja sirovih protein u poređenju sa WG obrokom, ali je to bilo niže u poređenju sa FM obrokom. Šaran hranjen WG obrokom je pokazivao jasne znake adipoze koje se pojavljuju u obrocima sa deficitom proteina. Međutim, sirovi pepeo je bio najviši u šarana hranjenog WG+AA obrokom što je znak ograničenosti sadržaja ili kvaliteta proteina. Kod tilapije je dodatak EAA rezultirao u nižem sadržaju sirovih proteina, kao i povećanom sadržaju sirovih lipida. I šaran i tilapija su iskoriščavali značajne količine dodatih EAA za prikupljanje rezervi lipida.

Potrebna su dalja istraživanja da bi se ispitalo ugrađivanje dodatih amino kiselina u tkivo riba. Ovo se može ostvariti tečnom hromatografijom/izotop masenom spektrometrijom sa $\delta^{13}C$ obeleženim amino kiselinama koja bi u budućim ispitivanjima mogla da dovede do boljeg razumevanja upotrebe protein biljnog porekla među različitim vrstama riba.

Ključne reči:*riblje brašno, biljni protein, ishrana, šaran, tilapija*

INTRODUCTION

Fish meal replacement in diets for various fish species has been one of the key topics in fish nutrition for the past few years. Due to its excellent composition in micro and macro nutrients and its high palatability, fish meal has been the ingredient of choice in aquafeeds. Between 1983 and 2005 international market prices for fish meal ranged between US$ 200 and 800 per metric ton (Tacon & Metian 2008). Ever since the El Niño year 2006, fish meal prices have been on a steady rise, up to almost US$ 2000 per metric ton during the first quarter of 2011. Today, up to 65% of the world's fish meal production is utilized in aquafeeds (Hardy 2010), of which roughly 80% are utilized in diets for carnivorous fish species and crustaceans. However, the remaining 20% are utilized in starter and fingerling diets for omnivorous species, such as carp, tilapia and catfish (Tacon & Metian 2008). Therefore, it is of major importance to increase the use of plant proteins as a fish meal substitute, to satisfy the increasing demand for aquafeeds from aquaculture farming practices. Nevertheless, most plant meals, concentrates or isolates are deficient in at least one or more of the ten essential amino acids (EAA) in fish. But even when these deficiencies are supplemented by crystalline amino acids, there are differences among fish species in the ability to utilize plant proteins. For instance, trout are able to utilize plant proteins supplemented with crystalline amino acids well (Rodehutscord et al. 1995, 1997; Dabrowski and Dabrowska 1981) while the utilization differs in carp. One reason may be the absence of a stomach in carp and hence their lack of acid and pepsin digestion.

Therefore, crystalline amino acids seem to be absorbed more quickly by the intestine, while larger proteins are broken down to di- and tripeptides and absorbed slower over the length of the intestine. This may cause peak situations of single amino acids in the blood plasma of common carp (Plakas et al. 1980), which cannot be sufficiently utilized for the assembly of new tissues, e.g. muscle, and are therefore utilized as an energy source by carp.

The purpose of this study is to compare carp and tilapia, two internationally very important aquaculture species with similar nutritional requirements but different anatomy and digestive physiology, in their ability to utilize plant proteins supplemented with EAA.

MATERIALS AND METHODS

Subsequently, two separate fully randomized feeding experiments with common carp and tilapia were conducted in the aquaculture facilities at the vTI-Institut, Institute of Fisheries Ecology, in Ahrensburg, Germany.

Prior to the feeding experiments, three iso-nitrogenous, iso-lipidic and iso-energetic (on the basis of digestible energy, DE) diets were composed for the two separate feeding experiments. EAA deficiencies (lysine, arginine and threonine) of the plant protein carrier wheat gluten (WG) were supplemented with crystalline amino acids to meet the requirements of common carp (NRC 1993). Table 1 shows the composition of the experimental diets: the diets were formulated to provide 5.6% nitrogen (= 35% crude protein), 10% crude lipids, and 11.1% crude ash. Nitrogen-free-extract was calculated to be 31% in diet FM (100% fish meal protein) and 34% in diets WG (100% wheat gluten protein) and WG+AA (wheat gluten + EAA) to adjust digestible energy.

The carp experiment was carried out in twelve 40 L-tanks, four tanks per diet, in a flow-through system supplied with vented well water for twelve weeks. Water temperature was held at 23°C ± 0.5°C by a heating element. Lighting regime was 12 h light: 12 h dark and the fish were hand-fed three times a day at 8:00, 13:00 and 17:00 hours. The tanks were stocked with seven carp with an initial body mass of 10.3 g ± 0.2 each. To test the effect of EAA supplementation all fish received the same amount of feed. Because feed intake of diet WG was lowest, all fish were fed accordingly with three times the maintenance requirement for common carp per day of 3.2 g $kg^{-0.8}$ (Becker et al. 1993), therefore, carp received a total of 9.6 g $kg^{-0.8}$ per fish per day.

The tilapia experiment was carried out in accordance to the carp experiment in a recirculating system. Twelve tanks were stocked with ten fish per tank with an initial body mass of 12.2 g ± 0.8. Water temperature was 24.8°C ± 1.0°C over the experimental period of 9 weeks. Since feed intake of the wheat gluten diets was very low, 20% of diet FM were added to 80% of diets WG and WG+AA (= WG_T and $WG+AA_T$) so a total amount of 10% fish meal was added to the wheat gluten diets (see table 1). Tilapia were fed a total amount of 10 g $kg^{-0.8}$ per day, supplied via three feedings by hand at 8:00, 13:00 and 17:00 hours.

At the end of both experiments all fish were sacrificed, after being starved for 24 hrs to evacuate guts from feed residues, with 8 g of 2-Phenoxyethanol (807291, Merck KGaA, Germany) per 10 L of water and stored at -20°C until proximate composition analysis. All fish from one tank were thoroughly homogenized using a Retsch Grindomix (GM 200, Retsch GmbH, Germany) at 3000 rpm for 3 minutes. Moisture content of the homogenized fish was determined in duplicates by mixing 5 g of the fresh material with approximately 15 g of sea sand (7712, Merck KGaA, Germany) in a crucible and drying to constant weight at 105°C in a drying oven. Afterwards, homogenized fish samples were freeze-dried for proximate composition analyses. Proximate composition analyses of fish and feeds were conducted according to AOAC (2000), all analyses were carried out in duplicates: dry matter was determined by drying sample material to constant weight at 105°C in a drying oven. Subsequently, ash content of samples was determined by incineration in a muffle furnace at 550°C for 5 hours. Nitrogen content was determined by varioMAX CN (Elementar Analysesysteme GmbH, Germany) and multiplied with the nitrogen conversion factor of 6.25 to calculate the protein content of fish samples. Lipid content was determined according to Smedes (1999, Koch et al. 2011). Differences between groups (FM/ WG, FM/WG+$AA_{(T)}$, WG/WG+$AA_{(T)}$) were analyzed by T-test for independent samples ($P < 0.05$). All statistical analyses were performed using Statistica Version 8 (StatSoft Inc., Tulsa, OK, USA). Values are expressed as means ± standard deviation.

Table 1 Composition of the experimental diets (g kg^{-1} diet) for common carp (*Cyprinus carpio*; FM, WG, Wg+EAA) and Tilapia (*Oreochromis niloticus*, FM, WG_T, WG+EAA_T).

Ingredients	FM	WG	WG+AA	WG_T	WG+AA_T
Wheat gluten	-	438.2	414.3	350.5	331.5
Fish meal	536.7	-	-	107.3	107.3
Fish oil	22.3	74.6	74.9	64.2	64.4
Sunflower oil	20.0	20.0	20.0	20.0	20.0
Wheat starch	329.8	269.5	274.5	281.5	285.5
Cellulose	103.7	106.7	107.1	106.1	106.4
Vitamin premix[1]	20.0	20.0	20.0	20.0	20.0
Mineral premix[2]	10.0	20.0	20.0	18.0	18.0
Additional minerals[3]	-	89.1	89.1	71.3	71.3
TiO_2	10.0	10.0	10.0	10.0	10.0
Lysine	-	-	11.6	-	9.2
Threonine	-	-	3.5	-	2.8
Arginine	-	-	2.0	-	1.6
Sum	1052.5	1048.1	1047.0	1049.0	1046.5
Sum DM	1000.0	1000.0	1000.0	1000.0	1000.0
Digestible Energy (MJ kg^{-1})	14.1	13.9	13.9	13.9	13.9

[1] Vitamin premix (per kg premix): 500000 I.U. retinol, 50000 I.U. cholecalciferol, 2500 mg tocopherol, 1000 mg menadione, 5000 mg thiamine, 5000 mg riboflavin, 5000 mg pyridoxine, 5000 µg cyanocobalamine, 25000 mg myo-inositol, 10000 mg pantothenic acid, 100000 mg choline chloride, 25000 mg niacin, 1000 mg folic acid, 250 mg biotin, 10000 mg ascorbic acid

[2] Mineral premix (per kg premix): 314.0 g $CaCO_3$, 469.3 g KH_2PO_4, 147.4 g $MgSO_4$ x $7H_2O$, 49.8 g NaCl, 10.9 g Fe(II)gluconat, 3.120 g $MnSO_4$ x H_2O, 4.670 g $ZnSO_4$ x $7H_2O$, 0.620 g $CuSO_4$ x $5H_2O$, 0.160 g KJ, 0.080 g $CoCl_2$ x $6H_2O$, 0.060 g NH_4molybdat, 0.020 g $NaSeO_3$

[3] Additional minerals (kg^{-1} mix): 337.4 g $CaCO_3$, 504.2 g KH_2PO_4, 158.4 g $MgSO_4$ x $7H_2O$

RESULTS

Table 2 shows the proximate composition of the experimental diets. Results for body mass gain and growth parameters are shown in table 3, while body composition of the experimental fish is shown in table 4.

Table 2 Proximate composition of experimental diets based on fish meal and wheat gluten.

Carp	Experiment	FM	WG	WG+AA
DM	(%)	95.6	92.2	93.7
N (% DM)*		5.6	5.7	5.7
CL (% DM)		10.1	10.3	10.6
CA (% DM)		12.4	10.6	10.2
Tilapia	Experiment	FM	WG_T	$WG+AA_T$
DM	(%)	94.5	92.9	93.1
N (% DM)*		5.6	5.6	5.6
CL (% DM)		10.3	10.2	10.1
CA (% DM)		12.7	11.2	11.9

DM = dry matter; N = nitrogen; CL = crude lipids; CA = crude ash; NFE = nitrogen-free extract

* Due to differences in N:P conversion factors of proteins from animal and plant origin (Sriperm 2011) comparisons are done on the basis of N for experimental diets

Final body mass, body mass gain, specific growth rate and metabolic growth rate are highest in carp fed diet WG+AA and accordingly feed conversion ratio is lowest. These parameters are significantly different ($P < 0.05$) from carp fed diets FM and WG, except for feed conversion ratio, where no significant difference was identified between WG and WG+AA. No significant differences for these parameters were identified between carp fed diets FM or WG. In tilapia the parameters final body mass, body mass gain, specific growth rate and metabolic growth rate are lowest in fish fed diet WG_T while they are highest in tilapia fed diet FM. All parameters shown in table 3 are significantly different ($P < 0.05$) between the three diets.

Table 3 Body mass gain, feed conversion, specific and metabolic growth rate of experimental carp and tilapia fed different diets based on fish meal and wheat gluten.

Carp	FM	WG	WG+AA	*P* FM/WG	*P* FM/WG+AA	*P* WG/WG+AA
$BM_{Initial}$ (g)	10.4 ± 0.3	10.2 ± 0.2	10.3 ± 0.2			
BM_{Final} (g)	19.7 ± 1.2	19.7 ± 1.8	25.3 ± 3.8		*	*
BMG (g)	9.4 ± 1.3	9.5 ± 1.8	15.1 ± 3.7		*	*
FCR	2.4 ± 0.3	2.4 ± 0.5	1.7 ± 0.3		*	
SGR (% d^{-1})	0.8 ± 0.1	0.8 ± 0.1	1.1 ± 0.2		*	*
MGR (g $kg^{0.8}$ d^{-1})	3.2 ± 0.4	3.3 ± 0.5	4.5 ± 0.7		*	*
Tilapia	FM	WG_T	$WG+AA_T$	*P* FM/WG_T	*P* FM/$WG+AA_T$	*P* WG/$WG+AA_T$
$BM_{Initial}$ (g)	12.5 ± 0.5	11.5 ± 1.4	12.6 ± 0.4			
BM_{Final} (g)	25.1 ± 1.5	16.1 ± 0.9	22.6 ± 1.3	*	*	*
BMG (g)	12.6 ± 1.2	4.6 ± 0.8	10.1 ± 1.0	*	*	*
FCR	1.5 ± 0.1	3.3 ± 0.7	1.8 ± 0.2	*	*	*
SGR (% d^{-1})	1.1 ± 0.1	0.5 ± 0.1	0.9 ± 0.1	*	*	*
MGR (g $kg^{0.8}$ d^{-1})	3.6 ± 0.2	1.7 ±0.4	3.1 ± 0.3	*	*	*

BM (g) = body mass; BMG (g) = body mass gain = BM_{Final} - $BM_{Initial}$; FCR = feed conversion ratio = feed fed (g)/ BMG; SGR (% d^{-1}) = specific growth rate = [(ln BM_{Final} - ln $BM_{Initial}$)/ experimental days] x 100; MGR (g $kg^{0.8}$ d^{-1}) = metabolic growth rate = BMG/ [{($BM_{Initial}$/ 1000)$^{0.8}$ + (BM_{Final}/ 1000)$^{0.8}$/ 2]/ experimental days

* Significantly different ($P < 0.05$), T-test for independent samples, (n = 4; carp 7 fish per replicate, tilapia 10 fish per replicate)

Table 4 shows a significant decrease ($P < 0.05$) in moisture and crude protein content in carp fed diet WG compared to diets FM or WG+AA, as well as a significant increase ($P < 0.05$) in crude lipids. Carp fed diets WG and WG+AA also show a significant increase in nitrogen-free extract when compared to diet FM. Tilapia fed diet $WG+AA_T$ show a significant decrease ($P < 0.05$) in moisture and crude protein. Crude lipids are highest in tilapia fed diet $WG+AA_T$ and lowest when fed diet WG_T, while crude ash is vice versa.

Table 4 Body composition of experimental carp and tilapia fed different diets based on fish meal and wheat gluten.

Carp	FM	WG	WG+AA	*P* FM/ WG	*P* FM/ WG+AA	*P* WG/ WG+AA
Moisture (%)	76.8 ± 1.1	74.2 ± 0.5	75.3 ± 0.8	*		
CP (% DM)	59.8 ± 3.9	43.9 ± 1.8	48.0 ± 1.6	*	*	*
CL (% DM)	28.8 ± 4.4	41.0 ± 1.7	37.0 ± 2.2	*	*	*
CA (% DM)	9.2 ± 1.0	10.2 ± 0.4	11.3 ± 1.0		*	
NFE (% DM)	2.2 ± 1.0	4.8 ± 1.6	3.8 ± 0.5	*	*	
GE (kJ)	114.5 ±13.3	130.7 ± 13.2	157.0 ± 17.6		*	
Tilapia	FM	WG_T	$WG+AA_T$	*P* FM/ WG_T	*P* FM/ $WG+AA_T$	*P* WG/ $WG+AA_T$
Moisture (%)	74.5 ± 0.2	75.6 ± 1.1	73.7 ± 0.7			*
CP (% DM)	56.8 ± 1.0	56.9 ± 1.5	54.8 ± 1.1		*	
CL (% DM)	15.5 ± 0.9	11.9 ± 2.6	18.8 ± 0.4	*	*	*
CA (% DM)	23.2 ± 0.7	26.1 ± 2.2	21.1 ± 0.6	*	*	*
NFE (% DM)	4.5 ± 0.4	5.1 ± 1.2	5.4 ± 0.7			

DM (%) = dry matter; CP (% DM) = crude protein; CL (% DM) = crude lipids; CA (% DM) = crude ash; NFE (% DM) = nitrogen-free extract; GE (kJ) = gross energy = [0.2431 x CP (% DM) + 0.3884 x CL (% DM)] x DM of fish (g) (Focken and Becker 1993)

* Significantly different ($P < 0.05$), T-test for independent samples, (n = 4; carp 7 fish per replicate, tilapia 10 fish per replicate)

DISCUSSION

Even though carp and tilapia have similar nutritional requirements, both species show a clearly different response to diets based on fish meal or wheat gluten. While highest body mass gain was obtained with diet WG+AA in carp, tilapia observed highest body mass gain with diet FM. Both carp and tilapia were able to utilize supplemented EAA: in carp, EAA supplementation resulted in higher crude protein content compared to diet WG, but it was lower in comparison to diet FM. Carp fed diet WG show clear signs of adiposis which is only likely to occur in highly protein deficient diets (Focken and Becker 1993). Nevertheless, crude ash is highest in carp fed diet WG+AA which is a sign of either protein limitation or poor protein quality (Focken and Becker 1993). In tilapia, EAA supplementation resulted in lower crude protein content, as well as higher crude lipid content. Both carp and tilapia seem to have utilized considerable amounts of the supplemented EAA for the assembly of lipid reserves. Further studies have to be performed to verify integration of supplemented amino acids into the fish tissue. This can be achieved by liquid chromatography/ isotope ratio mass spectrometry of $\delta^{13}C$ labeled amino acids (McCullagh et al. 2008, Gaye-Siesegger et al. 2011) and should be considered for further studies.

CONCLUSION

This study shows clear differences in plant protein utilization among carp and tilapia in spite of their similar nutritional requirements. Further studies have to be performed to come to a broader understanding of plant protein utilization among various fish species, why these differences occur and how they can possibly be overcome to increase the use of plant proteins in aquafeeds for omnivorous fish species.

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FATTY ACID PROFILE OF CARP FISH SPECIES FROM TWO AQUACULTURE SYSTEMS

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SASTAV MASNIH KISELINA ŠARANSKIH VRSTA RIBA IZ DVA SISTEMA GAJENJA

Abstrakt
Cilj ovog rada je bio ispitivanje masnokiselinskog profila mišićnog tkiva šaranskih vrsta riba gajenih u polikulturi u dva ribnjaka sa poluintenzivnim uzgojem. Poređen je profil masnih kiselina mišićnog tkiva šarana, tolstolobika i amura u vidu ukupnih zasićenih (ZMK), mononezasićenih (MNMK) i polinezasićenih masnih kiselina (PNMK), kao i odnosi P/S i n-3/n-6. Odnos P/S u lipidima mišićnog tkiva šarana kretao se od 0,26 (amur) do 0,80 (šaran). Veći sadržaj n-3 PNMK uočen je u mišićnom tkivu amura. Odnos n-3/n-6 iznosio je kod amura 2.15, dok je kod tolstolobika i šarana bio 0.07. Dobijeni rezultati ukazuju da je glavna razlika između šaranskih vrsta bila u sadržaju ukupnih PNMK, posebno u sadržaju n-6 masnih kiselina. Sastav masnih kiselina amura iz dva ribnjaka nije se statistički značajno razlikovao. Šarani sa dva ribnjaka su se značajno razlikovali u ukupnom sadržaju MNMK i PNMK. Da bi se procenio kvalitet slatkovodne ribe sa domaćeg tržišta, dobijeni rezultati su, takođe, poređeni sa gajenim vijetnamskim somom (*Pangasius hypophthalmus*) koji je uzet sa našeg tržišta. S obzirom na masnokiselinski sastav, *Pangasius* se ne može smatrati nutritivno vrednom namirnicom. Dalja ispitivanja su neophodna da se oceni kvalitet slatkovodne ribe sa našeg tržišta.

Ključne reči: *profil masnih kiselina, šaran, tolstolobik, amur, pangasius*

INTRODUCTION

Fish from the *Cyprinidae* family are the most widely cultured species. Common carp (*Cyprinus carpio*) in fish ponds is produced in polyculture with accompanying species such as bighead and silver carp and grass carp. Polyculture involves the culture, in the same pond, of several fish species that feed on different natural resources and thus improve water quality and increase fish production.

Grass carp *(Ctenopharyngodon idella*) is reared in fish ponds for regulation of overgrown aquatic plants like reed and rush. Silver carp (*Hypophthalmichthys molitrix*) is strong phytoplankton and zooplankton feeder. Polyculture in Europe expanded from the 1960s with the introduction of Chinese carp and often involves only two or three species (common carp, silver and grass carp) (Horvath *et al*., 1984; Olah, 1986).

The aim of this study is comparison of the fatty acid profile of carp fish from two semi - intensive culture ponds with different rearing system. Within the last few years farmed Asian white catfish, *Pangasius hypophthalmus,* has been introduced as a successful new fish species on the European market. *Pangasius* is fast growing fish species which reaches market size within eight months. In this study the fatty acid profile of Vietnamese catfish fillets from domestic market was investigated.

MATERIALS AND METHODS

Investigation was carried out on fifteen samples of marketable (two year old) common carp, silver carp and grass carp, collected in October and November 2010, from two semi-intensive fish pond with different feeding regime: in the first pond fish was fed with commercial extruded diet and in the second pond fish was supplementary feed with wheat only. Analysis was carried out on homogenized fish muscle samples after evisceration and deprivation of skin, tail, head, fins and bones. Five samples of *Pangasius* fillets were obtained from the market and analyzed too.

Total lipids for fatty acid determination were extracted from fish muscle tissue with hexane/isopropanol mixture by accelerated solvent extraction (ASE 200, Dionex, Germany). After evaporation of solvent until dryness under the stream of nitrogen total lipids were converted to fatty acid methyl esters (FAME) by trimethylsulfonium hydroxide. FAMEs were determined by using Shimadzu 2010 gas chromatograph equipped with flame ionization detector (FID) and cianopropyl HP-88 capillary column (100m x 0.25 mm x 0.20µm).

Statistical analysis

Data obtained for fatty acid composition were subjected to analysis of variance (ANOVA) with the least significant difference test at the level of significance of 5%.

RESULTS AND DISCUSSION

Fatty acid composition of muscle tissue of carp species is presented in Table 1.

Table1. Fatty acid composition (g100g^{-1} of total fatty acids) of muscle tissue of common carp, silver carp and grass carp (mean ± SD) from two aquaculture ponds.

	Common carp		Silver carp		Grass carp	
Pond / Fatty acid	I	II	I	II	I	II
SFA	27.21±1.56^{a}	27.02±1.93^{a}	29.40±1.93^{a}	32.61±1.23^{b}	34.48±0.94^{b}	35.49±0.64^{b}
MUFA	50.40±1.39^{b}	63.50±1.58^{d}	54.87±3.40^{a}	57.60±2.20^{c}	54.90±0.71^{a}	54.64±0.62^{a}
PUFA	21.67±1.12^{c}	8.91±0.44^{b}	16.52±1.63^{a}	9.15±1.23^{b}	9.83±1.04^{b}	9.23±1.02^{b}
n-3	1.43±0.13^{a}	0.63±0.01^{b}	1.14±0.34^{a}	2.66±0.24^{c}	6.70±0.74^{d}	6.14±0.54^{d}
n-6	20.12±0.98^{c}	8.28±0.44^{d}	15.39±1.38^{a}	6.50±1.18^{d}	3.12±0.35^{b}	3.09±0.25^{b}
n-3/n-6	0.07±0.01^{a}	0.08±0.01^{a}	0.07±0.01^{a}	0.41±0.01^{c}	2.15±0.16^{b}	1.99±0.11^{b}
P/S	0.80±0.01^{a}	0.33±0.04^{c}	0.56±0.07^{d}	0.28±0.02^{b}	0.28±0.03^{b}	0.26±0.03^{b}
UFA/SFA	2.65	2.68	2.43	2.05	1.88	1.80

I pond - extruded diet, II - grain (wheat, barley, maize)
Values in the same row followed by the same letters do not differ significantly ($p>0.05$)

Table 2: Fatty acid profile (g100g^{-1} of total fatty acid) of *Pangasius hypophthalmus* fillets from Serbian market (mean ± SD)

Fatty acid	*Pangasius fillets*
SFA	41.36±2.60
MUFA	42.36±1.78
PUFA	15.77±4.98
n-3	1.31±0.16
n-6	14.46±2.82
n-3/n-6	0.09±0.03
P/S	0.38±0.15
UFA/SFA	1.40

The quality of fat has been described by using different ratios, such as PUFA/SFA and n-3/n-6 (Ahlgren et al. 1994). Human diets with a P/S lower than 0.45 have been viewed as unfavorable, as they possibly promote the occurrence of hypercholesterolemia. In the present study, the lipid fraction of muscle tissue showed P/S values ranging from 0.26 (grass carp) to 0.80 (common carp). However, this index, which is based only on the degree of fatty acid saturation, also ignores the metabolic effects of MUFA. The ratio of unsaturated (UFA) vs. saturated fatty acids is of greater importance in edible fat. The value higher than 0.35 is usually believed to be beneficial. Higher ratio in this study was obtained for common carp (2.68) and silver carp (2.43).

The ratio between PUFA of the n-3 and n-6 groups is one of the indices used to evaluate the nutritional value of the lipid fraction present in foods. Henderson and Tocher (1987) reported n-3/n-6 value of 0.5-3.8 for freshwater and 4.7-14.4 for marine fish. The n-3/n-6 ratio in this study ranged from 0.07 (common carp) to 2.15 (grass carp).

A total PUFA content in the muscle tissue lipids of the investigated fish varied from 9.23% (grass carp) to 21.67% (common carp). The higher amount of total n-3 PUFA was observed in muscle tissue of grass carp (6.70%) as well as lower amount of n-6

PUFA (3.12%). The n-3 to n-6 ratio was 2.15 in grass carp. Fatty acid profile of grass carp from two fish ponds was not significantly different. Higher n-3 to n-6 ratio in grass carp depends on the type of diet consumed, i.e. feed on natural food that are richer in the n-3 families of acids (Steffens and Wirth, 2007).

Common carp from two fish ponds were significantly different in the total MUFA and total PUFA content. Supplementary feeding of carp with wheat results in a lower amount of n-3 essentially fatty acids in fish muscle (0.63% in common carp from II pond). This is due to the lower proportion of natural feed in the diet of the carp which received supplementary grain (Steffens, 1997). Similar results for common carp were previously reported (Trbovic *et al.*, 2009., Trbovic *et al.* 2010).

Obtained results indicate to different feeding habit of carp fish. The main difference is in the total PUFA content and, particularly, n-6 fatty acids.

As an omnivorous species, *Pangasius hypophthalmus* is fed agricultural by-products, mainly rice bran, soy and fish by-products during rearing. However, more recent information shows that farm-made feeds consist of 30-40% trash marine fish and 60-70% rice bran (Phu and Thanh Hien, 2003). The nutritional quality of catfish was not considered to be excellent due to their fatty acid profile. Total lipids were characterized by high percentage of saturated fatty acids (41.4%), monounsaturated fatty acid amounting to 42.4%. Total PUFA content was characterized by a high proportion of n-6 and low n-3/n-6 ratio (0.09). Similar results for the fatty acid profile of *Pangasius* fillets from the domestic market were reported by Orban *et al*., 2008. and Karl *et al.* 2010. Further investigations are needed to evaluate the quality of freshwater fish from Serbian market as well as more fish samples.

CONCLUSION

Fatty acid profile of common carp, silver carp and grass carp in terms of sum of total SFA, MUFA and PUFA as well as P/S and n-3 to n-6 ratios of carp fish meat were compared. The lipid fraction of muscle tissue showed P/S values ranging from 0.26 (grass carp) to 0.80 (common carp). The n-3/n-6 ratio in this study ranged from 0.07 (common carp) to 2.15 (grass carp). Common carp from two fish ponds were significantly different in the total MUFA and total PUFA content. Supplementary feeding of carp with wheat results in a lower amount of n-3 essentially fatty acids in fish muscle.

The nutritional quality of analyzed *Pangasius* fillets from Serbian market was not considered to be acceptable due to their fatty acid profile. Total lipids were characterized by high percentage of saturated fatty acids (41.4%), monounsaturated fatty acid amounting to 42.4%. Total PUFA content was characterized by a high proportion of n-6 and low n-3/n-6 ratio (0.09).

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UZGOJ MLADUNACA ŠARANA U RECIRKULACIJSKOM SUSTAVU I RIBNJACIMA

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BREEDING OF CARP FINGERLINGS IN RECIRCULATING SYSTEMS AND IN PONDS

Abstract
Taking into consideration high losses and long period required for carp breeding up to market size and weight, the research focused on assessment of possibilities of breeding carp larvae and fingerlings in recirculating system. Three-day larvae of the average individual weight of 1.3 mg·ind^{-1} were settled in three 150-liter tanks. Each thank contained 30 000 larvae. Water temperature, as an important factor for growing larvae and fingerlings, varied between 23-24°C throughout the breeding period, and concentration of dissolved oxygen varied from 4.7 to 8 mg·L^{-1}. Feeding of larvae started immediately after settling in with the nauplius larvae Artemia salina, which were decapsulated and prepared for feeding 1 day earlier. Afterwards, live feed was replaced with forage mixture. The daily amount of extra feed was given at 2-hour intervals. After the 31st farming day, mass of fingerlings bred in the recirculating system depended on feed type and varied from 855.3 to 1123 mg·ind^{-1}.

Key words: *recirculating systems, larvae, fingerling*

UVOD

Tradicionalan uzgoj konzumnog šarana u našim klimatskim uvjetima traje tri uzgojne sezone. Prva uzgojna sezona počinje nasadom i uzgojem ličinaka i mjesečnjaka, a nastavlja se uzgojem mlađa. Prvi mjesec uzgoja smatra se najtežim i odvija se kroz dvije faze i to podrašćivanjem ličinaka i uzgojem mladunaca. U tom razdoblju uzgoja gubici iznose 50 do 60 %, a ponekad i do 90 %. Uzroci gubitaka su: brojni predatori (mesojedni insekti, žabe, zmije, ribojedne ptice), nedgovarajuća veličina zooplanktona u prvim danima nakon nasada ličinaka, iznenadno zahlađenje vode, pogoršanje fizikalno-kemijskih parametara vode (nedostatak kisika, visoka koncentracija ugljičnog dioksida i nedisociranog amonijaka). Osim navedenog, na preživljavanje ličinaka i mladunaca šarana utječe raspoloživost prirodne i dodatne hrane te bolesti. Idealna hrana za uzgoj ličinaka i mladunaca šarana je prirodna hrana. Odgovarajućim agrotehničkim mjerama može se u ribnjaku uzgojiti veliki broj jedinki zooplanktonskih organizama. Međutim, gušćim nasadom ličinaka i mladunaca, prirodna hrana brže se troši nego obnavlja, stoga se pokušalo načiniti odgovarajuću industrijsku hranu koja će zadovoljiti hranidbene potrebe šarana. Jirásek i Mareš (2001a, 2001b) navode da bi takvo krmivo trebalo sadržavati oko 50% proteina, 10 do 15% ugljikohidrata i 12 do 15% masti. Međutim, autori ipak daju prednost prirodnoj hrani i navode neke bitne razlike koje su važne kod primanja i probave prirodne hrane u usporedbi s industrijski pripremljenom hranom.

Od zooplanktonskih organizama za hranidbu mlađih kategorija šarana značajni su najmanji razvojni oblici kolnjaka (*Rotatoria),* a zatim rašljoticalci *(Cladocera), veslonošci (Copepoda)* i ličinke trzalaca (*Chironomidae).* Prema istraživanjima koja su obavili Mann-a (1935); Wenig (1949); Farkas (1958); Farkas i Herodek (1960); Albrecht i Breitschprecher (1969); Bogatova i sur. (1971) utvrđeno je, da *Daphnia magna* u svježoj tvari sadrži 91,6 % vode, 2,98 % bjelančevina, 0,78 % hitina, 0,62 % masti, NET-a 2,62 % i 1,62 % pepela. Isti autori su utvrdili da mlađi primjerci *Daphnie magne* sadrže značajno veću količinu bjelančevina, dok se sadržaj masti povećava starenjem.

Bogut i sur. (2003); Bogut i Adamek (2005); Bogut i sur. (2007; 2008) su utvrdili da *Daphnia magna* i ličinke *Chironomidae* u optimalnim količinama sadrže sve esencijalne aminokiseline i masne kiseline te se smatraju idealnom hranom za najranije stadije šarana.

Primjenom tehnologije uzgoja ličinaka i mladunaca šarana u kontroliranim uvjetima, moguće je smanjiti gubitke za oko 50 % i povećati tjelesnu masu jednogodišnjeg mlađa sa sadašnjih 30 do 40 g na 100 do 150 grama. Na taj način, stvorili bi se preduvjeti za skraćenje uzgojnog razdoblja konzumnog šarana sa tri na dvije godine. Radi rješavanja navedene problematike, provedeni su pokusi uzgoja šaranskih ličinaka i mladunaca u recirkulacijskom sustavu.

MATERIJAL I METODE RADA

Osnovne značajke recirkulacijskog sustava

Snabdijevanje vodom osigurano je iz 70 metara dubokog bunara, kapaciteta 25 l/sek., i temeperature vode 14 ^{o}C. Iz bunara se voda radom crpke podiže u taložnik volumena 12 m^3. Brzina prolaza vode kroz taložnik je 0,1 m/min, što omogućava taloženje najvećeg dijela otpadnih tvari (oko 90 %). Taloženjem otpadnih tvari rasterećuje se biofilter. Iz taložnika se povremeno nategom i usisivačem odstranjuje nataloženi mulj. Preko crijevnih grijača voda iz taložnika gravitacijom prelazi u biofilter čija je zapremina 36 m^3.

Biofilter je građen od različitih granulacija riječnog šljunka, ispod kojega se nalaze snopovi plastičnih mreža čiji je zadatak povaćenje kontaktne površine između vode i zraka. Tjedan prije upotrebe, voda cirkulira preko biofiltera kroz cijeli sistem te se na taj način obavlja potpuno formiranje biofiltera (hladna proba). Voda se u bifilteru preko sistema plastičnih perforiranih cijevi skuplja u tzv bunaru. Voda se iz bunara crpi pomoću crpne stanice i tlači kroz injektor.

Injektor je suženo mjesto u sistemu koje na gornjoj strani ima dovodnu cijev za zrak. U tom dijelu se smanjuje volumen, a povećava tlak te se na taj način voda obogaćuje plinovima iz zraka. Na izlazu iz injektora (venturi cijev) voda sadrži suvišnu količinu plina. Ukoliko bi tako obogaćena voda došla u aparate za valjenje, mjehurići zraka izbacivali bi oplođenu ikru, predličinke i ličinke. Da bi se ti mjehurici eliminirali, voda se uvodi u uređaj za odstranjivanje viška plinova.

Ovaj uređaj se sastoji od dvije cijevi različitih dimenzija. Voda se tlači kroz unutarnju cijev. Na izlazu iz cijevi tlak je 1 bar. Kad voda dođe na drugi kraj, tlak pada na vrijednost atmosferskog te prema Hardijevom zakonu, kada se vodi smanji tlak, ona otpušta višak plinova poput otvorene boce mineralne vode. Ovako obogaćena voda kisikom, a oslobođena suviška plinova, slobodnim padom dolazi u visinski spremnik. Voda se podgrijava grijačem koji je ugrađen u taložnik, dok se u visinskom spremniku obavlja dogrijavanje na potrebnu temperaturu. Regulacija vode je automatska. Na spremniku je ugrađen alarmni uređaj koji signalizira poremećaj razine vode te pad ili povećanje temperature.

Iz spremnika voda gravitacijom odlazi u zugger posude i kade za smještaj matica. Ovdje se cijevna mreža grana i čini niz zatvorenih cirkulacijskih krugova s jednakim tlakom na svakom mjestu. U hidrološkom pogledu, ovo je jako važno jer omogućuje finu regulaciju uzgona vode u Zugger aparatima te se na taj način smanjuje gubitak ikre i predličinaki. Voda koja prođe kroz Zugger aparate odvodi se sistemom „čiste kanalizacije" u taložnik.

Kada su sve cijevi i uređaji napunjeni vodom zatvara se zasun, a daljnje dopunjavanje vodom se vrši automatski preko ventila s plovkom koji je ugrađen u visinski spremnik. Dio vode koji se uvodi u kade za matice jako je opterećen ugljičnim dioksidom i izmetom matica te se ne vraća u taložnik, nego se sistemom prljave kanalizacije odvodi u septičku jamu. Sistem prljave kanalizacije kupi i prolivenu vodu s podova valionice i odvodi je u septičku jamu.

Ribnjak površine 0,5 ha u kome je planiran nasad mlađa nakon uzgoja u recirkulacijskom sustavu dezinficiran je hidratnim vapnom, a tijekom zime ostavljen je na suhom. Prije upusta vode, ribnjak je pognojen kokošjim gnojem i plitko potanjuran.

Nasad i uzgoj ličinaka i mladunaca šarana

Ličinke šarana su nakon transporta nasađene u 3 protočne ležnice. Uzgoj je trajao 31 dan. U svaku ležnicu zapremine 150 litara nasađeno je po 30 000 četverodnevnih ličinaka. Nakon 10 dana uzgoja mladunci iz svake ležnice su razrijeđeni u dvije ležnice s po 15 tisuća. Nakon 20 dana uzgoja ponovo su razrijeđeni na polovicu i do kraja istraživanja se uzgajao u 12 protočnih ležnica.

Prvih 7 dana ličinke su u svim ležnicama hranjene Artemiom, a nakon toga jedna skupina je hranjena starterom (Dana feed), druga starterom i Artemiom, a treća samo Artemiom. Gotova krmna smjesa Dana feed (dan-ex karpfen 13/52) je prema deklaraciji proizvođača sadržavala 1,4 % sirove vlaknine, 10,3 % pepela, 17 % NET, 52 % bjelančevina i 16,4 MJ metaboličke energije.

Ličinke i mladunci su hranjeni 9 puta dnevno, a osvjetljenje je kombinirano (prirodno i umjetno) u trajanju od 17 do 18 sati tijekom 24 sata.

Tijekom istraživanog razdoblja svakodnevno je mjerena temperatura vode, koncentracija otopljenog kisika i pH vrijednost, a ostali fizikalno-kemijski parametri (prozirnost vode, organsko onečišćenje, amonijak, nitrati, nitriti i fosfati) jednom tjedno. Tjelesna masa ličinaka i mladunaca šarana mjerena je svakih 7 dana.

Dekapsulacija i priprema Artemije za hranidbu ličinaka i mladunaca obavljana je prema preporuci proizvođača.

REZULTATI I RASPRAVA

Temperatura vode jedan je od najvažnijih parametara koji utječu na rast riba. Tijekom naših istraživanja, temperature vode bile su ujednačene i varirale su od 23 do 24°C i bile su za 3 do 4°C niže u usporedbi s istraživanjima koja su proveli Kouril i Hamačkova (1982) i Prikril i sur. (1990). Niže temperature vode rezultirale su sporijim rastom u usporedbi s istraživanjima Kourila i Hamačkove (1982) koji navode, da je za brzi rast ličinaka i mladunaca šarana optimalna temperatura vode od 25 do 30°C. Temperature vode ispod 23°C i preko 32°C su nepovoljne za uzgoj ličinaka i mladunaca šarana.

Rezultati pH vrijednosti vode u našim istraživanjima varirali su od 6,6 do 7,5 (tablica 1.). Izmjerene vrijednosti kretale su se u optimalnim vrijednostima za uzgoj ličinaka i mladunaca šarana i u skladu su s preporukama koje navode Prikril i sur. (1990).

Koncentracija otopljenog kisika prikazanog u tablici 1. varirala je od 6,7 do 8 $mg \cdot L^{-1}$. Međutim, 8. i 9. dana nakon nasada došlo je do pada koncentracije kisika pa je 10. dana mlađ iz svake od tri protočne ležnice u koju je nasađeno 30 000 jedinki razrijeđen na polovicu. Ponovni pad koncentracije kisika i drugo razrjeđenje mladunaca obavljeno je 16. uzgojnog dana te je u svakoj ležnici do kraja istraživanja uzgajano oko 8 000 mladunaca. Ostali fizikalno-kemijski pokazatelji vode mijenjali su se u poželjnim i dopuštenim vrijednostima za uzgoj toplovodnih riba u recirkulacijskim sustavima.

Tabela 1. Fizikalno-kemijski pokazatelji vode u protočnim ležnicama tijekom uzgoja

Pokazatelj	Vrijeme uzgoja				
	1. dan	7. dan	14. dan	21.dan	30. dan
Temperatura vode °C	23,0	23,5	24,0	23,6	23,2
Prozirnost cm	do dna	do dna	do dna	do dna	do dna
$KMnO_4$ $mg \cdot l^{-1}$	4,9	8,5	9,0	12,1	14,3
pH	6,6	7,4	7,5	7,4	6,9
Alkalitet $mmol \cdot l^{-1}$	1,8	1,98	2,15	2,97	3,15
Kisik $mg \cdot l^{-1}$	8,1	6,2	6,4	6,2	6,0
PO_4^{-3}- P $mg \cdot l^{-1}$	0,2	0,3	0,5	0,6	0,6
NO_3 - N $mg \cdot l^{-1}$	0,15	1,51	2,18	3,24	3,98
NO_2 - N $mg \cdot l^{-1}$	0,00	0,12	0,09	0,09	0,09
NH_3 -N $mg \cdot l^{-1}$	0,001	0,009	0,017	0,015	0,021

Prosječna nasadna masa ličinaka bila je ujednačena i iznosila je 1,3 $mg \cdot ind^{-1}$. Nakon uzgoja koji je trajao 31 dan, najviša prosječna individualna masa utvrđena je u skupini koja je hranjena Artemiom, a iznosila je 1123 $mg \cdot ind^{-1}$. U skupini koja je hranjena Artemiom i starterom, prosječna individualna masa mladunaca iznosila je 914 $mg \cdot ind^{-1}$, što je za 18,1% niže nego se u skupini koja je hranjena živom hranom. Najniža individualna masa mladunaca utvrđena je u skupini koja je hranjena starterom, a iznosila je 855 $mg \cdot ind^{-1}$. Bolji rezultati u pogledu individualne mase utvrđeni su u istraživanjima Kourila i Hamačkove (1980) koji su ličinke i mladunce cijelo pokusno razdoblje hranili zooplanktonom, a temperatura vode je iznosila 26 do 27°C.

Kako bi se razumjele visoke vrijednosti dnevnog prirasta i specifične brzine rasta (tablica 2.) potrebno je poznavati ranu ontogenezu šarana. U fiziološkom pogledu, ličinački stadij obuhvaća ontogenezu koja počinje prelaskom na egzogenu hranidbu, a završava metamorfozom. To razdoblje karakterizira niz morfoloških i fizioloških promjena koje se očituju u načinu hranidbe, razvitku enzimatskog sustava, metabolizmu i hranidbenim potrebama. S gledišta fiziologije probave, ličinačko razdoblje završava razvitkom želuca u karnivornih riba ili u šarana potpunom funkcijom hepatopankreasa. Jedna od značajnijih osobina ličinaka i mladunaca šarana je visok potencijal rasta. Ličinke šarana nakon valjenja imale su masu od 1,3 mg. Za 30 dana masa je uvećana za 800 do 1000 puta. Za iskorištenje potencijala rasta potrebno je osigurati sve potrebne hranjive tvari i ekološke uvjete. Osim toga, preživljavanje ličinki i mladunaca ovisi o eliminaciji predatora.

Tabela 2. Kretanje individualna mase ličinaka i mladunaca šarana hranjenih različitim vrstama hrane

Pokazatelj	Hrana		
	Artemia	Artemia + starter	Starter
Nasadna masa ličinaka, mg	1,3	1,3	1,3
7. dan uzgoja	211	209	210
14. dan uzgoja	570	420	395
21. dan uzgoja	836	710	651
30. dan. uzgoja	1123	916	855

Nakon 31. dana uzgoja u recirkulacijskom sustavu ličinke su izlovljene i nasađene u ribnjak površine 0,5 ha. Desetog dana nakon nasada u ribnjake, primijećeno je da mlađ ne uzima hranu. Mjesec dana nakon nasada ispuštena je voda iz ribnjaka, a pri ispustu nije zabilježen niti jedan preživjeli mladunac.

ZAKLJUČAK

Na osnovi provedenih istraživanja uzgoja ličinaka i mladunaca u recirkulacijskom sustavu i nasada u ribnjak mogu se izvesti i definirati slijedeći zaključci:

Temperatura vode u recirkulacijskom sustavu kretala se od 23 do 24°C, a koncentracija otopljenog kisika od 6,7 do 8 $mg \cdot L^{-1}$. Ostali praćeni fizikalno-kemijski parametri (organsko onečišćenje, pH, alkalitet, posfati, nitrati, nitriti i nedisocirani amonijak) varirali su u optimalnim vrijednostima za uzgoj ličinaka i mladunaca

U sve tri pokusne skupine do 10. dana uzgoja ličinke su hranjene živom hranom (Artemiom)

Skupine ličinaka i mladunaca hranjene Artemiom salinom imale su najveću tjelesnu masu, prirast i SGR. Niže vrijednosti navedenih pokazatelja utvrđene su u skupini koja je hranjena kombinacijom žive hrane i startera. Najniže vrijednosti prirasta i SGR-a utvrđene su u skupini koja je hranjena od 10. dana do kraja uzgoja starterom.

Tijekom istraživanog razdoblja nisu utvrđeni gubitci ličinaka i mladunaca šarana u recirkulacijskom sustavu.

Mjesec dana nakon uzgoja u ribnjacima i nakon ispuštanja vode nije bilo preživjele mlađi.

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TENCH OOGENESIS: ULTRASTRUCTURAL ASPECTS OF THE OVARIAN FOLLICLES

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OVOGENEZA U LINJAKA: ULTRASTRUKTURNI ASPEKTI OVARIJALNIH FOLIKULA

Abstrakt
Ovaj rad predstavlja studiju folikula linjaka upotrebom transmisione elektronske mikroskopije (TEM), skening elektronske mikroskopije (SEM) i biohemijskih metoda. Studija ja fokusirana na morfološke aspekte previtelogenih, vitelogenih, postvitelogenih i atretičnih folikula ovarijuma. U folikularnoj fazi razvoja ovocit je okružen sa zona radiata, slojem folikularnih (granulosa) ćelija koje naležu na izraženu bazalnu membranu, vaskularizovani sloj theca folliculi sa brojnim kapilarima u mreži vezivnog tkiva i tankim epitelom na površini. Zabeležene su promene u folikulima uporedo sa pojavom proteina žumanceta. Antitela vitelogenina linjaka su pokazala prisustvo proteina žumanceta u krvnoj plazmi i vitelogenim folikulima.

Ključne reči: *linjak, ovarijum, folikul, ultrastruktura, vitelogeneza*

INTRODUCTION

The ovary structure and the stages of oocyte development in tench are well known. As in other teleosts, the oocytes grow within the ovarian follicles covered by cellular and acellular layers. Oocyte structure during oogenesis in tench has fragmently been studed by light microscopy (Epler et al., 1981; Horoszewicz, 1983; Pimpicka, 1986, 1989, 1990, 1995, 1997; Linhart, Billard, 1995). However ultrastructure of the ovarian follicles in tench appears unknown and it is upon this topic that we focused our research.

MATERIAL AND METHODS

Adult female tench were obtained from a fish farm. Blood and follicle samples were taken twice a month from April to October.

For scanning electron microscopy (SEM) the follicles were fixed on ice for one hour in 2% glutaralaldehyde in 0.1M sodium cacodylate buffer, dehydrated in an ethanol series, followed by critical point drying, mouting and gold sputtering. The samples were analyzed with a SEM LEO 420.

For transmission electron microscopy (TEM) and semi-thin sections, the follicles were embedded individually, after fixation in 2% glutaralaldehyde and in 1% O_sO_4 sodium cacodylate buffer, and in 1% agarose for orientation. Dehydration, embedding in LR-White and sectioning was done according to standard methods. Examination of ultra-thin sections stained with uranyl acetate was conducted with a Philips EM 208.

The electrophoretical separation of yolk proteins from follicles at various stages of ovarian development and from blood was done by SDS-polyacrylamide gel electrophoresis (SDS-PAGE).

The immunological identification of the yolk proteins in both follicles and blood was conducted with antibodies raised against vitellogenin from vitellogenic follicles. The yolk proteins were detected on ultra-thin sections from vitellogenic follicles using immunohistochemical techniques (rabbit anti-tench vitellogenin and goat anti-rabbit IgG, gold conjugate 10 nm) according to Liddell, Weeks (1996). Standard PAP-techniques were applied for immunological determination of the yolk proteins in blood plasma and follicle homogenate.

RESULTS

It is known that two stages are observed in oocyte development in teleosts: pre-follicle and follicle. This study only examines the latter, follicle stage of development or folliculogenesis. Nucleus changes accompanying cell divisions are not discussed either.

The follicle stage begins with the separation of each of the primary oocytes and their covering by the follicle, thecal and serosa cells, zona radiata and ends with ovulation or atresion. The folliculogenesis can be divided into several phases if the most important event in the oocyte development - the vitellogenesis - serves as a base. The following stages are distinguished: previtellogenic, vitellogenic, postvitellogenic and atretic.

A) Previtellogenic follicles

Before the beginning of vitellogenesis, previtellogenic follicles are built as a complex structure. They contain the oocyte in which the cortical alveoli (CA) develop, along with the adjoining cellular and acellular layers of the follicle wall which consist of the vitelline envelope or zona radiata (ZR), follicle cells (FC), basal lamina (BL), thecal cells (TC) and theca serosa (TS) (Fig.1).

1. Follicle wall formation

Follicle wall formation begins with the separation of the primary oocytes from the nest. Initially the follicle cells form a thick monolayer around the oocyte and are interconnected by desmosomes. They are separated from the thecal cells by the basal lamina, which is made up of multilayered lamellae, the most interior what serves as a base. Over the basal lamina are the thecal cells organized in two layers – theca interna (TCI) and theca externa (TCE). The cells of the TCI lie on the outer lamella of the basal lamina. They are large, polygonal cells, forming long overlapping outgrowths on the periphery (Fig.2). The TCE is made of connective tissue components, which provide mechanical support for the follicle, and blood capillaries. The theca serosa composed of thin polygonal cells, is the external cover of the follicles and it separates one follicle from another (Fig.3).

The zona radiata is an acellular layer of the follicle wall situated between the follicle cells and the oocyte (Fig.4). Its formation and the formation of microvilli are synchronous. With the appearance of follicle cells (FC) around the oocyte, many microvilli appear on the surface of the oocyte and the follicle cells (Fig.5). Initially there is a large perivitelline space between the follicle cells and the oocyte where the microvilli are situated. Electron-dense envelope material begins to accumulate between the microvilli that extend from the surface of the oocyte towards the overlaying follicle cells (Fig.6). This accumullation leads to the formation of canal pores in the zona radiata (Fig.7a,7b). Microvillar processes (microvilli) from both the oocyte and follicle cells are apparent within patent canals that traverse the developing zona radiata (Fig.4).

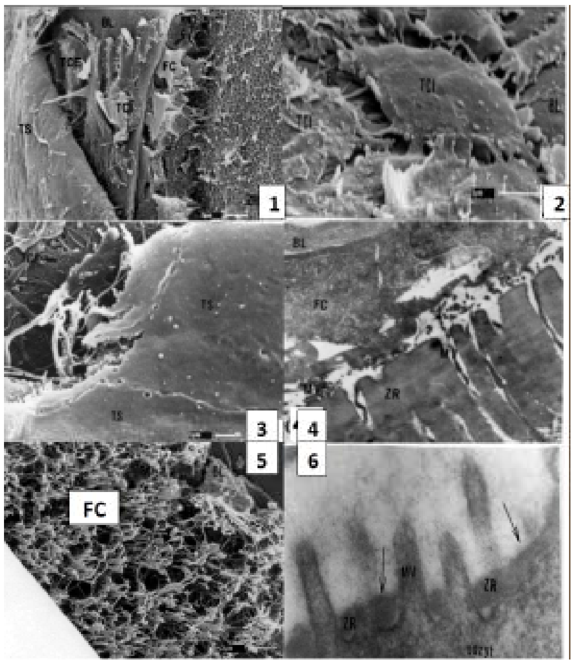

Fig.1. SEM of a portion of the previtellogenic follicle wall. Note the different cellular and acellular components of the wall. TS-theca serosa; TCE-thecal cells external; TCI-thecal cells internal; BL-basal lamina; FC-follicle cells; MV-microvilli; ZR-zona radiata (vitelline envelope).

Fig.2. SEM of a few thecal cells internal (TCI), which lie on the outer lamella of the basal lamina (BL).

Fig.3. SEM of part of the theca external (TCE) and theca serosa (TS). The theca externa comprises connective tissue components and the theca serosa comprises thin polygonal cells.

Fig.4. TEM of a section of the previtellogenic follicle wall. Elongate microvilli (MV) from both the oocyte (O) and overlying follicle cells (FC) are visible within patent pore canals of the developing zona radiata (ZR). x8000.

Fig.5. SEM of the apical side of the follicle cells (FC). The FC produce numerous microvilli (MV) on their apical side that traverse the pore canals of the zona radiata (ZR).

Fig.6. TEM of a section of the previtellogenic follicle wall. Note the zona radiata (ZR) formation. Electron-dense material (arrows) begins to accumulate between the microvilli (MV) that extend from the surface of the oocyte towards the overlying follicle cells. x31500.

2. Cortical alveoli formation

The first cortical alveoli are observed by light microscopy, within the periphery of the oocyte. Electron microscopy shows that their formation starts simultaneously with the formation of zona radiata. When zona radiata building material begins to accumulate around the microvilli, the first cortical alvaoli appear individually or in small groups in the ooplasm. Their size and number increase towards the oocyte cortex. In the immediate proximity of larger cortical alveoli, numerous small alveoli are observed, some of them in the process of fusion (Fig.8). Cortical alveoli comprised two different structures: medial, fine granulated, and lateral, coarse granulated.

B) Vitellogenic follicles

With the start of vitellogenesis, changes occur in the oocyte itself as a place of yolk protein accumulation, as well as in the follicle wall.

The first yolk granules appear between the cortical alveoli in the oocyte cortex. Intercellular spaces appear among the follicle cells (Fig.11) and between the cells of theca interna. Their sizes increase in proportion to intensity of the yolk accumulation. Such spaces are not seen in the previtellogenic follicles.

Antivitellogeninantibody marks selectively yolk proteins (vitellogenin) in the oocyte ooplasm (Fig.12), the cortical ooplasm close to the oolemma, the basal lamina, and the zona radiata, the latte was exceptionally strongly marked. The antibody did not detect vitellogenin in follicle cells

The SDS-PAGE showed that in the vitellogenic follicles numerous protein bands are present with molecular weights of 122 to 12 kDa (Fig.9). Antibodies against vitellogenin detect vitellogenic protein bands (P_1,P_2,P_3,P_4,P_5 and P_6) in the follicle homogenate from mature females during the reproductive season (Fig.10, lane 4). In the blood serum of mature females in the pre-spawning period (May) the antibody detects only one protein band P_0 with molecular weight 203 kDa (Fig.10, lane 1). In blood serum of mature females during the autumn-winter period the antibody does not detect vitellogenic bands (Fig.10, lane 3). In the blood serum of mature females with atretic follicles in the ovary during the reproductive season the antibodies detect vitellogenin protein fractions also, which results from the resorption of the vitellins from the vitellogenic follicles (line 2).

C) Postvitellogenic follicle

With the end of vitellogenesis in postvitellogenic follicles, withdrawal of follicle cell and oocyte microvilli from the zona radiata pore canals is observed and the canals are left empty (Fig.13). The follicle cells, which are cubic and bear numerous microvilli on their apical side during vitellogenesis become rounded and have no more microvilli during that period of follicle development. As a result of that, the intercellular spaces between them, typical for the period of vitellogenesis, have become very narrow or are absent.

The apex of the follicle cells shows exocytotic activity. A substance (mucopolysaccharide) is accumulated on the zona radiata and partially penetrates into its canal pores, forming the chorion layer of the follicle wall (Fig.14). The micropyle is the only area where this substance is absent (Fig.15).

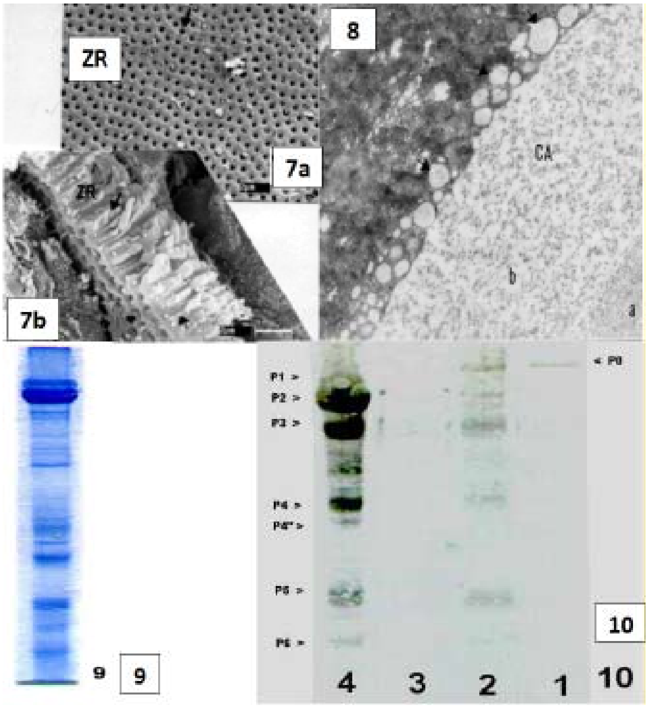

Fig.7a. SEM of a part of the zona radiata (ZR). Microvillar processes (microvilli) (arrows) are apparent within patent pore canals of the zona radiata (ZR).

Fig. 7b. SEM of a part of the outer side of the zona radiate (ZR). Pore canals (arrow) traverse zona radiata.

Fig. 8. TEM of a part of a cortical alveolus (CA) situated in the periphery of a previtellogenic follicle. Numerous small cortical alveoli (arrows) of variable size are closely associated with the surface of the larger alveolus. Note the two different structures in the CA: (a) medial, fine granulated, and (b) lateral, coarse granulated. x10000.

Fig.9. SDS-PAGE of extract of vitellogenic follicles. Note numerous protein bands with molecular weights from 122 to 12 kDa.

Fig.10. Western Blot. Antibodies against vitellogenin detect vitellogenic protein bands (P_1,P_2,P_3,P_4,P_5 and P_6) in the follicle homogenate from mature females during the reproductive season (lane 4). In the blood serum of mature females in the pre-spawning period (May) the antibody detects only one protein band P_0 with molecular weight 203 kDa (lane 1). In blood serum of mature females during the autumn-winter period the antibody does not detect vitellogenic bands (lane 3). In the blood serum of mature females with atretic follicles in the ovary during the reproductive season the antibodies detect vitellogenin protein fractions, which results from the resorption of the vitellins from the vitellogenic follicles (line 2).

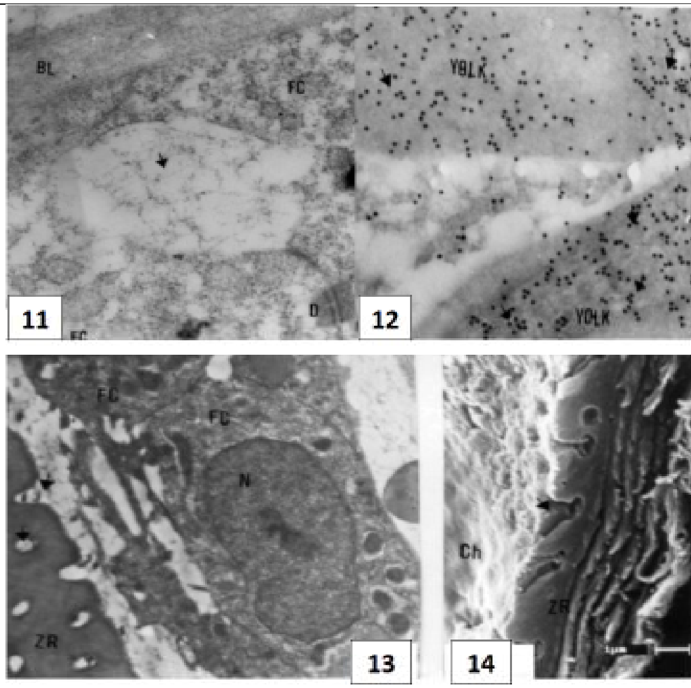

Fig.11. TEM of part of a vitellogenic follicle. Note the intercellular spaces (arrow) that appear between the follicle cells (FC) during vitellogenesis.(D) - desmosomes; (BL) – basal lamina. x25000.
Fig.12. TEM of parts of two yolk globuli in the ooplasm of a vitellogenic follicle. The antibodies mark vitellogenin substance (arrows) in the yolk globuli. (N) –nucleus from a FC. x40000.
Fig.13. TEM of part of the postvitellogenic follicle wall. Note the withdrawal of follicle cells (FC) and oocyte microvilli from zona zadiata (ZR) pore canals. x63000.
Fig.14. SEM of part of the postvitellogenic follicle wall. Note the empty pore canals (arrows) of the zona radiata (ZR) and the chorion layer (Ch) of the follicle wall.

D) Atretic follicle

After oocyte ovulation, only a part of the follicle wall remains in the ovarian stroma when the oocyte leaves the follicle with the zona radiata and the chorion layer. In this case the atretic follicle is built of follicle, thecal and serosa cells only. If ovulation does not occur, the destructive processes affect the whole follicle. Zona radiata loses its homogenous structure and numerous small “cracks” form in it (Fig.16). The follicle wall cells become hypertrophic, cell membranes perforate and become fragmented. Cortical alveoli lose their typical form and their composition mixes with cortical ooplasm and small yolk granules (Fig.17). Destructive processes occur in ooplasm and yolk also. Yolk proteins and cellular and acellular parts of follicle decompose very quickly and are conveyed through the blood vessels (Fig.18).

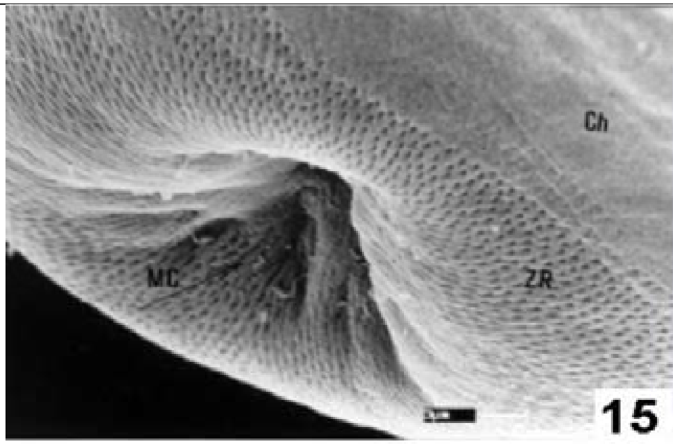

Fig.15. SEM of the micropyle area (arrow) of a postvitellogenic follicle after ovulation. (Ch) – chorion; (MC) – micropyle.

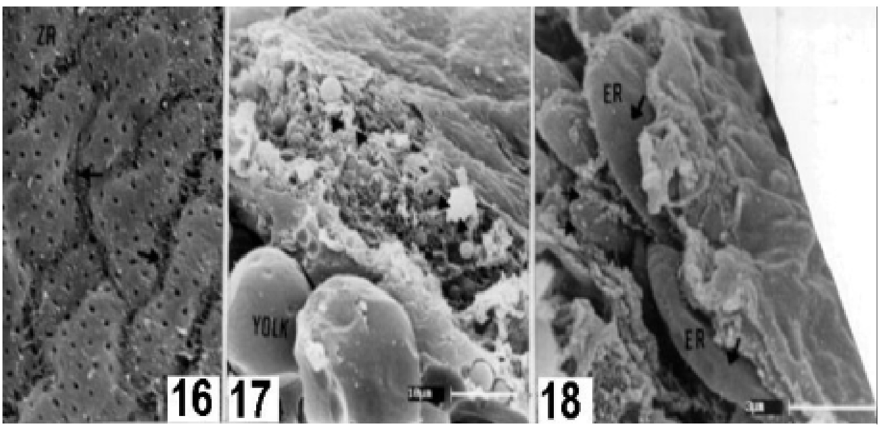

Fig.16. SEM of part of zona radiata (ZR) of an atretic follicle. Because of destructive processes zona radiate (ZR) loses its homogenous structure and small "cracks" (arrows) form in it.
Fig.17. SEM of part of the atretic follicle wall. Small yolk granules (arrows) mixes with cortical ooplasm and cellular and acellular parts of the follicle wall. Bigger yolk granules are seen in the periphery of the atretic oocyte.
Fig.18. SEM of part of the atretic follicle wall. Note that yolk proteins and other parts of the follicle wall are conveyed through the blood vessels (arrows: ER-erythrocyte).

DISCUSSION

The ultrastructural study has shown that the zona radiata is already differentiated as a structural element in the previtellogenic follicle. Through formation of numerous microvilli situated in the canal pores of the zona radiate, the oocyte surface is increased many times. The large area of the microvilli allows fast and intensive endocytosis of vitellogenin (Wallace et al., 1981).

When the oocyte increases during vitellogenesis, the zona radiata area should also increase at the same time. By using immunological methods, it has been shown that the basic proteins from which the zona radiate is assembled like vitellogenin, are synthesized in the liver and through the blood vessels they reach the follicles (Hamazaki et al., 1987, 1992; Nagahama et al., 1989). The authors propose that vitellogenin incorporation and zona radiata formation are parallel processes.

When the oocyte and zona radiata grow within the follicle, the follicle cells have to cover a larger area. We did not observe an increase in their number through mitotic division, thus we assume oocyte covering is achieved by means of follicle cells moving apart and forming relatively big intercellular spaces.

There is evidence of yolk protein synthesis in liver of teleost females (Ng, Idler, 1983; Wallace, 1985; Mommsen, Walsh, 1988; Wallace and Selman., 1990; Ding et al., 1994; Spannhof, 1995;) and its transport through the blood to the follicles (Wallace, Selman, 1981; Byrne et al., 1989). Our study showed that the final localization of blood capillaries in the follicle is the outer part of the basal lamina, between the TCI. This is where transition of vitellogenic macromolecules from blood capillaries should take place. That is possible for substances with a diameter of ≤10 nm, as case with the vitellogenic molecule (Wallace, Selman, 1990). Our findings support the hypothesis of Wallace and Selman (1990) about the pathway of vitellogenin into the follicle. We assume that it moves through the basal lamina, strongly marked by the antibodies, via the intercellular spaces between neighboring follicle cells. Vitellogenin is not found in the follicle cells but in the immediate proximity of them. In zona radiata canal pores, vitellogenic molecules contact the vitellogenic receptors in the plasmalemma of the microvilli and through receptor-mediated endocytosis they enter the oocyte (Opresko, Wiley, 1987, Tyler et al., 1991, Lancaster, Tyler, 1994, Manannos et al., 1997).

The results from our electrophoretical studies on vitellogenic follicles and blood serum of tench agree with the studies performed on other teleosts-*Carassius auratus* and *Archosargus probatocephalus* (Greely et al., 1986), *Fundulus heteroclitus* (Selman, Wallace, 1983), *Oryzias latipes* (Marakami et al., 1990), *Brachidanio rerio* (Selman et al., 1993), *Verasper moseri* (Matsubara, Sawano, 1995). Applying SDS-gels, a protein band is found in blood serum with molecular weight about 200 kD, and in the vitellogenic follicles typical bands are found with similar molecular weights of 122-197 kD, and three more in the lower molecular weight range. We have shown that in the tench, vitellogenesis occurs only in spring. Our antibody did not detect vitellogenin in the blood serum or the follicles in the autumn-winter period. Tench passes the winter with praevitellogenic follicles, in contrast to closely related species *C. carpio, C. carassius, H.molitri*).

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PROCESSING TRAITS OF EUROPEAN CATFISH (*SILURUS GLANIS*) FROM OUTDOOR FLOW-THROUGH AND INDOOR RECYCLING AQUACULTURE UNITS

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OSOBINE OBRADE SOMA (*SILURUS GLANIS*) IZ SPOLJAŠNJIH PROTOČNIH I UNUTRAŠNJIH RECIRKULACIONIH UZGOJNIH JEDINICA

Abstract

Wels (European) catfish, *Silurus glanis* L., is a high valued fish of European pond aquaculture. Recently, the quality of its flesh, suitability of very good growth performance in high stocking densities and ability to ingest artificial pelleted diets, led to its wider utilisation under conditions of intensive warm-water farming units including recycling systems. The evaluation of processing yields was performed using fish cultured in two different farming units – (1) outdoor pond aquaculture system (PAS) with flow-through regime (24.6±0.2°C) and (2) the indoor tank aquaculture system (TAS) with recirculation regime (26.0±1.0°C). Despite no significant differences appeared in their processing traits, the condition coefficients (based on eviscerated body weight) were significantly higher in PAS fish. However these coefficients were almost identical when calculated from the total weight of fish. Visceral, ventral and dorsal fat deposits were significantly higher in TAS fish in comparison to PAS fish and also in females as compared to males.

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Abstrakt

Evropski som, *Silurus glanis* L., je visoko vredna riba koja se gaji u Evropskim rubnjacima. U poslednje vreme, kvalitet njegovog mesa, pogodnost veoma dobrih performansi rasta u velikoj gustini nasada, kao i sposobnost korišćenja peletirane veštačke hrane, dovela je do povećanog obima gajenja u intenzivnim toplovodnim uzgojnim jedinicama, uključujući i recirkulacione sisteme. Procena prinosa prerade je obavljena ispitivanjem prerađenog proizvoda ribe gajene u 2 različita sistema: (1) ribnjačkom jezeru na o tvorenom (PAS) sa protočnim režimom (24.6±0.2°C) i (2) u tankovima u zatvorenom sistemu (TAS) sa recirkulacionim režimom (26.0±1.0°C). Iako nije bilo značajnih razlika u osobinama obrade, koeficijent kondicije (zasnovan na telesnoj masi bez viscere) je bio značajno viši kod riba iz PAS sistema. Ovi su koeficijenti bili gotovo identični kada bi se preračunali iz ukupne težine ribe. Visceralni, ventralni i dorzalni depoziti masti su bili značajno viši u riba iz RAS sistema u odnosu na ribe iz PAS, kao i kod ženki u poređenju sa mužjacima.

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IMPORTANCE AND USE OF GRAINS IN FISH NUTRITION

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ZNAČAJ I UPOTREBA ŽITA U ISHRANI RIBA

Abstrakt

Proizvodnja hrane za ribe, poslednjih godina beleži najbrži rast u industrijskoj proizvodnji hrane za životinje (Jovanović i sar. 2006). Ukoliko sektor akvakulture nastavi sa dosadašnjim prosečnim rastom od 8-10% godišnje do 2025. godine, i proizvodnja hrane moraće da prati taj rast (Tacon,2010). Danas se u hranu za ribe uglavnom uključuju riblje brašno i riblje ulje zbog visoke nutritivne vrednosti. Zbog ograničenosti prirodnih resursa. nameće se potreba za značajnijim uključivanjem biljnih hraniva, pre svega žita u ishrani riba. Žita prvenstveno predstavljaju izvor energije i služe za prihranu riba, međutim, njihovom preradom mogu se dobiti koncentati proteina koji se mogu koristiti kao delimična zamena za proteine animalnog porekla. Žita kao energetska hraniva čine osnovni deo obroka u poluintenzivnom sistemu gajenja riba. Proteinski deo potreba obezbeđuje im fauna dna i zooplankton.

U našoj zemlji kukuruz je najvažnije žito koja se koristi u ishrani životinja. Visok sadržaj skroba, srazmerno velika zastupljenost ulja i malo celuloze, čini kukuruz izrazito energetskim hranivom (Đorđević i Dinić, 2007). Kukuruz karakteriše nizak sadržaj proteina sa nepovoljnim aminokiselinskim sastavom. Protein kukuruza pretežno čini zein koji ima nisku nutritivnu vrednost, pre svega usled deficita lizina i triptofana. Proteini ječma su niske biološke vrednosti, ali nešto bolji u odnosu na proteine kukuruza (Perović, Janković et al.,2009). Siromašni su u lizinu i metioninu, mada su selekcijom dobijene sorte sa većim sadržajem lizina. U poređenju sa kukuruzom, po hranljivoj vrednosti, pšenica sadrži više proteina, a manje masti. Sadržaj proteina kod različitih sorata pšenice varira od 10-14% (Protić, Janković,1998).

Proteini pšenice su siromašni lizinom, zatim metioninom, treoninom, leucinom i izoleucinom (Janković et al.,2008). Količina lizina, koji je prva limitirajuća aminokiselina, iznosi 0,3-0,37%. Tritikale je hibrid pšenice i raži, koji se zbog relativno visoke nutritivne vrednosti sve više se koristi u ishrani svih kategorija životinja, pa i riba. Tritikale sadrži 11-20% sirovih proteina. Sadržaj aminokiselina je sličan sadržaju aminokiselina pšenice. Najsavremeniju tehnološku operaciju u procesu proizvodnje hrane za ribe predstavlja proces ekstrudiranja. Ekstruzionim kuvanjem na principu «visoka temperatura-kratko vreme» postižu se veca svarljivosti skroba, veća nutritivna vrednost, plutanje ili sporo tonjenje sto omogucava vece učesće žita u hrani za ribe. (Jovanović et al.2006).. Žita kao komponente u kompletnim smešama za ribe u intenzivnom uzgoju imaju izuzetno veliki značaj, posebno u ishrani šarana koji značajan deo energetskih potreba može zadovoljiti iz skroba. Proteinska hraniva, uz dodatak sintetičkih aminokiselina dobijena preradom žita, poput kukuruznog, pšeničnog glutena, kao i proteina pirinča, u značajnoj meri mogu zameniti, riblje brašno kao najkvalitetnije hranivo u ishrani riba. Selekcijom i genetskim modofikacijama treba stvarati žita sa povoljnim nutritivnim svojstvima potrebnim za hranu za ribe.

Ključne reči: *žita, hrana za ribe, ekstrudiranje*

INTRODUCTION

In recent years, production of fish feed is registering the fastest growth in industrial production of animal feed (Jovanovic et al,2006). If the aquaculture sector continues its present average growth from 8-10% annually until 2025, feed production will also have to follow this growth (Tacon, 2010). This poses the need for significant inclusion of plant feeds, primarily cereals in fish nutrition. The most important cereals in our country used in fish nutrition are: maize, wheat, barley and triticale. Cereal proteins have low biological value, primarily due to lysine deficit, but contain sufficient methionine and cystine, while soya is a rich source of the essential amino acids. If fish were fed only cereals, a series of health disorders would occur. Cereals are primarily an source of energy and serve for additional nutrition of fish, however, their processing can produce protein concentrates that can be used to partially substitute animal proteins. The yield of carp in fertilized fishponds is 390 kg/ha; if cereals and their byproducts are added to rations, the yield of carp increases up to 3000 kg/ha (Lovell et al.,1978). High energy feed with insufficient protein content leads to increased fat content in tissues (Cirkovic et al.,2011).

The protein level depends on the source and the quantity of energy in the ration. If these are fats, lower quantities of protein are required, than when energy is provided from carbohydrates. Protein digestibility is reduced when carbohydrate concentration grows (Smith and Lovell,1973). Carbohydrates contribute most to the nutritional value of cereal grains. Whole grains contain 62 to 72% of starch, digestible for warm water fish at a level of 60 do 70% (Cirkovic et al,2002), but much less for salmonids (Smith,1976). Starch from cereals is an important binding agent in pelleted and extruded fish feeds (Jovanovic et al.,2009; Cirkovic et al.,2010). Studies indicate that hormonal and metabolic carbohydrates regulation, as well as energy metabolism, vary in fish and can differ when compared to mammals (Cowey and Walton, 1989). California trout uses 30% glucose from 45% protein nutrition, while a 30% concentration of glucose in 30% protein nutrition has a negative effect on growth and efficiency of feed utilization (Bergot,1979). Fish do not secrete cellulase so, cellulose digestibility plays no significant role in their nutrition. Most fish can tolerate up to 8% fiber in rations, while concentrations from 8-30% decrease growth. High concentrations of digestible carbohydrates lead to liver enlargement and higher glycogen content in salmonids (Bergot,1979). Smith,1976 showed that for California trout only 49% of thermally unprocessed (raw) starch is digestible, while the carp family utilize over 70% of energy from raw starch.

Cereals in fish nutrition

Being energy feeds, cereals form the basic part of rations in a semi-intensive system of fish breeding. Protein requirements are provided by fauna populating the bottom and zooplankton.

In our country, maize (*Zea mays*) is the most important cereal used for animal nutrition. High starch content, a relatively high presence of oils and little cellulose, make maize a pronounced energy feed, with digestibility of organic matter of up to 90% due to poor utilization of starch in the diet, the share of cereals is expected to amount to 12% digestible carbohydrate The quantity of individual components varies depending on the type of hybrid and breeding conditions (Jelicic, Jankovic et al., 2009). The content of starch in maize is from 62-78%, raw protein from 7-14%, raw fat from 2.8-6% pentosanes from 4-5%, carbohydrates from 1-3% and cellulose from 1-3%. Maize is characterized by low protein content with an unfavorable amino acid composition. Maize protein is predominantly composed of zein, which has a low nutritional value. When fish are fed exclusively maize, the tryptophan deficit leads to niacin deficiency in the body. Geneticists have been trying for decades to create such forms that would have a better nutritional composition. Hybrids with increased oil and protein content and with altered starch composition were created.

Barley (*Hordeum vulgare*) is an important cereal in cold and humid areas and, after rice, it is the most widespread cultivated culture in the world. Barley proteins have low biological value, but they are better compared to maize proteins (Perovic, Jankovic et al.,2009). They are poor in lysine and methionine, although selection has created barley varieties with higher lysine content. Fat content in barley is around 2%, with a domination of palmitic and stearic acid. Raw fiber content is higher than in maize, wheat and rye, therefore its nutritional value is lower. Barley contains less starch then maize, and therefore it also has a lower energy content. Its vitamin content is similar as in other cereals.

Wheat (*Triticum sativum*) is primarily cultivated and used for human nutrition. Poorer quality wheat, as well as broken and inferior grain is used for nutrition of domestic animals when its price is lower than the price of maize. It is considered that of the total wheat production, 20-28% is used for nutrition of domestic animals. By its nutritive value, it is similar to maize. It has more protein, and less fat, as well as a somewhat lower level of digestible energy. Various wheat varieties contain from 10-14% of protein (Protic, Jankovic et al,1998). Wheat proteins are poor in lysine, methionine, threonine, leucine and isoleucine (Jankovic et al.,2008). The quantity of lysine, the first limiting amino acid, is 0.30-0.37%. It is poor in vitamins A and D, but contains high quantities of vitamins B1, B2, B6, nicotinic acid, pantothenic acid, folic acid and biotin. Wheat is poor in calcium, but contains more phosphorus (Prvulovic, Jankovic et al., 2009).

Triticale (*Triticale* sp.) is a hybrid between wheat and rye, which is due to its relatively high nutritional value used more and more in nutrition of animals, including fish. Its content of protein and lysine is higher compared to wheat and rye. Energy value is lower than for wheat and maize. Triticale contains 11-20% raw protein. Its amino acid content is similar to the amino acid content of wheat. Phosphorus utilization for triticale is much higher than for other cereals, owing to the presence of higher quantities of the enzyme phytase. A comparative review of cereal nutrients is presented in Table 1.

Complete mixtures, as additional feed in the form of pellets or granules, are becoming more and more important from the aspect of economically justifiable production, and are to a large extent replacing the traditional use of cereals in carp nutrition (Stankovic et al., 2011). In intensive forms of breeding, fish nutrition is supplemented by soya, peas, sunflower and synthetic amino acids, in pellet or extruded form. Intensive production, of over 3000 kg/ha, as well as production of noble predators, require the use of animal feeds.

Table 1. Chemical composition of triticale, maize, barley and wheat (Đorđević and Dinić, 2007)

Parameters	**Triticale**	**Maize**	**Barley**	**Wheat**
Raw protein,%	15.8	8.5	11.5	12.6
Lysine,%	0.52	0.25	0.40	0.40
Methionine,%	0.50	0.40	0.37	0.52
Threonine,%	0.51	0.36	0.36	0.46
Tryptophan,%	0.18	0.09	0.15	0.17
Raw cellulose,%	4.0	2.3	5.0	2.6
Oil, %	1.5	3.6	1.7	1.6
Calcium,%	0.05	0.03	0.05	0.04
Phosphorus,%	0.30	0.28	0.34	0.37
Digestible energy, DE MJ/kg	3299	3530	3120	3402
Metabolic energy, ME MJ/kg	3050	3420	3040	3300

Fish feed and the use of cereals

Intensive fish breeding, in addition to economy of nutrition, also poses strict requirements for the composition and physical characteristics of fish feed in order to minimize water pollution by remnants of unconsumed and/or undigested feed (Kiang, 2001). During the processing of fish feed, starch and proteins are exposed to chemical modifications, which include changes of molecular structure via starch gelatinization. By extrusion cooking based on the “high temperature – short duration” principle better digestibility of starch is achieved, higher nutritional value, as well as desirable physical characteristics – floating or slow sinking, which allows greater participation of cereal in fish feed (Jovanovic et al., 2006).

The additional feed for carp (*Cyprinus carpio*), in earlier times was to the highest extent or fully grainy feed, and to a high extent maize, wheat, barley, triticale. Today, more and more industrially produced pelleted or extruded feed containing required ingredients in accordance with nutritional requirements for certain ages of carp, is used. The diameter of pellets or granules depends on the age of fish, i.e. on the possibility to be consumed. Thus, literature mentions that a grain of maize can be consumed only when carp reaches 300 g (Đorđević and Dinić, 2007). Production of pelleted or extruded feed is desirable, to enable inclusion of cereals in nutrition for all categories of fish. In our country, mercantile carp is usually produced under semi-intensive conditions. For additional feeding of carp, until the first half of August, wheat (barley, triticale) is usually used, while maize is used in September and October. The feed conversion coefficient in a semi-intensive system is from 2.5 to over 6 kg. According to the statements of Mitrovic-Tutundzic et al., 1988, inadequate nutrition with maize, when natural food is lacking, can lead to a fatty degradation of the liver in carp and the depositing of fatty tissue of a soft consistency in subcutaneous connective tissue and in the stomach, as well as to degenerative lesions of the gonads.

From the aspect of nutrition, California trout is a carnivore. Trout are fed pelleted or extruded feed, prepared from flour type feeds originating from animals, and to a much lesser degree cereals (wheat and maize meal). Due to poor utilization of starch in the diet, the share of cereals should be up to 12% in the form of digestible carbohydrates (Đorđević and Dinić, 2007). When producing trout feed that should contain a high level of fats in granules, the problem of adding large quantities of fats is resolved using a vacuum device which enables distribution of oil throughout the entire volume of the pellet (Jovanovic et al., 2005). In the extrusion process, the share of carbohydrate feeds, (wheat or maize meal), enables the formation of puffy spaces within the granule into which oil is subsequently included by way of vacuum.

Expansion of starch simultaneously helps to obtain a physical structure slow to sink, which is decisive for adequate consumption by the trout. Cirkovic et al., 2005 stated that the density at which the extruded product 100% floats is below 550 g/l, while the density at which the extruded product 100% sinks is over 620 g/l, where the difference in water is only 8 liters. Fish meal is an adequate fish feed due to its high protein content, excellent amino acid profile, high digestibility. It is expected that in the near future the demand for protein feeds will exceed the annual offer of fish meal and induce greater use of plant proteins and protein isolates from plant feeds, protein from cereals, such as corn and wheat gluten and use of synthetic amino acids. Maize gluten meal is broadly used in fish feeds and the usual percent of in feeds is from 10-15%. Selection could create varieties and hybrids of cereals with favorable nutritional characteristics for use in fish feed. One of the most modern methods for achieving this goal is also genetic modification, but in this case the potential risks its entails must be taken into consideration.

CONCLUSION

The use of cereal grains in semi-intensive carp breeding systems is a realistic option in fish breeding in Serbia, because it simulates organic production. Cereals, as components in complete mixtures for fish under intensive breeding are very important, especially in the nutrition of carp, which is able to satisfy a significant portion of its energy needs from starch. By extrusion, as the most modern technological procedure for producing fish feed, starch is gelatinized, making cereal utilization much more efficient. Protein feeds, with the addition of synthetic amino acids obtained by processing cereals, such as maize and wheat gluten, as well as rice protein, can to a significant level substitute fish meal as the highest quality feed in fish nutrition. The process of selection and genetic modifications should be used to create cereals with favorable nutritive characteristics for fish feed.

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IMPORTANCE OF FISH MEAL AND OTHER ANIMAL FEEDSTUFFS IN PRODUCTION OF CONCENTRATE MIXTURES

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ZNAČAJ RIBLJEG BRAŠNA I DRUGIH HRANIVA ŽIVOTINJSKOG POREKLA ZA PROIZVODNJU SMEŠA KONCENTRATA

Abstrakt

U radu je prikazan značaj ribljeg brašna kao i drugih hraniva životinjskog porekla za proizvodnju smeša koncentrata, kao i mogućnost njihove susptitucije hranivima biljnog porekla u kombinaciji sa sintetičkim aminokiselinama, ili komercijalnim „zamenama" ribljeg brašna.

Riblje brašno je do sada najviše korišćeno hranivo životinjslog porekla. Zbog opasnosti od širenja bolesti *Bovine spongiform encephalopathy* – BSE, u Evropskoj uniji je regulativama 999/2001 i 1234/2003 zabranjena upotreba obrađenih animalnih proteina, u koje spadaju različite vrste mesno-koštanog brašna, za sve farmske životinje koje ulaze u lanac ishrane ljudi, izuzev ribljeg brašna za nepreživare. Kod nas je u skladu sa Zakonom o veterinarstvu iz 2005. uvedena obaveza za sve fabrike hrane za životinje da odvajaju linije u kojima se pripremaju koncentrati za preživare, ili da proizvodnju obavljaju na istoj liniji ali da se pri tome odreknu upotrebe hraniva životinjskog porekla. U skladu sa tim, vrši se stalni monitoring smeša za preživare.

Zadnjih godina je korišćenje ribljeg brašna u ishrani nepreživara jako smanjeno zbog navedenih zakonskih ograničenja, sve lošije hranljive i upotrebne vrednosti (zdravstvene ispravnosti), problema falsifikovanja (dodavanja hraniva niže hranljive vrednosti: sojine sačme, kukuruznog glutena, brašna od perja pa čak i uree) kao i zbog visoke cene. Osim toga, dobro je poznato da riblje brašno prenosi svoj specifičan miris na proizvode pa se obavezno isključuje iz smeša pri kraju tova brojlera.

Velike količine ribljeg brašna se i danas koriste pri proizvodnji peletirane hrane za pastrmke i druge karnivore ribe. Naime, ove vrste riba zahtevaju visok nivo proteina dobre biološke vrednosti u obrocima. Ranih 90-ih godina preporučivan odnos svarljivih sirovih proteina i svarljive energije u obrocima za pastrmke je bio 22-25 g/MJ. Nasuprot

tome, u zadnjoj dekadi eksperimentalno je dokazana efikasnost obroka sa većim sadržajem masti (>20%) i kada je odnos proteina i energije uži. Međutim, u tom slučaju treba obezbediti odgovarajući nivo esencijalnih aminokiselina. Objašnjenje je u specifičnom metabolizmu riba. Krajnji proizvod metabolizma proteina u riba je amonijak, za šta je potrebno manje energije. Nasuprot tome, krajnji proizvod metabolizma energije u svinja je urea, čime se objašnjava uži odnos proteina i energije (oko 14 g/KJ).

Zbog svega navedenog proizvođači teže da riblje brašno zamene (delimično ili potpuno) nekim drugim proteinskim hranivom (biljnog ili životinjskog porekla), pri čemu je jedan od glavnih ciljeva što niža cena proizvodnje. Do sada je najviše pažnje poklanjano proizvodima od soje, odnosno sojinoj sačmi. Ovo hranivo ima veliku biološku vrednost proteina ali i visok sadržaj različitih antinutritivnih materija. Nasuprot tome, efikasnijim se pokazao koncentrat proteina soje. To je hranivo koje se dobija uklanjanjem masti i rastvorljivih ugljenih hidrata. Eksperimentalno je utvrđena mogućnost delimične supstitucije ribljeg brašna suncokretovom sačmom, ali je glavni nedostatak ovog hraniva u velikoj količini nerastvorljivh ugljenih hidrata. Pored ovih hraniva, u eksperimentima su ispitivane sačme pamuka, kikirikija, uljane repice, brašno lupine, kukuruzni gluten, proteini krompira i dr. Međutim, nedostatak svih ovih hraniva je nizak nivo nekih esencijalnih aminokiselina kao i prisustvo antinutritivnih, štetnih i nesvarljivih materija. Jedno od potencijalnih proteinskih hraniva za ribe i domaće životinje je bakterijski protein. U pogledu brzine rasta i produkcije proteina, bakterije imaju prednost u odnosu na kvasce. Pored toga, bakterije sadrže i više proteina (do 80%), a aminokiselinski sastav je povoljniji i sličniji proteinima životinjskog porekla. Nedostatak je velika količina nukleinskih kiselina (do 18%) koje u sisara katabolišu do mokraćne kiseline.

U zaključku se ističe da su troškovi ishrane ključni za rentabilnost proizvodnje u stočarstvu i nekim oblicima ribarske proizvodnje. Supstitucija ribljeg brašna u obrocima za domaće životinje i ribe je neophodnost zbog visoke cene ovog hraniva, varijabilnog kvaliteta i eventualnog falsifikovanja jeftinijim hranivima. Za sada, najveći značaj pokazuju proizvodi prerade soje a određenu perspektivu imaju i bakterijski proteini.

Ključne reči: *riblje brašno, hemijski sastav, sintetičke aminoksieline, supstitucija.*

INTRODUCTION

According to modern concepts for nonruminant nutrition, the amount of essential amino acids is the most important criteria in formulating concentrate mixtures, compared to the amount of total or "crude" protein content. Aside from that, modern normatives for dairy cows take into account amino acid profile of the ration, considering that their microbial synthesis is not sufficient (Grubić and Đorđević, 2005). Feeds of animal origin were used for decades as essential source of amino acids in mixtures for almost all species of animals. However, in recent times the use of such feedstuffs is banned in European Union in the nutrition of all farmed animals which enter the human consumption chain, aside from fish meal used in nonruminants, in order to prevent spreading of the "mad cow disease" - *Bovine spongiform encephalopathy* (Đorđević et al., 2009). The utilization of animal feeds today is mostly restricted to the use of powdered milk in milk replacers for young ruminants (Đorđević et al., 2007), while meat meal is used for production of extruded pet feeds. According to the Veterinary Law of 2005, it is mandatory in Serbia that feed mills which use fish meal have separate production lines for mixing feeds for ruminants and nonruminants (Đorđević et al., 2010a). The production of mixtures for ruminants is regularly monitored according to this law (Nešić et al., 2010).

The possibilities to substitute fish meal and other animal feedstuffs

Feeds of animal origin are the main or secondary products of slaughterhouses, dairy, rendering industry and fish processing plants. They have high content of protein which may have variable biological value. Among these feedstuffs fish products have special place because of their high protein content with excellent biological value, and also high content of minerals and some vitamins. Fish and fish products are among least expensive feeds of animal origin. Because of that fresh fish was used for many years as feed for various animal species. However, due to the high risk of decaying fish and fish products should be conserved, which can be done by process of "ensiling" with direct acidification or stimulation of lactobacillus species, of which we poses or own results (Đorđević et al., 1998; 1999; 2000; Jokić et al., 2001). Fish conserved in such way have high moisture content and is not suitable for industrial production of concentrate mixtures. The other process, much more expensive, is fish drying and production of fish meal. Approximately 20% of the world's fish harvest is used for production of fish meal for animal feeding. In recent years the use of fish meal in nonruminant nutrition was reduced because of the explained lawful restrictions, and decreased nutritive and functional value (and health risks), problems with falsifying (adding feeds with lower nutritive value such as: soybean meal, maize gluten, feather meal and even urea), and also because of its high price. It is also well known that fish meal transfers specific odor on final products, and is excluded from concentrates in finishing broiler chick feeding (Đorđević and Dinić, 2007). This is why fish meal is today being replaced with combination of quality feeds of plant origin with added synthetic amino acids, or specific commercial products (Đorđević et al., 2010b). Such combinations can achieve similar nutritive value, with equal or even lower price (Đorđević et al., 2006).

High quantities of fish meal are used today in the production of pelleted feeds for trout and other carnivorous fish species. Such fish species require appropriate level of protein with high biological value in their diets (Đorđević et al., 2005). In the early 1990-s the recommended ratio of digestible protein and digestible energy in trout feeds was 22-25 g/MJ (Cho, 1992). However, during the last decade it was experimentally confirmed that diets with higher fat content (>20%) is more effective, when protein / energy ratio was narrower (Yamamoto et al., 2005). In such circumstances it is necessary to provide the appropriate level of synthetic amino acids. The explanation for that is in specific fish metabolism (Đorđević et al., 2005). The end product of protein metabolism in fishes is ammonia, and it requires less energy to produce than urea, which is produced in mammals, such as pigs, and that explains narrower protein / energy ratio (about 14g/KJ). Yamamoto et al. (2005) in their experiment with trout fry used diets with lower protein content and protein / energy ratio of 16-17 g/MJ, but the synthetic amino acids were added. It was confirmed that fish had better utilization of feeds and higher nitrogen retention when essential amino acids were added (table 1).

Table 1. Growth, feed performance and whole body proximate composition of rainbow trout (Yamamoto et al., 2005)

	A	B	C	D	P (anova)
Growth and feed performance					
Initial BW (g)	20.8±5	20.8±5	20.9±6	20.9±3	0.958
Final BW (g)	68.4±1.4	66.0±7.9	64.2±5.7	69.0±6.5	0.646
Gain, %	236±10	235±43	223±28	246±31	0.650
Feed intake, (%BW/day)	$1.91±0.09^{b}$	$1.80±0.09^{ab}$	$1.76±0.07^{a}$	$1.74±0.04^{a}$	0.026
DE intake (kJ/kg BW/day)	332±15	342±17	335±14	330±8	0.625
Feed efficiency	$0.94±0.03^{a}$	$0.99±0.06^{ab}$	$0.98±0.03^{a}$	$1.05±0.04^{b}$	0.016
N retention (%)	$35.4±1.5^{a}$	$44.4±3.1^{b}$	$44.5±1.8^{b}$	$49.7±1.6^{c}$	<0.001
Whole body proximate composition					
Moisture (%)	$69.3±0.5^{b}$	$66.6±0.5^{a}$	$66.1±0.6^{a}$	$66.5±0.2^{a}$	<0.001
Crude protein (%)	$16.6±0.2^{c}$	$15.8±0.1^{a}$	$15.9±0.2^{a}$	$16.1±0.0^{b}$	<0.001
Crude fat (%)	$12.1±0.7^{a}$	$15.5±0.4^{b}$	$15.7±0.6^{b}$	$15.1±0.3^{b}$	<0.001
Ash (%)	2.3±0.1	2.2±0.1	2.2±0.1	2.3±0.1	0.124

A = with fish meal (48%); B = with fish meal (35%) + non-essential amino acid mix (1.76%); C = with fish meal (35%) + non-essential amino acid mix (1.10%) + essential amino acid mix (0.74%); D = with fish meal (35%) + essential amino acid mix (2.25%)

Other feeds of animal origin (meat meal, poultry rendering meal, feather meal, blood meal and others) have also high protein content, but they are poorly utilized because of their unfavorable amino acid profile, and lower digestibility (Degani et al., 1997). Steffens (1994) discovered that fish meal in trout nutrition may be partially replaced with feather meal and poultry by-product meals, while for complete replacement it is necessary to add some essential amino acids (Lysine and Methionine).

Because of the explained reasons feed producers tend to replace fish meal (partially or completely), with some other protein feedstuffs (of plant or animal origin), and with the main concern for production price. The most consideration was given to soybean products, especially soybean meal. This feedstuff has high biological value of protein, but also high content of various anti nutritive substances. The most effective was soybean protein concentrate, which is produced by removing fat and soluble carbohydrates from soy grains (Montagne et al., 2001). For example, Kaushik et al. (1995) fed trout (starting mass 81±1 g) with protein sources such as fish meal, soybean meal, soy protein concentrate and casein. The substitution of fish meal with soy protein concentrate (33 and 100%) had no influence on body mass and utilization of feeds, while substitution with soybean meal and casein led to significant decrease in daily gain. It is experimentally confirmed that partial substitution of fish meal is possible with sunflower meal, but the main problem with this feedstuff is its high content of insoluble carbohydrates (Sanz et al., 1994). Some other feedstuffs were used in experiments, such as cottonseed meal, peanut meal, canola meal, lupine meal, maize gluten, potato proteins and others. However, the shortcoming of these feedstuffs is low level of essential amino acids and presence of antinutritive, harmful and indigestible substances (Đorđević et al., 2005). A possible potential source of protein for fish and other animals is bacterial protein (Aas et al., 2006). Đorđević and Dinić (2011) report that bacteria have certain advantages compared to yeasts. Also bacteria have higher protein content (up to 80%) and better amino acid profile, more similar to the profile of animal tissues. The problem is that such protein has high content of nucleic acids (to 18%) which are catabolised to uric acid in mammals.

CONCLUSION

Feed price is key factor in the production economy of all animal, including fishes. The substitution of fish meal in animal feeding is necessary considering the high price of this feedstuff, and also its variable and often unreliable quality. In the investigations conducted various feedstuffs of plant and animal origin were used to substitute fish meal. However, when they are used the care must be taken to provide optimal concentration of essential amino acids in concentrate mixtures. In practice it can be achieved by combining cheaper protein feedstuffs with synthetic amino acids (Lysine and Methionine on the first place). At this moment most promising appears to be soybean products and bacterial proteins.

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PERIPHYTON AS PARTIAL REPLACEMENT OF COMMERCIAL FEEDS IN THE CULTURE OF ORGANIC TILAPIA IN ISRAEL

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PERIFITON KAO DELIMIČNA ZAMENA KOMERCIJALNE HRANE U ORGANSKOM GAJENJU TILAPIJE U IZRAELU

Abstrakt

Cena hrane čini jednu od najvećih stavki u tekućim troškovima proizvodnje u akvakulturi. Usled potrebe za korišćenjem samo organskih sastojaka, cena koncentrovane hrane za uzgoj organske ribe je izuzetno visoka. Tokom petogodišnjeg perioda rađeni su eksperimenti kako bi se ispitala mogućnost upotrebe različitih supstrata za indukciju rasta perifitona koji bi služio kao prirodna hrana za tilapiju različite veličine, od mlađi do naprednih uzrasnih stadijuma. Kao supstrat, procenjivan je različit poljoprivredni otpad - plastične cevi, najloni i mreže. Različiti supstrati dali su različite prinose perifitona u zavisnosti od njihove površine (glatka ili hrapava) i boje. Rezultati rasta pokazali su da je ušteda hrane od 40% u naprednim fazama rasta dovela do svega 10% redukcije stope rasta tilapije u odnosu na kontrolna jezera, dok je u mladičnjaku moguće smanjiti količinu koncentrovane hrane do 50% bez ograničenja rasta riba. Ovo smanjenje količine hrane od 30-40% dovelo je do poboljšanja koeficijenta konverzije hrane (FCR) od barem 30% u jezerima sa perifitonom (45% u mladičnjacima).

Zaključak: upotreba supstrata hrapavih površina za indukciju rasta perifitona može pomoći u recikliranju otpadnih materijala i značajno redukovati troškove hrane u organskoj akvakulturi.

Ključne reči: *akvakultura, perifiton, tilapija*

INTRODUCTION

This is even more so in organic aquaculture due to the specific requirements to use only organic certified ingredients. Thus, the cost of organic pelleted feeds is almost double the cost of regular feeds used in aquaculture, hampering economic viability. To cope with this problem, a series of experiments aimed at improving natural food production for tilapia in the ponds while reducing added feed amounts were performed. Different hard surfaces were introduced in the water column of earthen fish ponds to induce the growth of periphyton on them and thus improve natural productivity of the water body. This presentation summarizes the results obtained over 5 years of research conducted under field tests in earthen ponds stocked with tilapia fish at stocking densities similar to those used in organic fish culture (1-1.5 fish per m^2.

MATERIAL AND METHODS

Tilapia culture experiments:

Five experiments were carried out in 6 earthen ponds of 300 m^2 area and 1 m depth at the Fish and Aquaculture Research Station Dor. The different experiments tested tilapia performance in 'periphyton+reduced feed' ponds (Periphyton) in relation to conventional ponds (Control), for tilapias of different stocking size and different substrates for periphyton. In all experiments 3 ponds were allocated to each treatment or control. The treatments consisted of the addition of underwater surfaces equivalent to 30-50 percent of the pond surface area with a simultaneous reduction by 30-40 percent in the amount of pelleted feed supplied to the fish. The substrates used and their location in the water column varied in each experiment. In the control ponds no underwater substrates were added and the full amounts of organic certified floating feed pellets were supplied. In each experiment the initial stocking weight of the tilapias was different (from fingerlings to advanced juveniles of 330g) but the fish in all ponds in the same experiment had the same initial weight and density. Experiments lasted 3-5 months and were conducted during the warm season of the year when the temperatures were optimal for fish growth.

Substrate experiments:

Three experiments were carried out in 1 m^3 cages placed within fish ponds of the tilapia culture experiments, to test growth of periphyton on materials with different characteristics. Strips of substrates were vertically placed in the epilimnion, and sub-sets of substrates were removed at sampling time to analyze chlorophyll and dry and organic matter attached on them. Measurements were all standardized on a cm^{-2} basis.

In the first experiment, periphyton growth on eight substrates with different textures was tested, including plastic smooth surface sheets and agricultural nets of different mesh and type of threads as rough substrates. In the second experiment the effect of the color of the substrate on periphyton development on it was tested using nets of the same type, differing only in their color (white, black or blue). In the third experiment the growth rate of periphyton development on a white rigid rough plastic substrate was measured through sampling at a several days intervals during a 3 week period.

RESULTS

Tilapia culture experiments:

In each of the experiments performed survival was similar in both treatments. In the periphyton ponds, reduction of 40% in the feed input did not negatively affect fingerling performance in the nursery. In early juveniles grow-out from 90g to 350g (exp. 1) and advanced growout from 320g to 520g, 40% feed saving led to a reduction of only 10% in tilapia’s growth rate in relation to the control ponds. This growth rate reduction did not result in significant differences between treatments in tilapia harvesting weight and biomass when the culture period was short (87 days), while a 10% reduction in tilapia harvesting weight and biomass in periphyton ponds took effect when the culture period was longer (135 days). When feed saving in periphyton ponds was reduced to 34% and 30%, even after a long culture period tilapia growth rate was not reduced and the performance was similar in Periphyton and Control ponds. This occurred when tilapia density was both lower than expected (~50% survival) and higher than expected (large amounts of wild spawning appearing in the ponds). In all the experiments the similar or just 10% reduced tilapia performance together with the 30%-40% decreased feed amounts supplied to the Periphyton ponds led to at least 30% improved feed conversion ratio (FCR) in the periphyton ponds (45% in the nursery).

Substrate experiments:

Results of the substrate experiment, which tested periphyton growth on 8 substrates of different texture, and color showed a marked difference in the periphyton among the different substrates. The amount of periphytic matter (measured as dry matter, DM, and ash free dry matter, AFDM) on fine nets, more than doubled that on coarse nets, and was about 4 fold increase over smooth plastic substrates. Chlorophyll was 60% higher in the fine mesh cylindrical thread net substrate than in the coarse mesh flat thread net and the white flexible smooth surface plastic sheets. Other rough and smooth substrates were intermediate and not significantly different from either. The color of the substrate did not affect the chlorophyll content of periphyton but did affect its dry and organic matter content. The white substrate had 40% more DM and 50% more AFDM than the blue and black substrates. Linear growth of periphyton on a white rigid rough plastic net substrate during 22 days was observed. The regression equations of the chlorophyll, DM and AFDM on time (number of days submerged) showed that periphyton increased daily by 3 mg chlorophyll, 2 g DM and 0.3 g AFDM per square meter of substrate.

CONCLUSIONS

The use of submerged substrates to allow periphyton development on these surfaces as a method to increase natural food resources for tilapia is an appropriate technology for organic tilapia culture that allows a decrease in feed inputs and reduction of costs.

INFLUENCE OF EXTRUDER SCREW SPEED ON PHYSICAL CHARACTERISTIC OF TROUT FEED

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UTICAJ BRZINE OBRTANJA PUŽNICE EKSTRUDERA NA FIZIČKE KARAKTREISTIKE HRANE ZA PASTRMKE

Abstrakt

Upotreba ekstrudera u tehnologiji proizvodnje svih vrsta hrane za ribe ubrzano se širi u celom svetu, što je u poslednje vreme evidentno i u domaćoj industriji. Danas se hrana za ribe uglavnom proizvodi u obliku ekstrudata čije fizičke karakteristike, pored ostalog, zavise od sastava hrane, ali i od procesnih parametara tokom ekstrudiranja, od kojih su najznačajniji: temperatura u cevi ekstrudera, geometrija matrice, površina otvora na matrici, tip ekstrudera, geometrija pužnice i brzina obrtanja pužnice. Sposobnost ekstrudata da pluta ili tone (brzina tonjenja) često je najkritičnija funkcionalna karakteristika hrane za ribe, jer utiče kako na ishranu riba i ostalih vodenih životinja, tako i na zagađenje vode koja predstavlja njihovo životno stanište. Plutajuće/tonuće karakteristike zavise od gustine ekstrudata, a gustina se reguliše upravo uslovima ekstrudiranja. U zavisnosti od vrste kojoj je namenjena, hrana za ribe se proizvodi kao: plutajuća (šaran, tilapia, som), sporo-tonuća (pastrmka, losos) i tonuća (grgeč, škampi).

Pastrmka je salmonidna vrsta ribe za čiji je opstanak neophodna sveža tekuća voda, a pripada porodici *Salmonidae*. Kako je grabljivica, "hvata" hranu dok sporo pada kroz vodu, pa je za njenu ishranu neophodno proizvesti sporo-tonuću hranu. S obzirom da slabo i teško vari skrob, osnovni izvori energije kod pastrmke su prvenstveno masti, a potom i proteini. Da bi se zadovoljili nutritivni zahtevi pastrmke, uz istovremeno obezbeđenje neophodnih fizičkih karakteristika hrane, uslovi ekstrudiranja se moraju strogo definisati i kontrolisati tokom čitavog procesa.

Brzina obrtanja pužnice, kako je već navedeno, predstavlja jedan od najznačajnijih parametara ekstrudiranja. Stoga se ovaj rad bavi ispitivanjem uticaja pomenute karakteristike procesa na kvalitet hrane za pastrmke. Na standardni sastav hrane za pastrmke primenjene su tri različite brzine obrtanja pužnice od 180, 300 i 420 obrtaja u minuti. Pri ekstrudiranju su upotrebljenje dve matrice sa ukupnom površinom otvora od 50 mm^2 i 100 mm^2. Tokom procesa, praćene su promene parametara: temperatura i pritisak u cevi ekstrudera, potrošnja energije, gubitak vode na izlazu iz ekstrudera i fizičkih karakteristika: nasipna masa i sposobnost absorpcije vode ekstrudata. Specijalna pažnja posvećena je promeni brzine tonjenja, veličini od naročitog značaja za ishranu pastrmke. Dobijeni rezultati brzine tonjenja izraženi su kao srednje vrednosti merenja za petnaest slučajnih uzoraka i upotrebljeni za izračunavanje jednostavnog linearnog modela zavisnosti brzine tonjenja hrane za pastrmke od brzine obrtanja pužnice ekstrudera i ukupne površine otvora upotrebljene matrice. Visoka vrednost koeficijenta determinacije (R^2 = 0.93) ukazuje na to da dobijena jednačina dobro opisuje gore pomenutu zavisnost.

Key words: *extrusion, physical characteristics, screw speed, trout*

INTRODUCTION

Extrusion can be defined as the process of forcing a food/feed material to flow under one or more of a variety conditions (i.e. mixing, heating and shear), trough a die which is designed to form and/or puff-dry the ingredients. In the extruder, the material is exposed to thermal and mechanical treatment, plasticizing and shaping the material from an initially powder to finished product (Riaz, 2010). Essential to any extrusion operation are feed delivery systems which provide uniform flow at any desired extrusion rate (Veenendaal, 1990). Material from the delivery system is fed into the pre-conditioner, where initial mixture is heated by the addition of steam and water into the dry mash (Čolović et al., 2010). In pre-conditioner, the material is heated up to 80-90ºC and moistened up to 22-28%. Preconditioning step improves extrusion process in many ways (Vukmirović et al., 2010).

Extrusion of aquatic feeds is a very broad topic, considering the number of different aquatic species being raised in the world today and the variety of feed formulations and product specifications (Lucht, 1991). Possibility to vary density of the extruded material in fish feed extrusion provides substantial advantage over simply manufactured pellets. In this way, the ability of the feed to sink in water can be specifically adapted to the eating habits of the fish, for example slowly sinking pellets for trout and salmon, with fat content up to 30%, or water-proof pellets for shrimps and other crustaceans (Phillips, 1989). Trout is freshwater and saltwater fish belonging to the Salmonidae family. It is predatory fish which "catches" feed while it is slowly sinking in the water (Plattner, 2007). Trout poorly digest starch and main energy sources in trout feeds are fats and proteins. Thus, adding fats in the feed for trout is very important (Aldrich, 2007).

The aim of this experiment was to investigate possibility of varying physical properties of extruded products when changing speed of extruder main screw. Number of rotations of extruder main screw were set on 180, 300, and 420 rounds per minute (rpm), and two different dies were used.

MATERIALS AND METHODS

Raw material formulation. Ingredient composition of dry mixture for trout was following: fish meal (610 g/kg), soybean meal (120 g/kg), corn gluten (120 g/kg), wheat flour (65 g/kg), yeast (20 g/kg), sunflower meal (20 g/kg), soybean oil (20 g/kg), vitamin and mineral premix (25 g/kg).

Processing. Muyang SLHSJ0.2A, China, double-shaft pedal mixer - steam conditioner was used for conditioning complete mixture, until material reached temperature of 80°C. Water was added directly into feed mash, to final moisture content of 23.5 ± 0.5 %. A single screw extruder, OEE 8, AMANDUS KAHL GmbH & Co. KG, Germany, with dies of 3 mm diameter openings and total openings' area of 50 and 100 mm^2, was used. The speed of material passage was 10 kg/h.

Physical parameters. Moisture of material was measured with infrared moisture analyzer, Ohaus MB45, United States. Water loss was calculated as a difference in moisture before and after extrusion process. Bulk density was defined as the weight of an experimental sample in a 1 L vessel. The expansion rate was calculated as ratio between die diameter and mean sample diameter (n = 15), multiplied by 100. A 125 cm length of 12 cm diameter perspex tube was used for assessment of pellet settling velocity (15 randomly chosen samples). Settling velocities were determined by timing the descent between two marks, 105 cm apart.

Data analysis. STATISTICA software version 9 (Statsoft, Tulsa, OK, USA) was used for analyzing variations (ANOVA) and least significant differences (LSD). The level of significance was set at $p < 0.05$.

RESULTS AND DISCUSSION

Influence of extruders screw speed on various physical properties is shown on Figure 1 (50 mm^2 die) and Figure 2 (100 mm^2 die).

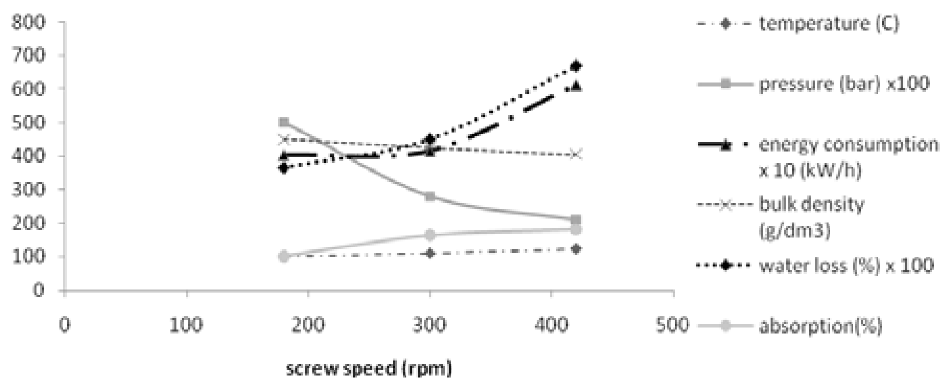

Figure 1. Influence of extruders screw speed on various physical properties (50 mm^2 die)

It can be noticed that bulk density and pressure in the barrel decreased with increasing extruder screw speed. In the same time, higher value of screw speed influenced on increasing of absorption, water loss, energy consumption, and temperature. At the end of extruder barrel, initial material changed into the plastic mass, and higher temperature caused lower dynamic viscosity of material, which is why pressure drastically decreased wit higher screw speed values. Higher temperatures also caused more intensive water evaporation, and therefore increase of water loss. The results were changing in the same way no matter 50 mm^2, or 100 mm^2 die was used.

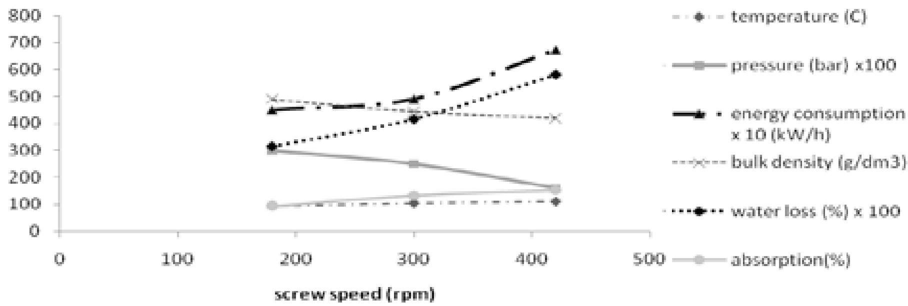

Figure 2. Influence of extruders screw speed on various physical properties (100 mm^2 die)

Table 1 shows influence of extruder screw speed on time of settlings of pellets, and consequently on settling velocity. Comparing mean values of settling velocity for 180, 300 and 420 rpm, it is clear that this physical quantity decreased with increasing of screw speed, as it was expected. Since material spent less time in the barrel, where pressure was also lower, density of pellets are lower as well, and pellets needed more time for settling.

Table 1: Influence of screw speed on time of settlings and settling velocity of pellets

	Die's total openings' area (mm^2)			
	50	100	50	100
Screw speed (rpm)	Time of settling (s)		Settling velocity (cm/s)	
180	11.02±0.77	10.78±0.42	9.57±0.67	9.76±0.38
300	11.52±0.84	11.32±0.53	9.15±0.68	9.29±0.44
420	13.21±1.36	12.15±1.00	8.02±0.80	8.70±0.70

The values are represented as mean ± SD, n = 15

Based on experimental results and by using mathematical regression, a simple linear equitation (Eq. 1) was determined, as a model for predicting settling velocity, if screw speed and die's total openings' area are known.

$$y = a * x_1 + bx_2 + c, \quad (1)$$

Where: x_1 is screw speed and x_2 is die's total openings' area.

Coefficients of regression equitation are given in Table 2.

Table 2: Regression equation coefficients for calculated model

Coefficients	Value	p -value
a	-0.005	0.009
b	0.007	0.159
c	10.207	< 0.001
R^2	0.95	< 0.05

Figure 3 shows commutative influence of screw speed and die's total openings' area on settling velocity.

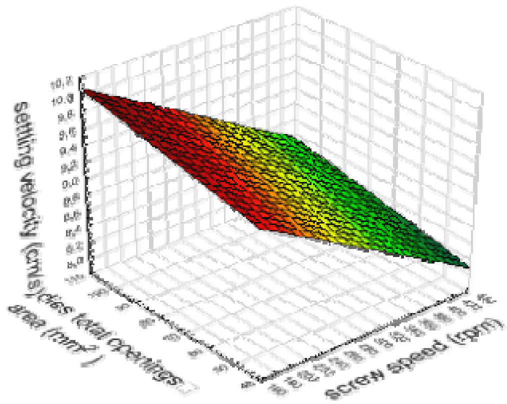

Figure 3. Influence of screw speed and die's total openings' area on settling velocity.

CONCLUSIONS

Extruders screw speed is important parameter in producing fish feed, and it must be strictly defined during whole extrusion process. By changing this characteristic, settling velocity can be controlled and modified. Settling velocity decreases with increasing of screw speed. Simple mathematical equitation was proposed for predicting settling velocity if screw speed is known, for 50, and 100 mm^2 dies.

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THE USAGE OF COTTONSEED MEAL INCLUDING DIFFERENT AMOUNTS OF GOSSYPOL IN DIET OF RAINBOW TROUT (*ONCHORHYNCUS MYKISS*)

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UPOTREBA BRAŠNA OD SEMENA PAMUKA SA RAZLIČITIM SADRŽAJEM GOSIPOLA U ISHRANI KALIFORNIJSKE PASTRMKE (*ONCHORHYNCUS MYKISS*)

Abstract

Cotton culture is common in many of area of Iran, especially Khorasan province. This study is considering the usage of cotton seed meal with different amount of Gossypol in rainbow trout (*Onchorhyncus mykiss*) diet.

In this examination, the effect of different amounts of Gossypol (common cotton seed, cotton seed with low Gossypol, and cotton seed without Gossypol in Rainbow trout growth in (GFT²) stage was examined. At the beginning of the experiment, the fish's weights were 140 gr. In this experiment, 240 Rainbow trout were kept in the training ponds with the 2.240×1.10 meter square dimensions and 0.75 meter depth. Each treatment consisted of 20 Rainbow trout fish. The required water for culturing was provided from a spring in a research center (near Tehran) which was close to the farm with mean temperatures of 14 Celsius.

We consider out four experimental diets including common cotton seed, cotton seed with low Gossypol, and cotton seed without Gossypol for feeding during a six week cultural period.

The diets which consisted of diverse levels of common cotton seed had significant differences (α=0.5%) on weight increasing, total length increasing, standard length increasing and conversion factor. In comparing between different treatments, fish fed with the diet which had common cotton seed had significant differences in the statically sight in weight, conversion factor, FER, total and standard length regarding the other diets and also had the worst condition.

Key words: *cotton seed meal, Gossypol, nutrition, rainbow trout, (Onchorhyncus mykiss)*

INTRODUCTION

Due to the increase in human population and the need for animal protein, especially white meat, fish has found its real position in the everyday diet of human beings. There is a lot of room for growth and profitability and using new alternative materials seems necessary. One of the materials which can be substituted for some of the imported and expensive food items is cottonseed meal. Cotton production in Iran, about 352 thousand tons is estimated that 37/97 percent of the irrigated cotton farms are derived. Annual production of seed cotton is more than 2 million tons (Iranian Agriculture Ministry, 2004). Production efficiency of irrigated and rained cotton farms in Iran has been alternative 2566 kg/hac & 1403 kg/hac (Samavat.2000).

Although different varieties of cotton have cultured without Gossypol, but lower yield per hectare means that they are not used largely in agriculture. So most of cottonseeds cultivated in the world still have a noticeable amount of Gossypol. Smith et al. (1988) reported that rainbow trout fish which was fed with plant or animal protein were not significantly different in growth, carcass composition and taste. Morales et al. (1994) reported that partial replacement of fishmeal (40%) by cotton seed meal had a good effect on growth, feed conversion ratio and energy utilization of rainbow trout. Dabrowski et al. (2000) reported that fishmeal replacement by solvent-extracted cottonseed meal had good results on reproductive and growth of adult rainbow trout fish. Dabrowski (2001) also reported that rainbow trout utilized cottonseed protein very efficiently and up to 75% and 25% of fishmeal protein can be safely replaced by cottonseed meal in diets for adult and juveniles rainbow trout, respectively. Lee et al. (2002) reported that rainbow trout fish can absorb proximately 35-50% of dietary Gossypol, and most of the absorbed Gossypol seemed to be excreted.

Lee et al., (2002) also reported that fishmeal could be entirely replaced by a mixture of plant proteins and animal by-product proteins. Their findings suggest that Cottonseed meal can be used as a good protein source at least 15% in juvenile rainbow trout diets. Lee et al. (2002) further reported that high levels of cottonseed meal had bad effects on the tissues of mature rainbow trout fish. Cheng and Hardy (2002) reported that cottonseed meal could be used at the inclusion rate of 10% in rainbow trout feed formulations. Lee et al. (2006) reported that in rainbow trout juveniles, cottonseed meal could be used as a plant protein source at the level of at least 15% of dietary component (25% of fishmeal protein replacement) without detrimental effects on fish growth. Rinchard et al. (2003) reported that cottonseed meal, which replaced 50% of fishmeal in diets, caused good growth in rainbow trout brood stocks.

MATERIALS AND METHODS

The experiments were carried out from April until May of 2009. The study was conducted at the Research Center of Agriculture and Natural Resources of Tehran, 13 km away from Tehran (Capital of Iran). Twelve channels (2.240 m long, 1.10 m wide and 75 m deep) supplied with flow through spring water (temperature14°C) were used. For experiments, 240 rainbow trout (with initial mean body weight of 140 ± 5 g) were transferred to 12 channels (20 in each) and fed with a commercial rainbow trout diet for an adaptation period of two weeks.

A one factor Completely Randomized Design (CRD) experiment was conducted for this experiment. Four treatments (diets) were tested; Diet -1(Control), Diet -2(with cottonseed meal and without Gossypol), Diet-3(with cottonseed meal and low Gossypol), Diet-4(with cottonseed meal and Gossypol). All treatments had three replicates. Experimental diets were set by the software, "U.F.F.D" and under tables (1994) NRC. The cottonseed meal diets were used in addition to other materials such as flour, corn flour, soybean meal, corn gluten, fish powder, soybean oil, vitamin and mineral supplements, soybean oil, vitamin and mineral supplements. Certain amounts of molasses were used for more consistency in the food pellets of different diets, amount of energy, protein, and etc. Diets were not significantly different from each other. Indicates measured in this study were: weight gain, feed conversion rate, body length, carcass quality (at the end were evaluated to determine the carcass quality of 5 fish of each replication) and percentage loss (It is worth mentioning that there were no casualties during the period.)

All the diets were pelletized using a meat grinder and dried using a laboratory oven-drier at 50 °C for 24 hours. The fish were fed with the test diets three times a day for 6 weeks. Proximate analyses were carried out for both ingredients and diets.

Fish were bulk weighted using a digital balance and counted fortnightly. Data on mortality, growth, and feed utilization were collected accordingly.

All statistical analyses were performed using SPSS. In order to compare the results of the statistical test with that of conventional ANOVA, one-way analysis of variance was performed. Duncan's multiple range (Duncan, 1955) test for means and LSD test to identify the significance of difference between any pair of treatment means were used. All differences were regarded as significant at $p<0.01$.

RESULTS

ANOVA shows that weight gain effect on weight gain in repeating the test levels not significant. However, between different treatments and also between different times according to test F, weight gain difference is highly significant ($\alpha=1\%$). ANOVA showed that the combined effects of time and treatment on weight gain is highly significant ($\alpha=1\%$). The combined effects of time and frequency levels on weight gain in the test is significant ($\alpha=5\%$). Comparison of different treatments indicated that the two treatments in terms of early SNK grouping has been weight gain. Control treatments (treatment A) treatments, respectively, three and four treatment groups are compared next ($\alpha=5\%$). It is noteworthy that the 1% level, two remain at the forefront of treatment SNK grouping in terms of weight gain has been treated and the control group is still but one and three treatments in a similar group (Group III) located Are ($\alpha=1\%$).

The effect of time (weeks) shows that week listed in terms of weight gain to be divided into six distinct groups. So that weight gain in six weeks, which reached its maximum more than a week, has been a significant increase. Fifth week, the fourth, third, second and first, are followed by ($\alpha=5\%$). The effect of time (weeks) on weight gain in the 1% probability level is also a similar situation ($\alpha=1\%$). ANOVA showed that among treatments and also between different times according to test F, the difference between conversion factors is highly significant ($\alpha=1\%$). Such a feature can be combined in time and treatment effect on the conversion ratio in the test levels to be quite significant ($\alpha=1\%$).

Comparison of the mean is concluded, the two treatments at 5 percent (α=5%) at the top category SNK, located in terms of conversion ratio compared with the treatments that are not desirable. Tuesday and one treatment, a treatment to a significant reduction in the level of 5 percent (α=5%) and the second group are in better condition than the ones treated four of his show. In the last four treatment groups are compared to other treatments that better feed efficiency shows (α=5%). It also can be in a situation likely to be seen as a percent (α=1%). The effect of time (weeks) shows that week listed in terms of conversion ratio of six distinct groups are divided. So that FCR in the sixth week to the minimum value reached more than a week has decreased significantly. Fifth week, fourth, second, third and first, respectively, are followed by (α=5%). The effect of time (weeks) on the conversion ratio at 1% probability level is also a similar situation (α=1%).

Conclusion of the ANOVA is repeated on the entire length of the test levels are not significant. However, between different treatments and also between different times according to test F, the total length difference is highly significant (α=1%). Such a feature can be combined in time and treatment effects on total length can be observed. However, the combined effect of time and repeated throughout the entire level on the test is not significant. Comparison of different treatments indicates that both treatment and control treatment (a treatment) SNK grouping at the top in terms of the total length have between them there is no significant difference (α=5%). Treatments three and four, in subsequent groups are located in this comparison (α=5%). At 1% probability level, treatments one, two and three are located in the top grouping in terms of total length SNK, but treatment four isolated in the next group (α=1%).

The effect of time (weeks) shows that the first weeks to sixth in terms of total length to be divided into six distinct groups. So that the total increase during the sixth week, which reached its maximum more than a week has been a significant increase. Fifth week, the fourth, third, second and first, are followed by (α=5%). The effect of time (weeks) over the entire length of the 1% probability level is also a similar situation (α=1%).

Analysis of variance concluded that the effect of repeated attributes, such as before, on the length of the standard test levels are not significant. However, between different treatments and also between different times according to test F, standard length difference is highly significant (α=1%). Such a feature can be seen in the combined effect of time and treatment effects on standard length. Combined effects of time and repetition, but on the whole length of the test levels are not significantly observed. ANOVA showed that both treatment and control treatment in an error of 5 percent (α=5%) at the top category SNK, in terms of standard length are located. Treatment three, compared to treatment with a significant reduction in the level of 5 percent (α=5%) and four treatments with a significant reduction in both levels have been tested. However, in testing a percent (α=1%), treatments 2 and 3 and the control treatment (a) in the top group and statistically significant differences do not show. However, the four treatments with other treatments are statistically different. (α=1% and 5%). Effect of time (weeks) suggests that such attributes as the previous standard length during the first weeks until the sixth is divided into six distinct groups. So that the increase in standard length in the sixth week, which reached its maximum more than a week has been a significant increase. Fifth week, the fourth, third, second and first, are followed by (α=5%). The effect of time (weeks) of standard length in 1% probability level is also a similar situation (α=1%).

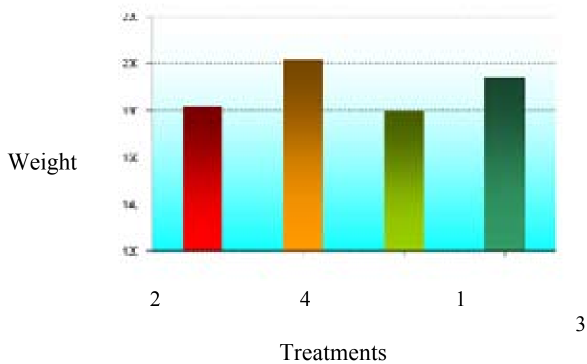

Figure 1. – The effect of treatments on weight gain

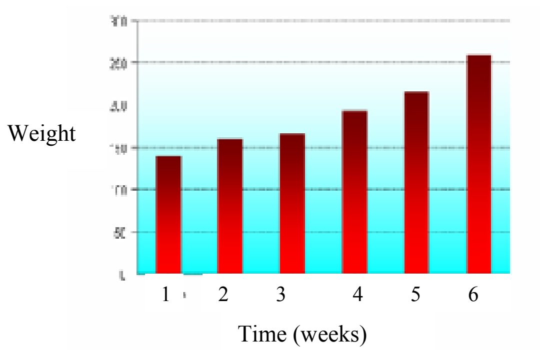

Figure 2. – The effect of time (weeks) on weight gain

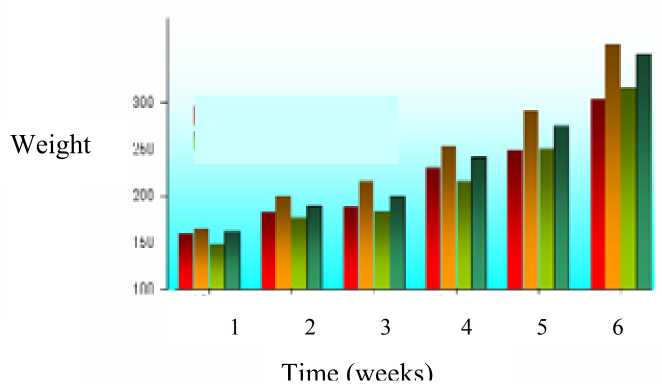

Figure 3. – The effect of interaction treatments and time (weeks) on weight gain

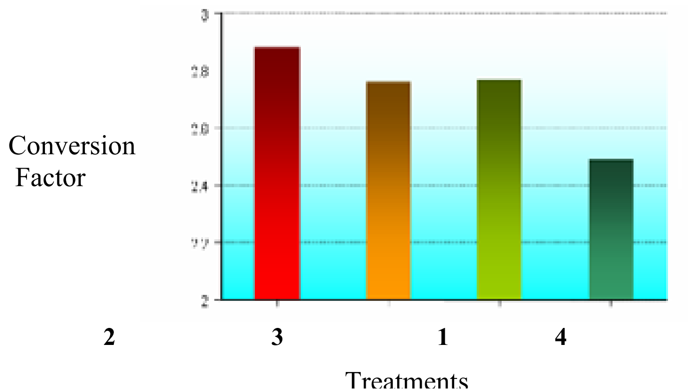

Figure 4. – The effect of treatments on the conversion factor

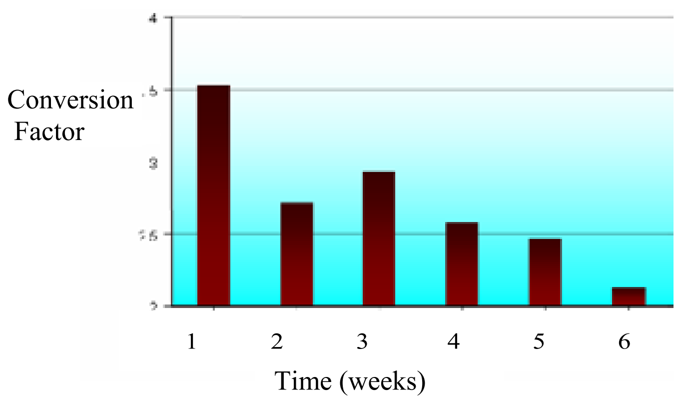

Figure 5.– The effect of time (weeks) on the conversion factor

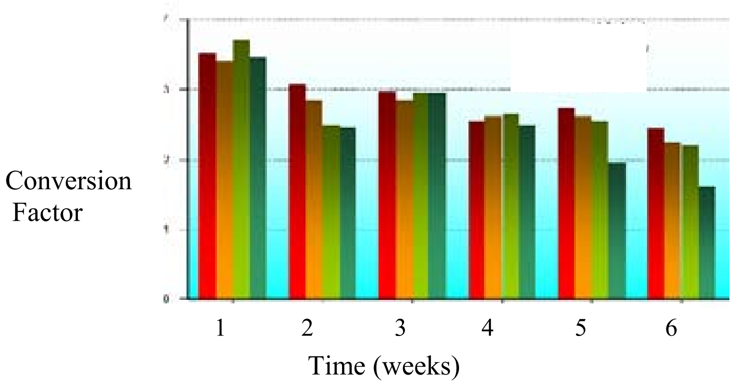

Figure 6. – The effect of interaction treatments and time (weeks) on FCR

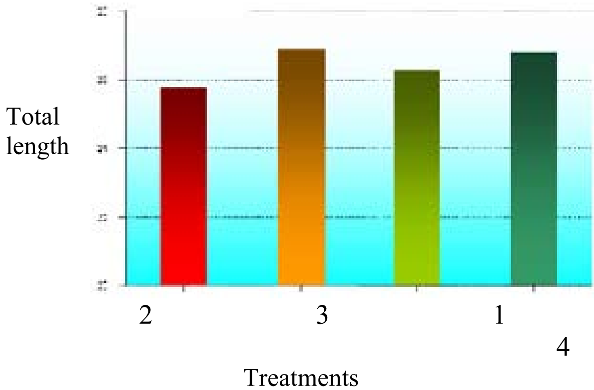

Figure 7. – The effect of treatments on total length

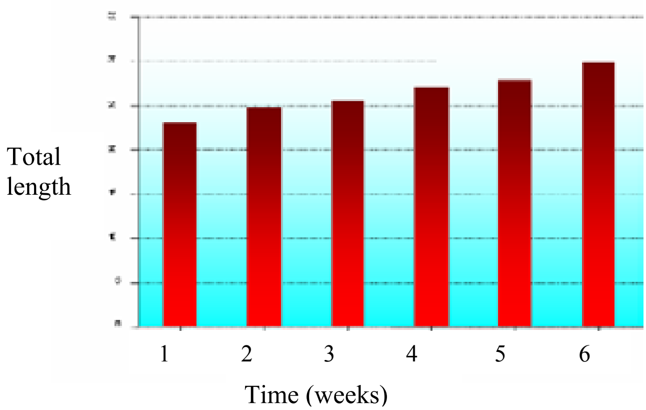

Figure 8. – The effect of time (weeks) on total length

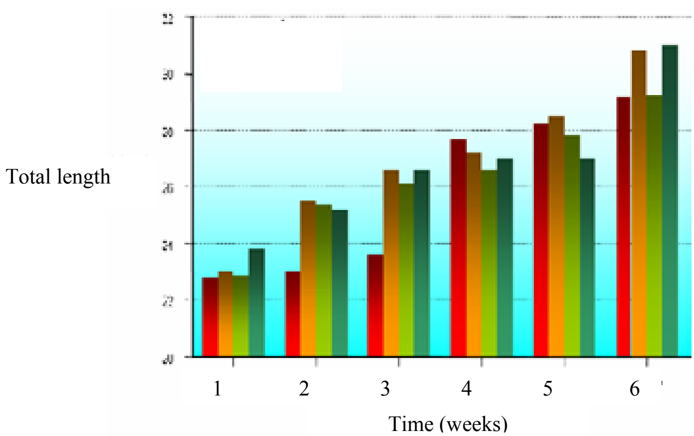

Figure 9. – The effect of interaction between treatments and time (weeks) on total length

DISCUSSION

Cottonseed meal is high in terms of nutrition value and can be used for poultry and fish diets (Cheng et al. 2002). In this current research, we examined the usage of different amounts of cottonseed meal in rainbow trout diets. Four experimental diets were considered: common cottonseed, cottonseed with low Gossypol and cottonseed without Gossypol for feeding during a six weeks cultural period.

The diets that were consist of diverse levels of common cotton seed had significant differences (α=0.5%) on weight increasing, total length increasing, standard length increasing and conversion factor. In comparing between different treatments, the diet which had common cotton seed had significant differences in the statically sight in weight, conversion factor, FER, total and standard length regarding the other diets and also had the worst condition.

This supports an earlier report by Robinson and Daniels (1987) that partial or complete substitution of soybean meal with cottonseed meal catfish diets resulted in similar growth, FCR, CF, survival and no Gossypol related mortality was observed. Lysine supplementation has been suggested when cottonseed meal replaced SBM completely to avoid impairing weight gain, FCR, and survival (Robinson, 1991). Fowler showed that, 34% cottonseed meal with gland was reported to induce the growth of Chinook salmon fingerlings more in comparison with 37% FM (Fowler, 1980). Coho salmon fed a diet containing 22% cottonseed meal with gland performed as well as fish fed a diet containing 37% FM.

Blom et al. (2001) reported that adult rainbow trout fed with diets in which cottonseed meal completely replaced FM had normal growth and survival percentage over the study period, but the reproductive performance of adult female trout was reduced. This was also reported by Lee et al. (2006) that female rainbow trout fertility and plasma testosterone levels were negatively affected by complete replacement of FM protein with cottonseed meal protein. In this chapter of the present study, the previously optimized levels of cottonseed meal (20%, 40% and 100%) were used as a protein source to replace soybean meal in rainbow trout diets.

A series of studies have been conducted on the utilization of cottonseed meal and toxicity of Gossypol to fish (Mbahinzireki et al. 2001, Dabrowski et al. 2000, Dabrowski et al. 2001, Blom et al. 2001). Reproductive efficiency and maternal-offspring transfer of Gossypol in rainbow trout (*Oncorhynchus mykiss*) fed diets containing cottonseed meal. However, the Gossypol level in liver was below the toxic threshold for fish fed with the experimental diets i.e., 100%, 40% and 20% cottonseed meal, indicating that Gossypol was not a barrier when feeding these diets containing Iranian cottonseed meal varieties at the levels mentioned. Moreover, this study does not support the findings of Hepher (1990) that cottonseed meal has a lower nutritional value than soybean meal.

CONCLUSION

A few studies have been conducted with cold-water fish using cottonseed meal as a protein source and, to the best of our knowledge; this study was the first one in Iran. The results of the present study indicated that Iranian cottonseed meal varieties could be used over a period of 6 weeks (from initial weight of 140 ± 5 g) to replace soybean meal without negatively affecting growth and survival of rainbow trout. In the present study, cottonseed meal varieties were used as a protein source to replace soybean meal in four separate experiments to investigate the various inclusion level of each variety followed by comparing the optimized levels in an independent study in rainbow trout diets.

The feeding experiment in this study was conducted for a short period. Long-term studies are suggested to closely monitor Gossypol level in fillets and to ensure that Gossypol level remains in safe ranges for fish growth and survival as well as human consumption. In conclusion, cottonseed meal is a promising candidate to replace soybean meal completely in rainbow trout fish farming in Iran.

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TANK DESIGN AND ACTION OF RAINBOW TROUT (*ONCORHYNCHUS MYKISS*)

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DIZAJN TANKOVA I PONAŠANJE KALIFORNIJSKE PASTRMKE (*ONCORHYNCHUS MYKISS*)

INTRODUCTION

Tank design can influence fish behaviour, water flow characteristics and quality, and biological performance. According to their shapes, rearing tank designs can be classified into circular and rectangular (raceway). Circular tanks with relatively high-velocity water flow provide uniform water quality, while raceways usually with low-velocity water flow generate a distinct water quality gradient from inlet to outlet. Furthermore, rate of aggression among fish in circular (mixed-flow) tanks with a uniform fish distribution tends to be higher than in raceways (plug-flow), where the distribution of fish is uneven. In this study action of the rainbow trout is addressed under circular tank and raceway culture.

MATERIALS AND METHODS

Rainbow trout, hatched and grown in captivity, were graded, randomly selected and transferred to six circular tanks and six raceways supported by a freshwater recirculation system in a temperature-controlled and insulated room. agonistic behaviour of each fish in the raceways was scored based on aggressive acts; these included chasing, nips and pushing.

RESULTS

As expected the frequency of agonistic acts among the fish aws more sever and intense in circular tanks than in raceway culture. A sort of dominat hierarchies was established in raceway culture. This was not obseraved in circular tank.

DUSCUSSION AND CONCLUSION

In any tank design however, attention is paid to water flow management, because current may affect feed distribution, fish swimming orientation and activity, schooling behaviour and agonistic responses. In the case of rainbow trout, tank design is one of the most important determining factors in fish distribution and orientation, total aggression and other physical and biological variables.

DNA BARCODING IN IDENTIFICATION OF RAW AND SMOKED SALMONID PRODUCTS

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DNK KODIRANJE U IDENTIFIKACIJI ŽIVIH I DIMLJENIH SALMONIDNIH PROIZVODA

Abstract

Identification of processed fish foods usually requires the application of a molecular tool because most of the morphometric keys used in species identification are lost during this process. Identification through DNA barcodes is being developed for all animal species and when used together with high throughput sequencing technologies, potentially provides a simple, common tool for the identification of raw and processed sea food. In this study, raw and smoked products of 8 species from genus Oncorhynchus were identified using DNA barcoding.

Raw and smoked products of all species (*Oncorhynchus clarkia, O. gorbuscha, O. keta, O. kisutch, O. masou, O. mykiss, O. nerka* and *O. tshawytscha*) were collected and samples of muscle tissue were taken for each species. Total genomic DNA was extracted and two regions of COI gene were amplified: DNA barcode region, which is approximately 650 base pairs long and a shorter part (307 base pairs) of DNA barcode region, which we used in amplification of heavily degraded DNA samples. PCR products were purified and DNA sequencing reactions were conducted on an automatic sequencing system. Alignment of sequences and phylogenetic analyses of the aligned sequences were conducted using MEGA 5. Sequences from the smoked eel products were aligned against GenBank COI entries for the species involved. Sequence divergences were calculated using Tamura-Nei distance model and a maximum likelihood (ML) tree using Tamura-Nei parameter was constructed with bootstrap tests of 1000 replicates for the reliability of the constructed tree.

Sequences of 307-652 base pairs in length were obtained from all raw and smoked fish products. Procedures used in smoked fish production do not appear to denature the DNA to an extent that eliminates recovery of short to moderate length sequences from processed fish food. Both universal primers (Fish F1-R1) and internal DNA Barcode region primers designed in this study successfully amplified the COI region from raw and smoked products of 8 different *Onchorhynchus* species. Raw and smoked products could be identified to the species level with 100% identity matches against sequences in GenBank. As high throughput sequencing tools become more accessible, DNA barcoding is becoming a standard tool for the identification of species and products.

Key words: *COI, DNA barcoding, Oncorhynchus, Smoked fish products, species identification.*

EFFECTS OF CARBOSULFAN ON SOME ENZYME ACTIVITIES IN BLOOD OF RAINBOW TROUT (*ONCORHYNCHUS MYKISS*)

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DELOVANJE KARBOSULFANA NA AKTIVNOSTI NEKIH ENZIMA U KRVI KALIFORNIJSKE PASTRMKE (*ONCORHYNCHUS MYKISS*)

Abstract

Detection of changes in enzyme activities is widely used as a quick method to determine the toxic effects of pesticides or other toxic chemicals. In this study, chronic toxic effects of carbamate insecticide carbosulfan (250 g/L, EC) used commonly in agriculture areas as well as in Eastern Black Sea Region on blood enzyme activity of rainbow trout (*Oncorhynchus mykiss*) were determined. For this purpose, rainbow trout (116.88±21.69 gr and 22.39±1.40 cm) acclimated to the laboratory conditions were exposed to carbosulfan within 60 days in flow-through system (6 l/h). According to preliminary tests, carbosulfan concentration of test water was designed to be 35 µg/l. During the chronic tests, erythrocyte acetylcholinesterase (AChE), erythrocyte δ-aminolevulinic acid dehydratase (ALA-D) and paraoxonase (PON) activities of rainbow trout were measured and inhibition rate of enzyme activities were determined. Changes in enzyme activities of rainbow trout were significant ($p < 0.001$). While increase of inhibition rates on AChE and PON activities lasted up to the 3rd week, δ-ALA-D activity lasted up to 4th week. Inhibition rates of AChE, PON and δ-ALA-D activities were determined as 41.32%, 16.67% and 26.24%, respectively and it was determined that change of enzyme activity affected fish behavior. Feed conversion ratio (FCR) was influenced from these behavior disorders during the experiment. Fish exposed to toxic carbosulfan had a 2% weight gain in the 60th day while the control group had a weight gain of 26%.

Keywords : AChE, δ-ALA-D, PON, *Oncorhynchus mykiss*

EFFECT OF DUO CULTURE ON GROWTH PERFORMANCE OF BROWN TROUT (*SALMO TRUTTA FARIO*) AND BLACK SEA TURBOT (*PSETTA MAXIMA*) IN TANK REARED CONDITION

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DELOVANJE DUO CULTURE NA PERFORMANCE RASTA POTOČNE PASTRMKE (*SALMO TRUTTA FARIO*) I CRNOMORSKE RIBE LIST (*PSETTA MAXIMA*) U USLOVIMA UZGOJA U TANKOVIMA

Abstrakt

Cilj ove studije je bilo poređenje performansi rasta i koeficijenta konverzije hrane potočne pastrmke i crnomorske ribe list u monokulturi i duo-kulturi u uslovima gajenja u tankovima u region Crnog mora, Turska. Eksperiment je trajao 84 dana od 3.marta do 26 maja 2009. na Karadeniz Tehničkom Univerzitetu. Ribe su bile 14 meseci stare potočne pastrmke, odgajene u mrestilištu, početne težine 54.81±6.53 g (n=72) i 9 meseci stare crnomorske ribe list, odgajene u mrestilištu, početne težine 50.80±3.14 (n=72) g. Ribe su držane u tankovima od fiberglasa zapremine 0.2 m^3, snabdevene bočatom vodom (17‰). Na kraju studije prosečna težina potočne pastrmke je uvećana za 104.46±1.69 g, a crnomorske ribe list za 100.15±6.71 g, u monokulturi, dok je uvećanje iznosilo 91.46±5.68 g za pastrmku i 91.71±3.77 g za ribu list u duo-kulturi, razlike su bile ststistički značajne između monokulture i duo-kulture za svaku vrstu ($P<0.05$). Specifične stope rasta u monokulturi su bile više nego u duo-kulturi ($P<0.05$). Finalni faktori kondicije za svaku vrstu su bili slični u monokulturi i duo kulturi. Koeficijent konverzije hrane crnomorske ribe list u monokulturi bio je bolji of potočne pastrmke u monokulturi nego u duokulturi ($P<0.01$). Ova studija je pokazala da je duokultura potočne pastrmke i crnomorske ribe list nepovoljna za ove vrste.

Ključne reči: *potočna pastrmka, crnomorska riba list, duokultura, parametri rasta, koeficijent konverzije hrane*

INTRODUCTION

Polyculture is the way of simultaneously producing more than one fish species in the same rearing space (Papoutsoglou et al., 1992; Papoutsoglou et al., 2001). The principle of polyculture is based on the fact that cultured fish species feed on different levels of food chain and environment (Milstein et al., 2002), but wild species can be stocked with domesticated species to get them used to artificial feed. In this case, increasing food intake due to competition and social hierarchy can affect fish growth positively. At the same time, uneaten feed by wild species consumed by domesticated fish, and feed efficiency is maintained (Okumuş, et al., 1999).

Culture possibilities of brown trout (*Salmo trutta fario; S.t.f.*) were evaluated by the Department of Fisheries Technology at the Faculty of Marine Sciences at Karadeniz Technical University, and Black Sea turbot (*Psetta maxima; P.m.*) by Ministry of Agriculture and Rural Affairs and the Central Fisheries Research Institute. The objectives of the present study are to compare growth performance and feed conversion ratios of brown trout and Black Sea turbot in monoculture and duo-culture tank reared conditions in the Black Sea Region, Turkey.

MATERIALS AND METHODS

The growth trial, which lasted 84 days, was carried out March 3rd – May 26th 2009 at the Prof.Dr. İbrahim OKUMUŞ Aquaculture Research and Production Unit at Karadeniz Technical University. The fish were about 14-month-old hatchery reared brown trout and 9-month-old hatchery reared Black Sea turbot with initial weights of 54.81±6.53 (n=72) and 50.80±3.14 (n=72) g, respectively. Fish were kept in 0.2 m^3 fiberglass tanks, and brackish water (17‰) supplied. Nine tanks were used and the fish were equally allotted to 3 groups with tree replicates. Each tank contained 16 fish from each species for monoculture, and 8+8 fish from each species for duo-culture.

Fish were fed to apparent satiation with commercial dry extruded pellets of 3 mm containing 48% crude protein and 18.0% crude lipid. Feed was manually given two times a day (at 8:30 and 16:30 h). Growth was followed by bulk-weighing the fish in each tank (within 0.1 g accuracy limit), and total lengths (±1 mm) and individual live weights (± 0.1 g) were noted down to determine condition factors.

The following parameters were calculated from the data collected: specific growth rate (SGR, %/day)=$[(\ln W_t - \ln W_0)/t] \times 100$; condition factor (CF)=$(W/L^3) \times 100$; feed conversion ratio (FCR)=Feed consumed/biomass increment; where W_0 and W_t are live weights (g) of the fish at the beginning and a successive weighing, respectively; t is number of days and L is total length of the fish (cm). The mean and standard deviation (±sd) were calculated for all parameters in each group and one-way analysis of variance (ANOVA) and Tukey were used to test for differences among the groups.

RESULTS

At the end of the study, mean live weights of brown trout and Black Sea turbot increased to 104.46±1.69 g and 100.15±6.71 g in monoculture, 91.46±5.68 g and 91.71±3.77 g in duo-culture, respectively, and significant differences were found between the monoculture and duo-culture groups for each species ($P<0.05$). The specific growth rates in monoculture were found higher than duo-culture ($P<0.05$). The final condition factors of species were similar for each species in monoculture and duo-culture. The feed conversion ratio (FCR) of Black Sea turbot in monoculture was the best than brown trout in monoculture, and duo-culture group (P<0.01) (Table 1).

Table 1. Mean growth (W_f: final weights; SGR: specific growth rate; CF: condition factor; and, feed conversion ratio (FCR) parameters (m: monoculture; d: duo-culture; *: duo-culture value of *S.t.f.* and *P.m.*).

	S.t.f. m	*P.m.* m	*S.t.f.* d	*P.m.* d	P
W_f (g)	104.46±1.69 [a]	100.15±6.71 [a]	91.46±5.68 [b]	91.71±3.77 [b]	<0.05
SGR	0.80±0.02 [a]	0.81±0.02 [a]	0.66±0.16 [b]	0.70±0.11 [b]	<0.05
CF	1.26±0,02 [a]	1.82±0,01 [b]	1.31±0,20 [a]	1.72±0,05 [b]	<0.01
FCR	1.29±0,14 [a]	0.80±0.10 [c]	1.11±0.14 [b]		<0.01

DISCUSSION

In this study, it was found out that both species had good FCR if the conditions are suitable. Feed conversion ratio, which fell below 1.3, in all three groups proves this. Since feed conversion ratio is affected by various such factors as biological value of feed, the ratio of main constituents of feed, stock density, genetic line of fish, fish size, basal metabolic rate, water temperature, feeding method and frequency (Jobling, 1995), comparing results of different trials may not have a practical value. The estimated feed use values during the study shows that there is no need to overfeed. As a result, this study has shown that duoculture of brown trout and Black Sea turbot is disadvantageous for each species.

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INFLUENCE OF DIETARY ADMINISTRATION OF THE β-HYDROXY-β-METHYLBUTYRATE (HMB) ON THE INNATE IMMUNITY AND RESISTANCE AGAINST BACTERIAL INFECTIONS IN PIKEPERCH (*SANDER LUCIOPERCA*)

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UTICAJ β-HIDROKSI-β-METILBUTIRATA (HMB) DODAVANOG U HRANU NA IMUNITET I OTPORNOST NA BAKTERIJSKE INFEKCIJE KOD SMUĐA (*SANDER LUCIOPERCA*)

Abstrakt

U ovoj studiji je ispitivan uticaj metabolita leucina β-hidroksi-β-metilbutirata (HMB) na imunitet i zaštitu od enterične bolesti crvenih usta (ERM) i furunkuloze kod smuđa (*Sander lucioperca*). Ribe su bile hranjene 18 sati dnevno komercijalnom hranom za pastrmke. Hrana je formulisana tako da obezbedi ili 0 (kontrolna grupa) ili 50 mg HBM/kg telesne težine na dan (grupa hranjena sa HBM) tokom 4 nedelje. Posle hranjenja sa HBM 20 zdravih smuđeva prosečne težine 35 g je anestezirano i uzeta je krv iz kaudalne vene u heparizovane špriceve. Takođe je aseptično izdvojen pronefros svake ribe i dobijena suspenzija pojedinačnih ćelija za izolovanje ćelija koristeći ili Gradisol (Polfa) ili Percoll (Pharmacia) gradijent. Test na izazivanje bolesti upotrebom *Yersinia ruckeri* ili *Aeromonas salmonicida* je obavljen 4 nedelje posle hranjenja. Ukratko, svakoj od 20 riba svake grupe data je jedna intraperitonealna injekcija 48 sati kulture *Y.ruckeri* ili *A. salmonicida* (0,2 ml). Zabeležen je mortalitet, a prisustvo patogena potvrđeno izolacijom iz bubrega. Rezultati ovog ogleda pokazuju da HBM u dozi od 50 mg/kg telesne težine statistički značajno stimuliše nespecifične ćelijske i humoralne odbrambene mehanizme i zaštitu od *Yersinia ruckeri* i *Aeromonas salmonicida*.

Ključne reči: *smuđ, HBM, specifični imunitet, enterična bolest crvenih usta (ERM), furunkuloza*

INTRODUCTION

The interaction between nutrition, defence mechanism and protection against diseases in fish has long been known, but this relation is far more complex than originally thought. Nutritional support is important for optimum health by providing the building blocks of nonspecific cellular and humoral defence mechanisms and thus protection against infectious diseases. Certain nutrients can be supplemented in the feed to stimulate or modulate directly host defence mechanisms. Several natural and synthetic drugs and biological modifiers have been tested in fish *in vitro* and *in vivo* and many of these products were applicated for stimulation of nonspecific defence mechanisms and protection against diseases (Siwicki et al. 1998). The substance β-hydroxy-β-methylbutyrate (HMB) is a catabolate of the amino acid leucine. In rainbow trout and tench HMB activated cell-mediated immunity (Siwicki et al. 2001; Siwicki et al. 2006). This study continues the examination of the influence of feeding the leucine metabolite HMB on the nonspecific defence mechanisms and on protection against bacterial diseases of pikeperch (*Sander lucioperca*) grown in an intensive system of culture.

MATERIAL AND METHODS

The fish were reared at the Department of Aquaculture, Inland Fisheries Institute in Olsztyn, Poland. The juvenile pikeperch were reared in circular fibreglass tanks with a water in volume of 200 litres each. The tanks were part of a recilculation system equipped with biological and mechanical filters. The water temperature was maintained at a constant level of about 22°C. Twenty-four hours illumination was applied with the light intensity at 30-80 lux. The fish were fed for 18 h per day with a commercial trout feed (TROUVIT, Nutreco Aquaculture, Holland) using automatic band feeders. The diets were formulated to provide either 0 (control-fed group) or 50 mg HMB kg^{-1} body weight per day (HMB-fed group) for 4 weeks. The leucine metabolite was obtained as a monohydrate calcium salt with a purity > 98 % (Metabolic Technologies, Ames, USA). The feed was distributed *ad libitum*, which was confirmed by observations of feed waste. The fish were observed daily for unusual behaviour, morphological changes and any mortality. Four weeks after feeding HMB, 20 healthy pikeperch of approximately 350 g were anaesthetised in Propiscin (IFI, Poland) and blood was drawn from the caudal vein into heparinized syringes. Also the pronephros of each fish was removed aseptically and single cells suspension were obtained for isolating individual cells using either a Gradisol (Polfa) or Percoll (Pharmacia) gradient. The metabolic activity of pronephros phagocytes by their respiratory burst activity (RBA) stimulated by Phorbol myristate acetate (PMA, Sigma) was measured by the technique presented by Siwicki et al. (2000). Potential killing activity (PKA) of the pronephric phagocytes was measured by the method presented by Siwicki and Anderson (1993). The lymphocytes proliferation (LP) was determined by the MTT colorimetric assay methods modified by Siwicki et al. (2000) for the fish species. The mitogens concanavaline A (ConA, Sigma) or lipopolisaccharide (LPS, Sigma) were used for the stimulation of lymphocytes. The lysozyme activity in the plasma was measured in a turbidimetric assay presented by Siwicki and Anderson (1993) and ceruloplasmine activity in the plasma was determined according to Siwicki and Studnicka (1986) which was modified for micro-methods. Total protein level in serum was measured by the colorimetric Lowry micro-methods (Sigma, Diagnostic Kits) and total immunoglobulin (Ig) levels in the serum were measured by spectrophotometric methods (Siwicki and Anderson, 1993). A disease challenge test using *Yersinia ruckeri* or *Aeromonas salmonicida* were conducted after 4 weeks of feeding. Briefly, 20 fish from each group were each given a single intraperitoneal injection of a 48 h growth of *Y. ruckeri* or *A. salmonicida* (0.2 ml). Mortalities were tabulated and the presence of pathogens was confirmed by isolation from the kidney. The data were statistically evaluated with the Student's t-test, and the results are presented as mean and standard deviations (SD). The significance level used was $P<0.05$.

RESULTS AND DISCUSSION

The results of this experimental study showed that HMB at a dose of 50 $mgkg^{-1}$ body weight statistically stimulated the non-specific cellular and humoral defence mechanisms (Table 1). The phagocytic ability (RBA) and potential killing activity (PKA) of pronephros phagocytes were statistically significant higher ($P<0.05$) from HMB-fed pikeperch, compared to fish from control group. The similar pattern was observed in proliferative response of pronephros lymphocytes stimulated by mitogens ConA or LPS. The lymphocytes proliferation was statistically significant ($P<0.05$) higher in pikeperch fed with HMB, compared to the control group of fish. The analyses of the study results showed that HMB activated nonspecific humoral-mediated immunity. The lysozyme activity in plasma and total Ig levels in serum were statistically significant ($P<0.05$) greater that in the control-fed pikeperch. The feeding of HMB decrease the mortality to 40 % after infection with *A. salmonicida* and 30 % after infection with *Y. ruckeri.*

The current study indicates that feeding HMB to pikeperch in intensive system of culture can improve the innate immunity and decrease in mortality after experimental infected fish with pathogenic bacteria *Y. ruckeri* and *A. salmonicida.* The application of HMB by feed demonstrated to have a practical and economical impact in intensive pikeperch culture.

Table 1. The nonspecific cellular and humoral defence mechanisms levels in control-fed and HMB-fed pikeperch (n = 20; mean ± SD, * statistically significant P<0.05

Immunological parameters:	Control group	HMB-fed group
RBA of phagocytes (OD 620 nm)	0.45 ± 0.04	0.59 ± 0.05*
PKA of phagocytes (OD 620 nm)	0.41 ± 0.04	0.62 ± 0.04*
LP stimulated by ConA (OD 620 nm)	0.49 ± 0.05	0.57 ± 0.05*
LP stimulated by LPS (OD 620 nm)	0.34 ± 0.04	0.51 ± 0.05*
Lysozyme activity ($mg\ l^{-1}$)	45.5 ± 5.8	57.5 ± 3.9*
Ceruloplasmine activity (IU)	22.8 ± 7.5	21.5 ± 2.5
Total protein in serum ($g\ l^{-1}$)	64.5 ± 4.0	67.7 ± 3.5
Total Ig in serum ($g\ l^{-1}$)	18.0 ± 1.4	21.5 ± 2.0*

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RESTORATION OF INNATE IMMUNITY IN FISH AFTER SUPPRESSION INDUCED BY XENOBIOTICS

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OPORAVAK PRIRODNOG IMUNITETA RIBA NAKON SUPRESIJE INDUKOVANE KSENOBIOTICIMA

Tokom procesa evolucije, biološki organizmi su razvili odbrambene mehanizme protiv opasnih bioloških, hemijskih ili fizioloških agenasa. Jedan od tih mehanizama u višim organizmima je kompleksni imuni sistem, čiji se fini detalji funkcionisanja postepeno otkrivaju. Razvoj i održavanje odbrambenih mehanizama je važno za dobrobit, i u nekim slučajevima i za preživljavanje organizama.

Veliki broj sintetički proizvedenih hemijskih materija se ispušta u okruženje. Mnoga istraživanja potvrđuju uticaj polutanata iz okruženja, naročito pesticida, metanih jona, hemoterapeutika i organskih polutanata na imuni sistem životinja, pokazujući da hemijski polutanti rezultuju mnogim imunomodulatornim efektima i na ćelijske i na humornalne imune funkcije, a na kraju i na zdravlje i otpornost na bolesti. Jedan od važnih problema je da imuosupresija indukovana kesnobioticima koji mogu favorizovati prisustvo i rast tumorskih ćelija. Premda još uvek postoji skepticizam u vezi hemijskih materija iz okruženja, istraživanja su pokazala da produžena supresovana imuna reakcija može da poveća opasnost od raka kod ljudi i vodenih životinja.

Prvo, direktno eksponiranje riba mnogim toksičnim materijama koje se ispuštaju u reke, jezera ili bare. Veliki broj ksenobiotika su takođe dodati u okruženje. Ksenobiotici imaju direktan uticaj na ćelijske i humoralne odbrambene mehanizme i zaštitu protiv infektivnih oboljenja.

Zatim, različiti polutanti imunokompetentnih ćelija imaju varijabilnu senzitivnost na ksenobiotike. Mnogi ksenobiotici su indukovali imunospresivne uticaje na nespecifičan imunitet i specifičan odgovor riba. Jedan broj istraživanja se bavio uticajima ksenobiotika na imuni sistem riba. Integritet imunog sistema je fundamentalan za dobru odbranu protiv različitih patogenih agenasa u okruženju, uglavnom parazita, bakterija i virusa. Glavne imune ćelije, limfociti, makrofage i neutrofili, su regulisane različitim stupnjevitim kontrolnim procesima ćelijskih kooperacija i interakcija. Makrofagi i neutrofili su važni faktori u ćelijskom imunom sistemu riba, s obzirom da su prva linija odbrane i deluju tako da štite domaćina fagocitozom stranog materijala, uključujući i patogene agense. Osim toga, različite populacije imunokompetentnih ćelija imaju varijabilni senzitivitet na ksenobiotike. Ova činjenica je veoma važna u istraživanju uticaja ksenobiotika na nespecifični ćelijski ili humoralni odbrambeni mehanizam i specifične zaštite protiv bolesti. U narednim primerima imuni testovi su generalno grupisani prema specifičnosti odgovora. Neki nespecifični testovi kao što je determinacija lizozimske aktivnosti, C-reaktivni nivo proteina (CRP), cerulopazmini (Cp) i dodatni nivoi su dosta korišćeni u prošlosti za analizu efekata kontaminacije okruženja na zdravlje riba. Lizozimi i Cp aktivnost su važni u inicijalnoj destrukciji invazivnih agenasa i u nekim slučajevima mogu poslužiti kao rani biomarkteri koji indikuju promene u odbrambenim mehnizmima.

Testovi sa humoranim imunitetom su korišćeni u mnogim studijama da se utvrdi izmenjen imuni kapacitet, koji je uslovljen ksenobioticima. U našim istraživanjima demostrirali smo imunosupresiju humoralnih antitela u ribama izloženim teškim metalima. Smanjenje broja ćelija koje produkuju antitela je usledila nakon imunizacione kupke kod riba koje su bile izložene fenolima, rastvorima deterdženata, pesticidima, herbicidima i antibioticima. Takođe su pentahlorfenol (PCP), polihlorovani bifenili (PCB) i dioksini indukovali jaku imunosupresiju ćelijskog i humoralnog imuniteta riba. Antibiotici i drugi hemoterapeutici korišćeni u ljudskoj i životinjskoj medicini imaju sposobnost akumulacije u vodi i direktan uticaj na odbrambene mehanizme vodenih životinja. Čini se da antibiotici menjaju imune reakcije organizama. Neki antibiotici (oksitetraciklin, florfenicol) mogu imati različite efekte na različite faze unutar imunog sistema. Na primer, mogu imati uticaj na povećanje senzitivnosti simultnano sa smanjivanjem ćelijskog imuniteta i proteinskom sintezom.

U našoj studiji smo ispitivali mogućnost oporavka ćelijskog i humoralnog odbrambenog mehanizma i specifičnog imunog odgovora nakon eksperimentalne supresije indukovane atrazinom i pinoksadenom (Axial 100 EC) na šaranu (*Cyprinus carpio*). Takođe smo istraživali mogućenost oporavljanja imuniteta kalifornijske pastrmke (*Oncorhynchus mykiss*) nakon supresiju indukovane deltametrinom i oksitetraciklinom. Zdrave ribe (šaran i kalifornijska pastrmka), težine od 50 do 60g su intoksikovane koncentracijama ksenobiotika koje indukuju imunosupresiju (preliminarno u *in vitro* and *in vivo* studijama). Nakon intoksikacije ribe su imerzijom hranjenje 4 nedelje peletama sa imunomodulatorima: 1,3-1,6-β-D-glucan (Leiber Beta S, Germany) u dozi od 200 mg ili 500 mg po kg hrane i β-hidroksi- β-metilbutirat (HMB, USA) u dozi od 20 mg ili 50 mg po kg mase tela po danu. Krv, slezina i pronefrosi 10 intoksikovanih i kontrolnih riba su uzeti pre i posle intoksikacije i 1, 2, 3 i 4 nedelje nakon hranjenja šarana i kalifornijske pastrmke sa Leiber Beta S. Takođe, nakon dve nedelje hranjenja šarana i kalifornijske pastrmke izvedeni su testovi veštačkih infekcija (challenge tests) sa *Aeromonas salmonicida* u grupama od 20 riba za svaki eksperiment i kontrolne grupe radi utvrđivanja da li je stvorena zaštita protiv furunkuloze šarana i kalifornijske pastrmke primenom dietetskog imunomodulatora. U našem istraživanju razmatrali smo imunotoksični efekat na ćelijski i humoralni odbrambeni mehnizam atrazina ili pinoksadena na šarana i deltametrina ili oksitetraciklina na kalifornijsku pastrmku. Kada su 1,3-1,6-β-D-glukan (Leiber Beta S, Nemačka) i β-hidroksi-β-metilbutirat (HMB, SAD) introdukovani u hranu u različitim dozama, nakon intoksikacije, svi imunološki parametri šarana i kalifornijske pastrmke su značajno porasli ($P<0.05$) u poređenju sa kontrolonom grupom riba. Fagocitarna aktivnost makrofaga i proliferativni odgovor limfocita su statistični značajno porasli u poređenju sa kontrom grupom riba. Osim toga, testovi veštačke infekcije na grupama su pokazale smtnost koja je u korelaciji sa rezultatima testova nespecifičnog odbrambenog mehanizma. Rezultati našeg istraživanja su pokazala da 1,3-1,6-β-D-glukan (Leiber Beta S, Nemačka) i β-hidoksi-β-metilbutirat (HMB, SAD), prirodni imunomodulatori, mogu da se koriste za oporavak ćelijskog i humoralnog odbrambenog mehanizma i zaštite protiv bakterijskog oboljenja (furunkuloze) nakon supresije indukovane različitim ksenobioticima u ribama. Ova eksperimentalna studija ima veoma važnu praktičnu primenu i novi efektivni koncept za redukovanje morataliteta nakon negativnog uticaja kontaminanata iz okruženja ili ksenobiotika na imuni sistem riba, posebno u intenzivnom sistemu gajenju riba.

RESTORATION OF INNATE IMMUNITY IN FISH AFTER SUPPRESSION INDUCED BY XENOBIOTICS

During the process of evolution, biological organisms have developed defence mechanisms for protection against assault biological, chemical or physical agents. One of these mechanisms in higher organisms is a complex immune system, the fine details of which are only gradually becoming understood. The development and maintenance of defence mechanisms are important for the well being, and in some cases survival, of the organisms.

A large number of synthetically created chemicals have been added to the environment. Many studies acknowledge the influence of environmental pollutants – with an emphasis on pesticides, metal ions, chemotherapeutics and organic pollutants on the immune system of animals, demonstrating that chemical pollutants result in a variety of immunomodulatory effects on both cellular and humoral immune function and, consequently, on health and resistance to diseases. One of the important problems is that immunosuppression induced by xenobiotics that can favour the existence and growth of tumour cells. Although still open to scepticism in the case of occupational or environmental chemicals, reports have indicated that the prolonged depression of immune responses can increase the hazard of cancer in human and aquatic animals.

Fish, directly exposed to many toxic chemicals from discharges to rivers, lakes or ponds. The large numbers of xenobiotics have been added to the water environment. The xenobiotics have a direct influence on the cellular and humoral defence mechanisms and protection against infectious diseases. Furthermore, different populations of immunocompetent cells have a variable sensitivity to the xenobiotics. Many of xenobiotics induced immunosuppressive influence on the nonspecific immunity and specific immune response in fish. A number of studies have been made on the influences of xenobiotics on the immune system of fish. The integrity of the immune system is fundamental for good defence against a variety of pathogenic agents in the environment, namely parasites, bacteria and viruses. The major immune cells – lymphocytes, macrophages and neutrophils – are regulated by a variety of multistep control processes of cellular co-operation and interactions. Macrophages and neutrophils are important factors in the cellular immune system of fish, since they are the first line of defence and act to protect the host by phagocytosis of foreign material, including pathogenic agents. Furthermore, different populations of immunocompetents cells have a variable sensitivity to xenobiotics. This fact is very important in the study of xenobiotics influence on nonspecific cellular and humoral defence mechanisms and specific protection against diseases. In the following examples, immune assays are broadly grouped according to the specificity of responses. Some nonspecific assays such as the determination of lysozyme activity, C-reactive protein (CRP) level, ceruloplasmine (Cp) and complement levels have been used extensively in the past for analysing the effects of environmental contamination on the health of fish. Lysozyme and Cp activities are important in the initial destruction of invasive agents and in some cases can serve as an early biomarkers indicating deterioration of some protective mechanisms.

The humoral-mediated immunity assays have been used in many studies to determine altered immune capacity, which is caused by xenobiotics. In our study we demonstrated immunosuppression of humoral antibody levels in fish exposed to heavy metals. A reduction in antibody-secreting cells following bath immunization was observed in fish that had been exposed to phenol, detergent solutions, pesticides, herbicides and antibiotics. Also the pentachlorophenol (PCP), polichlorined biphenyls (PCB) and dioxins induced strong immunosuppression on the cell-mediated and humoral-mediated immunity in fish. Antibiotics and other chemotherapeutics used in human and animal medicine has the possibility of accumulation in water and has direct influence on the defence mechanisms in aquatic animals. Antibiotics seem to modulate the immune responses. Some antibiotics (oxytetracycline, florfenicol) may have different effects on various steps within the immune system. For example, they may possess a sensitising property while simultaneously depressing cellular defence mechanisms and protein synthesis.

In the present study we examined the possibility of restorating cellular and humoral defence mechanisms and specific immune response after experimental suppression induced by atrazine and pinoksaden (Axial 100 EC) in carp (*Cyprinus carpio*). Also we examined the possibility to restoration of immunity in rainbow trout (*Oncorhynchus mykiss*) after suppression induced by deltamethrin and oxytetracycline. Healthy fish (carp and rainbow trout), weighing 50 – 60 g were intoxicated by examined xenobiotics at concentrations induced immunosuppression (preliminary *in vitro* and *in vivo* study). After intoxication by immersion fish were fed 4 weeks pellets with immunomodu**la**tors: 1,3-1,6-β-D-glucan (Leiber Beta S, Germany) at dose 200 mg or 500 mg per kg of feed and β-hydroxy- β-methylbutyrate (HMB, USA) at dose 20 mg or 50 mg per kg of body weight/day. The blood, spleen and pronephros from 10 fish of intoxicated and control groups were separated before intoxication, after intoxication and 1, 2, 3 and 4 weeks after feeding of carp and rainbow trout with Leiber Beta S. Also after 2 weeks of feeding carp and rainbow trout a disease challenge test using *Aeromonas salmonicida* were conducted using groups of 20 fish for each experimental and control groups to determine if protection was provided by the dietary immunomodulators against carp or rainbow trout furunculosis. In the present study, the immunotoxic influence of atrazine or pinoksaden in carp and deltamethrin or oxytetracycline in rainbow trout on the cellular and humoral defence mechanisms was observed. Also challenge test showed that examined xenobiotics decreased protection against furunculosis in carp and rainbow trout. When 1,3-1,6-β-D-glucan (Leiber Beta S, Germany) and β-hydroxy- β-methylbutyrate (HMB, USA) were introduced into the food at different doses after intoxication, all examined immunological parameters in carp and rainbow trout increased significantly ($P<0.05$), when compared with the control groups of fish. The phagocytic activity of macrophages and proliferative response of lymphocytes were statistically increased, compared to the control groups of fish. Also lysozyme activity and Ig levels in serum significantly ($P<0.05$) increased, compared to the control groups. Nevertheless, challenge tests on the groups showed mortality patterns that correlated with the results from the nonspecific defence mechanisms assays. The results of our study showed that 1,3-1,6-β-D-glucan (Leiber Beta S, Germany) and β-hydroxy- β-methylbutyrate (HMB, USA), a natural immunomodulators, could be used for restoration of cellular and humoral defence mechanisms and protection against bacterial disease (furunculosis) after suppression induced by different xenobiotics in fish. This experimental study has a very important practical application and a new effective concept for reduce mortality after negative influence of environmental contamination or xenobiotics on fish immune system, especially in intensive fish culture.

PRESENCE OF BACTERIAL DISEASES OF FISH IN THE SERBIA DURING THE PERIOD 2005-2010.

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PRISUSTVO BAKTERIJSKIH BOLESTI RIBA NA PODRUČJU SRBIJE U PERIODU OD 2005. DO 2010. GODINE

Abstrakt
U petogodišnjem istraživanju prisustva bakterijskih bolesti riba u Srbiji, ispitano je 28 ribnjaka na kojima se uzgaja kalifornijska pastrmka (*Oncorhynchus mykiss*) i 15 ribnjaka u kojima se uzgajaju šaran (*Cyprinus carpio*) i srodne vrste. Tokom istraživanja fenotipski smo identifikovali 120 izolata iz 2320 uzoraka riba, i utvrdili prisustvo jersinioze, furunkuloze, renibakterioze, eritrodermatitisa i septikemije izazvane pokretnim aeromonadama. Najčešće su detektovane infekcije uzrokovane sa *Yersinia ruckeri*, *Aeromonas hydrophila* i *A. salmonicida*, ali je utvrđeno i prisustvo infekcija uzrokovanih sa *Pseudomonas putida*, *Flavobacterium psychrophilum* i *Pseudomonas fluorescens* su također osnovane. Takođe, po prvi put je utvrđena pojava *Janthinobacterium lividum* infekcije i oboljenja crvenih pega.

Ključne reči: *bolesti riba, bakterijske bolesti*

INTRODUCTION

The disease is the primary limiting factor in aquaculture and may significantly affect the economic and socio-economic development in many countries (Subasinghe and Bernoth, 2000). The appearance and development of fish diseases is a consequence of the interaction of pathogen, host and environment. The relatively small number of pathogenic bacteria is responsible for major economic losses in cultured fish (Toranzo et al., 2005). Antibacterial therapy is usually administered through healing foods. Environmental contamination in the vicinity of ponds occurs mostly through feces and uneaten food (Hirsch et al., 1999). In addition to the release of active substances in aquatic ecosystems, the usual therapeutic interventions in aquaculture can lead to antibiotic resistance in bacterial pathogens of fish, but also in other bacteria present in the environment

(Alderman and Hastings, 1998). Many diseases affecting farmed fish also represent a threat to natural fish populations (Thoesen, 1994). Any systematic study of fish populations will certainly lead to establishing the presence of infectious disease. Whether this is indeed a newly diagnosed disease or disease has not previously observed, remains unclear. New pathogens can take the place of existing (Austin and Austin, 2007). Therefore, the ponds need regular control in order to establish the presence of pathogens. The aim of this study was to determine the presence of bacterial fish diseases in Serbia during the 2005-2010 in 28 trout and 15 carp farms.

MATERIAL AND METODS

Samples for bacteriological examination were collected from 28 trout and 15 carp farms. As a material for testing parenchymatous organs, skin and gills of clinically diseased fish were used. A total of 2320 samples of altered organs of fish were examined bacteriologically. Sampling was carried out through repeated visits to fish farms in situations in which it was necessary to solve existing health problems. Fish for examinations were transported to the laboratory and dissected for bacteriological testing. In all fish, external and internal examination was performed. Gills and body surface were examined microscopically for the presence of *Flavobacterium* sp. Then, the surface of the body was swabbed with 70% ethyl alcohol to prevent contamination. Liver, kidney and spleen were inoculated in trypticase soy agar (TSA), blood agar (BA), furunculosis agar, Mueller-Hinton agar (MH), Rimler-Shotts agar (RS), KDM-2 agar and brilliant green agar (BG), depending on the origin of fish. Samples from external lesions were inoculated on cytophaga agar and trypticase soy agar. After incubation at 20-25°C for 4 days or at 10-15°C for 10 days, isolated bacteria were subcultivated in order to test the purity of isolates. Pure culture of isolated colonies were biochemically characterized using API 20E, API 20NE (Biomerieux), and following biochemical tests were performed: Gram stain, cytochrome oxidase, catalase, beta-galactosidase, arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, citrate utilization, H2S production, urease, tryptophan deaminase, indole production, Voges-Proskauer, gelatinase, fermentation of glucose, mannitol, inositol, sorbitol, rhamnose, sucrose, amygdalin, methyl red, arabinose, lactose, esculin, xylose, mobility, and oxidative / fermentative test. The isolates were identified to the genus or species level based on standard bacterial taxonomic procedures (Krieg and Holt, 1984, Holt et al, 1994; Austin and Austin, 2007). The isolates were stored in broth with the addition of 15-20% glycerol at-80°C. For confirmatory identification of Renibacterium salmoninarum, direct immunofluorescence (FAT) and PCR were used.

RESULTS AND DISCUSSION

During the five-year study of presence of bacterial fish diseases in the Republic of Serbia, carried out in a total of 43 fish farms, following diseases were founded:

Yersiniosis - yersiniosis is a bacterial disease caused by gram-negative bacteria *Yersinia ruckeri*. The disease is widespread and occurs in most countries where salmonids are intensively cultivated. In Serbia, the disease was first diagnosed in 1987 (Ocvirk et al., 1987). In the period 2005 - 2010, yersiniosis was present on majority of examined trout farms in a one-year old rainbow trout. In addition to nonspecific symptoms (dark pigmentation, exophthalmia, ocular hemorrhages), subcutaneous bleeding in the mouth were present. The internal lesions were represented by petechial hemorrhage in pyloric caeca and perivisceral fatty tissue, edema of the kidney and spleen, as well as the absence of food from the intestines, which were filled with yellow, mucous content. *Yersinia ruckeri* was isolated from the spleen and kidney of diseased fish after incubation in aerobic atmosphere for 48 hours at 20°C. However, despite these cases, where the disease was caused by highly virulent "Hagerman" strain, with severe clinical and major morbidity (40%), in a few trout ponds we have isolated low virulent strains of *Yersinia ruckeri*, without the presence of specific clinical symptoms, and mortality did not exceed the technological limit.

Carp erythrodermatitis - Carp erythrodermatitis is a subacute to chronic skin disease that occurs at temperatures from 4 to 30°C (Pol et al. 1980). The etiologic agent of Carp Erythrodermatitis was isolated and described as an atypical, achromogenic variant of *A. salmonicida* (Bootsma et al. 1977; Bootsma and Blommaert 1978). The disease was first diagnosed in the former Yugoslavia in 1977 (Bootsma et al., 1977). In our study, disease was found in young carp in a number of fish farms. Chronic form of erythrodermatitis was diagnosed in carp yearlings during the spring period. At low temperatures in winter and spring disease develops slowly and runs with considerable losses. In summer, acute form of carp erythrodermatitis was present in a number of carp farms. Clinical signs consisted of deep ulcers accompanied by peripheral necrosis which was present in the epidermis and extended into the underlying musculature, hemorrhagic inflammation at the base of the fins, and slight to extreme exophthalmia. The symptoms were limited to external involvement only, and no pathologic signs were apparent on gross examination of the internal organs. Achromogenic atypical *Aeromonas salmonicida subsp. nova* was consistently isolated on trypticase soy agar containing 0.01% Coomassie brilliant blue from samples taken from the periphery of small ulcers.

Furunculosis - contagious disease caused by gram-negative bacteria *Aeromonas salmonicida subsp. salmonicida*. The first case of the disease in Serbia reported in 1972 (Snoj and Brglez, 1972). The disease is present in European countries with intensive Salmonids cultivation, and also in North America, South Africa and Japan. Improvements of cultivation technology and adequate health care have led to a reduction in mortality and incidence of clinically overt disease, although the disease is still present. In our study, furunculosis with characteristic clinical symptoms was detected in four examined trout pond. In one case the disease was diagnosed in grayling. *Aeromonas salmonicida subsp. salmonicida* was isolated from the lesions, spleen and kidney of diseased fish on TSA and furunculosis agar, after incubation under aerobic conditions for a 48^h at 20°C.

Bacterial kidney disease - Bacterial Kidney Disease (BKD) is a systemic infection of salmonids caused by the gram-positive diplobacillus *Renibacterium salmoninarum*. The disease has a wide geographic range. In our study, during the period 2005 - 2010, five cases of BKD were diagnosed. In all cases the disease was detected in a one-year old rainbow trout. Infected fish showed pale gills, exophthalmia, and abdominal distension. Turbid fluid was present in abdominal and pericardial cavities, and creamy-white granulomatous lesions were present in the kidney and, less frequently, in the spleen and the liver. Cumulative mortality was around 35%. Direct immunofluorescence test and PCR showed the presence of *R. salmoninarum*, a bacterium was isolated from the kidney of diseased trouts in KDM-2 agar.

***Janthinobacterium lividum* infection -** *Janthinobacterium lividum* is a Gram-negative bacteria, belonging to the family Oxalobacteraceae. Typical and atypical forms of

J.lividum is considered as normal microflora of water and soil (Sneath, 1984). However, several cases of the disease of rainbow trout caused by the bacteria, with a mortality rate about 30% was reported (Austin et al., 1992, Austin et al. 2003), and cases of human infection with fatal outcome were described (Patijanasoontorn et al., 1992). During december 2007 increased mortality (20%) in one-month old trout weighting 0.5 - 1.0 g, with lethargy, pale gills, dark skin pigmentation and abdominal enlargement was found. Pathoanatomical examination revealed oedema of kidney, liver and spleen ishemia, and presence of moderate amounts of clear liquid. Based on morphological, physiological and biochemical characteristics, isolated bacteria were identified as *Janthinobacterium lividum*.

Red mark syndrome (RMS) is a chronic and typically non-lethal skin condition affecting rainbow trout (*Oncorhynchus mykiss*) (Verner-Jeffreys et al., 2006). The condition is characterised by single to multiple skin lesions, typically found on the flanks. These lesions can affect carcass quality, which downgrades the product and lowers its market value. Morbidity ranges up to 80% and the disease affects fishes from 15cm in length to brood stock size (Pond, 2007). Results of field investigations indicated the condition had an infectious aetiology, but the cause could not be definitively proved (Ferguson et al., 2006; Verner-Jeffreys et al., 2008). Red mark syndrome was determined at a one trout farm in 2008, at two trout farms in 2009, and in four trout farms in 2010. The affected fish showed with a range of lesions of differing severity. Clinical signs include appearance of bright red, raised; circumscribed, ulcerated and indurated lesions in the skin that usually range up to 3 cm or more in diameter. Microscopically, the disease was characterized as a subchronic, focal, non–suppurative dermatitis with extensive lymphocytic infiltration. However, despite extensive analysis, including mycology, bacteriology and virology, no single potential disease agent was isolated from affected fish.

Motile aeromonad septicemia - Bacterial infections caused by motile members of the genus Aeromonas, are among the most common and troublesome diseases of fish raised in ponds and recirculating systems. The widespread distribution of these bacteria in the aquatic environment and the stress induced by intensive culture practices predisposes fish to infections. In our study, motile aeromonad septicemia in carp (*Cyprinus carpio*), grass carp (*Carassius carassius*), grass carp (*Chenopharyngodon idella*) channel catfish (*Ictalurus punctatus*) and rainbow trout (*Oncorhynchus mykiss*) was present in a number of fish farms. In addition, sporadic cases of infection with *Pseudomonas putida*, *Flavobacterium psychrophilum*, and *Pseudomonas fluorescens* in these species was detected.

CONCLUSIONS

The most commonly reported bacterial fish diseases in Europe are vibriosis, pasteurellosis, yersiniosis, furunculosis, flexibacteriosis, columnaris disease, motile aeromonad septicemia, pseudomonas infections, streptococcocis, mycobacteriosis. Most of these diseases were detected in this survey. The occurrence of diseases that previously did not report warns that the measures taken to protect the health of the fish are not enough. We need to invest additional effort in order to maintain the health status of fish populations through the use effective biosecurity measures.

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RECENT VIEWS ON POSTMORTEM AGING IN FISH

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NOVIJI POGLEDI NA POSTMORTALNE PROMENE MIŠIĆA RIBE

Prošireni abstrakt

Odmah nakon prekida cirkulisanja krvi, pa zbog toga i nedostatka izvora kiseonika, glikogen podlaže anaerobnom razlaganju i preko niza fosforilovanih derivata glukoze prelazi u mlečnu kiselinu, koja se nagomilava u mišićima, što rezultira smanjenjem pH vrednosti sa vrednosti blizu 7,4 na šest, ponekada i nižu. Mišićni osmotski pritisak raste u roku od sat vremena nakon smrti, sadržaj ATP (adenozin trifosfat) opada a lipidi oksidišu. Trimetilaminoksid (TMAO) prelazi u trimetilamin (TMA), usled dejstva endogenih enzima, a kasnije bakterijskih - kada počne mikrobiološka aktivnost. Količina azot-oksida i reaktivnih metabolita kiseonika se takođe povećava. Mitohondrije i sarkoplazmatski retikulum prestaju sa radom tokom pada pH vrednosti i promena osmotskog pritiska, što rezultira oslobađanjem kalcijumovih jona u citosol, gde njihova koncentracija može da dostigne i 0.2 mM slobodnog kalcijuma.

Proteoliza komponenata citoskeleta rezultira degradacijom miofilamenata. Kod ribe, u zavisnosti od vrste, ona može da obuhvati degradaciju titina, nebulina, distrofina, oslobađanje α-aktinina, proteolizu miozina i delokalizaciju tropomiozina. Sarkoplazmatski 16 kDa protein (označen kao nukleozid difosfat kinaza) takođe podleže proteolizi tokom skladištenja. Tropomiozin se oslobađa iz miofibrila, a sadržaj ekstrahovanog tropomiozina raste između 0 i 48 sati u prisustvu 5 mM EGTA. U ekstrakciji sa 5 mM Ca^{2+}, tropomiozin je pronađen u manjoj količini nakon 48 sati.

U mišićnim ćelijama postoje različiti proteolitički sistemi: multikatalitički kompleks, lizozomalni sistem koji uključuje aspartamske i cisteinske kisele katepsine, citosolni Ca-zavisni kalpaini i citoplazmatične aminopeptidaze, alkalne proteaze, kao i hidrolitični enzimi vezivnog tkiva, elastaze i kolagenaze.

Multikatalitički kompleks ili *proteazom* učestvuje u razgradnji hormona, antigena, transkripcionih faktora i ubikvitin konjugovanih i oksidovanih proteina. Za aktivnost 26S proteazoma protreban je ATP i hidrolizovani ubikvitin konjugovani proteini. Proteazom 20S, koji predstavlja deo 26S proteazoma, postoji u latentnoj formi koja može da se aktivira različitim jedinjenjima.

Katepsini su kisele proteaze, najčešće lokalizovane u lizozomima. Stoga su uglavnom neaktivni u tkivu živih organizama, ali bivaju oslobođeni usled povreda ili smrzavanja i odmrzavanja u mišićima postmortem. Katepsini mogu biti specifični po svom aktivnom mestu (aspartam, cistein ili serin proteaza), kao i po svojoj supstrat specifičnosti ili osetljivosti na određene inhibitore. U lizozomima se može naći najmanje 13 različitih katepsina. Pored njih katepsini B, D, L su već izolovani iz ćelija ribe. Katepsini B, D, L i H su najzastupljenije grupe katepsina u lizozomima mišićnih ćelija ribe.

Kalpaini su cistein proteaze, aktivne pri neutralnoj pH vrednosti i zavisne od kalcijuma. Ubikvitarni kalpain se sastoji od kalpaina I ili mikro(μ)-kalpaina, koji zahteva mikromolarne koncentacije kalcijuma za punu aktivnost (10 do 50 μM) i kalpaina II ili mili(m)-kalpaina, koji zahteva milimolarne koncentracije kalcijuma (300 μM do 1 mM). Kalpainski heterodimeri se razdvajaju u prisustvu kalcijuma. Mala subjedinica (30 kDa) je ista kod svih kalpaina, dok je velika subjedinica (80 kDa) specifična za određeni tip kalpaina i odgovorna za katalitičku aktivnost. Aktivnost kalpaina, aktivnih pri intracelularnoj fiziološkoj pH vrednosti, je u velikoj meri regulisana *in vivo*. Regulacija aktivnosti kalpaina je kompleksna i nije poznata u potpunosti. Četiri osobine kalpaina su kalcijum zavisne: vezivanje za celularne komponente, kao što su membrane, vezivanje za kalpastatin, proteolitička aktivnost i autoliza.

Metaloproteaze matriksa (MMP-Matrix metalloproteases) predstavljaju brojnu familiju strukturno sličnih endopeptidaza, odgovornih za katabolizam vezivnog tkiva. One su sposobne da razgrade različite tipove kolagena i proteine citoskeleta povezujući sarkolemu za ekstracelularni matriks. Oni su cink i kalcijum zavisni enzimi, klasifikovani u 4 podfamilije: kolagenaze, želatinaze, stromelizin i membranski tip MMP-a.

Postmortalne promene mesa ribe vezane su za metaboličke promene (prekid nukletida, hidroliza i oksidacija masti, pad pH, rast osmotskog pritiska, povećanje koncentracije Ca^{2+}, povećanje azot oksida i slobodnih radikala), za promene strukture miofibrila (oslobađanje i proteolizu α-aktinina, degradaciju titina, proteolizu nebulina i miozina, delokalizaciju tropomiozina, proteolizu troponina T, pristustvo 30kDa fragmenta, degradaciju aktina, dezmina i distrofina). U ovim promenama učestvuje brojni faktori,a promene neminovno dovode do gubitka svežine ribe.

Ključne reči: *riba, postmortalne promene, strukturne promene, svežina*

INTRODUCTION

Quality attributes of fish flesh, including food safety, organoleptic features, nutritive value quality and aptitude to industrial processing, influence on consumption and acceptability of fish as food. Fish sensorial changes and texture properties are closely linked to freshness. Along with

ante mortem muscle biochemistry, postmortem biochemical processes are directly linked to final quality attributes. The understanding of postmortem mechanisms is a prerequisite for an accurate control of commercialized fish quality by the identification of objective markers or indicators as measure of freshness.

Muscle metabolism postmortem

Immediately after the cessation of the circulation of the blood and, consequently of oxygen supply, the stored carbohydrate glycogen is anaerobically degraded and lactic acid accumulates in muscle (Watabe et al.,1989), resulting in a pH drop from a value close to 7,4 to 6 in fish, sometimes below (Church, 1998). Muscle osmotic pressure increases within hours postmortem, ATP (adenosine triphosphate) decreases and lipids are oxides. Trimethylamine oxide (TMAO) is changed to trimethylamine (TMA) by endogenous enzymes and later by bacteria when microbial activity begins. Nitric oxide and reactive species of oxygen also increase. Mitochondria and sarcoplasmic reticulum deterioration due to pH fall and osmotic pressure changes results in the release of calcium ions in the cytosol where concentration can reach 0, 2 mM free calcium.

The onset and extent of rigor mortis are biochemically characterized by a total exhaustion of energy-rich compounds. ATP depletion initiates the rigor mortis process and at less than 2 μM ATP, actin and myosin form inextensible actomyosin, which causes stiffness of the whole body in rigor mortis. The rigor generally begins one to six hours after death in fish (Watanabe i Turner, 1993), in particular, it is maximal one day and a half after death for sea bass muscle stored at 0^{o}C. This condition usually lasts for a day and then the resolution of rigor mortis makes the muscle less rigid and no longer elastic. The rate in onset and resolution of rigor mortis varies from species to species and is affected by temperature, handling, and stress before slaughter, size and physical condition of the fish, biological status of fish and temperature during storage before rigor onset. Also exists individual variations. Rigor is very dependent on stress before slaughter: control salmon shows maximum rigor at about 8 hours postmortem, but highly stressed fish can enter rigor as soon as 30 minutes postmortem (Skjervold et al., 2001).

Sometime after death, an opposing process called tenderization begins within hours postmortem and continues during postmortem storage. Several studies showed that tenderization begins in the early stage of postmortem storage of fish and mammals (Koohmaraie, 1996). The key structures which are degraded are the cytoskeletal links to sarcomeres and to the plasma membrane (Taylor et al., 2002). Tenderization rate and extent vary depending on species and other factors.

Proteolysis of cytoskeletal components results in myofilament degradation. In fish, depending on species, this may include degradation of titin, nebulin, dystrophin, α-actinin release, myosin proteolysis and tropomyosin delocalization (Ofsad et al., 1996, Papa et al., 1997). The costameres which link sarcomeres to the sarcolemma are also degraded within 24h postmortem, Most of the changes are common among different fish species but they may occur at different rates. In particular, sea bass muscle changes include the detachment of sarcolemma, the degradation of titin and nebulin as well as the release and proteolysis of α-actinin from the Z line and the degradation of dystrophin. Desmin remained unchanged after a 4 days cold storage in sea bass muscle. A sarcoplasmic 16 kDa protein (identified as nucleoside diphosphate kinase)

was also shown to undergo proteolysis during storage (Verrez-Bagnis et al., 2001). Tropomyosin was released from the myofibrils; the content of extracted tropomyosin increased with time between 0 and 48 h in the presence of 5 mM EGTA. In extracts with 5 mM Ca^{2+}, tropomyosin was observed in lower quantities after 48 h.

Connective tissue collagen is degraded in fish after death as shown by scanning electron microscopic analysis of muscle as a progressive breakdown of the collagen junctions between the myocommata and the muscle fibers during storage in ice. The structural change in collagen fibrillar network in fish correlates with the postmortem tenderization. Collagen fibrils in the pericellular connective tissue are disorganized and degraded and space between fibers increases. The decrease of type V collagen content has a correlation with the postmortem softening of fish meat during chilled storage (Shigemura et al., 2003).

ROLE OF PROTEASIS IN POSTMORTEM AUTOLYSIS OF FISH MUSCLE

Deterioration of fish flesh results from the complex combination of physical, chemical, biochemical and microbial processes. However, the first changes occurring in postmortem fish muscle are due to endogenous enzymes promoting proteolysis of muscle proteins and connective tissue as well as fat hydrolysis. Indeed, the muscle is not significantly contaminated by bacteria at this stage.

Proteases in fish muscle

Different proteolytic system exist within the muscular cell: a muticatalytic complex or proteasome, a lysosomal system including aspartic and cysteine acidic cathepsins, the cytosolic calcium dependent calpains, as well as cytoplasmic aminopeptidases, alkaline proteases and connective tissue hydrolytic enzymes such as elastase and collagenase.

The multicatalytic complex is involved in the degradation of hormones, antigens, transcription factors and ubiquitin-conjugated or oxidized proteins. The 26S proteasome requires ATP for activity and hydrolyses ubiquitin-conjugated proteins. The 20S proteasome, which in fact is also a part of 26S proteasome, exists as a latent form possibly activated by different compounds (Aoki et al., 2000).

Cathepsins are acid proteases usually located in organelles called lysosomes and thus are for the most part inactive in living tissue, but become released at sites of injury or upon freezing and thawing of postmortem muscle. Cathepsin can be distinguished by their active site (aspartic, cysteine, serine proteases) as well as by their substrate specificity and inhibitor sensitivity. Lysosomes are known to harbor at least 13 cathepsins.

Calpains are cysteine proteases active at neutral pH and are dependent upon calcium. Ubiquitous calpain include calpain I or micro (μ)-calpain which requires micromolar calcium concentration for full activity (10 to 50 μM) and calpain II or milli (m)-calpain requiring millimolar calcium concentrations (300 μM to 1 mM). Calpains are heterodimers dissociating in presence of calcium. The small subunit (30 kDa) is common to ubiquitous calpains and the large subunit (80 kDa) is specific to the calpain type and is responsible for catalytic activity. Calpains, active at intracellular physiological pH, are highly regulated in vivo. The regulation of calpain activity is complex and not fully understood. The regulation system is based on calcium binding, subunit association, interaction with calpastatin (the endogenous inhibitor specific to calpains) and cellular membranes as well as limited autolysis in the presence of calcium allowing proteolytic activity, but increasing instability. Calcium is binding in the calpain molecule resulting in a conformational switch allowing the alignment of the catalytic amino acid trio Cys, His, Asn. Four properties of calpains are calcium dependent: binding to cellular components such as membranes, calpastatin binding, proteolytic activity and autolysis (Hosfield et al., 1999).

Matrix metalloproteases (MMP) represent a large family of structurally related endopeptidases responsible for connective tissue catabolism. MMPs are able to degrade different types of collagen and cytoskeletal proteins connecting the sarcolemma to the extracellular matrix. They are zinc and calcium-dependent enzymes, classified into four subfamilies: collagenase, gelatinase, stromelysin and membrane type MMP.

Reports on other proteolytic activities such as serine proteases, neutral proteases or alkaline proteases from fish muscle are fragmented.

Contribution of proteases to postmortem autolytic changes

Until now an understanding of the complex mechanisms responsible for postmortem fish muscle changes hasn't reached general agreement. For fishes, of the different intracellular proteolytic systems identified so far, two major pathways were generally distinguished for muscle proteins degradation during postmortem storage: the cathepsins and the calpains. A rapid decrease in pH after death could indicate that lysosomal acidic proteinases may be active if they are liberated from lysosomes to reach the substrate. Cathepsins D and L are believed to play a major role in the autolytic degradation due to their wide pH range of activity while other cathepsins are active at pH values too low to be of physiological significance. The high cathepsin content in spawning fish and rapid degradation of postmortem muscle indicates a possible role also existing in normal fish. Also supporting a role for cathepsin is the degradation of connective tissue proteins within days in fish, connective tissue proteins are not susceptible to most proteases but are cathepsin substrates.

The second protease system which can degrade connective tissue is the metalloproteases. These proteases are constitutively inactive in mammals and require signal transduction pathway activation. There is report of their activity in some species of fish, but no studies of their role in texture.

A number of *in vitro* studies have clearly demonstrated the susceptibility of numerous myofibrillar proteins to proteolysis by calpains and lysosomal proteinases. In sea bass muscle, calpain was able to release α-actinin and tropomyosin from myofibrils in vitro. Calpains and cathepsins degrade myosin heavy chain, α-actinin and desmin while actin and tropomyosin appear to be sensitive to cathepsins B, D, L. Troponin T was degraded by cathepsins B and L. Minor changes of some other myofibrillar of cytosolic proteins were also observed (creatine kinase and other identified proteins).

Changes in collagen have been attributed to collagenases; they have also been related to the process responsible for gaping phenomenon in which the muscle fibers are gradually disconnected from the myocommata during chilled storage due to collagen fibers breakdown. It has been pointed out at role of matrix metalloproteases in the disintegration of the intramuscular connective tissue that induces the postmortem tenderization of fish muscle. It has been observed both quantitative and qualitative differences in collagenolytic activities in the muscle of different fish species (Kubota et al., 2003, Delbarre-Ladrat et al., 2004). None of these systems alone can explain all the changes observed postmortem, Synergy between proteases and other environment factors exists. During rigor mortis, since osmotic pressure is modified, ionic strength increases and may become high enough to weaken the myofibrillar structure, making it more susceptible to proteolysis.

CONCLUSION

Postmortem changes in fish are related to metabolic changes (nucleotides breakdown, lipid hydrolysis and oxidation, decrease of pH, increase in osmotic pressure, Ca^{2+} increase, increase in nitric oxide and free radicals) and structural changes (myofibrillar structure: α-actinin realease, α-actinin proteolyses, titin degradation, nebulin proteolysis, myosin proteolysis, tropomyosin delocalization, tropomyosin proteolysis, troponin T proteolysis, 30 kDa fragment appearance, actin degradation; desmin degradation, dystrophin degradation).

In these changes is involved big number of factors (nucleotide-degrading enzymes, phospholipases, oxidation status in cell, glycogen degradation and lactic acid accumulation, protein deterioration, increase in osmotic pressure and pH fall, calpains, cathepsins, calcium) and these changes will inevitably lead to loss of fish freshness.

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POTENTIAL USE OF AUXOTROPHIC AROA AND AROC MUTANT YERSINIA RUCKERI AS A LIVE ATTENUATED VACCINE

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POTENCIJALNA UPOTREBA AUKSOTROFNIH *aroA I aroC MUTANATA YERSINIA RUCKERI* KAO ŽIVE ATENUIRANE VAKCINE

Abstract

Yersinia ruckeri causes yersiniosis in rainbow trout (*Oncorhynchus mykiss*). Yersiniosis is the main causes of high mortalities and severe economic losses in freshwater and marine aquaculture. To treat and prevent yersiniosis, antibiotics and inactive vaccines have been used. However, use of antibiotics can cause antibiotic resistance in bacteria, while inactive vaccines do not provide prolonged protection against bacterial fish diseases. Formation of antibiotic resistance in bacteria against antibiotics, and chemical contamination of the environment are some of the undesirable outcomes. For these reasons, prevention of fish diseases using vaccination strategies is important for ensuring the profitability and sustainability of aquaculture production.

The objective of this research is to develop live attenuated vaccines against yersiniosis. To reach this aim, *aroA* and *aroC* genes of *Y. ruckeri* have been mutated and virulence and efficacy of these mutants are characterized. Our main hypothesis is that *Y. ruckeri* with mutations in their aromatic amino acid biosynthesis network (*aro*) will lose their ability to cause infections in fish and these will be used as live vaccines. To accomplish the aim of this research, 5' and 3' regions of *Y. ruckeri aro*A (5-enolpyruvylshikimate-3-phosphate syntheses) and *aroC* (2, 3-dihydroxybenzoic acid) genes are amplified and DNA fragments mutated by overlap extension PCR will be cloned into a suicide plasmid (pDS132). This plasmid will be transferred to *Y. ruckeri* for replacing wild type genes with mutated *aroA* and *aroC* genes via homologous recombination. Successful completion of this phase is expected to yield live attenuated vaccine candidates. It is expected that these live attenuated vaccines will provide resistance against the wild type *Y. ruckeri* infections in trout, and thus, prevent onset and progress

of diseases. Successful completion of this study is expected to prevent fish losses due to *Y. ruckeri* infections and increase the profitability of aquaculture. Contend with fish diseases using traditional methods is generally ineffective and expensive.

Key words: *Aromatic amino acid biyosenthesis network* (*aro*), *aroA*, *aroC*, *overlap PCR, live attenuated vaccine*

GROWTH PERFORMANCE AND SURVIVAL OF *CLARIAS GARIEPINUS* FINGERLINGS REARED IN PLASTIC BASINS AND CAGES

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PRIRAST I PREŽIVLJAVANJE MLAĐI *CLARIAS GARIEPINUS* U PLASTIČNIM BAZENIMA I KAVEZIMA

Abstrakt

Rast i preživljavanje mlađi *Clarias gariepinus* je ispitivana pod dva uslova sredine; u plastičnim bazenima i kavezima potopljenim u zemljane bazene.

Mlađ je hranjena u količini od 5% telesne težine. Nakon 8 nedelja (56 dana) određivane su jednom nedeljno masa i dužina. Krajnja srednja masa za plastične bazene i kaveze bila je 11,41 g i 17,63 g. Srednja ukupna dužina je bila za kaveze 13,53 cm a za plastične bazene 10,89 cm. Preživljavanje je bilo veće u kavezima (82%) nego u plastičnim bazenima (24%). Rast ribe procenjivan je u dva uslova sredine, ustanovljena je značajna razlika ($p < 0,005$) u porastu srednje mase i srednje dužine. Prema prirastu riba, rezultati ispitivanja govore da mlađ gajena u kavezima pokazuje bolji prirast i preživljavanje za mlađ afričkog soma *Clarias gariepinus*.

Ključne reči: *Clarias gariepinus, mlađ, plastični bazeni, kavezi*

INTRODUCTION

The goal of any aquaculture programme should be to adopt methods that will produce quality fingerlings which can survive better, grow faster and resist some routine or common disease and adverse environmental conditions.

Since the 1970s the African catfish *Clarias gariepinus* has been considered to be a fish of great promise for fish farming in Africa. Its growth rate is high; it is very resistant and appreciated in a wide number of African countries. The development of a reliable method for the production of *Clarias gariepinus* fingerlings is one of the priorities of agriculture research in Africa (Anonymous, 1987). *Clarias gariepinus* which is widely considered to be one of the most tropical catfish species for aquaculture has an almost Pan-African distribution, from the Nile to West Africa and from Algeria to Southern Africa. They occur in minor-Asia (Israel, Syria and South of Turkey) (FAO, 2003). In general *Clarias gariepinus* lives in most river basins.

There is a steady rise in human population in Nigeria and this has not been matched by corresponding increase in food production (Ekokotu and Ekelemu, 1999) while human population growth is rising at a rate of about 4-5% and live stock production is trailing behind at a rate of 2-3%. This shows that there is a wide gap between supply and demand of animal protein. As human population increase and natural fisheries resources diminish, knowing how to fish is not enough for today’s fishers and their families, many would be better off learning how to culture fish (Meryl, 1996).

The consequence of the sceneries is the soaring cost of animal protein needs. This has made it almost impossible for the poverty stricken Nigerians to meet their animal protein needs. Thus, the need arises to explore alternative avenues for accessing animal protein as a way of increasing protein consumption. This alternative source could be achieved if farmers could adopt better agriculture or fish culture methods.

This study present an analysis on growth response and survival of fingerlings of *Clarias gariepinus* reared in cages suspended in an earthen pond and plastic basins. The objective of this study is to determine the growth performance and survival of *Clarias gariepinus* fingerlings reared in plastic basins and cages suspended in an earthen pond.

MATERIALS AND METHODS

Experimental Site

The research project was carried out at the Teaching and Research Fish Ponds of The Department of Environmental Biology and Fisheries Adekunle Ajasin University Akungba Akoko, Nigeria.

Experimental Set Up

A total of one hundred (100) fingerlings of *Clarias gariepinus* were procured from Sunshine Fishery Alagbaka, Akure, Ondo State, Nigeria.

The initial average weight of the fish ranges from 0.643 g to 0.644 g. The fingerlings were randomly distributed into two plastic basins (25 fish per basin) and two cages (25 fish per cage).

The fingerlings were fed twice daily at 5% of total body weight with floating feed (copens). The experiment was carried out for a period of eight 8 weeks. (56days).

Maintenance of Fingerlings

The fingerlings were stocked in two replicated plastic basins A and B (25 per plastic basin) kept and reared in the hatchery (Picture 1, protected indoor rearing) and two replicated cages A and B (25 fingerlings per cage) suspended and reared in an earthen pond (Picture 2, protected outdoor rearing). The sizes of the plastic basin each is 2 x 1 x 1 m and the cages 2 x 1 x 1 m.

The plastic basins were filled to 2/3 of its depth and the cages were also suspended at the edge of the pond to the 2/3 of its depth. The fingerlings were fed twice daily in each plastic basin and cage with commercial feed (copens feed).

The left over feed was siphoned out of the base of each plastic basin and water was also replaced daily. As the water level reduced in earthen pond the cage were relocated to maintain their normal level of water.

Monitoring of Physico-chemical Parameters

Physico-chemical parameters like temperature and pH were monitored on a daily basis according to the methods of Boyd 1981 using pH meter and thermometer (Surgifield medical England SM-602 1A).

Data Collection

Weight and length measurements (standard and total length) were recorded weekly. The fingerlings were weighed to the nearest 0.1 g with an electronic weighing balance (Sartorius excellence scale). The standard and total lengths were measured to 0.1 cm using a standard metric ruler.

Growth and Survival Parameters

To determine growth response, fingerlings in each plastic basins and cages were weighed and measured weekly and readings obtained were used to compute parameters such as SGR (specific growth rate), DGR (daily growth rate), MWOF (mean weight of fish) MGW(mean weight gain) and Survival rate. The above parameters were calculated to determine the effect of the environment on the growth performance and survival of *Clarias gariepinus* fingerlings reared in plastic basins and cages suspended in an earthen pond.

Definition of the terms

Specific Growth Rate (SGR)

$$SGR = \frac{\text{Loge Wf - Loge Wi x 100}}{\text{Time (days)}}$$

Where

Wf = final average weight at the end of experiment

Wi = Initial average weight at the beginning of experiment

Loge = natural logarithm reading

Time = number of days for experiment

Mean weight of fish (MWF)

$$MWF = \frac{\text{Total weight of Fish}}{\text{Number of Fish}}$$

Daily growth rate (DGR)

$$DGR = \frac{\text{mean increase in weight per day}}{\text{Body weight}}$$

Mean weight gain (MWG)

$$MWG = Wt2 - Wt1$$

Where Wt1 = initial mean weight of fish at time T1

Wt2 = final mean weight of fish at time T2

$$\text{Survival rate (\%)} = \frac{\text{No of fish that survived}}{\text{Total no of fish Stocked}} \times 100$$

Statistical analysis

The data obtained were subjected to both descriptive and inferential statistics. T-test was then used to determine the significant difference in the environmental effect on fish growth rate and survival.

RESULTS

Table 1 shows the summary of the initial mean weight (g) and final mean weights (g) of fish, initial mean length and final mean length (cm), SGR (specific growth rate), DGR (daily growth rate) and survival rate at the end of experiment.

The percentage survival rate of fish in plastic basins and cages at the end of experiment were presented in Table 1. The survival rate was (24%) in plastic basins while the highest survival rate was recorded in cages (82%). The initial mean weight in plastic basin and cage are 0.64g ± 0.22. The final mean weight in cages 17.63g ± 6.12 was higher than that of plastic basins 11.41g ± 7.56. The plastic basins has a total number of 12 survived fish out of 50 stocked fingerlings while the cages has a total number of 41 survived fish out of 50 stocked fingerlings at the beginning of the experiment. Initial and final mean lengths recorded in cage are 2.55 cm ± 0.32 and 13.53 cm ± 1.63 while that of plastic basin were 2.55 cm ± 0.32 and 10.89 cm ± 2.69 respectively. Daily growth rate in plastic basin was 0.19 g and in cage as 0.30 g.

Table 1: Summary of growth performance of *Clarias gariepinus* fingerlings in plastic basin and cages

Parameters	Plastic Basin	Cage
Initial mean weight(g)	0.64 ± 0.22	0.64 ± 0.22
Final mean weight(g)	11.41 ± 7.56	17.63 ± 6.12
Initial mean length(cm)	2.55 ± 0.32	2.55 ± 0.32
Final mean length(cm)	10.89 ± 2.69	13.53 ± 1.63
SGR % day $^{-1}$	5.14	5.92
DGR (g)	0.19	0.30
Survival rate %	24	82

Table 2: Means and ranges of physicochemical parameters of water in plastic basins and cages for rearing *Clarias gariepinus* fingerlings

Parameter /week	pH Plastic basin	pH Cage	Temperature (^{0}C) Plastic Basin	Temperature (^{0}C) Cage
Wk 1	7.28 (6.90-7.80)	8.02 (7.30-8.50)	26.30 (25.10-27.50)	28.66 (27.20-30.20)
Wk 2	7.17 (7.10-7.60)	7.48 (7.20-8.50)	26.76 (25.20-28.70)	27.88 (25.30-30.10)
Wk 3	7.27 (6.70-7.80)	7.94 (7.10-8.50)	26.79 (25.20-29.20)	27.68 (26.20-28.70)
Wk 4	7.68 (7.30 7.80)	8.49 (7.90-8.70)	27.27 (24.20-29.20)	28.11 (25.20-31.80)
Wk 5	7.31 (7.10-7.60)	8.19 (7.80-8.50)	26.82 (25.2-28.1)	28.43 (27.2-30.00)
Wk 6	7.30 (7.10-7.70)	8.27 (8.00-8.60)	26.77 (25.50-28.60)	28.40 (27.20-30.10)
Wk 7	7.31(7.20-770)	8.33 (8.00-8.70)	26.42 (25.00-28.60)	28.40 (27.20-30.10)
Wk 8	7.35 (7.10-7.60)	8.38 (7.90-8.70)	26.83 (25.70-28.70)	28.46 (27.40-30.10)

Figures I and II showed the weight gain per week and survival rates per week for a period of 8 weeks. The means and ranges of physicochemical parameters of water inside plastic basin and cages for 8 weeks were shown in Table 2.

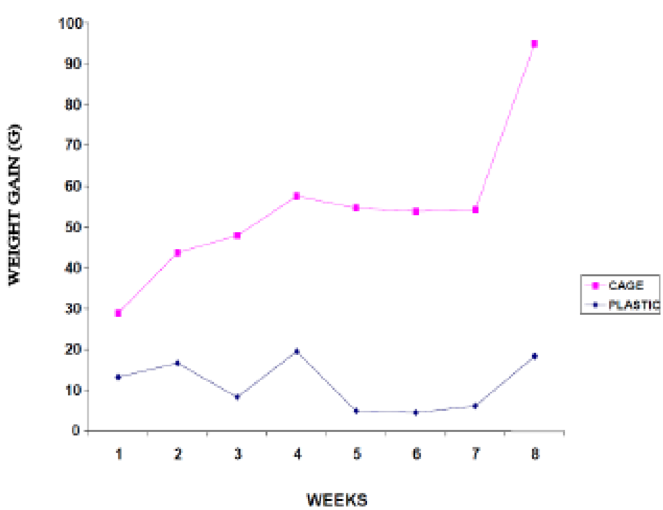

Figure 1. Weekly weight gain of *Clarias gariepinus* fingerlings in plastic basin and cages.

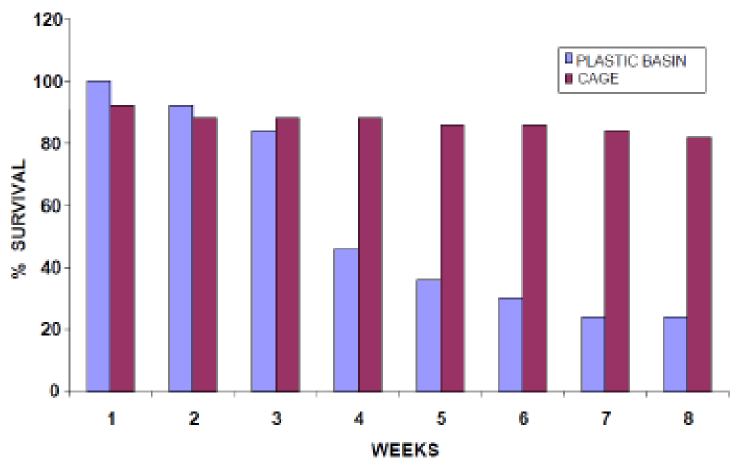

Figure 2. Percentage survival of *Clarias gariepinus* fingerlings in plastic basin and cage.

DISCUSSION

The growth response and survival of fingerlings in cages is clearly visible, compared to plastic basins. The cage had the highest survival rate of 82% which was comparable with survival for Tilapia (99%) cultured in cages by Alev and Dikel (2003) in Seyham Dam Lake. The results of this study indicate the survival ability of fingerlings reared in plastic basin and cages. The mortality rate was high in plastic basin (76%). This could be as a result of stress due to handling at short intervals which led to infection thus causing high mortality rate. It is an established fact that continuous stress affects the normal behaviour and development of fish with reduction in growth (Jobling and Reinsens, 1986) and increase in susceptibility to infection through immune-depression (Shreck, 1996). This may lead to mortality (Ajani *et al.,* 2006) as observed in plastic basins from 4-7th week in figure 2. The mortality in cage was very low (18%) and this could be attributed to the immunity derived from natural environment and less stress. The fingerlings in plastic basins had access to only to the supplementary feed given to them, while those of cages derives additional nutrient from the environment apart from the supplementary feed. This helps in the increase in growth performance of the fingerlings in the cage. Fingerlings size variations are common phenomenon during African catfish harvest in pond (Nwadukwe, 1995). This variation is known to be mainly as a result of both genetic and environmental factor (Nwadukwe, 1995). These could be the reasons for growth variation observed during this study. The recorded mean values of all the physico-chemical parameters in this study were within the acceptable range for fish growth and health (Boyd, 1979; Mazik *et al.,* 1991). Survival rate of *C. gariepinus* fingerlings in cages in this present study was higher than the range reported by Wesbster *et al.,* 2001 for juveline Sunshine bass, *Merone chrysops X M.saxotifilis* which had survival rate between 62 -75% raised in cages.

Based on the growth performance and survival rate of *Clarias gariepinus* fingerlings in this study, it is therefore suggested that rearing of *C.gariepinus* in cages is better than in plastic basins.

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THE EFFICIENCY OF ROTATING BIOLOGICAL CONTACTORS IN A CLOSED RECIRCULATING FISH CULTURE SYSTEM

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EFIKASNOST ROTACIONIH BIOLOŠKIH KONTAKTORA U ZATVORENOM RECIRKULACIJSKOM SUSTAVU ZA UZGOJ RIBE

Abstrakt

Recirkulacijska akvakultura predstavlja jednu od opcija okolišno održive i ekonomski isplative akvakulturne proizvodnje čije su beneficije jasno prepoznate. Pravilnom upotrebom različitih komponenti i tehnologija recikliranja vode, sustav omogućava kontrolu kvalitete vode u intenzivnim hranidbenim režimima. Biološka filtracija kao redukcija toksičnih dušičnih spojeva nastalih probavom proiteina jedan je od krtitičnih procesa koji određuje efikasnost cijelog proizvodnog sustava. Brojni tipovi biofiltera upotrebljavanih u različitim proizvodnim i istraživačkim pogonima razlikuju se po vrsti organizama, dizajnu, materijalu, cijeni i sl. Pri dizajniranju biofiltera trebaju biti uzeti u obzir svi kriteriji održavanja optimalne kvalitete uzgojne vode, kao i potrebni prostor te ekonomska isplativost. Rotirajući biološki kontaktor, od kojih je najčešći biodisk filter, sastoji se od okruglih ploča fiksiranih na centralnu osovinu. Diskovi se polako okreću da bi naizmjenice izložili aktivni biološki medij vodi koja sadrži otopljeni dušični otpad, i zraku kao izvoru kisika. Ovaj rad opisuje karakteristike rotirajućih bioloških kontaktora u komercijalnom recirkulacijskom sustavu i koristeći rezultate drugih autora daje usoredbu sa ostalim tipovima biofiltera. Rotirajući biološki kontaktor sa stopom nitrifikacije od 1,21 g/m^2/day pokazao se kao jedan od najefikasnijih biofiltera za upotrebu u akvakulturi.

Ključne riječi: *akvakultura, recirkulacija, biofilter, nitrifikacija.*

INTRODUCTION

Biological filtration is a critical determinant of the efficiency of a recirculating aquaculture system, and is essential to water treatment processes involving live organisms. Ammonia is the principal nitrogenous waste of fish, and results from the digestion of protein. Ammonia dissolved in the water exists as two compounds in equilibrium: ionized ammonium (NH_4^-) and unionized ammonia (NH_3). While unionized ammonia is extremely toxic to fish, the ionized portion is relatively harmless. The proportion of each is determined primarily by the pH of the water. The higher the pH, a measure of hydrogen ion (H^+) concentration, the higher is the proportion of unionized ammonia. Without significant dilution, as in recirculating aquaculture systems, ammonia must be removed by a two-step process called nitrification. Nitrifying bacteria, concentrated on the biofilter media surfaces, convert ammonia to nitrite and then to relatively harmless nitrate. Nitrate is allowed to accumulate to levels determined by the amount of dilution (defining the % recirculation rate of the recycle system). Since both ammonia and nitrite are toxic to fish, their levels must be managed through the efficient design of biofiltration systems. Biological filters must provide adequate surface area for the growth of nitrifying bacteria. *Nitrosomonas* and *Nitrosospira* convert ammonia to nitrite, and *Nitrobacter* and *Nitrospira* convert nitrite to nitrate. The water containing the dissolved waste must be brought in contact with the surface area supporting these populations of bacteria. During operation, the filter cannot be permitted to clog with fish wastes or the sloughing bacterial biomass. The filter media must therefore be self-cleaning, or involve manual or automated management technologies to remain unclogged.

Nitrite (NO_2) is the intermediate product of nitrification and the biofiltration process. Under normal operating conditions, biofiltration should maintain a balance of nitrifying bacterial populations which will control both ammonia and nitrite levels.

Biofilter design must take into account all of the stated water quality management criteria, as well as considerations of space and cost efficiency. A rotating biological contactor or biodisc filter is a fixed film bioreactor composed of circular plates aligned on a central axle. The discs are rotated slowly, to alternately expose the biologically active media to the water carrying the nutrients (the nitrogenous wastes of the fish), and to the air, providing an unlimiting source of oxygen to the bacteria.

Various mechanical designs of this biofilter configuration have been considered for recirculating aquaculture systems for decades (Lewis and Buynak, 1976). The RBC has been shown to outperform many other fixed film configurations applied to fish culture systems (Van Gorder and Fritch, 1980; Miller and Libey, 1984, 1985; Rogers and Klemetson, 1985). Wheaton *et al.* (1994) defines the inherent advantages of RBCs for aquaculture as: they are self-aerating, providing oxygen to the attached biofilm; they are a low-head device, minimizing pumping energy needs; they are a non-clogging device due to shearing of loose biofilm caused by the rotation.

The aim of this study is to describe the performance of rotating biological contactors as an integral part of a commercial closed recirculating fish production system. This paper also provides a practical comparison of RBC design and performance considerations with other biofilter options.

MATERIALS AND METHODS

The present recirculation system design includes side-by-side "cross-flow" concrete tanks, each tank measuring 30 meters long x 1.2 meters wide, with a volume of 57,000 liters. The two tanks share a central wall with a total volume of 114 m^3 of water, and are integrated with a filtration/pumping/oxygenation/ozonation system. The cross-flow tanks have influent and effluent pipe manifolds at floor level running their entire length. Perpendicular influent jets distribute water flow uniformly across a u-shaped tank floor, providing the appropriate cross-flow pattern and velocity, effecting the movement of solids to adjacent effluent ports. Each tank is divided by partitions into four increasingly large sections, each thereby receiving a proportionally increased volume of oxygenated water (Van Gorder and Jug-Dujaković, 1996). Water quality is maintained by integrated skid-mounted unit processes for the recirculation of the tank volume. Water returning from the tanks is mechanically filtered through a microscreen drum clarifier (60 micron). The flow then enters rotating biological contactors providing the required biofiltration (Van Gorder and Jug-Dujaković, 2005). A pump distributes the water to a carbon dioxide sparging chamber, and under pressure to an oxygen/ozone saturator, from which supersaturated levels of dissolved oxygen and ozone are distributed to the culture tanks. A chemical feed pump maintains pH, using NaOH dosing.

Computer telemetry systems monitor electrical status, flow rates, and temperature, while controlling feeding, clarifier function, emergency oxygen activation, pH, and oxygen and ozone flow. Computer control functions also include emergency response and notification for disruption or variance in flow rate, dissolved oxygen levels, pH and temperature. For this study, eight separate grow-out systems, using 16 RBCs, were studied, each system employing the RBC model described above in the table1.

Table 1. Description of the characteristics and dimensions of RBCs used in the study.

		Biofilter Specifications		
Tank Design	System Volume (liters)	Total Surface Area (m^2)	Total Specific Surface Area (m^2/m^3)	Total Flow Rate (liters/min)
Cross-Flow Raceways (8 systems)	115,000 (2 tanks/system)	1860 (2 RBC's)	258 (2 RBC's)	1900

For the culture systems observed in this study, the flow rates through the system components permit the tank water volumes to be circulated through the biofilters in an average of 55 minutes. The system was fed the same feed (40% protein, 16% fat) which was automatically administered several times daily over a sixteen hour light cycle. A theoretical level of TAN production is estimated as a function of the feeding levels. Biofilter efficiency is measured as a function of the removal of that ammonia, thus establishing a steady state TAN concentration within the culture tanks. The daily replacement of 5% of the water as a function of the recirculation % of the system, was also considered in the removal of ammonia.

Over a five-week period, the average level of feed per day was determined for each of eight production systems. This level of feeding was mathematically converted to levels of ammonia produced. Using Wheaton *et al* (1994), an ammonia production rate of 0.03 kg TAN/kg feed is assigned, and represents the mass of ammonia that must be removed by biofiltration and dilution, in order to maintain equilibrium.

RESULTS AND DISCUSSION

Table 2 lists the average feed levels administered to eight separate culture systems over a 5-week period, and the average levels of TAN produced by the fish. With a steady-state situation, the levels of TAN produced, less than 5% removed through water exchange, is assumed to indicate the levels of TAN removed by biofiltration.

Table 2. Average feed amount administered to eight separate systems and the average TAN removal rate for each system

	Average feed amount/tank/day kg	Average TAN removed/m^2/day kg
Tank		
1	33,60	1,19
2	31,16	1,15
3	35,04	1,27
4	38,80	1,17
5	29,60	1,08
6	30,92	1,14
7	35,42	1,29
8	30,62	1,12

Weekly average TAN removal rate (g/m^2/day) 1,21

A comparison of RBC design and performance considerations with other biofilter options was accomplished using data available from different authors (table 3).

Table 3. Comparative nitrification capacity for various types of biofilters:

Source	Ammonia removal rate
Submerged Filters (Wheaton *et al.,* 1994)	0.3-0.6 gms/m^2-day
Bead Filters (Wheaton *et al.,* 1994)	0.20-0.25 gms/m^2-day
Fluidized Sand Filters (Thomasson, 1991)	0.25-0.35 gms/m^2-day
Rotating Biological Contactor (this study)	1.21 gms/m^2-day

For fine media biofilters such as fluidized sand or bead filters, volumetric comparisons of nitrification efficiency are often used. By volume, this RBC, with 258 m^2/m^3, demonstrates a nitrification rate of 312 gms/m^3-day. Tsukuda *et al.* (1997) estimate nitrification rates for cold water fluidized sand filters at 150-410 gms/m^3-day. *Malone et al* (1993), citing data from Thomasson (1991) and Monaghan *et al.* (1996), reported ammonia removal rates of 630-800 gms/m^3-day in water.

Rotating Biological Contactors have been demonstrated to be one of the most efficient and robust biofilters available for nitrification of aquaculture wastes. They demonstrate extremely high nitrification rates, while providing additional qualifications for self-aeration, off-gassing, and low-head operation. An ammonia removal rate of 1.2 g/m^2-day surpasses all other biofilter configurations cited. With a volumetric nitrification rate of 312 g/m^3-day, comparisons to fluidized sand filters demonstrate a nearly equal volumetric nitrification rate and significant superiority in energy efficiency, ease of management, and reliability. Considering this, in addition to the positive considerations that have always been attributed to this biofilter, the RBC provides a reliable and effective alternative for consideration in commercial recirculating aquaculture systems.

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IN VITRO MODEL SYSTEMS; A VALUABLE TOOL TO STUDY BASIC BIOLOGICAL PROCESSES IN FISH

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KORIŠĆENJE IN VITRO MODEL SISTEMA ZA PROUČAVANJE OSNOVNIH BIOLOŠKIH PROCESA KOD RIBA

Abstract

With the increasing replacement of fish meal and fish oil with new ingredients in aquaculture diets, imbalances in amino acids, fatty acids, vitamins and minerals can occur. The metabolic and regulatory processes underlying these nutrition-induced imbalances in fish are still not fully understood. At the cellular level, essential dietary compounds and micro-nutrients have been shown to influence lineage determination, differentiation and proliferation of certain cell types, and hence the development of tissue structures and organogenesis. An improved understanding of cellular and molecular events occurring during development in teleosts will enable us to better characterize and define particular requirements, customize feed components, and thus enable development of sustainable feeds, minimize the occurrence of disorders as well as maintain the continuous growth of the fish. *In vitro* techniques have a great potential in experiments involving mechanism based hypothesis testing, where there is a significant need for a complete understanding of basic biological processes. Cell cultures provide means to study single-factor effects and the combinations thereof in detail, and further, to investigate the role of particular nutrients and their specific gene interactions, which are not possible when working at the organism level. In addition, whole organisms are complex and vary individually, depending on age, sex, health status, type of meal, genetics etc., which makes it difficult to accurately simulate nutritional processes. *In vitro* experiments offer the unique opportunity to develop standardized methods to study quality of novel and fortified feed products by studying the cellular and molecular effect of different types of food products, ranging from proteins to n-3 fatty acids (FAs) and from fat-soluble vitamins to minerals and trace elements. The system may also be used for studies on the development of functional feeds such as probiotics, prebiotics, bioactive peptides, lipase inhibitors, fat and cholesterol binders and antioxidants. Results from selected *in vitro* trials, showing how different nutrients may influence development of fat, bone and muscle cells and lipid metabolism in liver cells, will be presented.

Key words: *fish, development, cell culture, nutrients*

KORIŠĆENJE IN VITRO MODEL SISTEMA ZA PROUČAVANJE OSNOVNIH BIOLOŠKIH PROCESA KOD RIBA

Abstrakt

Zamena ribljeg brašna i ulja sa novim, alternativnim izvorima proteina i masti u hrani za ribe može dovesti do narušavanja odnosa amino kiselina, masnih kiselina i minerala kod ovih organizama. Promene u metaboličkim i regulatornim procesima koje mogu da nastanu loše balansiranom ishranom još uvek nisu dobro proučene kod riba. Esencijalne hranljive materije i mikronutrijenti mogu da utiču na razvoj pojedinih ćelijskih linija, njihovu proliferaciju, a samim tim i na pravilan razvoj tkiva i organa. Bolje razumevanje ćelijskih i molekularnih procesa, koji se dešavaju tokom razvića košljoriba će nam omogućiti da bolje razumemo i definišemo određene zahteve za ishranom na svakom stupnju razvoja riba, prilagodimo komponente riblje hrane, a samim tim i omogućimo normalan razvoj i rast riba, kao i da sprečimo pojavu različitih poremećaja. Korišćenje *in vitro* sistema predstavlja veliki potencijal za testiranje novih komponenata hrane i daje nam mogućnost za razumevanje osnovnih bioloških procesa kod riba. Prednost korišćenja ćelijskih kultura je što nam one omogućavaju da proučavamo uticaj pojedinačnih faktora, ali i kombinaciju dva ili više različitih faktora i njihov uticaj na gene, što nije moguće istraživati na nivou organizma. Pored toga proučavanje organizma kao celine je jako složeno, jer zavisi od pola, zdravstvenog stanja jedinke, ishrane, genetike, itd., te je na ovaj način lakše simulirati procese ishrane. *In vitro* eksperimenti nude jedinstvenu priliku da razvijemo standardizovane metode za proučavanje kvaliteta i efekata novih hranljivih materija: od proteina, preko masnih kiselina i minerala, do elemenata, koji se u hrani nalaze u tragovima. Ovi sistemi mogu se koristiti i za proučavanje novih, funkcionalnih hraniva, kao što su: probiotici, prebiotici, bioaktivni peptidi, masti, inhibitori lipaze, antioksidansi. Biće prikazani rezultati odabranih *in vitro* ispitivanja uticaja različitih hraniva na metabolizam masnog tkiva, kostiju i mišića kao i na sam metabolizam masti.

Ključne reči: *riba, razvoj, ćelijska kultura, hranljive materije*

THE EFFECT OF THREE DIFFERENT DIETS ON GROWTH AND SURVIVAL OF PERSIAN STURGEON (*ACIPENSER PERSICUS*) LARVAE

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EFEKAT TRI RAZLIČITE HRANE NA RAST I PREŽIVLJAVANJE LARVI PERSIJSKE JESETRE (*ACIPENSER PERSICUS*)

Abstract

A feeding experiment was conducted to evaluate growth and survival of Persian sturgeon larvae fed with live food (*Nereis diversicolor* and *Daphnia* spp.) and artificial diet. Diets were included: Diet 1: Dafnia (*Dafnia magna*), Diet 2: *Nereis diversicolor* worm; Diet 3: Mix of Daphnia (*Daphnia spp.*) (50%) and *Nereis diversicolor* (50%), Diet 4: Mix of *Nereis diversicolor* (50%) and concentrate food (50%) and *Nereis diversicolor* (50%); Diet 5: Mix of *Daphnia spp.* (33.33%) and *Nereis diversicolor* (33.33%) and concentrate food (33.33%). Persian sturgeon larvae were distinctly transferred to 15 tanks and fed for 15 days. For each treatment, 60 larvae were stocked into tanks. The total length and body weight of the fish were determined once before initiation of the experiment and at the end of the experimental period to assess their growth performance. Water quality parameters were recorded two times a day**.** There was significant difference ($P<0.05$) in survival of Persian sturgeon larvae fed diets 2 and 3 and the other diets. Significant differences ($P>0.05$) were not found between diets 2 and 3 and also between diets 4 and 5.

The value for BWI, PBWI, GR, DGI, SGR was higher in larvae fed mix of *Nereis diversicolor* and *Daphnia spp.* (diet 3) ($p<0.05$) and there was significant difference between all of groups. Except diet 3 there was no significant difference in the CF of fish fed the survey diets.

Key word**:** *Growth, Survival, Acipenser persicus, Nereis diversicolor, Daphnia spp., Diets*

INTRODUCTION

Rearing of sturgeon larvae has received increasing attention in recent years (Buddington and Doroshov, 1984), due mostly to the fact that in several countries the wild stocks of these migratory fish are severely depleted or in danger of disappearance.. Earlier Information on nutrition and feeding is scarce for most sturgeon species, being limited and deal mainly with Atlantic sturgeon (*Acipenser axyrhynchus*) (Kelly and Arnold, 1999), lake sturgeon (*A. fluvescense*) (Dilauro et al, 1998), Adriatic sturgeon (*A. naccrii*) (Randall et al, 1992), and also for Persian sturgeon (Ebrahimi and Zare, 2006) most information is generated from nutrition studies conducted on white and Siberian sturgeon, but this information is still incomplete (Webster and Lim, 2002). Some larval feeding studies have comparing growth performance of larvae fed different live organisms with those fed dry or semi moist feeds (Lutes et al., 1990).

The use of live food in aquaculture in feeding commercially important fishes such as sturgeons and salmons has gained considerable importance as compared to formulated diets, whereby at present the main food in early stages of sturgeon larvae culture is comprised of live food such as *Artemia, Daphnia*, and Oligochaeta worms and the other foods such as *Nereis diversicolor* were not applied. The study of other live food including *Nereis* is an important objective of aquaculture.

Nereis diversicolor belongs to phylum Annelid and class polychaeta, which can be used as a live food in aquaculture, in the world.

The rag worm *Nereis diversicolor* (O.F. Muller, 1776) is typically an inhabitant of estuarine mud flats in Europe where it is one of the commonest of all shore polycaetes (Chambers and Milne, 1975; Heip and Herman, 1979 ; Mettam, 1979; Olive and Garwood, 1981; Fidalgo e Costa, 1994; Fidalgo e Costa and Cancela da Fonseca, 1995).

The quest for these species is increasing rapidly, mostly due to their important role as a nutrient stimulating gonad maturation and spawning in hatchery-reared species, e.g. *Solea senegalensis* (Dinis, 1986), *Penaeus kerathurus* (Luis, 1989) and *Penaeus vannani* (Lytle and Ogle, 1990).

MATERIAL AND METHOD

After hatching, 900 larvae of Persian sturgeon (*Acipenser persicus*) were transported to the laboratory (international sturgeon research institute) obtained in Shahid Beheshtie Sturgeon Hatchery Center, Rasht, Iran. They were placed into fifteen 60-L fiberglass tanks, (60 cm diameter ; water depth 25cm ; 3 tanks per diet ; 3 replicates per treatment) and were carried out the experiments for 15 days. For each treatment, 60 larvae were stocked into tanks. Water quality parameters were recorded two times a day (morning and afternoon). The water temperature, dissolved oxygen (DO), pH and flow rate were 22-23°C, 6-6.8 mg/L, 7-7.45 and 2 L/min. The 15-tanks system was supplied with change of water continuously. Larvae were sampled randomly before initiation of the experiment and at the end of the experimental period. The total length and body weight of the fish were determined once before initiation of the experiment and at the end of the experimental period to assess their growth performance. Data represent means and standard error from 3 replicates with initially 60 individuals stocked per tank. Diets were included: Diet 1: Dafnia (*Dafnia magna*), Diet 2: *Nereis diversicolor* worm ; Diet 3: Mix of Daphnia (*Daphnia spp.*) (50%) and *Nereis diversicolor* (50%), Diet 4: Mix of *Nereis diversicolor* (50%) and concentrate food (50%) and *Nereis diversicolor* (50%);

Diet 5: Mix of *Daphnia spp.* (33.33%) and *Nereis diversicolor* (33.33%) and concentrate food (33.33%).

In this study, Live *Daphnia* spp. as a control diet (diet 1) and cut *Nereis* worm was cut *Nereis diversicolor* worm cultured in laboratory. concentrate food was a sturgeon larval diet which has been used with success for sturgeon starter diet. Larvae were fed five times daily based on 30% larvae body weigh. After 15 d, 30 individuals from each tanks. Wet weight of larvae was measured by using a laboratory scale (0.01 mg). The Condition factor CF=W(g)/L(cm)^3)×100) and specific growth rate (SGR) was calculated using the following formula; SGR (%day^{-1})=100×(lnWt-lnWo)/t where Wt and Wo represent final and initial mean body weights and t is the growing period in days (Gisbert and williot, 1997). Total length (TL) was defined to be the distance from the tip of the snout to end of the upper lobe of the tail. Food conversion ratio (FCR) was consulated: FCR= Total feed intake (kg)/weight gain (kg). Dead larvae were counted daily, and the number of larvae survival at the end of the experiment was expressed as a percentage of the initial number. Percent mortality was determined by hand-counting all dead fish at the periods.

The effects of the different feeding sequences on growth and nutrient utilization parameters (SGR, CF) and mortality between experimental tanks on different periods were examined by a one-way analysis of variance (ANOVA) (Zar, 1974).

RESULTS

Differences between the means were compared by Duncan test at a 95% confidence interval at the end of the feeding trials using SPSS software.

Significant differences in final weights of larvae were found amongst the experimental tanks. There was significant difference (P<0.05) in survival of Persian sturgeon larvae fed diets 2 and 3 and the other diets. Significant differences (P>0.05) were not found between diets 2 and 3. Also significant differences (P>0.05) were not found between diets 4 and 5 (Fig 1).

A comparison of mean weights shows that fish fed mix of *Nereis diversicolor* and *Daphnia spp.* (diet 3) had a significantly (P<0.05) higher weight than the other diets and significant differences (P>0.05) were not found between diets 2 and 3 (table 1). In diet 3 treatment, the mean final weight of larvae was 282.21±4.16 mg, while in the trial fed mix of three diets (diet 5) this value was 210.83±1.56 mg (table 1). The value for BWI, PBWI, GR, DGI, SGR was also higher in larvae fed mix of *Nereis diversicolor* and *Daphnia spp.* (diet 3) (p<0.05) and there was significant difference between all of groups. Except diet 3 there was no significant difference in the CF of fish fed the survey diets (Fig3).

There was significant difference (P<0.05) in FCR of Persian sturgeon larvae fed diets 2 and 3 and the other diets. Significant differences (P>0.05) were not found between diets 2 and 3. Also significant differences (P>0.05) were not found between diets 1,4 and 5 (Table 1).

Table 1. Effects of different diets on growth and survival of Persian sturgeon larvae for a period of 15 days.

	Diet 1	*Diet 2*	*Diet 3*	*Diet 4*	*Diet 5*
Initial wt (mg)	95.66±0.97 a	95.66±0.97 a	95.66±0.97 a	95.66±0.97 a	95.66±0.97 a
Final wt (mg)	239.75±0.86 c	278.82±3.13 d	282.21±4.16 d	223.87±3.81 b	210.83±1.56 a
BWI	144.08±1.12 c	183.16±3.69 d	186.54±3.98 d	128.20±3.98 b	115.17±1.05 a
PBWI	150.63±1.89 c	191.51±4.97 d	194.99±4.04 d	134.04±4.53 b	120.38±0.62 a
GR	9.60±0.07 c	12.21±0.42 d	12.43±0.26 d	8.54±0.26 b	7.67±0.12 a
DGI	10.63±0.08 c	12.71±0.37 d	12.88±0.19 d	9.72±0.24 b	8.94±0.04 a
FCR	9.53±0.45 b	5.80±0.02 a	5.94±0.24 a	9.36±0.40 b	10.15±1.06 b

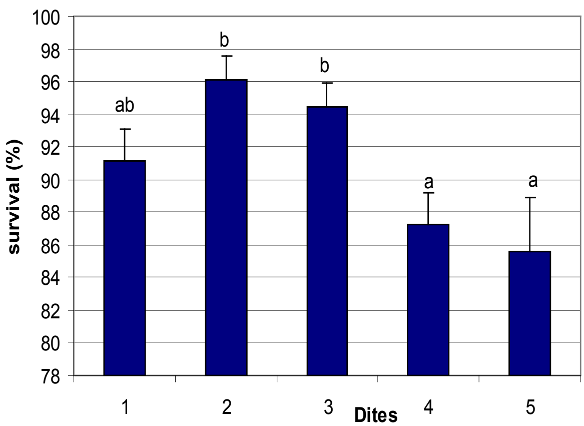

Figure 1. Effects of different diets on survival of Persian sturgeon larvae

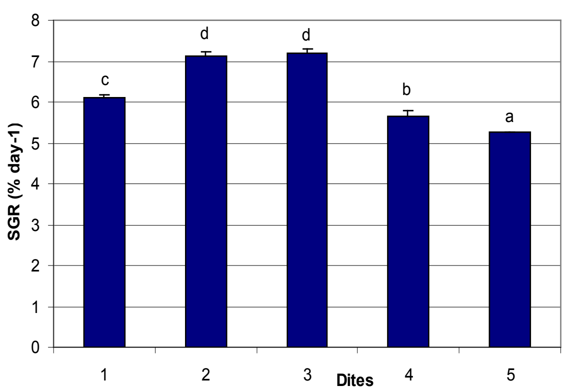

Figure 2. Effects of different diets on SGR of Persian sturgeon larvae

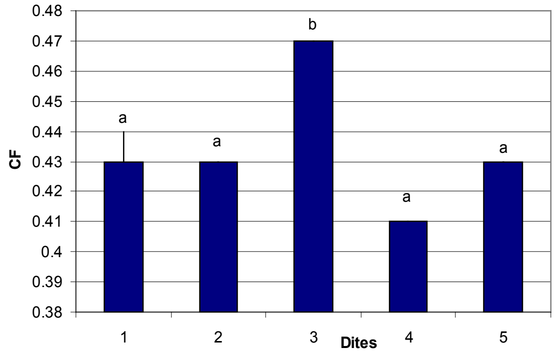

Figure 3. Effects of different diets on CF of Persian sturgeon larvae

DISCUSSION

Modern aquaculture is in need to improve the quality of the prepared fish feeds in particular for early life cycle stages, including start feeding. This problem is specially pertinent for sturgeons with due attention to an appropriate nutrient balance (Kasumyan et al., 1995).

Generally, our results indicate that Persian sturgeon larvae can be successfully weaned on prepared diets but loss in growth efficiency when compared to live food. Previous studies showed that the digestive system development of Persian sturgeon fingerling comes close to completion when reaching about 2.5 g (Soodakova, 1997).

Traditionally, hatchery-produced sturgeon larvae and fingerlings have been raised on live food organisms e.g. oligochaetes (*Enchytraeus* sp. and Tubifex sp.) and zooplanktonic organisms, such as *Daphnia* (*Daphnia* sp. and *Moina* sp.) or *Artemia salina* (Gisbert and Williot, 2002). Dabrowski et al. (1985) demonstrated that artificial larval diets could be used successfully for intensive commercial culture of several sturgeon species from the onset of exogenous feeding. These results are similar to those reported for *A. transmontanus* (Hung, 1991). An ideal larval sturgeon feed should be consisted of small (0.5- 1.4 mm diameter) soft pellets that sink rapidly to the bottom of a rearing tank and can be easily detected and ingested by larvae (Gisbert and Williot, 2002).

Laboratory experiments have demonstrated that food odour stimulates feeding responses in sturgeon larvae and juvenile (Kasumyan, 1999). As shown by Webster et al. (1991) who started feeding prepared diets after 17 days post-hatch in paddlefish and studies by Buddington and Doroshove (1984) comparing dry diets again *Tubifex*, we find in the literature similar results.

Mohler (2000) found that 26 days after feeding, the mean SGR in live *Artemia* feeds ranged between 4.9-11.1% per day. In our study, the mean SGR in live *Nereis* and Daphnia feeds was ranged between 5.26-7.21%.

Nathanailides et al. (2002) reported that on the third week after hatching, there were large and small size fish and that it was not possible to continue feeding all fish with the same size of dry feed diets. Furthermore, competition between the larger and smaller fish for any food supplied was obvious and too great to be ignored. In this study, we found in the literature similar results.

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COMMERCIAL FISHERIES ON DANUBE IN SERBIA

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PRIVREDNI RIBOLOV NA DUNAVU U SRBIJI

Abstrakt

U radu se daje pregled slatkovodnog ribarstva sa aspekta upravljanja, ekspolatacije ribljih resursa, tržišta ribom, legislative koja uređuje delatnost ribarstva, kao i problemi sektora ribarstva u Srbiji. Istraživanje i analiza privrednog ribolova na Dunavu kroz Srbiju (588 km toka) sprovedeno je prikupljanjem podataka ribarske statistike za period od 1948. do 2010. godine, njihovim unošenjem u elektronsku bazu i analiziranjem uz korišćenje različitih metoda. Statistički podaci uključuju ukupan ulov, ulov rekreativnog i privrednog ribolova, ulov privredno značajnih vrsta i alohtonih vrsta. Korišćenjem anketnog upitnika za ribare dobijeni su podaci o demografskoj strukturi ribara koji se bave ribarenjem kao osnovnom delatnošću, o sastavu ulova, koliko je ribarstvo perspektivna privredna grana i koji su problemi i potencijalna rešenja.

Većina ribara pripada starosnoj grupi koja je u opsegu od 45 do 50 godina. Privredni ribolov je važna ekonomska delatnost i predstavlja osnovni izvor prihoda za većinu ribara. Tržište slatkovodnom ribom je neuređena oblast koja zavisi od ličnog zalaganja i odgovornosti pojedinca koji ima interes u prodaji ribe. Ribarstvo na Dunavu u Srbiji je već dugi niz godina u procesu tranzicije ka tržišnoj ekonomiji.

Dugoročne fluktuacije hidroloških podataka (vodostaj) upoređivane su sa fluktuacijama godišnjeg ulova ribe iz Dunava kako bi se uočila eventualna međusobna zavisnost i uporedili njihovi trendovi.

Rezultati ovog istraživanja treba da posluže kao pregled stanja sektora sa svim postojećim nedostacima koje treba ispraviti u cilju što uspešnijeg upravljanja ribarstvom na Dunavu i ostalim rekama u Srbiji gde je zastupljen privredni ribolov.

Ključne reči: *ribarstvo, Dunav, ulov, hidrologija, upravljanje resursom*

INTRODUCTION

Fishery has a long tradition in Serbia, which is ingrained, expected, fixed activity of local population beside the rivers (Čaldarović, 2006). Irrational and disorganized utilization lead to the biological degradation and could disable the use of these resources in the form in which it existed prior to change (Simonović, 2005), which significantly threatens the economic function of resources. Commercial fishing in Serbia is allowed only on the Danube, Sava and Tisa Rivers, but there is the tendency to stream it to the Danube. Fishing waters in Serbia organizationally are divided in 6 areas (before 2007 there were 25 fishing areas), which are given to assigned users by the competition under defined conditions and legal obligations. Each fishing area is regulated by the type of fishing that can be performed, recreational and commercial, or only recreational. Fishing areas management is given to the public or private companies, fishing associations and fishing unions.

First statistic yearbook which included Yugoslav freshwater and marine fishery was published in 1953. Average annual freshwater catch in the years 1948 - 1952 in Serbia was 2284 t. Categorized fish species were common carp (*Cyprinus carpio*), wels (*Silurus glanis*), pike-perch (*Sander lucioperca*), sterlet (*Acipenser ruthenus*), northern pike (*Esox lucius*), nase (*Chondostoma nasus*), rudd (*Scardinius erythrophtalmus*), bream (*Abramis brama*) and second quality fish species as a separate group. According to findings restocking of the open waters have been done in the 1956, 1957. There were 1240 full time professional fishermen and 1023 half time fishermen. Statistics included market data like the quantities of fresh fish sold, processed, exported distribution of remaining stock of fish, and income from fishery. In 1969 there were 33 reporting units which collected information about fishery statistics which was submitted to the Statistical Office. Fish species included in the commercial catch statistics has been changed, some species were removed, but some introduced, alien species appeared. The list contained: danubian sturgeon (*Acipenser gueldenstaedtii*), stellate sturgeon (*Acipenser stellatus*), beluga (*Huso huso*), sterlet, common carp, bream, pike-perch, wels, asp (*Aspius aspius*) and second quality fish species. Alien species, prussian carp (*Carassius gibelio*), bighead carp (*Hypophthalmichthys nobilis*), silver carp (*Hypophthalmichthys molitrix*) first were noticed in the 1977 statistics data.

MATERIAL AND METHODS

Collecting of catch statistics implied importing of the basic historical data from the books of the catch statistics archived in the Statistical Office of the Serbia. From 2006 exists the full database about the catch of each fish species, number of recreational and commercial fishermen, and separated catch realized by each group. Introductory activities comprised field survey and interviews with commercial fishermen and employers in the fishmongers. 109 commercial fishermen found in 2010 and 2011 in the field were interviewed using questioners, in order to estimate the activity, intensity and mode of fish resources utilization in the River Danube and possible consequences on their abundance and structure. In addition, 35 fishmongers were interviewed. Acquired records were obtained by extraction of answers of interviewed fishermen and fishmongers processed using Excell and Statistica 5. analitical packages.

RESULTS AND DISCUSSION

Total catch statistics according to published data in the period between 1948 to 2010 is presented in Figure 1.

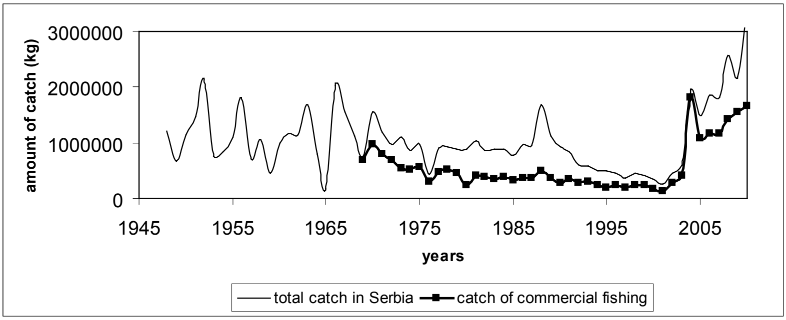

Figure 1. Trend in total fish catch in Serbia and catch of commercial fishing

The great oscillations characterize total catch in Serbia between 1948 and 1969. Reason for these oscillations may be natural fluctuations, but also insufficient data in some years. From 1969 commercial fishing is separately recorded from recreational. There is a clear follow-up trend and integration of both. 1990s decline in the catch may be, to some extent, the consequence of the political situation which led to a lack of statistical data. Poor catch statistics lasted until 2003. Since 2005, data collection methodology was changed, and recent reports showed bigger catches. Unfortunately, in the last 10 years fishermen were not obliged by the law to report their catches, so the statistical data are not quite reliable. Figure 2 presents species in the catch of commercial fishermen from 1969 – 2003. Second quality fish was removed because it substantially exceeds the average value of the catch of other species.

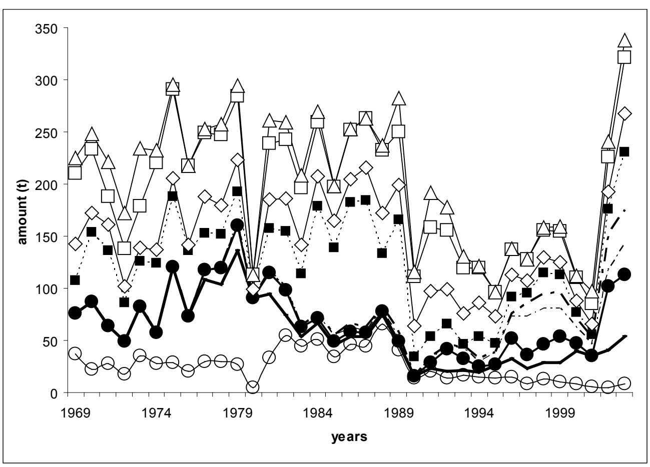

Figure 2. Commercial catch 1969-2003, Δ asp, □ wells, ◊ pike perch, - - ■ - - bream, – – bighead carp, – —– — silver carp, ▬ common carp, ● prussian carp, ○ sterlet

Statistics data for 2005 are missing, due to methodology changing, and the new catch data started from 2006. The increasing trend of second quality fish and alien species in the Danube catch in the last 5 years was perceived (Fig. 3). These species became common demand not only because of low prices but also because abundance and tasty meat.

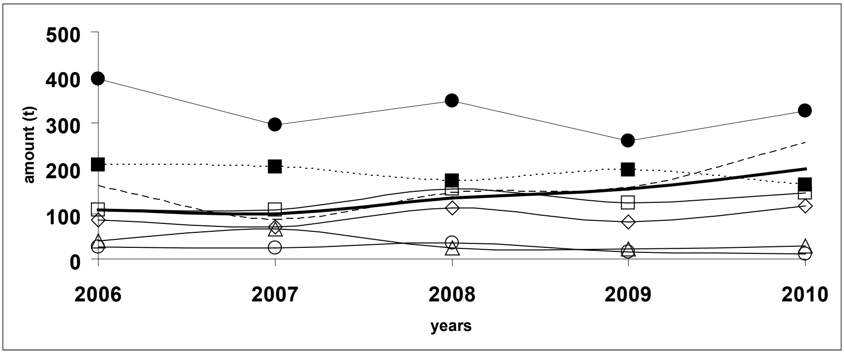

Figure 3. Commercial catch from 2006-2010, Δ asp, □ wells, ◊ pike perch, - - ■ - - bream, – – bighead carp, ▬ common carp, ● prussian carp, ○ sterlet

Data on total annual catch were compared with annual fluctuations of Danube water level. Analyses of the regression between Danube water level and total annual catch for the entire period of investigation (1948 – 2009) did not show significant relationship ($r = 0.172$; $P = 0.18$). On the other hand, when only data for the period before Đerdap dam construction (1948 -1975) were analyzed (Fig. 4), highly significant relationship between water level and catch fluctuations was found ($r = 0.468$; $P < 0.01$). The reason could be both impact of dam and unreliable statistics of the catch after 1990s. Regarding statistics, before 1990s almost entire catch has been redeemed by the authorized public companies for reasonable price in accordance with the price of fishing license, and the fishermen had an interest to give total catch to purchase. However, significant correlation indicates that statistical data for that period were reliable.

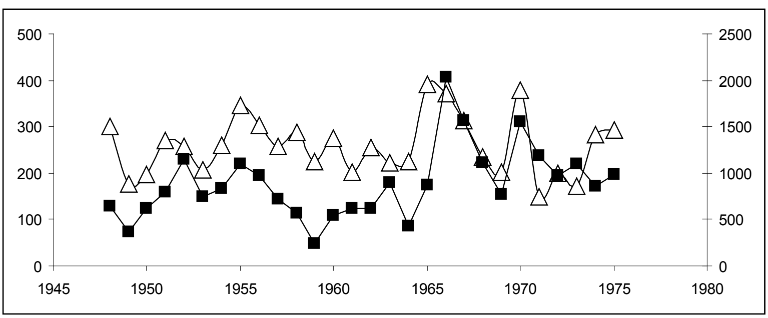

Figure 4. Δ Total catch (t) and ■ Danube water level (cm)

Number of commercial fishermen in Serbia is decreasing. In the period 1995-2004 numbers of registered commercial fishermen per year was around 1200 and more, but in the last years number falls rapidly. Number of registered commercial fishermen on Danube in 2010 was 511. By the establishment of the new law of the conservation and sustainable use of fish stocks in 2011 number of commercial fishermen has dropped even more, mostly because very expensive permit which has to be paid at the beginning of the season.

Regarding demography features of the commercial fishermen, majority of fishermen belong to the age group between 45 and 50.They are mostly local citizens of the towns and villages on the banks of the River Danube. Fishing is the only source of income for them. They sell their catch in different ways: to personal customers, fishmongers, restaurants, markets. Fishing market is more or less out of control and depends on the personal responsibility. Purchase of fish and processing of fish products doesn't exist almost 20 years. There are just individuals who produce small quantities for personal use.

Commercial fisheries in Europe show a downward trend. This is result of many factors: increasing operational costs which lead to decreased profitability, increasing uses of water, pollutions, habitat degradations, aspiring to protection and conservation character, increasing international trade, aquaculture (Pintér, 2004).

Analyzing commercial fisheries in the Serbia, especially on the River Danube we noticed some of the general problems of the fisheries sector in Serbia. Due to the shift from centralized to market economy, there has been more expressed the development of the gray fish market (Lenhardt *et al.,* 2006). The main problems are poor fishery resource management and lack of organized purchase of the catch, namely the complete absence of buying fish caught. Thus, the fishermen were forced to self-navigate in the sale. This lead to rise of the gray fish market; and on the other hand, fishery statistics has become unreliable.

Poaching, the high cost permits for commercial fishing versus prices for sport fishing, lack of organized purchase of fish and additional problem negatively affect the market. Measures that could be applied to overcome these problems are regulation and control of fishing intensity, as well as economic support and incentives of individuals in the organization of fishing and selling. Some countries organized fisheries in the way to support a commercial harvest that is exported and marketed away. But for quantities of fish consumed locally, economic importance is harder to measure.

ACKNOWLEDGEMENTS

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Fisheries, Fresh-Water – dam, river, sea, freshwater, effects, important, largest, system, plan http://www.waterencyclopedia.com/En-Ge/Fisheries-Fresh-Water.html

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THE RECENT STATE OF DISTRIBUTION OF ENDEMIC FISH SPECIES IN EASTERN HERZEGOVINA

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SADAŠNJE STANJE RASPROSTRANJENJA ENDEMIČNIH VRSTA RIBA ISTOČNE HERCEGOVINE

Apstrakt

Sadašnje stanje rasprostranjenja endemičnih vrsta riba Istočne Hercegovine. U radu je dat pregled istraživanja endemičnih vrsta riba na području Istočne Hercegovine na više lokaliteta. Prema ranijoj sistematici gaovice sa ovog područja su bile predstavljene vrstama: *Phoxynellus metohiensis, Phoxynellus pstrossi* i *Phoxynellus ghetaldii*, dok novija sistematika gaovice sa ovog područja svrstava u dvije vrste: *Telestes metohiensis* i *Delminichthys ghetaldii*. Pored najnovije promjene u sistematskom polažaju gaovica i u ranijem periodu bilo je promjena u nazivu roda. Tako je prvobitni naziv za gaovice bio je *Paraphoxinus*, potom *Phoxinellus*, a prema najnovijoj sistematici gatačka gaovica nosi naziv *Telestes metohiensis*, dok su trebinjska i popovska gaovica svrstane u rod *Delminichthys* i označene jednom vrstom *Delminichthys ghetaldii*. Naša istraživanja gaovice na području Istočne Hercegovine obuhvatala su više lokaliteta: Bilećko jezero, rijeke Mušnica, Vrijeka, Opačica, te Suški potok, Ljubomirski potok i Fatničko polje. U okviru istraživanja praćene su i određene ekofiziološke karakteristike gaovice, a u ovom radu dati su rezultati istraživanja o prisustvu ovih vrsta u pojedinim vodama, s obzirom da se u literaturi susreću različiti podaci o njihovoj rasprostranjenosti i prisutvu u određenim vodama. Neka ranija istraživanja navode da je po biomasi gaovica bila najzastupljenija vrsta Popova polja i da je imala veliki značaj za svakodnevni život ljudi. U okviru naših istraživanja praćeno je prisustvo gaovica na navedenim lokalitetima. Istraživanjem je konstatovano da nema pravilnosti u vremenu pojavljivanja i prisustvu određene na vrste na datom lokalitetu, što je vjerovatno uslovljeno vodnim režimom u ovom području i specifičnostima ekoloških uslova na malom prostoru. Rezultati pokazuju da je gatačka gaovica u značajnijem broju zastupljena u rijeci Vrijeci, Bilećkom jezeru, Suškom potoku i rijekama Mušnici i Opačici, dok je trebinjska gaovica konstatovana u Fatničkom polju nakon izlijevanja vode i Ljubomirskom potoku.

Ključne riječi: *gaovice, rasprostranjenje, vode*

INTRODUCTION

Fish, the most numerous group of Vertebrates, have significant role in aquatic environment. They are indispensable part of all biological variety and one of the best indicators of ecological state of aquatic habitats. Also, they are irreplaceable link in aquatic ecosystems food chains, but in the same time a link to terrestrial ecosystems food chains so, in that way, they are so valuable for humans (Jelić et al., 2008). Special group of them represent endemic fish species that usually have small and limited range of distribution, and sometimes they are found just in some specific regions. These species, in the same time, represent a specific genetic resource. Their research is very significant, because with adequate knowledge it is simpler to preserve them. Official scientific data about freshwater endemic fish species in Bosnia and Herzegovina were given at the end of the 90-is in several publications by Steindachner (Kähsbauer, 1959). More intense researches dating back to 1951 (Taler, 1951), and in the later period Sofradzija i Berberovic (1972) researched kariotype of freshwater fishes, and Ivanc et al. (1981; 1989; 1989a) their biochemistry and physiology. These researches are specially significant in edemic species studies, because with it a new perspectives were brought in to percieve their taxonomy.

The earlier used systematics is about species: striped pijor (*Paraphoxinus metohiensis,* Steindachner, 1901), trebinje minnow (*Paraphoxinus pstrossi* Steindachner, 1882) i popovo minow (*Paraphoxinus ghetaldii* Steindachner, 1882), that are then classified in order *Phoxinellus*, and had scientific names *Phoxynellus metohiensis* (Steindachner, 1901)*, Phoxynellus pstrossi* (Steindachner, 1882)*, Phoxynellus ghetaldii* (Steindachner, 1882).

Recent systematics classify minnows from this area in two separate orders. *Telestes*, with species *Telestes metohiensis* – striped pijor and *Delminichthys*, with species *Delminichthys ghetaldii* – trebinje and popovo minnow (Freyhof et al, 2006; Kottelat and Freyhof, 2007). It is sginificant that Freyhof and al. (2006), based on morphometric researches done by Zupančić and Bogutskaya (2003) and theirs own molecural-genetics research come to conclusion that separation of trebinje and popovo minnow in different species is not justified and that they represent just one species.

Minnows used to be the most numerous species in fields Dabarsko, Fatničko and Popovo by its biomass and also in freshwater in the area. They had big significance for daily lives of people (Glamuzina et al, 2007) and at the beginning was associated with underground fauna (Ćurčić 1913; Absolon 1916; Glamuzina et al, 2007).

Untill the hydropower system on Trebišnjica was done, minnows had significant economic values. In recent time significance of this species is very small, but its biological value is exceptional.

Before accumulations and system of canals and tunnels were made, minnows spent the biggest past of the year in underground water. In surface water they used to show out in the spring, in the period when fields of Eastern Herzegovina are flooded with underground water. As the rest of the species that inhabit karst water and have similar life cycle, minnows have specific complex of physiological and addaptive mechanisms that provide such way of survival (Ivanc et al, 1989; Lučić, 2009).

About global importance talks the fact that both of this species that inhabit water in Eastern Herzegovina are presented in red book of endangered species. Two greatest base of this data, WCMC and IUCN classify them in first category, species that are data defficient and threatened with disappearance, because of the changes in living conditions caused by anthropogenic influence.

In this research is given a review of recent state of distribution of minnows in Eastern Herzegovina.

MATERIAL AND METHODS

State of distribution of endemic species in Eastern Herzegovina was monitored in a several watercourses and accumulations in this area. For this research was used interviewing local fishermen about their observation and also our own fish collecting. For collecting was used transportable electroshocker IG 600. With power 1.2 kW and nets with different diameters. Fish collecting and monitoring of their distribution was done from year 2004 to 2010 in periods of year when individuals come out into surface waters. Beside determination of minnow presence in tested waters, eco-physiological researches were also done. Researches were done in Lake Bileća and in Rivers: Vrijeka, Opačica, Mušnica, also in streams Suško and Ljubomirsko, and in Fatničko field after water flood.

RESULTS AND DISCUSSION

Research of presence of certain fish species in karst regions depend on several factors, from which at first must be considered period of year and water level during research. The fact that minnows were found in certain area, while in another collecting their presence was not recorded, indicates previous statement.

These researches were conducted in periods from 2004 to 2010. During the entire researched period a presence of striped pijor was established (*Telestes metohiensis*, previous name *Phoxinellus metohiensis*).

Its presence was recorded in several researched regions in every trial catching. So, in this way, presence of numerous individuals of this species was determined in River Vrijeka, in lower part of its course, directly before the abyss. Striped pijor was also recorded in River Opačica during research in 2010, and in significant number in stream Suško, watercourse that is tributary of River Vrijeka in Dabar filed. Researches in River Mušnica during 2009 also showed presence of this species in significant number. Presence of striped pijor, based on many collected individuals, was recorded in accumulation Bileća during years 2004, 2006 and 2007.

There are still a massive appears of this species in spring in most of wells in Fatničo filed, but it certainly that big part of the population stays in accumulation Bileća throughout the year. The reason that is little known about minnow biology in the lake, in new environment, is its retention in the deepest parts of the lake, were it is not collected for obvious reasons – traditional way of fishing is unmanageable, and net collecting in the lake is forbidden. Beside that, based on data from survey, sporadic minnow catching in the lake show that it moves in shoals and choose certain zones. Significant means are necessary for minnow research in lake biotope, both in terms of equipment and methods of migration monitoring in the lake. Meanwhile, as the significance of this fish species is rising in the global scale, it is obvious that its research deserve full attention. This will enable our country to improve its status in scientific world, and beside that it can be valorized throughout scientific and educative tourism.

According to Vuković and Ivanović (1971) striped pijor is distributed in karst rivers and springs in regions of Gacko, Navesinje field, region of Cavtat and in some other waters in Herzegovina and Dalmatia.

In the same time Kottelat and Freyhof (2007) quote that striped pijor is presented in water in Nevesinje and Gacko, Carničko, Dabarsko and Lukovac fields in Bosnia and Herzegovina, and in River Ljuta in Croatia. Old findings in River Ljuta were not confirmed so it is consider regionally extinct (Jelic et al,2008).

Trebinje and popovo minnow according to new systematics are consolidated in one species *Delminichthys ghetaldii*, and in our research was recorded presence of trebinje minnow (previous name *Phoxinellus pstrossi*) in some of the researched water. A number of large specimens of trebinje minnow was collected in region of Fatničko filed after its outflow, and in significant number in Ljubomir stream.

Vuković and Ivanović (1971) quote that population of trebinje minnow is declining, and that was significantly present in lower water course of River Trebišnjica in recent period, while popovo minnow was distributed in waters in Herzegovina, Dalmatia and Bosnia. These authors quote that this species was also recorded in River Kasindolka which flows in River Željeznica, tributary of River Bosna.

Beside mentioned species in researches was recorded a presence of species *Phoxinellus alepidotus* in River Vrijeka during research conducted in March 2011, which presents a first finding of this species in this area. According to literature data this species is widespread in waters from Lavanjsko, Duvanjsko and Glamočko fields, lake Buško and Blidinje and in River Korana in Bosnia and Herzegovina, while in Croatia was recorded in River Cetina, in Sinjsko field and in lake Stipačevo (Jelić et al, 2008).

Also Vuković and Ivanović (1971) quote that this species is widespread in Duvanjsko, Sinjsko, Glamčoko and Livanjsko fields, in Buško and Mostarsko mud, in lake Blidinje and in River Neretva.

Our results indicate appearance of relative wide distribution of striped pijor (*Telestes metohiensis* (Steindachner, 1901) and a very narrow range of *Delminthis ghetaldii* (Steindachner, 1882) with sporadic appearance closely related with hydrological characteristics of groundwater.

CONCLUSIONS

Based on recorded data during the research, and comparison with literature data, it can be concluded that minnows are presented in researched areas in significant number, but with different distribution and specific phenology.

More complex eco-physiological and eco-biochemical researches are necessary for detailed insight in state and characteristic of minnows in Eastern Herzegovina waters.

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RECONSTITUTION OF TENCH NATURAL POPULATIONS (TINCA TINCA) BY APPLICATION OF LABORATORY SPAWNING

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REKONSTITUCIJA PRIRODNIH POPULACIJA LINJAKA (*TINCA TINCA*) PRIMENOM LABORATORIJSKOG MRESTA

Abstract

Linjak je jedina vrsta iz roda Tinca i živi u vodama Europe i Sibira do 61^0 sgš. Najčešće se sreće u manjim plitkim vodenim bazenima sa muljevitim dnom, prosečne dubine do 2 m, koji su dobro zagrejani preko leta i bogato obrasli vodenom vegetacijom. Aktuelna sistematska istraživanja ihtiofaune i ribljeg fonda brojnih, za linjaka, tipičnih hidroekosistema, ukazuju na njegovo sporadično prisustvo. Na osnovu analize brojnosti i gustine populacija u raznim tipovima stajaćih i tekućih voda, prirodnim i rečnim jezerima, akumulacijama, kanalskoj mreži hidroistema Dunav-Tisa-Dunav, kao i rekama i njihovim plavnim zonama ustanovljen je zvanični status kao nizak-LR (lc). Istovremeno, u Evropi se nalazi u kategoriji „intermediate", ali vrlo lako može biti prebačen u kategotije „redak" ili ranjiv", ako se nastavi sa devastacijom staništa. Na osnovu probnih lovova utvrđeno je individualno učešće u ukupnom ulovu na pojedinim lokacijama kanala HsDTD u Bačkoj i Banatu od 0.22 do 18%, dok je maseni udeo iznosio od 0.16 do 24%. U pojedinim rečnim sistemima Dunava, Tise, Save i Tamiša (obalni region, stara korita i plavna zona) brojni udeo linjaka se kreće od 0.37 do 25%, a težinski iznosi 1.2-17.3%. Tench are found in very small numbers in our waters. Sadašnja proizvodnja linjaka u toplovodnim ribnjacima Srbije je zanemarljiva, iako je u prvoj polovini prošlog veka ona bila na drugom mestu, odmah iza šarana.

U cilju uvećavanja brojnosti i gustina populacija linjaka sproveden je laboratorijski mrest matica odabranih iz divljih populacija na osnovu habitusa. Ženke su bile starosti 2-4 godine i težine od 400-1500 g. a mužjaci su imali tri godine i telesnu masu 250-700 g. Hranjeni su u dnevnoj količina 1-3 % u odnosu na ukupnu težinu. Hormonalna stimulacija izvedena je „kobarelinom", sintetičkim preparatom LH-RH (5 mg/kg) i jednokratnom hipofizacijom pri temperaturi vode 21-22 ^{0}C. Mešanje ikre i mleča se obavlja u odnosu 100g : 0,4 ml uz postupak odlepljivanja pomoću alkalaze. Do izvaljivanja, pri temperaturi 20-22^{0}C, došlo je za 126^{0} časova. Procenat oplođenja je iznosio 90%.

Izvaljene predlarve su prebačene u ležnice i posle 6-7 dana, pri temperaturi vode od 20^{0}C, razvile su se larve, sposobne za samostalnu ishranu u rastilištima i mladičnjacima, gde se gaje u mono- ili polikulturi (sa šaranom). U monokulturi (bez prelova) nasađuje se 100.000–400.000 kom/ha i na kraju sezone narastu 3-10g u zavisnosti od gustine nasada (gušći – 50.000-80.000, a ređi 30.000-50.000). U objektima sa prelovom nasađuje se 300.000-2.000.000 kom/ha. Preživljavanje se kreće 5-30%. Kod poluintenzivnog gajenja, u polikulturi sa šaranom, dobijen je prinos 300 kg/ha. Prezimljavanje jednogodišnjih mladunaca je sprovedeno u istom objektu, a nastavak gajenja je obavljen po ekstenzivnim principima uz dodavanje ugorelog stajnjaka (6000 kg/ha). Masa linjaka na kraju druge godine proizvodnje se kretala 60-120 g, a ukupna proizvodnja je iznosila 800 kg/ha dvogodišnjih riba uz utrošak kompletnih hraniva (25% sirovih proteina biljnog porekla i pivski kvasac) od 600 kg. Paralelno sa laboratirijskim, organizovan je i prirodni mrest u objektu od 0.2 ha optimalno obraslom vodenom vegetacijom, sa 5 matica.

Dobijeni dvogodišnji mladunci se koriste za poribljavanje otvorenih voda kao uspešna alternacija mrestu u prirodnim plodištima.

Ključne reči: *linjak, rekonstitucija, mrest, otvorene vode*

INTRODUCTION

Tench is the only species of the genus with the same name, which is widespread in the waters of Europe and Siberia (up to 61 north latitude), with the exception of Iceland, most of northern Scotland, Scandinavia, the Balkans of the Adriatic basin, Greece and Crimea. Tinca genus differs from other genera of Cyprinidae family and do not have close relative in the recent fish fauna (Brylinska et al. 1999). According to studies of genetic variation and differentiation of natural and ponds populations it is open the possibility that there are two kinds of tench in Europe, and it is necessary to protect their genetic resources (Kohlmann et al. 2008, Lajbner et al. 2008).

In the middle of last century tench was very populated in all types of standing and slow running waters, including warm-water pond where in the total production tench occupied the second place, immediately after the carp (Ćirković et al., 2008, 2009, 2010). However, due to increased hydro meliorative clutches, disorders of the hydrological regime and water quality deterioration during the recent decades, there was a loss of large complex of flood zone which is tench typical habitat what resulting in a drastic reduction of its population (Maletin et al, 2008a, b).

During the last few decades, by continuous monitoring of fish stocks of different types of aquatic ecosystems, especially the characteristic habitats of tench (flooded areas of big and small rivers, canals, hydro DTD, lakes and reservoirs) it was affirm a drastic reduction in the number and size of populations in time and space (Markovic et, 2008, 2010, Simic et al., 2010; Maletin et al., 2010). Among the main reasons for the reduction of flood zones are the deterioration of water quality and the negative impact of imported fish species (Lenhardt et al., 2010).

In order to increase the number and density of natural populations it is necessary to organize reproduction in the laboratory which includes the selection of parent materials, spawning and rearing of larvae and fingerlings which use for stocking of adequate hydroecosystems.

MATERIAL AND METHODS

By using different types of fishing gear (nets and electrofishing) and based on the evidence of sport fishermen it was ascertain the dynamic of changes with the tendency to reduce the number and density of populations in the typical habitats (flooded areas, canals Danube-Tisa-Danube, lakes and artificial reservoirs).

Artificial spawning of tench was conducted on hatcheries in the Trebon region in the Czech Republic and the experimental hatchery of Faculty of Science in Kragujevac. Selected females (average weight of 1000 g) originated from natural populations of reservoir Ćelije, were anesthetized by solution of 2 - phenoxyethanol (0.2 ml / l) and stimulated with the synthetic hormone (GnRH - 18-20 mg and dopamine-antagonists metoclopramide - 8-10 mg) at water temperature of 18-22 ° C. Breeding of larvae, one and two years old tench fingerlings was conducted at the experimental pond »Ribarska zadruga Mošorin« during the 2009 and 2010.

RESULTS AND DISCUSION

Conducting systematic surveys of ichthyofauna and fish in different types of aquatic ecosystem it was ascertain sporadic presence of tench in typical habitats inhabited by this species - river flood zones, natural and rivers reservoirs and canals, HsDTD. The river flows the Danube, Tisa, Tamiš and Sava with their flood zones are important habitats of tench. However, the density of population in these biotopes was very modest (0.37 - 1.2%.). The exception is Zasavica (the former bed of the river Sava) where the the individual and biomass presence of the tench amount 3.93-25%, and 6.91-17.3% respectively. In the lake Palic and Ludoš, especially in anthropogenic reservoirs, such as Zobnatica, Borkovac, especially Vlasina, tench populations are held by periodic stocking. In some channels of Backa and Banat parts of Hs DTD was noted sporadic presence of tench with the participation of 1-2% of the total catch. Exceptions are the oldest and purest shares where the share of tench can be over 10% (Maletin et al, 2008c). For laboratory spawning females were selected from wild populations according to habitus, females were 2-4 years of age and weighing 400-1500 g. and males with three years of age and weighing 250-700 g, were fed daily in amount of 1-3% in relation to body mass. Hatched pre-larvae were transferred to sieve-cloth boxes after 6-7 days, at water temperature of 20°C. Larvae were developed and capable of independent feeding in the nursery area and ponds where are grown in mono or polyculture (the carp). In monoculture (no overfishing) is stocked 100.000-400.000 units / ha and at the end of the season fish grow 3-10 g depending on the density of plantation (denser – 50.000-80.000 and less denser 30.000-50.000). In the facilities assigned for overfishing can be stocked 300.000-2.000.000 units / ha. Survival ranging 5-30%. Rearing in semiintensive system in polyculture with carp, resulted in the yield of 300 kg / ha. Overwintering of one year old tench fingerlings was conducted in the same pond.

Growing is continued according to extensive principles using mature manure (6000 kg / ha). The mass of tench at the end of the second year of production was 60-120 g, and total production was 800 kg / ha of fish with the consumption of 600 kg of complete feed (25% crude protein of vegetable origin and brewer's yeast). In parallel with laboratory was organized and natural spawning in the space of 0.2 ha covered with optimal emersal, submerged and floating vegetation. It was conducted by releasing five fish (2 females and 3 males) with the implementation of preventive measures and veterinary supervision (ectoparasites, wild fish, birds and minimum of stress).

Tench is a species of fish grown in aquaculture of several European countries (Steffens, 1995), mostly semi-intensive in polyculture with the cyprinid species. Besides Europe tench production increased dramatically in China since year 1998 (Wang et al. 2004). In order to perform adequately reconstitution of tench natural populations, which would have increased their number and density, it is necessary to master its controlled spawn in our best equipped hatcheries. Environmental conditions should be adjusted in accordance with literature data and knowledge in the technological processes of carp production. It is necessary to aspire how to balance the use of the natural capacity of the habitat in our environment and production where chemical substances are not in use. Thus the production of tench in our country would represent organic production and food which is safety for human consumption.

CONCLUSION

It was organized the artificial spawning of tench with the aim of increasing its populations in natural waters. Successful laboratory spawning was carried out thanks to quality parent materials. Larvae and one year old fingerlings were cultivated in semi-intensive system while two years old fingerlings were reared in extensive system. As feed were used pellets with 25% crude protein of vegetable origin and brewer's yeast. Yield for the first year amounted up to 300 kg / ha and in second year 800 kg / ha (with spent of 600 kg of food). Produced two-year old fingerlings can be used for stocking of open waters as successfully alternation of spawn in the natural places.

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HISTORICAL ASPECTS OF THE DEVELOPMENT OF FISH COMMUNITIES IN THE "PERUĆAC" RESERVOIR

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ISTORIJSKI ASPEKTI RAZVOJA ZAJEDNICE RIBA U AKUMULACIJI „PERUĆAC"

Abstrakt

Akumulacija „Perućac" je nastala pregrađivanjem rečnog korita reke Drine betonskom branom. Radovi na izgradnji brane vršeni su od 1952. do 1962. godine. Brana je duga 461 m i visoka 93 m. Izgradnjom brane stvoreno je akumulaciono jezero dugo oko 52 km, sa dubinom do 85 m i širinom od 60 do 1800 m. U jezerskom basenu akumulira se oko 340 miliona m^3 vode. Prosečan godišnji proticaj Drine na mestu gde se nalazi brana je 349 m^3/sek (Stanković, 2005). Sa druge strane, akumulacija „Perućac" predstavlja glavnu ribolovnu vodu na teritoriji Nacionalnog parka „Tara".

Struktura naselja riba u akumulaciji „Perućac" je tokom godina pretrpela mnoge promene. Naselje riba u akumulaciji u početku je formirano na bazi vrsta koje su naseljavale reku Drinu pre pregrađivanja i formiranja jezera. Njega su do 1978. godine činile autohtone salmonidne (5,1 %) i ciprinidne ribe (94,9 %). Prema raspoloživim podacima to naselje je gotovo u potpunosti devastirano prilikom pražnjenja jezera 1978. godine (Kosorić, 1979). Interesantno je da neke od tada registrovanih ciprinidnih vrsta (potočna mrena *Barbus peloponnesius*, nosara *Vimba vimba*, krkuša *Gobio gobio*, pliska *Alburnoides bipunctatus*, crvenperka *Scardinius erythrophthalmus*) više nisu beležene u kasnijim godinama. Može se reći da one danas ne naseljavaju jezero ili su vrlo retke. Stanje koje je zatečeno 2007. godine predstavlja riblju zajednicu koja je formirana na bazi ishodnih autohtonih salmonidnih i ciprinidnih vrsta i vrsta koje su u jezero unete poribljavanjima. Abundancija autohtonih ciprinida je značajno smanjena na račun akcidentalno unetih Percida (grgeč, *Perca fluviatilis*) i Centrarchida (sunčica, *Lepomis gibosus*). Stanje u 2010. godini predstavlja nastavak ovog procesa u kome Percide i Centrarhide preuzimaju dominaciju po brojnosti. Slična situacija se zapaža kada je u pitanju masena zastupljenost pojedinih familija riba. U početku u zajednici riba su po biomasi dominirale autohtone ciprinide i salmonide. Kasnije, nakon poribljavanja, značajnu ulogu u ihtiocenozi imaju Percidae, Siluridae (som *Silurus glanis*) i Centrarchidae.

Glavni razlozi za unošenje šarana i soma su svakako bili ribolovnog karaktera, a u cilju povećanja raznovrsnosti ribolovno značajnih vrsta. Međutim, poribljavanja šaranom vršila su se sa jednogodišnjom mlađi prosečne mase tela oko 50 g. Nažalost, praksa je pokazala da je sasvim uobičajeno da se prilikom isporuke takve mlađi u transportnim tankovima nađe nekoliko procenata jedinki vrsta koje nikako ne bi trebalo unositi u ribolovne vode (sunčica, grgeč, babuška *Carassius gibelio*). U tom slučaju bandar i sunčica, zahvaljujući velikom reproduktivnom potencijalu, nakon nekoliko godina postaju vrlo značajne i po brojnosti i po biomasi i igraju vrlo važnu ulogu u jezerskoj ihtiocenozi. Tako ove dve vrste po brojnosti 2007. godine čine oko 40 %, a 2010. godine i preko 50% naselja riba. I po biomasi ove dve vrste imaju velikog značaja za naselje riba čineći oko 21 % masenog udela. Negativan uticaj ovako formiranog naselja posebno se odražava na salmonidne ribe čija se zastupljenost konstantno smanjuje: 5,1 %, 2,7 %, 0,3 %, po brojnosti, odnosno 9,6 %, 3,5 %, 0,4 % po biomasi, respektivno po godinama. Som kao glavna predatorska riba, iako sa značajnim masenim udelom koji se u vremenu povećava, nema dovoljnog populacionog kapaciteta da značajnije utiče na regulaciju brojnosti nepoželjnih vrsta kao što su bandar i sunčica.

Ključne reči: *zajednica riba, brana, Akumulacija „Perućac“*

INTRODUCTION

In Serbia, a significant number of reservoirs with different primary purpose have been formed: exploitation of hydro power potential, water supply, protection from erosion, etc. Fish community in reservoirs are formed by species that inhabited the river that was dammed, and the species that were introduced by restocking. The „Perućac“ reservoir is no exception. This lake was created by constructing a concrete dam on the Drina river bed. The works on the construction of the dam were carried out between 1952 and 1962. The dam is 461 m long and 93 m high. The dam created a reservoir, 52 km long, between 60 m and 1800 m wide and with the maximum depth of 85 m. Around 340 million m^3 of water are accumulated in the lake basin. The average annual flow of the Drina river, in the area of the dam, is 349 m^3/sec (Stanković, 2005). On the other hand, „Perućac“ reservoir represents the main fishing water on territory of the National park „Tara“. This article analyses the development of the ichtyocenosis in this reservoir, since 1978 until the present.

MATERIAL AND METHODS

Samples of the fish fauna were taken during August, September and October 2007 and 2010. In order to determine the abundance (number proportion) and mass proportion in the fish community, the samples in the reservoir were collected using fish nets, with the following characteristics:

- pelagic gillnets 50 m x 6 m, mesh size 45 and 70 mm,
- pelagic trammel net 50 m x 4 m, mesh size 80 mm,
- benthic gillnets 20 m x 2 m, mesh size 20 mm, 60 m x 3 m, mesh size 30 mm,

30 m x 1,5 m, mesh size 50 and 60 mm,
- benthic trammel nets 30 m x 1,5 m, mesh size 40 and 60 mm.

Nets were set in the evening and raised the next morning (12 hours), and during the day (5 hours). Identification of species was made according to the key for identification of fish species „Fish of Serbia" (Simonović, 2001). The data about the fish community structure from 1978 were obtained from Kosorić (1979).

RESULTS

Over the years, the structure of the fish fond in the „Perućac" reservoir has undergone many changes. The fish community in the reservoir was initially formed from species that inhabited the Drina river before the construction of the dam and formation of the lake. Until 1978, the community consisted of native salmonid (5,1%) and cyprinid (94,9%) fish species (Fig. 1). According to available data, this community was almost completely devastated when the lake was emptied in 1978 (Kosorić, 1979). It is interesting that some of than registered cyprinid species (brook barbel *Barbus peloponnesius*, vimba bream *Vimba vimba*, gudgeon *Gobio gobio*, schneider *Alburnoides bipunctatus*, rudd *Scardinius erythrophthalmus*) are no longer recorded in the later years. It can be said that they do not inhabit the lake any more or that they are very rare.

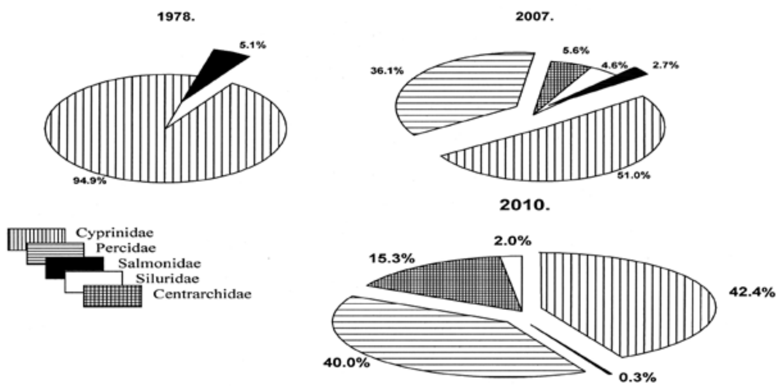

Figure 1. The structure of fish community in „Perućac" reservoir: the abundance of fish families between 1978 and 2010.

The data about the re-establishment and development of the fish community between 1978 and 2007 were not available, and the state of the fish community in 2007 was formed based on the original native salmonid and cyprinid species and the species who were introduced into the lake by restocking (Hegediš i Mićković, 2007). The abundance of indigenous cyprind species was significantly reduced due to the accidental introduction of percid species (perch, *Perca fluviatilis*) and Centrarchids (pumpkinseed, *Lepomis gibosus*). The state in 2010 demonstrates a continuation of this process in which percid and centrarhid species assume the dominance in number (Fig. 1) (Hegediš *et al.*, 2010). A similar situation is observed regarding the biomass distribution of certain fish families (Fig. 2). At the beginning, the fish community biomass was dominated by the indigenous cyprinids and salmonids. Later, after stocking, a significant role in ichthyocenosis have

Percidae, Siluridae (wells *Silurus glanis*) i Centrarchidae.

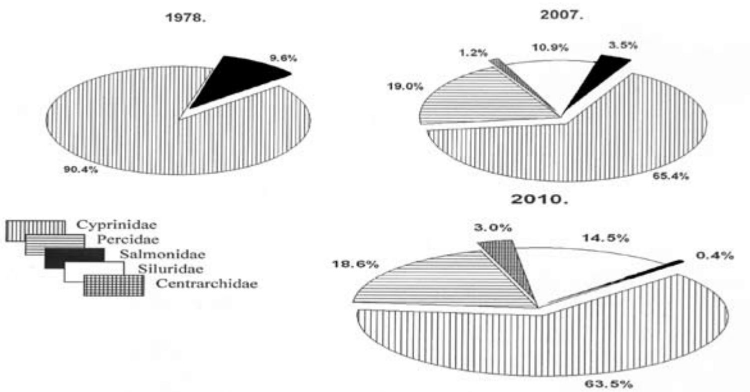

Figure 2. The structure of fish community in „Perućac" reservoir: mass proportion of the fish families between 1978 and 2010

Also, significant changes have occurred from the aspect of cyprinid species. It was mentioned that five of the original nine species from 1978 cannot be longer recorded in the community, but five new cyprinid species appeared, which had been introduced by restocking. Carp *Cyprinus carpio* and tench *Tinca tinca* have the status of target species, while prussian carp *Carassius gibelio*, whiteye bream *Ballerus sapa* i roach *Rutilus rutilus* were accidentally introduced into fish community of the lake.

DISCUSION

The construction of waterworks facilities for water supply or the exploitation of hydro power resources inevitably leads to significant changes in the conditions of inhabitation of all aquatic organisms, including fish. Damming rivers and forming reservoirs, in addition to fragmenting the river flows, leads to a series of consequences for the fish population: running waters are replaced by still waters; the migratory routes of local populations are disrupted; normal regimes of water temperature, pH values, oxygen levels are altered, and new aquatic organisms appear, thus changing the characteristics of trophic structure in aquatic ecosystems etc. Defining reservoirs as potential fishing waters directs fishing management towards restocking, according to species that are important for fishing and the economy: carp, catfish, pikeperch, northern pike. With the desired species, accidentally are introduced undesirable ones, and with the later developments of ichtyocenosis this could present a big problem, not only in terms of fishing use, but from the aspect of maintaining good water quality.

The main reasons for the introduction of carp and catfish were definitely because of the fishing, in order to increase the diversity of important fishery species. However, the restocking of carp was done with one-year fry of an average body weight about 50 g. Unfortunately, the practice has shown that is quite common that after the delivery of such fry in transport tanks, a small percentage of individuals of species that should not be introduced into the fishing waters could be found (pumpkinseed, perch, prussian carp *Carassius gibelio*). In this case, eurasian perch and pumpkinseed, due to a large reproductive potential, become very significant after a few years, both in number and biomass, and play an important role in the lake ichthyocenosis. Thus, these two species constituted 40% of the total number of fish species in 2007, and in 2010, that number was over 50%. The biomass of these two species has great importance for the fish community, making about 21% of total biomass. The negative impact of such a community has particularly been reflected apon salmonid fish, whose presence has been steadily reducing: 5,1 %, 2,7 %, 0,3 %, by number, or 9,6 %, 3,5 %, 0,4 % by biomass, respectively per year. Catfish, as a major predatory fish, although with significant biomass portion which increases in time, do not have such population capacity to significantly influence the number of non-target species, such as pumpkinseed and eurasian perch.

According to Richa *et al.*, (2009) ichtyocenosis od „Perućac" reservoir is now in dynamic and unstable „perch-phase", and the introduction of roach and whiteye bream could enhance the transfer of fish community, through a transit „perch-roach-phase" into a highly stable „cyprinid-phase".

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HEMATOLOGICAL STATUS OF DIFFERENT AGE CLASSES OF BARBUS BALCANICUS

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HEMATOLOŠKI STATUS RAZLIČITIH UZRASNIH KLASA *BARBUS BALCANICUS*

Apstrakt

U radu su istraživane vrijednosti osnovnih parametara crvene loze po starosnim kategorijama kod jedinki potočne mrene (*Barbus balcanicus*). Hematološki status predstavlja pouzdano sredstvo za utvrđivanje fiziološkog stanja organizma, a posredno i stanja životne sredine, jer njegovi parametri reaguju na promjene uslova sredine. Hematološki parametri su obuhvatali: broj eritrocita, koncentraciju hemoglobina, hematokrit, MCV, MCH i MCHC. Praćeni parametri su analizirani u toku dvije godine i kod populacija iz dvije rijeke (Suturlija i Jakotinska rijeka). Kod jedinki iz rijeke Suturlije u prvoj godini istraživanja bile su zastupljene jedinke starosti 1+ do 5+, dok je tokom druge godine uzorak bio predstavljen jedinkama starosti 1+ do 6+. Istovremeno su tokom 2004. godine kod riba iz Jakotinske rijeke bile zastupljene jedinke starosti 1+ do 4+, a u drugoj godini jedinke starosti 1+ do 5+. Analiza po starosnim kategorijama pokazuje postojanje značajnih razlika u vrijednostima pojedinih parametara, a razlika je izraženija kod jedinki potočne mrene iz rijeke Suturlije, kod kojih su razlike utvrđene u obje godine. Međutim, kod potočne mrene iz Jakotinske rijeke statistički značajne razlike između vrijednosti konstatovanih kod različitih starosnih klasa bile su statistički značajne samo u drugoj godini istraživanja.

Ključne riječi*: hematologija, potočna mrena, starosne klase*

INTRODUCTION

Hematological status represents reliable means for determining the physiological state of an organism and indirectly the state of its environment, because hematological parameters respond to environmental changes (Ivanc and Miljanović, 2001). The quantitative characters of red and white blood cells are used for detecting the haematological status. They provide insight in a number of processes in the organism. On this bases, analyses of different blood components give valuable information concerning changes that appear in certain systems influenced by external and internal factors. Determination of hematological parameters and blood plasma biochemistry is used to assess the health of wild and domestic animals. Values of these parameters are useful for interpretation of the results that are related to different diseases and environmental conditions (Seker et al., 2005). Prerequisite for such hematology application is satisfactory knowledge of reference range of values of fish blood parameters. Age-related values of hematological parameters are even less known and insufficient (Hrubec et al., 2001).

MATERIAL AND METHODS

Fish sampling was done by electro-shocker providing pulsed direct current and with ability to customise the output voltage. IG 600 brand, with 1.2 KW power. After appearance of galvanotaxis and galvano-narcosis, fish were collected with landing net. Fish sampling was made during two years in monthly intervals. Handbooks from Vuković and Ivanović (1971), Simonović (2001), Banaresku and Bogutavskaya (2003), Kottelat and Freyhof (2007) were used for taxonomic determination of collected fish. The fish were placed into containers with sufficient water of appropriate quality. After that were transferred in keepnets, placed in native stream and let to reanimate for one hour. River Suturlija, by its course and its basin is located in the area southwest of Banja Luka, and its mouth into Vrbas river, as a left tributary, is placed in Srpske Toplice (Gornji Šeher), at an altitude of 159 m. River Jakotina is a left tributary of river Vrbanja with length of 15 km, witch originates at an altitude of 670 m, with mouth on 260 m.

Hematological analysis

Blood for hematological analysis was collected by heart puncture using sterile needle (1.0 to 1.2 mm). Native blood, with anticoagulant is used for further analyses. Erythrocyte and leukocyte count was performed in hemocytometer using diluent by Kekić and Ivanc (1982). Hemoglobin concentration (Hb) was determined by hemiglobin cyanide method using Drabkin reagent (Blaxhall and Daisly, 1973). Hematocrit (Hct) was determined by microhematocrit centrifuge. Hematological parameters were calculated using values of hematocrit, erythrocyte number and hemoglobin concentration.

Mean corpuscular volume (MCV) $$MCV = \frac{Hct}{Br.eritrocita / l}$$

Mean corpuscular hemoglobin (MCH) $$MCH = \frac{Hb / l}{Br.eritrocita / l}$$

Mean corpuscular hemoglobin concentration (MCHC) $$MCHC = \frac{Hb / l}{Hct}$$

Age determination

Methods of scales observation and counting annual growth marks were used for age determination. Scales for age determination were collected from the shoulder area, above lateral line, and then, were placed in paper bags. Before age determination, scales were cleaned with NaCl and KOH solutions and water, and then were observed using binocular magnifier.

RESULTS AND DISCUSSION

Values of monitored hematological parameters were correlated with age groups. Mean values of erythrocyte parameters for different age classes, rivers and years of researches are shown in table 1. Blood values of Large-spot barbel (*Barbus balcanicus*) from River Suturlija in 2004. showed significant age-related differences. Erythrocyte number was the highest in age class 5+, and the lowest in class 2+. Significant difference was recorded between values of age classes 2+ and 4+ (0.021), 2+ and 5+ (0.020), as well as between 1+ and 5+ (0.029). The highest value of hemoglobin concentration was recorded in age class 4+, and the lowest in 1+ class.

Table 1. Mean values of erythrocyte parameters of *Barbus balcanicus* from Rivers Suturlija and Jakotina by age classes and years of research

River	Year	Age class	Mean value	Erythrocyte number x 10^{12}/l	Hb (g/l)	Hct (l/l)	MCV (fl)	MCH (pg)	MCHC (g/l eryt.)
Suturlija	2004	1+	x	1.254	69.02	0.398	318.750	55.241	175.272
		2+		1.246	72.13	0.422	341.563	58.393	172.825
		3+		1.294	71.64	0.431	333.855	55.609	167.768
		4+		1.310	73.61	0.411	315.757	56.490	180.797
		5+		1.390	69.44	0.416	301.609	50.139	169.562
	2005	1+	x	1.270	67.64	0.389	308.489	53.603	175.528
		2+		1.300	70.44	0.417	323.889	54.652	170.758
		3+		1.297	74.26	0.442	342.574	57.791	172.104
		4+		1.366	74.49	0.445	329.548	55.159	169.074
		5+		1.343	75.34	0.438	329.086	56.846	173.660
		6+		1.430	80.56	0.440	322.938	59.423	187.237
Jakotina	2004	1+	x	1.225	73.25	0.443	362.869	60.329	168.150
		2+		1.207	74.75	0.451	374.768	62.316	168.443
		3+		1.250	76.30	0.451	376.408	63.896	170.751
		4+		1.355	77.77	0.457	345.420	58.155	171.695
	2005	1+	x	1.258	72.49	0.437	349.536	58.081	168.989
		2+		1.240	75.10	0.453	375.276	61.671	168.131
		3+		1.273	75.87	0.466	371.632	60.594	165.223
		4+		1.282	73.56	0.451	360.319	58.366	164.954
		5+		1.269	71.91	0.450	358.376	57.126	161.549
		6+		1.245	70.37	0.317	256.317	56.585	223.562

Significant differences were established comparing values of class 1+ with values of age classes 2+ (0.014) and 4+ (0.013). Hematocrit values also were related with fish age. Significant differences in hematocrit values existed between class 1+ and 2+ (0.005) and 1+ and 3+ class (0.004). The highest value was in age class 3+, and the lowest in 1+. Values of mean corpuscular volume (MCV) ranged from 301.608 fl (age class

5+) to 341.563 fl (class 2+). Significant differences were between age class 2+ and 1+ (0.003) and 2+ and 4+(0.014). Values of MCH showed significant differences by comparing class 2+ with class 1+ (0.007) and 5+ (0.022). The highest value of this parameter was in age class 2+, and the lowest in 5+. In the same time, significant difference was recorded in MCHC values between age classes 3+ and 4+ (0.039).

In 2005 in research of *Barbus balcanicus* from River Suturlija, the individuals from age 1+ to 6+ were found. Analysis by age classes showed presence of significant difference in most of monitored parameters. The values of erythrocyte number ranged from 1.270 x 10^{12}/l (in age class 1+) to 1.430 x 10^{12}/l (class 6+). Relation of this parameter with fish age showed significant difference between age class 1+ on one side, and classes 4+ (0.000), 5+ (0.035) and 6+ (0.016) on the other side. Comparing values of class 2+ with values of 4+ (0.010) and 6+ (0.050). Differences were significant between values from class 3+ and 4+ (0.006) and 3+ and 6+ (0.044). Hemoglobin concentration was constantly increasing from classes 1+ to 6+. Significant differences were recorded comparing values from age class 1+ with classes 3+ (0.000), 4+ (0.000), 5+ (0.001) and 6+ (0.001). Values of class 2+ were significantly different from those in classes 3+ (0.012), 4+ (0.013), 5+ (0.024) and 6+ (0.017). The lowest hematocrit value was in age class 1+, and the highest in class 4+. Values from age class 1+ were significantly different from those in classes 2+ (0.015), 3+ (0.000), 4+ (0.000) and 5+ (0.003). Also, statistically significant difference was recorded comparing values from age class 2+ with values from classes 3+(0.030) and 4+ (0.021). Age class 3+ had signifaicantly higher values than class 1+ (0.002). This is also true rom MCH (0.013). MCHC values did not show significant difference by age classes. Correlation between hematological parameters and age was less expressed in barbel from River Jakotina.

In 2004, significant differences between age classes were not recorded. However, it should be pointed out that the highest values of erythrocyte number, hemoglobin concentration, hematocrit and MCHC were recorded in age class 4+, while the highest values of MCV and MCH were recorded in age class 3+. Lack of significant differencess in 2004 could be explained by different sample sizes. So, more than 50% of total sample belonged to age class 1+, followed by age class 2+, while the rest of age classes were presented by fewer individuals. Slightly different correlation between hematological parameters from barbel inhabiting River Suturlija was recorded in 2005. During that year, significant differences of hemoglobin concentration values were determined between age class 1+ on one side, and age classes 2+ (0.044) and 3+ (0.044) on the other side. The highest value of this parameter was in age class 3+, and the lowest in class 6+. The lowest hematocrit value was recorded in age class 6+, and it was significantly different from age classes 1+ (0.007), 2+ (0.002), 3+ (0.001), 4+ (0.003) and 5+ (0.005). Statistically significant difference was also recorded between age classes 1+ and 3+ (0.037).

Erythrocyte number did not differ significantly with age, while MCV values were different between age class 6+ (in which were recorded the lowest values) and classes 2+ (0.020), 3+ (0.027) and 4+ (0.046). Also, significant difference was recorded between age classes 1+ and 2+ (0.036). MCH differed significantly between classes 1+ and 2+ (0.030). MCHC values had highest values in class 6+ which was significantly different from classes 1+ (0.002), 2+ (0.002), 3+ (0.001), 4+ (0.001) and 5+ (0.001).

The other researches also showed presence of significant differences in values of hematological parameters between different age classes. Age-related analysis of blood parameters of hybrid *Morone chrysops* and *Morone saxatilis* showed presence of difference. In the research, the individuals from 4 to 19 month of age were presented, divided into age classes: 4 months, 6 months, 9 months, 15 months and 19 months. Values of hematocrit and MCV were significantly lower in the youngest individuals in comparison with other age classes. Individuals aged 6 months had significantly lower values of hemoglobin concentration, MCH and MCHC in comparison with other age groups. The values of erythrocyte number were the highest in individuals aged 9 and 15 months (Hrubec et al., 2001). Also, research regarding to correlation between hematological parameters and fish biology in *Silurus triostegus* showed that hemoglobin concentration and hematocrit values were growing with age, until the age of four, and then were decreasing. Research in correlation between hemoglobin concentration and hematocrit value showed high correlation coefficient (Abood, 1992).

According to Chanchal et al., (Chanchal et al., 1979), young fishes usually show high physical activity and intense feeding during growth period which explains high recorded values of hemoglobin concentration and hematocrit. However, the older fishes show slow metabolic activity after certain age (Joshi and Tandon, 1977). Research of erythrocyte parameters in species *Rhamdia quelen* showed that hematological parameters were also correlated with body length. Individuals of this species were divided in two groups, based on difference in standard body length. Standard body length values of fishes from the first group were ranged from 20.5 to 30.0 cm, and the second group from 30.5 to 40.0 cm. Analysis of hematological parameters showed significantly higher erythrocyte number in the first group, while the values of hemoglobin concentration, hematocrit and MCHC were approximately the same in the both groups. Higher values of MCV and MCH were recorded in fishes from the second group (Camargo and Pouey, 2004).

Results of haematological parameters in *Capoeta capoeta umbla* showed increased values of erythrocyte number, hemoglobin concentration and hematocrit with age (Örün and Erdemli, 2003).

Based on all presented hematological parameters it can be noted that they are different in different age classes, but specific for every fish species.

CONCLUSIONS

Erythrocyte parameters of Large-spot barbel (*Barbus balcanicus*) from researched rivers show significant difference with age.

Major differences of monitored parameters correlated with age groups were recorded in individuals from River Suturlija.

Differences were not recorded between barbel individuals from River Jakotina in the first year of research, while they were recorded in the second year.

It can be noted that all presented haematological parameters are different for different age classes, but specific for every fish species.

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EFFECT OF EXTENDER COMPOSITION AND CRYO-PROTECTANTS ON POST-THAW MOTILITY OF BROWN TROUT (*SALMO TRUTTA MACROSTIGMA*) SPERMATOZOA

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DELOVANJE EKSTENDERA I KRIOPROTEKTANATA NA MOTILITET SPERMATOZOIDA POTOČNE PASTRMKE (*SALMO TRUTTA MACROSTİGMA*) POSLE OTAPANJA

Abstrakt

Cilj ovog rada je identifikacija protokola zamrzavanja spermatozoida specifičnog za vrstu potočne pastrmke (*Salmo trutta macrostigma*) optimizacijom svih stadijuma tokom procedure krioprezervacije. U tom cilju su testirana dva različita ekstendera koji sadrže dva različita krioprotektanta.

U prvom stadijumu eksperimenta određen je kvalitet sperme zrelih mužjaka. Uzorci koji su pokazali >80 pokretljivosti su sakupljeni zajedno i razblaženi sa dva različita ekstendera koji su sadržali različite udele DMSO i glicerola na nivoima 10 i 15 %. Razblažena sperma je pakovana u zapreminu od 0.5 ml i ostavljena 30 min na 4 ºC. Potom je izlagana 10 min pari tečnog azota i uronjena u tečni azot. Zatim je krioprezervisana sperma otapana u vodenom kupatilu na 30ºC za 20 s da bi se odredila pokretljivost (%) i dužina pokretljivosti posle otapanja.

Uspeh zamrzavanja je procenjivan kroz motilitet sperme. Na osnovu dobijenih rezultata, pokretljivost zamrznute pa otopljene sperme je postignuta upotrebom glukoznog ekstendera sa 10 % glicerola od 40 %. S druge strane najbolje trajanje pokretljivosti od 42 s pokazali su uzorci sa Lahnsteiner ekstenderom koji je imao 10 % DMSO.

Ključne reči: *Salmo trutta macrostigma mleč, krioprezervacija, ekstender*

INTRODUCTION

Research on cryopreservation of fish sperm, with focus on cryopreservation protocols, has achieved great advances since the first successful cryopreserving of sperm in herring 50 yr ago (Blaxter 1953). It provides many benefits such as ease of global germplasm shipping and supply (Tiersch et al. 2004), selective breeding and hybridization with desirable characteristics (Kurokura et al. 1984), and conservation of genetic diversity (Ohta et al. 2001). Furthermore, a frozen sperm bank could maintain a continuous and stable supply of gametes for hatchery seed production or laboratory experimentation (Lubzens et al. 1997). Because of the advantages of this technique, fish sperm of over 200 freshwater and 40 marine species have been cryopreserved successfully (Gwo, 2000).

Cryopreservation of sperm in straws is advantageous for laboratory use such as gene banking, or small-scale commercial use, but it is impractical for largescale insemination at hatcheries. Therefore, much work remains to be done before cryopreservation of sperm can be successfully employed in large-scale application to fish species. There is a need to improve techniques on gamete storage and evaluation of sperm quality to facilitate optimization of controlled reproduction in fish (Alavi and Cosson, 2005). Successful sperm-freezing protocols depend on several factors such as sperm dilution rate, extender composition, cryoprotectant, freezing/thawing rates and handling factors (Bozkurt et al. 2005, Figiel and Tiersch 1998).

However, there is a little data on sperm quality in cnnection with cryopreservation. Motility is the most commonly used parameter to evaluate sperm quality in fishes (Billard et al. 1995). This parameter is acceptable so that spermatozoa must be motile to achieve fertilization. Sperm motility varies in vigor and duration not only among males but also within an individual male depending on its ripeness. Also, studies on most fish species showed that motility and motility of duration of semen may vary seasonally (Lahnsteiner and Patzner 1998). Therefore determining semen motility is an important component of a preservation program to prevent choosing poor quality semen prior to storage.

Furtherore, sperm motility is an important component of a cryopreservation program in order to prevent poor sperm quality sperm samples prior to freezing and to estimate the fertility of the stored sperm after thawing. On the other hand, differences in dilutents, cryoprotectants and freezing techniques make it difficult to achieve consistent estimates on the efficiency of cryopreservation procedure (Akçay et al., 2004). Most experiments in this field have focused on finding appropriate extenders and cryoprotective agents. Generally, two types of extenders have been used for the cryopreservation of fish spermatozoa such as: seminal plasma-mimicking media, and simple carbohydrate-based solutions (Ciereszko and Dabrowski, 1996).

Especially the use of an appropriate cryoprotectant solution prevents cells from cellular disruption and membrane damage during freezing and thawing. Usually dimethyl sulphoxide (DMSO) is applied as the internal cryoprotective agent, but other cryoprotectants such as dimethyl acetamide (DMA), ethylene glycol, glycerol and DMSO–glycerol mixtures are also considered as providing efficient results (Babiak et al., 2001). DMSO seems to provide salmonid sperm with the best protection and is used at concentrations between 5% and 15% (Tekin et al. 2007). In addition, glycerol has been successful for some species of fish; however, glycerol may be detrimental to salmonid sperm or may fail to provide adequate protection (Erdahl and Graham, 1980).

Salmo trutta macrostigma (known as mountain trout) is a salmonid species occurring in inland water habitats of Southern Europe, Western Asia, Northern Africa, and Anatolia (Geldiay and Balik, 1988). It is also critically endangered fish species in Turkish inland waters because of illegal fishing, overfishing, and other environmental changes, including hydroelectric plants and pollution. For this reason, *Salmo trutta macrostigma* has been considered for a biological conservation program in Turkey.

According to our knowledge, there is no information on cryopreservation of *Salmo trutta macrostigma* sperm. From this point of view, the main purpose of this study was to establish efficient method for cryopreservation of *Salmo trutta macrostigma* sperm and to compare the cryoprotective efficiency of cryoprotectants in different extenders.

MATERIALS AND METHODS

Broodstock management and sperm collection

During spawning season (November-December), mature brown trout (*Salmo trutta macrostigma*) males obtained from the farm of Directorate of Nature Conservation and National Parks, Turkey, were used for this research. In the pre-spawning period, the parenteral broodfishes were kept seperately in small ponds and fasted 48 h prior to sperm collection. For sperm collection, males were stripped by gentle abdominal massage and sperm was collected in dry plastic containers. Samples contaminated with blood, faeces, water or urine were discarded. Sperm was transported on ice to the laboratory within 15 min.

Evaluation of motility, density and pH of sperm

Sperm evaluation was carried out at the reproduction laboratory of the farm. For motility determination, 2 μL semen was activated on a glass slide with 50 μL of 4ºC sperm motility-activating solution (3‰ NaCl solution). The sperm motility rates (Table 1) were estimated within 10s following activation using a lightmicroscope (Olimpus, Japan) at x40 magnification. Motility determination was carried out in duplicate for each sperm sample. Sperm samples with a motility rating of ≥80% were pooled and used for cryopreservation. Time was recorded with a chronometer to determine motility period (s), from the time that spermatozoa starts moving until they stop or start to do local rotatory movement. All sperm manipulations were performed on ice (4ºC).

Table 1. Criteria used to assess the motility of brown trout (*Salmo trutta macrostigma*) sperm

Rating	Criteria
0 %	No motility (0%)
<10 %	Sperm cells are mostly immotile with few vibrations and <10% progressively motile sperm
10-25 %	Sperm cells are mostly immotile with few vibrations and 10–25% progressively motile sperm
25-50 %	Some sperm cells are immotile with some vibrations and 25–50% progressively motile sperm
50-75 %	Few sperm cells are immotile with some vibrations and 50–75% progressively motile sperm
75-90 %	Few sperm cells are showing vibrations with 75–90% progressively motile sperm
100 %	All sperm cells are progressively motile (100%).

Spermatozoa density was determined according to the haemacytometric method. Sperm was diluted at ratio of 1:1000 with Hayem solution (5g Na_2SO4, 1g NaCl, 0.5g $HgCl_2$ 200ml bicine) and mean spermatozoa count was calculated from three replicate samples for each fish at magnification of x40. Sperm density was expressed as $x10^9$ mL^{-1}. Counting chambers were always kept in a moist atmosphere for at least 10 min before cell counting. Sperm pH was measured using standard pH papers (Merck) within 30 min of sampling.

Extenders and cryopreservation procedure

The pooled sperm was diluted at a ratio of 1:3 (sperm:extender) with two different extenders that composing: (I) 75 mM NaCl, 70 mM KCl, 2 mM CaCl2, 1 mM MgSO4, 20 mM Tris, 0.5% BSA, 10% egg yolk, 0.5% sucrose (Lahnsteiner et al., 1998); (II) 300 mM glucose and 10% egg yolk (Tekin et al., 2003). Each extender contained DMSO and glycerol at levels of 10 and 15%. The control consisted of unfrozen and untreated sperm.

Using micropipettes, 0.5 ml plastic straws (IMV, France) were filled with the diluted sperm and sealed with polyvinile alcohol (PVA). The straws were equilibrated for 30 min at 4ºC, then suspended on a styrofoam raft that floated 3 cm above liquid nitrogen. After 10 min, the straws were plunged into liquid nitrogen where they remained until thawing. For the aim of thawing, the frozen straws were removed from the liquid nitrogen and immersed in a water bath at 30ºC for 20 s.

Determination of post-thaw sperm motility

For post-thaw motility determination, 2 μL of frozen-thawed semen was activated on a glass slide with 50 μL of 4ºC sperm motility-activating solution, covered with a cover-slip and the sperm motility rating was estimated within 10s after activation using a lightmicroscope at x40 magnification according to the sperm motility rating (Table 1).

Statistical Analysis

Results are presented as means±SEM. Differences between parameters were analyzed by repeated analysis of variance (ANOVA). Significant means were subjected to a multiple comparison test (Duncan) for post-hoc comparisons at a level of α=0.05. All analyses were carried out using SPSS 10 for Windows statistical software package.

RESULTS

The spermatological properties of the semen collected from seven males are shown in Table 2. Semen volumes were rather variable and ranged from 8 to 25 ml and mean volume was 13.7±2.24 ml. Motility values were ranged from 60% to 95%. Samples that motility values were below than 80% were not used for the cryopreservation. The mean motility value of sperm samples were 79.2±5.28%.

Table 2. Spermatological parameters of fresh brown trout *(Salmo trutta macrostigma)* semen.

	Body Weight (kg)	Body Length (cm)	Volume (ml)	Motility (%)	Mot. Dur. (s)	Density ($x10^9$/ ml)	Total Density ($x10^9$)	pH
Mean Value(±)	2.27±0.42	42.4±2.84	13.7±2.24	79.2±5.28	40.0±4.50	21.7±1.41	284.2±32.6	7.07±0.07
Highest Value	4.5	55	25	95	50	28.4	430	7.5

Frozen sperm showed a significant decrease in quality compared to fresh sperm (Table 3). The motility decrease was similar for the 2 types of extenders tested and varied from 10% to 40% except for glucose extender containing 15% glycerol. Semen frozen with glucose extender containing 15% glycerol showed lowest post-thaw motility than the other samples. Semen frozen with glucose extender containing 10% glycerol had the highest post-thaw motility. Differences between the post-thaw motility values were not significant (P<0.05). Sperm motlity of fresh samples (control group) was determined as 80%.

Table 3. Post-thaw spermatozoa motility of brown trout (*Salmo trutta macrostigma*) semen cryopreserved with two diluents containing different ratios of cryoprotectants.

Parameter	n	Lahnsteiner’s extender				Glucose extender			
		DMSO (10%)	DMSO (15%)	Glycerol (10%)	Glycerol (15%)	DMSO (10%)	DMSO (15%)	Glycerol (10%)	Glycerol (15%)
Motility (%)	3	30.4±2.27[a]	20.2±3.46[c]	20.8±2.18[c]	25.8±4.57[b]	20.2±2.46[b]	20.6±3.79[b]	40.8±2.37[a]	10.6±2.59[c]

Different superscripts indicate significant differences at $p<0.05$.

Motility durations (57 s) in fresh semen also showed significant differences with regard to the frozen spermatozoa (Table 4). It was observed that a decrease in motility duration occurred following cryopreservation. The longest post-thaw motility duration was achieved with Lahnsteiner’s extender containing 10% DMSO as 42 s. Differences between the means of motility durations were significant (P<0.05). The interaction between the extender and cryoprotectant was significant (F =2.279).

Table 4. Post-thaw spermatozoa motility durations of brown trout (*Salmo trutta macrostigma*) semen cryopreserved with two diluents containing different ratios of cryoprotectants

Parameter	n	Lahnsteiner’s extender				Glucose extender			
		DMSO (10%)	DMSO (15%)	Glycerol (10%)	Glycerol (15%)	DMSO (10%)	DMSO (15%)	Glycerol (10%)	Glycerol (15%)
Motility dur. (s)	3	42[a]	25[d]	30[c]	35[b]	27[b]	25[b]	37[a]	15[c]

Different superscripts indicate significant differences at $p<0.05$.

DISCUSSION

Post-thaw motility is one of the most important indicators of the success of a freezing protocol. Examination of the effect of extender constituents and cryoprotectants of diluted sperm on the motility of frozen semen yielded some interesting results. *Salmo trutta macrostigma* spermatozoa motility was affected during cryopreservation. Post-thaw motility in salmonid spermatozoa is low (Lahnsteiner et al., 1996) due to physical shocks during freezing and thawing (Babiak et al., 2002). This situation indicates a severe damage to the most cells.

The efficacy of a cryoprotectant depends on the delicate balance between its toxicity and its capacity to protect the cells from the damage caused by freezing and thawing. In this experiment, interactions between extenders and cryoprotectants were significant. Therefore the interaction between extender constituents is an important point to take into consideration.

In Lahnsteiner's extender the best post-thaw motility was determined when 10% DMSO was used. On the other hand, in the glucose diluent, frozen-thawed *Salmo trutta macrostigma* semen had a highest sperm motility when 10% glycerol was used as cryoprotectant than semen cryopreserved using DMSO. It can be concluded that glucose can act as semen diluent, protect the sperm from osmolality damage and also has an external cryoprotectant effect (Leung and Jamieson 1991). Another reason for better protection of glycerol may due to well penetratration and leaving the cells much faster than DMSO. However, glycerol is found to be toxic when used at concentrations above 10%.

The proportion of motile cells decreased faster with time in thawed sperm samples than in fresh ones. Similar motility values were obtained with Lahnsteiner's and glucose extenders in this study. Furthermore, motility duration was also affected from this process. One extremely important aspect of cryopreservation is the appropriate choice of the extender medium. In the present study, we have used the extenders proposed by Lahnsteiner et al. (1995) and Tekin et al. (2003) due to they reported good motility after freezing/thawing.

Strong cumulative effects of type of diluent and type of cryoprotectant demonstrate the multifactorial action of the extender on spermatozoal resistance against freezing injuries. These factors may not give general information about its effect on cryopreservation success but interactions might explain differences in the usefulness of extender constituents.

In conclusion as far as we know, this is the first report on successful cryopreservation of *Salmo trutta macrostigma* sperm with regard to post-thaw motility. On the other hand, additional studies are necessary to evaluate the viability, survival, and development of larvae produced from cryopreserved sperm.

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ULTRASTRUCTURAL VIEW OF THE MACROPHAGES OF TESTES OF TWO OHRID SALMONS, OHRID TROUT (*SALMO LETNICA* KAR.) AND OHRID BELVICA (*ACANTHOLINGUA OHRIDANA*)

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ULTRASTRUKTURNI IZGLED MAKROFAGA U TESTISIMA DVE OHRIDSKE PASTRMKE, OHRIDSKE PASTRMKE (*SALMO LETNICA* KAR.) I OHRIDSKE BELVICE (*ACANTHOLINGUA OHRIDANA*)

Abstrakt

U radu je data uporedna ultrastrukturalna analiza makrofaga testisa Ohridske pastrmke (*Salmo letnica* Kar.) i Ohridske belvice (*Acantholingua ohridana*) u periodu posle mresta. U semenifernim režnjevima ovih vrsta sa degenerisanim Sertolijevim materijalom može se opaziti prisustvo makrofaga. Oni se nalaze ili u zidu režnjeva, ili u njihovoj šupljini. Makrofagi poseduju karakterično produbljeno jedro. Neki makrofagi imaju proširen perinuklearni prostor. Makrofagi imaju segmentirano jedro, što je karakteristično za sve ćelije i citoplazmu bogatu fagolizozomima. Njihovo prisustvo u semenim režnjevima kada teče intenzivna involucija tj. degeneracija Sertolijevih ćelija može se opaziti po njihovoj eventualnoj fagocitozi, odnosno eliminaciji nekrotičnog materijala koji potiče iz Sertolijevih ćelija.

Ključne reči: *Ohridska pastrmka (Salmo letnica Kar.), Ohridska belvica (Acantholingua ohridana), testis, makrofagi, period posle mresta*

INTRODUCTION

In literature there are data wich describe macrophages as phagocytotic elements of testes in many Teleost fish (Chavez-Pozo et al., 2005, 2007;Hales, 2002; Hedger, 2002; Lahnsteiner & Patzner, 1990; Liarte et al., 2007; McClusky, 2005; Micale et al, 1987;

Sepulcre et al., 2002; Young, 2001). Also macrophages was noticed in Chondroichtyes, in the spotted ray (*Torpedo marmorata*) by Prisco et al. (2003). There are literature data which poin to the participation of the macrophages (besides Sertoli cells) in the elimination of the sperm residues with some species of Teleostea in the period after the spawning.This phenomenon with *Salvelinus fontinalis* was described by Henderson (1962), with *Rutilus rutilus dojranensis* K a r . by Dimovska (1965), with *Oryzias latipes* by Gresek et al. (1973) and also was stated with other Vertebrata, for example with swan (*Cygnus olor*) by Breucker (1978). The presence of macrophages with *Preca fluviatilis macedonica* K a r. in the period after the spawning was pointed out by Dimovska et al. (1986/87), Tavciovska-Vasileva (1992). According to Loir et al. (1995) with trout the interlobular macrophages can participate in the reinitiation of the spermatogonial proliferation. Macrophages was noticed in Salmonidae, in rainbow trout (*Salmo gairdneri*) by Billard et al. (1983); Scott & Sumpter (1989); Van den Hurk et al. (1978). The presence of these phagocytotic elements also was noticed with Ohrid trout (*Salmo letnica* Kar.) and Ohrid belvica (*Acantholingua ohridana*) in the period after the spawinig by Tavciovska-Vasileva (1999, 2001), Tavciovska-Vasileva & Rebok (2004). The presence of macrophages is connected with the phagocytosis of Sertoli cells, because they are present in interstitium, as well as in the seminiferous lobules.

MATERIAL AND METHODS

Testes of sexually mature Ohrid trout (*Salmo letnica* Kar.) and Ohrid belvica (*Acantholingua ohridana*) males caught in and Ohrid Lake were analysed. Analyses have been done with electronic microscope. Small parts of testes 1-2 mm big have been used for electronic microscopy. The material has been fixed by the following procedure: immediately after the tissue sections have been taken, they are fixed in 3% glutaraldehyde and then conserved in 0, 1 M phosphate buffer. After adequate fixation the material has been submitted to postfixation in 1% osmium tetraoxid (OsO4). In the further treatment the material has been washed in phosphate buffer, dehydrated in series of acetone and uranil acetate, and after that it has been dehydrated in dry acetone. The tissue sections have been infiltrated with Durcopan ACM mixture, a mixture of acetone-Durcopan, Durcopan No. 1, Durcopan No. 2, fit in Durcopan No. 2 and polymerised. For the ultrastructural analysis, ultrathin sections of 40-60 ηm tickness have been prepared, with the help of galss knives, on Reichert-Yung "Ultacut" ultramicrotome, installed on copper nets, contrasted with uranil acetate and lead cytrate. The sections have been observed on Tesla BS 500 and OPTON (Zeis) EM 109 electronic microscope. The microphotographs for electronic microscopy have been photographed with Agfa Scientia EM Film 23056/6, 5 x 9 cm, ORWO NP 20 panchromatic 120, KODAK 120 and made on Agfa Papirtone Paper P1-3.

RESULTS

In the seminiferous lobules of Ohrid trout (*Salmo letnica* Kar.) where Sertoli degenerated material is present, it is characteristic that there are macrophages located in the wall of the lobules, in their lumen or in the interstitium (Fig. 1). The macrophages possess a characteristic deepened nucleus. Some macrophages have widened perinuclear space (Fig. 2).

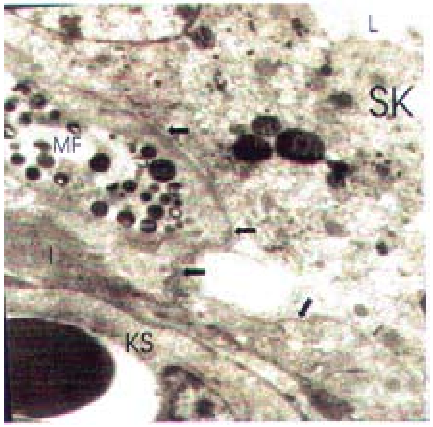

Figure 1. A part of the cytoplasm of Sertoli cell (SK) in degeneration. A part of the lumen (L) of the lobulus. Developed interstitium (I) with macrophage (MF) and blood vessel (KS). Well seen basal lamina (arrows) of the lobulus. Ultrathin section, x 4.400.

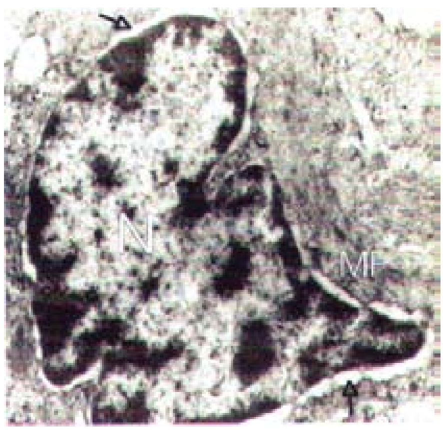

Figure 2. Macrophafe MF with deepened nucleus (N) with widened perinuclear space (arrows). Ultrathin section, x 12.000

The presence of macrophages in seminiferous lobules in the period of intensive degenetation, i. e. involution od Sertoli cells shows that these phagocyte elements probably participate in elimination (phagocytosis) of Sertoli necrotic material. Macrophages can be noticed in the lobules with the Sertoli degenerated material. With Ohrid belvica (*Acantholingua ohridana*) the macrophages have been noticed in the wall of the lobules, as well as in the lumen. At ultrastructural level we can see that macrophages possess a segmented nucleus (Fig. 3) which is characteristic for these cells, and a cytoplasm reach with phagolysosomes (Fig. 4, 5). Their presence in the seminiferous lobules when intensive involution, i. e degeneration of Sertoli cells goes on, can be connected with their eventual phagocytosis, i. e. elimination of the necrotic material, which originates from the Sertoli cells.

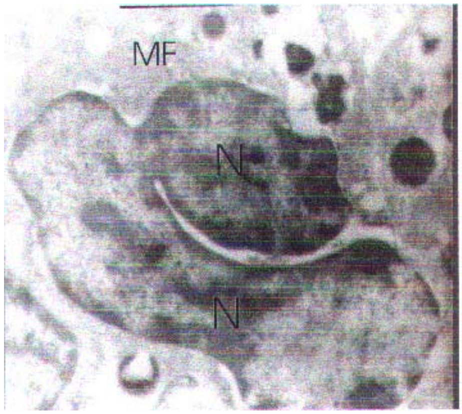

Figure 3. Macrophage (MF) with segmented nucleus (N). Ultrathin section, x 12.000.

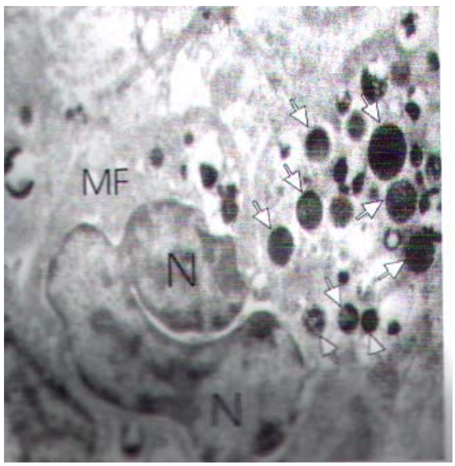

Figure 4. Macrophage (MF) and cytoplasm rich with phagolysosomes (arrows). Ultrathin section, x 7.000.

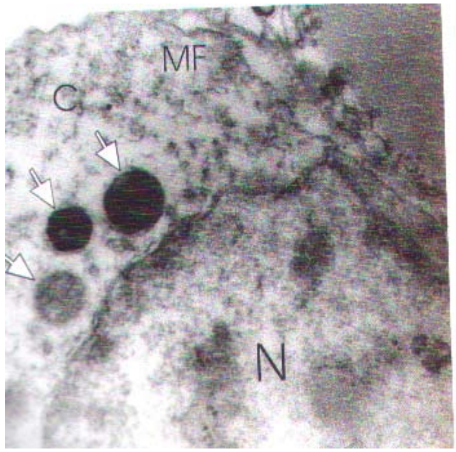

Figure 5. A part of macrophage (MF) with nucleus (N) and cytoplasm (C) with phagolysosomes (arrows). Ultrathin section, x 12.000.

DISCUSSION

In the testes of these two analysed species Ohrid trout (*Salmo letnica* Kar.) and Ohrid belvica (*Acantholingua ohridana*) the presence of macrophages have been stated on the level of interstitium, as well as in the wall or in the lumen of the seminiferous lobules by Tavciovska-Vasileva (1999, 2001), Tavciovska-Vasileva & Rebok (2004). Macrophages was noticed in Salmonidae, in rainbow trout (*Salmo gairdneri*) by Billard et al. (1983); Scott & Sumpter (1989); Van den Hurk et al. (1978). Their presence can point to their possible participation in the elimination of the Sertoli necrotic material. This phenomenon in *Salvenilus fontinalis* was pointed out by Henderson (1962), *Rutilus rutilus dojranensis Kar*. (Dimovska, 1965), *Oryzias latipes* (Gresek et al., 1973), *Perca fluviatilis macedonica Kar.* (Dimovska et al, 1986/87; Tavciovska-Vasileva, 1992). In literature there are data wich describe macrophages as phagocytotic elements of testes in many other Teleost fish (Chavez-Pozo et al., 2005, 2007;Hales, 2002; Hedger, 2002; Lahnsteiner & Patzner, 1990; Liarte et al., 2007; McClusky, 2005; Micale et al, 1987; Sepulcre et al., 2002; Young, 2001). Also macrophages was noticed in Chondroichtyes, in the spotted ray (*Torpedo marmorata*) by Prisco et al. (2003). It is also stated with other Vertebrata, for example with swan (*Cygnus olor*) by Breucker (1978). Because of the fact that they appear in the interlobular interstitium, as well as in the wall and in the lumen of the seminiferous lobules when degeneration, i. e. involution of Sertoli cells happenes, their presence can be connected with phagocytosis of the residues which originate from Sertoli necrotic (degenerated) material. With the trout, according to Loir et al. (1995) the interlobular macrophages can participate in reinitiation of the spermatogonial proliferation.

CONCLUSION

With the cytological analysis based on ultrastructural findings of the testes of Ohrid trout (*Salmo letnica* Kar.) and Ohrid belvica (*Acantholingua ohridana*) we can state that macrophages can be noticed in the wall and in the lumen of some seminiferous lobules, and their role can be connected with the elimination of Sertoli necrotic material.

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MARINE FISH EGG QUALITY INDICATORS IN AQUACULTURE, A REVIEW OF TECHNIQUES AND RESULTS

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KVALITET JAJA MORSKIH RIBA U AKVAKULTURI, PREGLED TEHNIKA I REZULTATA

Extended abstract

Background: Despite most of marine teleosts can produce large quantities of viable eggs in captivity, the quantity and quality of the larvae are low and variable, being the initial quality of the eggs one of the main causes of the variability. Considering that commercial hatcheries rely on good larval quality, the establishment of a series of criteria for egg quality is of paramount importance.

Objectives: This review presents an overview on some of recent researches carried out in IRTA and University of Salzburg (2004-2010) to address this bottleneck through correlating egg biochemical contents (BC) to embryo/larva success in a marine teleost considered as new species for Mediterranean aquaculture.

Target species: Besides production cost reduction and market enlargement, species diversification is one of the main strategies to ensure future expansion of aquaculture industry. A series of biological and commercial characteristics make the common dentex, *Dentex dentex*, a highly suitable species to enter mariculture systems as a new species to supplement the intensive sparids production and to increase diversification.

Applied methodologies: (i) Viable eggs were obtained from natural spawning of broodfish in captivity. (ii) Egg biometrical parameters (i.e., wet mass, dry mass, and water content) were recorded. (iii) Embryo/larva viability parameters (VPs, i.e., floating rate, hatching rate, and survival rate) were determined. (iv) Egg biochemical parameters (i.e., carbohydrate metabolites/enzymes, vitellogenin-derived proteins [VDP], non-Vtg-derived protein [non-VDP], free amino acid [FAA], proteinic amino acids [PAA], lipid classes [LC], fatty acids [FA], and morphological characteristics of lipid vesicle [LV]) were characterized and quantified. (v) A variety of statistical analyses were used to define the relationships existing between the egg BCs (as egg quality indicators) and the VPs (as criteria for embryo/larva success) that are currently used in mariculture systems.

Results: Although a combination of statistical methods were used to correlate the egg BCs to embryo/larva success in common dentex, the current review was prepared based on the results of regression models. Carbohydrate (CH) metabolite contents and enzyme activity of the egg together with VDP, non-VDP, FAA, PAA, LC , and FA contents for one side and lipid vesicle (LV) morphological characteristics on the other were significantly and strongly correlated to embryo/larva success through almost 350 simple regression model (11 [r^2=0.184–0.490] for CHs and enzymes, 16 [r^2=0.095–0.634] for VDPs, 55 [r^2=0.079–0.637] for non-VDPs, 49 [r^2=0.605–0.875] for FAAs, 19 [r^2=0.919−0.991] for PAAs, 5 [r^2=0.459–0.739] for LCs, 201 [r^2=0.640–0.948] for FAs, and 10 [r^2=0.293–0.480] for LV).

Conclusions: (i) Under a biological/physiological perspective the significant relationships found between egg BCs and embryo/larva success in common dentex highlight the importance of egg composition during the initial events of embryonic/larval development. The results obtained present a series of new relationships (either in agreement or against previous findings) that deserve further investigation to define their physiological ground. (ii) Under a more applied perspective these relationships indicate a potential use of egg BCs, as bio-markers, to predict egg quality in aquaculture. These markers can also be used as molecular probes to assess the improvements in broodstock management.

Key words: *Dentex dentex, egg quality, embryo/larva success, mariculture*

INTRODUCTION

Although most of marine teleosts can produce large quantities of viable eggs in captivity, the quality of larvae is low and variable (Samaee, 2010 and references therein). The initial quality of eggs is accounted for as one of the main causes (Brooks et al., 1997). The review aim at to present an overview on the results obtained (both published and new findings [unpublished data]) by the authors of the current article to address this bottleneck through correlating common dentex, *Dentex dentex*, (a new candidate for Mediterranean aquaculture to increase the diversification in mariculture systems) egg biochemical contents –BC- (as egg quality indicators) to a series of viability parameters –VP- (as criteria for embryo/larva success) that are currently used in mariculture systems.

2. DETERMINATION OF EMBRYO/LARVA VPs

Common dentex eggs were obtained from natural spawning of broodfish (Giménez et al., 2006). Floating rate –FR- (the ratio of floating eggs to the total eggs collected) was promptly determined after collecting egg samples while hatching rate –HR- (the ratio of hatched eggs to the incubated eggs), and survival rate –SR- (the ratio of larvae that survived at a certain day post-hatch [dph] to the incubated eggs) after incubating egg samples into 96 well microplates (Shields et al., 1997).

3. EGG BCs IN RELATIONSHIP WITH EMBRYO/LARVA SUCCESS

3.1. Carbohydrate metabolites/enzymes

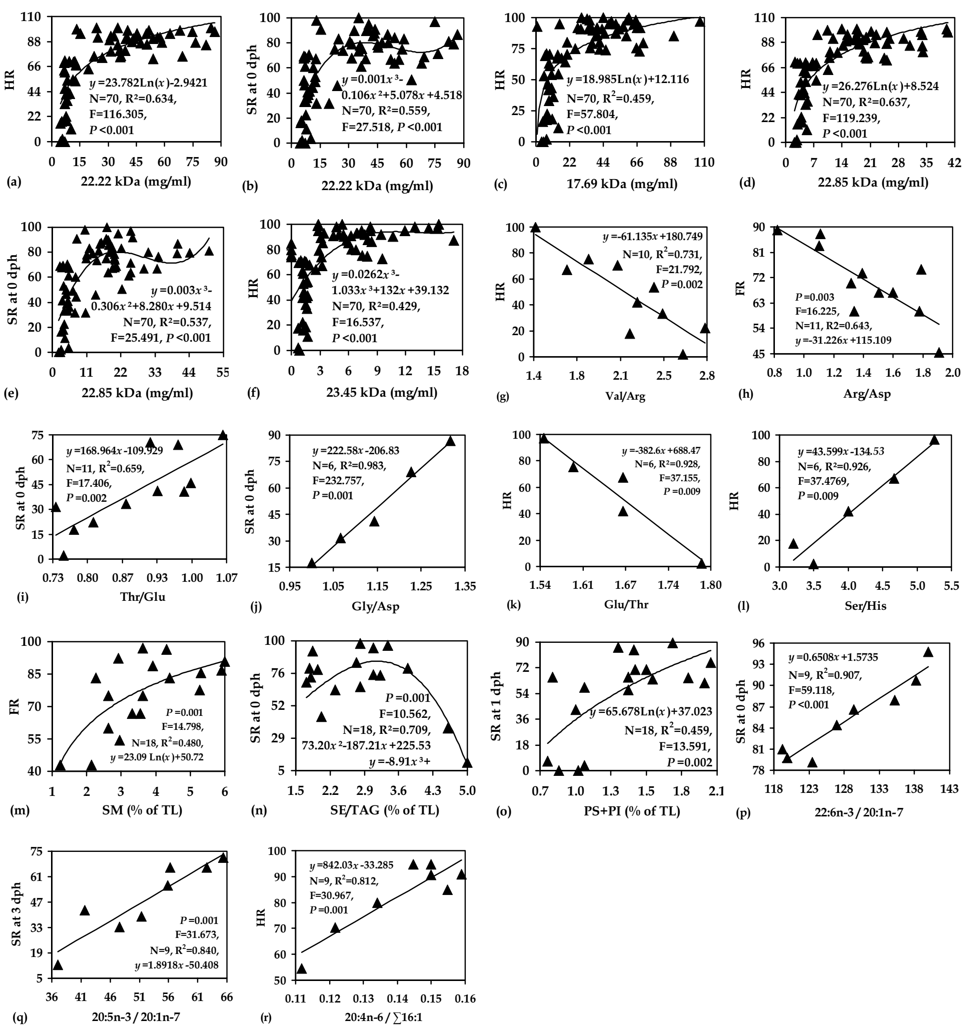
Spectrophotometry-based techniques were used to analyse metabolites and enzymes. The concentration of 4 metabolites (i.e., free 6-deoxyhexose, free monosaccharides, free ribose, and glucose) and 2 enzymes (i.e., pyruvate kinase and alkaline phosphatase) were found to be significantly linked to VPs through 11 simple regression models (SRM) with r^2=0.184□0.490 and p<0.001□p=0.053 (Giménez et al., 2006).

3.2. Egg yolk vitellogenin (Vtg)-derived proteins (VDP)

VDPs were purified (chromatography [Tsirogianni et al., 2004]), detected (SDS-PAGE), identified (LC-ESI-MS/MS), characterized (databank search), and quantified (densitometry). The concentrations of the 5 proteins (i.e., 77.97, 57.22, 17.69, 16.95 kDa [as VtgAb-derived proteins], and 22.22 kDa [as a VtgAa-derived protein]) were significantly correlated to VPs through 16 SRM (e.g., Fig. 1: a, b, and c) with r^2=0.095□0.634 and p<0.001□p=0.009 (Samaee et al., 2009a).

3.3. Egg non-vitellogenin-derived proteins (non-VDP)

Egg crude proteins were extracted, separated, and detected (SDS-PAGE). Then non-VDPs were characterized (specific-staining systems) and quantified (densitometry). The concentrations of 15 non-VDPs, with a frequency > 50%, (i.e., 105.43, 63.77, 50.48, 43.50, 33.59, 32.41, 31.40, 26.65, 25.56, 24.86, 24.01, 23.45, 22.85, 20.44, and 19.59 kDa) were significantly correlated to VPs through 55 SRMs (e.g., Fig. 1: d, e, and f) with r^2=0.079–0.637and p<0.001□p=0.001 (Samaee, 2010).



3.4. Egg free amino acids –FAA- (unbound amino acids)

FAAs were extracted, derivatised (Waters Pico-Tag method [Bidlingmeyer et al., 1986]), separated (HPLC), characterized (Spectra Physics SP4290 Integrator with WINner software), and quantified (Lyndon et al., 1993). The contents of 7 essential (i.e., arginine [Arg], valine [Val], isoleucine [Ile], leucine, tyrosine, threonine [Thr], and histidine [His]) and 8 non-essential (i.e., glutamic acid [Glu], asparagines, glutamine [Gln], aspartic acid [Asp], serine [Ser], glycine [Gly], proline, and cysteine) FAAs were found correlated with VPs through 49 SRMs (e.g., Fig. 1: g, h, and i) with r^2=0.605–0.875 and p<0.001□p=0.005(Samaee et al., 2010).

3.5. Egg proteinic amino acids –PAA- (bound amino acids)

Proteins were precipitated, and hydrolysed (incubation in acid [Finn et al., 1995]), and PAAs analysed as described for FAAs (Lyndon et al., 1993). The ratios of 7 essential (i.e., Thr, methionine [Met], Arg, Ile, Val, phenylalanine [Phe], and His) and 5 non-essential (i.e., Ala, Glu, Gly, Asp, and Ser) PAAs were correlated to VPs through 19 SRMs (e.g., Fig. 1: j, k, and l), with r^2=0.919−0.991 and p<0.001□p=0.009 (new findings [unpublished data]).

3.6. Egg lipid classes (LC)

After extraction of total lipid (Folch et al., 1957) LCs were separated, characterized (thin layer chromatography [Olsen and Henderson, 1989]), and quantified (densitometry [Henderson and Tocher, 1992]). Egg sphingomyelin (SM), phosphatidylcholin, phosphatidylserine (PS), phosphatidylinositol (PI), steryl+wax ester (SE+W), and triacylglycerol (TAG) contents were significantly correlated to VPs through 5 SRMs (e.g., Fig. 1: m, n, and o) with r^2=0.459–0.739 and p<0.001□p=0.002 (Samaee et al., 2009b).

3.7. Egg fatty acids (FA)

FAs were methylated (Christie, 1982), extracted (Tocher and Harvie, 1988), characterized and quantified (gas–liquid chromatography [Ackman, 1980]). The absolute concentrations, ratios, or combinations of 20 FAs (i.e., 14:0, 15:0, 16:0, 16:1n-7, 18:1n-7, 18:1n-9, 20:1n-9, 20:1n-7, 22:1n-11, 18:2n-6, 20:2n-6, 20:3n-6, 20:4n-6, 18:3n-3, 18:4n-3, 20:4n-3, 20:5n-3, 22:4n-6, 22:6n-3, and 24:1n-9) were significantly correlated to VPs through 201 SRMs with r^2=0.640–0.948 and p=0.0001–0.006 (Samaee et al., 2009b and new findings [unpublished data]).

3.8. Morphology of lipid vesicle (LV)

Since the shape of egg LV depends on its lipid composition, the characteristics of the egg factor is also considered in the review. Egg samples were photographed under a stereomicroscope at 25-fold magnification, pictures calibrated by means of micrometer scales, and the major and minor axes of the LV were measured. The ratio of the maximal to the minimal diameter of LV (RD) and lipid vesicle shape coefficient (SC), and embryo viability estimated from both RD and SC were significantly correlated to VPs through 10 SRMs with r^2=0.293–0.480 and p<0.001 (Lahnsteiner et al., 2008).

CONCLUSIONS

(1) Under basic aspects the significant and strong relationships found are addressing a crucial point of reproductive biology highlighting the importance of egg BCs during initial events of life in a marine pelagophil teleost such as common dentex. The results also present a series of new relationships (either in agreement or against previous findings) that deserve further investigation to define a physiological ground.

(2) Under applied aspects the efficiency of egg BCs to predict embryo/larva viability at critical stages of development indicates the potential use of egg parameters (that can be measured either in unfertilized eggs or at early developmental stages) as egg quality bio-markers that can also be used as molecular probes to assess improvements in broodstock management.

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SEA TURTLES IN IRAN, POPULATION ASSESMENT, ECOSYSTEM HEALTH AND CONSERVATION STATUS

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MORSKE KORNJAČE U IRANU, PROCENA POPULACIJE, ZDRAVLJA EKOSISTEMA I KONZERVACIONI STATUS

Globally, populations of sea turtles are declining. All species of sea turtles are listed in Appendix I of the CITES (1). This means that all turtle species are considered endangered by international trade to such an extent that if commercial trade is not eliminated with respect to these species, they will become extinct. Nesting density of hawksbill turtles is low throughout its range (4, 11). Over-hunting of marine turtles for their shells, meat and over-collecting of eggs from nests are some factors attributing to the endangered status of turtles. (5) It is believed that the declining turtle populations in the Iranian waters specially Persian Gulf has been accelerated by the breakdown of traditional conservation practices, the use of powered boats in turtle hunting, commercial sale, habitat degradation, incidental by-catch in fishing gear, and the large scale harvesting of eggs in rookeries. The factors that are known to cause decline in sea turtle populations are generally similar but differences do exist in terms of importance for different populations i.e. in different parts of the world, and with changing laws and technologies through time. For example, before the widespread use of trawlers and high seas gill-nets, turtle mortality caused by fishing was minimal but laws were not in force then to protect turtles and their products (5). Hence, there was widespread hunting of turtles for meat, shell and leather. Eggs were also collected extensively for food. Seas were not as polluted then, hence mortality caused by plastics, tar balls, pollutant induced diseases were not as extensive. Similarly, the degree of importance of factors threatening turtles in different parts of the world does differ. A constant cause for decline, independent of time, is when mortality is greater than recruitment. Mortality and recruitment vary, depending on predation, food availability and quality, habitat quality, and many other factors. Because the life cycle of a sea turtle is complex, and includes large periods of time and large expanses of the planet, mortality can occur at many places and many times during an individual turtle's life (2). Natural threats are indiscriminate and may affect any species. Natural predation on eggs and hatchlings is thought to be kept in check by natural balances of predator prey relationships (6). Predation is so high that it is obvious that a number of terrestrial, marine and avian species depend on sea turtles as a source of protein. Anthropogenic threats to nesting habitats are again indiscriminate and driven more by coastal development, industrialization and the recreational opportunities provided by coastal environments(6).

In Table (1) number of nests in some important site are shown. The numbers represent the actual tally of records recorded during the surveys, some of which may not represent a good estimate for the month due to limited surveys conducted on some beaches during some months.

Table1. Total hawksbill nests and nesting densities expressed as nest per kilometer

Island	Total nests	Beach length(km)
Shidvar	36	2.1
Lavan	24	3.2
Qeshm	56	6.7
Hormuz	34	3.4
Farour	35	4.6
Hendurabi	46	5.7
Overall	231	25.7

Threatening factors

Table 2. Current anthropogenic threat to sea turtle populations in Some of Iranian Islands

Threat	Shidvar	Lavan	Qeshm	Hormuz	Farour	Hendurabi
Habitat alteration and loss	Yes	Yes	Yes	No	No	No
Beach armouring (e.g., concrete sea walls)	No	Yes	Yes	No	No	No
Beach nourishment/sand mining	No	Yes	Yes	No	No	No
Beach cleaning and beach driving	No	No	Yes	No	No	No
Human presence on beach	Yes	Yes	Yes	Yes	No	Yes
Artificial light	No	No	Yes	No	No	No
Boat strikes	Yes	Yes	Yes	Yes	No	Yes
animal predation at rookeries	Yes	No	No	Yes	Yes	No
Oil pollution	?	Yes	Yes	?	No	No
Other pollution sources and entanglement	Yes	Yes	Yes	Yes	No	No
Fishing and incidental capture	No	Yes	Yes	Yes	No	No
Shrimp trawling	No	No	Yes	No	No	No
Pelagic fishing gear	Yes	Yes	Yes	Yes	No	No
Gill nets	No	No	Yes	No	No	No
Traditional and commercial fishing	Yes	Yes	Yes	No	No	No
Egg harvests	Yes	Yes	No	No	No	No
Adult harvests	Yes	Yes	No	No	No	Yes

In fallowing photos most important threatening factors for sea turtles are shown, these photos can say us everything about these valuable animals and their future.

Figure 1. Human activities in nesting sites of sea turtles (Oman Sea beach)

Figure 2. Natural parameters preventing from nesting activity of sea turtles (Shidvar Island)

Most important conservative programs in Iran are nomination of sea turtles as "Endangered animals" of the country, there was a fine of about 3,200,000 Rials for each killed turtle which doubled in the past year (6,400,000 Rials), about $US 700 and also fine for egg collection, about $US 233 for each, nominating of the nesting sites as" under management and control Area" like Mond protected area and Shidvar wildlife refuge and Monitoring of the sites by DOE guards.

All measures that prevent sea turtles from being killed would be of priority. These are:

- Conservation measures or techniques that reduce the incidental catch of adult and juvenile turtles in fishing gears e.g.: (i) use of TEDs in trawlers (shrimp and fishing); (ii) regulate or ban the use of high seas gill-nets; (iii) regulations to protect turtles or restrict the use of fishing methods harmful to turtles off their nesting grounds during the nesting season.

- Conservation measures to curb the hunting and trade of live turtles, adults and juveniles, for meat and other turtle products.
- Conservation measures to curb commercial exploitation of eggs, both legal and illegal.
- Conservation measures to curb the destruction of nesting grounds by beachfront development, seawalls, land reclamation, etc
- Conservation measures to curb the destruction of feeding grounds by trawlers, pollution, land reclamation, etc.
- Conservation measures to prevent the killing or drowning of turtles in man-made structures (e.g. oil rigs) or by powered watercrafts.

In order to respond to a critical conservation situation, as is the case of these sea turtle populations, an agreement must fulfill some requirements: (1) it must include all, or most of the countries involved in the problem; (2) it must be an "agile" organization, capable of facing a dynamic situation without getting bogged down with time consuming formalities; (3) it must turn words into actions very rapidly; and perhaps also, (4) it must have the capability to implement and execute a comprehensive program, and (5) if possible, it is preferable that the agreement is a binding one. It is quite clear that the institution must have a level of credibility with the different stakeholders.

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SEASONAL AND TEMPORAL DISTRIBUTION OF SOME POLLUTANTS IN SOUTH EASTERN BLACK SEA COAST

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SEZONSKA I VREMENSKA DISTRIBUCIJA NEKIH POLUTANATA U SEVERO-ISTOČNIM DELU CRNOG MORA

Abstract

The Black Sea coastal waters remain heavily impacted by sewage and marine activities. In this study, temporal and spatial distributions of some pollutants from marine and terrestrial activities were investigated in the water column of the southeastern Black Sea between November 2009 and October 2010.

Temperature, dissolved oxygen, pH, salinity, turbidity and alkalinity levels in surface water were 9.17-27.44°C, 7.28-10.90 mg/L, 7.79-8.51, 15.43-17.68 ppt, 14.5-20.20 NTU, and 150-185 mg/L, respectively. Maximum concentration of pollutants were found to be as 18.72 mg/L total suspended solids, 14.21 µg/L phenol, 28.80 µg/L anionic detergent, 102.90 mg/L oil and grease, <5 ppb Cd, 42 ppb Fe, 16 ppb Cu, 71 ppb Zn, and 23 ppb Pb in the water samples. Based on maximum concentration levels of contaminants observed in the present study, it can be concluded that phenol, copper, oil and grease values exceeded general marine water quality criteria, defined by general sea water quality standards in Turkish Water Contamination Regulations. The results indicated that seasonal and spatial distributions of pollutants were found to be irregular and the coastal waters of south eastern Black Sea impacted from marine and terrestrial activities.

Key words: *Southeastern Black Sea, Rize Port, Marine pollution, Metal pollution*

THE POTENTIAL OF COMMERCIAL FARMING OF THE EUROPEAN FLAT OYSTER, *OSTREA EDULIS*, IN THE KOTOR BAY (ORAHOVAC)

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MOGUĆNOST KOMERCIJALNOG UZGOJA ŠKOLJKE KAMENICE, *OSTREA EDULIS* U USLOVIMA KOTORSKOG ZALIVA (ORAHOVAC)

Abstrakt

Kamenica, ostriga, *Ostrea edulis*, je jadranska školjka, koja se već nekoliko decenija vještački gaji u Jadranu, najviše na hrvatskom primorju. Izuzetno je cijenjena, te stoga dostiže i visoku cijenu na tržištu. Nakon ranijih eksperimentalnih istraživanja Instituta sedamdesetih godina prošlog vijeka, ova vrsta je po prvi put, 2010. godine komercijalno uzgajana u uslovima Bokotorskog zaliva, u mjestu Orahovac kod Kotora. Institut i kompanija „D.O.O. Školjke Boke" su u julu mesecu uvezli 9000 jedinki mlađi iz poznatog uzgajališta u Stonu (HR). Školjke su nasađene na lokaciji Orahovac u neposrednoj blizini reke Ljute u severnom delu Kotorskog zaliva. Primjenjen je prirodno-veštački način uzgoja, gde se kamenice vešaju na brodske konope povezane sa polietilenskim bovama, na dubinu 4–7 metara. Mereni su osnovni hidrografski parametri, temperatura i salinitet, kao i dužinski rast na 100 slučajno odabranih jedinki iz uzgajališta. Maksimalne temperature 27.6 T°C izmerene su u julu a najniže 11.2 T°C. Vrednost saliniteta je bila najveća u avgustu i iznoslila je 27.8 ‰ a minimum je bio u decembru 6.3 ‰. Temperaturni opseg je uobičajen za područje Kotorskog zaliva, dok su vrednosti saliniteta bile neuobičajeno niske u jesenjem periodu, čak i na dubinama 4–5 metara, što je bilo uslovljeno izuzetno velikim količinama padavina. Istraživanja pokzuju da je rast od jula do oktobra bio ujednačen i ukupno je iznosio 1cm, odnosno u proseku 0.25 cm po mesecu. Od oktobra do januara nastupa nešto usporeniji rast i ukupno iznosi 0.3 cm. Od januara ponovo nastupa nešto pojačani intezitet rasta koji ukupno iznosi 0.5 cm. Dakle u periodu od 8 meseci (jul–mart) ukupni rast iznosi 1.9 cm.

Rast kamenica zavisi od mnogo faktora, a najviše od količine hrane (planktona) i temperature. Kako i naša istraživanja pokazuju, rast je najintezivniji bio u letnjim mesecima, kada su i povoljne temperature koje uslovljavaju u povećanu količinu planktonskih zajednica. Salinitet takođe igra značajnu ulogu u rastu kamenica, ali su one otpornije na kolebanja saliniteta od dagnje, koja se takođe veštački gaji. Do skoro identičnih istraživanja došao je Glamuzina u Malostonskom zlivu 2006, kada je konstatovao ukupni rast 1.7 cm za period jun–april. Merenja smrtnosti pokazala su nešto povećane vrednosti (15%) u julu neposredno posle postavljanja jedinki u more, što je verovatno uslovljeno transportnim šokom i prilagođavanju uslovima u vodi Kotorskog zaliva. U kasnijim mesecima on opada i od septembra do marta iznosi 0%. Onda se u martu 2011. pojavljuje uobičajeni problem predatora, kada ribe orade *Sparus aurata*, napadaju uzgajalište i čine veliku štetu, naročito na cementiranim nizovima kamenice, jer su više otvorene nego one u kašetama. Svi dosadašnji podaci pokazuju, da uslovi vode u Kotorskom zalivu, naročito na ušću rijeke Ljute, izuzetno pogoduju dobrom rastu i kondiciji ove vrste, što ide u prilog njenom komercijalnom uzgoju, ćime će se pored dagnje, *Mytilus galloprovincialis*, upotpuniti ponuda školjkaša, naročito u vrijeme turističke sezone na crnogorskom primorju.

Ključne reči: *Kamenica, marikultura, Kotorski zaliv, dužinski rast*

INTRODUCTION

European flat oyster, *Ostrea edulis,* Linnaeus, 1758 (ordo: Bivalvia) is a sea shell from the Ostreidae family, elongated and heart–shaped, whose natural habitat is in the Mediterranean, but is also found in the eastern Atlantic Ocean and the Black Sea. It lives in areas of brackish water up to 10 m in depth, attached to the sediment in sparsely populated colonies. Its body is located between two asymmetrically shaped shells connected by a strong muscle. The shells are stone–grey in colour, which helps the animal to stay hidden on the sediment. It feeds on plankton retained by filtering seawater. It can reach lengths of up to 13 cm and weights of up to 100 g. It is predated on by starfish, snails, crustaceans, and fish.

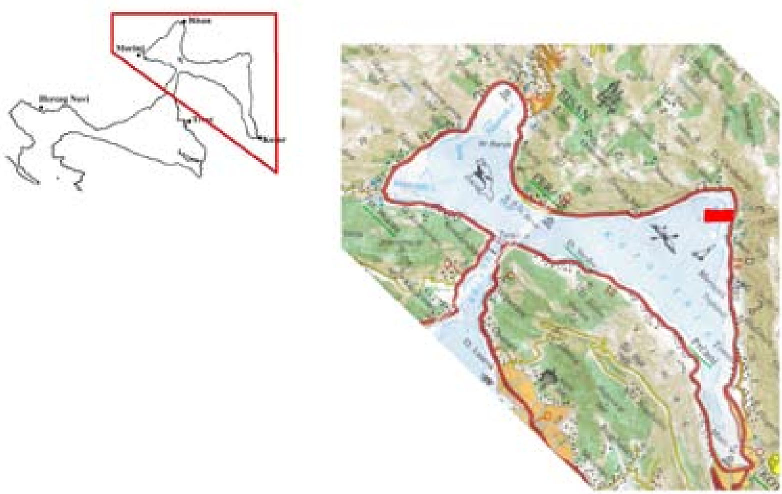

Figure 1. Map of Kotor Bay, locality of Orahovac (red)

European flat oyster has been considered a delicacy since the Roman times, due to the salty taste of its flesh, and is usually consumed raw. High levels of protein, carbohydrates and vitamins give the flesh its nutritional value (Tomšić & Lovrić, 2004). Due to this, it is artificially farmed in the Mediterranean, especially in Croatia in the Lim Bay and Bays of Pula and Mali Ston. The annual production in Croatia is about 4000 t (Statistical Yearbook Repablic of Croatia, 2009). Due to the negative influences it has been virtually wiped out from it natural habitat. It was once abundant in the Boka Kotorska Bay, especially in the Kotor Bay, an area with a high influx of freshwater from the Ljuta River. Unfortunately, specimens naturally found in the Kotor Bay are now few and far between. In the 1970s, the Institute of Marine Biology did some research on the European flat oyster (Stjepčević, 1974). Since then, 20 mussel (*Mytilus galloprovincialis*) farms were established in the Bay, but so far there have been no oyster farms. Due to this, the "ŠKOLJKE BOKE" company desires to re–establish oyster farming in the Kotor Bay area, under the expert guidance of the Institute of Marine Biology.

METERIALS AND METHODS

The individual oysters were imported from the oyster farm in Mali Ston in July 2010. All necessary permits for import of biological specimens were previously obtained under the surveillance of the Institute of Marine Biology in Kotor. A total of 9000 specimens were seeded at the newly founded private oyster farm of the "ŠKOLJKE BOKE" company in Orahovac, near the Ljuta river.

Figure 2. Oyster, *Ostrea edulis* and the oyster farm in Orahovac

Figure 3. Oysters attached to the ropey with cement

The basic hydrographical parameters were taken at the oyster farm, temperature and salinity, along with mortality and growth. A longline farming system was used, in which oysters are attached to ropes reaching 4–7 m in depth, which are then connected to polyethylene buoys. 80% of the total number were cemented to the surface, while the remaining 20% were grown in boxes. Length, height and width (thickness) were measured on a monthly basis on 100 randomly selected individuals. Visual control of the algae growth on the oysters was also done at the same time.

RESULTS AND DISCUSSION

The results of the hydrographical and meristic measurements from the July 2010–March 2001 period are given in Table 1.

Tabela 1. Results of the hydrographical and meristic parameters from the Orahovac locality

DATE	DEPTH (m)	T (°C)	SALINITY (‰)	LENGTH (cm)	WIDTH (cm)	HEIGHT (cm)	MORTALITY
06.07.2010	5	27.6	21.1	6.5	5	0.85	0%
04.08.2010.	5	21.3	12.51	6.9	5	0.87	7.5 %
01.09.2010	5	25.2	27.8	7.2	5.2	0.90	5%
02.10.2010.	5	17.7	25.4	7.5	5.3	0.92	0%
15.11.2010.	5	13.5	8.8	7.6	5.5	0.92	0%
10.12.2010.	5	11.3	6.3	7.6	5.5	1	0%
10.01.2011.	5	11.2	6.4	7.7	5.5	1.1	0%
24.02.2011.	5	14.1	8.2	8.0	5.7	1.2	0%
15.03.2011.	5	14.5	8.6	8.2	5.8	1.3	15%
19.03.2011.	5	12.1	6.4	8.4	6	1.4	0%

The data demonstrates that the temperatures ranged from the maximum of 27.6°C measured in July to the minimum of 11.2°C in January. The highest salinity levels were measured in August at 27.8‰, with the minimum recorded in December at 6.3‰. The temperature range is quite common for the Kotor Bay (Stjepčević, 1974), but the salinity levels are significantly different, especially during the autumn period. This can be explained by high rainfall during the previous autumn, reducing salinity in the process, even at greater depths.

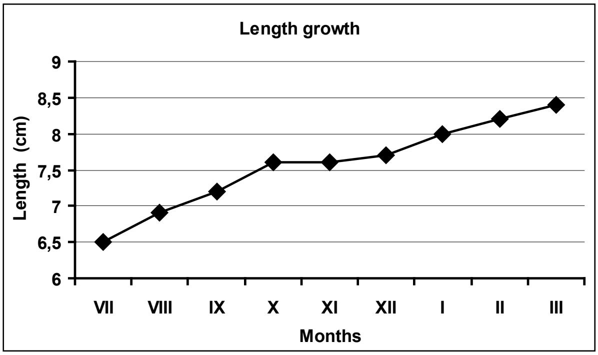

Figure 4. Graphic representation of the growth of oysters

As seen in Figure 4, growth in the July–October period was uniform. The research shows a uniform growth pattern in the July–October period. The total growth was about 1 cm, or approximately 0.25 cm per month. From October to January the growth slowed to a total of 0.3 cm. The growth intensity increased again in January, with a total of 0.5 cm. Therefore, the total growth for the entire eight–month (July–March) period was 1.9 cm. Oyster growth depends on a number of factors, but primarily on abundance of food (plankton) and temperature. As this research shows, the highest growth rate occurs during the summer months, when the temperatures suitable for plankton communities are the highest. Salinity also plays a significant role in the growth of oysters, but they are much more resistant to the salinity fluctuations than mussels, which are also farmed in the area (Stjepčević, 1974, Glamuzina, 2006, Marušić *et.al.,* 2010). The reduced growth during autumn months was mostly due to the high amounts of rainfall and increased freshwater influx. The autumn of 2010 had a high amount of rainfall, and the oysters had to be lowered deeper underwater to a depth of 7 m because of the low salinity levels (6.1‰) at the surface. The extremely low salinity values and low temperatures can cause negative growth, when the shell is forced to use its reserves for vital physiological processes. Glamuzina (2006) reported similar results from the Mali Ston Bay, where the total growth reported was 1.1 cm for the June–October period. He also reported a drop in growth rate, which was just 0.6 cm for the November–April period. This indicated similar hydrographical parameter changes in the Bays of Kotor and Mali Ston.

The mortality values were somewhat higher in July (15%), right before the seeding of the spat, probably caused by transport shock and subsequent acclimatisation to the conditions in the Kotor bay. The mortality later decreased, and was estimated at 7.5% in August and 0% in the September–March period. Higher mortality rate was avoided by lowering the oysters to greater depths during the rainy season and the reduced salinity caused by freshwater influx. In spring, oysters were exposed to gilthead seabream (*Sparus aurata*) predation, which can crush oyster's shell in order to get to the meat inside. This problem can be reduced by setting a protective net around the oyster beds, although nothing can guarantee a 100% protection.

The visual check–ups for the growth on shells determined that various organisms from the classes *Ascidiacea* and *Bryozoa*, *Polychaeta, Bivalvia* and various algae (*Phaeophyta* and *Rhodophyta*) can cause fouling of the oyster shells. This presents a problem as the presence of these organisms causes reduction in the available food for the oysters, and can reflect negatively on the growth of the shells.

CONCLUSIONS

Based on this preliminary research and comparisons with the earlier research done in the Kotor Bay, as well as the data from the Bay of Mali Ston, it can be said that hydrographical and biological conditions of the Kotor Bay offer extraordinary opportunities for oyster farming. The European flat oyster is highly priced, and along with the Mediterranean mussel could be considered a gastronomical part of the eco– and ethno–tourism, and could, in our humble opinion, play a significant role in the economy of Montenegro, as well as become a true Montenegrin brand in time. Of course, along with the great enthusiasm of the founders of this project, it is necessary to have the help of the Ministry of agriculture and rural development and the local authorities, who would have to recognise the importance and sustainability of the project.

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THE ESTIMATION OF SEA WATER QUALITY AT THE MONTENEGRIN COAST FOR MUSSELS FARMING

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PROCENA KVALITETA MORSKE VODE CRNOGORSKOG PRIMORJA ZA UZGOJ ŠKOLJKI

Abstrakt

Cilj ovoga rada bio je da se odredi kvalitet morske vode na Crnogorskog primorju vezano za uzgoj Mediteranske skoljke *Mytilus galloprovincialis* ili dagnje. Analizirana morska vrsta može se koristiti i kao bioindikatori zagađenja morske sredine. Uzorkovanje vode i divljih skoljki obavljeno je u periodu od jeseni 2005. do proleća 2007. godine, u četiri sezone, jesen i proleće, na četiri lokacije: u blizini Bar i Rt Đeran, na obali otvorenog mora, i u Bokokotorskom zalivu na lokacijama Sveta Stasija i Herceg Novi. Morske voda i škojlke uzorkovane su istovremeno na svakoj od ispitivanih lokacija u sve četiri sezone. U uzorcima morske vode određivani su fizičko-hemijski parametri, merenjem nutrienata (NO_3^- i PO_4^-), T, saliniteta i rastvorenog O_2, a u uzorcima dagnji teški metali: Hg, Cd, Pb, As, Cu i Zn.

Na osnovu izmerenih vrednosti nutrienata, temperature, saliniteta i rastvorenog kiseonika morske vode crnogorskog priobalja u četiri ispitivane sezone na četiri lokacije, može se zaključiti da je morska voda crnogorskog primorja optimalna za komercijani uzgoj školjki, jer se prvenstveno optimalni uslovi temperature i saliniteta morske vode nalaze u intervalu od 15 °C – 25 °C za temperaturu, i 20 ‰ – 35 ‰ za salinitet. Kiseonik je bio je najveći na lokaciji Sveta Stasija a bez velikih oscilacija na lokaciji Rt Đeran, u sve četiri ispitivane sezone. Sadržaj fosfata bio je veći u prolećnim uzorcima vode u odnosu na jesenje, dok je u slučaju nitrata bilo suprotno, njihov sadržaja bio je manji u prolećnim, a veći u jesenjim uzorcima morske vode.

Kada su u pitanju koncentracije teških metali u dagnji sa četiri lokacije u sve četiri ispitivane sezone, u odnosu na kriterijume po Airas-u (2003) koji morsku vodu u odnosu na koncentracije teških metala u školjki deli na pet klasa, od klase I, koja je nezagađena ili malo zagađena, do klase V koja je jako zagađena, razmatran je kvalitet morske vode na crnogorskom priobalju. Na osnovu ovog istraživanja, morska voda na lokaciji Sveta Stasija i Rt Đeran je I klase u odnosu na izmerenu koncentraju Hg, Cd, As, Cu and Zn u školjkama sa ovih lokacija u svim sezonama, međutim u odnosu na koncentraciju Pb u istim, morska voda je II klase ili umereno zagađena. Na osnovu izmerene koncentracije Hg u školjkama sa lokacija H. Novi i Bar, morska voda je zagađena na ovim lokacijama i pripada III klasi, dok u odnosu na izmerene koncentracije preostalih elemenata u školjkama sa ovih lokacija, morska voda je II klase ili umereno zagađena u sve četiri ispitivane sezone.

U odnosu moguće izvore zagađenja morske vode crnogorskog priobalja, posebno u Bokokotorskom zalivu gde su hirološki i biološki uslovi za njeno gajenje najpovoljniji, a u odnosu i na izmerene koncentracije teških metala u školjkama (Hg, Cd, Pb, As, Cu i Zn), školjke moraju biti prečišćene pre njihove komercijalne upotrebe kao hrane.

Ključne reči: *morska voda, fizičko-hemijski parametri, dagnja, tragovi metala, klase voda*

INTRODUCTION

The fact is that water and lands in the world today represent limited life resources which are more and more degrade and disturbed, mostly by anthropogenic influence. The investigation of Adriatic is more marked along the Italian coast than along the eastern coast of Adriatic (Joksimovic et al., 2011). Southeast part of Adriatic coast is very interesting for researches because Montenegrin and Albanian coast, i.e., sea and marine environment in those countries, are little investigated on possible pollutants (Babi et al., 1998; Celo et al., 1999; Cullaj et al., 2006, Jovic et al., 2011, Joksimovic et al., 2011, Stankovic et al., 2011). In common with the other coastline areas, the Montenegrin coastline is also under a great impact of anthropogenic factors and the activities on the shore. The Montenegrin coastal area receives a heavy influx of sewage, industrial effluents, domestic and agricultural wastes, all of which contain varying hazardous chemicals and can cause deleterious effects on an aquatic organism. Additionally, fishing and recreational activities in the coastal area further pollute the Montenegrin coastal waters.

The Mediterranean mussel *M. galloprovincialis* (L.) is widely distributed in the coastal waters of Montenegro and started to cultivate on farms for the market, while wild ones are still hand-collected for personal consumption. In light of this, cultivation of mussels along the Montenegrin coast has been increasing, 150 tones/per year in last decade (FAO, 2007b), particularly in the Boka Kotor Bay due to the good natural conditions, but it is still underdeveloped. The contribution of aquacultured mussels in Montenegro is insignificant, but there is potential for its development. Hence, data about basic physical-chemical factors (T, salinity, oxygen concentration and nutrients content) and trace metal concentrations in marine environment and their accumulation levels in mussels is essential to assess quality of sea water for mussels farming. This can also provide information about metal contents in marine organisms actively consumed by humans.

This study, initiated from fall 2005–spring 2007, is related to wild mussels because of their hand-collection for human consumption and the expansion of mussel aquaculture in Montenegro, especially in area of the Bay after the year 2000. The aim of this work was to determine the sea water characteristics and the trace metals levels of Zn, Cu, Pb, Cd, As and Hg in the soft tissue of wild mussels *M. galloprovincialis* collected on the Montenegrin costal area to estimate the sea water quality for mussels farming in this part of the Adriatic.

MATERIAL AND METHODS

Research presented in this paper includes four sites in the coastal water of Montenegro where sea water and wild mussels were sampled in the same time at the same place. Localities were chosen based on the intensity of pollutions influence from the land and the specific characteristics of locality: in the Boka Bay, sites Sveta Stasija, and Nerceg Novi, and at the open coastal area of Montenegro, port Bar and Rt Djeran, Fig. 1.

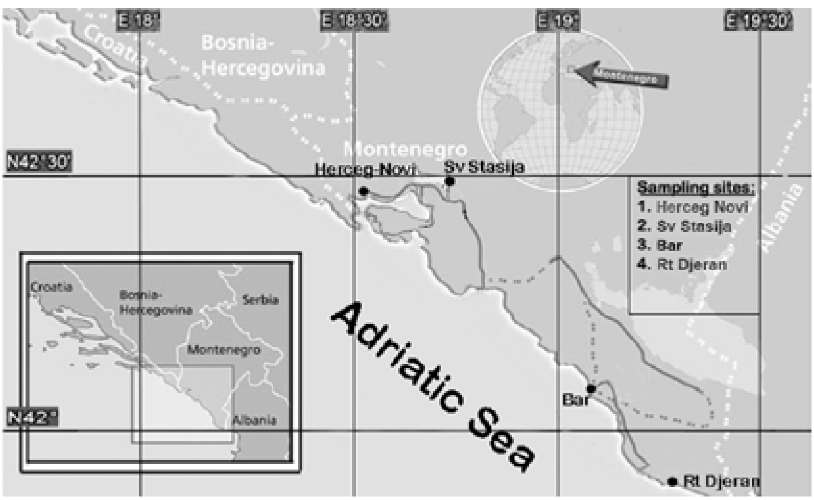

Figure 1. Sampling sites of the mussel *M. galloprovincialis* from the SE Adriatic, Montenegro

Samples of sea water were taken directly from the surface in the polyethylene bottles 0.5 L volume and physical-chemical factors (nutrients, T, O_2 and salinity) were measured at the sampling locality with MultiLine 4 labs. The mussel samples were collected seasonally from each site in each season; 25–30 mussels similar in length were selected, cleaned and rinsed with deionized water. All samples were measured before freeze-drying at – 40 ºC for 48 hours, and after, homogenized to a fine powder and stored at – 10 ºC until analyses. The average water content of the soft mussels tissues were: 81.8 % (spring) and 79.8 % (fall). Samplings were conducted for two years from 2005 to 2007, in spring and fall seasons.

Five replicates per site were analyzed for trace metals: approximately 0.5 g of the soft tissue was digested with a mixture of concentrated HNO_3 (65 % Merck, Suprapur) and H_2O_2 (30 % Merck, Suprapur) in a High Microwave Digestion System (CEM CORPORATION, MDS-2100). The digested samples were diluted to 25 ml with Milli Q water and stored in polyethylene bottles. The Fe, Mn, Zn, Cu, Ni and Co concentrations were determined by using F-AAS (PerkinElmer, AAnalyst 200) with an air–acetylene flame, but Pb and Cd by GF-AAS (Perkin-Elmer, 4100ZL, with Zeeman background correction) technique. HG/CV AAS technique was used for analyses of As and Hg (PerkinElmer, AAnalyst 200). The accuracy of the applied analytical procedure for the determination of heavy metals in mussels was tested using SRM 2976 (Mussel homogenate; NIST) material.

RESULTS AND DISCUSSION

The information related to surface water samples at the investigated sites is given in Table 1. The nutrients, NO_3^- and PO_4^-, are essential compounds for phytoplankton growth. Low nutrients in water leads to low phytoplankton density and therefore low feed level for mussels. The content of NO_3^- and PO_4^- was the highest in the spring 2006 in spring, with lowest NO_3^- range at harbor Bar (1.9 – 4.9 mg/L), but the PO_4^- content was always little be higher in spring seasons at the all locations and measured PO_4^- values were between 0.17 – 0.35 mg/L. Dissolved oxygen is needed for mussels respiration and its range was between 6.1 – 8.5 mg/L with the best oxygen water conditions at Sv. Stasija and H. Novi locations, Tab.1. Low dissolved oxygen levels can cause decline in mussels feeding and growth. Considering the temperature and salinity, the coastal water of Montenegro for mussels growth is optimal, since the mussel *M. galloprovincialis* grows to optimum size in sea water of temperature 15 °C – 25 °C and salinity between 20 ‰ – 35 ‰ (Braby and Somero, 2006).

Table 1. Sampling data of the sea water and the Mediterranean mussel *M. galloprovincialis* collected from the four locations of the SE Adriatic coast, Montenegro

No.	Location	Sampling Date	PO_4 (mg/L)	NO_3 (mg/L)	Disolved O_2 (mg/L)	Temperature T(° C)	Salinity (‰)
1.	Herceg Novi, in Boka Kotor Bay	fall 2005	0.16	2.1	7.8	19.9	29.6
		spring 2006	0.35	5.8	6.6	24.5	26.8
		fall 2006	0.12	1.6	7.4	19.6	36.3
		spring 2007	0.20	6.7	6.1	22.4	32.6
2.	Sveta Stasija, in Boka Kotor Bay	fall 2005	0.18	1.8	7.7	19.2	32.3
		spring 2006	0.34	6.0	8.2	26.0	15.9
		fall 2006	0.33	1.0	8.5	18.8	30.8
		spring 2007	0.17	1.1	8.1	18.1	16.2
3.	Bar, at the open coastal area	fall 2005	0.16	2.6	7.2	19.8	35.5
		spring 2006	0.33	4.9	7.3	24.0	29.3
		fall 2006	0.13	2.2	6.8	20.9	36.7
		spring 2007	0.30	1.9	7.3	20.5	35.2
4.	Rt Djeran, at the open coastal area	fall 2005	0.20	2.2	7.0	19.0	18.6
		spring 2006	0.32	3.9	7.1	24.8	28.6
		fall 2006	0.10	1.0	7.0	18.4	36.2
		spring 2007	0.17	1.8	6.9	20.2	30.5

Nos. follow those indicated in Fig.1.

Wild or aqua cultured mussels are usually collected for human consumption at the coastal area of Montenegro, especially in the Boka Kotorska Bay. The mussel soft tissues are typically eaten whole and after harvesting for human consumption they were not depurated on the possible pollutants. Coastal waters of Bokakotorska Bay, in regard to open sea, are most exposed to anthropogenic eutrophication (Joksimovic, 2010) with waste originated from the human activities on the coastlines land and sea water (Jovic et al., 2011). The Montenegrin sea water quality was determined on the basis of the Airas (2003) criteria which connects the concentration levels of trace metals in mussels with five different water quality classes: from class I, unpolluted, to class V, highly polluted. The concentrations of Hg, Cd, Pb, As, Cu and Zn in the investigated mussel samples are shown in Table 2.

Table 2. The concentrations (mean, range min–max, mg/kg) of mercury (Hg), cadmium (Cd), lead (Pb), arsenic (As), copper (Cu) and zinc (Zn) in the soft tissues of mussels collected at the Montenegrin coastal area from the fall 2005 – spring 2007

No	Location	N	WB	Hg (mg/kg)	Cd (mg/kg)	Pb (mg/kg)	As (mg/kg)	Cu (mg/kg)	Zn (mg/kg)
1	**H. Novi,** B. Kotor Bay	5	Dry	**0.59** (0.15-1.0)	**2.41** (1.7-2.9)	**7.0** (3.5 – 10.1)	**13.3** (8.5-17.8)	**8.0** (4.6-11.5)	**175.5** (151.9-190)
2	**Sv. Stasija** B. Kotor Bay	5	Dry	**0.27** (0.03-0.9)	**2.02** (1.4 -2.9)	**9.8** (8.1 -11.4)	**4.42** (1.9-7.4)	**7.5** (5.9-8.9)	**133.5** (82.0-205)
3	**Bar** at the open coastal area	5	Dry	**0.49** (0.25-1.0)	**2.13** (1.0-3.53)	**12.9** (8.5- 16.3)	**5.82** (2.7-8.0)	**14.5** (12.6-17.2)	**205.9** (101-300)
4	**Rt Djeran** at the open coastal area	5	Dry	**0.07** (0.03.-0.14)	**1.73** (1.0-2.3)	**4.7** (1.30- 7.9)	**8.9** (4.2-20.5)	**9.8** (7.4-12.4)	**188.3** (118.1-345)

Nos. correspond to locations indicated in Fig.1.
N – Number of observations

On the basis of the Airas (2003) criteria in the present study, the sea water from Rt Djeran and Sveta Stasija is unpolluted or slightly polluted by Hg, Cd, As, Cu and Zn related to their concentrations in mussels from these areas and belongs to the class I, but related to Pb concentrations in mussels, sea water from these areas belongs to the class II or moderately polluted. Related to the content of Hg in mussels from the Herceg Novi and Harbor Bar, these areas are polluted by Hg and sea water belongs to the class III, but related to the concentrations of rest elements in wild mussels, sea water in the area of Herceg Novi and Harbor Bar is class II or moderately polluted.

Comparing the metal levels in mussels from different Adriatic areas (Scancar et al., 2007; Cardellicchio et al., 2008; Stankovic et al., 2011), the obtained data indicates that the metal levels found in the wild *M. galloprovincialis* from the Montenegrin coastal area are similar to the metal levels reported from other Adriatic areas.

CONCLUSIONS

It is oblivious from water classifications related to the metal concentrations in wild mussels, before their consumption, that they have to be depurated. As this was a pilot study to evaluate the environmental quality of the coastal waters of Montenegro for future mussels farming, the continued monitoring of this sea area is necessary in order to control the water quality and amount of heavy metals in mussels for human consumption.

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ALIEN SPECIES AND THEIR IMPACTS IN THE BLACK SEA

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STRANE VRSTE I NJIHOV UTICAJ NA CRNO MORE

Abstract

The Black Sea, a unique fragile ecosystem, has been under the threat of pollution, climate change, invasive alien species and overfishing over many years. Combined impacts of these threats caused serious problems in the Black Sea ecosystem, fisheries economy and welfare of Turkish fishermen by reduction of the catch of commercial fish species, decrease in biodiversity, loss of habitats, increased food competition of endemic species and related problems in the food web by changes in various trophic levels. In this presentation the impacts of the alien invasive species will be explained on certain species basis in the Black Sea.

Unintentionally introduced new species to a certain area are known as invasive species. Alien species that become invasive are considered to be a main direct drive towards biodiversity loss across the globe. In addition, alien species have been estimated to cost global economies hundreds of billions of dollars each year (CBD, 2011).

Some of the alien species i.e. *Rapana venosa, Anadara inaequivalvis, Mnemiopsis leidyi, Beroe ovata, Mya arenaria, Balanus improvisus, Mugil soiuy, Potamopyrgus jenkinsi* caused significant impacts on the Black Sea ecosystem and fisheries. All kinds of impacts due to invasive species had a big effect on the reduction of total fish production in the Black Sea as predation, food competition and major destruction in the food web. There are many other species introduced to the Black Sea from phytoplankton to top predators. Some of the invasive alien species and their impacts in the Black Sea are summarized in Table 1.

Research studies on the impacts of alien species to the ecosystem and fisheries economy are very limited in the Black Sea. In order to support the ecosystem recovery in spite of all types of negative impacts for the sustainable management of the natural resources, it is very essential to understand, analyze and take urgent management measures regarding all aspects of the ecosystem. New research studies in the Black Sea should be focused on prey-predator relationships, reproduction and growth rates of invasive species, relationship between their distribution and environmental factors, assessment of limiting factors, natural barriers and mechanisms, etc.

***Key words**: Invasive species, impact, Black Sea, ecosystem, fisheries*

INTRODUCTION

Alien species that become invasive are considered to be a main direct drive towards biodiversity loss across the globe. In addition, alien species have been estimated to cost global economies hundreds of billions of dollars each year (CBD, 2011). Unintentionally introduced new species to a certain area are known as invasive species. Annually, more than 3000 species from the oceans change their locations via ca. 10 billion tons of ballast waters discharged from the ships (IMO, 1998).

Nearly 87% of the water volume of the Black Sea is anoxic and contains high concentrations of hydrogen sulphide (Bronfman, 1995; Zaitsev and Mamaev, 1997).

Therefore, living space suitable for the majority of aquatic organisms makes up to 10% of the total volume of the Black Sea. Since the early 1970s, noticeable changes have taken place in the Black Sea ecosystem because of anthropogenic euthrophication (Kideys, 2002). In the period from 1981-2000 the intensity of alien species appearance increased to about two species per year. The principal vector of alien species during this period was ballast water (Alexandrov *et al*. 2004).

The Black Sea is a recipient area for non indigenous species (NIS) native to very different geographical donor areas, i.e. North Atlantic (35%), East Atlantic-Mediterranean (23%), West Pacific (13%), South-East Asia (8%), South-West Pacific (1%), Indo-Pacific (6%), and cosmopolitan species (15%) (Zaitsev *et al*. 2004).

Invasive species are evolving very well in new areas by switching from their natural areas due to ballast water discharges of ships, aquaculture activities and environmental changes in the ecosystem. These species are in equilibrium with the existing predators and under the control of ecosystem relations in their natural environment. Generally, invasive species that occupy new ecosystems that are poor in biodiversity cause negative economical, environmental and ecological changes by competing with the local species in their transitional or new regions. Intentionally or unintentionally access of invasive species to the Black Sea ecosystem has started in the 19th century and significant effects to the ecosystem have emerged at present.

Zaitsev (1991) reported that more than 30 foreign aquatic plant and animal species were introduced to the Black Sea in 20th century. Some of these species (*Rapana venosa, Anadara inaequivalvis, Mnemiopsis leidyi, Beroe ovata, Mya arenaria, Balanus improvisus, Mugil soiuy, Potamopyrgus jenkinsi*) caused significant impacts on the Black Sea ecosystem and fisheries. All kinds of impacts due to invasive species had been very effective on the reduction of total fish production in the Black Sea as predation, food competition and major destruction in the food web. Some of the invasive alien species in the Black Sea are given in Table 1.

Table 1. Major alien species in the Black Sea and their impacts on the ecosystem

Species	**From**	**Native to**	**Way of Introduction**	**Impact**	**First discovered in**	**Reference**
Rapa whelk *Rapana venosa*	Gastropoda	Sea of Japan	Attach hulls Ballast UI*	Mussel banks, oysters and native bivalves collapsed	1947 Novorossiysk	Drapkin, 1953
Comb jelly fish *Mnemiopsis leidyi*	Ctephore	NW Atlantic	Ballast UI	Anchovy, horse mackerel and sprat stocks collapsed	1982 Black Sea 1988 Azov Sea	Vinogradov *et al.*, 1989
Ctenophore *Beroe ovata*	Ctephore	Mediterranean	I, UI ?	Sharp decrease *M. leidyi*, recover small pelagics	1997, Northern Black Sea, 1999, Azov Sea	Shiganova *et al*, 2001
Pacific mullet *Mugil so-iuy*	Mugilidae	Amur river estuary and Japan Sea	I**	Food competition with local mullet species which are disappeared	1972-1980, Azov Sea and the Black Sea	Zaitsev, 1991
Soft shell clam *Mya arenaria*	Myidae	Baltic Sea	Ballast UI	Formed new communities	1960	Strasser,1999
Invasive Blood cockle *Scapharca (=Anadara) inaequivalvis*	Arcidae	Indo-Pacific	Ballast UI	Depleting with mussels as a new filter feeder	late 1970s Black Sea	Zolotarev & Zolotarev, 1987
Bay barnacle *Balanus improvisus*	Balanidae	Atlantic, Pacific, Mediterranean	Ballast UI	Formed new communities	1844	Gomoiu & Skolka, 1996
New Zealand mud snail *Potamopyrgus jenkinsi*	Littoridinidae	New Zealand	Ballast UI	Formed new communities	1952, Razelm-Sinoe Lagoon 1960, Azov Sea	Bank, 2007
Common starfish *Asterias rubens*	Asteridae	Atlantic	Mediterranisation UI	Predating on mussels, baby clams, rapa whelk	2003, Bosphorus entrance	Karhan *et al.*, 2007

I*: Intentionally, UI** :Unintentionally

There are many other species introduced to the Black Sea from phytoplankton to top predators. When the food web was destroyed dramatically in late 1980's in line with collapse of anchovy stocks due to predation of comb jelly fish, a lot of other fish species from higher trophic levels, feeding on anchovies and other small pelagics, have also declined. After the intentionally or unintentionally introduction of *Beroe ovata,* that is still unclear, population of *Mnemiopsis lediyi* sharply declined and the anchovy fisheries started to recover.

The other ecological disaster was due to alien species *Rapana venosa*. After the first introduction in 1947 to Novorossiysk Bay, it has spread all over the Black Sea due to lack of predators, mainly sea stars. It destroyed all commercial mussel banks which are essential filter feeders acting as a fuse to control nutrient increase via river discharges. After the collapse of mussels, it started to feed on other mollusks like oysters, baby clams and carpet shells.

At present, the growth rate of rapa whelk considerably decreased due to lack of food but recruitment of the population is out of control and its impact continues at maximum level.

Pacific mullet *Mugil so-iuy* is one of the new introduced species in the eastern Black Sea. It was intentionally carried to the cages in the Azov Sea for aquaculture. However, it was accidentally released to the sea and has well adapted to the Black Sea since 1980. Recently it reached to the Mediterranean. It prefers the southeastern Black Sea coasts especially in the reproduction season starting in May and June. During spawning migration, it is harvested by gill nets all over the Black Sea coasts. Size of this fish is bigger than the local mullet species.

There were 5 endemic mullet species before its introduction. At present, all the native species almost disappeared due to high food competition. Fishermen also were obliged to change their gill nets to catch bigger sized Pacific mullet.

RECOMMENDATIONS

Research studies on the impacts of alien species to the ecosystem and fisheries economy are very limited in the Black Sea. In order to support the ecosystem recovery in spite of all types of negative impacts for the sustainable management of the natural resources, it is very essential to understand, analyze and take urgent management measures regarding all aspects of the ecosystem. New research studies in the Black Sea should be focused on prey-predator relationships, reproduction and growth rates of invasive species, relationship between their distribution and environmental factors, assessment of limiting factors, natural barriers and mechanisms, etc.

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THE EFFECTS OF DREDGE FISHING ON MARINE ECOSYSTEMS

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UTICAJ GRABULJANJA NA MARINSKE EKOSISTEME

Abstract
Fishing activity does not only impact on the fish stocks themselves, but also the ecosystem within which the fish live in. This paper presents an overview of knowledge on the effects of dredging activities on marine habitats. Dredging is used for harvesting bivalve mollusks such as oysters, clams and scallops from the seabed. The primary negative impact resulting from dredge fishing is a reduction in an abundance and diversity of seabed flora and fauna. Dredging operations inadvertently catch a range of unwanted species that are discarded into the sea. Identified dredging effects can include entrainment of organisms, increased turbidity at the dredging site, fish injury associated with exposure to suspended sediments and decreased dissolved oxygen, and fish behavioral effects due to the effects of noise. They reduce their abundance, spawning potential and, possibly, population parameters (growth, maturation, etc.). The impact on the habitat depends on the gear and sediment type. Highly dynamic, soft bottoms may suffer limited damage even when exploited by heavy (including hydraulic) dredges. On the contrary, stable, hard, and highly structured habitats will be easily damaged.

Key words: *fishing activity, marine habitats, dredging*

GROWTH OF DIPLOID (2N) AND TRIPLOID (3N) JUVENILE BLACK SEA TURBOT (*PSETTA MAXIMA*) UNDER DIFFERENT TEMPERATURE REGIMES

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RAST DIPLOIDNE I TRIPLOİDNE MLAĐI CRNOMORSKE RIBE LIST (*PSETTA MAXIMA*) U RAZLIČITIM TEMPERATURNIM USLOVIMA

Abstract

The present study was carried out to investigate growth and feed utilization of juvenile triploid Black Sea turbot under different temperature regimes. Diploid (D16) and triploid (T16) fish juvenile (4 g) were reared at 16 °C for 56 days (I. period) and at 21°C for 55 days (II. period), while diploid (D21) and triploid (T21) groups were reared at 21°C for whole period (111 days). Ploidi did not affect fish survival rates at all temperature regimes that was statistically significant ($P > 0.05$).

At the end of the first period, there was no difference in the specific growth rate (SGR) between D21 and T21 groups, but D16 group had a significantly higher SGR than T16 group. In the second period, there was no difference in SGR between D21 and T21 groups or D16 and T16 groups. SGR of D21 and T21 groups were significantly higher than D16 and T16 groups for both periods ($P < 0.05$). Triploid exhibited ssimilar weight with diploids reared at 21 °C but lower at 16 °C. It seams that triploid juvenile turbot may show lower performance than diploids at low rearing temperature. When lower rearing temperature (16 °C) was increased to 21 °C, D16 juveniles compensated their body weight and were similar to those of D21 group, but group T16 did not compensate their body weight to the body weight of T 21 group.

In conclusion, not only temperature but also ploidy influenced growth and feed utilization of juvenile turbot and similar growth can be obtained from juvenile triploid turbot by rearing them at 21°C.

Key words: *Black Sea turbot, Psetta maxima, ploidy, feed utilation, growth, temperature.*

THE EFFECET OF DEEP SEA DISCHARGE OF WASTEWATER OF CAYELI COPPER COMPANY IN THE MARINE ECOSYSTEM OF THE SOUTH EASTERN BLACK SEA

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EFEKTI DUBOKO MORSKOG UPUSTA OTPADNE VODE KOMPANIJE CAYELI COPPER NA MARINSKI EKOSISTEM JUŽNO-ISTOČNOG DELA CRNOG MORA

Abstract

The Black Sea receives considerable quantities of contaminants from urban and industrial waste water discharges. The impact of these contaminants on marine ecosystem should be monitored. Therefore, it is very important to determine the level of pollutants entering to the marine environment.

The aim of this study was to determine the impact of wastewater, discharged by Çayeli Copper Company with deep sea discharge system, to the coastal area of the south eastern Black Sea. The study was carried out between 1993-1995 and 2002-2006. Seven sampling stations were selected and samples were taken from surface, 75 m, 150 m, 200 m, and 320 m depth. Temperature, salinity, pH, dissolved oxygen, alkalinity, hydrogen sulfide, copper, zinc, lead, iron, cadmium, mercury, arsenic, and manganese levels were measured at each station. During the study period, temperature, dissolved oxygen, pH and salinity were found to be 7.94-27.33°C, 6.90-11.18 mg/L, 7.90-8.40 and ‰16.74-18.24, respectively at surface water. Maximum values of As, Hg, Pb, Cd, Mn, Fe, Cu and Zn were 7.32 µg/L, 1.36 µg/L, 17.83 µg/L, 0.80 µg/L, 571.60 µg/L, 44.49 µg/L, 11.55 µg/L, 112.27 µg/L, respectively. These results indicate that none of the findings are above the general sea water quality standard as stated in Turkish Water Pollution and Control regulations.

During the research period, seasonal and temporal distribution of heavy metals were not uniform, and there was no significant trends in the heavy metal pollution in the study area, therefore it can be concluded that discharge from Çayeli Copper Company has no noticeable effect on the water column of the receiving environment.

Key words: *Deep sea discharge, Heavy metal pollution, Black Sea, Çayeli Copper Company*

THE VARIATIONS IN PROXIMATE CONTENT AND FATTY ACID PROFILE IN DIFFERENT PARTS OF THE THORNBACK RAY (RAJA CLAVATA) CAUGHT FROM BLACK SEA, TURKEY

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VARIJACIJE U HEMIJSKOM SADRŽAJU I MASNOKISELINSKOM PROFILU RAŽE (*RAJA CLAVATA*) IZ CRNOG MORA, TURSKA

Abstract

Proximate and fatty acid (FA) composition of different parts and sexes of ray (*Raja clavata*) were compared. Significant differences usually occurred ($p<0.05$) between liver samples and others body parts. The highest moisture, protein and ash contents were found for pectoral fin 78.56, 20.52 and 1.21%, respectively while fat content represented liver as 39.71%. Sexual differences were also found for both proximate and FA values. The levels of saturated (SFA) , monounsaturated (MUFA) and polyunsaturated fatty acids (PUFA) varied as 27.65-32.10%, 14.93-19.01% and 34.29-39.50% in total FAME, respectively. The highest ∑PUFA and docosahexaenoic acid (DHA) values were observed in edible portion of liver for both sexes due to high contents of fat content despite the low values as % total FAME in comparison with other body parts. On the other hand, eicosapentaenoic acid (EPA) levels were found to be high in both calculated situation for liver samples. Significant variations among FA levels were observed for different body parts ($p<0.05$). The highest DHA and EPA values were in edible portion of liver were calculated as 6818 and 2331 mg/100 g, respectively indicating the importance of utilizing liver for this species.

Key words: *Raja clavata, fatty acids, proximate composition, sexual differences, liver*

AQUACULTURE IN AZERBAIJAN: THE RESULTS OF REARING OF COMMODITY FISH IN THE ADAPTED WATER BODY

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AKVAKULTURA U AZARBEDŽANU: REZULTATI GAJENJA RIBA U ADAPTIRANIM VODENIM POVRŠINAMA

Extended abstract

In the second half of the 20th century and in the beginning of the 21st century the intense development of industry and agriculture, the reduction of rivers flow, pollution of the Caspian sea by the industrial and domestic wastes and biological pollution led to the deterioration of the ecological environment, abrupt reduction of the fish stocks and decrease the catches of the food fish. While during the 70's and 80's of the last century in the Caspian sea the catches of fish were 450 thousand tones out of which 50 thousand tones of them were in Azerbaijan. Currently the catches are 39 thousand tones on average out of which 3.5 thousand tones are caught in Azerbaijan. The major part of fish catches consists of sprat which is mainly used for production of fish meal. At the same time, according to norms which are recommended by food specialists, the average annual consumption of fish products per person in the Azerbaijan republic should be 10.5 kg. Taking into account that the number of population of the republic is 9 million people at present, the catch of fish should increased to 95 thousand tones per year, which seems unrealistic taking into consideration the present condition of fish stock in the Caspian Sea. These figures powerfully illustrate the necessity of development of aquaculture and in particular marketable fish farming in the conditions of decreasing fish stocks of the Caspian Sea, reduction of arable lands and steady growth of a number of populations.

There are a lot of closed reservoirs in Azerbaijan, most of which were former stream canals of rivers. All of them practically are overgrown and don't have fish-farming importance. We have decided to use the potential opportunities of such ponds and to adapt them for breeding of marketable fish.

In order to realize the above-mentioned goal we have rented a drainless pond situated 120 km west from Baku city which was a former stream canal the Kura River. The reservoir with an area of 25 hectares stretches from south-west to north-east with the length of 1000 meters and the width of 250 meters and has a falcate contour. The reservoir was completely overgrown and during summer time algae were entirely covering its water surface. Canadian waterweed (*Elodea Canadensis*), coontail *(Ceratophyllum demersum)* and curly pondweed *(Potamogeton crispus)*, were prevailing from soft aquatic vegetation. Of hard aquatic vegetation the prevailing ones are reed mace *(Typha latifolia)* and reed (*Phragmites communis*).

Earlier the reservoir was a water supply and later on the water was used for irrigation of farmer's fields and vegetable gardens. When the water level in the pond declined, the smell of sulphuretted hydrogen and ammonia was appearing. In the beginning of 2009 that reservoir was used by us for fish farming purposes. Due to this, water was lowered as maximum as possible, and as a result at both ends of the reservoir ground appeared, one in the northern end with the area of 4 hectares and the other one in the southern end with the area of 2 hectares. All vegetation and roots left in the bottom were removed and pits were filled. Then the planning of pond bed was fulfilled and dams were constructed. Two ponds with the area of 2 hectares of each were built in the northern part with the purpose of breeding of marketable fish. In the southern part a pond with the area of 2 hectares was build with the purpose of breeding young fish.

The ponds are filled with water independently of each other. In the end of each ponds water discharging facilities were constructed. The water was not drained into a waste ditch, as the construction of such a ditch would be very expensive. Therefore it was decided the water from the ponds are drained into the to the reservoir and thereby supply them with water and improve hydro chemical regime.

For breeding of stocking material in May 2009 the fish farm purchased three-day larvae of common carp (*Cyprinus carpio*), silver carp *(Hypophtalmichthys molitrix)*, bighead carp (*Aristichthys nobilis*) and grass carp (*Ctenopharingdon idella*). In the first stage the larvae were bred during 14 days in 2 land fish-wells with 100 sq. m area of each. The larvae were fed every 2 hours during the bright part of the day.

The feed consisted of fish meal, dried milk and yolk. After 15 days the sluice was opened and larvae together with the water stream were transferred to the pond with 2 hectares area. In this pond young fish were bred until the stage of the under yearlings and were kept there for overwintering. Feeding tables made of galvanized iron were installed for feeding. Young fish were fed 2 times per day using previously wetted and fine splintered wheat and barley.

In the first days of March 2010 half of each grow-out ponds were filled in order to achieve fast heating of water. Previously bags made of kapron sieve with 2 mm mesh size were installed on the water delivering pipes in order to prevent the coarse fish, hardroe and larvae from getting into the pond. The ponds were kept without fish ten days in order to get rid of most of the parasites. At mid March 2010, the transfer of the yearlings to the grow-out ponds began. Before transfer the young fish was treated using against ectoparasites with metrifonate in the amount of $1g/m^3$.

The density of fish placed in the grow-out ponds was 2438 ind/hectare of common carp with the average weight of 46 g, silver carp 602 ind/hectare with the average weight of 60 g and grass carp 301 ind/hectare with the average weight of 55 g. In total 13 367 yearlings were stocked with the average density of 342 ind/hectares. Feeding places were marked in the ponds on the basis of 300 fish per one feeding point. Feeding of fish began from the 1st of May 2010 when the temperature of water reached +18ºC. The fish feeding norm was established taking into account the intensity of eating, water temperature and oxygen content. Common carp was fed with crushed wheat and barley.

During the first 10 days of feeding as preventive measure from parasites and bacteriosis vitamin additives with antibiotics "biovit 120" were added to the feed on the basis of 1 kg of biovit per 100 kg of carp. When fish increased to the average weight of 300 g, feed was done with previously wetted whole grains of wheat and barley. From the beginning of June 2010 carp was fed twice a day. The feeding of fish was conducted till the end of October 2010. In total 62 tones of wheat and barley was delivered. Taking into account the high placing of grass carp, they were fed in additional with freshly mowed alfalfa. For these purposes two walls with 15 m^2 surface were erected over the water using boards and canes in the middle of each pond. In order to prevent grass carp from eating feed of carp, first forage (alfalfa) was given 30 minutes after common carp was fed. Grass carp was also fed twice a day on the basis of 50% of the body mass.

In order to raise the natural food of silver carps and bighead carp the ponds were fertilized three times with ammonium nitrate, once at the end of April and twice in May with 25 kg/hectares. The overall amount of chemical fertilizers used within a season was 75 kg/hectares. Later on due to algal blooming, fertilizing was not possible. Liming was performed once, in August, using 100 kg of burnt lime per hectare. Two days a week flowage was happening in the ponds. On carrying out these measures as guidance the instructions were used (Vlasov, 2001; Ganizade et al., 2005). In order to follow the growth rate of carp and prevent the diseases two times per month test fishing of reared fish was carried out.

At the beginning of November 2010 the water was discharged from the ponds and the sale of reared marketable fish began. In total, 8551 kg of marketable fish, of which 4672 kg of common carp with the average weight of 0,542 kg, 1890 kg of silver and bighead carp with the average weight of 0,888 kg and 1989 kg of grass carp with the average weight of 1.730 kg were produced. The average fish capacity of reservoir was 2138 kg, of which carp was 1168 kg, silver and bighead carp 473 kg and grass carp 497 kg.

By piece outcome of marketable fish was equal to 88,3% on common carp, 88,3% on silver carp and bighead carp and 95,6% on grass carp. The consumption of the yearlings amounted to 156 pieces per 100 kg of reared fish. Feeding rate on common carp taking into account the deduction of placing weight was equal to 5,34 pieces. For grass carp 15 tones of alfalfa was brought.

The experience revealed that the given reservoirs can be used effectively for breeding of marketable fish. The development of commodity fish farming in the drainless reservoirs will allow using rationally the land resources. By using new spaces of drainless resservoirs and their relevant reconstruction the production of marketable fish can be increased in the republic for ten thousands of tones.

Key words: *drainless reservoir, pond, grass carp, bighead carp, silver carp.*

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FISHERIES INDUSTRY IN IRAN

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STANJE RIBARSTVA U IRANU

Prošireni abstrakt

Fisheries activities in Iran are conducted through an official agent called *SHILAT,* which is an affiliated body to the Ministry of Jihad-e-Sazandegi. As a post- revolution structure, the Ministry itself is structurally composed of four major divisions as: *Fisheries, Livestock* and *Poultry, Forestry* and *Pastures,* and *Rural Industries.* The official functions assigned to the Ministry center around rural and coastal developments. As it will be postulated, fisheries, inter alia, is of great significance in national economy.

Development of fisheries industry has been the focus of prime attention in the I.R. of Iran. To this end, respective development objectives have heed identified among which responsible fisheries management, as a unique concept, has increasingly been given a pivotal role. In particular, the national government, upon its Islamic teachings and international commitments has committed itself to observe such an international initiative and has strongly timed at the rehabilitation of God-given resources so as to ensure sustainability as well as accessibility of them to the future generations. Hence, my required measures are always taken to guarantee the maximum availability through either preventing over fishing or creating a balance between fishing efforts, and resource recruitments. To enlighten the readers, some of the major areas of the Iranian Fisheries success are briefly under pinned as follows:

Production

During the post-revolution era, fisheries production has had an ever- increasing trend. Fisheries production has now exceeded 400 thousand Mt.; indicating a l232% growth rate compared to that of 1978. Three major fisheries areas contributing a lot to the production of fisheries products are identifiable. Table 1 shows a cross-comparison of fisheries production as well as its future prospects.

cross-comparison of fisheries production as well as its future prospects

Area	1978	1998	by 2000
production in southern waters	25500	226500	350000
production in northern waters	3724	101500	142475
production in inland waters	3219	72000	875000
TOTAL	32443	400000	1367475

Employment

Creating employment opportunities mainly in fisheries areas is one of the paramount features of fisheries sector. Fisheries sector includes one percent of national employment, and about 122 thousand persons are directly engaged in this sector. It means that over 500 thousand people earn their living through fishing activities. (Table 2).

Table 2: Employment in Fishing (1989-1998).

Remarks	1989	1990	1991	1992	1993	1994	1995	1996	1997	1998
Inland farmers and fishermen	9579	9923	10934	10204	10532	10921	11004	11630	10250	16661
Northern waters’ fishermenn	11559	11330	11383	11772	11952	11610	7447	9864	11573	12515
Southern waters’ fishermen	39633	42855	45872	60434	70729	74850	91397	86904	90358	92994
Total No. Of employees	60771	64108	69189	82410	93213	97381	111848	108398	112161	122170

Exports

In line with the government’s economic policy to promote the exports of non-oil commodities, exports of fisheries products have received a prime importance. To meet the respective objectives, attempts are made to develop further marketing opportunities and processing industries so as to ensure the production of value-added products. Table 3 shows the details of fisheries exports.

Table 3: Value of Fisheries Exports (1989-I 998) $1000

Products	1989	1990	1991	1992	1993	1994	1995	1996	1997	1998	2020
Caviar	40718	53211	52077	42004	31050	31863	39620	34530	33683	38360	57500
Sturgeon	2190	3335	3071	2807	1384	68v7	396	1384	1023.5	609	6000
Shrimp	6075	6600	5574	7800	7000	9000	3588	8664	8986	9567	14000
Canned products	NA	58	92	185	1000	225	90	-	365	-	-
Others	NA	414	544	2590	4372	10160.7	1157.1	4160.3	5658	3785	-
Total	48983	63618	61358	55386	44796	51935.7	44851.1	48738.3	49715.5	52321	

Development Plans

The Islamic Republic of Iran has passed its 1st 5- year development plan and is now on the verge of the 3rd one. One of the fruitful achievements of these plans to the fisheries industry has been the unprecedented participation of people, which has resulted in huge private investments in fisheries development. These plans have revolved around a number of key issues such as: resource exploitation, fish consumption, fish marketing, aquaculture, infrastructure development, research and training, and development of international and regional cooperations. The achievements in all these areas are promising.

Following the various initiatives, per capita consumption of fish has increased from 1Kg in 1987 to 4.6 Kg in 1998. On the other hand, exports of fishery products have always been followed up in line with the export policies of the government

Aquaculture is mainly concerned with farming four main species including sturgeon, cold and *warm* water fish species, and shrimp. Breakthrough occurred in this area is shown in Table 1.

Another key development area is the *infrastructure* establishment. In this area, Shilat has undertaken the construction of 26 fishing harbors with a projected handling capacity of 500000 Mt. by the end of the 2nd 5-year plan (1999). Furthermore, *research and training* are the key issues in fisheries development which center on stock-assessment, selective fishing methods, hydrology, fish processing, fish disease, and holding long and short-term training courses.

Last but not least, authorities have made comprehensive attempts to develop a *25-year long term development plan* for fisheries industry which intends to achieve specific targets in three main fisheries areas by 2020 as: increasing fish production, consumption, and exports.

Fisheries Society

Sustainable development of fisheries industry lies globally on the development of fisheries communities. Such *Nongovernmental organizations (NGOs),* parallel to the public sector, participate in making fisheries policies and contribute to the materialization of a comprehensive development.

AQUACULTURE IN TURKEY

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AKVAKULTURA U TURSKOJ

Abstrakt
Turska je zemlja sa velikim potencijalom za dalji razvoj sektora akvakulture sa preko 8,300 km obale i 25 miliona ha upotrebljive površine mora. Akvakultura u Turskoj započinje sa gajenjem kalifornijske pastrmke (*Onchorhynchus mykiss*) i šarana (*Cyprinus carpio*) kasnih šezdesetih, dalje se razvija gajenjem orade (komarče, *Sparus aurata*) i brancina (lubina, *Dicentrarchus labrax*) sredinom osamdesetih. Proizvodnja tri najznačajnije vrste: pastrmke, orade i brancina je brzo rasla tokom devedesetih i sada dostiže 158 000 tona godišnje u 2009: pastrmka, orada, brancin, dagnje, šaran i ostale vrste na 1 855 farmi.

Trenutno, oko 25 procenata po zapremini (158 729 tona od 623 191 tona ukupne proizvodnje) dolazi iz akvakulture. Oko 98 % proizvodnje je iz intenzivnog uzgoja; pastrmka se uglavnom konzumira lokalno, dok se oko 75% orade i brancina izvozi u zemlje EU. Gotovo sva riba se prodaje u svežem stanju.

U ovom radu se daje pregled sadašnjeg stanja i ograničenja, zakonodavstva i administrativnih prepreka vezanih za akvakulturu u Turskoj.

Ključne reči: *akvakultura, Turska, proizvodnja riba, sistemi uzgoja*

INTRODUCTION

Turkey has rich and diverse aquatic resources ranging from fresh to brackish and marine that is 8.333 km of coastline, 151.080 square kilometres of economic coastal zone, 177.714 km total river length, around 900.000 ha natural lakes and 500.000 ha of dam reservoirs (Çelikkale et al., 1999). Despite of these large resources, Turkish fisheries has stagnated at an annual production of around 600.000 tons and depends mainly on small-scale and largely small pelagic species. Therefore, aquaculture has been seen more important than capture fisheries in Turkey.

Current per capita fish consumption in Turkey is very low (around 8 kg) comparing to many European countries but it is expected that the recent developments will lead to increases in domestic fish consumption. On the other hand, wild fish stocks are under pressure from over-fishing, environmental degradation and pollution. Therefore, the country needs aquaculture development for a number of reasons that are: Supporting increased per capita fish consumption and service export demand, rational natural resource utilization and to develop recreational and ornamental fisheries, restocking and ranching, stabilize the domestic market (Okumuş ve Deniz, 2007).

In Turkey, modern aquaculture began in the late 1960s; initially, the first species cultured was the rainbow trout (*Oncorhynchus mykiss*) from eyed eggs imported from Italy. Common carp (*Cyprinus carpio*) farming followed during the 1970s, but little development happened until 1985 which marked the beginning of gilthead seabream (*Sparus aurata*) and European seabass (*Dicentrarchus labrax*) farming (FAO, 2010).

The next major developments were commercial mariculture trials with rainbow trout and Atlantic salmon (*Salmo salar*) in the Black Sea during the early 1990s; only one experiment failed with kuruma prawn (*Penaeus japonicus*) on the Mediterranean coast and later mussels in the northern Aegean and the Sea of Marmara during the 1990s were introduced into the aquaculture activities. The Atlantic salmon farming initiative in the Black Sea failed, but rainbow trout mariculture is still practiced (FAO, 2010).

Production of the three major species, namely rainbow trout, seabass and seabream increased rapidly during the 1990s, with efforts having been given to the development of new species, such as the Black Sea turbot (*Scophthalmus maeoticus*) and some Mediterranean species such as sharpsnout seabream (*Diplodus puntazzo*), common seabream (*Pagrus pagrus*), common dentex (*Dentex dentex*) and groupers (*Epinephelus* spp.). Atlantic bluefin tuna (*Thunnus thynnus*) fattening, which started at the turn of the millennium has been the latest development in terms of species diversity. In this paper, the present status and constraints have been discussed.

PRESENT STATUS OF AQUACULTURE

Marine Aquaculture

Turkey has three distinct seas and coastal areas for marine fish production: The Black Sea, characterised by lower salinity levels, the Aegean Sea withwarmer waters and sheltered sites and the Mediterranean with warm waters but exposed sites. Most farming is on the Southern Aegean coast.

A wide diversity of aquatic species can be farmed in brackish or salt water using a variety of production systems. Today marine aquaculture plays an increasingly important role in the production of fishery products. The sector can be characterized by limited species and system diversity, small farms, a production oriented approach and export dependent (EU) markets (Okumuş ve Deniz, 2007).

Marine aquaculture on the Mediterranean and Aegean Sea coasts consists mostly of the cage culture of sea bream (*Sparus aurata*) and sea bass (*Dicentrarchus labrax*). The first cage farm was established on the Aegean Sea coast for the production of sea bream and sea bass in 1985 and was stocked with fry collected from natural stocks.

Marine culture of trout and salmon began in the late 1980s. The salmonid species, rainbow trout (*Oncorhynchus mykiss*) and Atlantic salmon (*Salmo salar*), were originally produced in the brackish waters of the northern coast of the Black Sea but because of the intolerance of salmon to the high water temperature, trout eventually replaced them. These conditions provide better growth conditions for rainbow trout, which are transferred directly from inland fresh water to the brackish waters of the Black Sea without any adverse effect.

In this environment, trout require 60-90 days to grow from 20-30 g to the market size of 200- 300 g or 300-360 days to the market size of at least 1,500 g (Çelikkale et al., 1999, Şahin et al., 1999). Fish are harvested during the summer when temperatures are relatively high. With the surprising appropriate ecological supply for trout culture in the marine environment thanks to low salinity the Black Sea has an enourmous potential. Shrimp farms in the Mediterranean Sea, established in the mid 1990s, encountered many critical problems that still need to be solved.

In addition, major new or alternative Mediterranean species cultured in experimental or pilot scales are common dentex (*Dentex dentex*), common sea bream (*Pagrus pagrus*), common pandora (*Pagellus erythrinus*), sharpsnout sea bream (*Puntazzo puntazzo*), white grouper (*Epinephelus aeneus*), shi drum (*Umbrina cirrosa*), striped sea bream (*Lithognathus mormmyrus*), meagre (*Argyyrosomus regius*), greater amberjack (*Seriola dumerili*), brown meagre (*Sciena umbra*), white sea-bream (*Diplodus sargus*), two-banded sea-bream (*Diplodus vulgaris*) (Okumuş ve Deniz, 2007).

Among these species only the common dentex and sharpsnout sea bream have been cultured and marketed; other species are still at the experimental stage. Considerable efforts have been made to farm common sea bream but abnormal pigmentation is the major limitation and production has ceased. Currently there several hatcheries trying to develop larvae and fry production, and four farms are registered to culture these new species in addition to sea bass and sea bream.

Efforts are also being made to develop the commercial production of species new to the Black Sea as well. The target species are turbot (*Psetta maxima*), sturgeons (*Acipenser* spp) and native sea going trout (*Salmo trutta*). Considerable progress has been achieved in the hatchery phase for turbot, but there is a need for considerable investment for on-growing (Okumuş ve Deniz, 2007).

Inland Aquaculture

The rainbow trout (*Onchorhychus mykiss*) has been cultured since the early 1970s and Turkey has become one of the top trout producing countries in Europe with an annual production of 75 567 tonnes, or 47 percent of the country's total aquaculture production (FAO, 2010).

Today there are more than 1 000 freshwater and 20 sea-based farms which are situated in the Black Sea. Approximately, half of the farms have an annual capacity of less than 10 tonnes with the rest producing usually less than 50 tonnes. The great majority of the farms (approx. 80 percent) are family-owned with almost two thirds of the production coming from the Black Sea, Aegean and Marmara regions; one third of the country's trout farms are located in the Central Anatolian region.

Apart from marine and some freshwater cage farms, the majority of the trout farms employ small concrete raceways mainly using stream waters. In the past ten years, trout cage culture in dams has reached a very important level of production. Today, 26 000 tonnes of trout production comes from cage culture at dam lakes (FAO, 2010).

Over 50 percent of the farms have their own hatcheries with eggs being produced during the natural breeding season, i.e. between December and February. Ongrowing in raceways lasts between 12 and 24 months. The majority of fish are sold locally as portion size white trout. In the Black Sea fish are reared in cages up to 0.5–1.5 kg and sold as "salmon". Common carp (*Cyprinus carpio*) is recorded as being cultured in 86 farms, however production has hardly exceeded 1 000 tonnes in recent years (FAO, 2010).

On the other hand, possibilities of culturing fish species such as tilapia *(Oreochromis* spp.), pike *(Esox lucius)*, European catfish *(Siluris glanis)*, eel *(Anguilla anguilla)*, etc., must be made to develop inland aquaculture.

CULTURE SYSTEMS AND PRACTICES

Turkey's aquaculture is mainly based on intensive finfish culture; extensive and semi-intensive aquaculture is limited to mussel and common carp production, totalling less than 1 500 tonnes/year. Different rearing systems are employed for intensive finfish production, the most common rearing system used in freshwater trout production is concrete raceways with some larger farms having modern circular concrete tanks; earthen ponds are also used for the intensive rearing of trout. Cages used in reservoirs for trout are generally simple wooden structures locally constructed. The use of semi-intensive earthen ponds is the most common practice for carps. In Table 1, number fish farms, farm capacities and production figures are shown. In addition, annual aquaculture production in Turkey by species and year are given in Table 2.

The most widely used intensive system for seabream and seabass is floating cages. These can be squares measuring from 5x5x5 m or circular, hexagonal or octagon shaped cages up to 12–50 m in diameter. More recently, marine farms have been relocating towards more exposed areas or secondary bays and thus, types and sizes of the cage systems used are changing. There are also seabass and seabream farms which utilize earthen ponds and only one high-tech (recirculation) land-based farm. Semi-intensive culture has also been practiced in some lagoons using large sized earth ponds. Large 50–75 m diameter cages are used for tuna fattening.

Table 1. Number fish farms, farm capacities and production in Turkey. (Anonymous, 2009).

Environment	No. farms	Farms capacity (tonnes/year)	Production (tonnes/year)
Inland aquaculture	1 499	104 629	76 248
Marine aquaculture	356	134 121	77 252
Total	1 855	238 750	158 749

Table 2. Annual aquaculture production in the past decade in Turkey by species and year (tonnes). (Anonymous, 2010).

Species	Trout	Seabream	Seabass	Mussel	Carp	Other	Total
2000	44 533	15 460	17 877	321	813	–	79 031
2001	38 067	12 939	15 546	5	687	-	67 244
2002	34 553	11 681	14 339	2	590	–	61 165
2003	40 868	16 735	20 982	815	543	–	79 943
2004	48 082	20 435	26 297	1 513	683	-	94 010
2005	49 282	27 634	37 290	1 500	571	2 000	118 277
2006	57 659	28 463	38 408	1 545	668	2 200	128 943
2007	61 173	33 500	41 900	1 100	600	1 600	139 873
2008	68 649	31 670	49 270	196	629	1 772	152 186
2009	75 657	28 362	46 454	89	591	2 247	158 729

MARKETS AND TRADE

The per capita consumption of fishery products in Turkey is around 8 kg, although this figure is declining steadily. Cultured fish products constitute only around 10 percent of the total domestic fish consumption, which is quite low in comparison to global and European average figures and also compared to Turkey's availability of aquatic resources. As a result, the government has shown a clear intention to increase the per capita fish consumption by increasing the production in the aquaculture sector which seems to be the only option for achieving this increase, rather than limiting options available to increase fishery production (FAO, 2010).

Appreciation and acceptance of cultured fish species is improving through a series of efforts by relevant government organizations and producers, however, there is an urgent need to improve the distribution infrastructure throughout the whole production chain. In addition, consumption of shellfish and cyprinid fish is low, due to a cultural preference, as well as, a perception of low quality affecting their appreciation among the consumers.

Rainbow trout is consumed almost entirely on the domestic market, while the Mediterranean marine species are exported across southern European countries. Fish are mainly sold as whole fresh and only negligible amounts of farmed fish products are imported into Turkey. In general, market prices, and as a result, profit margins, for all species are declining particularly at the wholesale level.

Trout depends completely on the domestic and in particular local markets. Fish produced from freshwater farms are marketed as portion-size fish, while those produced in sea cages are sold as "salmon" as a result of their larger sizes. No pigmentation has been used in Turkish trout farming; as a result, all trout produced are white fleshed. Similarly, it is rare to find value-added products such as filleted, smoked or frozen trout. Fish reared in freshwater farms are marketed during summer months, while fish grown in sea cages are either sold just before the summer or else they are transferred to freshwater farms. Fish are harvested daily and marketed as fresh product usually directly by the farmers to restaurants, hotels and factory catering services. Many farms have their own restaurants at or close to the farm.

CONTRIBUTION TO THE ECONOMY

Fisheries represent about 0.3 percent of Turkey's GDP (Gross Domestic Product) and 2.7 percent of the country's total agricultural production. Aquaculture represents 13.5 percent of the total production from the fishery sector by volume and approximately 25 percent by value. The contribution from the fisheries sector and particularly aquaculture to the national economy is not considered significant in financial terms. Fish is not an everyday food, but has great significance in coastal regions and restaurants serving local foods and fish (FAO, 2010).

Aquaculture has contributed significantly to rural development and will continue to do so in the future. Marine fish farming is mostly operated by large private enterprises with local communities rarely being involved; on the contrary, trout farming is distributed across the country and constitutes a valuable tool for the promotion of rural economic development. Although farmed fish are not a cheap food source, aquaculture can provide a supply of fresh fish in areas where normally no other fishery products would be available. Even in coastal regions and large cities farmed fish are the only seafood products that can be found in the markets during the late spring and the summer months (FAO, 2010).

Little concern has so far been shown regarding the social aspects of aquaculture development as the contribution of aquaculture towards food security and poverty alleviation has also been rather limited. Instead aquaculture is mainly aimed at the production of luxury food fish products and source of income.

Currently, aquaculture has no involvement in recreational fishery activities or restocking/ranching operations; however, these may become major development issues in the near future. Aquaculture and related services provide considerable employment opportunities both for local young people and graduates.

CONCLUSION

The aquaculture sector is very dynamic in Turkey and will continue to use its comparative advantage in terms of biological diversity, potential domestic market, climatic conditions and geographical position. Effective promotion and advertising campaigns to increase domestic demand, improving farm management and productivity to decrease production costs, research and development of value-added products and new species, concepts of quality management and product image, environmental considerations and sustainability will be key issues for the coming years.

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FURTHER CONTRIBUTION RELATED TO IDENTIFICATION OF CONDITIONS FOR THE USE OF RIVER HOPPER BARGES AS AQUACULTURE FACILITIES

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DALJI PRILOG U RAZMATRANJU IDENTIFIKACIJE USLOVA ZA UPOTREBU REČNIH BARŽI KAO RIBNJAČKOG OBJEKTA

Abstrakt

U poslednjim godinama, zasnovano na međunarodnim iskustvima, iskazuje se potreba za uvođenje novih tehnologija u sektoru akvakulture u Srbiji. Pokrenut je projekat sa ciljem da se ukaže na mogućnost korišćenja rashodovanih rečnih barži kao objekata za akvakulturu. Predloženi vid intenzivnog gajenja konzumnog šarana, upotrebom rečnih šlepova kao protočnih sistema, predstavlja novinu u Srbiji. Budući da predstavlja nov pristup, a u svrhu određivanja adekvatne tehnologije, istraživanja su fokusirana na uticaj osnovnih faktora sredine koji utiču na rast šarana. Praćen je uticaj sadržaja kiseonika i temperature na rast šarana u konkretnim uslovima uzgojnog procesa. Tehničkim rešenjem priključenja vazdušnih kompresora na tanjiraste fino perforirane difuzore fiksirane na dnu barže, u potpunosti je rešen problem oksigenacije uzgojne vode. Temperatura je, pošto nije dostizala optimalne vrednosti za rast šarana, bila limitirajući faktor. Za 100 eksperimentalnih dana (67 dana sa ishranom), prosečna težina nasađenih riba bila je 1350.5 ± 269.47 g, postignuti individualni i ukupni prirast iznosili su 65.81% i 59.61% (mortalitet 4.22%), dok je konverzioni faktor hrane iznosio 1.52. Budući da je barža porinuta u rečni tok, kao i da se radi o protočnom sistemu, celokupni uzgojni proces je u velikoj zavisnosti od klimatskih uslova., što ukazuje na sezonsku primenu ovog tipa uzgoja za intenzivan uzgoj konzumne ribe, ili za intenzivan uzgoj mlađi za potrebe nasađivanja tokom najtoplijeg dela godine.

Ključne reči: *rečna barža, šaran, temperatura, kiseonik*

INTRODUCTION

In recent years, based on international experiences, there has been indication of a need for introducing new technologies into the aquaculture sector in Serbia (Marković *et al.* 2009). Flow-through system is defined as an aquaculture water system in which water continuously flows through the culture area and is discharged after a single pass (Styckney, 1979). To the best of our knowledge, in Serbia this type of systems are not practised for warmwater fish farming. The proposed type of intensive farming of warm-water fish species by using river hopper barges as flow-through aquculture facility is new to Serbia. This fact was impetus for studies with goals of identifying and determining conditions for the growth and survival of carp as well as developing production methods under specific rearing conditions. Being the new approach and in order to determine proper technology, studies were focused on investigations of basic environmental factors which influence carp growth and survival.

MATERIAL AND METHODS

The river hopper barge (59.9 x 6.65 m) used as aquaculture faciliti is located on the left bank of the Danube (km 1168 + 800, locality Krnjača). The rearing unit is 40 m long, 4 m wide and 2 m deep (total volume 350 m^3), and can be separated in individual compartments at every 1.5 m. One compartment was established ($V = 40\ m^3$, $P = 29.4\ m^2$), and directly to it continuous water supply from Danube was applied by using submersible pump (10 – 20 m^3/h). The two air compressors, each of 170 l/min capacity, were used for aeration.

Rearing compartment was stocked with 479.43 kg of carps (*Cyprinus carpio*) obtained from a commercial fish pond. In total 355 individuals of average weight of 1350.5 ± 269.47 g were stocked on July 28th 2010. At the time of stocking a sample of 100 fish was taken and they were measured (total lenght – TL, ± 1 mm; body mass – W, ± g). The accomodation of the fish to the experimental conditions was monitored for 5 days and during this period fish were not fed. Fish feeding started on August 2nd 2010., and fish were fed with SOPROFISH 38/12 feed type produced by Veterinaty Institute Subotica (granulation 8 mm; 38% proteins, 12% fats, 10% water, 4% cellulose, 10% ashes, vitamins: A 15 000 IU/kg, D_3 2 500 IU/kg, E 90 mg/kg, C 200 mg/kg). Daily amount of food was determined using feeding tables, depending on temperature conditions and the weight of fish stock. Ration size ranged from 4 to 9.5 kg, and one self-feeding device was used for feed supply. Feed was offered at 10:00 a. m., and on the following morning the amount of consumed feed was recorded. Fish were not fed on Sundays and days prior to control sampling. Two control samplings and total harvesting were performed on experimental days 42, 72 and 100, respectively. Sampled fish (n = 51 and n = 53) were anaesthesised (alchocol solution) and individual weight (± 1 g) and total lenght (± 1 mm) were measured; same procedure was applied for total harvesting. Mortality was observed daily. The feed conversion ratio (FCR) was calculated based on the consumed feed/weight gain ratio (died fish were not considered). Water temperature, pH and dissolved oxygen concentrations were measured daily while concentrations of ammonia, nitrites and nitrates were determined weekly.

RESULTS

Range of variations in the environmental parameters were 9.5 – 23.8 ºC, 4.01 – 10.11 mg/l, 7.39 – 8.77, 331.5 – 357 mS/cm, 0.48 – 1.11 mg/l, 0.039 – 0.047 mg/l and 0.23 – 0.574 mg/l for temperature, dissolved oxygen, pH, conductivity, nitrate concentration, nitrite concentracion and ammonia concentration, respectively. Recorded ranges of temperature and dissolved oxygen content indicate high variations of these parameters, which are of primary importance for fish growth. Remaining water quality parameters were within ranges that are acceptable for rearing warm water fish species.

In Table 1, results of rearing trial are summaraized. Rearing trial lasted 100 days, however, uptake of food was recorded on 67 days which had significant consequences on the final results. Respective to the initial values, final average weight showed an increase of 65.81%, while due to mortality (4.22%) weight gain amounted to 59.61% in total. On the other hand, feed conversion ratio was 1.52 representing satisfactory result.

Table 1. Growth, production, feed conversion and mortality of carp after 100 days of rearing trial.

Indicator	Stocking	Harvest
N	355	340
Stocking density (ind./m^3)	8.88	8.55
Stocking density (kg/m^3)	11.99	19.13
Stocking density (ind./m^2)	12.07	11.56
Stocking density (kg/m^3)	16.31	26.02
W ± SD (g)	1350.5 ± 269.47	2239.25 ± 515.44
B (kg)	479.43	765.22
No. of experimental days		100
No. of feeding days		67
Weight gain (kg)		285.8
Consumed feed (kg)		434.2
FCR		1.52
M (n)		15
M (%)		4.22

Growth of reared fish and dissolved oxygen conditions for the entire experimental period are presented in Figure 1. Throughout the experimental period dissolved oxygen concentrations varied, but generally, an increasing trend for this water quality parameter was observed. Overall average value was 6.46 ± 1.30 mg/l, while in respect to the experimental phases (control and final mesurments) average values were 5.74 ± 0.92 mg/l, 6.69 ± 1.28 mg/l and 7.65 ± 1.04 mg/l, for 1-42, 43-72 and 73-100 days period, respectively. Concentrations bellow 5 mg/l were recorded only in 9 occasions.

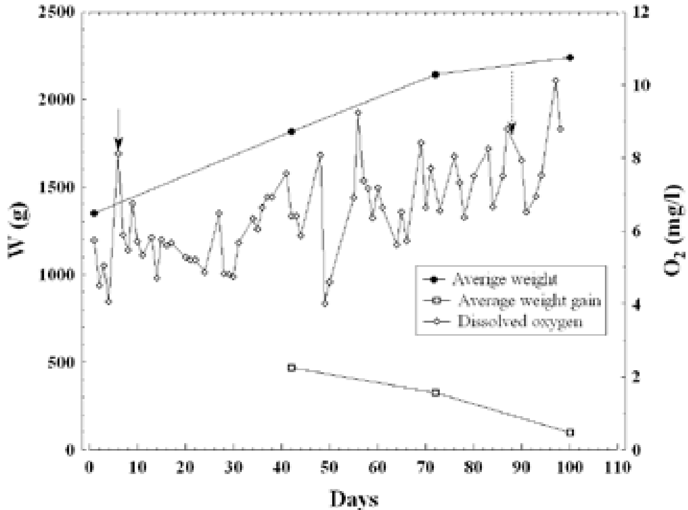

Figure 1. Growth of reared carp and variation of dissolved oxygen concentrations during the rearing period; solid arrow-start of feeding, dashed arrow-feeding cessation.

The relationship between growth of reared fish and temperature conditions for the entire experimental period is presented in Figure 2. Tempretarues above 20 ºC were recorded during 35 days from the beginning of experiment. On day 36 (1st September) temperature dropped by 1.6 ºC reaching 19.7 ºC, and from that point onwards a constant decrease of temperature was recorded. Overall average value was 18.29 ± 4.54 ºC, while in respect to the experimental phases (control and final mesurments) average values were 22.26 ± 1.49 ºC, 16.93 ± 1.45 ºC and 11.71 ± 1.36 ºC, for 1-42, 43-72 and 73-100 days period, respectively.

The increase in average weight and changes in weight gain were observed (Figs. 1, 2). Although positive growth has been obtained the slowing of growth rate was observed, particularly after 72 day of experiment, which is obviously the consequence of the temperature decrease.

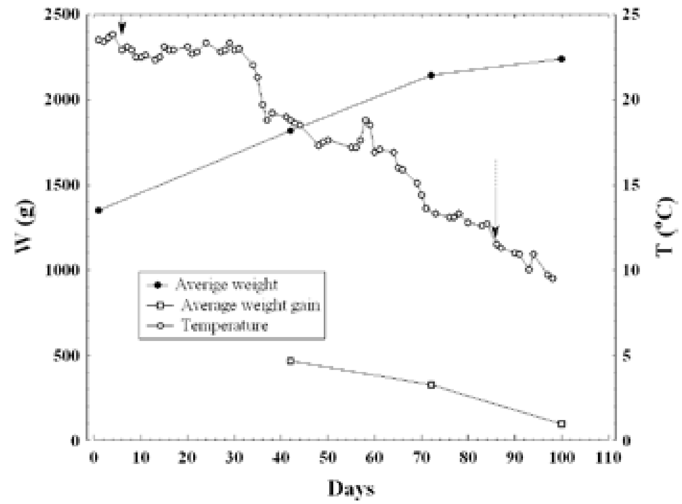

Figure 2. Carp growth and variation of temperature in the rearing water; solid arrow-start of feeding, dashed arrow-feeding cessation.

DISCUSSION

Temperature and dissolved oxygen content are among the most important factors affecting fish growth. The dissolved oxygen content during most of the rearing was, with some fluctuations, within the range which is considered suitable for intensive carp farming (Mitrović-Tutundžić *et al.* 1997). Air compressors were attached to the plate-shaped finely perforated air difussors fixed at the bottom of barge and this technical improvement solved the problems with dissolved oxygen content observed in pervious experiment (Regner *et al.* 2010). Such technical solution allowed relatively low water exchange rate, thus minimasing power demands. Huet (1994) stated that in Europe the optimum temperatures for carp growth being around 25 to 28 ºC, while the carps grow moderately well between 20 and 13 ºC, feebly between 13 and 5 ºC and not at all bellow that. Although optimal temperatures were not reached, our results were in agreement with reported ranges. Food uptake was continuosly recorded, and feeding activity was entirely dependent on water temperature. Feeding activity gradually weakened with lowering of temperature and actually ceased around 11ºC (day 88). This temperature is much higher in respect to literature reports that carp cease to feed when temperature falls bellow 5 ºC (Huet, 1994). Unfavourable temperature conditions and reduced feeding activity were reasons that production results were lower than expected. However, feed conversion ratio was considerably lower in comparison with data concerning literature reports on carp fed pelleted feed of the similar composition (Lukowicz, 1982; Mikavica *et al.* 2007; Bogut *et al.* 2007).

Barge is placed in river course and being flow-through system it is impossible to control temperature thus meaning that complete production process depends on climatic conditions. This fact indicates that suggested type of fish farming is resonable to apply on seasonal bases for fatenning marketable size fish, or for production of fingerlings for stocking purposes.

CONCLUSIONS

Studies on the possibilities of using river hopper barge as aquaculture facility are still in preliminary phase. The results obtained indicate that proposed type of fish farming might be applied on seasonal basis.

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INTRODUCED (NON-NATIVE) FISH SPECIES IN CENTRAL SERBIAN RESERVOIRS

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INTRODUKOVANE RIBLJE VRSTE AKUMULACIJA CENTRALNE SRBIJE

Prošireni abstrakt

U ihtiofauni Republike Srbije je registrovano prisustvo 23 introdukovane (alohthone) vrste iz 11 porodica. Načini i razlozi introdukcija su različiti – akvakulturno gajenje, spontano širenje areala, borba protiv preteranog razvoja makrofita, sportski ribolov i drugi. Posledice introdukcija se, uglavnom, mogu oceniti kao negativne – ugrožavanje autohtone ihtiofaune usled kompeticije za hranu, mrest, unos novih parazita i drugi neželjeni efekti.

Većina naseljava Dunav i neposredne pritoke. Međutim, pojedine vrste su ispoljile visok stepen aklimatizacije na specifične uslove akumulacija i postale su značajni članovi ribljih zajednica.

Hidrografsku mrežu Republike Srbije karakteriše postojanje oko 150 akumulacija različitih namena (vodosnabdevanje, proizvodnja električne energije, melioracija, odbrana od poplava, sportovi na vodi i turizam, ribolov......). Lociranost u različitim regionima uslovljava varijabilnost ekoloških uslova i diverzitet ihtiofauna.

U radu je dat osvrt na ulogu alohtonih vrsta u ihtiofauni tri akumulacije u slivu Zapadne Morave - Međuvršje na reci Zapadnoj Moravi, Gruža na reci Gruži i Ćelije na reci Rasini. Sve tri akumulacije karakteriše intenzivna eutrofikacija.

Rezultati ihtioloških istraživanja ukazuju na izrazito ciprinidan karakter analiziranih akumulacija. Akumulaciju Međuvršje naseljava 21 vrsta iz 7 porodica (Cyprinidae predstavljaju 90.64% brojnosti i 75.63% biomase), akumulaciju Gruža 19 vrsta iz 6 porodica (Cyprinidae su 80.94% brojnosti, 74.28% biomase) i akumulaciju Ćelije 16 vrsta iz 5 porodica (Cyprinidae su 77.5% brojnosti, 74.5% biomase). Među registrovanim ribljim vrstama, za vodotoke Srbije alohtone su *Carassius gibelio*, *Ctenopharyngodon idella*, *Hypophthalmichtys molitrix,* *Pseudorasbora parva*, *Lepomis gibbosus* i vrste roda *Ameiurus* (*A. nebulosus* i *A. melas*).

Srebrni karaš, babuška (*Carassius gibelio* Bloch 1782) je nakon II svetskog rata unet u vodotoke evropskog dela tadašnjeg SSSR, iz kojih u vodotoke širom Evrope (Holčik, 1991). Od prvog registrovanja u Srbiji, 60-ih godina prošlog veka, evidentirano

je širenje areala i ekspanzija populacione brojnosti u većini akumulacija Srbije. Vrsta preferira pliće vodotoke muljevitog dna, obrasle makrofitskom vegetacijom, sa razvijenim zajednicama planktonskih organizama i makrozoobentosom. Karakteriše je visoka tolerancija na promenljive ekološke uslove, širok trofički spektar i visoka plodnost.

Srebrni karaš je unet u sve tri akumulacije prikom poribljavanja kvalitetnijim vrsta riba, prvenstveno šaranom i somom. Vrsta je pokazala izuzetnu adaptibilnost, što potvrđuje činjenica da je dominantna u analiziranim ekosistemima sa tendencijom hiperprodukcije (posebno u akumulaciji Gruža).

Poznato je da su u populacijama srebrnog karaša koje naseljavaju pojedine ekosisteme nalažene samo ženke (partenogenetsko razmnožavanje). Prisustvo mužjaka ukazuje na mali stepen predatorstva i konkurencije u ishrani (Glaser, 1985). Registrovan je nalaz mužjaka u sve tri analizirane akumulacije. U akumulaciji Međuvršje brojčani odnos ženke : mužjaci iznosi 2.58 : 1, u akumulaciji Gruža 1.63 : 1 (Simović, 2001). Ova činjenica je još jedna potvrda povoljnih uslova za životnu aktivnost vrste u biotopima akumulacija.

Najintenzivniji tempo dužinskog rasta srebrni karaš ostvaruje u akumulaciji Gruža. Opšti ekološki uslovi (mala dubina, termika, visok organski produktivitet i drugi) favorizuju ovaj ekosistem. Omasovljenje vrste u analiziranim akumulacijama negativno utiče na autohtonu ihtiofaunu zbog konkurentskih odnosa sa komercijalno značajnijim ribama, prvenstveno šaranom. Bentofag način ishrane srebrnog karaša može usloviti mobilizaciju teških metala i drugih polutanata iz jezerskog sedimenta i pogoršati kvalitet vode akumulacija koje se koriste za vodosnabdevanje - akumulacije Gruža i Ćelije (Marković i Ivić, 2009).

Amur (*Ctenopharyngodon idella* Vallenciennes 1844) je introdukovan u veći broj akumulacija i kanala Srbije radi suzbijanja makrofitske vegetacije i njom izazvane eutrofikacije (Maletin et al., 1997; 2005). Brojčano prisustvo vrste u analiziranim akumulacijama je neznatno (izuzev u akumulaciji Ćelije). Omasovljenje amura može prouzrokovati smanjenje površina obraslim vodenim biljem na kojima se mreste šaran, linjak, deverika i druge autohtone fitofilne vrste (Janković, 1998). Kao biljojedu vrstu karakteriše je mala iskorišćenost unete hrane tako da deo unetog biljnog materijala biva nesvaren i može usloviti zagađenje vodotoka (Mitrović-Tutundžić i sar., 1996).

Beli tolstolobik (*Hypophthalmichthys molitrix* Valenciennes 1844) je planktofaga vrsta uneta zajedno sa *C.idella* u akumulacije Gruža i Ćelije. Retki nalazi ukazuju na mali uticaj na uspostavljene ekosistemske odnose.

Amurski čebačok, neriba (*Pseudorasbora parva* Temnick and Schlegel 1846) predstavlja invazivnu vrstu koja od prvog registrovanja u Srbiji (1978. godine) ispoljava tendenciju eksplozivnog proširenja areala (Cakić et al., 2004). Nema nikakvog ekonomskog značaja. Zbog kompetitivnih odnosa sa autohtonim vrstama, može pogoršati stanje lokalnih ihtiofauna. U analiziranim akumulacijama pokazuje velike fluktuacije brojnosti, sa tendencijom omasovljenja (posebno izražene u akumulaciji Ćelije).

Sunčica (*Lepomis gibbosus* Linnaeus 1758) ima severnoameričko poreklo. Karakteriše je širok trofički spektar u kome su prisutni, pored faune dna, riblja mlađ i ikra. Pokazuje velike oscilacije u populacionoj brojnosti, posebno ispoljene u akumulaciji Gruža. Poslednjih godina je primećeno opadanje brojnosti u akumulaciji Ćelije - moguća posledica omasovljenja predatorskih vrsta, prvenstveno smuđa.

Američki somići, cverglani (*Ameiurus nebulosus* Lesueur 1819) i **(*Ameiurus melas* Rafinesque 1820)** su vrste severnoameričkog porekla koje se hrane faunom dna, ribljom mlađi i ikrom što može imati negativan uticaj na autohtone ihtiofaune. Prime-

ćena je tendencija omasovljenja u akumulacijama Međuvršje i Gruža. Iako se njihovo prisutvo u šaranskim ribnjacima tretira kao neželjeno, poseduju određena gastronomska svojstva - vrsta *A.melas* se gaji u jednom ribnjaku (Lenhardt et al., 2010).

Najveću ekspanziju populacione brojnosti u ihtiofaunama analiziranih akumulacija ispoljava srebrni karaš *(C.gibelio)*. Vrsta je dominantna sa tendencijom hiperprodukcije (posebno u akumulaciji Gruža). Omasovljenje ove invazivne vrste, kao i prisustvo drugih, ugrožava autohtonu ihtiofaunu i remeti uspostavljene ekološke ravnoteže.

Ključne reči: *alohtone vrste, ihtiofauna, akumulacije, Centralna Srbija*

INTRODUCTION

The ichthyofauna of the Republic of Serbia includes 23 allochthonous (non-native) species belonging to 11 families (Simonović et al.,2010; Lenhardt et al., 2010). Methods of and reasons for fish introductions are diverse – aquaculture, spontaneous range expansion, struggle against excessive macrophyte growth, recreational fishing etc. The consequences of fish introductions tend to be negative - endangerment of native fish species due to competition over food, spawning, introduction of new parasites, and other undesirable effects.

Most non-native species dwell in the Danube and its immediate tributaries. However, some species have exhibited a high degree of acclimation to specific environmental conditions thus becoming important members of fish communities in the reservoirs.

The hydrographic network of the Republic of Serbia comprises about 150 reservoirs used for a variety of purposes (water supply, electrical energy production, land improvement, flood prevention, sports, tourism, recreational fishing). Reservoir locations within different regions involve variability of environmental conditions and ichthyofauna diversity. In addition to very large reservoirs that have a huge impact on the overall economic development of the country, such as Djerdap I-Iron Gates I (area: 235 km^2) and Djerdap II - Iron Gates II (area: 92 km^2), high importance in terms of human populations is also given to medium- and small-sized reservoirs. Such reservoirs in Central Serbia include the following reservoirs in the Zapadna Morava river basin – Međuvršje on the Zapadna Morava River, Gruža on the Gruža River and Ćelije on the Rasina River (Fig. 1).

Figure 1. Locations of the reservoirs analysed: 1 - Međuvršje; 2 - Gruža; 3 – Ćelije

The Međuvršje reservoir is among the oldest reservoirs in Serbia built in 1953 by a 31 m high dam for the purpose of electric power generation. The reservoir is 1.5 km^2 in area and 15.4 x 10^6 m^3 in volume. During more than half a century of its existence, the ecosystem has undergone large changes, primarily reflected in reservoir sedimentation (more than 70% of the original volume) and pronounced eutrophication (Lenhardt et al., 2009).

The Gruža reservoir was created in 1984 to supply water to the City of Kragujevac and surrounding areas. The lake ecosystem (9.34 km^2 in area and 64 x 10^6 m^3 in volume) located in the rural area is characterised by high organic productivity and occasional deterioration of water quality (Ostojić et al., 2007). Nevertheless, the Gruža reservoir biotope is extremely favourable for the development of different hydrobiont communities and high fish fauna production (Marković et al., 2007).

The Ćelije reservoir was formed in 1979 for the original purpose of flood control and land improvement. Since 1984, it has been used for the water supply of Kruševac and surrounding areas. The reservoir (3.35 km^2 in area and 41.1 x 10^6 m^3 in volume) has been affected by torrential watercourses (which cause biotope sedimentation) and municipal wastewaters discharged by communities upstream of the reservoir, which leads to intensive eutrophication of the ecosystem (Milenković-Anđelković et al., 2010).

MATERIAL AND METHODS

The ichthyofaunae of the reservoirs analysed have been occasionally monitored since their creation. This paper provides an overview of results of ichthyological studies conducted during 2000-2010. The fish fauna situation was analysed using not only research carried out by the present authors but also the available literature, fish catches by recreational fishermen and other relevant data. Determination of caught specimens was performed by standard methods (Vuković and Ivanović, 1971; Simonović, 2006). The presence of individual representatives within fish communities was evaluated using the scale defined by Šorić (1996).

RESULTS AND DISCUSSION

The results of ichthyological studies suggest that the reservoir is inhabited predominantly by cyprinids (Table 1). A total of 21 species belonging to 7 families (with Cyprinidae accounting for 90.64% of the total number and 75.63% of the biomass) were detected in the Međuvršje reservoir, 19 species of 6 families in the Gruža reservoir (Cyprinidae 80.94% of the total number, 74.28% of the biomass), and 16 species of 5 families in the Ćelije reservoir (Cyprinidae making up 77.5% of the total number and 74.5% of the biomass). The fish species non-native to Serbian watercourses include: *Carassius gibelio*, *Ctenopharyngodon idella*, *Hypophthalmichtys molitrix, Pseudorasbora parva*, *Lepomis gibbosus* and the genus *Ameiurus* (*A. nebulosus* and *A. melas*).

Prussian carp (*Carassius gibelio* Bloch 1782)

Carassius gibelio was introduced into the waters of the European part of the then USSR after World War Two and spread thereafter throughout Europe (Holčik, 1991). Since its first record in Serbia during the 1960's, expansion in both the range and population size of this species has been reported for most Serbian reservoirs. *C. gibelio* prefers shallow waters that have muddy bottoms covered with macrophyte vegetation, and host well-developed planktonic communities and macrozoobenthos. This species has a high tolerance to variable environmental conditions, a wide trophic spectrum and high fertility.

C. gibelio was introduced into the three reservoirs during stocking with fish species of higher quality, primarily *Cyprinus carpio* and *Silurus glanis.* The species has exhibited superb adaptabililty, as confirmed by the fact that it predominates the ecosystems analysed (Tab. 1), showing a tendency towards overproduction (particularly in the Gruža reservoir).

C. gibelio is well known for the fact that only females are found among the populations inhabiting some of the ecosystems (parthenogenetic reproduction). Male records in some populations serve as an indication of the absence of predators and food competitors (Glaser, 1985). The three reservoirs analysed were reported to contain male fish (the female : male ratio in Međuvršje and Gruža reservoirs was 2.58 : 1 and 1.63 : 1, respectively) (Simović, 2001). This is another confirmation of the favourability of the environmental conditions on the life activity of this species.

Table 1. Qualitative and semi-quantitative composition of the analysed reservoir ichthyofauna

TAXON	RESERVOIR					
	Međuvršje		Gruža		Ćelije	
	Number	Biomass	Number	Biomass	Number	Biomass
Fam. Cyprinidae						
Alburnus alburnus	ED	SD	D	R	ED	SD
Abramis brama	SD	SD	D	SD	ED	ED
Abramis sapa	SR	SR	-	-	SR	SR
Aspius aspius	SR	R	SR	SR	-	-
Barbus barbus	SR	SR	-	-	-	-
Carassius gibelio *	D	ED	ED	ED	ED	ED
Chondrostoma nasus	R	R	-	-	-	-
Cyprinus carpio	SR	R	SR	SD	R	R
Ctenopharyngodon idella *	SR	SR	SR	SR	SR	R
Hypophthalmichthys molitrix *	-	-	SR	SR	SR	SR
Gobio gobio	SR	SR	SR	SR	SR	SR
Leuciscus cephalus	R	R	SR	SR	-	-
Pseudorasbora parva *	SD	SR	SD	SR	R	SR
Rhodeus sericeus	R	SR	R	SR	-	-
Rutilus rutilus	D	SD	SD	SD	D	D
Tinca tinca	SR	SR	-	-	SR	SR
Fam. Siluridae						
Silurus glanis	SR	D	R	D	SR	R
Fam. Esocidae						
Esox lucius	SR	SD	SR	SD	SR	R
Fam. Percidae						
Perca fluviatilis	SD	SD	SD	SD	R	SD
Gymnocephalus cernuus	-	-	SR	SR	-	-
Sander lucioperca	-	-	SR	R	R	R
Fam. Cobitidae						
Cobitis taenia	SR	SR	-	-	-	-
Fam. Centrarchidae						
Lepomis gibbosus *	R	SR	R	SR	SR	SR
Fam. Ictaluridae						
Ameiurus (Ictalurus) sp. *	SD	R	SD	SD	-	-
Number of families / species	7 / 21		6 / 19		5 / 16	

* non- native (allochthonous) species
ED - eudominant (>20% of total) D – dominant (10-20%) SD – subdominant (4-10%)
R – recedent (1 – 3%) SR – subrecedent (<1%)

C. gibelio has the highest rate of growth in length in the Gruža reservoir (Figure 2). This biotope is favoured by overall environmental conditions (low depth, temperature regime, high organic productivity). The massive spread of this species within the ecosystems tested has an adverse effect on autochthonous ichthyofauna due to competitive relationships with commercially important species, primarily *C.carpio*. Moreover, the bentophagous diet of the species can induce mobilisation of heavy metals and other pollutants from the lake sediment and deterioration of the quality of reservoir water supplied (Gruža and Ćelije) (Marković and Ivić, 2009).

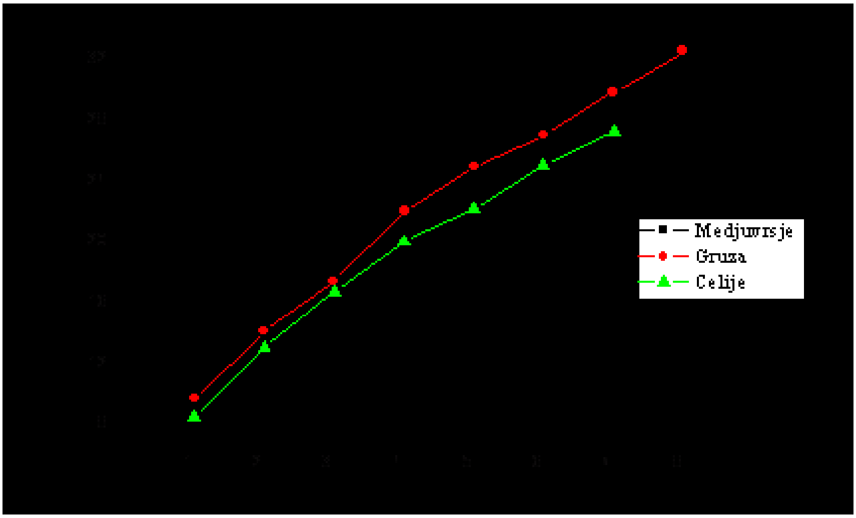

Figure 1. Length growth rate of *Carassius gibelio* in three Central Serbian reservoirs

The **grass carp (*Ctenopharyngodon idella* Vallenciennes 1844)** was introduced into a number of Serbian reservoirs and canals to control macrophyte vegetation and eutrophication (Maletin et al., 1997; 2005). The species presence is low in all the reservoirs excepting, to some extent, the Ćelije reservoir. It can lead to a reduction in areas covered with aqueous plants where *C.carpio, Tinca tinca, Abramis brama* and other autochthonous phytophilous species spawn (Janković, 1998). Being a herbivore, *C. idella* is characterised by low feed conversion efficiency, with most of the plant material ingested remaining undigested and, hence, likely to cause pollution of the water course (Mitrović-Tutundžić et al., 1996).

The **silver carp (*Hypophthalmichthys molitrix* Valenciennes 1844)** is a planktophagous species introduced into the Gruža and Ćelije reservoirs along with *C.idella*. Rare findings of the species suggest a low impact on established ecosystem relationships.

The **stone morocco, topmouth gudgeon (*Pseudorasbora parva* Temnick and Schlegel 1846)** is a highly invasive species that has shown an explosive range expansion tendency since its first record in Serbia in 1978 (Cakić et al., 2004). It has no economic importance. It can deteriorate local ichthyofauna situation due to its competitive relationship with autochthonous species. It exhibits high fluctuations in total number in the reservoirs analysed, tending towards a massive increase in its abundance (particularly in the Ćelije reservoir).

The **pumpkinseed (*Lepomis gibbosus* Linnaeus 1758)** is a North American species that shows a wide trophic spectrum involving fish fry and spawn along with the bottom fauna. It shows high oscillations in population numbers, notably in the Gruža reservoir. A decrease in its numbers has been observed in the Ćelije reservoir in the last years – a possible consequence of the massive spread of predators, *Sander lucioperca* in particular.

The **brown bullhead (*Ameiurus nebulosus* Lesueur 1819)** and the **black bullhead (*Ameiurus melas* Rafinesque 1820)** are North American species. Feeding on bottom fauna, fish fry and spawn induces an adverse effect on autochthonous ichthyofaunae.

Although their presence in carp fishponds is recognised as undesirable, these species have specific gastronomic properties (*A. melas* being farmed in one fishpond)(Lenhardt et al., 2010).

CONCLUSIONS

The ichthyofauna of the Republic of Serbia includes 23 allochthonous (non-native) species belonging to 11 families. Most non-native species inhabit the Danube and its immediate tributaries. Some species have shown a high degree of acclimation to specific environmental conditions thus becoming important members of fish communities in the reservoirs.

This study evaluates the importance of introduced fish species in the ichthyofauna of three major Central Serbian reservoirs (Međuvršje, Gruža and Ćelije). The highest expansion in population size has been exhibited by *Carassius gibelio*. This species predominates the reservoirs tested, showing a tendency towards overproduction (particularly in the Gruža reservoir). The massive spread of this invasive species within the ecosystems, along with the presence of other species, has led to endangerment of the autochthonous ichthyofauna and disturbance of the ecological balance.

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TREMATODE FAUNA OF SOME FISH SPECIES IN THE RIVER SPREČA

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FAUNA TREMATODA KOD NEKIH VRSTA RIBA U RECI SPREČI

Abstrakt

U radu su prikazane trematode koje su nađene kod riba u reci Streči tokom ihtioparazitoloških ispitivanja. Parazitološka ispitivanja su obavljena tokom 2010. godine i pokazala su prisustvo parazita kod 7 vrsta riba od 11 pregledanih u reci Streči tj. 144 primerka je bilo zaraženo trematodama od ukupnog broja (365) ispitanih riba. Kod pet vrsta riba nađene su dve vrste monogenih trematoda (*Dactylogyrus* sp., *Gyrodactylus* sp.) odnosno u crvenperki, deveriki, šaranu, ukliji i klenu. Digene trematode (*Diplostomum spathaceum, Posthodiplostomum cuticola*) zarazile su 6 vrsta riba u reci Spreči, odnosno crvenperku, deveriku, podusta, babušku, ukliju i klena. Najjača zaraza je nađena kod *Leuciscus cephalus* (63,63 %) i *Chondrostoma nasus* (54,54 %) Nešto slabiji stepen infekcije kod *Scardinius erythrophthalmus, Abramis brama* i *Alburnus alburnus.* Najmanja zaraženost je bila kod *Cyprinus carpio* i *Carassius gibelio*. Kod drugih vrsta riba nisu nađeni paraziti tokom perioda ispitivanja. Od pojedinačnih tipova trematoda najveći obim infekcije je bio sa *Diplostomum spathaceum* (14,79 %) i *Dactylogrus* sp. (12,86 %) a najmanji sa *Gyrodactylus salaris,* samo 1,91 %. Intenzitet zaraze kod ispitivanih riba varirao je od slabog pa sve do visokog stepena infestacije. Obim infestacije je zavisio od sezone ispitivanja.

Ključne reči: *reka Spreča, ihtioparaziti, trematode, ribe.*

INTRODUCTION

The tremendous wealth of our rivers, lakes and artificial reservoirs provide ideal conditions for existence of many different kinds of living beings. The survey of ichthyofauna of Spreča river has shown that it is a typical cyprinid watercourse in which there has been identified a small number of species, due to its high pollution, particularly in its lower reaches. Unlike ichthyologic researches, a research in diversity of invertebrates - fish parasites that inhabit the river Spreča - has never been conducted. In the world there is a relatively small number of published papers on the trematodes, especially on surveying trematodes in fish of open waters. Thanks to extensive testing of materials from most important rivers of Bosnia and Herzegovina, Čanković (1963) discovered the presence of 17 species of digenean trematodes in fish. Parasitological studies give us the possibility to achieve proper assessment of the validity of fish as food products, as well as to identify the parasites carried by fish which are dangerous to humans and animals. Therefore, the study of trematodes represents a special interest and among them there are many species which are fish parasites that are transmitted to humans.

MATERIALS AND METHODS

In order to assess the current state of fish parasitic fauna in the river Spreča, there were conducted parasitological surveys of 365 individual fish in the year 2010. The fish were caught at a number of locations, according to the needs of the research, with the standing triple fishing nets of the type gillnet with different mesh sizes, as well as by using other fishing tools and accessories. The largest number of parasitological tests was done on the spot, on the fresh material because the vegetative forms of trematodes very rapidly decline after the death of their hosts. Our studies were focused on examination of the skin, fins, gills, organs of the digestive system and lenses. After the determination, all the specimens of the identified fish species were thoroughly examined and analyzed for the presence of parasites.

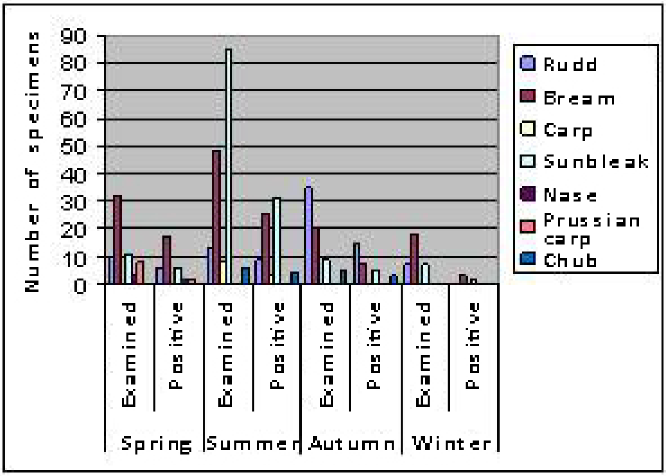
RESULTS AND DISCUSSION

Ichthyo-parasitological surveys of fish in Spreča river that were carried out during four annual seasons in 2010, have shown the presence of trematodes in a certain number of fish. Out of the total number (365) of the fish caught in river, the largest number belongs to the family *Cyprinidae* (333 individuals or 91.23%), significantly less percentage of the total ichthyo-sample is taken by *Percidae* (5.47%) and *Esocidae* (3.28%). (Table 1). The largest number within the total ichthyo-sample is taken by Bream with 118 individuals and Sunbleak with 112 individuals, whereas Ruffe was represented by only 2 individuals in Spreča river in the survey period.

Table 1. The total number of examined and infested fish species in the river Spreča

Nr.	Fish species	Family	Examined (365)		Posit.	%
1.	Rudd - *Scardinius erythrophthalmus*	*Cyprinidae* (91,23%)	65	17,80 %	30	46,15
2.	Bream - *Abramis brama*		118	32,32 %	52	44,06
3.	Carp - *Cyprinus carpio*		8	2,19 %	3	37,50
4.	Sunbleak - *Alburnus alburnus*		112	30,68%	44	39,28
5.	Nase - *Chondrostoma nasus*		11	3,01%	6	54,54
6.	Prussian carp - *Carassius gibelio*		8	2,19%	2	25,00
7.	Chub - *Leuciscus cephalus*		11	3,01%	7	63,63
8.	Ruffe - *Acerina cernua*	*Percidae* (5,47%)	2	0,54%	-	-
9.	Perch - *Perca fluviatilis*		9	2,46%	-	-
10.	Zander - *Sander lucioperca*		9	2,46%	-	-
11.	Pike - *Esox lucius*	*Esocidae* (3,28%)	12	3,28%	-	-

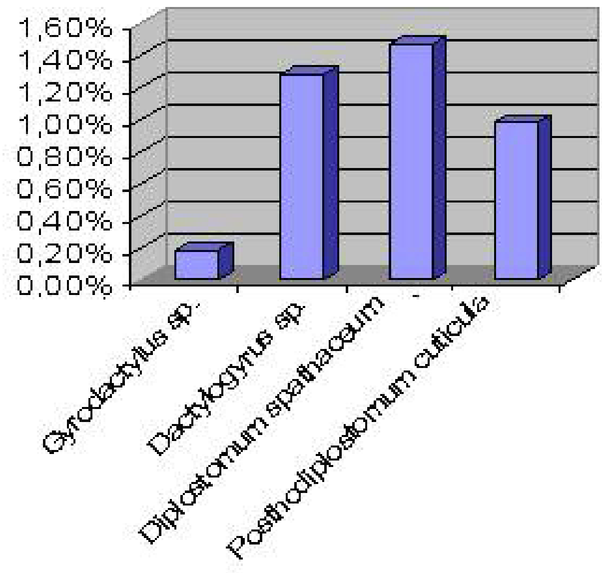
Of the 11 examined fish species in the river Spreča, 7 species were infested by trematodes. Fish with confirmed parasites belonged to the family *Cyprinidae*. The highest percentage of Trematodes (Graf 1.) was found among Chub individuals (63.63%) and Nase individuals (54.54%). In other studied species the extensity of infestation was lower. Our surveys showed certain regularity in the seasonal dynamics during the investigation period. Survey results in seasonal dynamics of trematodes found in fish of the river Spreča showed that during the spring the extensity of trematode invasion was the highest (57.81%), whereas it was slightly lower in the summer (54.60%) and autumn (50.70%). As expected, the extent of invasion during the winter period was the lowest, only 22.85%. Through parasitological examinations of fish in the river Spreča we found 4 species of trematodes so that two species of the order *Monogenea* belonged to the families *Dactylogyridae* and *Gyrodactylidae,* and two species of the order *Digenea* were classified in the family *Diplostomidae.*



Graph 1. Number of examined and trematode-infested fish by survey seasons

During the parasitological examinations of cyprinid fish from the river Spreča in 144 specimens were found parasites with the total extensity of infestation 39.45% and the intensity of infestation that ranged from low level up to a high degree of invasion. According to the data given by Čanković and et al. (1970), there was established a maximum extensity of trematode invasion in fish during the summer (58.5%), and during autumn and winter it gradually lowers and the next spring it amounts to only 7.36%. According to the data given by Komarova (1964) fish are invaded by parasites during the spring, and the infestation is reflected throughout the summer and autumn.

Byhovskaja - Pavlovskaja et al. (1963), give importance to the harmful effect of developmental forms of digenean trematodes on fish organisms. During our ichthyoparasitological (Graf 2.) surveys the ectoparasite *Gyrodactylus sp*. was found in 4 individuals of *Abramis brama* with the extensity of infestation 3.38% and a low intensity of infestation. In 3 specimens of *Leuciscus cephalus,* out of 11 examined, there was found the presence of *Gyrodactylus sp*. Overall, the extensity of infestation with *Gyrodactylus sp*. in the examined fish of Spreča river was 1.91%, with a low intensity of infestation. A significantly higher total extensity of infestation (12.86%) was determined with *Dactylogyrus sp*. also with a low intensity of infestation in the total ichthyo-sample. *Dactylogyrus sp*. was found in 4 species of fish in the river Spreča. Invasion degree of *Scardinius erythrophthalmus* with this kind of ectoparasites was 9.23%, with a low level of infestation. In *Abramis brama* was found the presence of *Dactylogyrus sp*. with the extensity of infestation 19.49% and with a medium-strong degree of infestation. The largest pathogenic impact had *Dactylogyrus sp*. (37,50%) in *Cyprinus carpio*, whereas the degree of infestation of *Alburnus Alburnus* by this parasite was significantly lower (13.39%) with a low infestation degree, as well as in the carp. According to the findings of Jažić, the largest presence of *Dactylogyrus* was in the period from May to October, which partially overlaps with our research results.



Graph 2. A comparative display of detected trematodes in fish of the river Spreča

For a five-year research period, with 5568 carps of different age categories, in all three fish farms in Bosnia and Herzegovina, Jažić (1995) found 21 species of parasites. The largest number of confirmed parasites belonged to helminths, 14 in total. Scrapings examination of fins, skin and gill of carp in the investigated fish farms discovered 11 species of monogenean trematodes, four of them belonging to the genus *Dactylogyrus*, six to the genus *Gyrodactylus* and one to the genus *Eudiplozon*. For species of the genus *Dactylogyrus* is known to extensively replicate at lower water temperatures, although it is possible to find them at higher teperatures as well, especially in the summer months. Of the total of 30 examined goldfish in three pet shops, Gjurčević et al. (2006) found that the parasite invasion degree in Carassius is for *Dactylogyrus sp*. 66.66% and for *Gyrodactylus sp*. 46.66%. During parasitological investigations of cyprinid fishes of Lake Prespa, Stojanovski et al. (2004) in three species of fish found in the gills 6 species of monogenean trematodes: *Dactylogyrus prostae, D. sphyrna, D. erhardovae, D. elegantis, D. vistulae* and *Paradiplozoon Zeller*. The largest extensity of infestation was by *Dactylogyrus sphyrna* 25.08%. With *Leuciscus cephalus albus* the extensity of infestation was 62.22%, with *Rutilus rubilio prespansis* 59.87%, and with *Chondrostoma nasus prespansis* it was 41.59 % - which is lower than the results that we achieved. In the examined specimens of fish from the river Spreča there was discovered the presence of *Diplostomum spathaceum* in five species.

The largest extensity of infestation was detected in Nase (54.54%) and Prussian carp (25%) with a low degree of intensity of infestation. In *Alburnus Alburnus* was detected the extensity of infestation 20.53% with *Diplostomum spathaceum* and a low degree of infestation. The extensity of infestation in *Abramis brama* was 14.40% with a medium-strong degree of infestation as well as in *Scardinius erythrophthalmus* but with significantly lower extensity of infestation (9.23 %). According to Wilfried Haas at al. (2002) the cercaria *Diplostomum spathaceum* parasitize in the skin of fish and other animals, depending on the appropriate stimulus on the basis of which they recognize the host. Studies have shown that stimulants which make possible prolonged contact of hosts and parasites are small molecules of carbohydrates, while amino acids, urea, electrolytes, and peptides do not enable contact. Parasitological examinations of fish caught in the river Spreča discovered the presence of *Posthodiplostomum cuticula* in 36 individuals or 9.86% of the total ichthyo-sample.

The largest extensity of infestation was found in *Leuciscus cephalus* 36.36% with a low degree of infestation. A significant presence of this parasite was found in Rudd with the extensity of infestation 27.69% and the highest intensity of infestation, with more than 30 examples of this endoparasite in each. Much less extensity of infestation was found in the examined specimens of *Abramis brama* (6.77%) and *Alburnus Alburnus* (5.35%) with a medium-strong degree of infestation. *Posthodiplostomum cuticula* shows the percentage prevalence of 73% in individual fish of the genus *Cobitis* (hybrid C. x Elongatoides Cobitis sp.) from the river Dyje in the Czech Republic, according to Halačka K. at al. (2000).

CONCLUSION

Exploring the presence of trematodes in fish of the river Spreča we found that 39.45% of the examined fish were invaded by parasites in the survey period. All the fish in which parasites were found belonged to the family *Cyprinidae*. The largest extensity of infestation was found in Chub 63.63% and Nase 54.54%. In Rudd, Bream, Carp and Sunbleak the extensities of infestation were lower whereas in Prussian carp it was only 25%. The highest intensity of infestation was found in Rudd. The parasitological examinations of fish in Spreča river discovered the presence of 4 species of trematodes, so that two species of the order Monogenea belonged to the families *Dactylogyridae* and *Gyrodactylidae,* and two species of the order Digenea were classified in the family *Diplostomidae*. Individually, we have found that the highest extensity of infestation was with *Diplostomum spathaceum* (14, 79%), slightly lower extensity was with *Dactylogyrus sp*. (12.86%) and 9.86% with *Posthodiplostomum cuticula*. The lowest invasion level in fish was with *Gyrodactylus sp*. as it was found to be only 1.91%. Invasion degree of the fish in Spreča river by trematodes shows proper seasonal dynamics.

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AN OVERVIEW OF BASIC DATA ON THE BOSNIAN-HERZEGOVINIAN PLECOPTERA SPECIES

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PREGLED OSNOVNIH PODATAKA O BOSANSKO-HERCEGOVAČKIM VRSTAMA PLEKOPTERA

Abstrakt
Bosna i Hercegovina se nalazi u središnjem dijelu sjevernog umjerenog pojasa. Ona se nalazi u jugoistočnom dijelu Evrope, u središnjem dijelu Balkanskog poluotoka. Najveći dio Bosne i Hercegovine hidrografski pripada crnomorskom slivu odnosno porječju rijeke Save. Sava svoje najveće pritoke prima upravo iz Bosne (Una, Vrbas, Bosna, Drina). Manji hercegovački prostor odvodnjava se prema Jadranskom moru.

U radu su analizirani postojeći literaturni podaci o zastupljenosti vrsta reda Plecoptera u Bosni i Hercegovini. Na temelju svih raspoloživih podataka, može se zaključiti da je u Bosni i Hercegovini vrlo širok stupanj biološke raznolikosti vrsta iz reda Plecoptera, zbog čega se Bosna i Hercegovina općenito svrstava u bogatija područja Evrope.

Zbog ekstremne osjetljivosti ličinki na smanjene koncentracije kisika, kao i osjetljivost na toksične tvari u vodi i promjene u strukturi staništa, upravo se ova skupina vodenih insekata koristi u biološkim procjenama kakvoće površinskih voda kao pokazatelj zdravih potoka i rijeka (metod biološkog indeksa).

Ključne riječi: *Bosna i Hercegovina, Plecoptera, bioraznolikost, endem*

INTRODUCTION

Plecoptera include soft body insects, which size can vary from 4 mm to 5 cm. They have two pairs of wings but they are weak fliers. Commonly found along the water in which they live their larvae stages, mostly in pure water, under rocks. To date, about 2000 described species of Plecoptera were classified into 15 families (Matoničkin, 2001).

Antennas Plecoptera are long, have complex eyes and two or three ocels. Lip of the appliance is adapted for nibbling. Of three thoracic segments protoraks is larger and mobile. The legs are strong with a pair of three-member tarsus and claws. Wings are membranacea; last are almost always larger than the front. The abdomen has 11 segments and a pair of multi daughter. (Krunić 1986, Brajković, 2004).

Intestinal system begins with ear hole, followed by long esophageal, stomach is rudimentisan or absent, and the mean and the rectum is short. The hose empties 20-60 Malpigijevih courts. Trachea system is open to the external environment over 10 pairs of stigmas. Nervous system consists of the brain, three pairs of thoracic and 6 to 8 pairs of abdominal ganglia. Male genital system is composed of pairs of testes which start seminal ducts joining the central ejaculation channel. Female reproductive system is composed of pairs, which are based on ovarian fallopian tubes that are opened through the gonopore and usually have spermateku (Krunić, 1986).

In former Yugoslavia by the number of species Plecoptera do not get behind vretenica (Odonata) or water flowers (Ephemeroptera), but still, it is little known about them. In our country, they have attracted attention in recent years even at the turn of this century. So far in Bosnia, there are 75 known Plecoptera species. This figure incorporates B&H in general among the richer areas of Europe.

MATERIALS AND METHODS

Information on the *Plecoptera* in Bosnia and Herzegovina in this paper are based on existing literature (Klapalek 1898, 1901, 1906, 1906a; Pongracz 1913, 1914; Aubert 1963, 1964; Kaćanski i Zwick 1970; Zwick 1978; Kaćanski 1970, 1971, 1978, 1983, 1989).

RESULTS AND DISCUSSION

This section presents the results of previous studies of order *Plecoptera* in Bosnia and Herzegovina. All data provided by districts to facilitate comparison of species composition.

All endemic species *Brachyptera* colonize springs and spring creeks, but they are more or less spread in the upper parts of ritrona. Slightly broader distribution in relation to habitat conditions are manifested by *Brachyptera graeca*. It was found at various altitudes of about 530-1400 m. Based on previous findings *Brachyptera graeca* is widespread in Macedonia, Kosovo, Montenegro and Bosnia and Herzegovina. *Brachyptera helenica* sites are known in Serbia, Bosnia and Herzegovina, Macedonia, at elevations 530-1150 m. *Brachyptera tristis* has been recorded in all parts of Yugoslavia except Macedonia. This species colonizes a strong karst springs in the karst area, and may be defined as species characteristic for the type of already mentioned streams. *Brachyptera beale* is widespread in the southern Balkans, and was observed in the mountains Maglić, Volujak and Zelengora at an altitude above 1000 m.

Table 1. Comparative review of the total number of species and subspecies and portion of Balkan endemic species in the basin of Bosnia and Herzegovina:

	Number of species	Number of subtype	Endemic species
BOSNA	51	1	*Brachyptera tristis* (Balkan)
			Brachyptera graeca (Balkan)
			Isoperla albanica (Albania)
			Chloroperla russevi (Balkan)
			Leuctra hippopoides (Greece, Yugoslavia)
			Leuctra signifera jahorinensis (Dinaridi)
DRINA	42	1	*Brachyptera beali* (southern Balkan)
			Brachyptera helenica (Balkan)
			Leuctra olympia (Greece)
			Leuctra aptera (BiH)
			Leuctra hippopoidea (Greece, Yugoslavia)
			Leuctra procera (BiH and Serbia)
			Isoperla albanica (Albania)
			Siphonoperla neglecta graeca (Balkan)
			Chloroperla russevi (Yugoslavia)
The upper DRINA flow	52	1	*Brachyptera graeca* (Balkan)
			Brachyptera helenika (Balkan)
			Brachyptera tristis (Balkan)
			Leuctra hippopoides (Greece,Yugoslavia)
			Leuctra olympia (Greece)
			Isoperla albanica (Albania)
			Siphonoperla n.graeca (Balkan)
			Chloroperla russevi (Yugoslavia)
SANA	1		
LASVA	5		
VRBAS	33	1	*Leuctra hippopoides* (Greece,Yugoslavia)
			Isoperla tripartita obliqa (Greece)
NERETVA	28	1	*Brachyptera graeca* (Balkan)
			Brachyptera helenica (Balkan)
			Brachyptera tristis (Balkan)
			Leuctra olympia (Greece)

Leuctra aptera is designated as endemic to the Dinaric Alps, but it is the same as *Leuctra jahorinensis* and it can be considered as an endemo of Bosnia and Herzegovina. Only typical habitats of this species are known so far (Kaćanski & Zwick, 1970; Kaćanski 1970). It was found in three small spring creeks that flow into Sutjeska at an altitude of about 900 m. A new sites of *Leuctra jahorinensis* species have not been found than those where the typical examples take their origin. They are the Praca Vrh and source Paljanska Miljacka (Kaćanski, 1972). The two mentioned localities belong to different basins, one in a mountain complex and another in the pinewood (*Picetum montanum*). *Leuctra hippopoides* type is quite widespread in streams of Bosnia and Herzegovina, it was found at several sites in Serbia. It colonizes clear streams primarily spring at an altitude of 550-1400 m. *Leuctra olympia*, according to previous data, is widespread in streams of Macedonia, Montenegro, Serbia and Bosnia and Herzegovina (Ikonomov, 1969; Kaćanski 1970, 1978, 1980).

Isoperla albanica is described from specimens collected in Skala Bicajt in Albania. According to the previously published data, it was found in Serbia, Montenegro and Bosnia and Herzegovina (Kaćanski, 1970,1971,1976; Sivec, 1980). It colonizes larger spring streams and those that match ritronu mountain streams, mostly at higher elevations 730-1650 m. *Isoperla intermis* species sites are known only in running the Dinaric mountain system. It has been recorded in the area holokarsta Bosnia and Herzegovina, and Croatian (Kaćanski & Zwick, 1970; Kaćanski, 1972, 1978). *Isoperla tripartita obliqa* is described accirding to the specimens collected in Greece. It is assumed to be distributed widely in the Dinarides, however, the material within this species requires revision, which will complement data on the distribution of the Balkan subspecies.

Siphonoperla neglecta graeca is recorded in Macedonia (Ikonomov, 1969), Montenegro (Kaćanski & Baumann, 1981) and Bosnia and Herzegovina, where it was found in the massif of Zelengora mountain (Kaćanski, 1970). Typical site of *Chloroperla russevi* is Rila Mountains in Bulgaria. In Yugoslavia, so far it is established in Macedonia (Ikonomov, 1976) and in a few places in Bosnia (Kaćanski 1970, 1971). It is a resident of the spring creeks in the higher altitudes of about 100 m to over 1600 m.

CONCLUSIONS

The results of this study can be summarized in a few conclusions:

- 75 species and subspecies have been found in the investigated catchments. Analyzing the data obtained by specifying materials for watershed Plecoptera, 51 species and one subspecies are related to the Bosna River Basin, at the confluence of the Drina (Maglić mountain resort, and Volujak Zelengora) 42 species and one subspecies, the upper basin of the river Drina 52 species and one subspecies , at the confluence of the Vrbas River, 33 species and one subspecies, the Neretva River basin belongs to 28 species and one subspecies, the basin Lasva 5 species and one type of basin drainage area. It can be concluded that in Bosnia and Herzegovina, there is a very wide degree of biological species diversity of Plecoptera order, which incorporates Bosnia and Herzegovina into the richer areas in Europe.
- So far in the villages of Bosnia and Herzegovina 13 Plecoptera species and subspecies have been recorded that are endemic to the Balkans. *Leuctra aptera* is designated as endemic to the Dinaric Alps, but as *Leuctra signifer jahorinensis* is considered as an endemo of Bosnia and Herzegovina

Nine species and two subspecies *Brachyptera beale*, *Brachyptera helenica*, *Brachyptera graeca*, *Brachyptera tristis*, *Leuctra hippopoides*, *Leuctra olympia*, *Leuctra procera*, *Isoperla albanians*, *Isoperla triparitia obliqa*, *Siphonoperla neglecta graeca* and *Chloroperla russevi*, according to today understanding of their distribution only inhabited the Balkan Peninsula, and are now considered endemic to this area. It is important to emphasize the site of *Isoperla intermis* species, endemic to the Dinarides, which can be treated as a characteristic species for sources in the Bosnian-Herzegovinian holokarsta, where in some places reaches a high density population. *Perla pallida* has also been recorded known in the Caucasus, the Carpathians and Asia Minor. Glogovka the first and so far the only site of its kind in Bosnia and Herzegovina has been recorded, too. Type *Taenopteryx kultreiberi* in Bosnia and Herzegovina has been recorded so far only in the Lasva basin.

- It is necessary to re-collect Plecoptera in the territory of Bosnia and Herzegovina, to make the determination, and then form a modern database of images, with emphasis on diagnostic characteristics of the studied taxa. Various aspects of human activity, such as for example deforestation, and various pollution greatly endanger the natural habitat types of the order Plecoptera. As a result of these and similar phenomena in some localities there is impoverishment of the population or even disappearance of *Plecoptera* including endemic species. Thus keeping the natural habitat is the most important measures to protect gene pool of these insects.

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QUALITATIVE AND QUANTITATIVE COMPOSITION OF BENTHOS COMMUNITY IN EVALUATION OF WATER QUALITY OF NERETVA RIVER AT VISICI AND ZITOMISLICI SITES

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KVALITATIVNI I KVANTITATIVNI SASTAV ZAJEDNICE BENTOSA U PROCENI KVALITETA VODE REKE NERETVE NA LOKALITETIMA VIŠIĆI I ŽITOMIŠLIĆI

Abstract

This paper is a result of the research of the river Neretva benthos at sites Zitomislici and Visici (downstream of Mostar) from 2005. to 2010. The sampling was done once a year, and 'kick-sampling' sampling was used for macrointervertebrates, while the samples for the analysis of the phytobenthos composition were scraped from the sediment with a scalpel or run-off from the sediment (standard EN 13946: 2003 Water quality – Guidance). Results of the analysis point to 62 algae taxa at site Zitomislici and 69 at site Visici. Macrointervertebrates benthos composition points on dominance of snails and sensible groups of larvae stages of the EPT insect groups. Saprobic values of both biological factors are relatively balanced and for the river Neretva, at site Zitomislici, point to oligo/betamesosaprobe level, while at site Visici they point to betamesosaprobe level of quality.

Key words: *benthos, degradation, saprobic index*

INTRODUCTION

The Neretva river originates at the top of the mountain Lebršnik, under the montain peak Grdelj, at 1227 meter above sea level, and its total length is 230 km. The largest part of the river basin is located in Bosnia and Herzegovina (90%). Neretva river flows through the valley that is surrounded on the north by the slopes of Mts. Bitovnja (1,700 m) and Vranica (2112 m), on the south side it is surrounded by the Mts. Prenj (2103 m) and Čvrsnica (2226 m), and in the west by the Mt. Bjelasnica (2062 m). The upper part of the Neretva river running parallel to the mountains, through the canyon that make Mts. Čvrsnica and Prenj. Characteristics of the mountain river, with a big drop and large mechanical strength are retained in central part of flow that is located in Mostar field. Neretva river, by the hydromorphological characteristics of the area in which it flows, is a unique complex nature of land and water. Benthic researches in the context of water quality of freshwater ecosystems dating from the period of the 20th century and are based on the composition of benthos and plancton biocenosis. Current data on the composition of these components points on the specificity and high degree of endemism (Kaćanski, 1978, Marinković-Gospodnetić, 1978, Trožić-Borovac, 2005) in this area. Last decade of intense researches in the implementation of the provisions of the WFD (2000/06/EC) were applied at the Neretva river basin. Activities are expressed in constant biomonitoring at specific sites. The aim of this paper is to display the results of biomonitoring at sites downstream from Mostar (sites Zitomislici and Visici) in period of 2005-2010.

Visici site is located at N 43° 4,58' 0,24"and E 17° 42,4' 01" at 0 meter above sea level in the Mediterranean area. Area of research is on the left side of the Neretva river bed, and on the right side of the bed the gravel factory is located. Depth at a given site ranged from 30 to 80 cm, the bottom is covered with plants up to 40%, the sediment is argyllal (Fig. 1). The shore is covered with bushes of white willow *Salix alba*.

Zitomislici site is located on the Neretva River at N 43° 20,1' 76,9"and E 17° 78' 57.2" at 16 meters above sea level. At this site sediment are microlital mesolital on the right side of the river bed. Overgrown sediment at the site is up to 60%, and the height of the coast is up to 40 cm. Coast on the right side is with restaurants and trees (Fig. 2). On the left side of the bed coast is the height of 150 cm with a well developed tree vegetation. Water is odorless and blue-green color. The depth of the sampling point is from 15 to 40 cm, and width of the bed is 4-5 meters.

Figure 1. Visici site

Figure 2. Zitomislici site

MATERIALS AND METHODS

Research of benthos at Visici and Zitomislici sites was made in the period from 2005.-2010., and sampling was done once in the year in June. Samples for analysis of phytobenthos. Macroscopically visible representatives of some departments of algae were determined using a binocular magnifier and removed from the sample, the remaining material was viewed under high magnification of light microscope.

Diatoms were determined from the permanent preparations that were made after the chemical processing of materials by Hustedt method (1930). Taxonomic background of microflora was determined with the help of keys and manuals for identification of diatoms: Hustedt (1930), Zabelina et al. (1950), Lazar (1960) and Hindak et al. (1978). The determination was done on light microscope *Olympus CX21FS1*, by immersing the lens (magnification 100*10). It was determined the presence of all the determined forms.

Given data were processed to determine the relative abundance of each taxon (Knopp, 1954), by which the relative frequency scale has values 1, 3 and 5, then, indicator values of species are defined by Wegl (1983). Using the following model, based on the indicator values and relative frequencies of indicator species, we calculated the Saprobic index (Pantle-Buck, 1955), at the individual study sites:

$$S = \sum_{i=1}^{n}(s_i . a_i) \Big/ \sum_{i=1}^{n}(a_i)$$

where:
S – saprobic index saprobity
s_i – saprobic value of taxon
a_i – relative abundance of taxon.

Based on saprobic indices (Pantle-Buck, 1955) the degree to saprobity was assessed (Libmann, 1962). For calculation of the saprobic index of phytobentos only the species which have saprobic value were taken into account.

For the qualitative and quantitative analysis of the macroinvertebrates, sampling of zoobenthos was performed at the same sites of the river Neretva. Sampling was performed by the "kick sampling" method (according to the provisions of the Official Journal of the European Community L 327 of 22.12.2000.). At each site was collected bulk sample with 12 subsamples. At the sites where this method could not be applied sampling was carried out according to ISO 7828-1985 (E) (International Standard 7828: Water quality - Methods of biological sampling - Guidance on handnet sampling of aquatic benthic macro-invertebrates) and ISO 8265 - 1988 (E) (International Standard: Water quality - Design and use of quantitative samplers for benthic macro-invertebrates on stony substrates in shallow freshwaters). Samples were fixed at the site by 4% formaldehyde and in the laboratory was done washing and separation of the organisms in 70% alcohol. Identification of the species was made using the keys: Aubert, 1959, Bole, 1969; Consiglio, 1980, Karaman, 1998, Eliot at all., 1988, Studemann, 1992, Waringer and Graf, 1997.

In addition to biological parameters physical/chemical parameters of water that were measured by Institute of Public Health in Mostar between 2009 and 2010 years are presented too.

RESULTS

The results of analysis of selected physical and chemical parameters of water in the localities of the Neretva River (Table 1) show elevated levels of total phosphorus at the Zitomislici site for both years. Among the other parameters at the Visici site high concentration of oxygen and saturation is measured. During 2009 the BOD_5 values show that the water is in the second category of quality. Other parameters during the two years of research have been in a category under the applicable law on the categorization of water in the FBiH (Official Papers of FBiH nr.70/06).

Table 1. Values of physical and chemical parameters of water of the Neretva River at the Zitomislici and Visici sites between 2009. and 2010.

Parameter	MDK	Neretva River - Zitomislici		Neretva River - Visici	
		2009	2010	2009	2010
T°C		13,3	13,90	17,3	16,80
Cond. µS/cm	<400	297	316	329	360
pH	6,80-8,50	8	7,7	8	7,6
O_2 mg/l	≥8,00	10,86	13,23	14,24	13,16
O_2 %	90-105	113,2	128,0	148,2	135,5
BOD_5	2	1,43	1,34	2,28	1,32
P mg/l	0,100-0,250	0,120	0,160	0,380	0,404
N mg/l	1,0	0,280	0,319	0,050	0,065

The results of the analysis of qualitative and quantitative composition of benthic organisms show a high diversity of algae from the departments of Cyanophyceae, Chlorophyceae, Bacillariophyceae, Rhodophyceae, Xanthophxceae and Conjugatophyceae. At the site Zitomislici, during six years of study, 62 species were collected. Species belonging to department of Bacillariophyceae were dominant. At the site Visici greater diversity was registered (69 taxa), and the greatest diversity was registered in samples in 2008th (47 taxa). Within phytobenthos that belongs department of Bacillariophyceae at the site Zitomislici highest abundance show species *Cocconeis pediculus* (oligo/betamesosaprobic indicator) *Cocconeis placentula* (oligo/betamesosaprobic indicator) and *Fragilaria construens* (oligo/betamesosaprobic indicator). From the other groups of phytobenthos the greatest abundance show *Nostoc sp*. and *Oscillatoria limosa*, while other types are poorly represented. The results indicate the diversity and the unequal representation of species of lower plants during the period of research, and the increased presence of indicators of organic pollution. At the site Visici (Dračevo) species *Cocconeis pediculus* and *Cocconeis placentula* had the greatest abundance. Among the other groups *Nostoc sp*. *Chamaesiphon incrustans*, *Ulotrix zonata* in 2010. and *Vaucheria sp*. in 2006. year shows large abundance.

Whithin samples of zoobenthos from the site Zitomislici 51 taxa was identified with dominance of larval stages of orders Ephemeroptera, Plecoptera and Trichoptera. The highest diversity was registered in samples from 2005. (27 taxa) and lowest in 2010. (11 taxa). Number of snails and caddisflies that are registered stand out with nine species of snails and 16 species of caddisflies. Amphipod shrimp *Gammarus fossarum* was numerous in composition of zoobenthos at the studied sites. Samples of zoobenthos at the site Visici was noted with 35 taxa and dominance by snails (13 species). The highest diversity was registered in samples from 2007, and the largest number of individuals of macroinvertebrates were in 2005. Saprobic index values for the phytobenthos at the site Zitomislici were between 1,68 in 2006. and 1,82 in 2009 (Table 2). Values of sapribic index for macroinvertebrates ranged from 1,53 in 2010. to 1,87 in 2005. According to the values of both parameters saprobic index ranged from 1,67 in 2010 to 1,82 in 2005. and 2008. Comparison of the values of saprobic index for both biological parameters indicates the relative uniformity (Figure 3).

Table 2. Values of saprobic index for the phytobenthos and macroinvertebrates composition sampled in the Neretva River at the site Žitomislici from 2005. to 2010.

	2005		2006		2007		2008		2009		2010	
	Zoo.	Phyt.	Zoo.	Phyt.	Zoo.	Phyt.	Zoo.	Phyt.	Zoo.	Phyt.	Zoo.	Phyt.
SI	1,87	1,77	1,74	1,68	1,74	1,73	1,84	1,78	1,72	1,82	1,53	1,81
Both	1,82	II	1,71	I/II	1,74	I/II	1,82	II	1,80	I/II	1,67	I/II
Cat.	βmesosaprobna		Oligo/ βmesosaprobna		Oligo/ βmesosaprobna		βmesosaprobn		Oligo/ βmesosaprobna		Oligo/ βmesosaprobna	

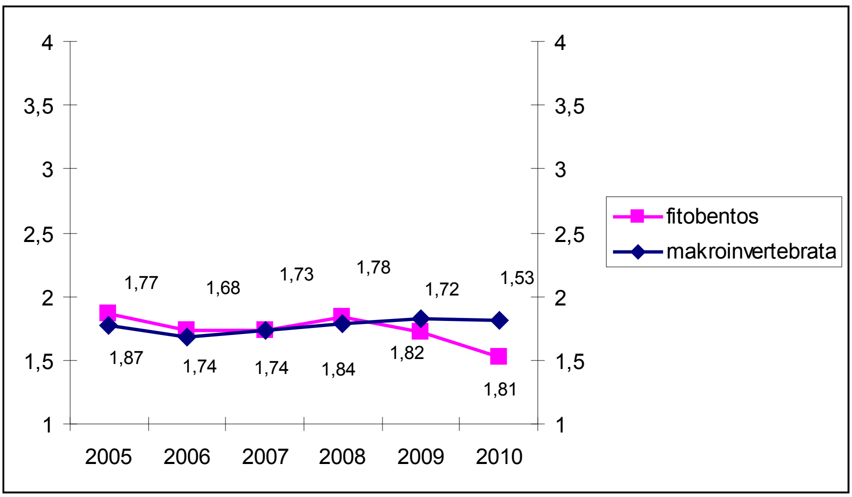

Figure 3. The ratio values of saprobic index for the composition of macroinvertebrates and phytobenthos at the site Zitomislici in the period from 2005. to 2010.

At the site Visici the values of both analyzed biological parameters show that the water is the second category of quality. The saprobic values varied from 1,84 in 2010 to 2,14 in 2009 (Table 3). Values of saprobic index for phytobenthos indicate relatively favorable conditions, while there were some significant differences in the composition of macroinvertebrates (Figure 4).

Table 3. Values of saprobic index for the phytobenthos and macroinvertebrates composition sampled in the Neretva River at the site Visici from 2005. to 2010.

	2005		2006		2007		2008		2009		2010	
	Zoo.	Phyt.	Zoo.	Phyt.	Zoo.	Phyt.	Zoo.	Phyt.	Zoo.	Phyt.	Zoo.	Phyt.
SI	1,86	1,86	2,01	1,75	1,95	1,79	2,06	1,87	2,39	1,90	1,89	1,79
Both	1,86	II	1,88	II	1,87	II	1,96	II	2,14	II	1,84	II
Cat.	βmesosaprobna		βmesosaprobna		βmesosaprobna		βmesosaprobna		βmesosaprobna		βmesosaprobna	

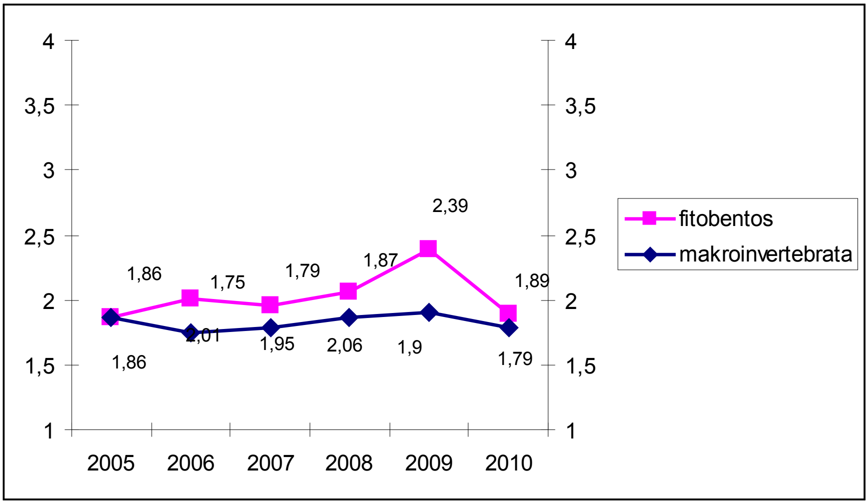

Figure 4. The ratio values of saprobic index for the composition of macroinvertebrates and phytobenthos at the site Visici in the period from 2005. to 2010.

DISCUSSION

The results of the qualitative and quantitative analysis of composition of the benthos (phytobenthos and macroinvertebrates) in the Neretva River and the Zitomislici and Visici sites, indicating a high degree of variations during the reasearch period. According to an analysis of basic chemical parameters (2009. and 2010.) the concentration of oxygen at both sites were the first category , according to BOD_5 values, waters of the Neretva River at the site Visici was in the second category 2009. (2,28 mg/l), and 2010 in the first category (1,32 mg/l). At the site Zitomislici it was registered a slight increase of total phosphorus as a result of the influx of waste water rich detergents.

During the research biological parameters showed little variations both in species composition as well as the total number of taxa identified by samples. In the past studies in Neretva River was recorded 104 species of caddisflies (Marinkovic-Gospodnetić, 1978; Trožić-Borovac, 2005), as a result of the diversity of geological and climatic characteristics of the space on which spreads the flow. Analyzed community composition illustrates overall state of watercourses, which is under direct anthropogenic influence. Variation of qualitative and qualitative composition of the benthos is caused by the existence of hydroelectric power plants upstream from the sites of investigation. During the day there is a marked variation in water levels caused by power plant operations. At the site Zitomilsići sediment shows distinct differences on the left and right side of the waterbed. On the right side of the waterbed on the coast there is a catering facility, and the left banks are covered with natural vegetation. At the site Visici on the right waterbed there is gravel factory, and the sediment on the left bank is fital and sludge (Trožić-Borovac et al., 2010).

Organisms identified in benthic communities with their presence and abundance objectively reflect the situation in the Neretva River at sites Zitomislici and Visici. By analyzing the composition of the benthos in the assessment of water quality in the area of the Vrelo Bosna and river Fojnica were obtained similar results (Trožić-Borovac, Hafner, 2005, Trožić-Borovac, Hafner, 2010). Increased use of hydropower, residential areas, unsolved treatment of the waste waters irreversibly damage aquatic ecosystems (Neumann et al., 2003a, 2003b).

CONCLUSIONS

On the basis of the analysis of quantitative and qualitative composition of river Neretva benthos, downstream of Mostar (Zitomislici and Visici) from 2005. to 2010., saprobic values point to oligo/betamesosaprobic to betamesosaprobic category of water quality. During the period of research, oscillation in abundance and species composition of benthos was expressed as a result of hydroelectric power plant work upstream of the site. The benthic organisms with their presence and abundance proved to be reliable indicators of general ecological state in the watercourse of Neretva at research sites.

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PHYLOGENETIC RELATIONSHIP AMONG INTENSIVELY FARMED CARP SPECIES THROUGH DNA BARCODES

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DETERMINACIJA FILOGENETSKIH VEZA IZMEĐU INTEZIVNO GAJENIH ŠARANA UZ POMOĆ DNK BARKODINGA

Abstract

Many carp species have been domesticated and cultured as food all around Europe and Asia for thousands of years. Aquaculture of carp still exceeds the total amount of farmed fish volume of intensively farmed marine species, especially in Asia. In this study, mitochondrial cytochrome c oxidase subunit I gene (COI) sequences, known as DNA barcode, was used to clarify phylogenetic relationship among 7 cultured carp species.

Specimens of all species (Bighead carp (*Hypophthalmichthys nobilis*), Catla (*Gibelion catla*), Common carp (*Cyprinus carpio*), Grass Carp (*Ctenopharyngodon idella*), Mrigal carp (*Cirrhinus mrigala*), Rohu (*Labeo rohita*), Silver carp (*Hypophthalmichthys molitrix*)) were collected and tissue (muscle and liver) samples were taken from each specimen and stored at -20°C until DNA extraction. Mitochondrial COI gene was amplified using polymerase chain reaction (PCR), with universal fish DNA barcoding primer pairs (Fish F1-R1 and Fish F2-R2). Amplified DNA's were purified and DNA sequencing reactions were conducted on an automatic sequencing system. Alignment of sequences was performed using ClustalW. Phylogenetic analyses of the aligned sequences were conducted using MEGA 5.

Analyses of nucleotide composition, variable/conserved sites and parsimony informative sites were conducted on each species. Tamura-Nei model was used for pairwise distance calculation. Maximum likelihood (ML) and unweighted pair-group method of arithmetic average (UPGMA) methods were used in construction of consensus tree, with bootstrap tests of 1000 replicates for the reliability of the constructed tree.

Including all species studied, there were 76.0% conserved sites and 24.0% variable sites along with 16.1% of parsimony informative sites. Average of A-T contents was found as 55.2%. Rate of transitionsal pairs over transversional pairs (R) was calculated as 2.84. Estimates of evolutionary divergence between sequences were between 0.040-0.171 among the species with an average distance of 0.132. In conclusion, DNA barcode analysis of seven intensively farmed carp species provided us some useful information about effectiveness of using DNA barcodes in estimating phylogenetic relationship among these species, in congruence with the existing taxonomy.

Key words: *Carp, Cyprinidae, cytochrome c oxidase subunit I, DNA barcode, phylogeny.*

ONTOGENY OF THE FEEDING APPARATUS OF HATCHERY-REARED *SALMO FAROIDES* AND *SALMO MACEDONICUS* DURING THEIR EARLY STAGES

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ONTOGENIJA USNOG APARATA *SALMO FAROIDES* AND *SALMO MACEDONICUS* GAJENIH U MRESTILIŠTU TOKOM RANIH FAZA RAZVITKA

Abstrakt

Continuing losses of natural production from over harvesting, habitat degradation and disappearance of spawning habitat due to hydroelectric development, irrigation, logging and transportation are increasingly showing the importance of hatchery operations in many countries. Few years ago, the Republic of Macedonia started with captive breeding programs for salmons. This program involves capturing wild fish of species as *Salmo faroides* and *Salmo macedonicus* from their native habitats and subsequent culturing the offspring from captive broodstocks which are then stocked into ancestral streams at the juvenile stage. From a practical point of view, the importance of study on how a developing larva copes with the changing functional demands during ontogeny, especially when being reared under artificial conditions, is obvious. Understanding how the locomotor and feeding apparatus is formed during early ontogeny can assist in improving the success of artificial propagation in terms of effective production of high quality juveniles. This would especially be valuable when offspring would be re-introduced into the river ecosystem.

On the other hand knowledge on the ontogeny of fishes, especially for the early development of the skeletal system, provides information that can also be useful for solving some taxonomic problems and unravel phylogenetic relationships. For example, it is well known that morphological variation is commonly observed in salmonids. These fishes often form reproductively isolated populations across a diversity of environments and exhibit high levels of phenotypic variation. The final form of a phenotype and its life history are determined during early ontogeny. To better understand the relationship between morphology and ecology studies on the effect on environmentally induced variation in early life stage development within a single species, or study differences in the effect of a single environment in closely related species. Among the *Salmo* species that are present in the Balkan Peninsula, there is a high level of phenotypic variability, where also phenotypic plasticity is problematic for demarcate species boundaries between previously defined salmon species. Molecular data have confirmed the existence of previously defined species but several nominal species and populations of Balkan trout still remain unresolved. Still, understanding patterns of phenotypic variation that underlies molecular affinities remains essential. Within this context, we analysed the ontogeny of the skeletal system in *Salmo faroides* and *Salmo macedonicus*, two species of a still uncertain taxonomic status, reared under controlled condition. We wanted to test to what degree ontogeny of these closely related species is similar. In this study we focus on the early development of the feeding apparatus, from hatching till beginning of the exogenous feeding.

Key words: *Salmo faroides, Salmo macedonicus, ontogeny, feeding apparatus*

DISTRIBUTION OF *POSTHODIPLOSTOMUM CUTICOLA* (DIGENEA) METACERCARIAE IN CYPRINIDS OF THE MODRAC RESERVOIR

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DISTRIBUCIJA *POSTHODIPLOSTOMUM CUTICOLA* (DIGENEA) METACERCARIAE KOD CIPRINIDA AKUMULACIJE MODRAC

Abstract
During the ichthyofaunistic research of the Modrac reservoir near Tuzla in Bosnia and Herzegovina, there were registered 22 fish species and one natural hybrid *(Rutilus rutilus* x *Abramis brama),* which are classified into seven families of fish. The total sample is dominated by representatives of cyprinid species. For a large number of individuals of certain species of fish caught, it was noticed the presence of a large number of parasites Metacercaria *Posthodiplostomum cuticola* (Nordmann, 1832). Metacercaria were present in all parts of the body. The presence of parasites was registered in Bream *(Abramis brama)*, Roach *(Rutilus rutilus)* and Sunbleak *(Alburnus alburnus)*. The largest number of metacercaria was present in roach, and the least in sunbleak.

Key words: *Posthodiplostomum cuticola, Ciprinidae, Modrac*

INTRODUCTION

Fish and fish products in many countries occupy an enviable position in the human diet. Thus, in Bosnia and Herzegovina from year to year increases consumption of fish. Until now, fishing in Bosnia and Herzegovina had only sporting character, while a very small part of it belonged to industrial. The Modrac hydro-reservoir is one of the largest water facilities of this kind in B&H. Water basin at the profile of Modrac dam amounts to 1189 km^2 in total. In this water basin there are many rural settlements, which greatly burden the reservoir with various wastes, mostly with public utility wastewaters. In order to provide water quality in Lake Modrac, it is necessary in a quality manner to stop the entry of sewage and wastewaters into the river basins of rivers Spreča and Turija, and into the hydro-reservoir itself. This can be achieved by construction of sewage networks in populated locations. It is also necessary to prohibit the use of vessels that use liquid fuel. In this hydro-reservoir the water quality is currently in a very critical condition as a result of multi-year devastation of the area as a whole.

Socio-economic development of Tuzla region and the spatial distribution of water resources have caused that already in the seventies of the last century there began consideration about a common regional approach to solving the problem of drinking water. As a solution to this problem, there was built facility for purification of water from the Modrac reservoir and its use for drinking.

In the postwar period, it was noted that the number of precious species of fish constantly decreased. Among the negative effects that lead to a reduction in the number of these fish are fish diseases. Among the causes of fish diseases that can lead to morbidity and mortality, in addition to viruses, bacteria, mycorrhizae, the most important role is played by parasitic diseases. For these reasons, parasitological researches are important and justified because they give insight into the prevalence status of parasites in economically important fish as well.

MATERIALS AND METHODS

In the aim of determining the current state of fish diversity in the Modrac reservoir, during the year 2006, there have been carried out ichthyological surveys. Simultaneously, surveys were conducted on the presence of the parasite *Posthodiplostomum cuticula* as a typical ectoparasite which is very common in fish populations. Ichthyological surveys of the Modrac aquatic reservoir were conducted in the period from February through November 2006, that is during the four seasons: spring, summer, autumn and winter. The sample was obtained by combined standing triple fishing nets of the type gillnet, length 30 meters and with mesh sizes 10-30 mm. At the same time there were used „barukude" of 50 meters in length and a 50 mm mesh size. In the littoral part of the reservoir fishing was carried out from a boat using a power generating set for electrofishing of the type " ELT 61 II" and "Honda", strength of 2 kV and manual nets.

After catching and taking morphometric data there followed determination of the presence of cysts of the mentioned parasite in the sample examined, and their counting. The presence of Metacercaria manifests as black stains on the body and can be easily noticed.

RESULTS AND DISCUSSION

During the surveys on fish diversity in the Modrac aquatic reservoir there was a total of 1104 individuals of different species of fish caught, which are classified into seven families of fish. After the determination of fish we registered a high diversity of species expressed through the presence of 22 fish species and one natural hybrid - *Rutilus rutilus x Abramis brama* (Adrović, 2007).

Looking at the overall sample, it is evident that *Posthodiplostomum cuticula* appears in the following three fish species: *Abramis brama, Rutilus rutilus, Alburnus alburnus*. (Table 1). Its presence was the biggest in bream. Bream was represented with 216 samples (19.56%). Of the total number of caught specimens, the parasite was present in 16 specimens (0.74%) of roach (*Rutilus rutilus*), which participates in the sample with 345 individuals, which is 31.25% of the total catch. Of the total number of roach individuals caught, the parasite was present in 106 individuals (0.31%). The parasite was least present in Sunbleak (*Alburnus alburnus*), where in the sample of 174 (15.76%) individuals registered in the catch, the parasite was present in three specimens (0.017%).

We also tested the number of cysts present in some individuals of those fish species in which was confirmed their presence.

Table 1. Diversity of ichthyofauna in the Modrac reservoir and the presence of parasites in individual fish species

Family	Fish species	N	%	Number and % of individuals with cysts	
Esocidae	*Esox lucius*	47	4,26	-	-
Cyprinidae	*Abramis brama*	216	19,56	16	0,74
	Rutilus rutilus	345	31,25	106	0,31
	Hybrid	96	8,70	-	-
	Aspius aspius	2	0,18	-	-
	Alburnus alburnus	174	15,76	3	0,017
	Tinca tinca	21	1,90	-	-
	Scardin. erythrophthalmus	17	1,54	-	-
	Carassius carassius	3	0,27	-	-
	Carassius gibelio	1	0,09	-	-
	Leuciscus cephalus	15	1,37	-	-
	Gobio gobio	7	0,63	-	-
	Barbus petenyi	4	0,36	-	-
	Cyprinus carpio	1	0,09	-	-
	Rhodeus amarus	1	0,09	-	-
Cobitidae	*Cobitis elongata*	6	0,55	-	-
	Cobitis elongatoi.	4	0,36	-	-
Ameiuridae	*Ameiurus nebulosus*	9	0,81	-	-
Siluridae	*Silurus glanis*	1	0,09	-	-
Percidae	*Perca fluviatilis*	89	8,06	-	-
	Sander lucioperca	17	1,54	-	-
	Gymnocephalus cernuus	1	0,09	-	-
Centrarchidae	*Lepomis gibbosus*	27	2,45	-	-
Total		1104	100,00	10,82	

The number of cysts in the $\bar{x}$ species *Abramis brama* ranged between 1 and 24, and the average value was = 6,55. In roach, the number of cysts ranged from 2 to 130 cysts per one individual, and the average value was = 14.98. In Sunbleak (*Alburnus Alburnus*) was least number of cysts, only one to two cysts. (Figure 1).

During the research it was observed that Metacercaria of parasites were present at all parts of the body. A similar case is found in the work of Rolbiecki (2004), who examined the distribution and number of Metacercaria of ***Posthodiplostomum cuticula*** in some parts of the body and fins of cyprinid fishes in Poland. It can also be concluded that the parasite is present in fish of all age categories and that its presence significantly affects the conditional state of fish. We could not see any particular regularity in the distribution of Metacercaria on the body, neither that it, perhaps, prefers certain parts of the body, or some fins. Therefore, in a future analysis this problem should be given greater attention.

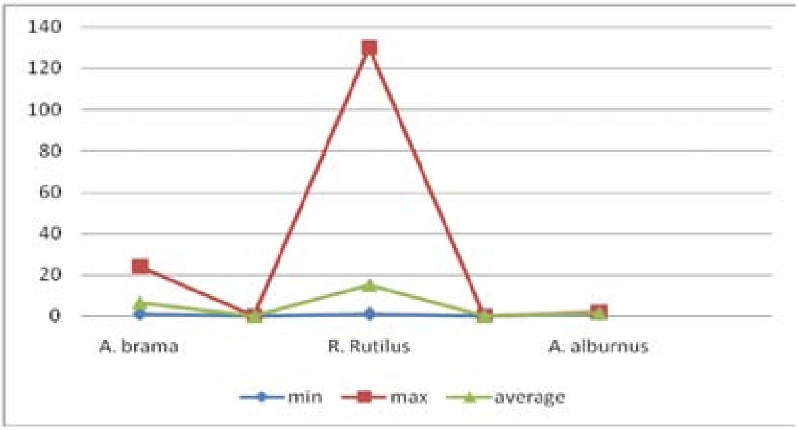

Figure 1. The range of variation and average values of parasite cysts numbers on the body of fish examined

The presence of Metacercaria of this parasite we found in the research of Marković (2004) and Jurišić et al. (2007), conducted on fish in the Međuvršje reservoir. In part of the collected fish sample, there was registered the presence of ***Posthodiplostomum cuticula*** in the following species: *Abramis brama*, *Rutilus rutilus, Squalius cephalus, Alburnus alburnus* and *Cyprinus carpio* and *Chondrostoma nasus*. The aforementioned researches state that the highest degree of infection show individuals of Nase and Chub, and as reasons for development of the parasite are given good thermal conditions of reservoirs, richly developed macrophyte flora, the presence of transitional hosts and numerous population of the final host - Gray Heron *Ardea cinerea*. Having in mind the relatively small spatial distance between the two surveyed reservoirs, it can be concluded that very similar ecological conditions prevail in the Modrac reservoir, which may be given as the reason for presence of the aforementioned parasite. It is noticeable that in the two investigated hydro-systems the same parasite infected different species of fish. Therefore, it remains a dilemma, whether this parasite prefers certain types of fish or its presence in certain species of fish is determined by some other environmental factors.

Figure 2. Infected roach from the Modrac reservoir

Similar studies of parasites in fish from the Modrac reservoir (Skenderović et al., 2005) included testing on the presence of endoparasite *Trianophorus nodulosus,* where it was noted that the mentioned endoparasite was present only in the species *Esox lucius*. Shukerova (2004), in *Carassius gibelio* in the area of Bulgaria, identified the presence of two trematodes and two nematodes characteristic for this species. According to Rukavina (1959), the harmful activity of *Cestoda*, *Cyathocephalus truncates* on more strongly attacked trout manifests in a reduced vitality and a significant negative impact on the fullness and weight of fish.

All these studies point to the need to give the study of fish parasites a greater attention.

CONCLUSION

Ichthyofaunistic surveys of the Modrac reservoir show a relatively high diversity of species which is characteristic for hydro-systems that are very loaded with different pollutant materials and exposed to severe pollution. Environmental conditions prevailing in such hydro-systems are favorable for successful existence and development of fish ectoparasites, among which is frequent *Posthodiplostomum cuticula*. This parasite, although present in only three fish species (bream, roach and sunbleak), is a relatively large threat to the successful maintenance of populations of the surveyed species, particularly roach. Metacercaria of parasites are present in all parts of the body and in fish of all age categories, but there can not be determined the regularity of their distribution on the body or a stronger manifestation in some parts of the body. Modrac is characterized by adverse environmental conditions, and that is a prerequisite for the successful existence of the investigated parasite. In the future, researches on this parasite and other parasites as well, should be given greater attention.

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STUDY ON INFECTION WITH SOME PROTOZOAN PARASITES OF THREE ENDEMIC FISHES IN THE ZAYANDEHROOD RIVER, IRAN

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STUDIJA INFEKCIJE PARAZITSKIM PROTOZOAMA KOD TRI ENDEMIČNE VRSTE RIBA U RECI ZAYANDEHROOD U IRANU

Abstract

Various parasites (protozoan and metazoan) can reside either inside or on the surface of the host. Pathogens or parasites do not always cause disease in fish, but may be present in a subclinical or carrier state. Some of parasites can infect all or most species of freshwater fishes. For example *Ichthyophthirius multifiliis* can infect almost all freshwater fish. External parasites may cause secondary fungal, bacterial and viral infections or act as carriers of bacteria, virus and other pathogens. In this study samples were collected from five stations in summer of 2010. Overall 71 native fish from 3 species had been caught from Zayandehrood River, including 18 *Capoeta damascina* (62.4 ± 27.5 g), *20 Chondrostoma regium* (34.8 ± 10.4 g) and 33 *Leuciscus Lepidus* (43.7±11.9 g). Wet mount from skin and gills were prepared and studied under a light microscope, after that gills were studied with a stereo microscope. Results of our study showed that 7 out of 18 *Capoeta damascina* (38.88%), 4 out of 20 *Chondrostoma regium* (20%) and 5 out of 33 *Leuciscus Lepidus* (15.15%) were infected with *Ichthyophthirius multifiliis.* 3 out of 18 *Capoeta damascina* (16.66%), 5 out of 20 *Chondrostoma regium* (25%) and 5 out of 33 *Leuciscus Lepidus* (15.15%) were infected with *Trichodina spp.* None of 18 *Capoeta damascina* and 20 *Chondrostoma regium* (0%) was infected with *Chilodonella sp*. However, 2 out of 33 *Leucisus Lepidus* (6.06%) were infected with *Chilodonella sp*. In our study 3 protozoan parasites were collected from 3 species and in total *Ichthyophthirius multifiliis* (22.53%) was most common parasite. Environmental factors such as temperature can effects on prevalence rate of external protozoan parasites.

Key words: *Zayandehrood River, Ichthyophthirius multifiliis, Chilodonella, Trichodina*

INRODUCTION

Fish can serve in the life cycle of parasites as definitive, intermediate or paratonic hosts. Both adult and larval parasites can be found in almost every tissue of the host fish. Various parasites (protozoan and metazoan) can reside either inside or on the surface of the host. Pathogens or parasites do not always cause disease in fish, but may be present in a subclinical or carrier state (Barber, 2007). Parasites sometimes can cause serious diseases in result of mass epidemics. Some of parasites can infect all or most species of freshwater fishes. For example *Ichthyophthirius multifiliis* can infect almost all freshwater fish (Ventura and Paperna, 1985). Itch or white spot disease (*Ichthyophthiriasis*) due to *I. multifiliis* is widespread and has been reported from different countries (El-Dien et al., 1998). Sometimes, external parasites like *Ichthyophthirius multifiliis* or *Trichodina sp*. can cause high mortalities in freshwater fish. Naturally occurring outbreaks of *Ichthyophthiriasis* in wild fish populations can cause devastating effects. For example, natural outbreak of the Ich caused the deaths of 18 million *Orestias agassi* in Lake Titicaca, Peru (Wurtsbaugh and Tapia, 1988). External parasites may cause secondary fungal, bacterial and viral infections or act as carriers of bacteria, virus and other pathogens (Azari Takami, 1997). Identification of fish parasites and an understanding of parasite life cycles are prerequisites to prevention or management of parasitic disease outbreak. Zayandehrood River is the second constant river and also the main resource of agriculture and drinking water of central regions of Iran. Considering the importance of the Zayandehrood river fishery, research and continuous monitoring of fauna and flora are necessary. This study was conducted to determine the prevalence rate of protozoan parasites of three endemic fishes (*Capoeta damascina*, *Chonrostoma regium* and *Leucisus Lepidus*) of Zayandehrood River (Chaharmahal and Bakhtyari Province), Iran.

MATERIAL AND METHODS

Samples were collected from five stations in summer of 2010. Overall 71 native fish from 3 specimens had been caught from Zayandehrood River including 18 *Capoeta damascina* (62.4 ± 27.5 g), *20 Chondrostoma regium* (34.8 ± 10.4 g) and 33 *Leuciscus Lepidus* (43.7±11.9 g). Fish were caught using net and were placed in plastic tanks with local water and immediately were transferred to the research laboratory. After that they were kept in an aquarium with local waters of Zayandehrood River until parasitic evaluations were done. After biometry, species were examined for parasitic infection. Wet mount from skin and gills were prepared. During the dissection, the gill filaments, the fins and the skin were thoroughly examined under a light microscope and a stereo microscope and parasite specimens were identified by using of identification keys.

RESULTS

Results our study showed that 7 out of 18 *Capoeta damascina* (38.88%), 4 out of 20 *Chondrostoma regium* (20%) and 5 out of 33 *Leuciscus Lepidus* (15.15%) were infected with *Ichthyophthirius multifiliis.* 3 out of 18 *Capoeta damascina* (16.66%), 5 out of 20 *Chondrostoma regium* (25%) and 5 out of 33 *Leuciscus Lepidus* (15.15%) were infected with *Trichodina spp.* None of 18 *Capoeta damascina* and 20 *Chondrostoma regium* (0%) were infected with *Chilodonella sp*. However, 2 out of 33 *Leucisus Lepidus* (6.06%) was infected with *Chilodonella sp*. (Fig 1, 2 and 3). All of these parasites were found from skin and Ich was found in both skin and gills of these 3 species. Generally, results showed that 16 fish were infected to *Ichthyophthirius multifiliis* (22.53%) and 13 fish were infected to *Trichodina sp.* (18.3%) and 2 fish were also infected with *Chilodonella sp.* (2.81%).

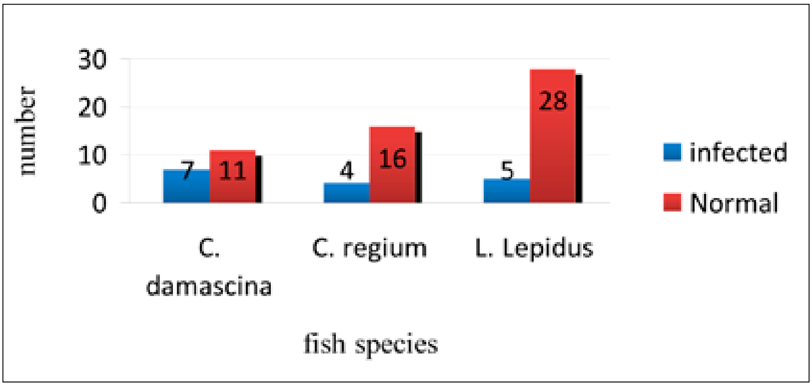

Figure 1. Number of normal and infected fishes with *Ichthyophthirius multifiliis*

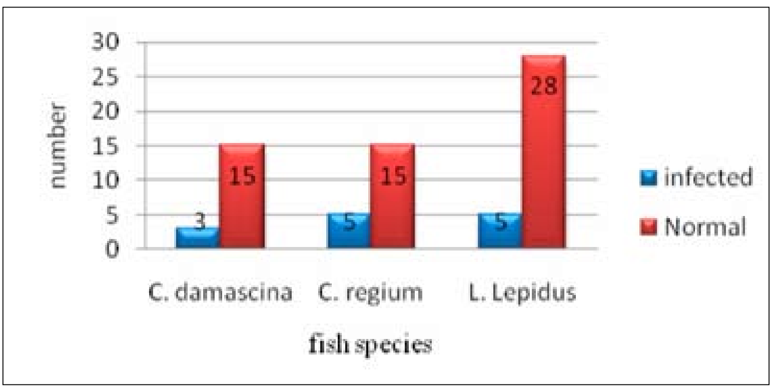

Figure 2. Number of normal and infected fishes with *Trichodina sp.*

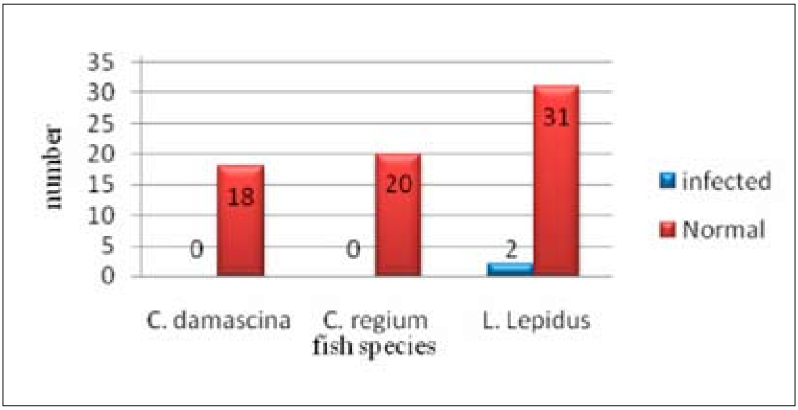

Figure 3. Number of normal and infected fishes with *Chilodonella sp.*

DISCUSSION

Different records of external protozoan parasites were reported from cultivated and non cultivated fishes of Iran. Bazari Moghaddam et al (2010) reported *Trichodina reticulate* from fingerlings of the Persian sturgeon (*Acipenser persicus*). *Ichthyophthirius multifiliis* from Spiny eel (*Mastacembelus mastacembelus*) and *Trichodina pediculus* from Spiny eel (*M. mastacembelus*) and common carp (*Cyprinus carpio* Linnaeus) were reported in Zarivar Lake that is situated in North of Kurdistan Province, Iran (Jalali and Barzegar, 2006). In our study 3 protozoan parasites were collected from 3 species and in total *Ichthyophthirius multifiliis* (22.53%) was most common parasite. In a study that was performed about parasites of some fish species in Gandoman Lagoon (Chaharmahal and Bakhtyari Province, Iran) 14 *Chondrostoma regium* were evaluated. In that study 85.7% of *C. regium* were infected with *Trichodina sp*. (Raissy et al, 2010). In our study 25% of *C. regium* were infected with *Trichodina sp*. However, none of 18 *Capoeta damascina* was not infected with *Trichodina sp.* (Raissy et al, 2010). But in our study prevalence of *Trichodina sp.* in *C. damascina* was 16.66%. And in our study both of *C. regium* and *C. damascina* were infected with *I. multifiliis*, but on that study no *I. multifiliis* was reported from these two fishes.

Reason of these deference between prevalence of these parasites, maybe was in result of different natural resources (Lagoon and river) or because of different temperature. In another study that was performed in October of 2010 about epizootic of *Ichthyophthiriasis* among 6 fish spicies in Armand River, Chaharmahal and Bakhtyari Province, Iran, prevalence of *I. multifiliis in C. damascina* was 33% (Raissy et al, 2010). Prevalence rate of *I. multifiliis* in that study was near to our study. In both studies *C. damascina* had more prevalence rate than other fish species. Environmental factors of Armand River and Zayandehrood are similar, relatively. It could be the reason of these similarities of prevalence rates.

CONCLUSION

Prevalence of some external protozoan parasites can depends on some of environmental factors such as temperature. Kind of environment that fish live there like river, lake or lagoon can effects on prevalence rate of parasitic infections.

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MORFOPHYSIOLOGICAL AND HEMATOLOGICAL FEATURES OF RECOVERY–MATERNAL STOCK OF STURGEON FISH REARING IN STURGEON FISH HATCHERY OF AZERBAIJAN

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MORFOLOŠKI I HEMATOLOŠKI POKAZATELJI MATIČNOG JATA ZA OBNOVU JESETARSKIH RIBA GAJENIH U JESETARSKIM MRESTILIŠTIMA U AZERBEJDŽANU

Abstract

In Azerbaijan the forming of recovery-maternal stock of sturgeon fish is carrying out since 2004 year and breeding of fish from the stage of larvae till the adult conditions is fulfilled by using of pool method. Meanwhile it is known that quality of a breeding material in many respects depends on conditions of their maintenance. The task of our researches included studying on the basis of mofophysiological and hematological parameters to the extent of forming of the major functional systems of uneven-aged species of recovery-maternal stock of sturgeon fish during their long growing in bondage.

Persian sturgeon (*Acipenser persicus*) of ages 1 to 5 served as study objects in 2009. Directly after collecting blood from a tail vein content of hemoglobin and ESR (erythrocyte sedimentation rate) were defined, and also smear of blood samples were made by classical technique of Romanovsky-Gimza. Calculation of blood corpuscles was carried out in chamber of Goryayeva with staining blood cells by neutral read and crystal violet. 200 leukocyte cells were counted in each test. For differentiation of cells of the red blood 500 erythrocytes of different age groups of experimental fish were counted on smear. Index of leucocytes shift is determined according to commonly accepted methodology.

As researches have shown, on the first to year of a life of young fish of the Persian sturgeon erythropoiesis is generated completely. It has been defined that mature erythrocytes (76,5 - 81,5 %) prevail in peripheral blood at all age groups of young fish of researched kind. Ratio of basophilic normoblasts has comprised 0,8-1,4%, and hemolyzed erythrocytes 0,4-1,6 %. The insignificant number of pathological forms of erythrocytes, in our opinion, testifies to natural dying off of cells of erythrocyte series.

The structure of blood corpuscle of uneven-aged species of repair broodstock of the Persian sturgeon, grown up on industrial conditions, testifies that function of leukopoiesis is generated at them. Lymphoid nature of leukocytes was determined in researched fish of Persian sturgeon which was reared in the pool conditions of Sturgeon Fish Hatchery. The obtained information about the right (lymphoid) shift in leukogram of the blood of the uneven-aged young fish witnesses the normal developing leukopoiesis. Index of leucocytes shift has a direct dependence on the age of reared fish: yearlings – 0,54; two-year old – 0,40; three-year old – 0,38; four year old 0,37 and five-year old– 0,32.

Making preliminary conclusions of hematological researches of the Persian sturgeon as the modeling object used for formation of recovery-maternal stock on Khilly Sturgeon Fish Hatchery, it is possible to draw following conclusions: - 1. In process of growth of young Persian sturgeon the number of erythrocytes on unit of volume of blood increases; 2. On the first year of a life of young fish of the Persian sturgeon the erythropoiesis is generated completely; 3. The number of leukocytes on unit of volume of blood of the Persian sturgeon has seasonal variability and has no direct dependence on age of fish; 4. The quantity of immune cells (lymphocytes) has direct dependence on age of fishes.

Key words: *Persian sturgeon, recovery-maternal stock, erythrocyte, leukocyte, lymphocytes*

INTRODUCTION

The abrupt decrease of scales of industrial reproduction of the sturgeon fish happened in whole Caspian region during recent years. The main reason of fall of scales of artificial reproduction of the sturgeon fish is shortage of pisciculturally quality producers. In this regard the forming of reproductive broodstock of sturgeon fish in conditions of Sturgeon Fish Hatchery based on fish of artificial generation and natural complex is priority trend of modern sturgeon-farming. In Azerbaijan the forming of recovery-maternal stock of sturgeon fish is carrying out since 2004 year and breeding of fish from the stage of larvae till the adult is fulfilled by using of pool method.

Formation of recovery-maternal stock of sturgeon fish «from roe» assumes long growing of fish industrially. Meanwhile it is known that quality of a breeding material in many respects depends on conditions of their maintenance. Considering that the morphological analysis of blood is one of delicate and objective methods of an estimation of a physiological condition of an organism (Ivanova, 1983), the task of our researches included also studying on the basis of mofophysiological and hematological parameters to the extent of forming of the major functional systems of uneven-aged species of recovery stock of sturgeon fish during their long growing in bondage.

MATERIALS AND METHODS

Persian sturgeon (*Acipenser persicus*) of ages 1 to 5 served as study objects in 2009. Ten experimental fish of each age group were sampled. Directly after collecting blood from a tail vein content of hemoglobin and ESR (erythrocyte sedimentation rate) were defined, and also smear of blood samples were made by classical technique of Romanovsky-Gimza. Hemoglobin content was determined in a hemoglobinometer GF-3, and ESR was studied using the micromethod of G.P.Panchenkova (Musselius et al., 1983). Calculation of blood corpuscles was carried out in chamber of Goryayeva with staining blood cells by neutral read and crystal violet (Ivanova, 1983). 200 leukocyte cells were counted in each test. For differentiation of cells of the red blood 500 erythrocytes of different age groups of experimental fish were counted on smear. Index of leucocytes shift is determined according to commonly accepted methodology. Obtained data were processed by the standard methods of the statistical analysis and software package Stadia is used.

The global practice of fresh-water aquaculture proves a possibility of the maintenance and growing of sturgeon fishes in artificial conditions (Stroganov, 1968; Smolyanov, 1987; Popova et al., 2004; Chebanov et al., 2004; Cotenev et al., 2005). The principle of a bundling of maternal stock of sturgeon fish from spawn up to spawn is based on selection of elite posterity with the subsequent growing within 8-10 years up to mature sires. On the basis of posterity of artificial generation the recovery-maternal stock of sturgeon fish is formed with the purpose of reception of own sires on Khilly Sturgeon Hatchery since 2004 (Mamedov and Salmanov, 2009; Mamedov et al., 2009; 2010). At the moment five-year old- (427 pieces), four-year old– (250 pieces), three-year old- (200 pieces), two-year old- (1260 pieces) sturgeons and one-year old Persian sturgeon (*A.persicus)*, starred sturgeon (*A.stellatus*) and Kura-river ship (*A.nudiventris*) (more than 1000 pieces) of industrial manufacture are grown as an experiment in conditions of the Khilly Sturgeon Hatchery.

RESULTS AND DISCUSSION

Some ten of specimen of Siberian sturgeon (*A.baerii*), sterlets (*A.ruthenus*), beluga (*Huso huso*), besters (*H.huso x A.ruthenus*) and Russian sturgeons (*A.gueldenstaedtii*) are grown as an experiment in the Khilly Sturgeon Hatchery. The general quantity of recovery–maternal stock of different kinds of sturgeon fish on Khilly Sturgeon Hatchery constitutes about 4000 specimens. The dynamics of development of young sturgeons grown in Khilly Sturgeon Hatchery since 2004 is shown at the figure 1.

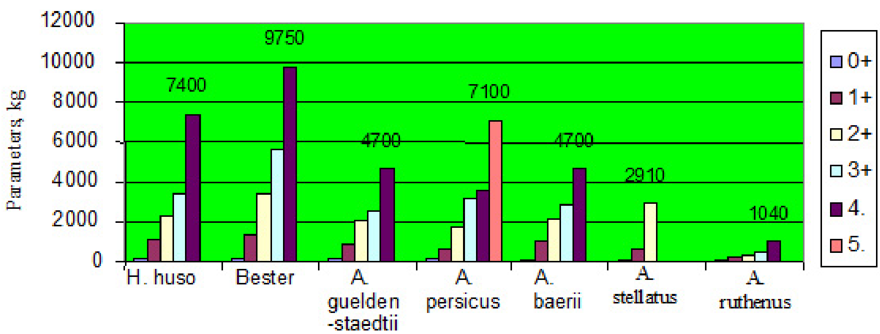

Figure 1. The dynamics of development of young sturgeons grown in the Khilly Sturgeon Hatchery since 2004.

The system of blood forming of fish sensitively reacts to changes of factors of an environment. The content of hemoglobin in blood of the Persian sturgeon was defined in five age groups during the summer period. Concentration of hemoglobin of blood was in limits from (41,5±0,9g/l) to (54,0±1,1g/l). The quantity of erythrocyte at considered age groups in process of growth of grown up fishes has increased (Table 1).

Table 1. The hematological features of uneven-aged recovery specimens of the Persian sturgeon, rearing on Khilly Sturgeon Hatchery (2009)

Age of fishes	Amount of hemoglobin, g/l	ESR, mm/h	Amount of erythrocyte, mln./mm^3	Amount of leukocyte th.sp./mm^3
1.	41,5±0,9	4,3±0,4	0,365±0,062	11,7±2,1
2.	42,0±0,8	3,4±0,4	0,422±0,164	13,7±2,4
3.	47,0±0,9	2,5±0,5	0,509±0,036	14,3±1,9
4.	52,0±1,3	3,2±0,4	0,512±0,066	13,5±2,3
5.	54,0±1,1	2,9±0,5	0,4195±0,054	14,9±1,8

As researches have shown, on the first to year of a life of young fish of the Persian sturgeon erythropoiesis is generated completely. It has been defined that mature erythrocytes (76,5 - 81,5 %) prevail in peripheral blood at all age groups of young fish of researched kind.

Ratio of basophilic normoblasts has comprised 0,8-1,4%, and hemolyzed erythrocytes 0,4-1,6 %. The qualitative structure of erythrocyte at uneven-age individuals of the Persian sturgeon is presented in the table 2.

The insignificant number of pathological forms of erythrocytes, in our opinion, testifies to natural dying off of cells of erythrocyte series.

The structure of blood corpuscle of uneven-aged species of repair broodstock of the Persian sturgeon, grown up on industrial conditions, testifies that function of leukopoiesis is generated at them. It is necessary to note also that weight of separate elements of leukocytes formulas is of interest for an estimation of a physiological condition of grown up fish during the various periods of life cycle, both in norm and under influence of various factors.

Table 2. Differentiation of erythrocyte at uneven-age individuals of the Persian sturgeon

<table>
<tr><th colspan="2" rowspan="3">Age of fishes</th><th colspan="5">Number on 500 erythrocyte, piece</th></tr>
<tr><th colspan="3">Normoblasts</th><th rowspan="2">Normocytes</th><th rowspan="2">Pathological forms</th></tr>
<tr><th>Basophilic</th><th>Polychromotophilic</th><th>Oxyphilic</th></tr>
<tr><td rowspan="2">1</td><td>piece</td><td>7,2</td><td>6,4</td><td>101,8</td><td>382,5</td><td>2,1</td></tr>
<tr><td>%</td><td>1,4</td><td>1,3</td><td>20,4</td><td>76,5</td><td>0,4</td></tr>
<tr><td rowspan="2">2</td><td>piece</td><td>4,3</td><td>11,4</td><td>89,6</td><td>392,3</td><td>2,4</td></tr>
<tr><td>%</td><td>0,8</td><td>2,3</td><td>17,9</td><td>78,5</td><td>0,5</td></tr>
<tr><td rowspan="2">3</td><td>piece</td><td>-</td><td>3,1</td><td>81,3</td><td>407,5</td><td>8,1</td></tr>
<tr><td>%</td><td>-</td><td>0,6</td><td>16,3</td><td>81,5</td><td>1,6</td></tr>
</table>

Lymphoid nature of leukocytes was determined in researched fish of Persian sturgeon (*A. persicus*) which was reared in the conditions of pool of Sturgeon Fish Hatchery. The functional activity of lymphocytes consists of realization of immunological response of organism.

The number of immune cells is affected not only by seasonal changes. The obtained information about the right (lymphoid) shift in leukogram of the blood of the uneven-aged young fish witnesses the normal developing leukopoiesis (Table 3) but also in direct proportion depends on the age of reared fish bred in artificial conditions. Index of leucocytes shift has a direct dependence on the age of reared fish: yearlings – 0,54; two-year old – 0,40; three-year old – 0,38; four year old 0,37 and five-year old– 0,32.

Table 3. The leukogram of uneven-aged young fish of Persian sturgeon, %

<table>
<tr><th colspan="3" rowspan="2">Parameters</th><th colspan="5">Age of fishes</th></tr>
<tr><th>1</th><th>2</th><th>3</th><th>4</th><th>5</th></tr>
<tr><td rowspan="6">Neutrophils</td><td rowspan="6">Переводи прямо с мобильного:
----
http://m.translate.ru
Нейтрофилы</td><td>blastical forms</td><td>-</td><td>-</td><td>-</td><td>-</td><td>-</td></tr>
<tr><td>myelocytes</td><td>5,9</td><td>7,0</td><td>6,5</td><td>6,4</td><td>6,1</td></tr>
<tr><td>metamyelocyte</td><td>7,0</td><td>5,2</td><td>2,6</td><td>2,4</td><td>2,5</td></tr>
<tr><td>Stab</td><td>9,5</td><td>4,4</td><td>8,4</td><td>7,5</td><td>5,7</td></tr>
<tr><td>segmentonuclear leukocyte</td><td>2,5</td><td>1,5</td><td>1,9</td><td>2,6</td><td>3,1</td></tr>
<tr><td>pathological forms</td><td>-</td><td>-</td><td>-</td><td>-</td><td>-</td></tr>
<tr><td colspan="3">eozinophils</td><td>10,0</td><td>10,6</td><td>8,0</td><td>8,1</td><td>6,7</td></tr>
<tr><td colspan="3">monocytes</td><td>-</td><td>-</td><td>-</td><td>-</td><td>-</td></tr>
<tr><td colspan="3">lymphocytes</td><td>65,1</td><td>71,3</td><td>72,6</td><td>73,0</td><td>74,1</td></tr>
<tr><td colspan="3">ISL (Index of leucocytes shift)</td><td>0,54</td><td>0,40</td><td>0,38</td><td>0,37</td><td>0,32</td></tr>
</table>

CONCLUSIONS

Making preliminary conclusions of hematological researches of the Persian sturgeon as the modeling object used for formation of maternal stock on Khilly Sturgeon Fish Hatchery, it is possible to draw following conclusions:

1. In process of growth of young Persian sturgeon the number of erythrocytes on unit of volume of blood increases;
2. On the first year of a life of young fish of the Persian sturgeon the erythropoiesis is generated completely;
3. The number of leukocytes on unit of volume of blood of the Persian sturgeon has seasonal variability and has no direct dependence on age of fish;
4. The quantity of immune cells (lymphocytes) has direct dependence on age of fishes.

The data of hematological analysis allow to drawing a preliminary conclusion that functional development of the recovery species of the sturgeons on Khilly Sturgeon Hatchery runs without significant deviations from physiological standards.

Creating of spawning school in the sturgeon fish rearing station allows the save the gene pool of the sturgeon fish and enlarge the opportunities of their artificial reproduction for releasing to natural reservoirs. The schools which are formed in artificial way are not alternative for the natural reproduction, but are the assured source of receiving of sturgeon young fish.

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THE ISOLATION AND STUDY OF FISH DISEA SE-CAUSING *VIBRIO SPP.* AND *AEROMONAS SPP.* AND THEIR SPECIFIC BACTERIOPHAGES FROM GEORGIAN AQUATIC ENVIRONMENT

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IZOLACIJA I ISPITIVANJE *VIBRIO* SPP. I *AEROMONAS* SPP. PROUZROKOVAČA BOLESTI RIBA I NJIHOVIH SPECIFIČNIH BAKTERIOFAGA IZ VODENE SREDINE GRUZIJE

Abstrakt

Infekcije povezane sa *Vibrio* spp. i *Aeromonas* spp. koje se javljaju kod riba iz morske i slatkovodne sredine se navode širom sveta. Bolest, kada izbije, može izazvati znatan mortalitet u ribnjacima.

Cilj ovih ispitivanja je bila izolacija i identifikacija *Vibrio* spp. i *Aeromonas* spp. izazivača bolesti riba iz gruzijske obalske zone Crnog mora i slatkovodnih akumulacija, kao i njihovog diverziteta i kvantitativnog učešća.

Sezonski monitoring je izvođen u morskim (2006 - 2009) i slatkovodnim akumulacijama-Lisi jezeru, Tbilisi moru i Kumisi jezeru (2006 - 2010). 223 izolata *Aeromonas* spp. i 858 izolata *Vibrio* spp. je ispitano. Ukupno 8 vrsta *Aeromonas* i 14 vrsta *Vibrio* pronađeno je u vodenoj sredini Gruzije. *V. parahaemolyticus, V. natriegens, V. pelagius* preovlađuju u morskoj vodi. Veći deo *V. mimicus, V. vulnificus, V. alginolyticus, V. cholerae, V. nigripulchritudo, V. nereis, V. campbellii, A. media, A. veronii, A. eucrenopila, A. sobria, A. schuberti, A. salmonicida* su nađene u slatkovodnim akumulacijama. *V. orientalis, V. metchnikovii, V. splendidus, V. marinus, A. hydrophila, A. caviae* se mogu naći u i morskim i u slatkovodnim akumulacijama. Sojevi su podeljeni u grupe po sličnosti zasnovanoj na njihovim zahtevima za salinitetom.

Drugi cilj ovih ispitivanja je bilo izolovanje soja specifičnih bakteriofaga iz uzoraka vode. Dobijeno je 49 primarnih fag izolata, većina iz slatkovodnih rezervoara. Litični spektar bakteriofaga je ispitivan koristeći izabrani set sojeva domaćina. U rezultatima kloniranja 9 primarnih mešavina faga dobijeno je 13 klona bakteriofaga aktivnih za *A. sobria, A. caviae, A. salmonicida, A. eucrenophila*.

Ključne reči: *Aeromonas, Vibrio, infekcije riba, bakteriofagi*

THELOHANELLUS HOVORKAI - IN FEMALE FISH AND CARP FINGERLINGS

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THELOHANELLUS HOVORKAI-KOD ŽENKI I MLAĐI ŠARANA

Abstrakt

Telohaneloza je obolenje šaranskih riba koje se klinički uočava na perajima i krljuštima (Thelohanellus nikolskii) i svim drugim tkivima a naročito u centralnom nervnom sistemu (Thelohanellus hovorkai). Na prisustvo parazita Thelohanellus nikolskii pregledani su mladunci i matične ribe šarana (Cyprinus carpio) koji potiču sa ribnjaka u Srbiji. Prisustvo ovog parazita zabeleženo je kod mladunaca šarana starijih od 90 dana i kod matica šarana. Intenzitet infekcije razlikovao se u zavisnosti od ribnjaka na kojima je obolenje utvrđeno. Ustanovljeno je da su najintenzivnije infekcije kao i najveći broj inficiranih jedinki poticali sa ribnjaka gde su gajeni sa starijim kategorijama, jezera sa prirodnim mrestom, i objekata koji su služili kao skladištbeni prostor tokom zimskog perioda. Spore Thelohanellus hovorkai nađene su na škrgama, koži, muskulaturi, jetri, slezini, bubrezima, crevu, žučnom i vazdušnom mehuru, kičmenoj moždini i mozgu gde je bio prisutan najveći broj parazita. Spore su bile obavijene mukofilnim omotačem. Prosečne veličine spora T. hovorkai iznosile su: dužina spore 20-21µm, širina spore 14µm, dužina polarne kapsule 12.5-14µm, širina polarne kapsule 11.5-12.5µm, dužina spore sa sluzavim omotačem 22.5-25µm i širina spore sa sluzavim omotačem 14.5-17.5µm.

Dijagnostika ovog obolenja vezana je za detekciju spora čija se veličina razlikuje u opisima istraživača kod riba iz otvorenih voda i šaranskih riba gajenih u akvakulturi. Da bi utvrdili da li je u pitanju možda druga vrsta ili podvrsta parazita to možemo učiniti primenom molekularnih tehnika koje pokušavamo da uvedemo u dijagnostiku. Obolenje se manifestuje poremećajima u centralnom nervnom sistemu, malokrvnosti i slabijem napredovanju mladunaca a naročito kada se dijagnostikuje veći broj spora u tkivima riba. Specifične terapijske mere nisu poznate ali opšte preventivne mere vezane za pripremu objekata za gajenje mladunaca šarana daju povoljne rezultate.

Predmet istraživanja je bio detekcija miksosporidia kod šarana. U ovom radu, opisana je telohaneloza kod jednogodišnje mlađi i matičnih jedinki šarana uzrokovana sa parazitom Thelohanellus hovorkai. Istraživanja su sprovedena u severnom delu Srbije (Vojvodina) na 15 ribnjaka u razdoblju 2007-2010. Svi ispitivani ribnjaci snabdevaju se vodom iz mreže kanala, reka Tisa, Tamiš, i Dunava, i bunara. Metode koje su korištene su klinička posmatranja, svetlosna mikroskopija i klasična patohistologija s H & E bojenjem.

Ono što se pokazalo veoma bitno jeste da prirodni mrest riba nije pogodan za intenzivniju proizvodnju šarana i da se preporučuje gajenje mladunaca samo ako su iste starosne grupe. Tokom zime, ribnjačko tlo treba isušiti i izamrznuti a nakon 3-5 godina eksploatacije, površinski sloj tla od ribnjaka treba ukloniti. Tokom pripreme ribnjaka preporučuje se korišćenje rotacionih plugova a za dezinfekciju ribnjaka treba koristiti gašeni (1000 kg / ha) ili negašeni kreč (2000 kg / ha).

Ključne reči: *telohaneloza, šaranske ribe, Thelohanellus hovorkai, spore*

INTRODUCTION

Telohanelosis is disease of cyprinids that are clinically observed in the fins and scales *(Thelohanellus nikolskii)* and all other tissues and especially in the central nervous system (*Thelohanellus hovorkai*). The biology and histopatohology of *Thelohanellus hovorkai* was described by Molnar and Kovacs-Gayer (1986) and Ćirković (1986). Its pathology was described by Yokoyama *et al.* (1998) who found severe haemorhages on the body surface of the affected common carp and designated disease as “haemorhages thelohanellosis”. Diagnosis of this disease is related to the detection of spores, whose size varies according to researches descriptions in fish from open water and carp fish raised in aquaculture. Application of molecular techniques can help in diagnosis and can determine whether the parasite correspond to this species or another species or subspecies Anderson *et al.* (2000). Disease is manifested by disturbances in the central nervous system, anemia and absence of progressin of fingerlings when are diagnosed numerous spores in the tissues of fish. Beside some investigations Yokoyama *et al.* (1999) efficacious therapeutic measures are not known but the general preventive measures related to the preparation of facilities for the cultivation of carp fingerlings give favorable results.

MATERIALS AND METHODS

The subject of the investigation was myxosporidia detection in carps. In this paper, thelohanellosis of one-year old common carp fingerlings and spawning carp fish caused by *Thelohanellus hovorkai* is described.

Investigations were conducted in the northern Serbia (Vojvodina province) in 15 fish ponds during the period 2007-2009. All investigated fish ponds are provided by water from channel network, rivers Tisa, Tamiš, and Danube, and wells. Methods that have been used were clinical observations, light microscopy and classical pathohistology with H&E stain.

RESULTS AND DISSCUTION

Presence of *Thelohanellus hovorkai* was detected in common carp fingerlings older than 90 days and female carp fish. Infection intensity and number of *T. hovorkai* carriers differed among ponds. It has been noted that the most serious infections and the majority of infected fingerlings were from ponds where they were reared with older fish, ponds with natural spawning, and ponds which served for older carp winter storage.

The spores of T. hovorkai were present in gills, skin, muscules, liver, spleen, kidneys, intestines, bile and swim bladder, spinal cord and brain where were present the highest number of spores (Fig. 4). Spores were rounded by mucoid layer (Fig. 3.)

Microscopic observation of compressed samples of brain tissue reveals small or large groups of spores (Fig. 1., 2.). According to our investigations spores show next values: spore length 20-21 µm, spore width 14 µm, polar capsule length 12.5-14µm polar capsule width 11.5-12.5µm, spore length with mucal layer 22.5-25 µm, spore width with mucal layer 14.5-17.5µm.

Disease was manifested with disturbances in central nervous system, anemia, and absence of fingerlings progression especially when higher number of spores is diagnosticated.

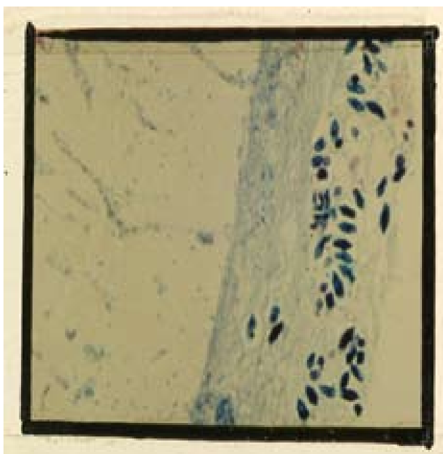

Figure 1. Spores of *T. hovorkai* in brain

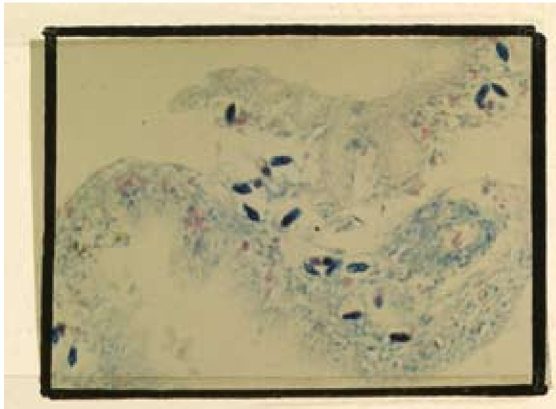

Figure 2. *T. hovorkai* spores

Figure 3. Fresh spores of *T. hovorkai*

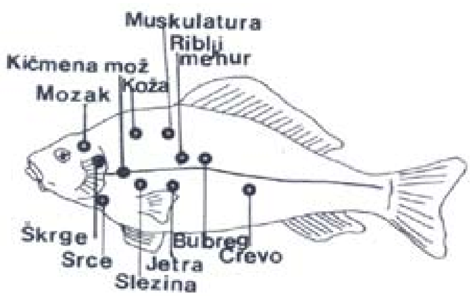

Figure 4. Organs where spores of *T. hovorkai* were detected

CONCLUSIONS

Natural spawning of fish is not convenient for more intensive common carp production. It is recommended to rear carp fingerlings of the same age group. During the winter, fish ponds bottom soil should be dried up and frozen. After 3-5 years of exploitation, the surface layer of the soil from ponds should be removed. During ponds preparation it is recommended use rotary ploughs. For fish ponds disinfection, burnt lime (1000 kg/ha) or hydrated lime (2000 kg/ha) should be applied.

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THEORETICAL MODEL CALCULATION OF CONCENTRATION OF AMMONIA AND OXYGEN IN WATER POND

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TEORIJSKI MODEL IZRAČUNAVANJA KONCENTRACIJE AMONIJAKA I KISEONIKA U VODI RIBNJAKA

Abstrakt

Od kvaliteta vode u velikoj meri zavisi uspeh u proizvodnji riba. U gajenju pastrmki to znači da normalan rast, prirast, konverzija hrane, zdravstveni i reproduktivni status zavise osim od genetskih osobina i tehnologije proizvodnje i od ambijetalnih faktora, koji mogu direktno uticati na postizanje boljih ili slabijih rezultata u akvakulturnoj proizvodnji. U ovom radu su sa aspekta utvrđivanja koncentracija kiseonika i amonijaka u vodi istraživani teorijski modeli izračunavanja produkcije amonijaka i potrošnje kiseonika, kao jednih od najvažnijih parametara vode.

Utvrđeno je da potrošnja kiseonika i ekskrecija amonijaka neposredno zavise od mase riba, temperature i protoka vode, vrednosti pH, količine hrane i sadržaja proteina u njoj. Prosečna razlika između teorijski očekivane i utvrđene koncentracije kiseonika iznosila je 5,72%. Teorijski dobivene prosečne vrednosti produkcije amonijaka, razlikovale su se od laboratorijski utvrđenih za 7,66% (metod Piper-a i sar.), odnosno za 11,16% (metod Colt-a).

Ključne reči: *voda, riba, kiseonik, amonijak*

INTRODUCTION

Starting from the fact that intensive aquaculture inevitable burdens by toxicants the aquatic environment not only fisheries, but also the recipients of water from the pond, the knowledge and understanding of the physical and chemical properties of water for growing fish is critical to achieving cost-effective and successful production.

The content of individual fractions in water depends primarily on the total weight of fish, the technological process of growing, intensity of the biological autopurification, the quantity of the food and physical-chemical characteristics of the incoming water (Boaventura et al.,1997). Inadequate use of food for fish, creating a metabolic excretory products of the fish and the presence of organic fraction of nutrients that are subject to microbial oxidation, inevitably cause the reduction of dissolved oxygen and increased concentrations of harmful substances dissolved in water. This may call into question the sustainability of fish farming (Liao et al., 1974). According to Neor et al.,(1991) high protein diet food contributes to the body tissues of fish uses about 30% of the total nitrogen ingested by food, and up to 12% nitrogen of dry matter of food due to the water. Non-consumed nutrients, metabolic products, and faeces of fish in the aquatic environment are subject to intensive biochemical processes, resulting in reducing the concentration of dissolved oxygen and increases the concentration of ammonia, phosphate, carbon and suspended matter.

For successful aquacultural production of special importance are the amount of dissolved oxygen, temperature, concentration of nitrogen fractions and pH value of water, which affect the synergistic effect of certain toxic substances and gases dissolved in water. The intensity of the effects of chemical parameters of water in fish depends primarily on the density of plantation, age, nutritional state and condition factor of fish (Pillay, 1992).

Modern concepts of sustainable aquaculture are based on ecological principles of breeding and feeding fish, where emissions created by organic matter and gases have little effect on the capacity utilization of the water environment and the environment.

The aim of this study is a mathematical assessment of oxygen consumption and creation of ammonia in water to classic trout pond and a comparison of the calculated theoretical values and the values of laboratory results.

MATERIAL AND METHODS

The experiment was carried out on trout pond near Čačak, which is located at an altitude of 320 feet, for a period of 90 days during the summer period. The volume of experimental pool was 25,2 m^3 with a water flow rate of 41 changes of water for 24 hours. The pool is set on the 3370 rainbow trout individuals, aged 12 + months, average weight of 65,2 g and total body length of 17,92 cm. During the experiment, we used the extruded trout food, manufactured by "BioMar", that contained 45% crude protein. The amount and number of daily meals were determined according to table food manufacturers, which were adapted to water temperature and fish mass.

Water samples were taken twice a day every day for two control profiles, before entering the water in the pool and the release of water from the pool. For each studied parameter was found the average value for a period of seven days. Water parameters were determined using an automatic multiparametric photometer "MultiDirect", by Lovibond company.

In parallel with examining the characteristics of water, the growth of fish and feed conversion with a seven-day monitoring of health status and mortality were measured, which presents the average values on a monthly basis. Representative control sample of 50 individuals in the pool to determine the individual body weight and linear dimensions is taken by random sampling method. Individual weight of fish was determined by measuring with the decimal technical scale, and total body length was determined by using ichthyometer. Theoretical values of certain production parameters were determined using the formula:

Fulton’s condition factor (Pravdin, 1966): **$Fk = M(g) / L^3$ (cm)**

Index density (Piper et al.,1982): **$Ig = [M(kg) / L(mm)] \times Q(m^3)$**

Flow Index (Piper et al.,1982): **$Ip = [M(kg) / L(mm)] \times P(l/sec)$**

Where: M - mass of fish, L - total length of fish, Q - the volume of pond; P - water flow

Specific growth rate (Brown, 1957): **$G = [(MT-Mt) - (T-t)] \times 100$**

Where: G - specific growth rate,%; MT - final weight of fish, g; Mt - initial weight of fish, g, T - number of days at the end of the experiment, t - start of the experiment

Oxygen saturation of water, depending on altitude, temparature and partial pressure, was calculated using the following formulas (Boyd et al.,1998; Yin-Han, 2006).

$$Nv = \text{altitude (m)} \times 3{,}28 \qquad PP\ (\text{mm Hg}) = 10\wedge \frac{2{,}880814 - Nv}{64790{,}7}$$

$$Bk(L/L\text{-atm}) = 0{,}00099902 \times EXP \; \frac{9{,}7265 - 5268{,}95}{t^0C + 273{,}15} + \frac{1004170}{(t^0C + 273{,}15) \times (t^0C + 273{,}15)}$$

$$ZO_2\ (\text{mm Hg}) = 760 * EXP \; \frac{11{,}8571 - 3840{,}7}{t^0C + 273{,}15} - \frac{216961}{(t^0C + 273{,}15) \times (t^0C + 273{,}15)}$$

$$Pp\ O_2\ (\text{mm Hg}) = \frac{O_2}{Bk} \times 0{,}5318 \qquad O_2\ (\%) = \frac{Pp\ O_2}{0{,}20946 \times (PP - ZO_2)}$$

Where: Nv-altitude; PP-partial (barometric) pressure; Bk-Bunsen’s coefficient; ZO_2-saturation O_2 in water (mm Hg); Pp O_2-partial pressure O_2 in water; O_2 -oxygen concentration (mg/l); O_2 (%) – saturation O_2 in water

The amount of oxygen consumed (Liao, 1971): **$PO_2 = K_2 \times T^a \times M^b$**

Where: PO_2 - oxygen consumption (Ib/100 Ib mass of fish/day); K_2 – the ratio of oxygen consumption and water temperature; T – water temperature ; T^a – numerical decline in oxygen consumption by reducing the temperature at the same weight of fish;M – mass of fish (g); M^b - numerical decline in oxygen consumption by the same temperature when increasing the mass of fish

The values of constant coefficients at different temparatures of water, according to Liao (1971), are: water temperature is less than or equal to 50 ^{0}F: $K_2 = 1{,}9 \times 10^{-6}$ (b = -0,138; a = 3,130); water temperature is more to 50 ^{0}F: $K_2 = 3{,}05 \times 10^{-4}$ (b = -0,138; a = 1,855).

Modification of the constant coefficients was performedin accordance with the SI system (Kulišić, 1989), and oxygen consumption of PO_2 was expressed in g/100g weight of fish per day.

Water temperature is less than or equal to 10^0C: **PO_2=0,05676 x $T^{1,2100199}$ x $M^{-0,1355334}$**
Water temperature is more to10 ^{0}C: **PO_2 = 0,1310712 x $T^{0,8843114}$ x $M^{-0,146128}$**

The amount of available oxygen was calculated by the formula: **R_{O2} = Pv x (O_2 – 5) / 1000**

Defined formulas do not show oxygen consumption depending on the used amount of food. According to various authors coefficients amount of oxygen consumed per kilogram of used food range from 0,20 to 0,40, depending on water flow (Wheaton, 1977; Soderberg, 1994; Timmons, 2001).

In this paper, the ratio of oxygen consumption of 0,40 is used in accordance to a pound of food, because the insufficient number of changes of water for 24 hours is taken into account:

$$\mathbf{PO_2} = \frac{0{,}40 \text{ x kg food}}{Pv} \mathbf{\ x\ 1000}$$ Where: Pv – water flow l/24 hours

Concentracion of dissolved oxygen in water (Ce_{SL}) at normal atmospheric pressure of 760 mm Hg (101,325 kPa) and different temparatures were calculated according to the modified formula by Soderberg (1982, 1994):

Ce_{SL}=14,161 – 0,3943 t + 0,0077147 t^2 - 0,0000646 t^3

Correction factor for altitude is calculated using the formula:

$$Ce_k = \frac{760}{760 + (3{,}28xNv) / 32{,}8} \quad \text{or} \quad Ce_{k(a)} = \frac{Nv}{760}$$

Steady state of dissolved oxygen with atmospheric pressure, at different water temparature and altitude, is obtained by Soderberg equation (1982, 1994):

$$Ce_{SL}{}^1 = Ce_{SL} \text{ x } Ce_k \quad \text{or} \quad Ce_{SL}{}^1 = Ce_k - Ce_{k(a)}$$

The concentration of ammonia (Piper et al., 1982) based on the quantity of food and water flow:

$$\mathbf{Fn} = \frac{NH_4 - N \text{ x } Pv}{\text{food (kg/day)}} \qquad \mathbf{NH_4\text{-}N\ (g)} = \frac{Fn \text{ x food (kg/day)}}{Pv}$$

Depending on water temperature and pH values, the concentration of nonionic NH3 is calculated, compared to the total ammonia concentration of NH4-N to the tabular values (Emerson, 1975; Piper et al., 1982):

$$\mathbf{NH_3\,(mg/l)} = \frac{NH_4 - N \times \%NH_3}{100}$$

Where: NH4-N-total ammonia; Fn-factor of the ammonia; Pv-flow L/24 hours

Predicting ammonia is also calculated based on concentrations of total protein content in foods and modified conversion factor (Colt, 1986):

$$\mathbf{NH_4\text{-}N\,(mg/l) = (1\text{-}Fp) \times 1000 \times} \frac{\%\,\text{protein}}{6{,}25}$$

Where: Fp- conversion factor (0,65-0,80)

RESULTS AND DISCUSSION

The results achieved by weight increase, weight gain and linear increase in linear dimensions (Table 1) indicate the normal dynamics of achieving production parameters during the test. This fact confirms the value of total specific growth rate that was 120% during the test.

Mortality during the experiment had a minimum value, because of total cultivated fish until the end of the experiment died 52 individuals or 1,57%. From the results of fish mortality in this study, it can be concluded that the applied ichthyohygienic measures had a positive effect on reducing mortality of cultivated fish. Flow index (Ip) and the density index (Ig) are among the most important ichthyosanitary parameters because according to their values the optimum of density plantation of fish is estimated. Flow index indicates the amount of fish per unit volume compared to the flow of water, and the index of density is expressed as a ratio of weight of fish per unit area. Practically, this means that in spite of adequate water flow and adequate oxygen concentration too many fish in relation to the area can cause many adverse situations. By most authors,the determined Ip values of 1,021 to 1,917 and Ig values of 0,048 to 0,091 are in accordance with recommended standards of fish planting (Piper et al., 1982; Meade, 1989; Klontz, 1991)

Table 1. The average values of productions parameters during the test

Parameters	**days**			
	1	**30**	**60**	**90**
The total number of fish	3370	3347	3330	3318
Average length,cm	17,92	19,10	23,20	24,80
Average weight,g	65,20	81,10	134,50	172,00
Mortality %	-	0,687	0,510	0,361
The average weight gain,g	-	15,90	53,40	37,50
The total gain weight,kg	-	51,72	176,44	122,81
Total gain kg/m3	-	2,05	7,00	4,87
The average increase in length,cm	-	1,18	4,10	1,60

The average increase in length per day,cm	-	0,039	0,136	0,053
The averaga weight gain per day, g	-	0,53	1,78	1,25
Condition factor, FK	0,0113	0,0116	0,0108	0,0112
The total mass of fish, kg	219,72	271,44	447,88	570,69
Number of fish, m^3	133,73	132,81	132,14	131,66
Density index, Ig	0,048	0,056	0,076	0,091
Flow index, Ip	1,021	1,184	1,608	1,917
The average feed intake, kg/day	2,63	3,53	5,82	7,42
Feeding coefficient, HK	-	0,068	0,032	0,060
Daily food consumption per fish, g	0,78	1,05	1,74	2,23
Specific growth rate, G %	-	54,82	178,00	125,00

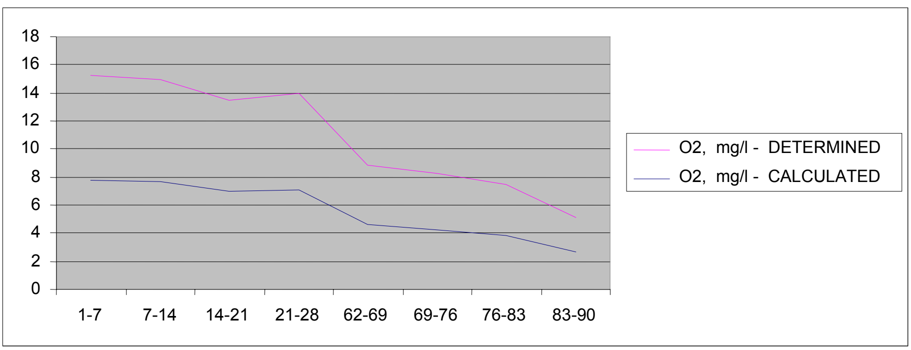
Conduction of basic biological and biochemical processes directly dependent on water temperature, and which is particularly important when converting ammonia into nitrites and in which the intensity of transformation increases with increasing temperature. For trout, the optimum temparature at which they maximum grow, take adventage of food and have the greatest immune potential is from 12-16 ^{0}C, but the tolerant values of the temparature are from 10 to 18 ^{0}C (Sedgwick, 1990). Water temparature values (Table 2) during the study period ranged from 12,30 ^{0}C to 14,00 ^{0}C. The determined pH values had a lot of consistent values and ranged from pH 7,40 to pH 7,90. The concentration of dissolved oxygen inlet water was 10,00 mg/l to 10,90 mg/l. Most authors found (Piper et al., 1982; Boyd et al., 1982; Meade, 1989; Rounds, 2003) values of these parameters of water were in the optimum range for trout water.

Available oxygen for fish is the difference between the amount of dissolved oxygen in the incoming water and physiological minimum concentration, which, for the trout, is 5 mg / l (Wheaton, 1977; Walker, 1994). Exposure of trout to concentrations of dissolved oxygen from 3 to 5 mg/l increases food consumption, interferes with general health and survival, and decreases of oxygen levels below 3 mg/l for trout has a lethal effect.

The amount of oxygen consumed during the experiment was directly proportional to the number or weight of fish, then the water temperature, intensity of metabolic processes and quantity of meals. These data (Table 2, Graph 1) indicate that oxygen consumption increased with increasing water temperature and fish biomass.Some authors, such as Pillay (1992), reported that the cultivation of trout in 1m^2 pond water at the temparature of 10 ^{0}C spends about 70 mg of oxygen, and at the temparature of 20 ^{0}C to 150 mg.

Table 2. Parameter values of oxygen consumption by periods

PARAMETERS	DAYS							
	1-7	**7-14**	**14-21**	**21-28**	**62-69**	**69-76**	**76-83**	**83-90**
temperature ^0C	12,50	12,30	13,20	13,50	12,80	13,10	13,90	14,00
pH	7,70	7,60	7,40	7,55	7,80	7,75	7,80	7,90
Input-determin. O_2, mg/l	**10,90**	**10,70**	**10,20**	**10,40**	**10,60**	**10,30**	**10,10**	**10,00**
satiety O_2, %	106,24	103,82	100,99	103,66	104,02	101,75	101,57	100,78
O_2 Ce_{SL}, mg/l	10,31	10,36	10,15	10,08	10,24	10,17	9,98	9,97
O_2 Ce_{SL}^1, mg/l	9,89	9,94	9,74	9,68	9,83	9,76	9,59	9,57
available O_2, g/day	6117	5961	5391	5598	5806	5495	5287	5184
consumption O_2, g/day	1804	1769	1927	1980	2860	2941	3160	3929
consumption O_2, mg/l	1,74	1,71	1,86	1,91	2,76	2,84	3,05	3,79
consumption O_2 fish,g/day	0,54	0,52	0,57	0,59	0,86	0,88	0,95	1,18
% utilization O_2	29,49	29,67	35,74	35,37	49,26	53,53	59,77	75,79
consumptionO_2+food,mg/l	3,10	3,06	3,22	3,27	5,99	6,04	6,23	7,38
Out-calculated O_2, mg/l	**7,80**	**7,64**	**6,98**	**7,13**	**4,61**	**4,26**	**3,87**	**2,62**
Out- determin. O_2, mg/l	**7,45**	**7,30**	**6,50**	**6,85**	**4,20**	**4,00**	**3,65**	**2,50**



Graph 1. Determined and calculated values of O_2 mg/l at the exit of water from the pool

The calculated concentration of dissolved oxygen at the exit from the pool have showed higher values in control sections compared with the measured values. The average difference between the expected and theoretically determined oxygen concentration was 5,72%, on this basis we can conclude that the empirical oxygen consumption was greater than the theoretical and the numerical differences ranged from 4,09% (21-28 days) to 9,76% (62-69 days).Most authors found (Boyd, 1979; Meade, 1989; Summerfelt, 1990; Klontz, 1993) oxygen consumption and ammonia excretion in fish subject to a variety of daily variations and change of values over an hour depending on the mode of nutrition and overall physical and chemical properties of water. For comparison, 1 kg of fish food to feed the fish bass affects the creation in water: 0,28 kg CO_2; total 0,03 kg of ammonia; 0,3 kg of suspended solids, and also 0,2 kg of dissolved oxygen is spent (Colt, 1986).

Based on these results, which are presented in Table 3 and Graph 2, it can be concluded that the established concentration of NH_3 at the exit of the pool had a higher value of the used theoretical models. The average total difference between the established and the obtained values ranged from 7,66% for the method of Piper and associates, (1982) to 11,16% for the Colt's method (1986). According to the shown results it is noticed the highest concordance of the results between theoretical methods of Piper et al.,(1982) and determined concentration of NH_3.

The creation of nitrogen in fish pond water is directly dependent on water temperature, pH fluctuation, water flow, number of changes of water for 24 hours, the protein content in food, number and weight of fish and quantity of meals. In aqueous solution there is a constant balance between nonionic form NH_3 and ammonium ions NH_4^+ depending on other parameters of water, where special significance is given to pH and temperature, because with increasing pH value of water increases the concentration of ammonia, and reduces the amount of ammonium ions (Thurston, 1988).

Table 3. Production of ammonia by periods

PARAMETERS	DAYS							
	1-7	**7-14**	**14-21**	**21-28**	**62-69**	**69-76**	**76-83**	**83-90**
input-set NH_3, mg/l	for all days <0,0001							
min. conc. NH_4, mg/l	0,050	0,050	0,050	0,050	0,080	0,080	0,080	0,080
max. conc. NH_4, mg/l	0,075	0,075	0,080	0,080	0,185	0,185	0,190	0,190
NH_4-N g/day - **Piper**	54,88	55,15	64,81	76,26	116,69	130,33	144,84	151,36
NH_4-N mg/l/day**Piper**	0,0529	0,0532	0,0625	0,0735	0,1125	0,1257	0,1397	0,1459
NH_3, mg/l - **Piper**	**0,00059**	**0,00047**	**0,00037**	**0,00057**	**0,00164**	**0,00145**	**0,00220**	**0,00289**
NH_4-N g/day - **Colt**	52,07	53,46	59,40	69,89	115,23	128,70	140,58	146,92
NH_4-N mg/l/day- **Colt**	0,0502	0,0515	0,0572	0,0674	0,1111	0,1241	0,1356	0,1417
NH_3, mg/l - **Colt**	**0,00055**	**0,00045**	**0,00034**	**0,00053**	**0,00162**	**0,00143**	**0,00214**	**0,00280**
out-set NH_3, mg/l	**0,0006**	**0,00049**	**0,00042**	**0,00061**	**0,00176**	**0,00158**	**0,0024**	**0,0031**

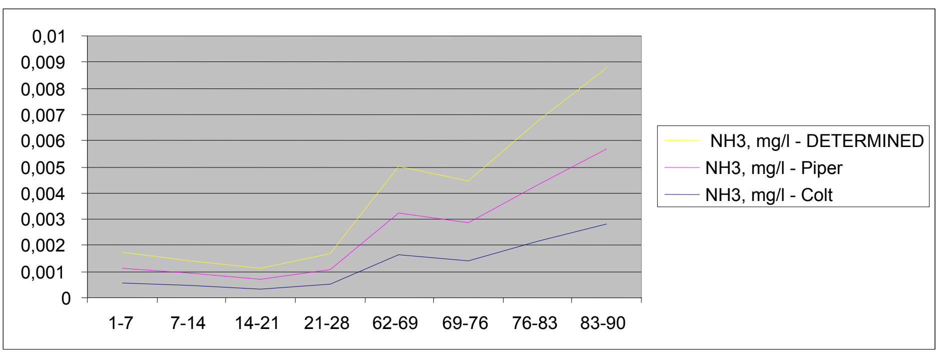


Figure 2. Determined and calculated (method of Piper and Colt) values of NH_3, mg/l at the exit of water from the pool

Excessive concentrations of ammonia cause damage to gills, reducing growth and increasing mortality, but thanks to the developed adaptive organ systems, trout relatively well tolerate more higher concentrations of ammonia than a critical value. The information on limit values of ammonia are different and range from 0,0125 mg/l to 0,025 mg/l (Boyd et al., 1992; Klontz, 1991). Production of toxicants in the aquatic environment is directly correlated with the amount of the daily meal. Coefficients that create important nutritiens in water at fed trouts per kilogram of pellet food at a water temperature from 10 to 15 ^{0}C are: for total amount of ammonia 0,289, for phosphates 0,0162 for suspended solids 0,52 for biochemical oxygen consumption 0,60 for chemical oxygen consumption 1,89 (Haskell, 1959; Liao et al., 1974; Soderberg, 1994).

Bearing in mind the presented results, there is the further task of determining the precise calculation procedures for obtaining more closer values compared with the results of laboratory analysis, which would allow their wider application in the intensive breeding of fish.

CONCLUSION

On the basis of the test the following conclusions can be done:

a) oxygen consumption and ammonia excretion directly depend on the temperature and water flow, pH, the amount of food and protein content in it and the weight of fish.

b) average difference between the expected and theoretically determined oxygen concentration ranged from 4,09% (21-28 days) to 9,76% (62-69 days), or an average of 5,72%.

c) The value of production of ammonia applied by mathematical models differed from the established laboratory values. Closest match was found between the calculated value method of Piper et al.,and determined the concentration of NH3, where the average difference was 7,66%.

d) The theoretical values obtained by the method of production of ammonia by Colt's method most varied from laboratory values, where the average difference was 11.6%.

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CAPILLARIOSIS IN BREEDER DISCUS (*SYMPHYSODON AEQUIFASCIATUS*) IN IRAN

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KAPILARIOZA KOD GAJENIH DISKUS RIBA (*SYMPHYSODON AEQUIFASCIATUS*) U IRANU

Abstrakt

Svetska trgovina dekorativnim ribama je industrija koja brzo raste. Gajenje i razmnožavanje dekorativnih riba se poslednjih dvadeset godina povećava u Iranu. Diskus (*Symphysodon aequifasciatus*), predstavnik Cichlidae je jedna od najpopularnijih i skupih akvarijumskih riba. Poslednjih nekoliko godina gajenje ove ribe je dobro razvijeno u Iranu. Dva odgajivača diskus ribe iz dva različita centra obratila su se laboratoriji za vodene bolesti Veterinarskog fakulteta Univerziteta u Teheranu zbog znakova anoreksije, gubitka ravnoteže, slabosti i tamljenja kože (sa velikim mortalitetom). Posle pretrage na ektoparazite obavljen je postmortem pod optimalnim uslovima, napravljena bakterijska kultura sa standardnim medijumom i izdvojen alimentarni kanal. Kod obe ribe ektoparaziti nisu rađeni i bakterije nisu rasle na standardnim medijima. Pretragom unutrašnjosti kod svake ribe nađeno je 5-25 nematoda. Prema morfološkim karakteristikama nematoda i njihovih jaja, identifikovane su kao *Capillaria* sp. Tretiranje drugih riba sa levamisalom je bilo efikasno i gubici su bili zaustavljeni. Neke gliste kao *Capillaria pterophylli* Heinze, 1933 mogu da izazovu veliki mortalitet kod ciklidnih akvarijumskih riba. Ova ispitivanja su pokazala da infekcija sa nekim vrstama *Capillaria* može da izazove velike gubitke kod ciklida. Dijagnostifikovanje parazita kod ovih riba može da pomogne sprečavanje velikog mortaliteta.

***Ključne reči**: diskus, nematode, Capillaria, levamisol, Iran*

INTRAMUSCULAR ANESTHESIA OF BELUGA (*HUSO HUSO*) WITH KETAMINE

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INTRAMUSKULARNA ANESTEZIJA MORUNE (*HUSO HUSO*) SA KETAMINOM

Abstrakt

Sredstva za anesteziju se kod riba obično daju kupkama, sa IM, IV, IP injekcijama. Ketamin ima anestetičke i analgetičke osobine i široku primenu u humanoj i veterinarskoj medicini. U ovoj studiji posmatrano je 50 *Huso huso*, prosečne težine 550 $\pm$ 20 g. Tokom ispitivanja ribe su držane u bazenu (1000 l) koji je bio napunjen aerisanom bunarskom vodom koja recirkuliše jednu nedelju pre eksperimenta. Ribe su podeljene u 5 različitih koncentracija (70, 75, 80, 85 i 90 mg/kg). Stanja analgezije i anestezije su konstatovana. Oporavak se javljao kada su ribe stavljane u vodu bez anestetika i beleženo je vreme oporavka. Rezultati su pokazali da je prosečno indukciono vreme u obrnutom odnosu sa dozom ketamina. Sa povećanjem doze intramuskularnog ketamina smanjivalo se indukciono vreme, a vreme oporavka povećavalo. Vreme oporavka je bilo 25 minuta kada je *Huso huso* anestezirana sa 90 mg/kg. Povećano vreme oporavka može biti povoljno u nekim slučajevima, kao u hirurgiji, ali nije korisno za rutinsko korišćenje. U ispitivanjima, anestezija je davana *Huso huso* putem injekcija sa 30 mg/kg ketamina u dorzalnu ili kaudalnu arteriju. U poređenju sa našim ispitivanjima doza ketamina u onoj situaciji (30 mg/kg) bila je manja od naše minimalne doze (70 mg/kg), ali je oporavak bio duži nego u našim ispitivanjima, što je možda je zavisno od načina iniciranja (IM i IV) ili individualnih razlika kod riba. Uopšte, neki značajni faktori za procenu individualnog anestetika kod riba su brzo indukciono vreme anestezije (manje od 4 minuta) i kratko vreme oporavka (manje od 8 minuta). Tako ovi opšti faktori i dobijeni razultati naših ogleda pokazuju da je 80 mg/kg ketamina sa 2,4 ili 8,0 minuta za indukciju i vreme oporavka najpogodnija anestezija za *Huso huso*.

Ključne reči: *ketamin, Huso huso, anestezija, intramuskularno.*

INRODUCTION

Several different chemicals such as MS-222 and clove oil have been used as anesthetics in fish farms and fish biology researches to reduce handling stress on fish. Anesthetic agents are commonly administered by immersion, with IM, IV, and IP injection and oral administration are used less commonly. Ketamine (Vetalar) is available either as a white powder or as a readymade injectable solution in saline. It has anesthetic and analgesic properties and has been widely used in human and veterinary medicine. Ketamine is the major drug in current use with crocodilians. It is effective at intramuscular weight-related doses between 12 and 80 mg kg−1 in a range of species (Ross and Ross, 2008). Ketamine is a rapid-acting, nonnarcotic, nonbarbiturate anesthetic agent that induces a state of unconsciousness known as dissociative anesthesia (Graham and Iwama, 1990). It is thought to induce anesthesia and amnesia by functionally disrupting the CNS through over stimulation induction of a cataleptic state (Williams et al. 2004). The most common injection route of ketamine, IM, requires high doses when used alone (Fleming et al. 2003; Bruecker and Graham, 1993**.** Ketamine, used alone or in combination with α2-agonists is effective for a number of species and has been shown to have minimal hematological effects in mammals and fish (Harms, 1999). Beluga (*Huso huso* (Linnaeus, 1758)) has a wide distribution. It is distributed in the Caspian Sea, Black Sea, and the Sea of Azov and many of the tributaries of these seas. Beluga is the largest species of Acipenseriformes reaching a length of six meters and a weight of more than one tone (Berg 1948; Sudagar et al 2010). The aim of this study was to determine the best dose of ketamine for anesthesia of Beluga (*Huso huso*) in intramuscular (IM) injection.

MATERIAL AND METHODS

For this study 50 *Huso huso* with average weight 550±20 g were chosen. During the study, the fish had been kept in a tank (1000-liters), containing aerated re-circulating well water for one week prior to experimentation. Water temperature ranged from 18 to 22^{0}C, pH ranged from 7.5 to 8.2, and minimum oxygen concentration was > 6 mg/L. After a period of one week, experiments were started with the Ketamine anesthesia. Fish were divided into five groups of 10 fish. Drug used in the study included ketamine (50 mg/ml) diluted with sterile saline [0.9% NaCl] solution and injections were performed with 2-mL Syringes and 22-gauge needles. Five groups anesthetize with IM injection method by ketamine in different concentration (70, 75, 80, 85, and 90 mg kg^{-1}). Stages of analgesia and anesthesia such as ataxia and reduced response to stimulation, ventilation decreased, erratic swimming, partial loss of equilibrium, and reduced activity and finally absent respiration were monitored and also ability of fish handling for injection (total loss of reaction to surgical stimulation) were assessed. Recoveries were occurred when the fish were placed in anesthetic-free water and recovery times were recorded.

RESULTS

Results showed that average of induction and recovery times were different in 5 groups. Result showed that average of induction time had a reverse relation with increase in dose of ketamine. And recovery time had a direct relation with increasing in dose of ketamine. With increasing in dose of intramuscular ketamine, times of induction decreased and times of recovery increased. In first and last groups average of induction and recovery times were 6.45, 5.48 minutes (for 70 mg kg^{-1}) and 1.04, 25 minutes (for 90 mg kg^{-1}) respectively. Induction and recovery times of 5 groups have been showed in figure 1.

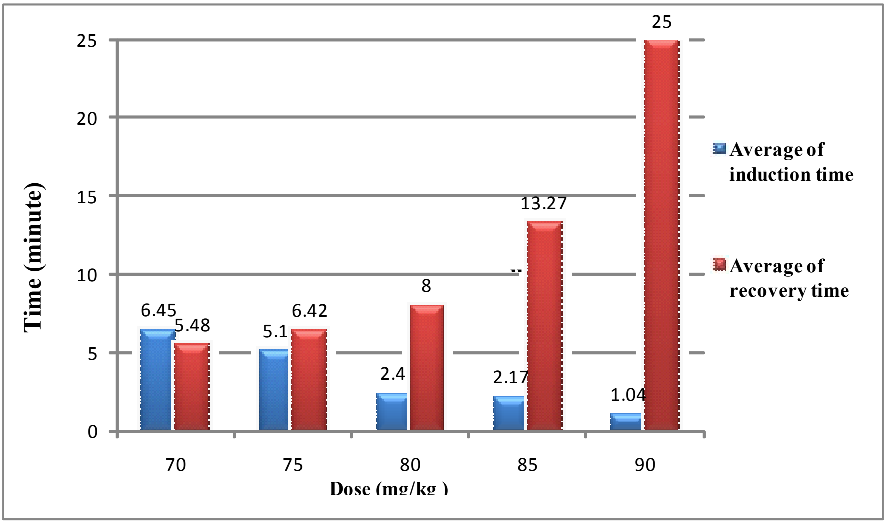

Figure 1. Average of induction and recovery times in 5 groups of *Huso huso*

DISCUSSION

Anesthesia with Ketamine at 30 mgL^{-1} in *Oncorhynchus mykiss* and *Oncorhynchus kisutch* was successfully used (Graham and Iwama, 1990). In another study rainbow trout (*Oncorhynchus mykiss*) and brown trout (*Salmo trutta*) were injected intra-muscularly with ketamine at 130 mg kg^{-1}, after that, anesthesia persisted for only 20 minutes, but anesthesia with 150 mg kg−1 (IM) persisted for 50–80 minutes. Recovery time was prolonged, taking up to 90 minutes, during which there was notable hyperactivity and ataxia (Oswald, 1978). In our study, recovery time was 25 minutes when *H. huso* was anesthetized with 90 mg kg^{-1}. In both studies, recovery was prolonged, it can be useful for some purposes such as surgery, but it is not useful for routine uses. Anesthesia in *Heros citrinellum* via injection of 30 mg kg^{-1} ketamine into the dorsal aorta or caudal vein was induced and was very fast with considerable reduction in ventilation after 1minute. Anesthesia lasted for up to 40 minutes and recovery required up to 4 hours (Bruecker and Graham, 1993). In comparison with our study dose of ketamine for anesthesia in that study (30 mg kg^{-1}) was less than our minimum dose (70 mg kg^{-1}), but recovery time was longer than in our study. It maybe dependant on method of injection (IM and IV) or on individual differences of fishes. Use of 12–20 mg kg^{-1} ketamine in combination with xylazine (Rompun) with grey nurse sharks, *Carcharias taurus* was successful (Smith, 1992). Induction took 10–20 minutes.

Ketamine was successfully used in combination with the analgesic and sedative medetomidine in bonitos (*Sarda chiliensis*) and Pacific mackerel (*Scomber japonica*) (Williams *et al.* 2004). These two studies show that ketamine can be used in combination with other sedative and analgesic drags. Maybe we can use ketamine with some other drugs such as xylazine or medetomidine in anesthesia of Acipenseridae such as *H. huso*. It need next studies to find that best form of anesthesia with ketamine is use alone or is in combination with other drags in *H.huso*. In general, the important factors for evaluating an ideal anesthetic in fish include rapid induction time of anesthesia (less than 4 minutes), short recovery time (less than 8 minutes), low toxicity for humans (at the concentrations used for fish), and availability and handler safety (Neiffer, 2007). So these general factors and the results that were obtained from our investigation, suggest that 80 mg kg^{-1} ketamine with 2.4 and 8.0 minutes respectively for induction and recovery times is the most suitable dosage for anesthetize of *Huso huso*.

CONCLUSION

In conclusion, results of our study showed that the longest and the shortest induction and recovery times were seen in 70 mg kg^{-1} ketamine (6.45 and 5.48 min) and 90 mg kg^{-1} (1.04 and 25 min). There were direct relations between increasing dose of ketamine and recovery time, so with increasing of anesthetic dose, recovery time was increased. But with increasing of anesthetic dose, induction time was decreased. So average dose (80 mg kg^{-1}) is the most suitable dosage for anesthetize of *Huso huso*.

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MICROBIOLOGICAL ASPECTS OF THE CARP POND ECOLOGICAL STATUS DURING THREE YEARS PERIOD

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MIKROBIOLOŠKI ASPEKTI EKOLOŠKOG STATUSA ŠARANSKOG RIBNJAKA TOKOM TROGODIŠNJEG PERIODA

Apstrakt

Šaranski ribnjaci su, pored pastrmskih ribnjaka jedan od dva najzastupljenija vida akvakulture na našim prostorima. Konzumni šaran može da dostigne dužinu od preko 30 cm i masu preko 6 kg. Meso šarana bogato je polinezasićenim masnim kiselinama koje imaju korisno delovanje u ishrani. Šaran iz akvakulture, dobro je prilagođen na uslove životne sredine, koji su slični onima u prirodnom staništu. Naseljava bentos i pelagijal, rastresiti mulj i vegetaciju širokih, usporenih vodotokova. Šaran je omnivor i hrani se rečnim rakovima, insektima, crvima, larvama i submerznim biljkama. Pored makroozooplanktna i makrozoobentosa, mikroskopski vidljive alge, bakterije, gljive i virusi prisutne su u ekosistemu šaranskih ribnjaka. Bakterije mogu biti korisne, bezopasne, štetne ili patogene. *E. coli* i *Enterobacteriaceae* važni su pokazatelji fekalnog zagađenja vode i rizika po zdravlje gajenih životinja. Zdrave jedinke šarana u svom crevnom sistemu imaju korisne bakterije, saprofite, koje u uslovima optimalnog metabolizma, postaju dominantne. Saprofitnu mikrofloru crevnog sistema šarana čine, sa više od 99 % *Bacillus spp., Bifidobacterium spp., Lactobacillus spp., Saccharomycetes*, itd. Aerobne i fakultativno aerobne bakterije čine oko 1 % crevne mikroflore. Različite su bakterije koje izazivaju oboljenja gajene ribe. *Aeromonas spp.* su Gram- negativne bakterije koje naseljavaju prirodno stanište slatkovodnih i morskih ekosistema a i deo su oportunističke saprofitne mikroflore gastrointestinalnog trakta. *Aeromonas spp.* vrlo je čest izolat iz hemoragičnih lezija i nekrotičnih delova tkiva. Kod šarana, *Aeromonas salmonicida* dovodi do oboljenja, poznatijeg kao eritrodermatitis. Septikemije kod šarana mogu izazvati pokretljive aeromonade, *A. hydrophila, A. sobria* i *A.caviae.* U slučaju oboljenja šarana, gde je veoma velika gustina populacije ribe u ribnjaku, lako dolazi do širenja bolesti, a sama bolest teško se iskorenjuje, uz velike ekonomske gubitke.

Enterobakterije obuhvataju rodove *Escherichia, Salmonella, Klebsiella, Edwardsiella* i *Yersinia.* Ukupno je ispitivano po 6 uzoraka ribe (koža i mišić), hrane za ribe, mulja i vode, tokom 4 godišnja doba, tokom trogodišnjeg perioda.

Uzorci kože i mišića šarana ispitivani su prema metodama propisanim u Pravilniku o metodama vršenja mikrobioloških analiza i superanaliza životnih namirnica (Sl. list SFRJ br.25/80). Na ovaj način uzorci su ispitani na prisustvo *Salmonella spp., Proteus spp., E.coli, Clostridium spp. i Staphylococcus aureus.* Odgovarajućim ISO i ostalim mikrobiološkim metodama utvrđivano je prisustvo *Aeromonas hydrophila, Vibrio parachaemoliticus*., *Yersinia enterocolitica*., *P. aeruginosa,* odnosno *E. coli*, koliformnih bakterija i intestinalnih enterokoka. Ukupan broj bakterija izolovanih iz kože i mišića kretao se od 3.11 $\log_{10}$ CFU/g do 4.72 $\log_{10}$ CFU/g. *E.coli* izolovana je iz barem jednog uzorka kože od šest ispitivanih, tokom svih uzorkovanja. Ostali patogeni mikroorganizmi nisu izolovani iz tkiva. Ukupan broj koliformnih bakterija u vodi nije prelazio 198 bakterija/100 ml, dok su u mulju nađeni predstavnici rodova *Aeromonas* i *Clostridium*, pored *E.coli*. Hrana za ribe bila je mikrobiološki ispravna po kriterijumima važećeg Pravilnika. Na osnovu ovih pokazatelja može se zaključiti da je proizvodnja šarana u veštačkom sistemu akvakulture omogućila dobijanje mikrobiološki ispravnog mesa iz ujednačenog ekosistema najsličnijeg prirodnom staništu rečnog šarana *C. carpio.*

Ključne reči: *Cyprinus carpio, carp mikrobiologiaja šarana, ribnjak, ekoistem*

INTRODUCTION

Carp from our aquaculture, is derived from the Asian wild strain that is selected for certain characteristics (Kohlmann et al., 2003). Cultivated carp can reach a length of over 30 cm and weight over 6 kg. Carp meat is rich in polyunsaturated fatty acids (Djinovic et al., 2010) that have beneficial effects in nutrition. Favorable effect of n-3 polyunsaturated fatty acids from fish meat, on human health has been demonstrated in many studies (Von Shacky, 2001, Mozaffarian et al., 2004). Carp inhabits benthos and the pelagic, loose sand and vegetation of broad, slow streams. In terms of reduced concentrations of dissolved oxygen in the water, the fish rises to the surface. Carp is omnivor and feeds on river crabs, insects, worms, larvae and submerged plants. In addition cultivated carp is fed by plant feed. Bacteria from feed and water can be useful, harmless, harmful or pathogenic. Near the habitat of carp there are a number of settlements that are the sources of fecal and other pollution (Harnisz M., et al. 2010.). *E. coli* and *Enterobacteriaceae* are important indicators of faecal contamination of water and health risk of animals. Healthy individuals in their intestine have beneficial bacteria, saprophytes, which in terms of optimal metabolism, become dominant. Saprophytic microflora of carp intestinal systems are, with more than 99%: *Bacillus spp*. *Bifidobacterium spp*., *Lactobacillus spp*. *Saccharomycetes*, etc.. Aerobic and facultative aerobic bacteria are about 1% of the intestinal microflora (Zhou Q., et al. 2009). In carp, *Aeromonas salmonicida* causes disease, known as erythrodermatytis (Gostin I., et al., 2010.). Septicemia in carp can be caused by movable aeromonads, *A. hydrophila, A. Sobria* and *A.caviae,* even when they do not cause disease of fish, can cause gastrointestinal diseases in humans, when eating inadequately prepared meat fish. In the case of carp disease, where there is very high population density of fish in the pond, disease spreads easily and is difficult to eradicate, followed by economic losses. *Enterobacteriaceae* include genera

Escherichia, *Salmonella, Klebsiella, Edwardsiella* and *Yersinia.* Enteropathogenic *E. coli* has been isolated from clinically healthy fish (Kurtovic B. et al., 2001.).

MATERIALS AND METHODS

A total of 6 samples of the fish (skin and muscle), fish feed, mud and water, have been tested during each season, for three years. Samples of skin and muscle of carp were examined by the methods prescribed in the Regulation[1]. In this way the samples were tested for the presence of *Salmonella spp*., *Proteus spp*., *Escherichia coli*, *Clostridium spp*. and *Staphylococcus aureus*. Samples of skin and muscle of fish were tested for the presence of *Aeromonas hydrophila* using method for isolation and identification of moving aeromonads, and method for Isolation of *Vibrio parachaemolyticus* for isolation of *Vibrio* spp.. In order to determine the presence of pseudomonads, and the presences of the genus *Yersinia*, samples were analyzed using Method for isolation and identification of *P. aeruginosa*, or Method for isolation and identification of *Yersinia enterocolitica*.

Samples from the environment, mud and water, were analyzed by the relevant ISO methods the presence of *Enterobacteriaceae*, *Salmonella* spp., *E. coli* and total viable count. Water from the lake was examined by membrane filtration method for determining *E. coli* bacteria and coliforms: EN / ISO 9308-1:2000 Cor 1:2007, and intestinal enterococci EN ISO 7899-2:2000. Samples of complete feeds for carp, were analyzed for the presence of pathogenic bacteria, yeasts and molds, and total aerobic bacteria, the relevant ISO methods.

RESULTS AND DISCUSION

The results are shown in Tables 1, 2, 3 and 4 .

Table 1. The three-year overview of microbiological findings in samples of muscle and skin of carp from aquaculture

	FISH									
Examination	**sep.08**	**dec.08**	**mar.09.**	**jun.09**	**sep09.**	**dec.09**	**mar.10**	**jun.10**	**sep.10**	**dec.10**
TVC (log CFU/g)	4,72	3,76	3,92	4,08	4,25	3,66	3,45	4,22	4,58	3,11
Salmonella	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø
E.coli	+ (2/6)	+ (3/6)	+ (3/6)	+ (1/6)	+ (4/6)	+ (2/6)	+ (2/6)	+ (1/6)	+ (4/6)	+ (1/6)
L.monoc.	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø
Vibrio	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø
Yersinia	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø
Aeromonas	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø
Clostridium	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø

Most commonly isolated bacteria in fish muscle is *E. coli*, during all the seasonal study. In raised carp, the majority of the members of the species E. coli and 64%, following 4.2% of *Salmonella spp*., 17.6% *Vibrio spp*. and 3.3% *L. monocytogenes* (Basti A., et al., 2004). According to other findings mostly representatives were isolated of the genus *Flavobacterium* 37% of isolates, following family *Enterobacteriaceae* (15.6%) and *Vibrionaceae* (37.8%) and others families with less than 1% of the total number of isolates (Mahmoud B., et al., 2004.). As for the total number of aerobic bacteria, its maximum value is 4.72 log_{10}CFU/g in autumn sampling. Similar growth of TVC in the fall was recorded with other authors, where the TVC was 9.4 log_{10}CFU / g (Al-Harbi A.A. et al., 2004). Bacteria of the genus *Aeromonas*, *Yersinia* and *Vibrio*, were not isolated during any season or year of sampling. Species of the genus *Vibrio*, they are much more common isolates from aquaculture brackish water ecosystems, which are as high as 58% of total isolates (Al-Harbi A.A. et al., 2004). In water and sludge, other than coliforms, intestinal enterococci, *E. coli* and *Aeromonas* spp., as is the case with our samples, it is possible to isolate *Pseudomonas spp*., *Vibrio spp*. and *Bacillus* spp., also nonsporogenic and Gram- positive bacteria (Kennedy B. et al, 2006). The total number varies from 10^1 to 10^5 cfu / ml, slightly more than the total number of coliform bacteria in water (Table 2 and Table 3).

The most common isolate in our case is *E. coli*, when it comes to mud, while in some findings, the most frequent isolate of Gram-negative bacteria is *Aeromonas*. The number of bacteria *Vibrio* ranged from 10^1/ml to 10^3/ml. (Kennedy B. et al, 2006).

Table 2. The three-year overview of microbiological status of sludge from the carp pond

	M U D									
Examination	**Sep-08**	**Dec-08**	**mar.09.**	**Jun-09**	**sep09.**	**Dec-09**	**Mar-10**	**Jun-10**	**Sep-10**	**Dec-10**
Salmonella	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø
E.coli	+ (4/6)	+ (3/6)	+ (5/6)	+ (2/6)	+ (3/6)	+ (4/6)	+ (4/6)	+ (5/6)	+ (6/6)	+ (3/6)
L.monoc	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø
Vibrio	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø
Yersinia	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø
Aeromonas	+ (2/6)	+ (3/6)	+ (3/6)	+ (2/6)	+ (4/6)	+ (4/6)	+ (3/6)	+ (2/6)	+ (4/6)	+ (5/6)
Clostridium	+ (2/6)	+ (5/6)	+ (4/6)	+ (4/6)	+ (5/6)	+ (3/6)	+ (4/6)	+ (1/6)	+ (5/6)	+ (1/6)

Table 3. The three-year overview of microbiological status of water from the carp pond

	W A T E R									
Examination*	**Sep-08**	**Dec-08**	**mar.09.**	**Jun-09**	**sep09.**	**Dec-09**	**Mar-10**	**Jun-10**	**Sep-10**	**Dec-10**
Total col. number	115	101	129	187	88	97	122	120	101	108
Intestinal enterococci	47	33	104	94	39	45	15	9	25	42
E.coli	98	82	67	122	74	86	54	14	75	55

Table 4. Microbiological safety of fish feed during three year period

				F	E	E	D			
Examination	Sep-08	Dec-08	mar. 09.	Jun-09	sep 09.	Dec-09	Mar-10	Jun-10	Sep-10	Dec-10
Salmonella	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø
L. monoc.	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø
E.coli	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø
Yeasts and moulds	2.95	2.85	2.7	4	2.95	4.3	5	3.25	2.87	3.14
TVC	3.85	4.7	3	4.9	4.48	4.77	7	5.52	6.82	4.14
C.botulinum/ C.perfringens	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø
S.pyogenes	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø
Proteus	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø	Ø

When it comes to feed that has been used for carp (Table 4.), microbiological safety of the analyzed samples corresponds to the current Regulation[2]. Pathogenic microorganisms were not detected, and the total number of aerobic bacteria did not exceed 6.82 $\log_{10}$ cfu/g.

It is very important not to have *Salmonella* and other pathogens in feed, because they are cause of numerous outbreaks, that can not be controlled easily (Moretro T. et al., 2004.). Once it enters into a factory for the fish food production, these bacteria are very difficult to eradicate, and it becomes so called "domestic strain" (Lunestad B.T., et al., 2007.)

CONCLUSIONS

Regarding obtained results, it can be cocncluded that during three year long period of examination, common carp from aquaculture was microbiologicaly safe, its water habitat was microbiologicaly safe and it wasn`t threatened with pathogens. Based on these results, it could be concluded that the production of carp in artificial aquaculture system allowed obtaining microbiologicaly safe food from balanced ecosystem most, that is most similar to natural habitats of river carp *C. carpio*.

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COMPARISION OF STOCKING DENSITY FOR NEREIS DIVERSICOLOR UNDER CULTURAL AND NATURAL (ANZALI LAGONA) CONDITIONS

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POREĐENJE GUSTINE NASDA *NEREIS DIVERSICOLOR* U PRIRODNIM I UZGOJNIM (ANZALI LAGONA) USLOVIMA

Abstract

Nereis diversicolor is regarded as a live food and is significantly effective in increasing stocks, enhancing tolerance in sturgeons and also increasing survival of sturgeon fry. Research conducted indicates that *N. diversicolor* is more abundant in May as compared to other months of the year. In February, only breeders of this species are found in the environment. *N. diversicolor* was found in four different weight classes from March 2009 to February 2010. They showed decrease in density with increase in temperature and reached the lowest numbers in February. In years 2009 and 2010, 200 sampling conducted monthly at the point where Caspian Sea mixes with the Anzali lagoon. Sampling was performed by Van Veen sampler with 400 cm^2 cross section. Sediments were washed through a sieve with 0.5 mm mesh size. The residue along with *Nereis* was transferred to a dish, worms separated and transferred to lab. The density of worms per m^2 was calculated according to the density formula. Two experiments were conducted to determine the best stocking density for the culture of *N. diversicolor.* Stocking density of 381-6350 worms m^{-2} were used in 6 trials initially and best growth was recorded in trial with density of 381 worms m^{-2}. In the second experiment using 381-3175 worms m^{-2} in 7 trials, trial with 381 worms m^{-2} again showed better growth as compared to other trials. These results obtained were almost similar to those obtained for *N. diversicolor* is in its natural environment (447 worms m^{-2}).

Key words: *Density, Nereis diversicolor, Anzali lagoon, Caspian Sea*

INTRODUCTION

Nereis diversicolor belongs to the phylum Annelida, class ploychaeta and plays an important role in the nutrition of commercially valuable fishes like sturgeons. Considering the distribution of this worm in the Sea of Azov that served as a feed for sturgeons inhabiting this sea (Nikolsky, 1963) Russian scientists transferred about 65000 of this benthic organism from the Sea of Azov to the Caspian Sea during 1939-1941 to increase the food resources of sturgeons in this sea. In 1948 they had spread through an area more than 30,000 Km^2 particularly in the western part of the Caspian Sea (Clark, 1989).

As a result of the introduction of benthic organisms including *N. diversicolor*, *A. stellatus* began to feed on this (Holcik, 1989) and thus nereis and abra constituted the basic food for this species. *A. gueldenstaedtii* also usually feed on *N. diversicolor* and abra (Kazenchev, 1971). According to Holcik (1989) in October 12.3 % of the stomach contents of *A. gueldenstaedtii* was reported to be composed of *Nereis*. *N. diversicolor* is widely developed in sediments with high organic content where they feed on organic matter. Juvenile and adult worms can survive easily in salinities of 1 ‰. These worms live for up to one year and die after breeding. They are also capable of tolerating oxygen deficits for relatively long periods (Zenkenvitch, 1963). Zenkenvitch (1963) reports that 66 % of dry weight in nereis is made up of protein, 7.73 % fat and 13.83 % ash. *N. diversicolor* has a nutritive value of 5.58 kilo calorie g^{-1}.

MATERIAL AND METHODS

Sampling stations were located at the lagoon estuary of the Caspian Sea where the highest abundance of *Nereis* was found. *N. diversicolor* worms were collected using a Van Veen grab with 400 cm^2 cross section (20x20x10 cm) to determine biomass per square meter. Sediments were washed on a sieve (mesh size 0.5 mm) and the remaining matter on the sieve was collected in a plastic container and transferred to the laboratory where the live worms were separated. This procedure was repeated once every month through a period of one year. The worms were counted and divided into different weight classes and maintained to conduct experiments to determine their density for culture. Dissolved oxygen, water and air temperature, salinity and pH were recorded at the sampling site at each sampling.

At first about 3-50 *N. diversicolor* were placed in 1 L beakers containing 500 cm^3 of sediment collected from the sampling site with a surface area of 78.5 cm^2 and height of 6.5 cm. 500 cc of water was added to each beaker. The experiment was run with six trials (3, 9, 15, 25, 40 and 50 worms) using three replicates for each trial. Each worm was weighed prior to the experiment. No feed was given to the worms through the experiment period, however the worms fed on detritus found in the sediment. After one month the worms in each beaker were weighed again and their relative growth was calculated. The experiment was repeated using seven trials (3, 5, 7, 10, 15, 20 and 25 worms) under the same conditions.

RESULTS

Results obtained from the studies conducted on abundance of *N. diversicolor* in the Anzali Lagoon indicate that Class I (1-9 mg) of *N. diversicolor* that was found during five months of the year showed highest density in May (236 worms m^{-2}). Also density of *N. diversicolor* belonging to class II (11-50 mg) and class III (51-200 mg) showed highest density during April (113 worms m^{-2}) and May (127 worms m^{-2}), respectively. *N. diversicolor* of class IV (>200 mg) were found almost throughout the year except in October and November with the highest density of 92 worms m^{-2} in May.

Experiments on culture of *N. diversicolor* under experimental conditions showed that trial with 381 worms m^{-2} using three replicates under similar conditions showed increase in weight from 2.7 to 3.2 g whereas 0.5 g increase in weight was observed in trial using 3175 worms m^{-2}. Trials with higher densities showed negative growth as well as mortality.

In the second experiment also trials using 381 worms m^{-2} with three replicates under similar conditions showed increase in weight from 2.2 to 3.7 g and trials using 3175 worms m^{-2} showed weight gains of less than 0.5 g.

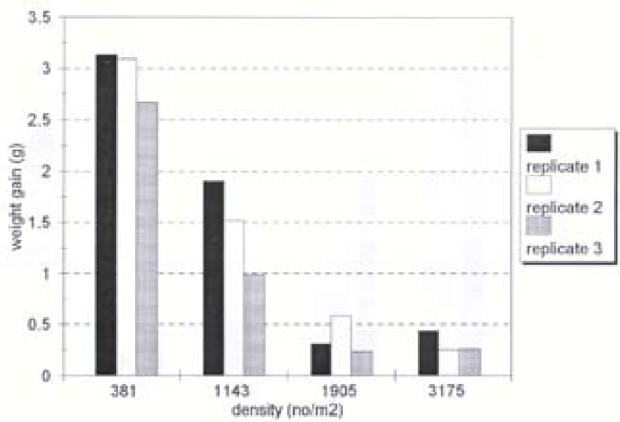

Figure 1. Stocking density of *Nereis diversicolor* in first experiment

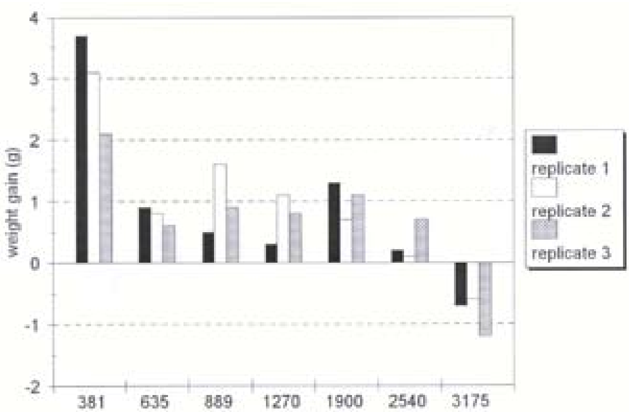

Figure 2: Stocking density of *Nereis diversicolor* in second experiment

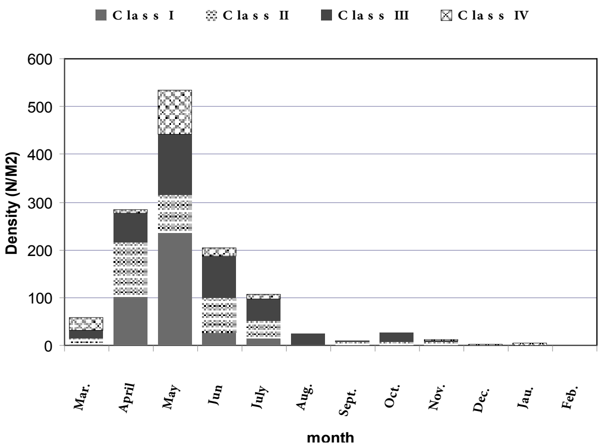

Figure 3. Density of *Neries diversicolor* in different weight classes in the lagoon estuary of Caspian Sea (March 2009-Febraury 2010)

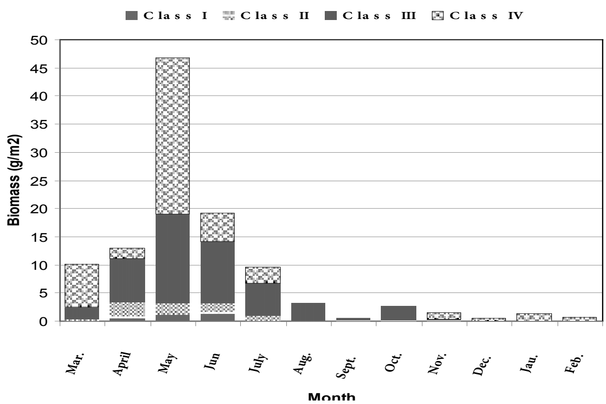

Figure 4. Biomass of *Neries diversicolor* in different weight classes in the lagoon estuary of Caspian Sea (March 2009-Febraury 2010)

DISCUSSION

The study of *Nereis diversicolor* in Anzali Lagoon indicate that this organisms can be a usefulness live food for economical Fish nutrition.

On the basis of results obtained it is evident that *N. diversicolor* occupies a wide area in the lagoon estuary of the Caspian Sea and are easily collected at 4.5 m depth. In the first experiment using six trials the density at the end of the experimental period was 381 worms m^{-2}, although negative growth rates were seen in trials 5 and 6 due to high stocking densities. In trials with lower stocking density, the *Nereis* worms not only showed increase in body weight without being fed diets but also showed no mortality. Trial number one in the first experiment showed the highest growth rates with an increase in biomass from 2.6 g m^{-2} to 3.3 g m^{-2} (Fig. 1). However increase in stocking density resulted in decrease in growth. As seen from results obtained in the second experiment it is clear that highest growth was obtained in trial using 381 worms m^{-2} (2-1-3.7 g). Trials with higher stocking density showed negative growth during the experimental period (Fig. 2).

In studies conducted in Gorgan Bay in 1964 the biomass of *N. diversicolor* was reported as 7 g m^{-2} (Ghasemov & Bagherov, 1983).

Results from studies conducted in the central region in the Sulak Bay indicate that *Nereis* worms are distributed at depths between 0.3 to 4 m in marshy sandy bottoms of this region with a biomass of 4.5 g m^{-2} (Latipov *et al*., 1994).

The biomass of *N. diversicolor* was reported as 0.01 g m^{-2} in 1995 in the Kura River. However in 1979 the biomass at 10 m depths was 0.8 g m^{-2} and in 1980 biomass in that region at similar depths was 1.17 g m^{-2} (Ghasemov, 1987).

Studies conducted to determine the density of *N. diversicolor* in the lagoon estuary of the Caspian Sea indicate that highest abundance in these worms is attained in May (Fig. 3). In February, only breeders of this species are found to remain in the environment that migrate to the deeper layers of the sediment and thus are not trapped in the Van veen grab.

Decrease in abundance in class I (1-9 mg) as compared to class II worms from July to December and again their increase from March to May indicate that the worms start breeding when temperature increases. Density of worms in their natural environments (447 m^{-2}) with that in the experimental medium (371 m^{-2}) were almost similar.

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SHELLFISH FARMING AND PREPARATION FOR THE MARKET

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UZGOJ I PRIPREMA ŠKOLJKAŠA ZA TRŽIŠTE

Abstrakt

Ovaj rad predstavlja kratki prikaz proizvodnje školjkašana istočnoj obali Jadrana. Na ovom se prostoru komercijalno uzgajaju dvije vrste školjkaša, kamenica *Ostrea edulis* i dagnja *Mytilus galloprovincialis*, dok se ostale vrste izlovljavaju iz prirodnih populacija. Pored tehnologije uzgoja, opisani su i zakonski uvjeti potrebni za plasman školjkaša na tržište. To su u prvom redu monitoring uzgojnih područja, potrebna infrastruktura (otpremni i centri za pročišćavanje) i te pravilan postupak sa školjkašima nakon izlova.

Ključne reči*: uzgoj školjki, ostriga, dagnja, centri za prečišćavanje*

INTRODUCTION

The increasing demand for protein rich food sources have led to a significant increase in aquaculture production, including shellfish culture. Higher demand for healthy food sources have also increased the consumption of fish and shellfish. In comparison to intensive shrimp and fish culture, shellfish cultivation is simpler and less demanding.

Commercial production of the european flat oyster *Ostrea edulis* and the black mussel *Mytilus galloprovincialis* is carried out on the East Adriatic Coast. Several other shellfish species (*Venus verrucosa*, *Tapes decussatus, Chamelea gallina, Arca noae* and *Pecten jacobeus*) are available to the local market, but they are derived from the wild catch.

Shellfish are filter feeders, feeding on phytoplankton and zooplankton, dissolved mineral salts, and organic detritus, so no additional artificial feeding is required (Gosling, 2003; Gavrilović, 2011). However, different harmful microorganisms (bacteria, viruses, parasites, aquatic biotoxins, etc.) and chemical pollutants can be present in the sea water surrounding the cultured shellfish populations. These contaminants can accumulate in the shellfish soft tissue during the cultivation process and cause food borne diseases (Huss et al., 2004). The most common pollutant and the cause of most foodborne diseases is fecal contamination of the sea water (Jackson i Ogburn, 1999). The most effective protection of consumer health is with the control of seawater quality through the regulation of the shellfish farming/collecting areas, as well as during the handling of shellfish after harvest (on-shore infrastructure, such dispatch and depuration centers).

FARMING TECHNOLOGY AND WATER QUALITY

Shellfish farming on the eastern adriatic coast is still based on the collection of larvae from their natural habitat. Collectors with an attached spat of oysters and black mussels remain in the water for an average of six months. They are then placed on growout installations until the shellfish reach market size. The growout phase in the eastern adriatic region is carried out between the sea bottom and surface (considered the mediterranian method), on fixed or floating installations. Lately, floating long lines are the most commonly used installations (Marguš i Teskerdžić, 1993; Gavrilović and Petrinec, 2003).

In this region, shellfish farms are usually situated within in-shore locales: shallow productive coastal areas, protected coves, lagoons, and river estuaries. Besides having suitable geomorphological and ecological characteristics (optimal temperature, oxygen saturation, salinity, pH, suitable concentration of nutrients and plankton, etc.) (Hrs-Brenko, 1973), the shellfish farming aquatoria must be of satisfactory sanitary quality. This is especially important for the shellfish that are consumed raw, such as oysters. This is also a priority for the other species as well, since they are usually consumed after minimal termical processing.

With reference to the microbiological water quality, the farming areas in the EU and most other european countries are divided into three categories:

- Category A : less than 230 *Escherichia coli* per 100g of shellfish meat and intervalval fluid
- Category B: 230-4600 *E. coli* per 100g of shellfish meat and intervalval fluid
- Category C (> 4600 *E. coli* per 100g of shellfish meat and intervalval fluid).

The specific procedures followed with the shellfish after harvesting depends on the microbiological quality of the cultured sea water. By regulation, following harvest and before distribution to the market, all shellfish must pass through a dispatch center, where they are cleaned, sorted, packed and labeled. Shellfish from Zone B, have to undergo a depuration process. The depuration unit can be located within a separate facility, or within the dispatch center. Only after depuration can shellfish from Zone B pass through the prescribed procedures within the dispatch center, and be placed on the market. Direct harvesting of the shellfish from zone C is not permited. In order to be harvested, the shellfish would be required to relay for a longer period within an approved area of proper water quality, and then sent to the depuration/dispatch center (Regulation EC 854/2004).

The legal requirements for the construction and operation of dispatch and depuration centers, for storage and transport of shellfish to the market, and the obligatory application of good hygienic practices, HACCP and traceability in all facilities that deal with food, are clearly defined. According to the legislation, the responsibility for food safety assurance rests with the commercial food handler, who must satisfy prescribed requirements (Regulation EC 852/2004; Regulation EC 853/2004; Regulation EC 854/2004; Regulation EC 882/2004).

In addition to the selection of an optimal culture location with frequent monitoring of the farmed area, and using adequate farming techniques, procedures for the handling of the shellfish after harvest are also of great impotance.

SHELLFISH HANDLING AFTER HARVESTING

According to the legislation, those involved in the shellfish business, and with harvesting or handling of live shellfish after harvest, are required to fullfill all prescribed legal requisites. All harvesting methods and techniques, and handling following harvest, must be carried out carefully to prevent physical damage (braking, scraching, vibration etc.), or contamination. Once harvested, live shellfish cannot be returned to the culture water. Transport vehicles must be equipped with proper drainage outlets for the waste water, provide optimal conditions for shellfish survival (maintaining an optimal temperature regime), and assure effective protection from contamination (Regulation EC 852/2004; Regulation EC 853/2004; Regulation EC 854/2004; Regulation EC 882/2004).

Improper handling during harvest, or during delivery to the dispach center, can lead to damage of the shell, stress, or a multiplication of the bacterial populations (exposure to high temperatures, repeated placement within the culture area after harvest, or contact of the shellfish with infected or ill workers), resulting in the product being unfit for human consumption, and negating the depuration process (Jackson i Ogburn, 1999; Huss at al., 2004).

LEGAL REQUIREMENTS FOR DEPURATION AND DISPATCH CENTERS

Design and construction of dispatch and depuration centers is regulated by law. Shellfish producers are responsible for the appropriate design, and the maintenance and operation of these centers, as well as the proper education of their employees. Only correctly labeled shellfish, with a proper declaration, can be distributed from the dispatch center to the market. Transport vehicles also must satisfy the hygienic regulations, with the proper documentation accompanying the transport of all shellfish.

According to the prescribed requirements for the dispatch centers and depuration facilities are:

1. They must be located on sites that are not endangered by possible flooding due to tidal changes or water runoff.
2. Water tanks and reservoires must meet the following requiremants:

a) Inner surfaces has to be smooth, rigid and water proof, and must be easy to clean

b) They must be designed to allows for easy water drainage

c) Water inlets must be positioned in such a way as to avoid water contamination.

3. In the depuration centers, reservoirs for depuration must be sized to accomodate the requirements and quantities of the treated species.

Basic hygienic requirements and procedures (good hygiene practices, HACCP, traceability) for the dispatch/depuration centers are also defined and regulated by law (Regulation EC 852/2004; Regulation EC 853/2004; Regulation EC 854/2004; Regulation EC 882/2004). These are basic requrements described for the design, construction and organization of activities within the dispatch/depuration center .

SHELLFISH DEPURATION

Depuration (purification) is a process by which live shellfish harvested for market are placed within a facility where the contents of their digestive tract are purified using clean sea water under controlled conditions (Jackson i Ogburn, 1999). In this manner, bacteria are eliminated from the gut, and a significant reduction in the presense of viruses is also achived. By the application of this process, which is prescribed by law for shellfish from Zone B, the most common human pathogens are eliminated.

The depuration process is carried out untill the microbiologically correct product is achieved, usually within 48 hours. CEFAS recommends a minimal period for purification of 42 hours, whereas the Canadian regulatory body recommends 44 hours (Jackson i Ogburn, 1999.

Theoretically, the depuration process seems simple, but several factors influence its efficiency: the handling of shellfish after harvest, the design and efficiency of the depuration system components, water quality, sliminess of the shell, the initial number and species of the microorganisms present within the shellfish, and their physical condition. For this reason, a knowledge of the local farming conditions is necessary in order to achieve maximal efficiency of the depuration process (Jackson i Ogburn, 1999; Jug-Dujaković i sur., 2010).

Basic components of the depuration system include those that achieve: mechanical filtration, biological filtration, temperature control, salinity control, oxygenation, and chemical purification. Factors that have to be taken into account in the process of designing the depuration system are: efficiency, legal requirements, characteristics of the aquatoria, profitability, cost, and the ease of construction and maintenance. As an example, the depuration system designed in the Technology and Business Innovation Center for Mariculture (MARIBIC) is presented. For reasons of efficiency, a vertical depuration design is used. From the live shellfish holding tanks, sea water flows to a reservoir, and is then pumped through a sand filter which removes particles larger than 2-5μm. After the sand filter, the water passes through a diatomaceous earth (DE) filter which separates particles as small as 1μm. The mechanically treated water then flows through a UV reactor for sterilization. A side-stream chiller is also included in the system to maintain coolwater conditions during summer. A biofilter within the main reservoir removes ammonia, a product of shellfish metabolism, and a protein skimmer, also included in the system, removes very fine and dissolved organic solids. The entire process, and the proper functioning of the system components, is monitored or controlled by computer. The computer also records all important water quality parameters of the depuration process (Jug-Dujaković i sur., 2010).

CONCLUSION

Shellfish are mostly consumed raw or after minimal thermal processing. For this reason, they can easily cause food borne diseases. In order to protect consumers health, the legal requirements for the construction and operation of dispatch and depuration centers, for storage and transport of shellfish to the market, and the obligatory application of good hygienic practices, HACCP and traceability in all facilities that deal with food, should be followed carefully.

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HEAVY METALS (CU, PB, CD, ZN) MONITORING IN WATERS OF THE SHAHID BEHESHTI STURGEON HATCHERY, RASHT, IRAN

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MONITORING TEŠKIH METALA (CU, PB, CD, ZN) U VODAMA SHAHID BEHESHTI MRESTILIŠTA JESETRI, RASHT, IRAN

Abstract

The presence of heavy metals; copper (Cu), lead (Pb), cadmium (Cd) and zinc (Zn) in water was monitored during different stages of breeding and rearing of *Acipenser persicus* at the Shahid Beheshti Sturgeon Hatchery. Water samples were collected (3 samples from each point) from the main pond, Kurenski pond, incubators, vniro tanks, earthen ponds and at the river estuary where fingerlings are released into the sea. Water parameters such as pH and water and air temperature were measured and recorded at the time of sampling. Heavy metal concentrations in water samples were determined using Atomic Absorption Spectrophotometer FAAS-Varian AA-220.

In the present study the distribution pattern for heavy metal concentrations in different stages of breeding and rearing was as follows: Zn>Cu>Pb>Cd. Highest concentrations of heavy metals belonged to the element Zn (36.0±3.6 ppb) which was determined in the water sample collected from the inlet of master pond. Lowest concentration for Zn recorded was 0.98±0.26 ppb. Highest concentration for Cu was 10.5±1.18 ppb and the lowest concentration for this element was 0.65±0.26 ppb. The highest and lowest concentrations recorded for lead were 9.33±1.19 and 0.15 ±0.01 ppb, respectively, while those for cadmium were 0.116±0.019 and 0.016±0.003 ppb, respectively. The results obtained indicate the presence heavy metals studied in the water samples although they were lower than the maximum permissible concentration for these pollutants for the breeding and rearing of *Acipenser persicus* in sturgeon hatcheries.

Key words: *Sturgeon fingerlings, heavy metals, incubation, vniro tanks, rearing ponds*

INTRODUCTION

In addition to the type and level of contaminant and its behavior in the environment (air, water, sediment and their interfaces), the response of the organisms will also depend on its developmental stage and the interaction between it and its environment. For example, the consequences of any impact of genotoxic compounds is likely to be far more severe if the impact is on germ cells than if it is on adult somatic cells. The organism’s health, age, reproductive state and nutritional state will all affect its response to pollution load (Lawrence & Hemingway, 2003).

In an extensive study, Nriagu (1979) studied the concentrations of heavy metals in water, sediments and organisms in ponds and lakes and reported various effects of these heavy metals such as decline in growth, changes in behavior, genetic changes and mortality in aquatics. These changes may also imperil aquatic life completely. Similar studies on the effects of heavy metals in rivers are available (Mortazavi, 1994; Barak and Mason, 1989; Dixit and Witcomb, 1983; Mason, 1981).

Sturgeons are among the aquatic organisms that are affected by pollutants entering the Caspian Sea. They are the most valuable commercial species of the Caspian Sea and are being protected in different ways. Although the concentrations of heavy metals in Caspian Sea sturgeons are low, similar to that of freshwater fish, they are higher than the levels reported for marine species. It was recently reported that muscle atrophy and abnormality of gonad development in sturgeons was caused by increasing pollution levels in the Caspian Sea (Khodorevskaya et al., 1997).

The I. R. Iran contributes 5% of the freshwater flowing into the Caspian Sea. About 128 large and small rivers flow into the Caspian from Iran. About 36 rivers are considered as major rivers of the basin. The total average flow of these main rivers exceeds 16839×10^6 m^3. More than 90% of pollution is brought by rivers. Hence, continuous monitoring of rivers can provide us with information on their hydrological cycles as well as on the pollutant load entering the Caspian Sea through these rivers (Sadeghi, 2005).

Study of rivers of the coastal regions of the Caspian Sea is very important in that rivers are the main source of water flowing into the Caspian Sea. Hence any decrease or increase in the volume of water entering the sea will have direct effects on the amount of nutrients transferred and on the productivity of the sea. Secondly most fish are anadromous in nature, and rivers serve as the spawning ground for them. In the recent decades, rivers have been confined and their flow has been deviated and their water is used for agricultural and industrial purposes, causing the deterioration of many spawning grounds and decreasing natural reproduction in fish (including commercially important sturgeon species). The worldwide drastic decline in sturgeon stocks, particularly in the Caspian Sea necessitates the development of aquaculture of these species (Jenkins, 2001).

Development of artificial breeding operations and rearing of native species to produce fingerlings tolerant to culture conditions and the supply of suitable diets during larval and fingerling stages and ultimately releasing fingerlings into the sea at higher weight are among the objectives of conservation programs. Nowadays experts are of the opinion that the current stocks are dependant on artificial breeding and rearing programs of these species under suitable and controlled conditions. Hence a suitable culture environment is the main factor to enhance resistance and survival of larvae and fingerlings in artificial breeding programs.

The Shahid Beheshti Sturgeon Hatchery water is obtained from the Sepidrud River, which is exposed to heavy metal pollution from agricultural wastes and drainage. The aim of this study was to determine the concentrations of heavy metals such as Zn, Cu, Cd and Pb in water used in different stages of sturgeon breeding and rearing.

MATERIALS AND MATHODS

Water samples were collected from different sections of the Shahid Beheshti Hatchery including the master pond (2 samples collected one at the inlet and the other at the outlet), incubators, vniro tanks, earthen rearing ponds and the point of release of sturgeon fingerlings into the Sepidrud River.

Water parameters such as pH and water and air temperature were measured and recorded at the time of sampling. About 50 liters of water was collected in plastic containers from each sampling point and transferred to the laboratory.

To prepare water samples for the determination of heavy metals, about 3 liters of each sample was taken and heated gently for about 15 h until about 10 ml of sample remained. Heavy metal concentrations in the water samples were determined following standard analytical procedures using a FAAS-Varian Atomic Absorption Spectrophotometer (detection limit of Pb was 0.3-25 ppm, 0.1-15 ppm for Cu, and 0.3-3 ppm for Cd). Standards for each element were used to establish the stability and for calibration of the apparatus. Using the calibration curve the concentration of heavy metal in each sample was calculated (ASTM, 1996).

Data obtained were analyzed using computer based statistical software SPSS and Excel**.**

RESULTS

Mean concentrations of heavy metals in water in different stages of breeding and rearing in the Shahid Beheshti Hatchery are presented in Table 1.

Table 1. Mean concentrations (n=3) of heavy metals in water used at the Shahid Beheshti sturgeon hatchery

Sample	Pb (ppb)	Cd (ppb)	Zn (ppb)	Cu (ppb)
Master pond (inlet)	9.33±0.019	0.116±0.019	36.0±3.6	10.5±1.18
Master pond (outlet)	4.66±0.098	0.083±0.098	18.83±2.84	6.5±0.95
Kurenski Pond	0.016±0.003	0.016±0.003	11.16±2.48	8.33±1.46
	0.11±0.035	0.11±0.035	0.98±0.26	1.01±0.17
Incubator	0.15±0.014	0.15±0.014	10.66±1.31	3.66±0.4
	0.16±0.007	0.16±0.007	22.33±5.64	2.9±0.36
Vniro	0.007±0.1	0.007±0.1	2.0±0.32	1±0.45
	0.15±0.021	0.15±0.021	11.66±2.89	2.83±0.17
Earthen Ponds	0.15±0.028	0.15±0.028	19.15±3.92	0.65±0.26
Point of Release	0.20±0.035	0.2±0.035	5.15±1.8	1.66±0.036

Highest concentrations recorded belonged to Zn while the lowest concentrations were recorded for Cd. Highest concentrations for Zn (36 ppb) belonged to water sample collected from the inlet of the master pond, while Zn concentrations (18.83 ppb) at the outlet of the master pond were much lower. Lowest Zn concentrations (0.98 ppb) were recorded water samples collected from the Kurenski ponds.

Highest concentrations for Cu (10.5 ppb) belonged to the water sample collected from the inlet of the master pond, and the lowest Cu concentrations (0.65 ppb) belonged to the water sample from the earthen rearing pond. Negative correlation (-87) was detected among the samples studied. No significant differences were observed for Cu concentrations among the various samples (t=0.061, sig. lev=0.95) and their replicates (t=2.2, sig. lev=0.040).

Highest Pb concentrations (9.33 ppb) belonged to the water sample collected from the inlet of the master pond, while water samples from the vniro tanks showed the lowest Pb levels (0.15 ppb). Negative correlation (-24) was detected among the different samples studied. No significant differences were observed among the samples (t=5.14, sig. lev=20.101) and replicates (t=1.9, sig. lev=0.08).

Similar trends were observed for Cd concentrations in the different samples studied. Highest Cd concentrations (0.116 ppb) were found at the inlet of the master pond, and lowest concentrations of Cd (0.016 ppb) were recorded in the Kurenski pond. Positive correlation (0.63) was found for Cd concentrations among the samples. Significant differences were detected for Cd among the various samples (t=683, sig. lev=0.0001) and among the replicates studied (t=7.66, sig lev=0.001).

DISCUSSION

Considering that the Shahid Beheshti Sturgeon Hatchery water is obtained from the Sepidrud River, the contamination of this water with various chemicals including pesticides and heavy metals is inevitable.

In the present study the distribution pattern for heavy metal concentrations in different stages of breeding and rearing was as follows: Zn>Cu>Pb>Cd. Zn showed the highest concentration (36 ppb), and Cd showed the lowest concentration (0.016 ppb) determined. Pb, Ni, Cr and Cd concentrations in water monitored in seven sampling points located between the Sepidrud estuary and behind the Manjil Dam during the spring, summer and autumn of 1993 demonstrate significant variations in the concentrations of these elements in the Sepidrud River in the different seasons studied. These variations are mainly due to the volume of wastes entering this river and the sources which produce these wastes. Nickel showed the highest concentrations (51-200 ppm) in all the stations studied. Highest concentrations were recorded in autumn which is associated with the activity of factories in that region. The lowest and highest concentrations for Cd and Pb in this study were 0.9±0.05 ppb, 7.4±0.557 ppb, 12.7±0.35 ppb and 260±94 ppb, respectively (Mortazavi, 1994).

In the present study, the lowest and highest concentrations for Cd were 0.016±0.003 ppb and 0.116±0.019 ppb, respectively, while those for Pb were 0.15±0.01 and10.33±0.13 ppb, respectively which were lower than the estimated concentrations in previous studies (Mortazavi, 1994). This could be due to the lower concentrations of these heavy metals from the source. The estimated highest maximum allowable concentration (MAC) for Pb, Zn, Cu and Cd for *A. persicus* fingerlings are 12.84, 0.97, 0.0025 and 0.41 mg/l, respectively and those for *A. stellatus* are 12.061, 0.865, 0.0018 and 0.51 mg/l, respectively. The LC50 96h for Pb, Zn, Cu and Cd for *A. persicus* fingerlings were 128.4, 9.7, 0.025 and 4.1 mg/l, respectively while that for *A. stellatus* fingerlings were 120.61, 8.65, 0.018 and 5.1 mg/l, respectively (Mirzaie, 2004). Except for copper, the estimated concentrations for heavy metals in the present study were lower than the MAC for aquatic organisms (Table 2) and water quality (WHO, 1993). The effects of heavy metals on aquatic ecosystems is a matter of serious concern and Nriagu (1979) conducted a comprehensive study on the sediments and organisms of ponds and lakes. Similar studies have been carried out on heavy metals in rivers. The main factors influencing the concentrations of heavy metals in living organisms in freshwater systems have been documented by Mason (1981), Hellawell (1986), Connel & Mill (1984) and Kelly (1988).

Table 2. Comparison of maximum levels of heavy metals in the present study with standard MAC values

Reference	Zn	Pb	Fe	Cu	Cr	Cd
Present study	36	10.33	-	10.5	-	0.116
MAC for aquatic life (Gardiner and Mance, 1984)	40	25	-	5	15	5
Water quality standards (WHO, 1993)	3000	10	-	-	50	5
Protection of life in seawater (Anon, 1998)	2.5 (sea) 5 (estuary)	15	5	1000	25	40

The bioaccumulation of heavy metals in organisms maybe the result of direct absorption from the external environment through the cell membrane or food and/or a combination of both. It is not clearly known which of the two routes is more important because the toxicity of heavy metals may spread and affect the internal organs. In terrestrial environments food is the main route of absorption (Ireland, 1983), whereas in aquatic systems heavy metals are mainly absorbed from the body surface (Oze et al. 2006) because heavy metals are always suspended in the water and a large volume of this water enters the body of organisms during respiration and exchange of gases and thus provide the route for the entry of heavy metals. Most invertebrates and vertebrates (fishes and aquatic mammals) are not able to absorb heavy metals directly from water.

In another study, Russian sturgeon fingerlings were exposed to different concentrations of heavy metals, pesticides and petrochemical compounds for two months. Being in their early stages of exogenous feeding, muscle tissue in Russian sturgeon fingerlings showed severe degradation after exposure to these pollutants (Adeli, 1999).

Comparison of heavy metal concentrations determined in this study with those reported by Mirzaie, 2004) revealed that they were below the lethal concentrations for sturgeon fingerlings. However further studies are needed to demonstrate if these levels can cause pathological and histological effects on these fingerling**s.** Therefore careful monitoring of pollutants and their effects in the environment is suggested for successful rearing of sturgeons.

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SHORT-TERM PRESERVATİON OF BROWN TROUT (*SALMO TRUTTA MACROSTİGMA*) SPERM: EFFECT OF EXTENDERS ON MOTİLİTY

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KRATKOTRAJNO OČUVANJE SPERME POTOČNE PASTRMKE (*SALMO TRUTTA MACROSTİGMA*): DELOVANJE EKSTENDERA NA POKRETLJİVOST

Abstrakt
Cilj eksperimenta je bila procena spermatoloških parametara kratkotrajno čuvane sperme potočne pastrmke (*Salmo trutta macrostigma*) korišćenjem različitih ekstendera. Mleč je uzorkovan od odraslih mužjaka istiskanjem rukom, bez anestezije. Po određivanju najvažnijih karakteristika sperme (volumen, pokretljivost, trajanje pokretljivosti, gustina, pH) uzorci sperme koji su pokazali >80 pokretljivosti su prikupljeni i razblaženi u odnosu 1:3 sa tri različita ekstendera.

Razblažena sperma je čuvana 72 sata na 4°C. Tokom čuvanja na svaka 24 h je procenjivan motilitet spermatozoida (%). U zaključku, rezultati studije su pokazali da se ekstender I pokazao boljim od druga 2 za kratkotrajno očuvanje sperme potočne pastrmke.

Ključne reči: *Salmo trutta macrostigma, kratkotrajno očuvanje, pokretljivost, mleč.*

INTRODUCTION

In combination with illegal methods and heavy fishing pressure, spawning success was reduced caused by pollution of streams, degradation of spawning habitats, river damming and interspecific competition with introduced rainbow trout has caused a decline in the stocks or extinction of native trout populations in Turkey. Brown trout (*Salmo trutta macrostigma*) is endangered by illegal catching methods, human pressures and degradation of spawning habitats (Alp et al., 2005). Consequently, there is need for good management practices for the conservation of brown trout stocks in Turkey. To fulfill this task, it is necessary to achieve a good knowledge of the reproduction of this subspecies.

Short-term preservation of fish sperm have many applications. Collection, evaluation, and storage of sperm for several days enable choosing the highest quality semen for desired pair matings. Freshly collected and stored semen can be shipped to other locations for fertilization or cryopreservation (Bozkurt and Seçer, 2005). The basic objective of sperm preservation is to reduce spermatozoa motility during storage. Spermatozoa motility is the most commonly used criterion to evaluate semen quality. However, in numerous fish species with external fertilization, duration of sperm motility is very short. Also, studies on most fish species show that the duration and motility of semen may vary seasonally. Therefore determining semen motility is an important component of a preservation program to prevent choosing best quality semen prior to freezing and to determine fertility of the stored semen following thawing.

The objective of this experiment was to determine the main spermatological parameters of brown trout (*Salmo trutta macrostigma*), short term preservation of semen in different extenders and to use these results in feasible mass production of this subspecies.

MATERIALS AND METHODS

Broodstock management and collection of sperm

This experiment was carried out at the Çamlıyayla Fish Production Station during the spawning season of brown trout (*Salmo trutta macrostigma*). In the spawning period, adult males were kept seperately in small ponds under constant environmental condition. Water temperature varied between 7 and 11°C during the spawning period (December-January) and adult males were fasted 48 hour prior to semen collection.

Semen was collected into the 20 ml calibrated glass beakers by abdominal massage from 10 adult males (aging between 2 and 5 years old) by the hand stripping method without anesthesia and samples contaminated with faecal material or urine were discarded.

Evaluation of sperm

Semen volume was measured with ml in calibrated glass beakers. Motility was evaluated using a light microscope at x40 magnification and was expressed as percentage of motile spermatozoa before cooling. 0.3 % NaCl was used to estimate motility as activating solution. For the evaluation of motility, about 5 μl semen was placed on a cold glass microscope slide and 100 μl activation solution was added, mixed and covered with a coverslip. For each sample, at least five microscopic fields were observed. The sperm motility was observed by two observers. Only samples showing high motility (>70 %) were pooled and used for storage at 4ºC.

Motility and movement duration were evaluated based on following criterias: 1) Mass progressive motility when most of the spermatozoa were still actively swimming with progressive movement 2) Total duration of movement until most spermatozoa stopped swimming. Movement duration of spermatozoa was estimated using a sensitive chronometer. Semen density was estimated by using the hemocytometric method and expressed as spermatozoa x10^9/ml. pH was measured by using indicator papers.

Dilution of sperm and short-term preservation

The pooled samples were diluted at a 1:3 ratio with three different extenders. Extender I contained 600 mg NaCl, 315 mg KCl, 15 mg $CaCl_2.2H_2O$, 20 mg $MgSO_4.7H_2O$, 100 ml distilled water, 1.5 g BSA, 0.5 g sucrose, 7% egg yolk, 1% methanole (modified Lahnsteiner et al., 2000). Extender II contained 4.68 g/lt NaCl, 2.98 g/lt KCl, 0.11 g/lt $CaCl_2$, 3.15 g/lt Tris, 1% DMSO (modified Billard and Cosson, 1992) and extender III contained 600 mg NaCl, 315 mg KCl, 15 mg $CaCl_2.2H_2O$, 20 mg $MgSO_4.7H_2O$, 100 ml distilled water, 1.5 g BSA, 0.5 g sucrose, 7% egg yolk, 10% methanole (Lahnsteiner et al., 2000). Only samples with good initial motility (total motility >70 %) were chosen for experiment. The diluted semen was stored for 72 hours at 4°C. During the cool storage, motility of the spermatozoa was evaluated every 24 hours.

Statistictical analysis

All data were expressed as mean±standard deviation. Relative quantities were transformed by angular transformation and metric data were tested for normality. Data were analyzed one-way multifactorial variance (ANOVA) with subsequent Tukey's-b test.

RESULTS

Average fresh semen motility was 80.4%. According to the results of the experiment, the highest motility (58% after 24 h, 46% after 48 h, 24% after 72 h) and movement duration (60 s after 24 h, 42 s after 48 h, 21 s after 72 h) were determined by using first extender after 24, 48 and 72 hours storage. Spermatological parameters of fresh semen from brown trout are shown in Table 1. Motility values of fresh and short-term stored semen for 72 h are presented in Table 2.

Table 1. Spermatological parameters of brown trout (*Salmo trutta macrostigma*) semen.

	Volume (ml)	Motility (%)	Movement Duration (s)	Density ($x10^9$/ml)	Total Density	pH
X± Sx (n=30)	8.4±0.4	84.7±1.4	68.1±1.3	21.9±0.3	657±..5	7.0±0.5

Table 2. Effects of short-term preservation on sperm motility of brown trout (*Salmo trutta macrostigma*).

Extender Type	Motility (0 h) X ± Sx	Motility (24 h) X ± Sx	Motility (48 h) X ± Sx	Motility (72 h) X ± Sx
I	79.5±5.4 ^{a}	61,4±5.7 ^{a}	49,4±3.1 ^{a}	21.5±3.1 ^{a}
II	20.3±6.3 ^{b}	5.1±3.1 ^{b}	0 ^{b}	0 ^{b}
III	61.4±3.1 ^{c}	21.4±2.4 ^{c}	0 ^{b}	0 ^{b}
Control	81.5±2.4 ^{a}	0 ^{d}	0 ^{b}	0 ^{b}
p	**	**	**	**

Data expressed as the mean ± SD (n=30)
a, b, c, d : Different superscripts within lines denote significant differences.
** : p< 0,01 : Difference between among groups were significant.

DISCUSSION

Limited amounts of data are available on short-term preservation of brown trout sperm. Most of experiments in this field focused on rainbow trout. The present paper describes the first attempt in Turkey to preserve the brown trout sperm for short periods.

Mean semen volume was similar to the results reported by Gjerde (1984), Munkittrick and Moccia (1987) but different from those reported by Erdahl et al. (1984) and Tekin et al. (2003b). The difference may be due to differences in breeding, feeding conditions and regime, environmental factors, or spawning time. The mean spermatozoa motility observed in this study was similar to the findings of Bozkurt and Secer (2006) but different from those of Schmidt-Baulain and Holtz, (1989). Spermatozoa motility varies in vigor and duration not only among males but also within an individual male depending on ripeness (Akçay et al., 2002). Most studies on fish species have shown that the duration and motility of semen can vary seasonally (Akçay et al., 2004).

Results regarding post-activation motility and duration of movement are supported by similar studies (Kumar, 1988; Jayaprakas and Lal, 1996; Mansour et al., 2004). Both properties decreased with time but the proportion of motile cells decreased faster in fresh semen samples than in activated ones. Movement duration was similarly affected. Similar results were reported by Lubzens et al. (1997), and Tekin et al. (2003a). Post-activation motility is one of the most important indicators of the success of a preservation protocol. Spermatozoa motility was affected during preservation in this research. The proportion of motile cells decreased faster with time in undiluted sperm samples than diluted ones. Similar results for the motility parameters of chilled stored spermatozoa were reported in fish in some experiments (Stoss and Holtz 1983, Bozkurt and Secer 2005). It is possible to enhance the fertilizing capacity of fish by using suitable activating mediums that increase motility duration.

Knowledge of the factors influencing spermatozoa motility has tremendous importance in fish breeding and aquaculture. In this research spermatozoa stored without oxygen supply at 4°C showed a rapid decrease in motility (Table 2). It can be concluded from the results of this research that, aerobic conditions are necessary for maintaining the viability of spermatozoa during in vitro storage. Similarly, previous studies have shown that short-term fish sperm preservation can be improved with the addition of oxygen. According to Billard (1981), rainbow trout sperm survival was improved after storage in an oxygen atmosphere in comparison with storage in air. Under suitable conditions, salmonid sperm can be stored for a few weeks in unfrozen form. This methodology is important because it is not always practical or even possible to harvest sperm and use it to fertilize eggs immediately (Bencic et al. 2000). Previous investigations showed that successful short-term storage of salmonid sperm depends on numerous factors such as temperature, fluid volume, and gaseous environment (Rana, 1995). The most commonly used method of short-term storage has been under an atmosphere of 100 % O_2 at low temperatures (Billard, 1981, Rana, 1995).

Storage temperature is also important factor affecting viability of fish gametes in vitro experiments. Viability can be prolonged by maintaining gametes and embryos close to 0°C to reduce metabolic rate. However, the ability to tolerate low temperature may vary between temperate and tropical species (Leung and Jamieson 1991). According to Carpentier and Billard (1978) sperm stored in vitro conditions could be survived for one to several days at 1–4°C. (Buyukhatipoglu and Holtz (1978) also reported that the spermatozoa of rainbow trout were fertile for 21 days at 4°C under oxygen or air. However, the motility percentage and beat frequency of the flagellum continued to decline with an increase in storage period and the fertilizing capacity of sperm was completely lost after 6 days of storage. Harvey and Kelley (1984) found that post-activation motility of undiluted Sarotherodon mossambicus sperm stored at 5°C declined to zero in 60–120 h.

The efficiency of spermatozoa storage was affected by individual sample variability, but not by the genetic source of donors. This individual variability of samples, usually named ''individual male variability'' results from biological variation among individuals, as well as from collection techniques (Piironen, 1985). In the present study, collected semen from 10 males were pooled in equal amounts to eliminate the effect of individual variability of gamete donors. Contamination with urine is considered as an important interference factor, randomly affecting quantitative characteristics of spermatozoa (Glogowski et al, 2000). This may play an important role in short-term storage of spermatozoa. The method of storage showed a highly significant effect on fertilization ability, as well as on motility and concentration of refrigerated spermatozoa. In addition different storage methods (bags, foils, glass and plastic tubes) may affect motility and fertility in cooled semen samples. Also the changes in spermatozoa concentrations during storage indicate that a desiccation process has likely been the reason for differences among different storage methods (Bozkurt et al. 2005).

CONCLUSIONS

In this experiment, significant differences in motility of sperm stored were observed in different extenders. The best motility rates were obtained by modified Lahnsteiner solution. In conclusion, the present study indicates that brown trout (*Salmo trutta macrostigma*) semen can be successfully preserved for 48 h at 4ºC prior to fertilization. However, further investigations are needed to determine the optimal semen/egg ratio and to evaluate the viability, survival, and development of larvae produced from short-term stored semen.

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NUTRIENT ASSESSMENT OF SEDIMENTS OF CARP FISH PONDS

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PROCENA NUTRIJENATA U SEDIMENTU ŠARANSKOG RIBNJAKA

Abstract

The aim of this study is related with the characterization of chemical parameters of organic nitrogen and biogenic elements in sediments of carp ponds, permitting an assessment of their nutritional value.

A vertical differentiation was established in the levels of the surveyed chemical indicators of the sediments. In the 0 cm - 15 cm horizon the content of organic nitrogen is on average 47.95% higher, and that of ammonium and nitrate ions is on average of 34% - 37.85% higher compared to that registered in the 15 cm - 30 cm horizon. The content of phosphate ions is 13.1% higher in 15 cm - 30 cm horizon in comparison with the 0 cm - 15 cm horizon.

Based on the reported levels of organic nitrogen, soluble inorganic forms of nitrogen and phosphorus, the reserve of bottom sediments is determined as average to good. This characterizes them as a natural source of nutrients with the possibility of their integration in agriculture.

Key words: *fish ponds, sediments, organic nitrogen, soluble inorganic forms of nitrogen, soluble inorganic forms of phosphorus*

INTRODUCTION

In fish ponds, the bottom sediments are a natural biological product, formed during their long term exploitation. As a result of allochthonous and autochthonous organic matter mineralization and technology used in fish farming, they form different in chemical composition and quantity slime layer.

Bottom sediments are rich in organic matter, nitrogen, phosphorus, potassium and other macro and micro biogenic elements accumulated in fish ponds during the vegetation period (Briggs and Funge-Smith, 1994; Hopkins et al., 1994; Krom et al., 1985; Smith, 1996; Jamu and Piedrahita, 2001; Boyd et al., 2002). In China, Thailand and Vietnam, the pond sediments are used in agriculture as fertilizer for the field crops, whereas higher yields have been recorded (Little and Muir, 1987; Prein, 2002).

The integration of aquaculture with agriculture is not widespread yet, due to the lack of scientific researches in this field (Hopkins and Bowman, 1993; Lin and Yi, 2003). In most cases the fish is taken as the only product of fish ponds and the role of fish pond water and bottom sediments is neglected (Brankeret, 1979; Little and Muir, 1987; Pillay, 1994).

In this context, the aim of this study is related with the characterization of chemical parameters of organic nitrogen and biogenic elements in sediments of carp ponds, permitting an assessment of their nutritional value.

MATERIAL AND METHODS

The study was conducted in carp ponds, situated in the Experimental Base of the Institute for Fisheries and Aquaculture, Plovdiv, which have different characteristics in terms of the area size. A traditional technology for growing warm-water fish species in polyculture is applied. The scheme is shown in Table 1.

Taking of samples was carried out during 2009 vegetation season, whereas the sediment samples are taken once per month by applying the modified version of the Kachinski probe from 2 horizons at a depth of 0-15 cm and 15-30 cm. The analysis of the sediments include the determination of organic nitrogen (%) by the Kijeldahl method after selenium mineralization by using VELP - Scientifica semiautomatic analysis system type DK-6 for decomposition and UDK - 132 for distillation; of nitrogen's mobile forms, determined spectrophotometrically during their preliminary extraction from the soil solution of KCl; of phosphorus' inorganic forms, determined according to double-lactate method of Egner-Reem (Tomov et al, 1999). The data were statistically analyzed using the Microsoft Office 2007.

Table 1. Scheme of study

Pond No	Area, ha	Type of farmed fish
8	0.38	Common Carp, Silver Carp
28	2.4	Common Carp, Silver Carp, Grass Carp
29	7.0	Common Carp, Grass Carp, Silver Carp, Northern Pike, Wels

RESULTS AND DISCUSSION

Data obtained by surveys of the organic nitrogen (**ON**) content in bottom sediments of the examined fish ponds in 0 cm - 15 cm and 15 cm - 30 cm horizons are shown in **Table 2**. In the period 31.03.- 06.10.2009, the variation in the values of the ON in 0 cm - 15 cm horizon is 0.2198 % to 0.4252 % with average seasonal value of 0.3264 %, while in 15 cm - 30 cm horizon - respectively 0.0979 % to 0.2759 % with average seasonal value of 0.1699 %. In the surveyed ponds is reported trend of higher values of the organic nitrogen content in the bottom sediments in 0 cm -15 cm horizon - average 47.95 percent, compared with those reported for 15 cm - 30 cm horizon. In seasonal aspect is determined gradual increase in the organic nitrogen level up to August, with pronounced peaks in pond 8 and pond 29.

The research results on nitrogen soluble inorganic forms in the bottom sediments of the surveyed fish ponds are shown in **Table 3**.

N-NH$_4$. The variation in the values of N-NH$_4$ in pond 8 at 0 cm - 15 cm horizon is 42.5 mg.kg^{-1} to 76.25 mg.kg^{-1}, with average seasonal value of 61.67 mg.kg^{-1}, and at 15 cm - 30 cm horizon - respectively 11.25 mg.kg^{-1} to 68.75 mg.kg^{-1}, with average seasonal value of 38.33 mg.kg^{-1} In the surveyed ponds is recorded a trend of higher values of the content of ammonium ions in the bottom sediments of 0 cm - 15 cm horizon on average 37.85 %, compared with those reported for 15 cm - 30 cm horizon. In pond 28 and pond 29 the average seasonal levels of ammonium ions are lower than those recorded in pond 8 with 43.4 % - 66.73 % for 0 cm - 15 cm horizon and by 64.57% - 72.65% for the 15 cm - 30 cm horizon. Their absolute values vary from 6.25 mg.kg^{-1} to 58.75 mg.kg^{-1} for 0 cm -15 cm horizon and 3.75 mg.kg^{-1} to 33.75 mg.kg^{-1} for the 15 cm - 30 cm horizon. In the seasonal aspect is established a gradual increase in the level of ammonium nitrogen from June to August, whereas they are very specific for the different ponds.

Table 2. Average values of organic nitrogen (%) in sediments of the fish ponds in 0 cm - 15 cm and 15 cm - 30 cm horizons

2009	n=3	Pond 8		Pond 28		Pond 29	
months	horizons	0-15 cm	15-30 cm	0-15 cm	15-30 cm	0-15 cm	15-30 cm
April	**X**	**0,304**	**0,1399**	**0,4019**	**0,1775**	**0,3926**	**0,21**
	Sx	0,0031	0,0001	0	0	0,0001	0,0034
	Cv	1,46	0,11	0	0,03	0,04	2,29
June	**X**	**0,2663**	**0,1493**	**0,317**	**0,1354**	**0,3145**	**0,1743**
	Sx	0,0035	0	0,0004	0,0029	0,0036	0,0042
	Cv	1,88	0	0,19	2,99	1,63	3,38
July	**X**	**0,2991**	**0,1801**	**0,3223**	**0,0981**	**0,3691**	**0,2148**
	Sx	0	0,0004	0,0032	0,0033	0,0033	0
	Cv	0,02	0,33	1,4	4,79	1,26	0,03
August	**X**	**0,3691**	**0,2148**	**0,2997**	**0,1307**	**0,4252**	**0,2293**
	Sx	0,0033	0	0,0252	0,0001	0,0034	0,0034
	Cv	1,26	0,03	11,89	0,13	1,14	2,07
September	**X**	**0,285**	**0,182**	**0,2198**	**0,1166**	**0,383**	**0,2759**
	Sx	0,0034	0,0035	0,0033	0,0034	0,0001	0,0033
	Cv	1,68	2,72	2,12	4,12	0,05	1,7
October	**X**	**0,3269**	**0,1635**	**0,229**	**0,0979**	**0,3503**	**0,1678**
	Sx	0,0068	0,0034	0,0033	0,0032	0,0033	0,0001
	Cv	2,92	2,91	2,03	4,65	1,34	0,09

N-NO$_3$. The variation in the values of N-NO$_3$ in pond 8 at 0 cm - 15 cm horizon is 5.14 mg.kg^{-1} to 17.56 mg.kg^{-1}, with average seasonal value of 10.35 mg.kg^{-1}, and at 15 cm - 30 cm horizon - respectively 1.22 mg.kg^{-1} to 16.37 mg.kg^{-1}, with average seasonal value of 6.83 mg.kg^{-1}. In the surveyed ponds is recorded a trend of higher values of the content of nitrate ions in the bottom sediments of 0 cm - 15 cm horizon on average by 34% compared with those reported for 15 cm - 30 cm horizon. Similar trend of nitrates reduction in depth is reported in Bharmal and Laurent (2004) research. For pond 28 and pond 29 the absolute values vary from 1.49 mg.kg^{-1} to 8.19 mg.kg^{-1} for 0 cm - 15 cm horizon and by 1.08 mg.kg^{-1} to 5.06 mg.kg^{-1} for the horizon 15 cm - 30 cm. In the seasonal aspect is established a gradual increase in the level of nitrogen nitrate in June (pond 8) and July-August (ponds 28 and 29), whereas they are very specific for the different ponds.

The research results on soluble inorganic forms of phosphorus (**P-PO$_4$**) in bottom sediments of the surveyed fish ponds are shown in **Table 3**. The variation in the values of phosphate phosphorus in the 0 cm - 15 cm horizon is from 15.0 mg.100g^{-1} to 105.5 mg.100g^{-1} sediment with average seasonal value of 50.31 mg.100g^{-1} and at 15 cm - 30 cm horizon - respectively 20.13 mg.100g^{-1} to 115.0 mg.100g^{-1}, with average seasonal value of 57.91 mg.100g^{-1}. A trend of higher values of the content of phosphate ions in bottom sediments is recorded for 15 cm - 30 cm horizon on average by 13.1% compared to the 0 cm - 15 cm horizon. This pattern is probably a consequence of the higher level of organic matter in this layer, the mineralization of which releases phosphates.

In a seasonal aspect is monitored natural increase of phosphate phosphorus with registered characteristic peaks in August at 15 cm - 30 cm horizon for pond 8 and in the entire analyzed horizon for the other two ponds. There are specific relations between the horizons in the sediments of the surveyed ponds in the period from July to August for pond 8, August to September for pond 28 and pond 29.

Given that the bulk of nitrogen in the soil (98% - 99% of its total volume) is represented by organic compounds, it is essential to help their mineralization for the purpose of release the accessible forms of the nitrogen. Thus they are included in the natural food chain in the ponds (Tomov et al, 1999). In fresh deposits, for as such could be considered bottom sediments of fish ponds, a large part of organic nitrogen gets mineralized in depositions through nitrification and denitrification (Bharmal and Laurent, 2004).

Summarizing the results of the concentration of soluble inorganic forms of nitrogen and phosphorus indicated that the content of ammonium and nitrate ions in bottom sediments of operational fish farming ponds at the 0 cm - 15 cm horizon is on average 34% - 37.85% higher compared with that recorded in 15 cm - 30 cm horizon. The dynamics of the nitrate nitrogen content follows that of the ammonium nitrogen, especially regarding the 0 cm - 15 cm horizon, whereas the concentration of the ammonium forms is higher than nitrate forms.

The content of phosphate ions in the 15 cm - 30 cm horizon is on average 13.1% higher - than that recorded in 0 cm - 15 cm horizon. Overall the reserve of the ponds in the analyzed 0 cm - 30 cm horizon is determined as good (Tomov et al, 1999).

Table 3. Average values of soluble inorganic forms of nitrogen ($mg.kg^{-1}$) and phosphorus ($mg.100\ g^{-1}$) in sediments of the fish ponds in 0 cm - 15 cm and 15 cm - 30 cm horizons

2009	Pond 8	$N\text{-}NH_4$, $mg.kg^{-1}$		$N\text{-}NO_3$, $mg.kg^{-1}$		$P\text{-}PO_4$, mg/100 g	
months	horizons	0-15 cm	15-30 cm	0-15 cm	15-30 cm	0-15 cm	15-30 cm
April		53,75	36,25	12,8	8,63	22,50	32,50
June		42,5	18,75	10,12	4,47	21,25	37,63
July		73,75	68,75	17,56	16,37	105,50	52,00
August		76,25	51,25	8,25	5,55	15,00	93,75
September		76,25	11,25	8,25	1,22	26,25	32,63
October		47,5	43,75	5,14	4,74	42,63	52,63
Average of season	**X**	**61,67**	**38,33**	**10,35**	**6,83**	**38,85**	**51,19**
	Sx	6,93	9,47	1,9391	2,3435	15,18	10,37
	Cv	25,14	55,25	41,88	76,72	33,94	23,18
	Pond 28	**$N\text{-}NH_4$, $mg.kg^{-1}$**		**$N\text{-}NO_3$, $mg.kg^{-1}$**		**$P\text{-}PO_4$, mg/100 g**	
months	horizons	0-15 cm	15-30 cm	0-15 cm	15-30 cm	0-15 cm	15-30 cm
April		11,25	8,75	2,68	2,08	33,75	39,50
June		23,75	21,25	5,66	5,06	45,00	77,50
July		32,5	10,63	7,74	2,53	37,00	58,75
August		33,12	6,25	7,88	1,49	87,00	115,00
September		6,25	5,63	1,49	1,34	62,50	41,25
October		16,25	11,25	1,67	1,22	65,00	40,20
Average of season	**X**	**20,52**	**10,63**	**4,52**	**2,29**	**55,04**	**62,03**
	Sx	4,98	2,5368	1,3213	0,6469	9,07	13,37
	Cv	54,29	53,38	63,36	63,26	20,28	29,91
	Pond 29	**$N\text{-}NH_4$, $mg.kg^{-1}$**		**$N\text{-}NO_3$,* $mg.kg^{-1}$**		**$P\text{-}PO_4$, mg/100 g**	
months	horizons	0-15 cm	15-30 cm	0-15 cm	15-30 cm	0-15 cm	15-30 cm
April		34,38	14	8,19	3,33	48,00	70,00
June		20	8,75	4,76	2,08	56,25	38,75
July		52,5	33,75	5,68	3,66	40,25	59,00
August		58,75	3,75	6,36	1,41	70,00	96,25
September		21,88	11,25	2,36	1,22	87,68	20,13
October		21,88	10	2,36	1,08	40,20	85,00
Average of season	**X**	**34,9**	**13,58**	**4,95**	**2,13**	**57,06**	**61,52**
	Sx	7,5892	4,6699	1,029	0,4993	8,38	12,76
	Cv	48,63	76,86	46,47	52,16	18,73	28,52

CONCLUSION

A vertical differentiation was established in the levels of the surveyed chemical indicators of the sediments. In the 0 cm - 15 cm horizon the content of organic nitrogen is on average 47.95% higher, and that of ammonium and nitrate ions is on average of 34% - 37.85% higher compared to that registered in the 15 cm - 30 cm horizon. The content of phosphate ions is 13.1% higher in 15 cm - 30 cm horizon in comparison with the 0 cm - 15 cm horizon.

A trend of higher levels of organic nitrogen in ponds with a larger area (2.4-7.0 ha) is recorded during the period from July to September in the surveyed horizons up to 30 cm depth of the bottom layer, compared to ones with area of max 0.5 ha. More pronounced dynamics in the quantities of mineral forms of nitrogen are recorded for ammonium compared to nitrate ions.

Based on the reported levels of organic nitrogen, soluble inorganic forms of nitrogen and phosphorus, the reserve of bottom sediments is determined as average to good. This characterizes them as a natural source of nutrients with the possibility of their integration in agriculture.

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ARTIFICIAL SPAWNING OF CARP IN A SUBTROPICAL CLIMATE CONDITIONS

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VEŠTAČKI MREST ŠARANA U USLOVIMA SUBTROPSKE KLIME

Abstrakt

Najefikasniji i najpouzdaniji način proizvodnje ikre i ribljeg podmlatka je veštački mrest. Matične jedinke se drže u vodi koja je zasićena sa kiseonikom i čija je temperaura u rasponu 20-24 ºC. Daju im se dve doze injekcije hormona hipofize ili mešavina GnRH/dopamin antagonista da bi se indukovala ovulacija i spermatogeneza. Otkriće da dopamin deluje kao inhibitorni faktor za sintezu gonadotropina dovelo je do revolucije u razvoju veštačkog mresta. Od sredine osamdesetih, hipofizacija je unapređena uvođenjem standardizovanog suvog ekstrakta hipofize šarana u kojem je sadržaj i aktivnost luteinizirajućeg hormona (LH) kalibrisana (cCPE). U suptropskim klimatskim uslovima, šaran sazreva u starosti od šest meseic, a sezona mresta šarana počinje krajem februara i početkom marta, kada temperatura vode dostigne 19-21°C.

Ogled je izveden u Gan Shmuel mrestilištu i centru za gajenje riba, u Izraelu, u decembru. Trideset ženki KOI šarana (Cyprinus carpio L.) bilo je smešteno u deset plastičnih bazena, zapremine oko 450 litara. U svakom tanku je bilo po tri matice. Mužjaci su držani odvojeno od matica da bi se sprečila nekontrolisana reprodukcija. U svakom bazenu aeracija i stalno snabdevanje svežom vodom su bili kontrolisani. Pošto je veštački mrest sproveden van sezone, 21 dan pre izvođenja mresta ribe su držane u vodi na temperaturi od 24°C. Urađena je biopsija po jedne ženke iz svake grupe. Anestezija ženki je izvršena sa 99% 2 phenoxyetanolom i biopsija jajnika je urađena preko genitalnog otvora, uvođenjem plastičnog katetera do gonada. Procenat oocista koje su bile u stadijumu I (centralno postavljen GV) i procenat oocista u stadijumu II (ekscentričan GV) je određen posmatranjem pod binokularnim mikroskopom. Pozicija germinalnog vezikula i zrelost matica je određena i broj oocisti u svakon stadijumu je izračunat. Ukupna telesna masa svih matica je izračunata i iznosila je oko 40 kg. cCPE koncentarcije 1 ml/kg je pripremljen za sve jedinke zajedno. Hipofizacija riba je izvršena u dva koraka. Primarna doza je iznosila 20% od ukupne doze, dok je druga doza iznosila 80% od ukupne doze. Deset mužjaka je dobilo po jednu dozu cCPE, 70% doze od one koja je data maticama. Sledećeg dana, došlo je do mrešćenja nešto kasnije, nego što je očekivano. Ovo se može objasniti time što je ogled sproveden izvan sezone mresta. Period latence je takođe visoko zavisan od temparature vode. Tokom ogleda došlo je do malog problema sa sistemom za grejanje, pa je to još jedan od razloga za nešto produžen period latence. Jaja su nežno istisnuta u suvu posudu i oplođena primenom "suve metode", a adhezivnost jaja je eliminisana korišćenjem mleka ili tretiranjem sa solima uree, a zatim sa kupkom u taninskoj kiselini ("Woynarovich method"). Inkubacija je sprovedena u Cuger bocama.

Za statističku obradu podataka korišćen je SPSS za Windows i Excel (MS Office). Uspešnost mresta je iznosila 43 %. Izračunat je Spearmanov koeficijent korelacije između mase ženki i mase dobijene ikre, korelacija između mase riba i broja jaja i korelacija između procenta migriranih oocita i uspešnosti mresta. Uočena je značajna korelacija (F=0,709) između telesne mase matica i mase ikre i značajna korelacija (F=0,642) između telesne mase matica i broja jaja. Negativna korelacija (F=-0,530) između procenta migriranih oocista i uspešnosti mresta je u suprotnosti sa mnogim ranijim istraživanjima. Petpostavka je da bi razlog ovome mogao biti u faktorima okoline, kao i u kvalitetu samih matica. Iako je procenat izmrešćenih matica bio niži od normalnog procenta uspešnosti veštačkog mresta šarana, uspešnost je bila vrlo zadovoljavajuća imajući u vidu da je mrest izvršen van sezone mresta.

Ključne reči: *veštački mrest, KOI šaran, cCPE, vansezonski mrest, suptropska klima*

INTRODUCTION

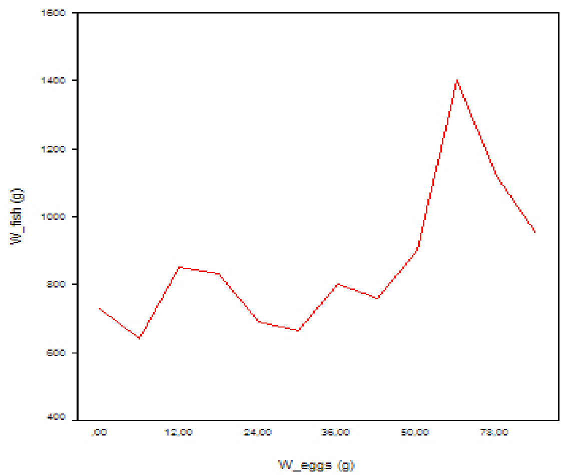
Artificial spawning is the most effective and the most reliable method of eggs and fingerlings production and control of infectious and parasitic diseases. Controlled spawning began around year 1725th when L. Jacobi succeeded to control fertilization of eggs of salmon and trout (Hristić and Bunjevac, 1991; Treer et al, 1995). The major breakthrough in fish breeding came in with the finding that dopamine acts as inhibitory factor for synthesis of gonadotropin (Peter et al, 1986). This breakthrough led to the development of the artificial spawning. Common carp matures in subtropical climate zone at six months (Sarig, 1966). An adult common carp may spawn four or five times per year in subtropical conditions if maintained at 20 to 22°C (Horvath, 1986). Diversity in fish reproductive strategy involves diversity in the timing of fish spawning (Webb and McLay, 1996). Thus, it occurs in late spring to summer in carp in Europe (Billard, 1995). In Israel the spawning seasons starts with common carp at the end of February and beginning of March, when the water temperatures reach 19-21°C. (Jovanović and Ristić, 1960). Since the middle 1980s, hypophysation has improved through the introduction of a standardized dry carp pituitary extract in which the luteinizing hormone (LH) content and activity have been calibrated (calibrated carp pituitary extract = cCPE) (Yaron et al, 2009). Approximately 300 000 to 800 000 newly hatched fry can be expected from a single female (Ćirković et al, 2002; FAO, 2006). The aim of this study was to investigate the possibility and efficiency of out off season artificial spawning of KOI carp, the impact of body mass of females on the number of eggs, as well as the correlation between the percentage of migrated oocysts and spawning success.

MATERIALS AND METHODS

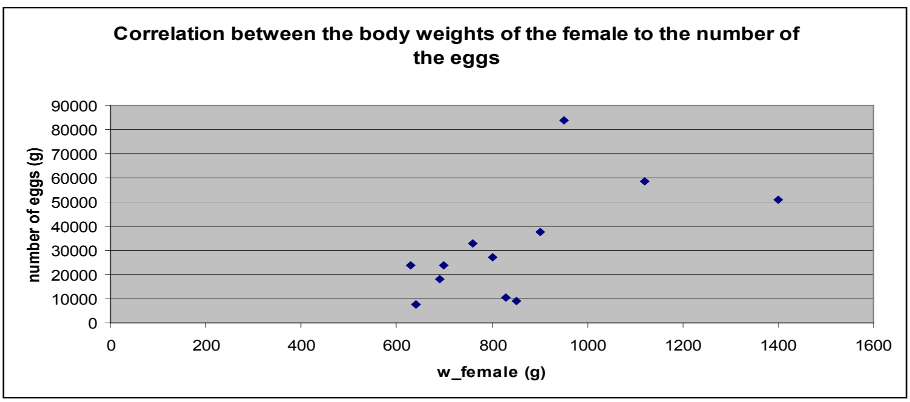
Spawning was done at Gan Shumel Fisher Hatchery and Breeding Centre in Israel in December. Thirty females KOI-carp (*Cyprinus carpio L.*) were placed in 10 plastic tanks, cca 450 liter. Carp males were kept in separated ponds of carp female brood fish, in order to avoid uncontrolled reproduction. Brood fish were kept in water saturated with oxygen, within the temperature range of 20-24 ºC. The tanks were covered with a net, to prevent the fish from jumping out of the tank. From each tank, one female was biopsied. Females were anesthetized by 99% 2 phenoxyethanol and the biopsy of ovary was done via genital opening by inserting a 3 mm plastic catheter into the gonad. Carp eggs are opaque and the only way to examine the position of GV (germinal vesicle) under the binocular microscope is to make them transparent. The ovarian sample (of about 100 oocytes) was cleared in SERA solution (ethanol 60%, formalin 30% and acetic-acid 10%). Within 3 min the oocytes became translucent and remained so for an additional 5 min. The position of the germinal vesicle and ripeness of female was determined and the number of oocytes at each stage was recorded. Total weight of all females was calculated and it was around 40 kg. Hormones for injection were prepared for all fish together. 10 ml of 0,9 %NaCl saline was added to marked vial with "10 kg of CPE" (CPE-carp pituitary extract) and was mixed by hand. Final concentration was 1 kg/ml for each kg of fish that was injected with 1 ml of solution. The dose of hormones was divided in two doses. After the anesthesia of the fish, around 11 00h, intramuscular injection of cCPE hormone was given in the base of the dorsal fin - priming injection. Around 24 00 h fish received a second - inducting injection. The area of injecting was gently massaged followed with the withdrawal of the needle after injection to aid distribution of the extract into musculature and prevention of its backflow. 10 males were injected with 70% of the dose CPE that used for females. The day after, the spawning was occurred little bit later than we expected. Eggs were gently squeezed in the dry bowl and letter a small volume of sperm was added and mixed carefully together „the dry method". Physiological solution-saline was used to prolong the fertilization and rinsing solution (dilution of milk) to remove the stickiness of common carp eggs was added afterwards. Incubation was carried out in Zoug jars. For statistical analyses of correlations were used SPSS for Windows and Excel (MS Office).

RESULTS AND DISCUSION

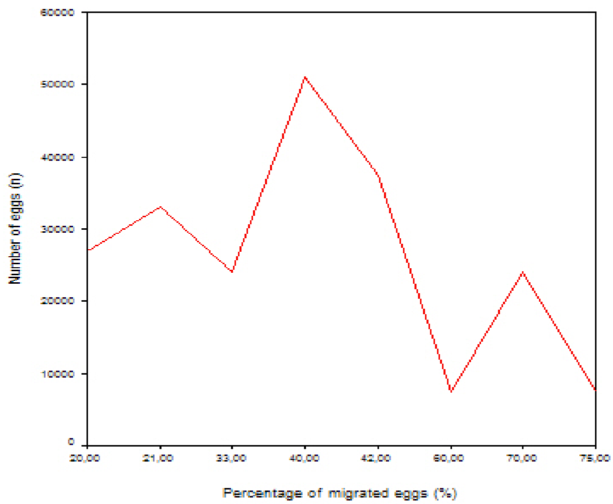
With total number of fishes n = 30 and number of spawned females n = 13 we had 43.33 % of spawning success. We measured the Spearman's correlating coefficient between body weight of fish and weight of eggs (Graph 1), correlation between body weight of fish and number of the eggs (Graph 2) and the correlation between the % of migrating oocytes to spawning success (Graph 3). In correlation between the percent of migrating oocytes to spawning success, only eight of the females with biopsy spawned (Table 1). There is significant correlation (F=0.709) between the body weight of the female to the weight of eggs and a significant correlation (F=0.642) between the body weight of the female to the number of the eggs. We found negative correlation (F = -0.530) between the percentage of migrating oocytes to spawning success which contradict with many spawning researches. We assume that the reason can be related due to environmental circumstances, as well as the quality of spawners.



Graph 1. Correlation between the body weight of the female to the weight of eggs (SPSS for Windows); F = 0,709



Graph 2. Correlation between the body weights of the females to the number of the eggs F = 0,642657



Graph3. Correlation between the % of migrating oocytes to spawning success; F=-0.530

Table 1. Percentage of migrated oocytes and number of eggs

N of females	% of migrated oocytes	N of eggs
1	70	24000
2	60	7500
3	40	51000
4	75	7500
5	20	27000
6	21	33000
7	33	24000
8	42	37500

To induce and synchronize ovulation and spermiation by hormonal stimulants, fish receive injection of pituitary gland, calibrated pituitary extract or a synthetic GnRH (Drori et all., 1994). It is recommended to use cCPE at the beginning and end of the spawning season when the LH content in the pituitary is low, and synthetic GnRH in mid-season and in field spawning. (Yaron et all., 2009). In our trial of induced spawning of carp, we used cCPE because of winter time and out off-season of spawning. Concerning hatching performance, hatching rate of 43.33% was slightly lower compared to 95% reported by Horvath and Lukowicz (1982) and 90-100% reported by Billard et all. (1995). Latency time is also highly dependable on water temperature. Latency time of our treated carps was little postponed due to small system heating error. Even though the percentage of spawning was lower than normal percentage of spawning for carps we had a very big success because it was done out off-season. Carp pituitary extract (CPE) have been used in most hatcheries, however the increased production targets and the cost of this biological material led to consider alternative approaches (Yaron 1995).

CONCLUSIONS

Concerning hatching performance, hatching rate of 43,33 % was slightly lower compared to results reported by others. This could be attributed to immature of some individuals, since the experiment was performed before the reproductive period, in order to secure the spawning response in fully mature fish. The recognition of the best moment for applying hormonal induction in cyprinid artificial spawning is very important. Before any action on fish, it must be anesthetized and handling must be done very gently. Even though the percentage of spawning was lower than normal percentage of spawning for carps we had a very big success because it was done out off-season.

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ESTABLISHING SELECTIVE BREEDING PROGRAM FOR RAINBOW TROUT (*ONCORHYNCHUS MYKISS*, WALBAUM) IN SERBIA

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USPOSTAVLJANJE PROGRAMA SELEKTIVNOG UZGOJA KALIFORNIJSKE PASTRMKE (*ONCORHYNCHUS MYKISS*, WALBAUM) U SRBIJI

Abstrakt

Zahtev za sve većim količinama hrane za ljudsku upotrebu, efikasnost iskorišćavanja hraniva i prostora za gajenje domaćih životinja, kao i pojava mnogih bolesti doveli su do intenzivnog razvijanja selekcionih programa u svim granama poljoprivrede. Za razliku od ostalih gajenih životinja, programi selekcije u akvakulturi su se razvili nešto kasnije usled nedostatka potrebnih procena heritabilnosti, koeficijenata varijacija i korelacija za ekonomski bitne osobine. Trenutno se u akvakulturi samo 1-2% od ukupne proizvodnje zasniva na genetički unapređenim vrstama riba (Gjedrem, 1997). Korišćenjem selektovanih linija riba postiže se više korisnih stvari. Na prvom mestu, programi selekcije riba predstavljaju jedini način stalnog unapređenja ekonomski bitnih osobina kao sto su prirast, konverzija hrane, otpornost na bolesti, smanjenje masnoće u mesu riba. Ribe iz selektovanih linija efikasnije iskorišćavaju hraniva i na taj način smanjuju pritisak na divlje populacije riba koje se koriste za proizvodnju ribljeg brašna, kao glavnog izvora proteina u smešama za ishranu pastrmki. Ribe koje potiču iz divljine ne napreduju zadovoljavajućim brzinom, što znači da su konstantno pod stresom, za razliku od riba poreklom iz selekcionih programa koje pokazuju manji uticaj stresa usled gajenja u zarobljeništvu. Tako se na ovaj način korišćenjem selektovanih linija riba postiže efikasna i održiva proizvodnja uz vođenje računa o dobrobiti životinja. Drugi dobar razlog za uspostavljanje programa selekcije pastrmke u Srbiji je dobro dokumentovano postojanje interakcije genotip x okolina kod kalifornijske pastrmke, kao što su pokazala istraživanja (McKay et al., 1984; Hanke et al., 1989; Sylven et al., 1991; Winkelman and Peterson, 1994; Kettunen et al., 1999; Kause et al., 2003). Na kraju, ali ne i najmanje važna je i kontrola bolesti kod kalifornijske pastrmke i mogućnost stvaranja otpornih linija pastrmki na neke bolesti putem selektivnog uzgoja (Henrion, 2005). Jedno od rešenja za ovaj ozbiljan problem, pored boljeg sistema kontrole pastrmske ikre poreklom iz uvoza, je i stvaranje lokalnih selektovanih matičnih jata sa proverenim poreklom i povećenom otpornošću na bolesti.

Obzirom da za kalifornijsku pastrmku postoje pouzdani podaci o varijabilnosti i heritabilnosti proizvodnih osobina ovo pruža mogućnost efikasne selekcije, a samom tim i značajnog unapređenja proizvodnje uz smanjen ukupni mortalitet, putem odgovarajućeg programa selekcije u Srbiji.

Ključne reči: *kalifornijska pastrmka, program selekcije, genetički parametri.*

INTRODUCTION

The theory of selective breeding of plants and animals used for human consumption first appeared in the early 20th century. Request for larger quantities of food for human consumption, feed utilization efficiency and lack of space for breeding of domestic animals, as well as the occurrence of many diseases has led to intensive development of breeding programs in all branches of agriculture. Unlike at the other cultivated animals, selective breeding programs in aquaculture have been developed later due to lack of heritability estimates, coefficient of variation and correlation of economically significant traits. Nowadays, only 1-2% of total aquaculture production is based on genetically improved fish species (Gjedrem, 1997).

The selective breeding programs are the only way to consistently improve economically important traits such as growth, feed conversion and disease resistance. In some fish species, heterosis effect also can be used in production, but this requires further detailed investigation of interaction among local line fish. Thus, a studies in rainbow trout like Gall (1975), Ayles and Baker (1983) and Gjerde (1988) showed significant effect of heterosis for body weight of fish obtained by the crossing of different lines of cultivated trout. Fish from selected lines more efficiently utilize nutrients and thus reduce pressure on wild fish populations used to produce fishmeal as the main protein source in diets for feeding trout. Fish originating from the wild are not progressing at a satisfactory pace, which means they are constantly under stress, in contrast to the fish originated from breeding programs that show less effect of stress due to breeding in captivity. At this way, by using the selected lines of fish, we achieve efficient and sustainable production with due consideration for animal welfare.

Another good reason to establish a trout-breeding program in Serbia is well-documented existence of genotype by environment interaction in rainbow trout, as evidenced by research (McKay et al., 1984, Hanke et al., 1989; Sylven et al., 1991; Winkelman and Peterson, 1994, Kettunen et al., 1999, Kaus et al., 2003). This phenomenon, together with the possibility of using heterosis effect, shows the need to create local rainbow trout lines and need for detail examination of these important influences concerning each selective breeding program. Finally yet importantly are the control of diseases in rainbow trout production and the potential for creating resistant trout races against some diseases through selective breeding (Henrion, 2005). Occurrence of some diseases outbreaks in trout farms and hatcheries, some of which were recorded for the first time in Serbia, as shown by Jeremic and Radosavljevic (2009), represent a growing problem for trout producers in Serbia. One solution for this serious problem, together with better system of disease control of imported trout eggs, is to create a local trout lines with proven background and increased resistance to specific diseases. As there are reliable data on genetic parameters of rainbow trout, we are expecting a good response to selection, increase of production and reduced impact of some diseases on rainbow trout farms in Serbia.

Phenotypic and genetic parameters in rainbow trout

For some fish species, such as rainbow trout, there are sufficient genetic parameters for traits such as growth rate, feed conversion, percentage of body fat, meat color and resistance to some diseases (Gjedrem, 2000). Well-performed experiments provided us good estimates of these parameters, with a sufficiently large number of specimens examined, so we can use these data as very reliable.

For the specific trait as weight of rainbow trout, heritability estimates are above 0.2 and coefficient of variation is above 20, which means that one can expect a good response to selection ranging from 10-15% per generation. For traits related to the quality of meat, such as the percentage of fat and meat color, heritabilities are 0.47 and 0.27 respectively, also with high average values for the coefficients of variation (Gjerde and Schaeffer, 1989). Trait resistance to diseases such as infectious pancreatic necrosis (IPN), (Wetten et al., 2011), viral hemorrhagic septicemia (VHS), enteric red-mouth disease (ERM) and the rainbow trout fry syndrome (RTFS), (Henryon et al., 2005) show a high heritability in the range 0.42 to 0.57, and suggest the possibility of a successful response to selection.

The correlation coefficient between traits growth rate and feed conversion ratio for rainbow trout is high Rg =- 0.87 (Gjoen et al., 1993) and between growth rate and overall survival Rg = 0.23 (Rye et al., 1990). These correlation values indicate positive indirect impact on feed conversion ratio and overall survival (as a measure of resistance to disease) when selecting for improved growth rate. Between traits body weight and fat content there is a negative correlation, which prevents successful selection for weight gain and low fat content at the same time (Gjedrem, 2000).

Objectives and selection method

Before defining the objectives of selective breeding program, it is necessary to construct a breeding plan of selection. Basic breeding plan would, in principle, look like this: create the base population, define the goals of the selection, choose the method of selection, predict breeding values, choose candidates for the next generation, calculate the selection response and commercialize a program of selective breeding for rainbow trout. The objectives of the selection of rainbow trout, which would be conducted at the Center for Applied Hydrobiology and Fisheries (CRPH), Agricultural Faculty in Belgrade, are as follows:

- faster pace, with the indirect reduction in feed conversion ratio;
- increased resistance to certain diseases of trout;
- improvement of meat quality;

Taking into account the heritabilities for these traits the primary method of selection would be the selection of families combined with methods of individual selection and selection within the families of rainbow trout. Family selection assures the greatest accuracy in predicting the breeding value of future broodstock candidates. Challenge testing for particular disease of some individuals from each family will give us wanted data, without challenging the potential broodstock for next generation. Methods for improving the meat quality are requesting sacrifice of individuals; in this case, the selection of families is the only suitable method for assessment of needed estimates. Method of individual selection would have an advantage only in case of selection for faster growth rate of rainbow trout.

The breeding scheme would use factorial design as crossing method in order to achieve higher selection intensity of females, for given number of tanks and the number of individuals per family (Martinez et al., 2006, Boring and Knudsen, 2007).

Base population and experimental matings

Central place of implementation of selective breeding program for rainbow trout are the objects at the Centre for Applied Hydrobiology and Fisheries (CRPH), Faculty of Agriculture, University of Belgrade.

In the first phase of the project, technological equipment at the Center was modernized by adding additional equipment for water enriching with oxygen to provide optimum environmental conditions for rearing rainbow trout. In the second phase, we collected broodstock from various trout ponds in Serbia (Table 1.) which we used for mating trials and for investigation of technological possibilities of selective breeding at CRPH. In the process of gathering the broodstock primary criteria was diversity of trout farms and that this farms did not had a practice of importing eggs and fry from abroad (in order to avoid the possibility of transmission of infectious disease).

Table 1. Origin of rainbow trout broodstock for mating trials

Name of pond-location	Origin of broodstock	Number of individuals
Žubor (Lisine)	Istok (Kosmet)	4
Lazic (Resava)	Tropik ribarstvo	5
Bast komerc (Resava)	Imported	12
Jablanica (Boljevac)	Soko Banja	10

The third phase is experimental mating, spawning and breeding of rainbow trout and rearing fry up to the age of 10g when hatchlings will be tagged with PIT tags (passive integrated transponders) and transported to one of partnership trout farm. There we will continue to monitor and record the production traits of fish, as planned in selective breeding program.

In the period from 08.12.2010. to 25.02.2011., mating the broodstock individuals at the Center for Applied Hydrobiology and Fisheries, we created the 20 families of rainbow trout. Until the moment of crossing, we kept the potential candidates for spawning in 1000 liters capacity tanks and we were checking readiness for spawning every 5 days. Before spawning all broodstock candidates were marked and their body weight and body length was recorded. During the incubation, hatching and rearing of fry we recorded the following parameters: egg diameter and average weight, water temperature, oxygen saturation of water, duration of incubation and overall mortality of rainbow trout fry.

Future steps in selective breeding of rainbow trout

At the Center for Applied Hydrobiology and Fisheries, as Breeding organization with a special authority, we planned to produce 70 families of rainbow trout annually. Number of fish per family should be 50, which means that at the end of the season the number of potential breeding candidates would be 3500 individuals. Objectives of breeding program would be as indicated above: faster growth, increased resistance to certain diseases and improvement of meat quality. The goal of increasing the growth rate is to obtain a commercial size (250g) within 12 months (from the current average period over 15 months), which would significantly reduce production costs and thus made this type of fish more accessible to end users. The aim of increasing resistance to some disease at rainbow trout at Serbia is to increases survival from current 30 - 40% (from eggs to commercial fish) to over 50%, which would further reduce costs in production and increase production efficiency. The goal of improving meat quality should reflect the fat percentage, color and taste of fillet.

Fulfillment of long-term goals of the selective breeding program would provide genetically improved races of rainbow trout, which would form the basis for economically efficient and sustainable production of this fish species in Serbia.

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STUDY OF VIRAL DISEASES OF FISH IN THE REPUBLIC OF SERBIA DURING THE *PERIOD 2005-2010*.

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ISPITIVANJE VIRUSNIH BOLESTI RIBA NA PODRUČJU REPUBLIKE SRBIJE U PERIODU OD 2005. DO 2010.GODINE

Abstrakt
Tokom petogodišnjeg istraživanja prisustva virusnih bolesti riba kao što su PVŠ, VHS, ZHN, EHN, ZNG ispitano je 56 šaranskih i 32 pastrmska ribnjaka. Virusne bolesti riba su dijagnostikovane pomoću modernih laboratorijskih metoda: izolacija virusa na kulturi tkiva, PCR, ELISA i test fluorescentnih antitiela. U radu su opisane bolesti riba koje su dijagnostikovane pre 2005., kao što je prolećna viremija šarana, ali i pojava novih bolesti, nakon uvoza živih riba i njihovih proizvoda u Srbiju, i to zarazne nekroze gušterače pastrmki i epizootske hematopoezne nekroze američkog somića.

Ključne reči: *ribe, virusne bolesti, izolacija i identifikacija*

INTRODUCTION

Aquaculture is one of the fastest growing food production industries with an average growth rate of 9.2% per year. Fish diseases represents significant obstacle for development of aquaculture, which annually causes losses that are measured in billions of dollars. The intensive aquaculture process is often characterized by high density of fish, poor water quality, and accumulation of pathogens in the production systems and in the environment. As a result, most populations of fish from intensive rearing systems are characterized by chronic stress. Stress leads to increased susceptibility to disease, and prevalence of disease depends on the interaction between fish pathogens and the environment (Jeremić, 2003). Also, international trade of live fish and their products is a major hidden cause of many outbreaks. Damages caused by the disease significantly delay the development of fisheries and prevent its transition to modern forms of intensive aquaculture. This paper describes the most common and also the latest viral diseases diagnosed in fish farms in Serbia during the period 2005 - 2010.

MATERIAL AND METODS

During the five years 7000 samples of all fish categories from 56 carp and 32 trout farms were examined for the presence of viral diseases. Clinical examination and selection of samples for laboratory was done on the fish farms. For histopathological examination, altered organs were stained with hematoxylin and eosin. For virological investigation, homogenates of kidney, spleen, liver and gills were used. Pools of parenchymatous organs and gills were homogenized with MEM and centrifuged at 2500 x g, 20 minutes. For isolation, supernatants were inoculated at 24 hours old culture of EPC, FHM, RTG-2 and BF-2 cell lines. Inoculated cultures were incubated at 15 - 20°C for 7 days and observed daily by the appearance of cytopathic effect. Virus was identified performed by PCR, ELISA and fluorescent antibody test. As a material for PCR extracted organ homogenate and the first or second passage of the appropriate cell lines were used. DNA was extracted using a DNA mini kit according to the manufacturer (QIAGEN, Valencia, CA, USA). RNA was extracted using RNA mini kit according to the manufacturer (QIAGEN, Valencia, CA, USA). PCR products (for SVC, IPN, ECV) were sequenced directly using Big Dye Terminator v1.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) and ABI PRISM 3100-Avant Genetic Analyzer (Applied Biosystems). The obtained sequences were analyzed using Sequencing Analysis Software 5.1 (Applied Biosystems).

RESULTS AND DISCUSSION

Viral infections represent a constant danger for fish breeding. These diseases are often related to stressful situations and spread to a greater or lesser extent in all ponds (Jeremić and Radosavljević 2007). After wintering, in early spring and summer period, mortality often occurs in juvenile trout and carp caused by various infectious agents, due to the unfavorable conditions of environment and international trade in live fish and their products. During five years following diseases were identified:

Spring viremia of carp - acute contagious viral disease caused by the *Rhabdovirus carpio*. Carp of all ages are affected and also other cyprinid fish species. Due to seasonal occurrence of the disease, the most vulnerable are 9-12 and 21-24 months old carp. Physiological status of carp after wintering significantly contributes to the occurrence of disease in the spring, having in mind that at a similar temperature conditions are present in autumn, but without big losses. Mortality caused by spring viremia occurs from November to July with a peak between April and June. The disease usually occurs at temperatures between 11-17^{o}C, and very rarely at temperatures lower than 10^{o}C. SVC outbreaks first occurred in the spring of 1986 in Serbia, with water temperature between 13 and 15°C. Severity of disease varied from pool to pool in the same pond, or from pond to pond, and some ponds were viral positive in two consecutive years. Previous investigations of prevalence and incidence showed that in 1950 samples from 38 carp farms in Serbia, *Rhabdovirus carpio* was determined in 256 samples as follows: 124 samples in April, 112 in May, 19 in June, 4 in December and 3 samples in February (Jeremic et al. 1997). Keeping fish during the winter in large densities of fish without food, during the initial rise of temperature in spring, with all the factors that provoke stress, favoring the occurrence of this disease and high mortality (Jeremić et al. 2005). The occurrence of mortality with clinical symptoms of the disease is found in 1 pond with a two-year old carp in 2005, reared in intensive production, after the transport from the winter quarters (Jeremić et al. 2008). Clinical and pathomorphological examination of the skin and gills of diseased and moribund fish revealed an increased amount of mucus, exoftalmia, enlarged abdominal cavity and reddening of the vent. Gills were ishemic, with petechial haemorrhages on fin and gills. In internal organs, notable changes were found: peritonitis, petechial haemorrhages and miliary necrosis of the liver, hemorrhages and necrosis of the kidneys, bleeding in the anterior part of the air sac, oedema of the spleen. Mucous membranes of the intestine were hemorrhagic, and the lumen filled with a yellow, gelatinous fluid (Jeremić et al. 2005). The most frequent histopathological changes were found in liver, kidney and spleen. In the liver hepatocytes light vacuoles of various sizes were noticed. Besides fatty changes, liver tissue shows a blue dye mononuclear cellular infiltration (hepatitis). In renal interstitium, mononuclear cellular infiltration was clearly evident. Reticular hyperplasia of spleen and discrete blood extravasated were also present (Jeremic et al. 1999).

Rhabdovirus carpio was isolated from the gills and parenchymal organs of diseased carp on EPC and FHM cell lines. In the first passage within 7 days of incubation FHM and EPC cell lines inoculated with filtrate of pooled samples of diseased carp cytotoxic effects occured. Control cells were normal, and cell culture inoculated with the reference virus SVC gave a clear cytopathogenic effect of the CPE. Cytopathic effect appeared 48-72 hours after subcultivation in the form of rounding EPC and FHM cells. After 72 hours good CPE appeared. Identification of virus isolates was performed using ELISA and indirect immunofluorescence test (IFAT). By RT-PCR with specific primers, presence of nucleic acid of the virus of spring viremia of carp was demonstrated. After purification, sequencing of obtained PCR products was done. Comparing the sequences of the glycoprotein with NCBI sequences previously isolated virus showed that the isolates belong to the group Id of the SVCV isolates.

Infectious pancreatic necrosis (ZNG/IPN) - acute, infectious, highly contagious disease of trouts, which are clinically manifested in the first 20 weeks of the feeding, with sudden onset of mortality (10-90%). Mortality depends on many factors, such as virus strain, host and environmental conditions (Dobos & Roberts, 1983). Cause of the disease is Aquabirnavirus, family Birnaviridae (Dobos & Roberts, 1983). The most important source of infection is affected fish and those that remain lifelong carriers after they recover from infection. Carriers excrete virus through feces and sexual products. Detection of such carriers is important for disease control, because the virus in addition to the horizontal is vertically transmitted, via eggs (Jeremic et al. 2008; Dorson & Torchy, 1985), and surface disinfection of eggs is not completely effective in preventing this type of transmission (Bullock et al., 1976).

The disease was first noticed in Serbia in 1989 (Jeremić et al. 1989) and did not emerge until 2007, when the disease was confirmed at a trout farm in Mačva district, in diseased rainbow trout, derived from fertilized eggs imported from USA. In 2008, the disease was diagnosed in three trout ponds in Zlatibor, Mačva and Pirot district, in diseased trout fry. In all cases this was clinically manifest disease with high mortality in rainbow trout (Radosavljević et al. 2009). Diseased fry showed characteristic clinical signs, with dark body pigmentation, swelling of the abdominal region and moderate exophthalmos. In the abdominal cavity red serous fluid was present. Liver and spleen were pale in color, and bile sac was enlarged. The intestines were devoid of food, with a large amount of mucous content.

IPN virus was isolated in 2009 from clinically healthy one year old rainbow trout, which was in quarantine at the trout farm in Mačva district, and in 2010 in the same pond; virus was isolated from rainbow trout that showed no clinical symptoms of disease. In 2010, infectious pancreatic necrosis virus of trout was isolated from clinically diseased rainbow trout in three ponds in the region of Zlatibor. Also in 2010 disease spread to one fish farm in Pomoravski region.

The most frequent histological changes were found in the exocrine pancreas and intestine. Stained sections of the exocrine pancreas showed pronounced signs of necrosis of pancreatic tissue and the presence of inclusions. In the lamina epithelial mucosae tunicae the individual and the multiple cells with eosinophilic cytoplasm were present. These cells were seen in the intestinal lumen as a result of desquamative process (Jeremić et al. 1998). For virus isolation, 24^{h} old RTG-2, EPC, BF2 cell culture were used. Inoculated cultures were incubated at 15^{o}C for 7 days and controlled daily for appearance of cytopathic effect (CPE). After the appearance of CPE identification of the virus was carried out by ELISA and indirect immunofluorescence test (IFAT). RT-PCR demonstrated the presence of the nucleic acid of the virus infectious pancreatic necrosis.

Epizootic haematopoietic necrosis (EHN) - acute viral disease of perch, rainbow trout, catfish and ictalurids caused by Iridoviruses from genus Ranavirus. The disease is caused by three similar viruses: hematopoietic necrosis virus (EHNV), european sheatfish virus (ESV) and european catfish virus (ECV). The disease is characterized by hemorrhages, edema and necrotic changes in liver, spleen, kidney and hematopoetic tissue. Epizootic hematopoietic necrosis was first reported in perch (*Perca fluviatilis*) in Australia (Langdon & Humphrey, 1987). After the outbreak of the diseases caused by the EHNV in Australia, epizootic haematopoietic necrosis was found in France in *Ictalurus melas* (Pozet et al., 1992), in Germany in *Silurus glanis* (Ahne et al., 1990.), Denmark and Finland (Ariel et al, 1999). The disease occurs in all age categories of fish . Cyprinid species are not susceptible to EHN virus. During the August of 2008, high mortality appeared in brown bullhead (*Ameiurus nebulosus*) in one carp farm located in north-western Serbia. The outbreak occurred after a increase of water temperature in the end of July, when the ambient temperature ranged from 30 to 36^{o}C, and water temperatures around 28^{o}C. Mortality lasted until mid-September (Jeremic et al. 2009). Fish presented clinically with exophthalmia, pale gills, and fin and skin haemorrhages. Gross necropsy findings included pale livers, swollen spleens and petechial haemorrhaging of mesenteric fat and internal organs. For virus isolation, 24h old EPC and FHM cell cultures were used. Inoculated cultures were incubated at 20°C during 7 days and observed daily for appearance citopatogenog effect (CPE). An extensive cytopathic effect (CPE) was observed 48^{h} after inoculation. Diagnosis was based on clinical signs, virus isolation in the EPC cell line and polymerase chain reaction (PCR).

CONCLUSIONS

Viral diseases represent a constant threat for fish breeding. Clinically manifested, their participation in the pathology and economical production is of high importance. Their harmful effects are manifested in increased morbidity and mortality, the weakening of the fish, reduced growth, poor feed utilization and lack of breeding material. The appearance of a number of diseases that had not occurred earlier, warns that the measures taken to protect the health of the fish are not enough. Given that most new diseases causes high mortality, it is necessary to invest additional effort in order to maintain the health status of fish populations through the use of effective biosecurity measures, primarily regardingthe purchase of fertilized eggs and fry outside Serbia. In the carp farms the number of cases of spring viremia of carp is reduced.In the trout farms the number of cases of infectious pancreatic necrosis in trout is increased.

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SOCIO-ECONOMIC DRIVERS AND NON-INDIGENOUS FRESHWATER CRAYFISH SPECIES IN EUROPE

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SOCIOEKONOMSKI UZROCI I ALOHTONE VRSTE SLATKOVODNIH RAKOVA U EVROPI

Abstrakt

Strane vrste slatkovodnih rakova (NICS) prelaze po brojnosti autohtone u mnogim evropskim zemljama, predstavljajući pretnju biodiverzitetu zbog širenja "kuge" rakova, agresivne invazije i antagonističkog delovanja. Iako je biološka osnova ovog stanja dobro proučena, moguća uloga socijalnih, ekonomskih i demografskih činilaca za ovu situaciju je praktično ignorisana. U pokušaju da se ispitaju takvi odnosi, mi predlažemo da je gustina ljudske populacije i ekonomski rast meren kao rast domaćeg proizvoda (GDP) per capita pozitivno povezan sa povećanim brojem NICS-a u Evropskoj Uniji. Ovo je očigledno u zemljama sa većim ukupnim ekološkim tragom. U gornjem kontekstu hitno su potrebne akcije da se povrate/uravnoteže postojeće i projektovane ekološke promene prouzrokovane prisustvom NICS-a.

Ključne reči: biodiverzitet, demografski pritisci, ekološke promene, invazivne vrste rakova.

INTRODUCTION

Social forces are widely accepted as the dominant factor shaping natural environment (Moore, 2000). Accordingly, 'modernization' processes (e.g., economic growth, industrialization, urbanization and demographic pressures) are considered as critical to biodiversity, climate change and ecological health of the natural environment (Clausen & York, 2008). This is particularly true for aquatic environment where degradation is continuously documented (Kottelat & Freyhof, 2007). Only recently, the influences of economic growth have started to be examined against the possibilities of further widening existing ecological rifts in the aquatic environment (Clausen & Clark, 2005; Perdikaris & Paschos, 2011).

The populations of the five European indigenous crayfish species (ICS) once abundant were seriously devastated by 'crayfish plague' (Alderman, 1996). Moreover, pollution and eutrophication, overharvest, agro-chemical runoffs, alteration/destruction of natural habitats and water abstraction are the major anthropogenic pressures leading to the extirpation of populations and disjunctive distribution of the remaining ones (Koutrakis et al., 2007). These diversity losses are expected to develop further in the future, due to current spatial expansion and competitive advantages of the nine established non-indigenous crayfish species (NICS) in Europe. Moreover, the practically uncontrolled globalized aquarium trade of live ornamental NICS possesses additional threat to ICS (Chucholl, 2010). Therefore, economic incentives and human-generated impacts appear to determine significantly the future status of ICS in a Pan-European level.

The present work aims to examine possible relations and trends between demographic factors (human population density), economic factors (urbanization level, GDP *per capita*), ecological status (biocapacity/footprint) and the number of established NICS in the EU area.

MATERIALS AND METHODS

Data on the number of NICS present in 26 EU countries were supplied by Holdich et al. (2009). All data relevant to population density, urbanization percentage and GDP *per capita* came from the World Bank (2009). Finally, biocapacity and ecological footprint data were supplied by World Wide Fund (2008). The number of NICS was plotted against each one of the above independent variables and coefficient *r* of the best fitted curve was calculated in each case.

RESULTS & DISCUSSION

A. Demographic effects on the number of NICS

Human population density positively correlates (r=0.67) with the number of NICS (Figure 1). In fact, nine out of ten species introduced in European freshwaters have been successfully established (Holdich et al., 2009). Similar studies (Hoffman, 2004; Clausen & York, 2008) suggested that demographic factors are related to environmental degradation as the most densely populated nations have higher rates of threatened species and certainly more hobbyists possessing and culturing crayfish.

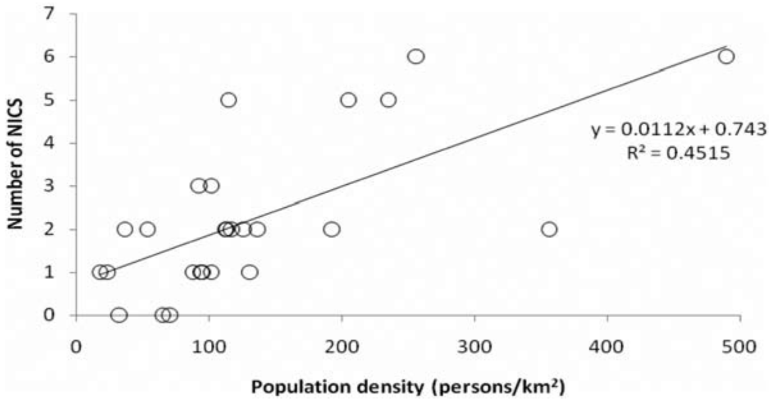

Figure 1. Graphic plot of the number of NICS against population density in the EU countries inhabited by freshwater crayfish (Austria, Belgium, Bulgaria, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Spain, Latvia, Lithuania, Luxembourg, Netherlands, Poland, Portugal, Romania, Slovakia, Slovenia, Sweden, United Kingdom)

B. Economic effects on the number of NICS

Urbanization level seems to have a moderate effect on the number of NICS in EU area (r=0.39) (Figure 2). It is a fact that cities especially close to airports and ports are the main entrance gates of international trade and also the biggest trading places themselves; however this does not necessarily imply that ornamental NICS released deliberately by urban citizens have more chances to survive compared for example to the escapees from a crayfish farm in the countryside (depending primarily on culture density, the number of stocked animals, the number of escapees and finally the species concerned).

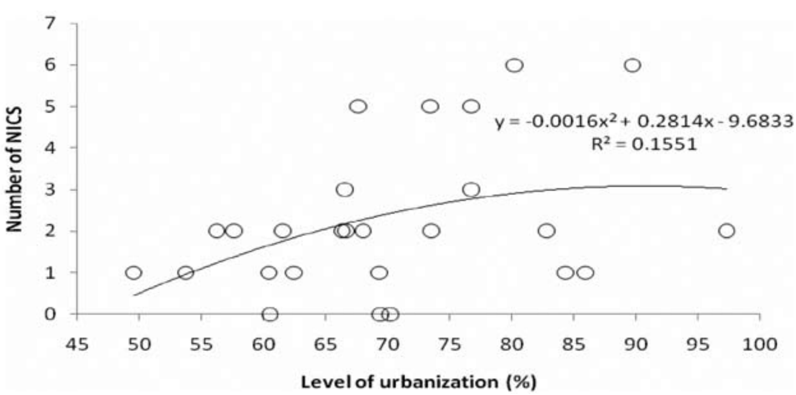

Figure 2. Graphic plot of the number of NICS against urbanization level in the EU countries inhabited by freshwater crayfish (countries as in Figure 1)

According to the neo-liberal theories, early stages of economic development goes parallel to environmental degradation, but eventually economic improvement (e.g., >$9000 measured as GDP *per capita*; Hoffman, 2004) leads to 'ecological rationalization' and amelioration of the impacts. In the case of NICS, there is a moderate tendency (*r*= 0.47) to gradually incease their numbers in the EU countries up to $40,000 GDP *per capita* before start falling (Figure 3). This picture suggests that economic growth contributes to the amplification of the pressures faced by the ICS in their habitats due to the increased numbers of NICS. Similar outcomes have been reported for the observed and expected decline of endangered species for developed nations (Czech et al., 2000; Naido & Adamowicz, 2001) and cross-nationally (Clausen & York, 2008).

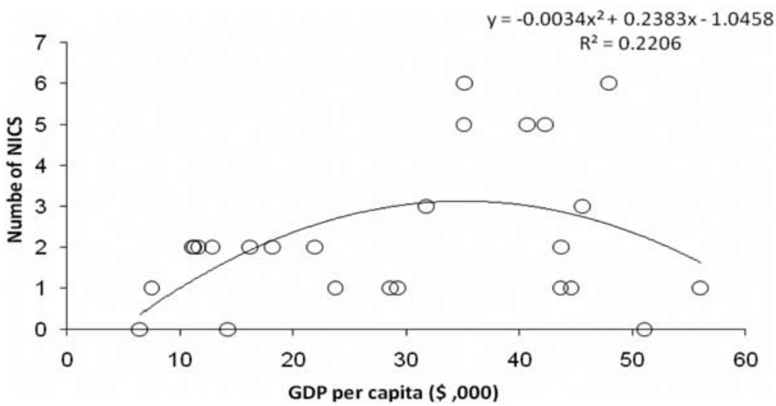

Figure 3. Graphic plot of the number of NICS against GDP *per capita* in the EU countries inhabited by freshwater crayfish (countries as in Figure 1 excluding Luxemburg)

C. Ecological status and NICS

Ecological footprinting tools revealed that European *per capita* ecological footprint during 2005 was about double compared to the available biocapacity (WWF, 2008). In this context, the number of NICS increases as we move from the ecological creditor to ecological debtor countries (*r*=0.45) (Figure 4). This trend can be either explained by the long history of trade dependency for natural resources in many European countries or the absence or weak enforcement of environmental protection, taking in account the risks generated for the ICS. Overall, the presence of NICS correlates well with unsustainable practices in resource management and implies possible ecological rifts generated in many production sectors.

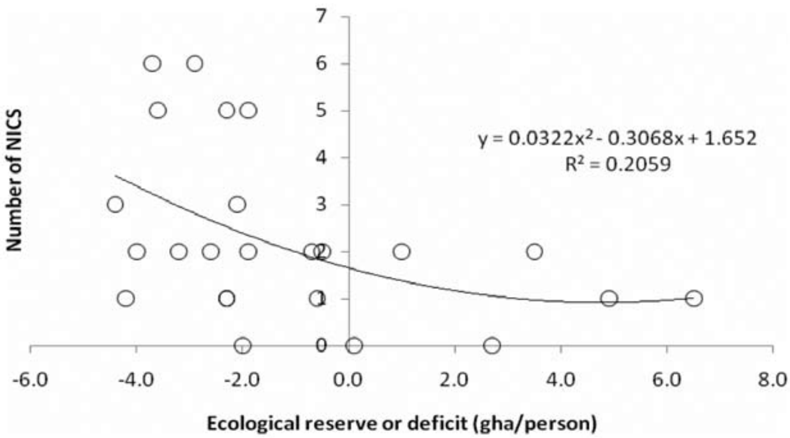

Figure 4. Graphic plot of the number of NICS against ecological reserve/deficit values in the EU countries inhabited by freshwater crayfish (countries as in Figure 1)

CONCLUSIONS

Managing the spread of NICS is not an easy task given the human-assisted introductions (Peay, 2009). It is obvious thus, that profit generation and accordingly strong lobby interests in aquarium trade is a major obstacle against the introduction and enforcement of legal instruments. Indirect metabolic (ecological) rifts are continuously created by the spread of NICS through direct impacts on ICS and other aquatic biota and structural modification of habitats (Nyström et al., 1996). EU legislative frameworks, recent actions to adopt strategies against alien species and the development of risk screening tools of NICS are expected to assist in some extent to the protection of ICS. Moreover, there is a need to address the fundamental causes of NICS spread in the EU territory in a more holistic way taking in account impacts generated by the social, demographic and the economic environment.

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INTESTINE PARASITES OF BREAM *ABRAMIS BRAMA* (LINNAEUS, 1758) OF THE DANUBE IN BELGRADE AREA

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PARAZITI CREVNOG TRAKTA DEVERIKE *ABRAMIS BRAMA* (LINNAEUS, 1758) DUNAVA U BEOGRADSKOM DELU TOKA

Abstrakt

Izučavanje parazitofaune riba prirodnih voda od izuzetnog je značaja, ne samo u naučnom pogledu, već i za njihov uspešan intenzivan način gajenja u akvakulturi. U radu su predstavljeni rezultati istraživanja crevnih parazitskih vrsta deverike *Abramis brama* L. 1758. Primerci riba su sakupljeni tokom perida 2007–2009 na dva lokaliteta uzorkovanja duž toka reke Dunav koji protiče kroz beogradski region. Ukupno je pregledano 177 jedinki deverike različite starosti (2^+ to 6^+). Prtisustvo crevnih parazitskih vrsta identifikovano je kod 97 pregledanih primeraka deverike, sa intenzitetom infekcije u rasponu 1–165, predstavljajući 54.80% od ukupnog broja sakupljenih i pregledanih primeraka riba. U inficiranim jedinkama deverike utvrđeno je prisustvo 27 taksona endoparazita (helminta) iz četiri klase: jedanaest vrsta pantljičara (Cestoda), pet vrsta i jedan takson metilja (Trematoda), dve vrste klase Nematoda i osam vrsta Acanthocephala. Sprovedena istraživanja su pokazala da je inficiranost deverike Dunava u beogradskom regionu značajna, s obzirom na brojnost i raznovrsnost identifikovanih crevnih parazita. Buduća istraživanja su neophodna da bi se utvrdila uloga crevnih parazita u regulisanju brojnosti populacija deverike Dunava.

Ključne reči: *Deverika, Abramis brama L. 1758, crevni paraziti, beogradski region, Dunav*

INTRODUCTION

The middle course of the Danube runs through the territory of Serbia or along its border. The Serbian stretch of the Danube, which is 588 km long (20.6% of total length), begins at the border with Hungary (rkm 1433) and ends at the mouth of the Timok River, at the border with Bulgaria (rkm 845). With its main tributaries, the Danube represents the most significant Serbian water resource (Babic-Mladenovic et al., 2010). In the Belgrade region, the Danube is constantly subjected to heavy loading with matter of mainly organic origin.

The bream *Abramis brama* Linnaeus 1758 is a benthopelagic, potamodromous fish, member of the carp family (Cyprinidae), growing up to 82.0cm and to 6.0kg. Living at the muddy and sandy bottom of still and slow-running rivers, as well as brackish waters. Feed on insects, particularly chironomids, small crustaceans, mollusks and plants. Larger specimens may feed on small fish. The juveniles feed on zooplankton. The area of it`s distribution extends in Europe and Asia, also present in rivers belonging to the basins of the North, Baltic, Black, Caspian and Aral seas (http://www.fishbase.org). In Serbia, bream *Abramis brama* lives in open waters of the drainage system of the Danube River, i.e. in surface waters in Vojvodina area/region, as well as in the main course of the Velika Morava River (Simonović, 2006). The bream was the subject of many ichthyological studies in Serbia (Janković, 1965; Marković, 1962; Simonović, 2006) contributed to the recognition of the ecology of this fish species and its populations in the Serbian part of the Danube River.

In the present paper the results of a parasitological survey of the intestinal helminth fauna of *Abramis brama L. 1758* the Belgrade region of the Danube River are reported.

MATERIAL AND METHODS

The breams were collected during the period 2007–2009 along the course of the Danube River through the Belgrade Region, by nets of different size of mesh (32 – 50 mm). Samples were taken from two sampling sites, Zemun (1.171,5 rkm; 44° 50′ 21"N 20° 24′ 02"E) and Visnjica (1.162 rkm; 44° 49′ 51″N, 20° 32′ 52″E).

The total length, weight, sex and age were recorded for each fish specimen on both sampling sites. Fishes were transported to the laboratory and immediately examined for parasites. During the parasitological examination, the intestines were cut open and examined under stereomicroscope. Parasites found were bleached, prepared and fixed for species identification and subsequent storage. Parasites were identified using identification keys (Bauer, 1987; Kakacheva-Avramova, 1977; Lom and Dykova, 1989; Moravec, 1994). Statistical non-parametric analysis, Mann-Whitney U test (comparing two independent groups) and Kruskal-Wallis ANOVA and Median test (comparing multiple independent groups) were used to determine the significant differences comparing the number of parasites per individual in regard to sampling locality, apropos Fulton's body conditional factor (CF) and values of parasitic number per locality and months of sampling (statistical significant differences on level possibility of 5%).

RESULTS AND DISCCUSSION

A total of 177 fish specimens of different age (2^+ to 6^+) were examined at two sampling sites in the Belgrade area of the Danube River. By the examination of bream`s intestine a total of 27 endoparasites belonging to 4 classes were found in 97 bream specimens, which was 54.80% of all captured bream specimens. The number of parasites per individual was varied in range from 1 up to 165. The highest value was found in bream caught on locality Visnjica, in May 2009, infected with parasitic species *Caryophyllaeus laticeps* Pallas 1781 (Cestoda). Number of examined breams in Zemun locality was 54 and in Visnjica locality 123, while number of infected specimens was 19 and 78, respectively (Table 1).

Table 1 Number of examined breams, average values of length and weight, as well as number of infected breams, intensity of infestation and parasitic species on sampling sites, per months

Locality	Number of examined breams	Average value of length (cm)	Average value of weight (g)	Number of infected breams	Infestation intensity	Parasitic species
ZEMUN						
November 2007	1	25.9	200	/	/	
December 2007	4	22.6±2.093	162±34.05	/	/	
January 2008	5	25.52±5.359	240.6±131.4	/	/	
February 2008	3	20.87±2.285	116.7±13.87	1	0-2	*Apophallus müehlingi* Jagerskiold 1899 *Sphaerostomum bramae* Müller 1776
March 2008	1	21	143	/	/	
April 2008	5	23.2±2.332	172±58.08	3	3-7	*Allocreadium* sp. Looss 1900 *Sphaerostomum sp*. Rudolphi 1809 *Acanthocephalus sp*. Koelreuter 1771 Trematoda juv.spp
Jun 2008	3	24.43±4.244	258.3±87.8	1	0-6	*Caryophyllaeides fennica* Schneider 1902 *Triaenophorus sp.* Rudolphi 1793 *Acanthocephalus sp*. Koelreuter 1771
July 2008	1	19	225	/	/	
September 2008	3	22.83±2.15	210±78.58	2	1-3	*Pomphorhynchus sp*. Monticelli 1905 *Caryophyllaeus sp*. Gmelin 1790
October 2008	2	26.55±2.475	262.5±123.7	1	0-1	*Acanthocephalus sp*. Koelreuter 1771
December 2008	1	25	200	/	/	
January 2009	3	24.2±1.572	170±34.64	2	0-1	*Ligula intestinalis* Linnaeus 1758
February 2009	4	24.38±3.097	181.3±51.54	2	1-2	*Caryophyllaeus laticeps* Pallas 1781
March 2009	2	24.35±0.495	201.7±27.54	/	/	
April 2009	1	27	230	1	3	*Caryophyllaeus brachycollis* Janiszewska 1951 *Caryophyllaeus laticeps* Pallas 1781
May 2009	4	22.43±0.995	181.3±23.94	3	2-6	*Caryophyllaeus laticeps* Pallas 1781 *Caryophyllaeus sp*. Gmelin 1790
Jun 2009	2	22.7±0.141	150±35.36	1	0-3	*Pomphorhynchus laevis* Zoega in Müller 1776 *Metechinorhynchus sp*. Petrochenko 1956
July 2009	2	21.45±0.354	117.5±10.61	1	0-7	*Pomphorhynchus laevis* Zoega in Müller 1776 *Metechinorhynchus sp*. Petrochenko 1956
September 2009	4	24.02±1.307	162.5±32.27	1	0-2	*Pomphorhynchus laevis* Zoega in Müller 1776 *Acanthocephalus anguillae* Müller 1780
November 2009	3	25.5±1.48	193.3±40.41	/	/	
VISNJICA						
December 2007	1	35.2	610	/	/	
January 2008	1	28.5	255	/	/	
February 2008	7	32.06±3.25	437.86±163.86	5	1-17	*Caryophyllaeides fennica* Schneider 1902 *Caryophyllaeus brachycollis* Janiszewska 1951 *Caryophyllaeus laticeps* Pallas 1781 *Caryophyllaeus sp.* Gmelin 1790 *Triaenophorus nodulosus* Pallas 1781

March 2008	10	35.89±5.05	600±228.51	7	1-17	*Hysterothylacium bidentatum* Linstow 1899 *Rhabdochona* sp. Railliet 1916 *Sphaerostomum bramae* Müller 1776 *Allocreadium isoporum* Looss 1894 *Allocreadium sp.* Looss 1900 *Caryophyllaeides fennica* Schneider 1902 *Caryophyllaeus sp.* Gmelin 1790
April 2008	6	30.95±1.97	379.17±90.41	2	4-25	*Triaenophorus sp.* Rudolphi 1793 *Caryophyllaeus sp.* Gmelin 1790 *Acanthocephalus sp.* Koelreuter 1771
Jun 2008	8	33.61±1.88	500±63.13	6	1-21	*Proteocephalus torulosus* Batsch 1786 *Ligula intestinalis* Linnaeus 1758 *Caryophyllaeus sp.* Gmelin 1790 *Triaenophorus sp.* Rudolphi 1793 *Acanthocephalus lucii* Müller 1776 *Acanthocephalus sp.* Koelreuter 1771 *Echinorhynchus sp.* Zoega in Müller 1776 *Pomphorhynchus laevis* Zoega in Müller 1776
July 2008	9	33.48±1.56	499.44±40.34	5	1-4	*Caryophyllaeus sp.* Gmelin 1790 *Acanthocephalus sp.* Koelreuter 1771 *Pomphorhynchus sp.* Monticelli 1905 *Echinorhynchus sp.* Zoega in Müller 1776
September 2008	13	33.48±3.22	546.92±149.07	9	1-7	*Caryophyllaeides fennica* Schneider 1902 *Triaenophorus sp.* Rudolphi 1793 *Pomphorhynchus laevis* Zoega in Müller 1776 *Pomphorhynchus sp.* Monticelli 1905 *Acanthocephalus sp.* Koelreuter 1771 *Echinorhynchus cinctulus* Porta 1905
October 2008	13	31.08±1.45	416.15±47.965	10	1-11	*Pomphorhynchus laevis* Zoega in Müller 1776 *Pomphorhynchus sp.* Monticelli 1905 *Acanthocephalus* sp. Koelreuter 1771 *Echinorhynchus sp.* Zoega in Müller 1776
November 2008	9	30.06±0.752	360.56±49.96	3	1-10	*Ligula intestinalis* Linnaeus 1758 *Proteocephalus torulosus* Batsch 1786
December 2008	7	30.44±1.434	370±50.415	3	1-3	*Caryophyllaeides fennica* Schneider 1902 *Caryophyllaeus brachycollis* Janiszewska 1951
February 2009	6	34.32±2.499	520±78.486	3	1-2	*Caryophyllaeus sp.* Gmelin 1790 *Pomphorhynchus sp.* Monticelli 1905
May 2009	8	34.95±1.92	601.25±104.565	6	2-165	*Caryophyllaeides fennica* Schneider 1902 *Caryophyllaeus laticeps* Pallas 1781 *Caryophyllaeus brachycollis* Janiszewska 1951 *Proteocephalus sp.* Weinland 1858 *Pomphorhynchus laevis* Zoega in Müller 1776
Jun 2009	5	28.18±0.766	325±17.68	4	2-11	*Caryophyllaeus laticeps* Pallas 1781 *Caryophyllaeides fennica* Schneider 1902 *Proteocephalus sp.* Weinland 1858 *Diphyllobothrium sp.* Cobbold 1858
July 2009	13	32.43±1.73	506.15±83.52	10	1-22	*Caryophyllaeides fennica* Schneider 1902 *Caryophyllaeus laticeps* Pallas 1781 *Caryophyllaeus fimbriceps* Annenkova-Khlopina 1919 *Pomphorhynchus laevis* Zoega in Müller 1776 *Metechinorhynchus sp.* Petrochenko 1956
September 2009	7	33.16±1.46	495±88.46	4	2-7	*Caryophyllaeides fennica* Schneider 1902 *Caryophyllaeus laticeps* Pallas 1781 *Caryophyllaeus fimbriceps* Annenkova-Khlopina 1919 *Pomphorhynchus laevis* Zoega in Müller 1776

Breams from the Danube river in Belgrade region contained, in their intestines, numerous and diverse helminth representatives belonging to four classess – Cestoda (11 species), Trematoda (5 species, one taxa), Nematoda (2 species) and Acanthocephala (8 species).

According to results of Mann-Whitney U test when independent group (variable) was the locality of sampling in regard to number of parasites per individual, significant statistical differences has been found (p=0.000504). Non-parametric Kruskal-Wallis ANOVA and Median test shows that there are dependence of number of parasites and Fulton's body conditional factor (CF) in regard to sampling months (Fig. 1).

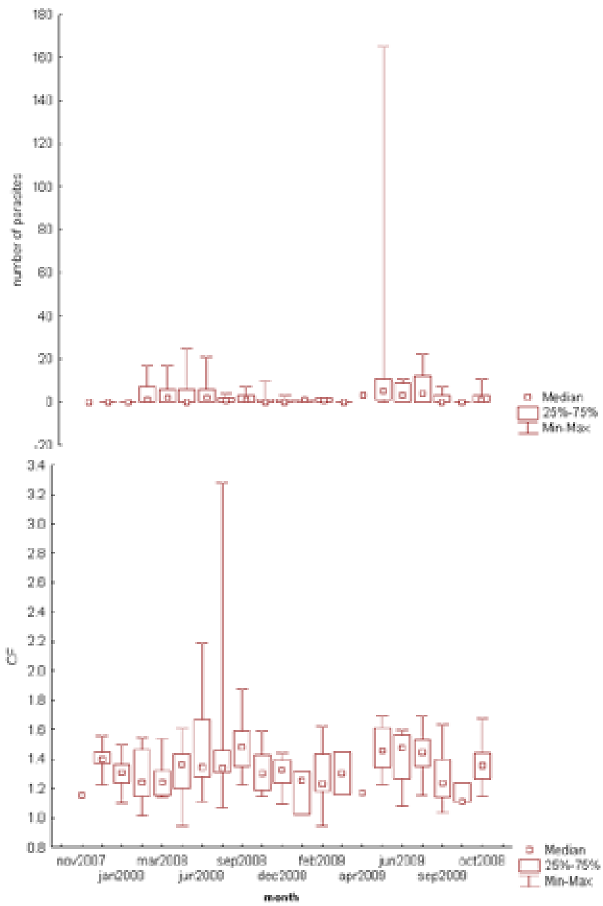

Figure 1. Correlation between the number of parasites and CF per months/locality

Within ichthyoparasitological surveys of surface waters in Serbia (Djanić, 1979; Kiškarolj and Tafro, 1988; Cakić, 2002; Cakić et al., 2001, 2007) there is no data for parasitofauna of bream. The main purpose of this study is to present the results of helminth fauna on *Abramis brama* L. along the Danube River in Belgrade region, as a contribution to their better knowledge.

CONCLUSIONS

Presented results show that the degree of bream parasite infestation is notable and contribute to the knowledge of bream`s intestinal parasites distrubution. By further investigations is needed to determine the role of endoparasites in regulation of bream`s population density.

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THE EFFESTS OF METACAIN ANESTHESIA ON COD BLOOD PARAMETERS

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DELOVANJE ANESTEZIJE METAKAINOM NA PARAMETER KRVI BAKALARA

Abstract

Anesthetics are commonly used in aquaculture to immobilize fish before handling in order to minimize stress responses that may influence fish quality negatively. However, anesthetics may actually trigger secondary stress responses by affecting blood respiratory properties and disturbing acid-base and electrolyte balance. In this respect we can monitor possible stress responses caused by anesthesia by measuring key blood parameters. In the present investigation we have studied the effect of metacain anesthesia on these blood parameters in Atlantic cod (*Gadus morhua*).

In total, 6 fish with an average length of 45 cm and weight of 1 kg were used in this study. The fish were kept in 2 m^3 tank with air saturated running sea water (34 ppt, 8.5°C) at natural light conditions (November 2010, Bergen, Norway) and fed with commercial cod feed. The test fish were anesthetized with metacain (1g/10L diluted in sea water) until fully immobilized prior to blood sampling from ductus cuvieri. These fish recovered after blood sampling. The control fish were killed with a sharp blow to the head and blood was sampled immediately after by cardiac puncture. Ice-cold syringes with Heparin lithium salt were used for blood sampling from both groups. The following parameters were measured: pH, Hct, Hb, mLO_2/gHb, Glu, Lac and plasma ions (Na^+, K^+, Li^+, Mg^{2+}, Ca^{2+}, Cl^-, F^-, Br^-, NO_3^-, PO_4^{3-} and SO_4^{2-}) for both groups.

The blood parameters were measured at 15°C and 100% air saturation after being equilibrated for 20 min. Oxygen carrying capacity was measured using a modified Tucker chamber technique. The other blood parameters were analyzed by standard techniques (pH electrode/meter, photometer, hematocrit centrifuges) as well as using i-STAT hand-held analyzer (used cartridges CG4+ and CHEM8+).

The results show a significant acidification of the blood from metacain anesthetized fish compared to control fish. This pH decrease is partly caused by the acidity of meta-

cain itself since there were no significant changes in Lac, which probably is due to the equilibration of blood with air for 20 minutes. This effect may cause arterial desaturation and reduced metabolic scope. Hct, Hb, mLO_2/gHb, Glu and Lac showed no significant differences between the two groups except for a larger variability of these parameters (higher standard deviation) for the metacain anesthetized fish. However, there was a significant increase in plasma Na^+ cations, which could be a direct effect of metacain on the Na-K-transport mechanism in gills leading to an ion imbalance.

In conclusion, the acidification caused by metacain itself, during anesthesia, appears to be the most serious problem for the fish during anesthesia and precaution should be taken in order to avoid metabolic stress until full recovery.

LIVE FOOD IN *ACIPENSER PERSICUS* REARING PONDS

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PRIRODNA HRANA *ACIPENSER PERSICUS* GAJENOG U RIBNJAČKIM JEZERIMA

Abstract

A study on live food in *Acipenser persicus* rearing ponds was carried out In four rearing ponds; two in the Shahid Beheshti hatchery and two ponds in the Yousefpour hatchery. Samples were collected from three points in each pond on a weekly basis. Samples were collected using plankton nets (mesh size 50 µm), Ruthner sampler and Ekman grab. Zooplankton and benthic samples were fixed in 4 % formalin solution for later analysis. Zooplankton species identified in water samples collected at all four ponds belonged mainly to two phyla; Arthropoda and Rotifera. Total abundance of zooplankton species was estimated 87576 individuals L^{-1} in the Yousefpour hatchery and 136626 individuals $^{L-}1$ in the Shahid Beheshti hatchery. Insect larvae, oligochaeta worms and mollusks were found in the sediment samples collected from the Yousefpour hatchery while sediment samples collected from the Shahid Beheshti hatchery contained only insect larvae and oligochaeta worms. The total abundance of benthic organisms was 0.343 g m^{-2} in the Yousefpour hatchery and 1.28 g m^{-2} in the Shahid Beheshti hatchery. Condition factor in fishes ranged from 0.29-0.54 in the Yousefpour hatchery and from 0.30-0.47 in the Shahid Beheshti hatchery.

Key words: *Rearing ponds, sturgeon fry, planktons, benthic organisms, condition factor*

INTRODUCTION

Lack of the required environmental potential in the Sepidrud, Volga and Kura Rivers has posed problems to the natural reproduction of sturgeons in these rivers (Kuliyev and

Kasimov, 1989; Keyvan, 1994) to the extent that it is not observed in the Sepidrud River (Fadaee et al., 1998). With regard to the negative impacts of anthropogenic activities on the river ecosystem, artificial reproduction in sturgeons is on the increase and thus earthen rearing ponds in sturgeon hatcheries are of significant importance (Isa Ava & Kabacchova, 1989). At the end of the last century Russian scientists turned to artificial breeding programs in sturgeons as the only solution to sturgeon conservation. Although instructions for sturgeon culture were prepared in 1900, artificial breeding in these species commenced only in 1916 in the Volga River. With the increasing trends in artificial breeding programs today, the study of biotic and biotic factors in sturgeon rearing ponds is essential for the sustainable use of these valuable species.

An understanding of the different components in an aquatic ecosystem is essential for the quantitative and qualitative study of productivity in an aquatic ecosystem. Determination of biomass of zooplanktons and benthic organisms is necessary as it is directly related to condition factor in sturgeon fingerlings. Placed at the apex of the energy pyramid, phytoplanktons are of significance in every aquatic ecosystem. All other organisms in the food cycle depend on each other and also on phytoplanktons directly or indirectly (Davis, 1955). Zooplanktons in ponds include cladocerans, ephemeptera and crustaceans. The study of benthic communities and determination of biomass and secondary productivity of these organism is considered very essential. The objective of this study was to determine the biomass of plankton species and benthic organisms and to assess the suitability of growth in fingerlings.

MATERIAL AND METHODS

This study was conducted on two rearing ponds with a surface area of 4 hectares and a maximum depth of 2 m each in the Dr. Yousefpour Hachery (No. 9 and 16) and 2 rearing ponds with a surface area of 2 hectares each in the Shahid Beheshti Hatchery (No. 27 and 35). Samples were collected from three points in each pond; pond inlet, pond outlet and center of the pond. Sampling was carried out on a weekly basis. Water samples were collected between 9 and 11 hours in the morning using a Ruthner sampler.

At each sampling point two samples were collected to estimate zooplankton biomass, one from 0.5 m depths and the other from a depth of 20 cm above the pond bottom. Sampling at 0.5 m depths was done using a plankton net with a mesh size of 50 µm whereas samples collected 20 cm above the pond bottom were collected in a Ruthner sampler and filtered through a plankton net. The water sample remaining in the collected at the end of the plankton net was transferred to a plastic container. Samples were fixed in 4 % formalin solution (ASTM, 1996). Identification and counting of zooplanktons was done in a chamber under an invert microscope. The zooplankton number was calculated using the formula:

$$N = \frac{axc}{vxL}$$

where N= total number of zooplankton specimens m^{-3}, *a*= mean number of zooplankton specimens counted per sample, *c*=volume of sample in ml, *L*=volume of water (m^3), *v*=volume of counting chamber in ml.

Benthic organisms were collected using an Ekman grab with a surface area of 225 m^2. Sediments collected were washed over a sieve and the benthic organisms remaining on the sieve were collected and transferred to the laboratory in plastic containers (Holme & MacIntire, 1984).

To determine condition factor in sturgeon fry, fish samples (n=10) were collected from each pond on a weekly basis using trawl nets. Length, and weight of each fish was measured and recorded and condition factor was calculated following Sabrowski & Bachholz (1996) using the formula:

$CF = W \times L^{-3} \times 100$ where:

CF= Condition factor, W=weight of fish (g), L= Total length of fish (cm)

RESULTS

Water temperature during the study period ranged from 19 to 27 ºC in the Shahid Beheshti hatchery and from 19 to 25 ºC in the Yousefpour hatchery. Zooplankton species identified in water samples belonged to the genera *Cyclops, Daphnia*, and *Moina* of the phylum Arthropoda and the genera *Brachionus, Keratella, Pedalia* and *Polyarthra* of the phylum Rotifera. Rotifera belonging to the genera *Synchaeta* and were only observed in water samples from the Yousefpour ponds. Overall average zooplankton abundance and biomass in pond no. 27 was 120527 individuals L^{-1} (n=6) with copepoda showing the highest biomass. Cyclops sp. and its nauplius with a biomass of 90.3 g m^{-3} showed the highest abundance. The biomass of Daphnia sp. was 2.9 g m^{-3}. Zooplanktons in pond no. 35 belonged to 5 genera. Copepoda comprised 79 per cent of the zooplanktons. Daphnia sp. and Moina sp. with a mean biomass of 5.7 g m^{-3} (n=7) comprised 19.5 per cent of the zooplankton community. Abundance of zooplanktons in this pond was recorded as 54624 individuals L^{-1}. Zooplankton abundance in pond no. 9 was calculated as 63125 individuals L^{-1} with copepoda comprising 68 per cent of the zooplanktons. Cyclops sp. and nauplius of Cyclops were dominant species with a biomass of 44.9 g m^{-3}. Seven zooplankton genera were identified in pond no. 9 and in pond no. 16. Abundance in pond no. 16 was 210215 individuals L^{-1} with copepoda being the dominant species. Biomass of cladocera and ploima showed lower abundance. Seven genera of zooplanktons were identified in this pond. Zooplankton genera identified in the ponds under study are presented in Table 1. Mean biomass of zooplanktons in the ponds under study are presented in Fig. 1.

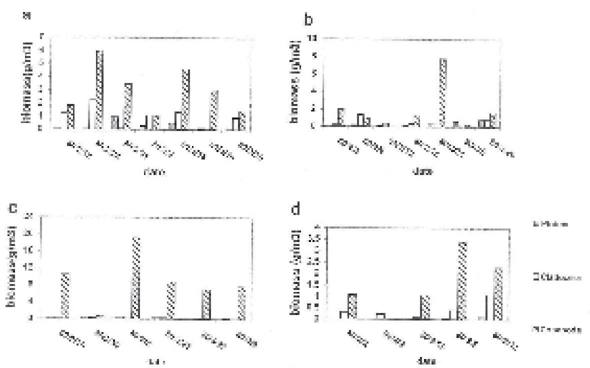

Figure 1. Comparison of zooplankton biomass (wet weight) found in the rearing ponds in the Yousefpour (a=Pond No. 9, b=Pond No. 16) and in the Shahid Beheshti (c=Pond No. 27, d=Pond No. 35) Hatcheries

Benthic organisms found in the sediment samples collected from the Yousefpour hatchery were mainly insect larvae, oligochaeta worms and mollusks whereas sediment samples collected from the Shahid Beheshti hatchery only contained insect larvae and oligochaeta worms (Table 1). The mean total biomass (n=7) of benthic organisms in pond nos. 9, 16, 27 and 35 was 1.53, 1.37, 2.9 and 2.2 g m^{-2}, respectively.

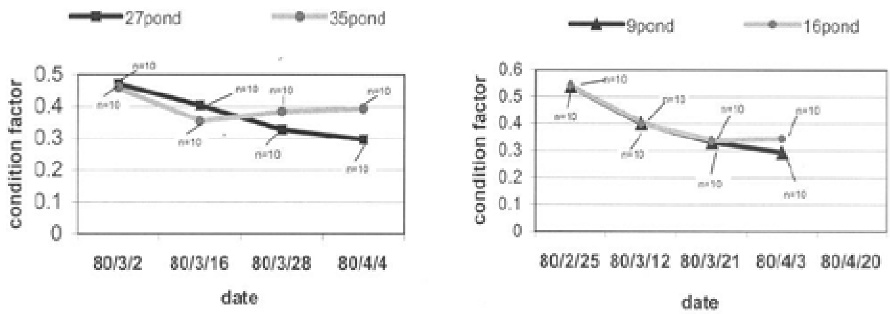

Figure 2. Condition factor of sturgeon fingerlings in a) The Yousefpour hatchery, b) The Shahid Beheshti hatchery

DISCUSSION

Percentage survival of fingerlings is to a great extent influenced by live food available in the pond. In the four ponds studied highest biomass values belonged to copepoda and while variations were observed in biomass of cladocera. According to Badenko and Bakhtenia (1962) long intervals between application of fertilizers results in irregular variations in zooplankton growth (Kohne Shahri and Azari Takami, 1974). 25-40 days sturgeon fingerlings feed on chironmomidae in addition to daphnia (Kohne Shahri and Azari Takami, 1974). In the present study chironomidae comprised only 6-15 % of the stomach contents of sturgeon fingerlings examined. Due to low biomass of chironnomidae in the rearing ponds sturgeon fingerlings feed more on daphnia in the ponds According to Strogonov (cited in Martyshev, 1983) benthic biomass of 7 g m^{-2} particularly that of chironomidae and oligochaeta is suitable for growth in sterlet fingerlings. Studies conducted in this regard indicate that this species feeds on benthic organisms (52.12 % of feeds consumed) mainly chironomidae. Condition factor of sturgeon fingerlings during early rearing was good, however showed decreasing trends towards the end of the rearing period. Mean condition factor for sturgeon fingerlings in the two hatcheries was <0.5. Condition factor in the range of 0.5-0.6 is considered intermediate for sturgeon fingerlings (Krupi, 1995). On the basis of studies conducted by Fadaee et al in 2001 (cited from Chubian et al., 2001) survival of sturgeon fingerlings in ponds 9 and 16 were 36.5 and 45.3 %, respectively. 62.5 % of fingerlings released from pond 9 and 80 % of fingerlings released from pond 16 were above 3 g in weight. Survival rate for fingerlings in ponds 27 and 35 were 77.1 and 54.2 %, respectively. 73.3 % of the fingerlings released from pond 27 and 86.7 % of fingerlings released from pond 35 were above 3 g in weight.

Information related to sturgeon fingerlings stocked in the ponds under study is shown in Table 3.

Table 3. Data on sturgeon fingerlings stocked in rearing ponds studied at the Yousefpour and Shahid Beheshti Hatcheries (N_1=number of fingerlings stocked; N_2= number of fingerlings released; W_1=Mean initial weight; W_2= mean final weight)

Hatchery	Pond No.	N_1	N_2	W_1 (mg) (n=10)	W_2 (g) (n=10)	Survival rate (%)
Dr. Yousefpour	9	415000	151720	73	3.8	36.5
	16	400000	181195	64.5	4.9	45.3
Shahid Beheshti	27	200000	154250	96	4.4	77.1
	35	200000	108450	86	5.5	54.2

Cited from Fadaee et al., 2001

It is evident from the results obtained that survival rates for fingerlings in the Shahid Beheshti hatchery were higher than those in the Dr. Yousefpour hatchery indicating suitable rearing conditions in the rearing ponds studied in this hatchery. Condition factor in sturgeon fry was satisfactory at the beginning of rearing and decreased gradually with decrease in food resources in the rearing ponds. Therefore sturgeon fry become weaker and weaker when the rearing period becomes prolonged. With regard to the role of life food in survival rates in sturgeon fry in rearing ponds it is necessary to improve live food production in rearing ponds through suitable fertilization. An alternative to this would be to decrease the stocking density of fry in rearing ponds.

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PROXIMATE COMPOSITION AND CHOLESTEROL CONTENT IN COMERCIAL IMPORTANT FRESHWATER FISH SPECIES IN SERBIA

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HEMIJSKI SASTAV I SADRŽAJ HOLESTEROLA U KOMERCIJALNIM SLATKOVODNIM VRSTAMA RIBA U SRBIJI

Apstrakt

Riba se, pripremljena na različite načine, konzumira u celom svetu, zahvaljujući činjenici da riblje meso sadrži veoma značajne komponente za ishranu ljudi. Riba je bogat izvor visokovrednih proteina, vitamina i esencijalnih minerala, ali iznad svega predsavlja veoma bogat izvor omega-3 i omega-6 polinezasićenih masnih kiselina, koje su veoma značajne za zaštitu zdravlja potrošača.

Holesterol je, takođe, značajan za organizam čoveka. Potrošnja namirnica sa visikom sadržajem holesterola može da ima za posledicu nastajanje brojnih kardiovaskularnih oboljenja.

Cilj ovih ispitivanja je bio da se odredi hemijski sastav i sadržaj holesterola u komercijalnim slatkovodnim vrstama riba (amur, tolstolobik i šaran), gajenim u dva objekta za akvakvakulturu u Srbiji. Sa druge strane, da bi se poredili parametri kvaliteta (proteini, voda, mast, pepeo) i sadržaj holesterola u ribljim vrstama, gajenim u Srbiji sa uvoznim, u poslednje vreme veoma zastupljenim na našem tržištu, uzorci pangasiusa iz Vijetnama takođe su analizirani.

U ovom radu analizirani su uzorci amura, tolstolobika i šarana gajenih u dva ribnjaka sa poluintezivim uzgojem ali različitim načinima ishrane. U prvom ribnjaku riba je hranjena komercijalnom ekstrudiranom hranom, dok su u drugom ribnjaku, kao dodatna hrana prirodnoj, korišćene žitarice. Uzorci pangasiusa iz Vijetnama uzeti su sa tržišta.

Statističkom evaluacijom dobijenih rezultata za hemiski sastav (proteini, voda, mast, pepeo) i sadržaj holesterola u amuru, tolstolobiku i šaranu iz prvog ribnjaka ustanovljeno je da postoji statistički značajna razlika ($p<0,005$) u sadržaju istog parametra kvaliteta kod najmanje dve vrste ribe. Sadržaj holesterola u šaranu se statistički značajno razlikovao ($p<0,005$) u odnosu na sadržaj holesterola u tolstolobiku i amuru, dok ta razlika nije bila statistički značajna ($p>0,005$) između sadržaja holesterola u tolstolobiku i amuru.

U drugom ribnjaku, sa izuzetkom sadržaja proteina, takođe je ustanovljeno da postoji statistički zanačajna razlika ($p<0,005$) u sadržaju istog parametra kvaliteta kod najmanje dve vrste ribe. Statistički značajna razlika u sadržaju holesterola ustanovljna je između tolstolobika i amura.

Dobijeni rezultati ovih istraživanja ukazuju da se amur, u poređenju sa šaranom i tolstolobikom iz oba ribnjaka, može smatrati nutritivno najkvalitetnijom vrstom ribe.

Ispitana riba iz familije ciprinida (šaran, tolstolobik i amur) iz domaće akvakulture ima veći sadržaj proteina u odnosu na pangasiusa iz Vijetnama, tako da ona predstavlja nutritivno kvalitetniju namirnicu u ishrani ljudi.

Ključne reči: *hemijski sastav, sadržaj holesterola, riba iz akvakulture*

INTRODUCTION

Fish is consumed all over the world in various forms, considering the fact that fish meat contains a lot of important nutritional components (Vranic et al., 2010). Accordingly, studies on food composition and its nutritional implication (Ramos Filho et al., 2010) is important as it is known that a high consumption of fish meat has a benefic role on human health (Friesen and Innis, 2009). Fish are a source of high quality proteins, vitamins and essential minerals but, above all, a virtually unique, rich source of omega-3 long-chain poly-unsaturated fatty acids (PUFA). Fish also contains significant amounts of essential amino acids, particularly lysine which is low in other animal tissues. Fish protein can therefore be used to complement the amino acid pattern and the overall protein quality of a mixed diet (FAO, 2005).

Cholesterol is also very important in the human organism. A correlation between serum cholesterol level and mortality rate on the cardiovascular diseases in humans was proved in many studies (Griffin, 1999). Lower consumption of foods with high cholesterol content was the consequence of higher incidence of many cardiovascular diseases.

The aim of this study was to determine proximate composition and cholesterol content in muscle tissue (filet) in three fish species (common carp, silver carp – *Hypophtalmichthys molitrix* and grass carp – *Ctenopharyngodon idella*) reared in two aquaculture fishponds in Serbia. On the other hand, in order to compare chemical composition and cholesterol content of fish species reared in Serbia with some commercial, marketable fish, filets of pangasius imported from Vietnam were analyzed too.

MATERIALS AND METHODS

In this study marketable size common, silver and grass carp from two fish farms (I and II) from Serbia in a semi-intensive rearing system, were analyzed. These semi-intensive fish farms had different feeding regime. In the first fish farm fish was fed commercial extruded diet, while in the second fish farm as supplementary feed only wheat was used. Although all these three fish species belong to the same fish family (Cyprinidae), they were feed different kind of feed. On the other hand, marketable size pangasius imported from Vietnam was analysed too.

Samples were collected from October and November 2010. The collected fish of each category ($n=6$) were stored at +4°C before being filleted. Fish fillets were homogenized in a laboratory blender (Braun CombiMax 600), separately placed in plastic bags and stored at -25°C until analyzed. A day before the sample preparation, samples were defrosted overnight, at +4°C.

Analyses of moisture, fat and ash were preformed according to ISO standard procedures (ISO 1973, 1996, 1997, 1998). Protein content (Kjeldahl nitrogen) was determined by using Kjeltec Auto 1030 Analyzer. Cholesterol determination in all samples was performed by using HPLC/PDA system, according to the method described by Maraschiello et al. (1996).

The data were statistically analyzed by using MINITAB Statistical Software (Release 14 for Windows) in order to compare the effect of feed on proximate composition and cholesterol content in the three fish species reared in the same fish pond. This effect was declared significant if $P<0.05$. Analysis of variance (ANOVA) with Tukey's test (95% confidence intervals) was applied.

RESULTS

Chemical composition and cholesterol content in the analysed fish samples are given in Tables 1-3.

Table 1. Chemical composition and cholesterol content in filets of three marketable size carp species from first fish farm (mean ± standard deviation, n=6)

I Fish farm	*Common carp*	*Silver carp*	*Grass carp*
Proteins (%)	$15.92^a \pm 0.54$	$18.69^b \pm 0.18$	$16.41^a \pm 0.13$
Moisture (%)	$75.59^a \pm 1.07$	$75.04^a \pm 0.15$	$71.22^b \pm 0.15$
Fat (%)	$6.99^a \pm 0.15$	$4.39^b \pm 0.13$	$11.59^c \pm 0.35$
Ash (%)	$0.96^a \pm 0.07$	$1.16^b \pm 0.04$	$0.95^a \pm 0.10$
Cholesterol (mg / 100g)	$50.55^a \pm 2.73$	$42.27^b \pm 1.21$	$40.12^b \pm 0.72$
Energy value (kcal /100g)	127.07 ± 1.63	117.40 ± 0.14	168.75 ± 1.99
Energy value (kJ /100g)	531.53 ± 6.66	493.33 ± 0.53	702.78 ± 8.10

Values in the same row followed by the same letters do not differ significantly (p>0.05)

Table 2. Chemical composition and cholesterol content in filets of three marketable size carp species from second fish farm (mean ± standard deviation, n=6)

II Fish farm	*Common carp*	*Silver carp*	*Grass carp*
Proteins (%)	17.41 ± 0.44	17.46 ± 0.43	16.74 ± 0.70
Moisture (%)	$76.14^a \pm 2.01$	$74.02^b \pm 1.32$	$71.90^c \pm 0.15$
Fat (%)	$5.00^a \pm 1.56$	$7.44^b \pm 1.53$	$8.87^b \pm 0.65$
Ash (%)	$1.06^a \pm 0.05$	$1.06^a \pm 0.04$	$1.79^b \pm 0.39$
Cholesterol (mg / 100g)	50.19 ± 10.10	$66.69^a \pm 20.60$	$43.82^b \pm 5.91$
Energy value (kcal /100g)	115.23 ± 17.47	134.93 ± 11.04	144.42 ± 4.14
Energy value (kJ /100g)	483.49 ± 72.06	564.61 ± 45.62	603.52 ± 16.36

Values in the same row followed by the same letters do not differ significantly (p>0.05)

Table 3. Chemical composition and cholesterol content in filets of marketable size pangasius (mean ± standard deviation, n=6)

Marketable size fish	**Pangasius**
Proteins (%)	11.67 ± 0.09
Moisture (%)	85.78 ± 0.06
Fat (%)	0.94 ± 0.08
Ash (%)	1.36 ± 0.01
Cholesterol (mg / 100g)	47.14 ± 3.31
Energy value (kcal /100g)	56.14 ± 0.71
Energy value (kJ /100g)	237.50 ± 3.03

DISCUSSION

By statistical evaluation of the obtained results for proximate composition (proteins, moisture, fat, ash) and cholesterol content for the three investigated fish species (Common, Silver and Gras carp) from first fish farm (Table 1) it was established that there was a significant difference ($p<0.05$) in the content of the same parameter at least between two fish spaces.

In the second fish farm (Table 2), with exception of protein content, it was observed that there was a significant difference ($p<0.05$) in the content of the same parameter at least between two fish spaces too.

In the first fish farm (Table 1), when comparing the individual parameters, the highest content of proteins contained silver carp followed by grass carp and common carp. Content of fat ranged from 4.39% (silver carp) to 11.59% (Grass carp). In the second fish farm (Table 2), the content of protein was similar for all three species (average value – 17.20%), while the content of fat was 5.00%, 7.44% an 8.87% for common carp, silver carp and grass carp, respectively.

Comparing cholesterol content in the same fish species only in the case of silver carp from the second fish farm higher cholesterol content was determined. The lowest cholesterol content was established in crass carp from both aquacultures rearing systems (40.12% - I Fish farm; 43.82% - II Fish farm). The highest energy value (kcal/100 g) was determined in crass carp (168.75 - I Fish farm; 144.42 - II Fish farm).

Marketable size pangasius contained only 11.67% of proteins followed by 85.75% of moisture and only 0.94% of fat. The calculated energy value was only 56.14 kcal/100 g. These values indicate to low quality fish when comparing with common carp, silver carp and grass carp from the national aquaculture.

CONCLUSIONS

The obtained data indicate that crass carp compared with common carp and silver carp from both fish farms might be considered as most valuable fish.

Marketable size Cyprinidae fish family (common carp, silver carp and grass carp) from domestic aquaculture contain higher value of proteins than pangasius from Vietnam what makes these fish more adequate from nutritional point.

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TEMPERATURE DIFFERENCES OF SPRING WATER THE RIVER ZDENA AND DABAR

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TEMPERATURNE RAZLIKE U IZVORSKOJ VODI REKA ZDENA I DABAR

Abstrakt

Prema mjerenju parametara izvorskih voda rijeka Zdena i Dabar, analizirane su moguće temperaturne razlike na uzorcima koji su uzimani u isto doba dana i pri istoj temperaturi zraka. Kod ovih rezultata mjerenja upoređivanjem temperatura konstatuje se da postoje bitne temperaturne razlike ovih dviju voda.

Na osnovu ovih rezultata može se zaključiti da ove vode ipak imaju drugačije uslove tečenja i zadržavanja unutar različitih struktura terena kroz koji prolaze.

Ključne reči: *Zdena, Dabar, kraš, temperaturna razlika.*

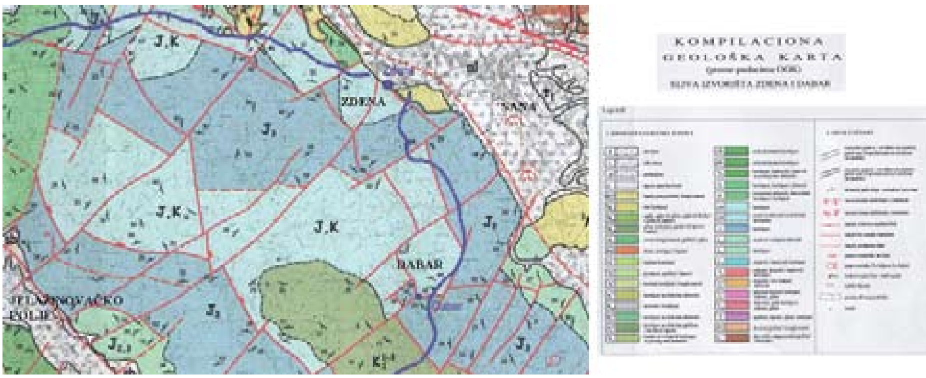
Extended abstract

By measuring the parameters of spring waters of rivers Zdena and Dabar, an analysis of possible temperature differences in the samples were taken at the same time of day and at the same temperature air. When these test results comparing the temperature it is concluded that there are significant temperature differences between the two waters. Based on these results, it can be concluded that these waters do have different conditions of flow and retention within the various structures which pass through the courts.

Zdena source, the central and the second largest source of drinking water, and located northwest approximately 2 km from the city center, a karst spring Zdena river which flows through the city. For many years this source has been the most dominant source of water supply throughout the municipality Sanski Most. Significant water resource in this region is a large karst spring Dabar, located from the city center about 10 km, with a minimum estimated yield of approximately 400 l / s. The development of long-term projections of Sanski Most, provides the abstraction and hot water supply for Dabar *(Institute of Hydro-GF in Sarajevo (1998))*.

Citing the earlier measurements of parameters of spring water river Zdena and Dabar, analyzed the possible temperature difference measurements on samples taken at the same time of day and at the same temperature air.

In taking on some samples was recorded to assess the profile of water flow. Measurement and assessment of profiles highlighting the sources was done in extreme cases. Recorded profiles and flows are different for different situations. Monitoring and analysis of the temperature of water in karst allows answering numerous questions related to the circulation of water, its origin, time spent in the underground, etc. In the rubble, there are favorable conditions for the application of geothermal research because the existence and functioning of karst channels through which the transport takes place underground water causes the temperature anomalies can be observed even on the surface. *(Bonacci, O. (2001))*



Slika 1. Kompilaciona Geological Map Zdene i Dabra
(*Đerkovic B., Đordevic D., Hohrajn J., i ostali, Sarajevo, (1975))*

Tabela 1. REZULTATI FIZIČKO KEMIJSKIH ANALIZA VODE IZ IZVORIŠTA "ZDENA" - SANSKI MOST

Parametar Uzorak	MDK	1	2	3	4	5	6	7	8	9	10	11
Datum		30.05.97	24.06.97	15.07.97	08.08.97	22.01.98	01.04.98	23.04.98	15.05.98	26.05.98	16.06.98	26.06.98
Vrijeme uzimanja uzorka		13:00	12:00	10:00	10:00	10:00	14:00	10:00	14:00	15:00	14:00	14:00
Protok Q (m^3/s)		0.827	0.484	0.355	0.205	1.280	0.710	2.1		1.91	1.48	1.19
Temperatura zraka (°C)						-1.0	9.1	15.2	21.0	20.5	18,0	28,0
Temperatura vode (°C)	8 - 12	11.7	11.5	11.8	11.6	10.4	10.2	10.7	11.0	11.0	11,0	11,3

Tabela 3. REZULTATI FIZIČKO-KEMIJSKIH ANALIZA VODE IZ IZVORIŠTA "DABAR" - SANSKI MOST

Parametar Uzorak	MDK	1	2	3	4	5	6	7
Datum		08.08.97	01.04.98	23.04.98	16.05.98	26.05.98	16.06.98	26.06.98
Vrijeme uzimanja uzorka		12:00	13:00	11:00	11:00	13:00	12:00	12:30
Protok Q (m^3/s)				29.6		21.80	8.35	4.61
Temperatura zraka (°C)		24	9.0	15.5	21,2	19,5	17,0	28,5
Temperatura vode (°C)	8 - 12	9.4	8.9	9.5	9.5	9.9	9,9	11,8

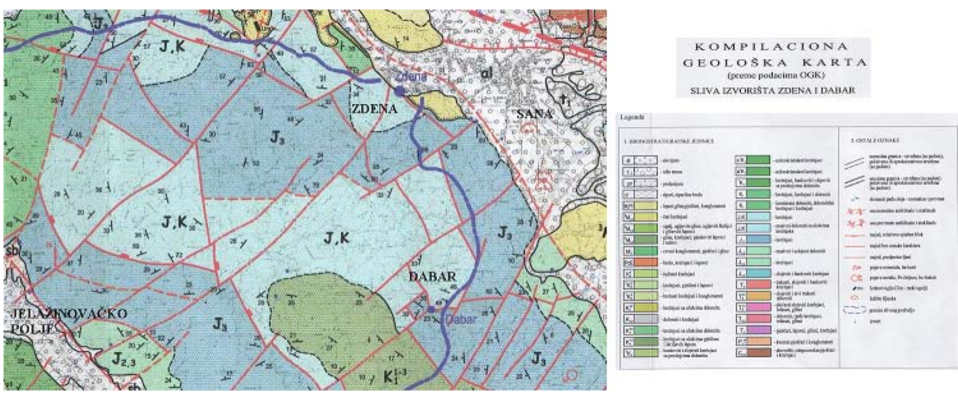
Slika 2. Table of results of some source parameters Zdene i Dabar
(*Institut za hidrotehniku GF u Sarajevu, (1998)*

The available hydrogeological background can be concluded that the structure and tectonic layers of the same origin. According topografik map shows that these two sources apart barely 10 kilometers and that are on the same left bank of the river Sana, and that they and the length of the pouring into the river Sana both short and less than 3 kilometers. For the same substrate show that faults that are found on this site are interchangeable between the reticulated Jelašinovačko field that has multiple sinks and sources Zdene and Dabar. Inserting fluoroscentih color was proved by the connections between sinks and sources Jelašinovačkom field Zdene and Dabar. What is interesting here, and it is often the case with the source that pass through the karst terrain, is that the water temperature at the exit from these sources differ.

Comparing the results of measuring the temperature of spring water Zdene and Dabar for the same day at the same time and at the same temperature it is concluded that there are significant temperature differences between the two waters. Based on these results, it can be concluded that these waters do have different conditions of flow and retention within the various structures which pass through the courts.

INTRODUCTION

Zdena source, the central and the second largest source of drinking water, and located northwest approximately 2 km from the city center, a karst spring Zdena river which flows through the city. For many years this source has been the most dominant source of water supply throughout the municipality Sanski Most. Significant water resource and karst spring Dabar, which is far from the city about 10 km, with an estimated minimum yield of about 400 l/s. The development of long-term projections of Sanski Most, provides the abstraction and hot water supply for Dabar. (*Institut za hidrotehniku GF u Sarajevu, (1998)*)

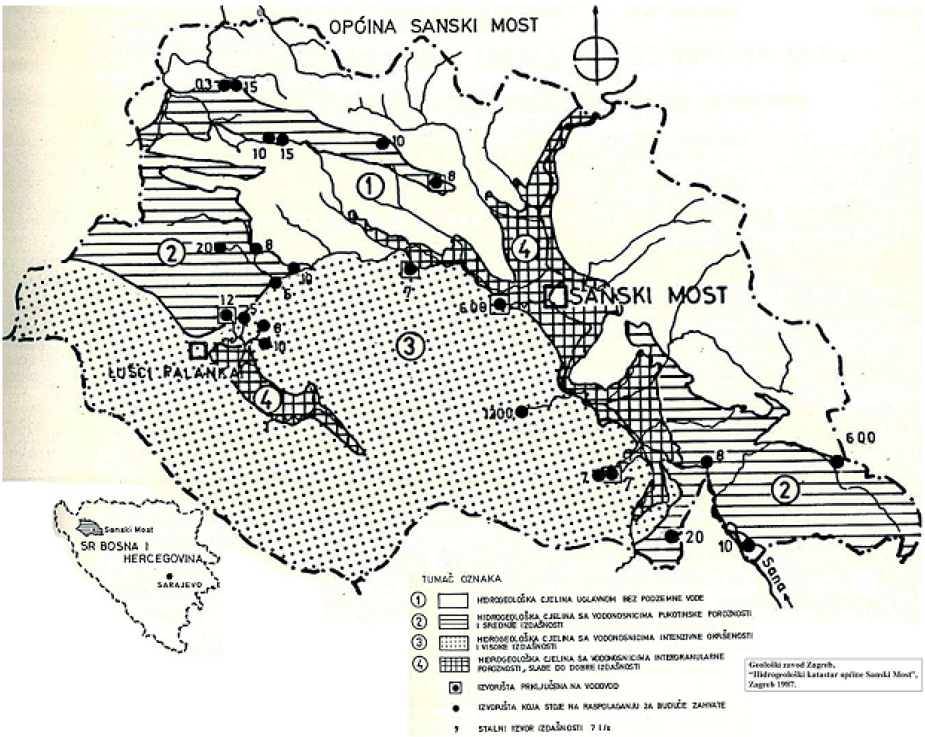


Slika 1. Kompilaciona Geological Map Zdene i Dabra (*Đerkovic B., Đordevic D., Hohrajn J., i ostali, Sarajevo, (1975)*)

Sources Zdena and Dabar are fed from the same basin, with very Grmec karst massif, in which, despite all the typical karst forms, exists a large karst depression Lušci palanka field.

Given that this is a karst springs is often blurs in periods of intense rainfall and melting snow, when in the field of water poured through the multitude of periodic sources. Swelling of the water from this region is predominantly done through a large zone of sinking in the southwest edge, near the village Jelašinovci and two small sinks near Lušci Palanka.

Injecting solutions of fluorescent colors in the abysses Jelašinovci demonstrated a direct relationship with water from the fields hot Zdena and Dabar. The mechanism of swelling and sinking in the basin, as well as the mechanisms of water in the aquifer, and given the way the use of critical areas in the hinterland of the spring, indicating a significant threat to water quality of water resources, the uncontrolled use of certain parts of the immediate catchment.



Slika 2. Hydrogeological sketch of Municipalities Sanski Most, (*Geološki zavod Zagreb, (1987*))

Tabela 1. REZULTATI FIZIČKO KEMIJSKIH ANALIZA VODE IZ IZVORIŠTA "ZDENA" - SANSKI MOST

Parametar Uzorak	MDK	1	2	3	4	5	6	7	8	9	10	11
Datum		30.05.97	24.06.97	15.07.97	08.08.97	22.01.98	01.04.98	23.04.98	15.05.98	26.05.98	16.06.98	26.06.98
Vrijeme uzimanja uzorka		13:00	12:00	10:00	10:00	10:00	14:00	10:00	14:00	15:00	14:00	14:00
Protok Q (m³/s)		0.827	0.484	0.355	0.205	1.280	0.710	2.1		1.91	1.48	1.19
Temperatura zraka (°C)						-1.0	9.1	15.2	21.0	20.5	18,0	29,0
Temperatura vode (°C)	8 - 12	11.7	11.5	11.8	11.6	10.4	10.2	10.7	11.0	11.0	11,0	11,3

Tabela 3. REZULTATI FIZIČKO-KEMIJSKIH ANALIZA VODE IZ IZVORIŠTA "DABAR" - SANSKI MOST

Parametar Uzorak	MDK	1	2	3	4	5	6	7
Datum		08.08.97	01.04.98	23.04.98	16.05.98	26.05.98	16.06.98	26.06.98
Vrijeme uzimanja uzorka		12:00	13:00	11:00	11:00	13:00	12:00	12:30
Protok Q (m³/s)				29.6		21.80	8.35	4.61
Temperatura zraka (°C)		24	9.0	15.5	21,2	19,5	17,0	28,5
Temperatura vode (°C)	8 - 12	9.4	8.9	9.5	9.5	9.9	9,9	11,8

Slika 3. Table of results of some source parameters Zdene i Dabar (*Institut za hidrotehniku GF u Sarajevu, (1998))*

MATERIALS AND METHODS

Citing the earlier measurements of parameters of spring water river Zdena and Dabar, analyzed the possible temperature difference measurements on samples taken at the same time of day and at the same temperature air. In taking on some samples was recorded to assess the profile of water flow. Measurement and assessment of profiles highlighting the sources was done in extreme cases. Recorded profiles and flows are different for different situations.

Slika 4. The results of measurements of temperature and flow

Temperature differences of spring water the river Zdena and Dabar

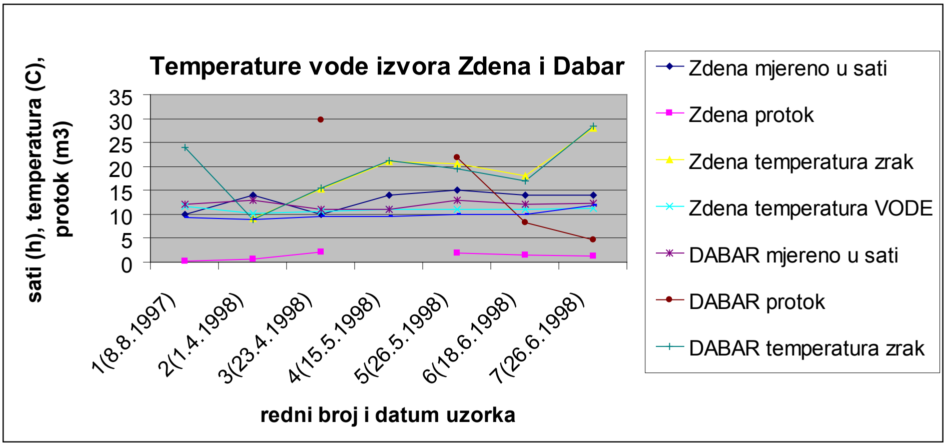
Naziv parametra	datum uzorka	8.8. 1997	1.4. 1998	23.4. 1998	15.5. 1998	26.5. 1998	18.6. 1998	26.6. 1998
redni broj uzorka		1	2	3	4	5	6	7
Zdena mjereno u sati (h)		10	14	10	14	15	14	14
Zdena protok Q (m^3/s)		0,205	0,71	2,1		1,91	1,48	1,19
Zdena temperatura zrak (ºC)			9,1	15,2	21	20,5	18	28
Zdena temperatura VODE (ºC)		11,6	10,2	10,7	11	11	11	11,3
DABAR mjereno u sati (h)		12	13	11	11	13	12	12,3
DABAR protok Q (m^3/s)				29,6		21,8	8,35	4,61
DABAR temperatura zrak (ºC)		24	9	15,5	21,2	19,5	17	28,5
DABAR temperatura VODE (ºC)		9,4	8,9	9,5	9,5	9,9	9,9	11,8

Monitoring and analysis of the temperature of water in karst allows answering numerous questions related to the circulation of water, its origin, time spent in the underground, etc. In the rubble, there are favorable conditions for the application of geothermal research because the existence and functioning of karst channels through which the transport takes place underground water causes the temperature anomalies can be observed even on the surface. *(Bonacci, Ognjen, (2001))*

RESULTS AND DISCUSSION

The available hydrogeological background can be concluded that the structure and tectonic layers of the same origin. According topografkoj map shows that these two sources apart after only 6 kilometers and is located on the same left bank of the river Sana, and that they and the length of the flow entering the river Sana both short and less than 3 kilometers.

For the same substrate show that faults that are found on this site are interchangeable between the reticulated Jelažinovačko field that has multiple sinks and sources Zdene and Dabar. Inserting fluoroscentih color was proved by the connections between sinks and sources Jelašinovačko field Zdene and Dabar. What is interesting here, and it is often the case with the source that pass through the karst terrain, is that the water temperature at the exit from these sources differ.



Slika 5. Graph of temperature and flow

Slika 6. Photo source Dabar i Zdena (*Geološki zavod Zagreb, (1987*))

When defining the size of the basin water resources Zdena and Dabar calculated the total balance of the fallen and swollen water sources Zdena and Dabar, which was calculated to be served and the size of catchment area to form:

$$F_{s} = \frac{Q_{s\ Z+D} \cdot T}{\eta \cdot P_{s}}$$

where:

$Q_{sr\ Z+D} = 4{,}86\ m^3/s$ - cumulative average flow Zdene and Dabar determined by comparison with r. Sana on V.S. Ključ.

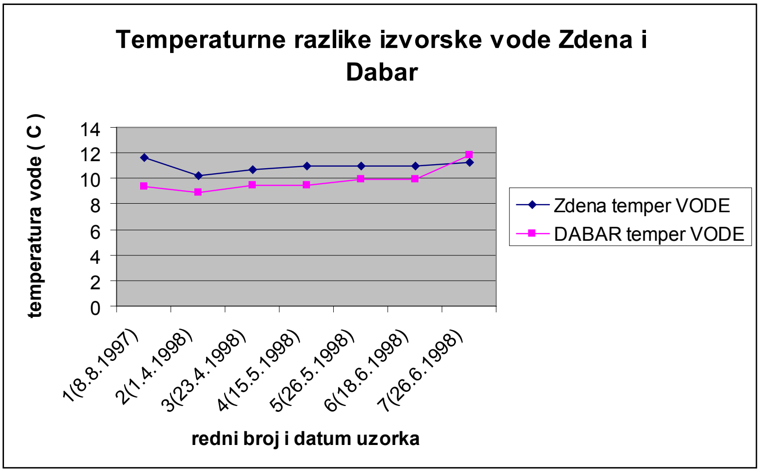
Average flow only Zdene $Qsr_Z = 0{,}4\ m^3/s$,

and Dabra $Q_{sr\ D} = 4{,}44\ m^3/s$;

$P_{sr} = 1170$ mm - The average height of precipitation throughout the basin;

$\eta = 0{,}5$ - runoff coefficient for the medium water (passed)

Using these values was obtained by a joint catchment area Zdene and Dabar, F = 262 km2, which is approximately the size of the estimated basin F = 270 km2, which is obtained by analysis of geological and hydrogeological relations. (*Institut za hidrotehniku GF Sarajevo, (2004.))*.



Slika 7. Graph of water temperature

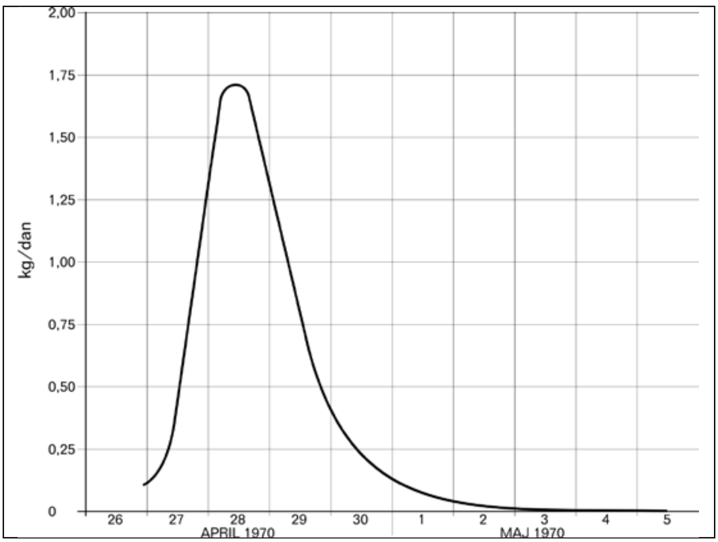
The effective rate of groundwater flow and the flow velocity of water particles is considered to be equivalent to the transport speed of a known content of the gripper through the underground facility. Velocity of groundwater in the aquifer Zdena calculated in April 1970th year, by the Republic Hydrometeorological Institute from Sarajevo, through the implementation of the project defining the Una River basin. On 23.04.1970. , in the sinking zone located in the southeastern part Lušci palanaka fields near the village Jelašinovci, inserted the 40 kg of sodium fluorescein, which was previously dissolved in an appropriate ratio of alcohol, ammonia and water. At the time of launch colors, the abyss Jelašinovci, penetrated volume of 3.62 m3 / s, while at the same time the source Zdena stressed volume of 1.15 m3 / s. Any color is countersunk into the abyss of 15-17 hours in the day. Zdena on hot colors appeared 27.04.1970. in 14 hours or 93 hours after insertion into the abyss. Coloration spring lasted until 05.05.1970. in 10 hours or seven days and 20 hours. Color appeared before the boiling Dabar, where he expired, and the greatest amount of color. Zdena the source, the maximum appeared after 4 hours of the first appearance of color and it is up 3.23 kg color or 8.1% of the total amount injected. Straight-line distance from the oblivion to hot Zdena is 12 kilometers and 10 kilometers Dabar. (*Republicki hidrometeorološki zavod, Sarajevo, (1970))*

The effective rate of groundwater wells in the basin Zdena i Dabar

Slika 8. The effective rate of flow of groundwater, obtained from the results described staining sinks Jelašinovci (*Institut za hidrotehniku GF Sarajevo, (2004.))*

Lokacija bojenja	Datum bojenja	Mjesto pojave	Udaljenost (m)	Vrijeme putovanja (sat)	Efektivne brzine (m/sat)
		Zdena	12 000	97	123,7
Ponor Jelašinovci	23.04.1970.	Dabar	10 000	81	123,4

The diagram shows changes in dye concentration in time, registered at the source Zdena (*Republicki hidrometeorološki zavod, Sarajevo, (1970)*).



Slika 9. Diagram of changes of concentration of Na-fluorescein at a time when the source Zdena staining sinks Jelašinovac (*Institut za hidrotehniku GF Sarajevo, (2004.))*

CONCLUSION

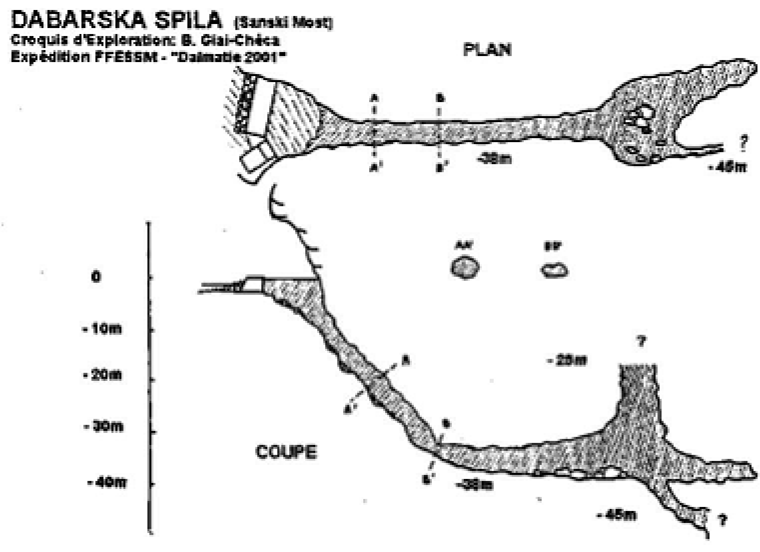
Comparing the results of measuring the temperature of spring water Zdene and Dabar for the same day at the same time and at the same temperature it is concluded that there are significant temperature differences between the two water.

Slika 10. Table of temperature difference

Temperature differences of spring water the river Zdena and Dabar

datum uzorka	1 (8.8. 1997)	2 (1.4. 1998)	3 (23.4. 1998)	4 (15.5. 1998)	5 (26.5. 1998)	6 (18.6. 1998)	7 (26.6. 1998)
Zdena temperatura VODE (ºC)	11,6	10,2	10,7	11	11	11	11,3
DABAR temperatura VODE(ºC)	9,4	8,9	9,5	9,5	9,9	9,9	11,8
razlika temperatura (ºC)	2,2	1,3	1,2	1,5	1,1	1,1	-0,5

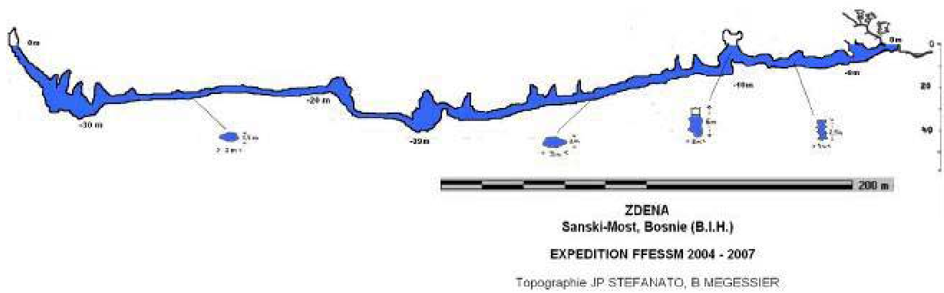
Based on these results, it can be concluded that these waters do have different conditions of flow and retention within the various structures which pass through the courts.



Slika 11. Dabar caves (*F.F.E.S.S.M., (2009))*

The direction of further research is necessary to make a comprehensive action plan on what would be any monitoring and recording of new data. This is certainly not enough to consider the abyss in Jelašinovačko field. On the south side of the field there estavelles from which the water rapidly and in large quantities appears somewhere after the fifth day after the sudden melting of snow or heavy rain. For this estavelles is interesting that during calm weather the water is withdrawn at a certain constant amount below which you never fall, regardless of summer heat. For this water estavelama is interesting to them and teperatura constant 9°C, or something deeper 8ºC, and abundant flora, such as Proteus and other fish. Slightly more than Jelašinovačko fields located in the south of the mountain behind Trovare Petrovačka field, and the West fewer Radanovo field with abysses and other karst forms. In further research should take into account the possible paths of underground water and the chemical composition of rocks where the water comes in contact.

We should not exclude the possible effects of thermal springs are located east and west at a distance of about 15 to 20 kilometers. It is certainly a bit close to mine coal and iron ore 50-10 kilometers northwest of the catchment area Blihe, Zdena, Dabar and Sane.



Slika 12. Underground Stream Zdene (*F.F.E.S.S.M., (2009))*

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ABREEDING OF TENCH FISH (*TINCA TINCA*) IN LABORATORY

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UZGOJ LINJAKA (*TINCA TINCA*) U LABORATORIJSKIM USLOVIMA

Abstrakt

Istraživanja biodiverziteta kopnenih voda Srbije sa ciljem konzervacije u in situ i/ili ex situ uslovima sprovode se u proteklih 10 godina u okviru više istraživačkih projekata. Kao rezultat ovih istraživanja formirana je aplikaciono-informativna baza podataka pod nazivom: Biodiverzitet akvatičnih ekosistema Srbije-ex situ konzervacija „BAES ex situ". Na osnovu podataka iz baze, „BAES ex situ", sprovedena su istraživanja tokom 2008. godine sa ciljem izrade Programa za unapređenje ribarstva u slivu Velike, Zapadne i Južne Morave (i dela toka Dunava, Save, kanala hidrosistema DTD i voda Pančevačkog rita). Dobijeni podaci jasno ukazuju da je linjak (Tinca tinca) u odnosu na istraživanja od pre 10 i 20 godina znatno manje zastupljen u slivu Velike Morave. Svi nalazi linjaka južno od Save i Dunava tokom ovog istraživanja su iz hidroakumulacija Srbije (Vlasina, Ćelije) i uneseni su poribljavanjem iz uzgajališta ili prirodnih voda sa područja Vojvodine. Stanje populacija linjaka prema literaturnim podacima Kirchhofer & Hefti (1996), Kottelat & Freyhof (2007) ukazuju da bez obzira na njegovo široko geografsko rasprostranjenje svuda su u manjem ili većem opadanju, a kao osnovni uzrok se navodi degradacija i uništavanje vodenih staništa, pre svega onih koja su bogata makrovegetacijom. Konstatovano stanje populacija linjaka u vodenim ekosistemima Srbije nameće potrebu konzervacije, veštačkog mrešćenja i repopulacije ugroženih prirodnih populacija. Cilj rada jeste istražiti mogućnosti uzgoja linjaka u laboratorijskim uslovima, u pogledu različite ishrane, u cilju uspešne konzervacije.

Istraživanje je sprovedeno u Akvarijumu ″Kragujevac″, koji je deo Instituta za biologiju i ekologiju Prirodno-matematičkog fakulteta u Kragujevcu. Eksperiment je trajao 11 meseci (januar-novembar 2010.). Nasadni materijal činile su jedinke donete iz ribnjaka ″Mošorin″, uzrasne kategorije 0^+.

Formirane su tri eksperimentalne grupe sa po tri akvarijuma, dimenzija 100 x 40 x 40 cm, zapremine 160 l. Ukupan broj nasađene riblje mlađi iznosio je 30 individua (≈160

g) po grupi, odnosno 10 jedinki po akvarijumu, prosečne telesne mase 5,52 g. Ishrana je vršena na sledeći način: prva grupa hranjena je kombinovanom hranom - peletirana i *Tubifex*, druga grupa animalnom hranom – *Tubifex* sp. i treća grupa peletiranom hranom. Peletirana hrana, korišćena u ishrani riba, proizvedena je u fabrici Royal optima 1P, uvoz iz Italije. Animalna hrana - Tubifex sp.donošena je iz toka reke Lepenice. Na početku eksperimenta po akvarijumu nasađeno je 10 riba prosečne mase 5,52 g, odnosno 30 jedinki po formiranoj odgovarajućoj grupi ishrane. Ishrana riba zasnivala se na količini hrane koja iznosi 1-3 % ukupne mase riba u formiranoj grupi. Premda je masa riba u akvarijumima svih formiranih grupa bila ista, bila je predviđena ishrana istom količinom hrane.

Prema literaturnim saznanjima, ali i iz godišnjih statističkih pregleda gotovo je sigurno da je proizvodnja linjaka u potpunosti potisnuta. Najčešće se ističe da je razlog za napuštanje gajenja slaba konverzija hrane.

Poređenje dužinskih i težinskih parametara na početku i na kraju eksperimenta pokazuje značajno povećanje mase riba, kao i njihove totalne dužine. Na osnovu dobijenih rezultata konstatovano je da je linjak riba koja se može uspešno gajiti u laboratorijskim uslovima, kao i da su najbolji prirast ostvarile jedinke hranjene kombinovanom hranom - peletiranom i animalnom.

Ključne reči: *Tinca tinca, uzgoj, dužinski i težinski prirast, laboratorija.*

INTRODUCTION

Tench, *Tinca tinca* (L. 1758) is one of the cyprinid fish species widespread in Europe and surrounding regions (Ćirković et al., 2009). It lives in the waters of the Danube Basin, and it is artificialy transmitted in an basin of the Beli Drim (Simonović, 2001).

Research of biodiversity of inland waters of Serbia with the aim of preserving the in situ and / or ex situ conditions were implemented in the past 10 years across multiple research projects. As a result of these studies the application-information database called: Biodiversity of aquatic ecosystems of Serbia-ex situ conservation „BAES ex situ" was formed (Simić et al., 2006). Analysis of data from the database, changes were seen in the number of populations and ranges of distribution of macroalgaes, macroinvertebrates and fishes. Based on data from the database, „Baes ex situ", research were conducted in 2008. years, to complete a program to promote fishing in the basin of Velika, Zapadna and Južna Morava (and part of the Dunav, Sava, channels DTD hydro and water of Pančevački rit). The data clearly indicate that the tench (*Tinca tinca*) with respect to research from 10 and 20 years, much less represented in the basin of the Morava. All findings of tench south of the Sava and Danube rivers in this study were from the accumulations of Serbia (Vlasina, Ćelije) and included the stocking of farms and natural waters in Vojvodina. State of populations of tench to literature data shows that regardless of its wide geographic distribution everywhere are a greater or lesser decline, and as the main cause states degradation and destruction aquatic habitats especially those that are rich macrovegetation (Kirchhofer & Hefti, 1996; Kottelat & Freyhof, 2007).

Stated condition of tench populations in aquatic ecosystems of Serbia imposes the necessity to implement conservation projects, artificial spawning and repopulating endangered natural populations (Simić et al., 2009).

The aim of this paper is to explore the possibility of breeding tench under laboratory conditions, in terms of different nutrition, in order to successful conservation.

MATERIALS AND METHODS

The research was conducted in the Aquarium „Kragujevac”, which is part of the Institute of Biology and Ecology of Faculty of Science in Kragujevac. The experiment lasted 11 months (january-november 2010). Material consisted of specimens taken from the pond „Mošorin”, age groups 0^+.

Three experimental groups with three tanks, measuring 100 x 40 x 40 cm, capacity 160 l were formed. Set on the total number of juvenile fish was 30 individuals (≈ 160 g) per group, and 10 individuals per tank, average body mass of 5,52 g. Nutrition is performed as follows: the first group was fed with combined food - pelleted and *Tubifex* sp., another group with animal food - *Tubifex* sp. and the third group with pelleted feed.

Pelleted food, used in fish nutrition, was produced by the factory of Royal Optima 1P, imported from Italy. Animal food - *Tubifex* sp. (Oligochaeta) was taken from the river Lepenica.

The percentage composition of pelleted food is shown in Table 1. Energy value of Tubifex varies depending on season and sampling location (Bakrač – Bećiraj, 2008). Considering that Tubifex we used for feeding tench is not always being taken from the same location, it is not possible to express the true energy value of foods and therefore analysis of animal food - *Tubifex* sp. is not done.

Table 1. Analytical value of pelleted food

Percentages of food	Components (%)
Crude protein	47,0
Crude fat	24,0
Crude ash	8,0
Crude fiber	1,0
Phosphorus	1,1

During the experiment, according to the plan, we have measured the following parameters:

a) chemical composition and nutritive value of food was established at the beginning of the experiment. Food consumption was recorded daily. Once a month was determined conversion of food and nutrients based on food consumption and performance measurement of weight gain in length of individuals of tench,

b) control of growth is carried out once a month and included all individuals from each of the experimental tank. Each individual was measured body weight (W) and total length (SL),

c) fish health was monitored daily and mortality noted,

d) once a month physico-chemical parameters of water (water temperature, pH, conductivity, water hardness, oxygen saturation and oxygen concentration, the concentration of phosphate, nitrate and ammonia) were measured.

RESULTS AND DISCUSSION

Satisfactory growth of fish can be achieved by proper diet and knowledge of the needs of fish for certain nutrients to their maximum utilization. In addition to quality fish food is necessary good water too. That quality includes a series of physical-chemical parameters whose values must not deviate from the boundary of the specified type (Ivance et al., 2007).

During the experiment, the physico-chemical characteristics of water have been successfully maintained in the optimal values for the tench. The values shown in Table 2 were proved as suitable for the breeding of tench. Although the values of measured parameters during the year were relatively uniform and no major variations should be noted that the tench fish which in certain conditions may be submitted by the adverse physical and chemical environmental factors. In winter, holding up to pH 4.6 and decrease the concentration of O_2 to 0.3 mg/l (Ćirković et al., 2009).

Table 2. The average annual value of physico-chemical parameters of water

Physico-chemical parameters	Values
T°	20,19
Ph	7,68
Ep (μS)	402,78
Dh (ppm)	201,45
O_2 (mg/l)	7,28
O_2 (%)	81,92
NO_3 (mg/l)	6,58
PO_3 (mg/l)	4,37
NH_4 (mg/l)	0,27

At the beginning of the experiment the tank was set on 10 fish of average weight 5.52 g as for 30 individuals per group, formed an appropriate diet. Fish feeding was based on the quantity of food which is 1-3% of the total weight of fish in the formed group. Although the mass of fish in tanks of all formed groups was the same, food was provided the same amount of food. At the end of the experimental part, the following results were related to the total length (SL) and weight (W) growth of tench (Table 3).

Table 3. The movement of the average total length and weight of fish

Date	Group I		Group II		Group III	
	length	mass	length	mass	length	mass
January	6,18	8,37	8,15	5,83	7,45	4,52
February	8,49	9,05	8,46	6,8	7,55	4,96
March	9,6	10,3	8,62	7,53	7,88	5,94
April	10,34	13,5	9,6	11,32	8,22	7,16
May	11,63	19,66	10,84	15,09	8,73	8,07
June	11,34	21,06	10,48	12,93	8,43	9,27
July	11,91	21,88	10,65	16,63	8,44	7,93
August	12,28	21,19	11,0	15,51	8,93	8,35
September	12,53	23,04	10,9	17,1	9,0	9,67
October	12,63	25,6	11,03	19,05	9,49	10,7
November	12,96	25,54	11,27	18,74	9,39	10,72

The next part presents the charts in length and weight growth of fish in the experimental groups (Fig. 1, 2, 3, 4, 5).

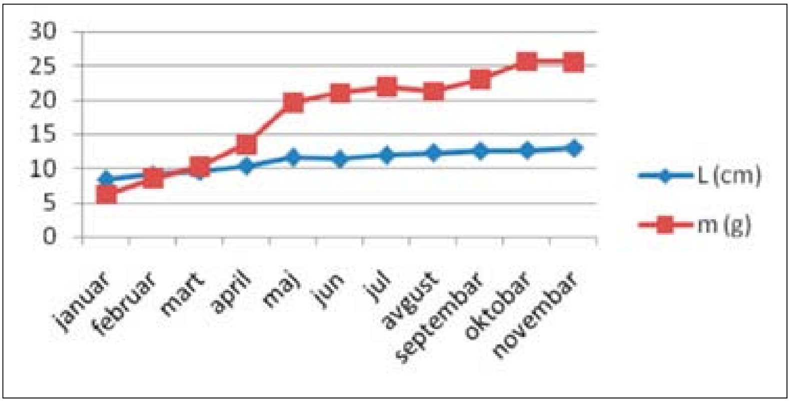

Figure 1. Graphic representation of growth in total length (SL) and weight (W) of fish in the first group (combined food - peletted and *Tubifex* sp.)

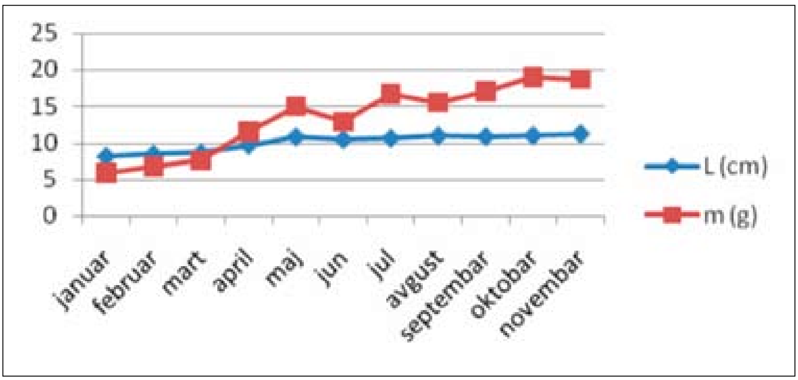

Figure 2. Graphic representation of total length (SL) and weight (W) of fish growth in the second group (animal food - *Tubifex* sp.)

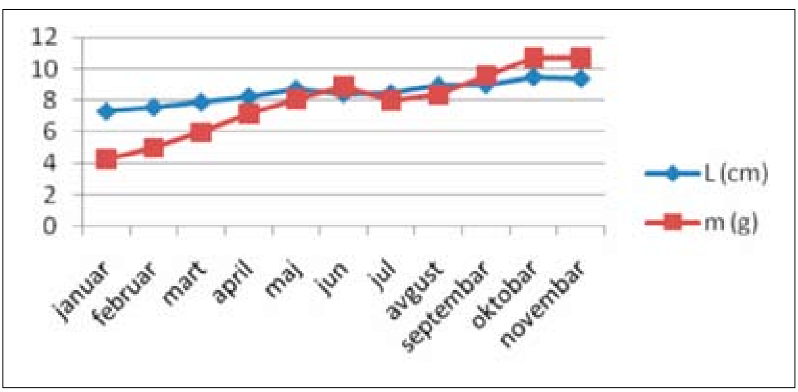

Figure 3. Graphic representation of total length (SL) and weight (W) of fish growth in the third group (pelleted food)

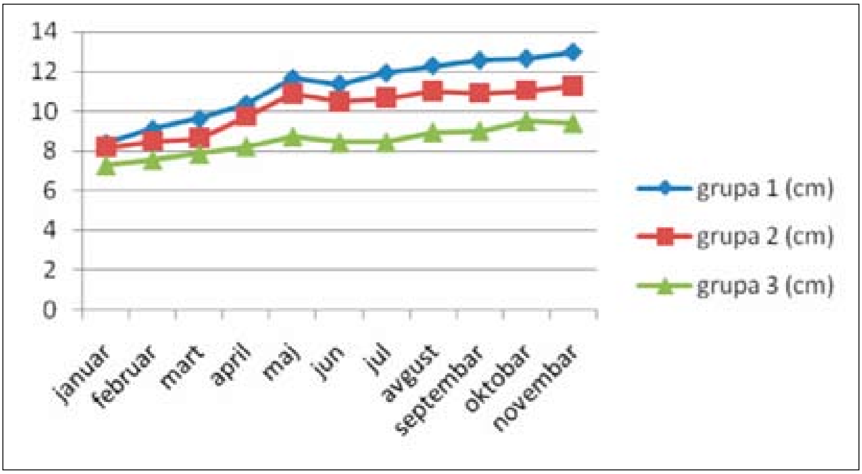

Figure 4. Comparative graphic of total length growth of fish in the experiment groups

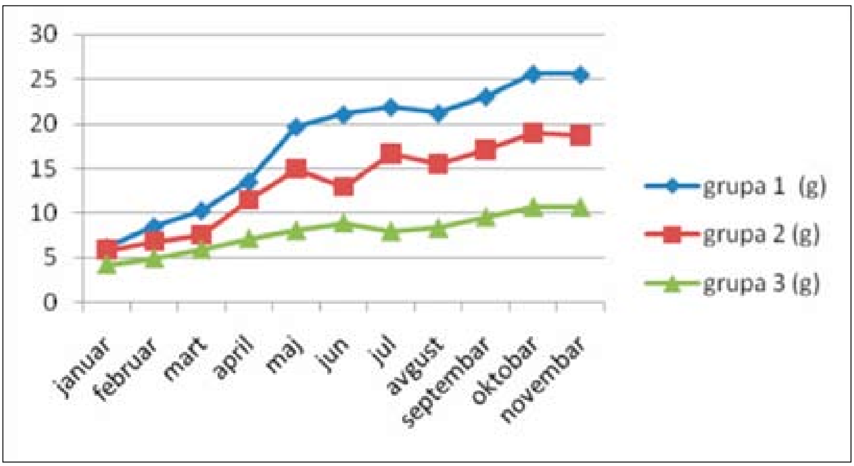

Figure 5. Comparative graphic of weight increase of fish in the experiment groups

According to information from the annual statistical review, the production of tench is almost completely suppressed. The most common stresses that the reason for leaving the poor feed conversion of growing (Ćirković et al., 2009). Comparison of lenght and weight parameters at the beginning and the end of the experiment shows a significant increase in weight of fish, and their total length. On the basis of individual and comparative graphical length and weight growth of fish (Fig. 1, 2, 3, 4, 5), it can be concluded that the greatest length and weight gain was recorded in the first experimental group, where it was applied a combined diet – pelleted and animal food.

CONCLUSIONS

During the period of eleven months an experiment aimed to determine the best type of food which affects weight gain and length of tench (*Tinca tinca*) in laboratory conditions was performed. Based on these results it was concluded that the tench can be successfully grown in laboratory conditions, and that the best gain is achieved in individuals fed with combined food - pelleted and animal. In our opinion, considering the successful breeding in laboratory conditions, we have to work on the reintroduction of tench in the aquaculture of our country.

ACKNOWLEDGEMENTS

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SEASONAL AND VERTICAL VARIATIONS OF WATER TEMPERATURE AND OXYGEN CONTENT IN THE DOSPAT RESERVOIR, BULGARIA

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SEZONSKA I VERTIKALNA VARIJACIJA TEMPERATURE VODE I SADRŽAJA KISEONIKA U AKUMULACIJI DOSPAT, BUGARSKA

Abstract

The changes in water oxygen content depending on the water temperature and depth of Dospat dam lake for the period May 2009 –April 2010 were investigated. The dam lake is situated in the Western Rhodopi Mountain. With it`s altitude of 1200 m above sea level it is the highest dam lake in Bulgaria, with a total of 22 000 dka of flooded area. The average surface layer temperature values vary from 9.6 °C (April) to 22.74°C (July). In the summer months these values are within the limits of 17.26 °C – 22.74°C (0.5 m dept) – 5.26 °C -5.47°C (30 m dept). Lower temperature values were reported during the spring (April-May) and autumn (November). The horizontal differentiation indicates that the average surface layer values of the oxygen content in the water vary from 4.22 $mg.1^{-1}$ to 11.48 $mg.1^{-1}$. In the summer months sampling points the values were within the limits of 4.22 $mg.1^{-1}$–7.47 $mg.1^{-1}$ (0.5 m depth) and respectively of 1.12 $mg.1^{-1}$ - 1.60 $mg.1^{-1}$ (30 m depth).These investigations gave the possibility to determine the periods and the depth zones with optimum values of temperature and oxygen content for trout rearing, which can be used for optimization of the technological process of the net cage aquaculture in Dospat dam lake.

Key words: *monitoring, water, temperature, oxygen content, Dospat reservoir*

INTRODUCTION

Dospat Reservoir is an artificial dam lake with considerable depressions in the bottom terrain, great depths and characteristic temperature stratification. It is situated upon a total area of 22 000 dka (22 km^2) in the Western Rhodopi Mountain, with an altitude of 1200 m above sea level and it is the highest dam lake in Bulgaria. This also determines the prolonged period of time, during which the ice cover of the dam is preserved (2-4 months) (Naumova and Zivkov, 1988).

Dospat Reservoir is a part of the hydro-energetic complex, the cascade of Dospat - Vatcha. The complex utilization for the needs of energy production, hydro-meliorations and aquaculture has motivated a number of investigations on the ecosystem and water quality of Dospat Reservoir during different periods of time after it was put in operation in 1967 (Naidenov & Sais, 1977; Zivkov, 1987; Naumova & Zivkov, 1988; Ivancheva & Sirakov, 2003).

Naumova & Zivkov (1988) have outlined two periods in the fisheries utilization of Dospat Reservoir. The first one – up to 1978-1980, which is characterized by a successful introduction and acclimatization of carp (*Cyprinus carpio*) and peled (*Coregonus peled Gmelin)* as well as with the relatively low production catches (5-10 t annually), in which the fish species of little value were dominant. The second period – after 1978-1980 is distinguished by its high production catches of a rainbow trout and by the powerful development of the intensive trout-fisheries in net cages.

The aim of this investigation was to determine the seasonal and vertical dynamics of water temperature and the quantity of the oxygen dissolved in it as limiting ecological factors affecting the processes in the aquatic environment.

MATERIALS AND METHODS

The investigation was carried out in Dospat Reservoir, with geographic co-ordinates at $41^{o}\,41^{I}\,54^{II}$ Northern latitude and at $24^{o}\,05^{I}\,10^{II}$ Eastern longitude, for the period May 2009 – April 2010. The water samples were collected from 6 stations located along the wall (1 station), around the net cages (3 stations) and on the tail (2 stations) of the Reservoir. The water temperature and the oxygen content ($mg.1^{-1}$) were determined ***in-situ*** per each meter from the surface layer (0.5 m) to the bottom layer, which depending on the dam lake bottom and the level of waters in the dam lake reaches up to 30-40 m. The measurements were done by a submerged oximeter WTW Oxi 1970i.

The data were statistically analyzed using the Microsoft Office 2007.

RESULTS AND DISCUSSION

The average surface layer temperature (Table 1) values vary from 9.6 ^{o}C (April) to 22.74^{o}C (July). In the summer months these values are within the limits of 17.26 ^{o}C – 22.74^{o}C (0.5 m dept) – 5.26 ^{o}C -5.47^{o}C (30 m dept). Lower temperature values were reported during the spring (April - May) and autumn (November). The differences between the average seasonal temperature values in the separate stations are insignificant.

The vertical temperature differentiation depending on the depth (0.5-40 m), season (spring, summer, autumn) and stations position for taking samplings are shown in Table 2. There was a larger variation of the temperature in the surface layer (up to 0.5 m) with recorded values between 9.2°C – April 2010, 16.5 °C - 17.4 °C – September and 10.0 °C - 10.3 °C November 2009, and from the 5th to the 10th meter, the values vary between 8.8 °C to 9.6 °C – May, 16.2 °C -16.5 °C – September and 9.9 °C -10.0 °C – November. The lowest temperatures were measured just above the bottom.

The highest absolute value, of 23°C, for this index, was reported on 23/24.07.2009 at Station 2 with variations of 22.1 °C to 22.9°C in the remaining stations of the epilimnon layer. These values are close to the values reported for the same month (22°C) by Naumova & Zivkov (1988).

The temperature stratification for the separate stations varies from 4.5 °C to 10.3°C (May) to 0.9 °C -1.9°C (0.5 - 5 m) – 11.1 °C - 12°C (0.5-30 m) (July - September). The zone of the temperature cline (termocline) of the stratification lake is particularly clear during the months of July-September – (8 - 15 m).

Table 1. Average values of temperature (T⁰C) and oxygen content ($mg.l^{-1}$) in Dospat reservoir in period 2009-2010

Depth 0.5 m	2009	**Average values of temperature**				Average of year
Months	May	July	September	November	April	
X	13.94	22.74	17.26	10.18	9.26	14.67
Sx	0.2387	0.1833	0.321	0.1194	0.0758	2.759
Cv	3.23	1.60	3.72	2.35	1.63	37.59
n	5	5	5	5	5	25
Depth 30 m						
X	4.52	5.26	5.47	6.15	4.70	5.22
Sx	0.055	0.0837	0.0553	0.1374	0.0354	0.3249
Cv	2.42	3.18	1.74	3.87	1.5	12.45
n	5	5	4	4	5	23
Depth 0.5 m	2009	**Average values of oxygen content**				Average of year
Months	May	July	September	November	April	
X	10.26	4.22	7.47	8.42	11.48	8.37
Sx	0.1016	0.2076	0.3757	0.1752	0.0503	1.3982
Cv	1.98	9.84	10.06	4.16	0.87	33.41
n	5	5	5	5	5	25
Depth 30 m						
X	8.05	1.60	1.12	1.24	9.08	4.22
Sx	0.3786	0.1772	0.2763	1.1137	0.1741	1.9944
Cv	9.40	22.15	42.53	155.25	3.83	94.57
n	5	5	4	4	5	23

In November the autumn homothermy was reported for stations 1-3, at water temperature of 8.9 °C - 10.2°C and depth up to 20 m. For the bottom layer (20-30 m) colder waters were reported of 6.1 °C - 6.2°C and 5.7 °C on the depth 40 m.

In the region of the net cages the surface layer temperature varies within the limits of 9.1 °C - 9.2 °C (April), 13.4 °C - 14.7°C (May), 22.8 °C - 23.0 °C (July), 16.7 °C - 17.7°C (September) and 9.90 °C - 10.3°C (November). At the 5th meter the values vary within the limits of 8.7 °C - 9.1°C (April), 8.8 °C – 10.0°C (May), 21.0 °C -21.6 °C (July), 16.4

^{o}C -16.5^{o}C (September) and 9.9 ^{o}C -10.0^{o}C (November). In general, the dam lake offers favorable conditions for trout rearing.

Table 2. Values of water temperature in Dospat reservoir (T^{0}C)

Date	depth	sampling points (station)					
	m	1	2	3	4	5	6
14/15.05.09	**0.5**	14,0	13,8	13,4	14,7	13,8	
23/24.07.09		22,1	23	22,8	22,9	22,9	
24/25.09.09		16,5	16,7	17,7	17,4	18,0	
11/12.11.09		10,2	10,0	9,9	10,3	10,5	
26/28.04.10		9,5	9,2	9,2	9,1	9,3	9,3
14/15.05.09	**5**	9,5	8,8	10	9,6	9,4	
23/24.07.09		21,2	21,1	21	21,6	21,1	
24/25.09.09		16,2	16,5	16,4	16,4	16,4	
11/12.11.09		10,0	9,9	9,9	10,0	10,1	
26/28.04.10		9,1	9,1	8,7	8,7	9,1	7,3
14/15.05.09	**10**	6,2	6,4	6,5	8,6	6,2	
23/24.07.09		12,7	13,1	13,6	13,8	12,8	
24/25.09.09		16,2	16,3	16,0	16,2	15,7	
11/12.11.09		10,0	9,9	9,8	9,8	10,1	
26/28.04.10		8,7	8,5	8,4	6,6	7,6	6,8
14/15.05.09	**20**	5,2	4,8	5,3	5	4,7	
23/24.07.09		6,6	6,2	6,2	6,2	6,6	
24/25.09.09		6,4	6,1	6,1	6,3	6,4	
11/12.11.09		9,5	8,9	9,4	7,4	6,7	
26/28.04.10		5,9	5,1	5,0	5,2	4,7	4,7
14/15.05.09	**30**	4,5	4,5	4,7	4,5	4,4	
23/24.07.09		5,4	5,2	5,4	5	5,3	
24/25.09.09			5,5	5,4	5,4		
11/12.11.09			6,1	6,2	6,1		
26/28.04.10		4,8	4,7	4,8	4,7		4,6
14/15.05.09	**40**	4,5	4,3		4,3	4,4	
23/24.07.09		4,9	4,9		4,9	4,9	5,1
24/25.09.09		5,2	5,2		5,2		
11/12.11.09			5,7		5,7		
26/28.04.10			4,5		4,5	4,6	

The vertical distribution of the oxygen content depending on the depth, season and position of stations in which samples were taken (parallel with temperature records) are shown in Table 3.

Table 3. Values of oxygen content in Dospat resevoir (mg.l^{-1})

Date	depth	sampling points (station)					
	m	1	2	3	4	5	6
14/15.05.09	**0.5**	10,16	10,25	10,07	10,2	10,6	
23/24.07.09		4,44	4,50	4,43	4,24	3,5	
24/25.09.09		7,42	6,34	8,41	7,77	7,40	
11/12.11.09		8,34	8,31	7,94	8,64	8,86	
26/28.04.10		11,58	11,45	11,53	11,55	11,33	11,11
14/15.05.09	**5**	11,06	10,1	12,13	10,76	11,53	
23/24.07.09		4,08	3,95	3,90	3,95	2,67	
24/25.09.09		5,23	7,86	8,70	7,86	6,38	
11/12.11.09		8,20	7,95	7,53	8,28	8,43	
26/28.04.10		11,34	11,3	11,41	11,24	11,22	10,86
14/15.05.09	**10**	9,80	9,26	9,95	10,76	9,76	
23/24.07.09		4,33	4,25	3,86	4,06	2,46	
24/25.09.09		4,53	6,98	3,24	7,24	3,91	
11/12.11.09		8,12	7,75	7,12	7,75	8,30	
26/28.04.10		10,99	11,15	11,29	10,6	10,95	10,53
14/15.05.09	**20**	8,82	9,09	11,46	9,29	8,67	
23/24.07.09		3,43	2,50	2,46	2,59	1,57	
24/25.09.09		3,40	3,40	2,02	4,07	3,62	
11/12.11.09		6,68	4,37	5,75	1,03	0,76	
26/28.04.10		10,56	9,99	10,03	9,80	8,76	8,59
14/15.05.09	**30**	7,88	7,06	7,88	8,82	7,69	
23/24.07.09		1,98	1,62	1,98	1,59	1,03	
24/25.09.09		1,38	1,49	1,38	0,43		
11/12.11.09		4,08	0,06	4,08	0		
26/28.04.10		9,30	9,20	9,30	9,37	8,50	
14/15.05.09	**40**	8,02	7,03		3,42	6,83	
23/24.07.09		0,99	0,21		1,10	1.00	1,02
24/25.09.09		0,30	0,24		0,06		
11/12.11.09			0,04		0,02		
26/28.04.10			6,45		6,10	8,36	

The oxygen content within the region of net cages, as well as outside them, is comparatively high during the spring (IV-V) and autumn (XI) samples, in all sampling points at depth of 0.5 m to 10 m (7.12 mg.1^{-1} – 12.13 mg.1^{-1}) and it is within the optimal limits for trout rearing. In the epilimnion layer, in July, the oxygen content in the region of net cages (stations 2, 3, 4) was in range of (3.90 mg.1^{-1} – 4.5 mg.1^{-1}). These values are lower in comparison to those reported for September (4.53 mg.1^{-1} – 7.86 mg.1^{-1}), as well as compared to the spring and autumn levels. At a greater depth, in the region of termocline and hypolimnion, oxygen deficiencies were registered and the oxygen level decreases sharply up to values below 1 mg.1^{-1}. A similar tendency is also reported by other authors (Borovec et al., 1998). In such situations the processes of biological oxidation of organic substances in the water are disturbed and conditions for accumulation of organic matter in the bottom layer of water and on the bottom are created.

The horizontal differentiation (**Table 1**) indicates that the average surface layer values of the oxygen content in the water vary from 4.22 mg.1^{-1} to 11.48 mg.1^{-1}. In the summer months sampling points the values were within the limits of 4.22 mg.1^{-1}–7.47 mg.1^{-1} (0.5 m depth) and respectively of 1.12 mg.1^{-1} - 1.60 mg.1^{-1} (30 m depth). The level of oxygen during the spring and autumn varies from 8.42 mg.1^{-1} - 11.48 mg.1^{-1} (0.5 m depth) to1.24 mg.1^{-1} - 9.08 mg.$^{-1}$ (30 m depth). In the spring samples the average values are significantly higher (P<0.001) than those registered for sommer and autumn up to depth of 30 m.

Taking into account the fact that the optimal values of temperature and oxygen content for trout fish rearing (Zaikov, 2009) are respectively in the range of 12-16^{o}C, and above 8.0 mg.1-1, and according to Directive 2006/44/EC above 9.0 mg.1^{-1} and 50% of saturation (Petrescu-Mag, 2008), then the results obtained from the research give the possibility to detemine the periods and the depth zones with optimal temperature and oxygen content within the area of the net cages (stations 2, 3, 4) in 2010.

In regard to temperature these are the months of May and September, at water layer depth of 0.5-5.0 m, and respectively in July-September, at water layer depth of 10 m. The oxygen levels were optimal in the spring and autumn for all sampling points at water layer depth up to 10 m.

The water temperature at 20th meter during the seasons investigated and for all stations was below the optimum for trout fish rearing. At this depth, only during the spring (April and May) the oxygen content was within the optimal levels. Having in mind that the net cages are located at a depth of up to 20 m, then the results from the present investigation can be used for technological process optimization of the net cage aquaculture in Dospat dam lake.

CONCLUSIONS

The dependencies between water temperature, oxygen content of the water and depth in seasonal aspect were determined.

For the summer period (July to September) there are clearly defined termocline and areas of temperature stratification for the separate sampling points. The heterograde profile of the oxygen content describes its specific alteration in the epilimnion.

The investigation gives the possibility to determine the periods and the depth zones with optimal values of the temperature and oxygen content of the water for trout rearing, which can be used for optimization of the technological process of the net cage aquaculture in Dospat dam lake.

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FISH AS A BIOLOGICAL INDICATOR IN ASSESING WATER QUALITY OF THE RIVER TAMIŠ

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RIBE KAO BIOLOŠKI INDIKATOR U PROCENI KVALITETA VODE REKE TAMIŠ

Abstrakt

Materijal za istraživanje ihtiofaune Tamiša sakupljen je avgusta i oktobra 2009. u periodu srednjeg vodostaja kao i aprila 2010. godine u periodu visokog vodostaja na lokacijama Sečanj, Banatski Despotovac i Opovo. Prikupljanje uzoraka vršeno je pomoću aparata za elektroribolov i stajaćim mrežarskim alatima dužine od 37 do 100 metara, promera okaca od 45 do 100 mm i dubine 3 do 5 metara. Ovom prilikom evidentirano je 28 vrsta riba iz 8 familija. Prema sastavu ihtiofaune određen je indeks saprobnosti prema Pantle-Buck-u koji iznosi 2.15 i koji ukazuje na to da voda Tamiša pripada drugoj klasi boniteta.

Ključne reči: *ihtiofauna, Tamiš, indeks saprobnosti*

INTRODUCTION

Fish are highly sensitive to changes in the river flow, damming of watercourses, habitat destruction, temperature increase and intensive use of water. Therefore, the water flow, oxygen levels, temperature, aeration and pollution determine the structure of fish fauna. Any change in ecological conditions results in changes within the fish population. Changes in species diversity clearly indicate that significant changes in one or more factors have occurred in the aquatic ecosystem. At the same time the rare and vulnerable species are always in the greatest threat. Endangering or extinction of some species reduces the gene pool of a certain area and for this reason more attention is given to the protection of ecosystems in order to preserve and maintain them for as long as possible.

This paper gives the qualitative and quantitative analysis of the ichthyofauna.

Given the complex research of the Tamiš River (Pujin et al., 1987; Marković et Svirčev, 1998) and the statement that, in the earlier period, there have been changes in the physical, chemical, and biological data, the aim of this paper is to show if the qualitative and quantitative composition and the structure of ichthyofauna have changed, and to indicate the state of water quality based on these data.

MATERIAL AND METHODS

Description of sites

The Tamiš is the longest river in Banat and its basin has the total of 10.352 km^2 (without the Brzava River). After the Tisa River, the Tamiš is the most significant left tributary of the Danube in the Danube basin in Serbia. The Tamiš rises in the north-eastern slopes of Semenic Mountains in Romania and empties into the Danube near Pančevo. The length of the whole river flow is 339.7 km, of which 118 km is in Serbia. The surface of the basin area in Serbia covers 5104 km^2 (Prohaska, 1998).

The Tamiš is characterized by a very imbalanced flow. This can be seen within one year period, as well as by comparing specific years. High water levels appear in late winter and early spring as a result of snow melting, and in summer as a result of heavy rains. At its lower course, the Tamiš is under the influence of the Danube's high water levels. The low waters usually occur in the period from September to October. In the period of low water levels, with its connecting channel near Kuštilj, the Tamiš complements the Begej low water levels. In the dry season, in the whole sector of the Tamiš in Serbia, the water flow decreases to a minimum so that the bed is almost dried out (Prohaska, 1998).

The Tamiš is included in the Main Channel Network of the Danube-Tisa-Danube Hydro-system (OKM HS DTD), which significantly improves its water regime, especially concerning the low water levels.

Material for the research of the Tamiš ichthyofauna has been collected in August and October 2009, during the medium water levels period and in April 2010, during the high water levels period at the locations Sečanj, Banatski Despotovac and Opovo. The samples were collected by using the electrofishing device PERM-MB with an output power voltage 115-565 V (pulse frequency 20-200 Hz; direct current power 650 W, pulsed current power 1200 W), according to electrofishing standards "Water Analysis-Fishing with Electricity" (EN 14011, Cen, 2003) and stake fishing nets 37-100 meters long, having a mesh diameter 45-100 mm and 3-5 meters deep.

For the determination of fish species the following key was used (Vuković et Ivanović, 1971; Simonović, 2001; Pinter, 2002; Harka et Sallai, 2004).

The saprobity index was calculated by the Pantle & Buck Method (1955) (Grginčević et Pujin, 1998) on the basis of the ichthyofauna structure according to the following formula:

$$S = \frac{\sum(h \times s)}{\sum h}$$

where h – estimated frequency of occurrence of each species, and s – saprobic index of each individual species.

RESULTS AND DISSCUTIONS

During the Tamiš research in August-October 2009 and in April 2010, the total of 28 species of fish from 8 families were registered at 3 sites (Sečanj, Banatski Despotovac and Opovo), which is nearly the half of the recorded species in the waters of Vojvodina.

Of the total number of recorded species in Tamiš, 21 are indigenous, and 7 are allochthonous (*Lepomis gibbosus*, *Ameiurus melas*, *Carassius gibelio*, *Hypohthalmichthys molitrix*, *Arystichthys nobilis, Pseudorasbora parva* i *Percottus glenii).* The first two species were introduced from North America and the remaining from the Far East and all have successfully acclimatised.

Table 1. The total proportional ichthyofauna composition of the Tamiš River 2009-2010.

FAMILY AND SPECIES OF FISH	%
Fam. Esocidae	
Esox lucius – pike	1.81
Fam. Percidae	
Sander lucioperca – pike-perch	1.57
Sander lucioperca – Volga pikeperch	0.18
Perca fluviatilis – European perch	0.48
Fam. Centrarchidae	
Lepomis gibbosus – pumpkinseed sunfish	1.57
Fam. Siluridae	
Silurus glanis – wels catfish	1.02
Fam. Ictaluridae	
Ameiurus melas – black bullhead	12.28
Fam. Cyprinidae	
Cyprinus carpio – common carp	1.32
Carassius gibelio – Prussian carp	4.09
Abramis brama – common bream	2.83
Abramis ballerus – blue bream	1.02
Abramis sapa – white-eye bream	0.06
Rutilus rutilus – common roach	6.08
Scardinius erythropthalmus – common rudd	0.72
Alburnus alburnus – common bleak	42.75
Leuciscus cephalus – European chub	0.96
Leuciscus idus – ide	0.42
Hypophthalmichthys molitrix – silver carp	2.71
Arystichthys nobilis – bighead carp	0.18
Aspius aspius – asp	1.99
Blicca bjoerkna – silver bream	4.58
Vimba vimba – vimba bream	0.06
Rhodeus sericeus – Amur Bitterling	7.10
Gobio albipinnatus – white-finned gudgeon	0.06
Pseudorasbora parva – topmouth gudgeon	3.79
Fam. Gobiidae	
Neogobius fluviatilis – monkey goby	0.06
Neogobius marmoratus	0.06
Fam. Odontobutidae	
Perccottus glenii – Chinese sleeper	0.24
TOTAL	100.00

By comparing the Tamiš ichthyofauna composition for the 2009-2010 research period with the data of commercial or sport fishing on the "Tamiš I" in the period 1977-1986, which were published in the plan for improving and exploitation of this fishing area (Pujin et al., 1987), it can be stated that in 2009-2010 following species were not registered: *Leuciscus leuciscus* – common dace, *Ctenopharyngodon idella* – grass carp, *Tinca tinca* – tench, *Chondrostoma nasus* – common nase, *Gobio gobio* – gudgeon, *Barbus barbus* – common barbel, *Pelecus cultratus* – sabre carp, *Carassius carassius* – crucian carp, *Barbatula barbatula* – stone loach, *Misgurnus fossilis* – European weatherfish, *Cobitis elongatoides* and *Gymnocephalus cernua* – Eurasian ruffe; but the following species were recorded which were not present in the previous period: *Pseudorasbora parva*, *Sander volgensis*, *Gobio albipinnatus*, *Neogobius fluviatilis* and *Neogobius marmoratus*. *Carassius carassius* – crucian carp and *Tinca tinca* – tench are almost completely extinct in the waters of Serbia and they are on the list for the Red Book for Serbia.

In comparison to the 90s of the 20th century (Maletin et al., 1998), during the entire research period 2009-2010 *Ctenopharyngodon idella* and *Tinca tinca* were not registered, but *Pseudorasbora parva*, *Percottus glenii*, *Sander volgensis*, *Gobio albipinnatus*, *Neogobius fluviatilis* and *Neogobius marmoratus* were recorded.

Ecological analysis of ichthyofauna in terms of individual contribution showed domination of common bleak – *Alburnus alburnus* (42.75 %) and black bullhead – *Ameiurus melas* (12.28 %). The dominant species are Amur Bitterling – *Rhodeus sericeus* (7.1 %) and common roach – *Rutilus rutilus* (6.08), and they are followed by silver bream *Blicca bjoerkna* (4.58 %), silver Prussian carp – *Carassius gibelio* (4.09 %), topmouth gudgeon – *Pseudorasbora parva* (3.79 %), white-eye bream – *Abramis brama* (2.83 %) and silver carp – *Hypophthalmichthys molitrix* with 2.71% in the individual contribution. The species that have share less than 2% are: asp – *Aspius aspius*, pike – *Esox lucius*, pike-perch – *Sander lucioperca*, pumpkinseed sunfish – *Lepomis gibbosus*, common carp – *Cyprinus carpio*, blue bream – *Abramis ballerus* and wels catfish – *Silurus glanis*.

Based on the ichthyofauna structure, the saprobity index according to Pantle & Buck is 2.15, which indicates that the Tamiš water is in the second class.

CONCLUSION

Based on the research of Tamiš ichthyofauna in the period August-October 2009 and in April 2010 at three sites (Sečanj, Banatski Despotovac and Opovo), the following can be conluded:

-There was total of 28 fish species from 8 families recorded. Of these, 21 are indigenous, while 7 are allochthonous and all have acclimatized successfully.

-Ecological analysis of ichthyofauna in terms of individual contribution showed domination of common bleak – *Alburnus alburnus* and black bullhead – *Ameiurus melas*.

-The saprobity index on the basis of ichthyiofauna structure is 2.15 according to Pantle & Buck, which indicates that the Tamiš water is in the second class.

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PRELIMINARY RESULTS ON SUCCESSFUL STOCKING OF PIKEPERCH (*SANDER LUCIOPERCA* L.) IN THE ZLATAR RESEVOIR

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PRELIMINANARNI REZULTATI O USPEŠNOM NASAĐIVANJU SMUĐA (*SANDER LUCIOPERCA*) U AKUMULACIJU ZLATAR

Abstrakt

Zlatarsko jezero je nastalo izgradnjom hidroelektrane „Kokin Brod" koja je počela sa radom 1962. godine. Brana izgrađena na reci Uvac dugačka je 1264 m i visoka je 83 m. Jezero se nalazi na nadmorskoj visini od 880 m. Prosečan godišnji doticaj Uvca je 12.45 m^3/s, a površina sliva jezera je 1057 km^2. U basenu jezera akumulirano je 250 miliona m^3 vode. Voda jezera je visokog kvaliteta i kreće se u granicama druge klase (Stanković, 2005). Riblje naselje koje se danas sreće u akumulaciji je nastalo na bazi ribljih vrsta koje su ishodno naseljavale tok Uvca i na bazi vrsta koje su unete različitim višekratnim poribljavanjima (Mićković i Hegediš, 2007). U cilju regulisanja brojnosti i gustine prenamnožene populacije ukljeve (*Alburnus alburnus*) u jezero je 2005. godine introdukovan smuđ (*Sander lucioperca*).

Na bazi uporednog pregleda sastava ribljeg naselja u akumulaciji „Zlatar" u periodu od 2003. do 2010. godine konstatovano je da je introdukcija smuđa donela značajne promene u strukturi zajednice riba: jasna dominacija domaćih (klen, plotica, skobalj, rečna mrena) i unetih (šaran, babuška) ciprinidnih riba po abundanciji (72,7% i 86,9% u 2003. odnosno 2007.) i masenom udelu (51,4%, odnosno 82%) je značajno smanjena u 2010. godini (abundancija 40,3%, maseni udeo 58,3%), zastupljenost salmonidnih riba (jezerske zlatovčice) kontinuirano opada (abundancija 27, 3%, 10,9%, 0,6%, maseni udeo 48,6%, 17,9%, 1,8%, u 2003, 2007 i 2010, respektivno).

Karakteristike dužinsko-težinskog odnosa i rasta smuđa u Zlatarskom jezeru su takve da se u uzrastu 3+ dostiže lovna veličina od 40 cm sa masom tela od oko 450 g, dok se uzrast 4+ karakteriše prosečnom dužinom oko 50 cm i masom oko 1 kg, što se može

oceniti kao sasvim zadovoljavajućim odlikama rasta. Koeficijent alometrije od 3,081 ukazuje na proporcionalan prirast mase u odnosu na dužinu tela.

Inspekcijom digestivnog trakta utvrđeno je da 95,3 % ishrane smuđa u jezeru čini uklјeva: od ukupno 21 primerka riba u ishrani bilo je 20 uklјeva i jedan primerak jezerske zlatovčice.

Prema Richa *et al.*, (2009) ihtiocenoza akumulacije „Zlatar“ se, sa aspekta stabilnosti zajednice riba, do unošenja smuđa nalazila u visoko stabilnoj „cyprinid-phase“, odnosno „riverine species phase“ u skladu sa Kubečka (1993). Introdukcija i aklimatizacija smuđa je tu stabilnost pomerila ka tranzitnoj „percid-cyprinid-phase“ (Kubečka, 1993). Dalja istraživanja pokazaće da li se zajednica riba kreće ka visoko dinamičnoj i nestabilnoj „perch-phase“ ili se vraća stabilnoj „cyprinid-phase“ (Richa *et al.*, 2009). Od toka pomenutog procesa zavise mere ribarstvenog gazdovanja koje će se primeniti u akumulaciji „Zlatar“.

***Ključne reč**: introdukcija, akumulacija, „Zlatar“*

INTRODUCTION

Zlatar reservoir was created by constructing a hydro power plant “Kokin Brod” that began working in 1962. The dam that was built on the Uvac River is 1264 m long and 83 m high. The reservoir is situated at an altitude of 880m. The average annual flow of Uvac is 12.45 m^3/s, and the area of the lake basin is 1057 km^2. The lake basin has accumulated 250 million m^3 of water. The area of the Zlatar reservoir is 7.25 km^2, and the maximum depth is up to 75m. During the year, the amplitude of water level can reach 45m (Stanković, 2005). Fish community, which is now present in the reservoir, was created based on fish species that initially inhabited Uvac river and species which were several times introduced by restocking (Mićković and Hegediš, 2007). Nikčević *et al.* (2003) and Mićković and Hegediš (2007) reported that the reservoir is inhabited by a numerous population of bleak (*Alburnus alburnus*). Pikeperch (*Sander lucioperca*) was introduced into the reservoir in 2005, in order to regulate the population density of this species. This article presents the preliminary results of the pikeperch’s successful acclimatisation into the Zlatar reservoir.

MATERIAL AND METHODS

Samples of the fish fauna were collected in October 2010. For sampling the following fishing nets were used:

- benthic gillnets 20 m x 2 m, mesh size 20 mm; 60 m x 3 m, mesh size 30 mm; 30 m x 1.5 m, mesh size 50 and 60 mm;
- benthic trammel nets 30 m x 1.5 m, mesh size 40 and 60 mm.

Nets were set in the evening and lifted the next morning. Length-weight relationship, growth and diet of the pikeperch were analyzed based on the length frequency distribution and stomach content analysis in 62 caught specimens. The data about the fish community structure from years 2003. and 2007. were obtained from Nikčević *et al.* (2003) and Mićković and Hegediš (2007).

RESULTS

Comparative review of the fish community composition in the Zlatar reservoir for the 2003.-2010. period (Table 1.), showed that the introduction of pikeperch has induced significant changes to the fish community structure. Clear domination of cyprinids in abundance (72.7% and 86.9% in 2003 and 2007. respectively) and mass proportion (51.4% and 82% in 2003 and 2007. respectively) has significantly decreased in 2010. (abundance 40.3%, mass proportion 58.3%). Participation of salmonid species has been continuously declining (abundance 27.3%, 10.9%, 0.6%, and mass proportion 48.6%, 17.9%, 1.8%, in 2003., 2007. and 2010., respectively). However, data from 2010 should be taken with caution, because the nets were placed in the shallow zones (< 10 m) of the reservoir, while most of the Arctic char population is usually found at depths exceeding 20 m.

Characteristics of the length-weight relationship and growth of the pikeperch in the Zlatar reservoir are shown in Figure 1. At the age 3^+ specimens reach minimum landing size of 40 cm, while the age 4^+ is characterized by average length of about 50 cm and 1 kg mass, which can be described as quite satisfactory growth characteristics. Alometry coefficient of 3.081 indicates a proportional increase in the mass relative to body length.

Table 1. Review of the composition of the fish community between 2003 and 2010.

Fish species	2003.		2007.		2010.	
	% number	% biomass	% number	% biomass	% number	% biomass
Arctic char *Salvelinus alpinus*	27,3	48,6	10.9	17.9	0.6	1.8
Danubian roach *Rutilus pigus*	4,5	2,6	17.4	6.6	20.5	17.6
Chub *Squalius cephalus*	45,5	37,7	8.7	28.2	8.7	12.1
Nase *Chondrostoma nasus*	-	-	23.9	16.9	8.1	18.5
Barbel *Barbus barbus*	18,2	9,4	28.2	1.8	1.2	4.8
Carp *Ciprinus carpio*	4,5	1,7	2.2	26.1	1.9	1.7
Prussian carp *Carassius gibelio*	-	-	6.5	2.4	0.6	1.6
Bleak *Alburnus alburnus**	*	*	*	*	19.3	2
Pumpkinseed *Lepomis gibosus*	-	-	2.2	0.1	0.6	0.1
Pikeperch *Sander lucioperca*	-	-	-	-	38.5	39.8
* - in all of the three given years, a very high representation of the bleak population has been acknowledged in the reservoir, which is expressed in tens of kilogrames per hectare						

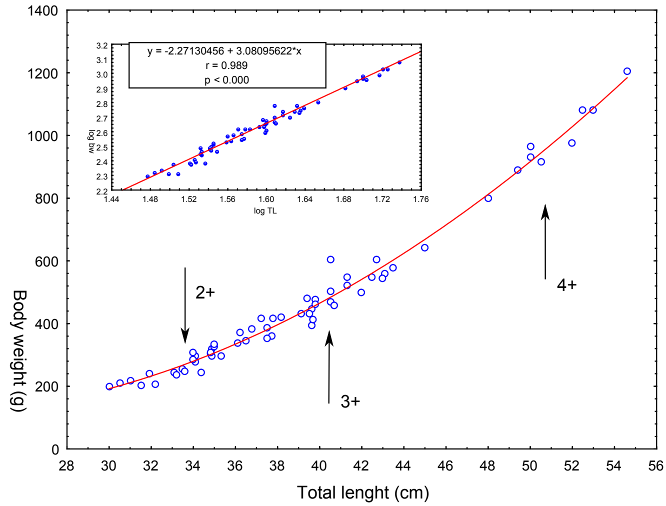

Figure 1. The length-weight relationship and growth of the pikeperch in the Zlatar reservoir

Analysis of stomach content showed that 95.3% of the pikeperch diet consisted of bleak, the remaining being Arctic char.

DISCUSSION

Lake biomanipulation theory (Shapiro and Wright, 1984) assumes that the introduction and subsequent population increase of piscivorous fish species in the reservoir will induce abundance planktivorous fish species. Although there are some doubts about the effects of biomanipulation (De Melo *et al.,* 1992), this restoration technique was applied in a number of cases in European stagnant waters (Jacobsen *et al.,* 2004; Gulati and Van Donk, 1989; Salonen *et al.,* 1996). Drenner and Hambright (1999), based on experiences from 39 lakes worldwide, defined 5 types of biomanipulative techniques and determined their efficiencies: piscivore stocking (28.6%), piscivore stocking + partial fish removal (60%), partial fish removal (90%), elimination of fish (40%), and elimination of fish followed by restocking (66.7%).

In the time scale, the observed changes in the fish community of the Zlatar reservoir followed similar pattern which was reported for considerable number of highland reservoirs in Serbia: 1) the dominance of autochthonous cyprinids indigenous in the river course accompanied with significant presence of autochthonous salmonids; 2) introduction of autochthonous cyprinids and allochthonous salmonids as well as unintentional introduction of autochthonous and allochthonous species. After that, a rapid population growth of some unintentionally introduced species occurs (bleak in the Zlatar reservoir) accompanied by declining participation of autochthonous salmonids. With aim to regulate overcrowded bleak population in the Zlatar reservoir, the "piscivore stocking" type of biomanipulation techniques was applied. The stocking of pikeperch was conducted in 2005 (930 kg, age 1^{+}, average weight 250 g) and the first positive results were noted in 2008. In 2010 the presence of stable and reproductively successful population was recorded. Diet analysis showed that pikeperch feed almost exclusively on bleak thus indicating that growth of pikeperch population was directly related to the state of bleak population, while there were no significant effects on the rest of the species.

The fish community in the Zlatar reservoir before the introduction of pikeperch was in a highly stable cyprinid phase (Richa *et al.,* 2009), i.e. "riverine species phase" in accordance to Kubečka (1993). Introduction and acclimatization of pikeperch has pushed this stability towards a transit "percid-cyprinid-phase" (Kubečka, 1993). Additional studies are needed in order to monitor further development of fish community. According to Richa *et al.,* (2009), two processes may occur, developing of fish community towards a highly dynamic and unstable "perch-phase", or its returning to the stable "cyprinid-phase". Fishery management measures that should be applied will depend on the aforementioned processes.

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RELATION BETWEEN AUTOCHTHONOUS AND ALLOCHTHONOUS FISH SPECIES IN SOME SERBIAN RESERVOIR

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ODNOS AUTOHTONIH I ALOHTONIH RIBLJIH VRSTA U NEKIM AKUMULACIJAMA SRBIJE

Abstrakt

Poslednjih godina u svetu je veoma aktuelno pitanje alohtonih ribljih vrsta. Bilo da su unete slučajno ili namerno, alohtone vrste najčešće uspevaju da opstanu u novom okruženju, u kojem zbog degradiranosti staništa i narušenih biocenoloških veza vremenom postaju dominantne. Alohtone vrste često imaju invazivan karakter jer se brzo i nekontrolisano šire u nove ekosisteme gde se ponašaju kao kompetitori za prostor i hranu autohtonim vrstama i polako ih potiskuju. Ovim radom prikazan je odnos u brojnosti i biomasi autohtonih i alohtonih ribljih vrsta u nekim akumulacijama u Srbiji.

Tokom istraživanja obraćena je pažnja na veličinu i starost akumulacija u kojima je praćena ihtiocenoza. Istraživanje je obuhvatilo 15 akumulacija različite starosti, tipa postanka, morfometrijskih, fizičkih i hemijskih osobina.

Konstatovano je prisustvo 20 vrsta riba iz 9 familija, sa izrazitom dominacijom porodice Cyprinidae. Od ovog broja 8 vrsta su alohtone i pripadaju familijama Salmonidae (Oncorhynchus mykiss), Cyprinidae (Carassius auratus, Arystichtys nobilis, Hypophthalmichthys molitrix, Ctenopharyngodon idella, Pseudorabora parva), Ictaluridae (Ictalurus nebulosus - cverglan) i Centrarchidae (Lepomis gibbosus - sunčica) (Simić i Simić, 2009). Cverglan i sunčica potiču iz Severne Amerike, dok su ostale azijske vrste.

U najvećem broju akumulacija, po brojnosti i biomasi, zastupljenije su autohtone riblje vrste u odnosu na alohtone. Izuzetak je Oblačinsko jezero, koje pripada grupi malih i starih jezera, gde brojnošću dominiraju alohtone vrste sa 61,63%, kao i biomasom sa čak 80,18%. Velika zastupljenost biomase alohtonih ribljih vrsta je i u jezeru Buljanka, 53.43%, dok njihova brojnost iznosi 17.07%. Najveći udeo u biomasi ima sivi tolstolobik. Autohtona ihtiocenoza dominira u jezeru Ćelije i Zavojskom jezeru koja su u grupi starijih i većih jezera. U ovoj grupi su i akumulacije Gruža, Vlasina i Bovan, gde se zbog neplanskog poribljavanja brojnost alohtonih vrsta približava abundanci autohtonih. Sa druge strane, planskim poribljavanjem očuvana je autohtona ihtiofauna u Miloševom i Ribničkom jezeru, koja su male veličine i stara.

Konstatovano je da u najvećem broju jezera i dalje dominiraju autohtone vrste u odnosu na alohtone. Uticaj alohtonih ribljih vrsta je uvek veći u manjim i starijim akumulacijama, upravo zbog većeg diverziteta autohtone zajednice u njima. Poznajući invazivni karakter i negativan efekat alohtonih vrsta na ekosisteme u koje su unete, kao i na faunu, neophodno je stalno pratiti stanje na terenu.

Ključne reči: *akumulacije Srbije, autohtone riblje vrste, alohtone riblje vrste.*

INTRODUCTION

For the last few years the worlds highly topical issue was question of alien fish species. Whether, they have been entered accidentally or intentionally, because of the greater degradation of habitat and degradation in community relations, they usually manage to survive in the new environment. Some introduced species are often invasive character, because of the rapid and uncontrolled spread into new ecosystems, where they act as competitors for space and food with autochthonous species, slowly pushing them. Ciruna et al. (2004) considered invasion phenomenon that affects autochthonous biodiversity, particularly in the fresh waters of temperate climate.

In Serbian landwater ecosystems were entered by accident or on purpose 22 non-native fish species, four of which occupy 51% of the inland waters of Serbia, and five between 21-50% of that territory (Lenhardt et al., 2010).

The aim of this paper is to show the relation between autochthonous and non-autochthonous fish species in some reservoirs in Serbia. During the research, we followed by the numerical ratio of native species and non-native and the relation of biomass.

MATERIALS AND METHODS

Qualitative and quantitative analysis of the ichthyocenoses were performed since 2003 to 2010 year. The research included 15 reservoirs different ages, type of formation, morphometric, physical and chemical properties. Reservoirs greater than 20ha are: Lake Gruza, Vrutci, Potpec, Celije Lake, Barije, Bovan, Zavojsko and Vlasinsko Lake, and smaller reservoirs are: Ribnicko, Rastovnicko, Bagrdan, Milosevo, Lake Buljanka, Oblacinsko and Jovacko Lake. Young reservoirs are Vrutci, Barije, Bagrdan, Buljanka and Jovacko Lake, while the other reservoir are older then 20 years.

Fishes were collected using nets in length from 10 to 400m, a width of 2 to 10m with mesh size 10mm and 100mm. Part of samples were collected by angling and electrofishing..

Identification of the ichthyologic material was carried out in the field using standard methods (Ladiges und Vogt, 1979; Wheeler, 1983; Simonović, 2006).

RESULTS AND DISCUSSION

The results of this study are presented in Table 1. During the research we determined the presence of 20 fish species from nine families, with a distinct dominance of the family Cyprinidae. Eight species are non-native and belonging to the families Salmonidae

(1 species), Cyprinidae (5 species brown bullhead (*Ameiurus nebulosus*)), Ictaluridae (1 species) and Centrarchidae (1 species) (Simić i Simić, 2009). Brown bullhead (*Ameiurus nebulosus*) and pumpkinseed (*Lepomis gibbosus*) are from North America, while the others are Asian species.

Through the analysis of ichthyocenoses was made a conclusion for the dominance of native species: bleak (*Alburnus alburnus*), bream (*Abramis brama*) and perch (*Perca fluviatilis*), as a subdominant species there are carp (*Cyprinus carpio*) and chub (*Squalius cephalus*). Among the allochthonous species dominates the prussian carp (*Carassius auratus*) in 12 of 15 lakes, and with little difference followed by pumpkinseed and bullhead.

Table 1. The aspect of quantitative composition and biomass of native and non-autochthonous fish species in some reservoirs of Serbia

	% native species		% non-autochthonous species	
	number of species	biomass	number of species	biomass
Gruza Lake	56,53	71,54	43,46	28,46
Vrutci Lake	73,81	83,62	26,19	16,38
Potpec Lake	81,59	90.59	18,75	9,41
Celije Lake	98,59	99,96	1,41	0,03
Vlasinsko Lake	63,68	71,32	36,32	28,68
Barije Lake	97,06	95,01	2,94	4,99
Bovan Lake	82,77	76,38	17,23	23,62
Zavojsko Lake	95,24	99,63	4,76	0,37
Bagrdan Lake	64,71	47,11	35,29	42,89
Milosevo Lake	75,00	96,20	25,00	3,80
Buljanka Lake	82,93	46,57	17,07	53,43
Oblacinsko Lake	38,37	19,82	61,63	80,18
Jovacko Lake	61,91	95,50	38,09	5,50
Ribnicko Lake	94,23	95,62	5,77	4,38
Rastovnicko Lake	90,37	74,76	9,63	25,24

In most reservoirs, the abundance and biomass, the autochthonous fish species are more represented than non-native species (Fig. 1 and 2). The exception is Oblacinsko Lake with abundance domination of the introduced species with 61.63%, and even biomass with 80.18%. Among them, in the greatest number, sunfish is represented by 18.6%, while white carp (*Hypophthalmichthys molitrix*) is dominated by biomass with 51.48%. Carp is numerically the most common species in this lake (37.21%).

The specific situation was observed in Lake Buljanka. Native species are dominated by abundance (82.93%), but when it comes to biomass, non-native species are represented with 53.43%. The largest contribution, to the high values of biomass, has been given by a gray carp (*Arystichtys nobilis*), with a share of 41.88%. Both reservoirs are part of a group of small reservoirs and generally, the presence of alien species is more pronounced in small than in large reservoirs. In addition, Oblacinsko Lake belongs to a group of old reservoirs which is largely the reason of the state of ichthyocenoses in it.

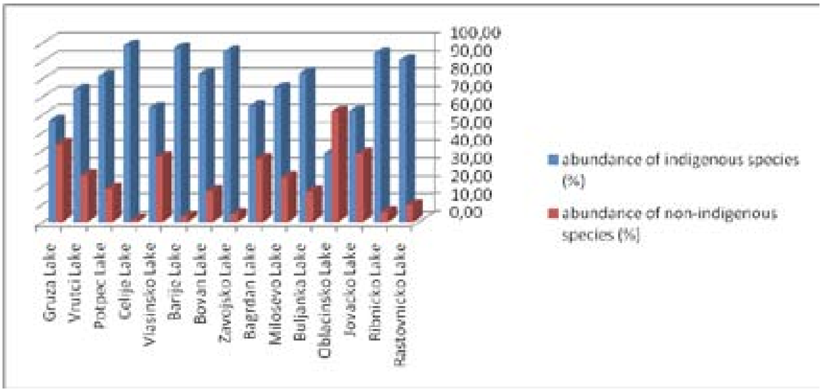

Figure 1: Graphic review of abundance of autochthonous and non-autochthonous species

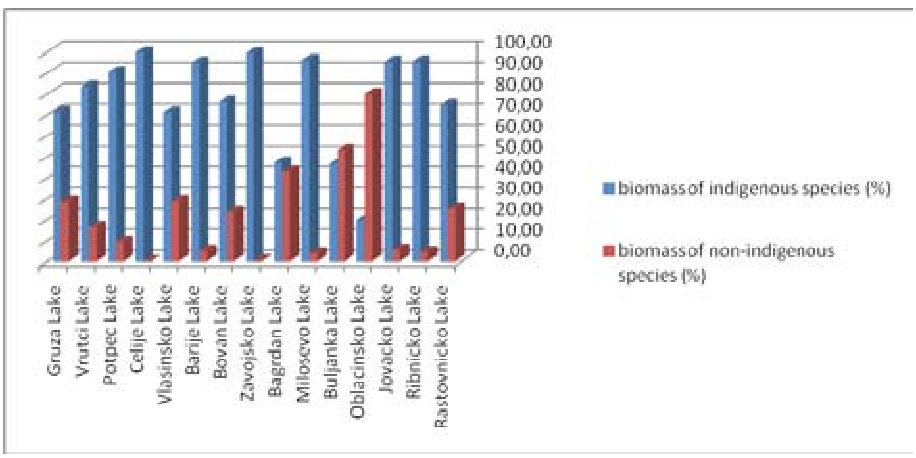

Figure 2: Graphic review of biomass of autochthonous and non-autochthonous species

Autochthonous fish species were dominated in Lake Celije and Zavojsko Lake which belong to the group of older and larger reservoirs. This group includes the reservoir of Gruza, Vlasina and Bovan in which are introduced non-native fish species by unplanned stocking and they become closer to the autochthonous community abundance. Planned stocking preserved the autochthonous ichthyofauna in Milosevo and Ribnicko Lake, which are small and old. In the Celije Lake bream is dominant with 67.61% and with the abundance was followed by roach with 19.72%. Besides being the numeros, bream dominated also with biomass (40.57%). Ichthyofauna of Ribnicko Lake are characterized by domination of nase (*Chondrostoma nasus*), numerically (86.54%) as well as with biomass (76.41%). Perch is the dominant native species in Lake Barije, Milosevo and Zavojsko Lake. In terms of biomass in the Lake Barije perch (*Sander lucioperca*) dominates with 50.49%, in Milosevo Lake that is northern pike (*Esox lucius*) with 20.52%, and in Zavojsko Lake catfish (*Silurus glanis*) with 57%.

CONCLUSION

During this research the relation between autochthonous and non-autochthonous fish species was analyzed in 15 reservoirs in Serbia. It was found that in most lakes native fish species still dominate in relation with non-autochthonous species. Impact of alien fish species was always higher in smaller and older reservoirs, in relation to larger, because of greater diversity of native fish species. Knowing the invasive character and the negative effects of alien species on ecosystems that have been made, it is necessary to continue monitoring the situation on the ground.

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STRUCTURAL CHROMOSOME DAMAGES IN THE CARP (*CYPRINUS CARPIO,* L.) FROM SOME LOCALITIES OF THE RIVERS KOLUBARA, SAVA, DANUBE AND TAMIŠ

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STRUKTURNA OŠTEĆENJA HROMOZOMA ŠARANA (*CYPRINUS CARPIO,* L.) SA NEKIH LOKALITETA REKE KOLUBARE, SAVE, DUNAVA I TAMIŠA

Abstrakt

U rekama Kolubari, Savi, Dunavu i Tamišu, tokom višegodišnjih istraživanja, utvrđivano je prisustvo genotoksičnih i potencijalno genotoksičnih materija: nafte, derivata nafte, fenola, pesticida, teških metala, među kojima su Hg i Cd detektovani ne samo u sedimentu reka, već i u ribi i školjkama. U ribi su utvrđeni policiklični ugljovodonici, od kojih su mnogi kancerogeni i genotoksični; pesticidi, među kojima: heksahlorocikloheksan-HCH i njegov izomer lindan, kao i DDT i njegovi metaboliti. Kako je povišena učestalost hromozomskih oštećenja-prekida i gapova, pokazatelj genotoksičnog dejstva, citogenetičke metode je moguće primeniti na organizme iz prirode, radi utvrđivanja prisustva genotoksičnih agenasa u životnoj sredini. Tokom ranijih istraživanja, na osnovu rezultata dobijenih u laboratorijskim kontrolama, kao i na osnovu učestalosti promena kod riba iz čistih voda, pretpostavljena je oblast kritične zone-iznad beckgraund-a ili nivoa “spontanih” promena, pri čemu su sve vrednosti iznad 3,5 %, tj. iznad oblasti zone, one koje ukazuju na nesumnjivo prisustvo i delovanje genotoksičnih agenasa. Cilj istraživanja je bio da se putem analize hromozoma šarana-*Ciprinus carpio,* L., odredi učestalost oštećenja tipa prekida i gapa, radi procene eventualnog genetičkog rizika na nekim lokalitetima reka Kolubare, Save, Dunava i Tamiša.

Citogenetički su analizirani metafazni hromozomi šarana-*Cyprinus carpio,* koji su dobijeni klasičnom metodom preparacije iz tkiva bubrega. Kod svake jedinke je pregledano 30-50 metafaznih figura hromozoma. Utvrđivan je broj hromozomskih prekida i gapova, a dobijeni rezultati su analizirani primenom statističkih metoda.

Najviše vrednosti su ustanovljene, redom, u reci Kolubari, Tamišu, Dunavu kod Višnjice i Savi kod ušća i bile su iznad nivoa "spontanih" promena i iznad predpostavljene kritične zone (3,0-3,5 %). Značajne statističke razlike (p <0.001) se javljaju poređenjem lokaliteta sa najnižom učestalošću promena, kao što su Dunav kod Beške i Slankamena, sa lokalitetima: Kolubare, Tamiša, Dunava kod Višnjice i Save kod ušća. Rezultati pokazuju da postoji genetički rizik od stalnog ili povremenog zagađenja genotoksičnim materijama na lokalitetu Kolubare kod Obrenovca, Tamišu kod Pančeva, Dunavu kod Višnjice, kao i u Savi kod njenog ušća.

Ključne reči: *genotoksični agensi, hromozomi riba, prekidi i gapovi, Cyprinus carpio*

INTRODUCTION

The largest group of genotoxis agents comprises *industrial genotoxic agents* and within the group oil and oil derivates are of the greatest importance (IARC, 1989; Vanzzella et al., 2007; Kolwzan, 2009; Olieira - Martins & Grisolia, 2009; Araújo et al., 2010). Genotoxic and carcinogenic properties were found in *phenols* (Michalowicz, 2004) and *pesticides,* as *hexachlorocyclohexane* – HCH and its γ – isomer, *lindane* (Kalanz et al., 2004), whereas genotoxicity of DDT and its metabolites is controversy: no effect detected in bacterial test systems, but found in mammal cell cultures (ATSDR, 2002). These, and similar chemicals and heavy metals, like Hg and Cd, have been found in Kolubara, Sava, Danube and Tamiš waters as in shell and fish bodies (Fingler & Drevenkar, 1988; Janković & Jovicic., 1994; Maljević, 2004; Ekostatus of the river Tamiš, 2010).

Increasing frequency of chromosome breaks and gaps is a useful indicator of genotoxic actions (Bröger, 1982). Cytogenetic methods are commonly used in laboratory conditions (Preston et all., 1981) and are possible to apply the same methods in organisms in nature.

This study was performed to detect chromosome breaks and gaps frequencies in the carp – *Cyprinus carpio* L., in specimens which were caught in three consecutive years on some localities of the rivers: Kolubara, Sava, Danube and Tamiš, with the aim to evaluate eventual genetic risk of genotoxic agents presence in the river water.

MATERIAL AND METHODS

Carp specimens were collected during three consecutive years, 1986, 1987 and 1988, on different localities of the rivers Kolubara, Sava, Danube and Tamiš, and cytogenetically analyzed. Metaphase chromosomes were obtained by preparation of kidney tissue according the classical methods (Fontana et al., 1970). Procedure involving 6 – 12 fishes from the same locality simultaneously. In the every individual fish, 30 – 50 mitoses were examined. Breaks and gaps were detected and scored, and results were statistically analyzed.

RESULTS AND DISCUSSION

Results of cytogenetic analysis of carp specimens are presented in Table 1.

Lower level of breaks and gaps has been registered in fish from the Danube near Beška and Slankamen (Table 1). Higher frequencies of breaks and gaps were detected in fish from Danube caught by Zemun and Grocka, and Sava by NT – B ($p < 0.05$). The highest values, above the 3.5 % changes, i.e. above the level of proposed critical zone (Fišter, 1992), were detected in river Kolubara near Obrenovac, Tamiš near Pančevo, Danube by Višnjica and Sava near the mouth, respectively. Statistically significant differences ($p < 0.001$) were found in comparison localities of Danube near Beška and Slankamen with Kolubara, Tamiš, Danube by Višnjica and mouth of the river Sava. Very high frequencies of chromosome breaks and gaps obtained in fish from the last announced localities indicate to genetic risk of permanent or periodical presences of genotoxic agents in river water.

Table 1. Frequency of breaks and gaps in carp – *Cyprinus carpio*

Locality	***Year***	***Number of individuals***	***Mitoses examined***	***Breaks and gaps***	***Breaks and gaps %***	***$\bar{x}$ for three years (%)***
Lokalitet	Godina	Broj jedinki	Pregledanih mitoza	Prekida i gapova	Prekida i gapova %	$\bar{x}$ za tri godine (%)
Kolubara – Obrenovac	**1986**	8	260	10	**3,84**	**3,7845**
	1987	7	210	8	**3,80**	
	1988	7	217	8	**3,68**	
		Σ=22	Σ=687	Σ=26		
Sava – "NT" B	1986	6	212	6	2,83	**2,7536**
	1987	6	236	6	2,54	
	1988	7	242	7	2,89	
		Σ=19	Σ=690	Σ=19		
Sava – ušće	**1986**	9	280	10	**3,57**	**3,4246**
	1987	9	331	11	**3,32**	
	1988	8	265	9	**3,39**	
		Σ=26	Σ=876	Σ=30		
Dunav – Beška	1986	10	346	7	2,02	**2,0250**
	1987	11	339	8	2,35	
	1988	11	352	6	1,70	
		Σ=32	Σ=1037	Σ=21		
Dunav – Slankamen	1986	12	384	8	2,08	**1,9090**
	1987	12	373	7	1,87	
	1988	11	343	6	1,74	
		Σ=35	Σ=1100	Σ=21		
Dunav – Zemun	1986	12	372	10	2,68	**2,6022**
	1987	10	340	9	2,64	
	1988	12	364	9	2,47	
		Σ=34	Σ=1076	Σ=28		
Dunav – Višnjica	**1986**	12	351	13	**3,70**	**3,5989**
	1987	12	396	14	**3,53**	
	1988	12	420	15	**3,57**	
		Σ=36	Σ=1167	Σ=42		
Dunav – Grocka	**1986**	11	363	12	**3,30**	**2,9439**
	1987	12	375	10	2,66	
	1988	10	315	9	2,85	
		Σ=33	Σ=1053	Σ=31		
Tamiš	**1986**	10	365	14	**3,98**	**3,7442**
	1987	11	354	13	**3,67**	
	1988	12	376	14	**3,72**	
		Σ=33	**Σ=1095**	**Σ=41**		

Sometimes, genotoxic contamination is difficult to prove immediately, especially in cases of some substances which are not genotoxic, but become genotoxic in metabolic detoxication processes – metabolic activation (Magee, 1982). Genotoxic agents could arise from biological degradations, or could be found after remediation of oil contaminated soil (Kolwzan, 2009). From the soil, chemicals could penetrate into underground waters and by that way, to the rivers flow. Many of the oil products are known as carcinogens, mutagen and genotoxic (IACR, 1989; Vanzzella et al., 2007; Kolwzan, 2009; Olieira-Martins & Grisolia, 2009; Araújo et al., 2010). Some chemicals, similar to DDT, could accumulate in animal tissues and long retained in the food-chains (ATSDR, 2002).

Examined carp specimens were collected from localities where oil products, phenols, pesticides and heavy metals, especially Hg and Cd have been continuously detected in the river water (Fingler & Drevenkar, 1988; Janković & Jovicic, 1994; Maljević, 2004; Ekostatus of the river Tamiš, 2010). In additions, findings in carp are in concordance with results which were obtained in other fish species: *Esox lucius, Carassius auratus gibelio, Tinca tinca, Alburnus alburnus, Stizostedion volgensis* and *Perca fluviatilis* (Fišter, 1992; Fišteret al., 1994; Fišter & Soldatović, 1996; Fišter et al., 1996).

CONCLUSION

High frequencies of chromosomal damages, breaks and gaps detected in the carp-*Cyprinus carpio,* L. and significant statistical differences among localities examined, indicated to periodical or permanent presence of genotoxic chemical agents in the rivers Kolubara near Obrenovac, Tamiš near Pančevo, Danube near Višnjica and Sava near the mouth.

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IMPACTS OF SMALL HYDRO POWER STATIONS ON STREAM ECOSYSTEMS AND FISHES IN STREAMS OF THE NORTH EASTERN ANATOLIA

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UTICAJ MALIH HIDROCENTRALA NA RIBE I EKOSISTEM VODOTOKA U SEVEROISTOČNOJ ANADOLIJI

Abstract

Caucasian region is the twenty five terrestrial “Ecological Region” and “under threat “rich biological diversity area in the world. South west part of Caucasus includes north east part of Turkey. This part is mostly mountainous and has many streams. In the region many small hydroelectric power plants are constructed on natural mountainous stream ecosystems. Aquatic habitats of fish have been seriously affected due to river construction works, closing of the upstream migration passages, sand removals, construction of dams and regulators. Road and other constrictions works in or near the stream cause soil erosion which shifts turbidity in stream water affecting fish species including brown trout populations. Hydroelectric stations (HES) cause some environmental and social problems in the region. Small HES constructions pollute stream water and damage natural fish stocks as well as fish from trout farms.

Key words: Hydroelectric station (HES), Ecological impacts, South West Caucasus, stream ecosystem, brown trout

PRODUCTION EFFICIENCY OF RAINBOW TROUT DEPENDING ON THE TYPE OF FEED

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EKONOMIČNOST PROIZVODNJE DUŽIČASTE PASTRMKE U ZAVISNOSTI OD VRSTE KORIŠĆENE HRANE

Abstrakt

Dužičasta pastrmka (*Oncorhynchus mykiss*) predstavlja dominantnu hladnovodnu vrstu ribe u komercijalnom gajenju na području Balkana, zbog relativne tolerantnosti na kolebanja kvaliteta vode i brzog rasta. Pastrmka se intenzivno gaji u kaveznom sistemu sa osnovnim ciljem postizanja maksimalnog prirasta uz što niže troškove hrane.

U radu su predstavljeni razultati ekonomičnosti proizvodnje dužičaste pastrmke u uslovima kaveznog gajenja u zavisnosti od tipa i cijene korišćene hrane na bazi rezultata iz eksperimenta sa upotrebom šest različitih vrsta hrane u kaveznom ribogojilištu „Tropic ribarstvo" na jezeru Bočac, u 2 ciklusa po 90 dana (ukupno 180 dana) i 6 tretmana/ciklusu. Računat je parcijalni koeficijent ekonomičnosti riblje hrane u šest tretmana, kao odnos vrijednosti ostvarenog prirasta ribe i troškova upotrebljene hrane za svaki od šest kaveza posebno, za dva proizvodna ciklusa (jesen-zima i proljeće-ljeto).

U jesenje-zimskom režimu ishrane najveća parcijalna ekonomičnost riblje hrane ostvarena je u 2. tretmanu (E_{pm}=3,51), koji potvrđuje da se na 1 KM troškova riblje hrane ostvaruje 3,51 KM po osnovu ostvarenog prirasta ribe. Gotovo istu ekonomičnost je imala i hrana u 6. tretmanu. Hrana u 2. tretmanu izbila je na prvo mjesto zbog povoljnog faktora konverzije, a hrana u 6. tretmanu zbog najniže cijene. Najmanje ekonomična hrana je bila ona korišćena u 1. tretmanu (E_{pm}=2,29).

U proljetno-ljetnom režimu ishrane potrošnja riblje hrane i prirast mase ribe su bili intenzivniji. Najpovoljniji parcijalni koeficijent ekonomičnosti riblje hrane u ljetnom periodu ostvaren je (ponovo) u 2. tretmanu (E_{pm}=3,07 odnosno recipročno E_{pm}=0,33). Iako je ovaj koeficijent niži nego u jesenje-zimskom periodu, treba imati u vidu da je ostvareni prirast mase pastrmke, a time i ukupan prihod, u proljetno-ljetnom periodu 2,5 puta veći nego u jesenje-zimskom periodu. Na drugom mjestu po isplativnosti je hrana u 3. i 4. tretmanu čija primjena u jesenje-zimskom periodu nije bila isplativa, dok je najmanje ekonomična bila hrana korišćena u 1. tretmanu (E_{pm}=2,45 odnosno recipročno E_{pm}=0,41).

Proizvodnja ribe u uslovima intenzivnog gajenja mora biti ekonomski isplativa da bi se održala na duži rok. Sa ekonomskog stanovišta najpovoljniji način ishrane ne mora biti onaj koji ima najniži faktor konverzije, isto kao ni onaj sa najjeftinijom hranom. To je potvrdio i eksperiment ispitivanja efikasnosti šest tipova riblje hrane u uslovima kaveznog uzgoja dužičaste pastrmke u različitim temperaturnim uslovima, koji kao optimalnu kombinaciju ishrane preporučuje onu sa najpovoljnijim odnosom vrijednosti oswtvarenog prirasta ribe i troškove njene ishrane, uz pretpostavku nepromjenljivosti svih ostalih troškova proizvodnje.

Ključne riječi: *dužičasta pastrmka, riblja hrana, ekonomičnost proizvodnje.*

INTRODUCTION

One of the ways to increase bioproductivity of water resources is the intensive cultivation of fish in cages. Rainbow trout (*Oncorhynchus mykiss*) is the dominant type of coldwater fish in the commercial growing in the Balkans, due to the relative tolerance to fluctuations in water quality and rapid growth. The main objective of the intensive growing of fish is to achieve maximum growth with minimum cost, and critical analysis used the food which is the major portion of the costs (Hardy, 1989). Determining the food in aquaculture, it is evident that it is one of the more expensive animal feed on the market, because of higher nutritional content and it is produced by using expensive processes (extrusion, pelleting). Nutritional and biological efficiency of feed for salmonids is constantly improving, and its share makes fish meal and high-quality power components that contain a high amount of usable energy. Food with high content of digestible nutrients (40-45% SP-CP and 20-30% SM-DM) results in a conversion ratio from 1 to 1.2, even below 1 (Bureau, 2004).

MATERIAL AND METHODS

The aim of this paper is to analyze the production efficiency of rainbow trout in cage farming conditions depending on the type (content of fat and protein) and the cost of feed used. Experiment with 6 different types of nutrients is carried out in a cage fish farm "Tropic Fishing" - Lake Bocac, at 2 cycles per 90 days (180 days) and 6 treatments per cycle. The first cycle was carried out in the autumn-winter 2005/06, and another in spring-summer 2006 cages in production volume of 162.5 m^3/cage. Average weight of rainbow trout at the beginning of the cycle was 95.3 grams, and second cycle 96.1 grams. Set on a 400 kg fish/treatment, a total of 2,400 kg/cycle. Used the 6 types of extruded fish feed (Table 1), 3 mm grit (Savic, 2008).

Table 1. Composition of feeds in experiment

Nutritients/treatment	1.	2.	3.	4.	5.	6.
Crude protein/crude fat, SP/SM (%)	44/14	48/26	42/22	42/23	44/26	42/18
Total energy (MJ)	20,4	23,8	21,8	22,3	23,2	21,0
Digestible energy (MJ)	17,7	21,9	19,3	20,3	20,9	19,1
Metabolizable energy (MJ)	15,7	19,6	17,4	18,3	18,9	17,2
Nitrogen free extract (NFE) %	21,0	17,0	15,0	17,2	13,0	21,5

Statistical data processing (8400 fish) included the arithmetic mean, coefficient of variation, analyzing the impact of the growing season and type of nutrients on the growth of body weight by two-factorial model, 2x6 (Statistical Analysis-ANOVA).

Production efficiency is an indicator of the relationship between actual performance and used elements of production to achieve this effect. In the elements of production, there are occurring three primary factors: labor, capital goods and materials, so the efficiency can be measured as partial efficiency of: labor, capital goods or materials (Andric, 1998, Berberovic, Todorovic, 2009). Fattened trout in cage breeding system assumes the same expenditure of capital goods and labor, the research focused on only one factor of production - fish food and its consumption and costs (similar like Vasko, Drinic, 2009). For this reason, the determination of the economic effect of using different types of food made by calculating the coefficient of partial material efficiency (E_{pm}) for each type of fish food, and especially their mutual comparison of two different feeding regimes. Partial efficiency ratio was determined from the ratio of increment fattened trout and value of food in each of the six treatments.

Besides food, significant material cost of fattening of trout is a fish fry. Its quantity (400 kg) and cost were the same for all treatments, so it is logical that the value of production valued only for the achieved gain weight of fish as a result of the use of fish food during the experiment. Production value was evaluated as the product of gain weight and prices of fish. To acualize the results of the analysis in relation to the time of performing the experiment in the model the current prices of cleaned trout in early 2011 have been taken into consideration in the market of Banja Luka (8 BAM/kg). As in the case of the selling price of fish, in the model, there were used the current retail market price of the appropriate type of fish franco-Banja Luka (1.85 to 2.83 BAM/kg). Consumption of fish food was not the same in all six cages because of its different energy values, but also not the same in the autumn-winter and spring-summer regime fattening as a result of different temperature regimes of water.

RESULTS AND DISCUSSION

The low water temperatures in autumn-winter period affect the lower level of the diet that causes fewer effects of nutrients on growth of rainbow trout. Drop in water temperature does not affect the growth dynamics of change in the relationship of mass and type of nutrients, nutrients in treatment 2 and 5 have more evident effects on the growth of body mass, but does not cause significant differences in mean body weight compared to other treatments.

Table 2. Efficiency of trout production in autumn-winter nutrition regime

Diet in spring-summer		Treatment					
Element		1.	2.	3.	4.	5.	6.
1.	Avg. body weight at the beginning (g)	96,28	94,75	97,95	94,29	94,56	93,97
2.	Coeff. var. body mass at the beginning	16,83	22,99	19,39	22,63	19,65	21,59
3.	Avg. body mass at the end (g)	125,23	131,50	131,60	128,26	131,47	127,35
4.	Coeff. var. body weight at the end	24,75	22,93	21,53	23,56	19,60	19,69
5.	The total mass at the beginning (kg)	400	400	400	400	400	400
6.	The total mass at the end (kg)	516,3	551,7	534,2	540,8	553,8	539,9
7.	Total fish weight gain (kg)	116,3	151,7	134,2	140,8	153,8	139,9
8.	Price of fish (BAM/kg)	8,00	8,00	8,00	8,00	8,00	8,00
9.	The value of increment of fish	930,6	1.213,7	1.073,2	1.126,4	1.230,6	1.119,0
10.	Quantity of feed (kg)	172,4	138,9	172,4	172,4	138,9	172,4
11.	The coefficient of conversion	1,48	0,92	1,29	1,22	0,90	1,23
12.	Price of feed (BAM/kg)	2,36	2,49	2,50	2,13	2,83	1,85
13.	The costs of feed (BAM)	406,9	345,9	431,0	367,2	393,1	318,9
14.	Partial coefficient of efficiency (E_{pm})	2,29	3,51	2,49	3,07	3,13	3,51
		0,44	0,28	0,40	0,33	0,32	0,29

Biggest partial efficiency of fish food has been achieved in the second treatment, although the economy had virtually the same feed in the 6th treatment. Feed in the second treatment emerged in the first place because of the favorable conversion factors, and the feedd in the 6th treatment for the lowest prices. This example illustrates that a conclusion about the viability of the use of fish feed based on the lowest conversion factor would not be right because the most favorable factor in the 5th treatment, but also the feed is the most expensive. The least favorable option is in the first treatment, because the conversion factor is high, and feed expensive. Significant differences in mean body mass in spring-summer period, under the influence of different foods, come to the fore the growth of water temperature >9^{o}C . Season of feed and type significantly influence the high differences in mean values (p>0,01) of mass of rainbow trout, as well as interactive relationship of rainbow trout as well as interactive showing the high degree of dependence (p>0,01) of growth of body mass of the season and type of feed nutrients.

The low water temperatures in autumn-winter period, affecting the lower level of the diet that causes fewer effects of nutrients on growth of rainbow trout. Drop in water temperature does not affect the growth dynamics of change in the relationship of mass and type of nutrients, nutrients in treatment 2 and 5 have more obvious effects on the growth of body mass, but does not cause significant differences in mean body weight compared to other treatments.

Table 3. Production efficiency of trout in cage in spring-summer diet regime

Diet in spring-summer		Treatment					
Element		1.	2.	3.	4.	5.	6.
1.	Avg. body weight at the beginning (g)	94,08	97,32	95,04	99,43	96,33	94,39
2.	Coeff. var. body mass at the beginning	21,19	20,14	27,76	21,68	21,56	24,20
3.	Avg. body mass at the end (g)	167,67	191,84	192,01	185,26	195,95	161,23
4.	Coeff. var. body weight at the end	18,03	25,35	19,59	23,54	18,15	22,42
5.	The total mass at the beginning (kg)	400	400	400	400	400	400
6.	The total mass at the end (kg)	713,6	781,0	800,8	741,5	808,1	680,2
7.	Total fish weight gain (kg)	313,6	381,0	400,8	341,5	408,1	280,2
8.	Price of fish (BAM/kg)	8,00	8,00	8,00	8,00	8,00	8,00
9.	The value of increment of fish	2.508,4	3.048,3	3.206,3	2.732,3	3.265,0	2.241,9
10.	Quantity of feed (kg)	433,6	398,6	433,6	433,6	398,6	433,6
11.	The coefficient of conversion	1,38	1,05	1,08	1,27	0,98	1,55
12.	Price of feed (BAM/kg)	2,36	2,49	2,50	2,13	2,83	1,85
13.	The costs of feed (BAM)	1.023,3	992,5	1.084,0	923,6	1.128,0	802,2
14.	Partial coefficient of efficiency (E_{pm})	2,45	3,07	2,96	2,96	2,89	2,79
		0,41	0,33	0,34	0,34	0,35	0,36

In spring-summer dietary, consumption of fish food and fish weight gain were more intense. The most favorable partial coefficient of efficiency of food fish in the summer period was realized (again) in the second treatment (3.07 or reciprocal 0.33). Although this coefficient is lower than in autumn-winter period, we should bear in mind that the achieved weight gain of trout, and thus the total income, in the spring-summer period, 2.5 times higher than in autumn-winter period. The second most cost effectiveness is the food in the 3 and 4 treatment, whose application of the autumn-winter period was not profitable. In the spring-summer it is the least economically viable to use the fish food that was used in the first treatment. From the standpoint of the results from Table 3 it should be emphasized the observation that the cheapest food is not necessarily the best solution for breeding fish, because the food in 6 treatment was the cheapest, and from the stand point of conversion and fish increment was the worst. Generally, the conclusion is that during the year for breeding trout in conditions similar to those which are characterized by experimental conditions are most favorable to use food that has been used in the second treatment. As an alternative to the autumn-winter period was equally profitable food used in the sixth, and in spring-summer period it is approximately the cost-effective food used in the third and fourth treatment. For the realized cost of production of rainbow trout in cage conditions thee were not comparative results found in the available local literature. Similar studies on the economic efficiency (profitability) for fish production were found in foreign literature (Ugvumba and Okoh, 2010, Bozoglu et al., 2009, Kudi et al., 2008), but it is mainly related to other fish species and farming systems.

CONCLUSIONS

Even the cheapest fish food, or the lowest conversion factor do not have to provide the most favorable economic results in the fattening of fish. This is confirmed by the example of experiment in fattening rainbow trout in cage system using six different types of food. The coefficient of conversion of food in the autumn-winter conditions ranged between 0.90 and 1.48, while the spring-summer conditions between 0.98 and 1.55. Food prices ranged from 1.85 to 2.83 BAM/kg. Economic efficiency of production is measured using the partial coefficient of efficiency as the relative values of gain and cost of fish feed used. In autumn-winter conditions of growing the largest coefficient of efficiency (E_{pm2}=3.51) had the food used in treatment 2, although it is not even the cheapest food or food with the lowest conversion ratio. The same food was confirmed as the most cost-effective and economical spring-summer conditions of the experiment (E_{pm2}=3.07) in which the consumption of food and fish growth were more intense than in autumn-winter period.

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MORPHOMETRICAL STUDY OF INTESTINAL FOLDS OF CARP FED DIFFERENT ADDED FEED IN SEMIINTENSIVE SYSTEM

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MORFOMETRIJSKO ISPITIVANJE NABORA CREVNE SLUZOKOŽE ŠARANA HRANJENOG RAZLIČITOM DODATNOM HRANOM U POLUINTENZIVNOM SISTEMU

Apstrakt

U poluintenzivnom sistemu gajenja šarana u poslednjoj deceniji dolazi do promena u vrsti dodatne hrane koje povećavaju održivost proizvodnje. Sa žitarica koje su se godinama koristile kao dodata hrana, sada se prelazi na peletiranu i ekstrudiranu hranu. Ova zamena uzrokuje i promene u fiziologiji varenja riba, a kao posledica se dešavaju morfološke promene na crevima. Najčešće se na organima za varenje riba gajenih u akvakulturi ne nalaze teže histopatološke promene, pa se pribegava kvantifikaciji i morfometriji histoloških parametara koje mogu da ukažu na odstupanja od normalne građe/funkcije. Metod evaluacije dužine crevnih nabora je korišćen u velikom broju studija koje su se bavile efektima različitih tipova hrane i/ili aditiva na crevo riba. Cilj rada je da se utvrdi efekat različitih tipova hrane (žitarice, peletirana, ekstrudirana hrana) na crevo šarana merenjem dužine crevnih nabora kod riba koje su gajene poluintenzivno. Dužina crevnih nabora je korelisana sa prirastom ribe.

Kretanje dužine crevnih nabora po jezerima/vrsti dodate hrane je imalo pravilan tok (Fig. 1). Najkraći crevni nabori su izmereni kod riba hranjenih žitaricama. U sva tri jezera primetan je pad dužine nabora u junu. U ovom mesecu su vrednosti najmanje, manje čak i od inicijalnih na početku eksperimenta (u aprilu), kao i vrednosti dobijenih u maju. Od juna dužina nabora creva konstantno raste kod riba u svim jezerima, bez obzira na tip dodate hrane.

Rezultati su pokazali visok stepen statistički značajne korelacije između mase tela ribe i dužine crevnih nabora u svim jezerima (Tab. 2). Ukoliko se posmatraju pojedinačna jezera, vrednosti se kreću između 0.82 kod riba hranjenih peletiranom hranom, preko

0.83 kod riba hranjenih žitaricama do 0.86 kod riba hranjenih ekstrudiranom hranom. Dužina crevnih nabora je koristan parametar u eksperimentima ishrane riba i često se primenjuje u proceni efekta određene hrane na crevo riba. Smanjenje dužine nabora može da ukaže na pojavu enteritisa, na nedovoljnu ishranu ili gladovanje. Naši rezultati pokazuju pozitivnu korelaciju između mase tela ribe i dužine crevnih nabora. Oni su u skladu i sa sličnim istraživanjima koja su sprovedena na drugim životinjskim vrstama, mahom na domaćim životinjama. Crevni nabori na početku eksperimenta ne rastu, čak pokazuju trend pada sve do juna meseca i to iako ribe pokazuju konstantan prirast u svim jezerima, što se objašnjava količinom prirodne hrane koja je u to doba najveća u jezerima. Naime, na osnovu analize prirodne hrane tokom ovog perioda možemo videti da postoji velika biomasa zooplanktona u toku aprila, maja i juna dok je zoobentos na niskom nivou u svim jezerima u toku celog eksperimenta i ne prelazi 10 g/m^2. Ovo uzrokuje promene u načinu ishrane ribe od juna meseca. Do tada se šaran hranio uglavnom zooplanktonom, koga je bilo u izobilju, a nakon toga, početkom juna, zbog nedostatka prirodne hrane,riba počinje da sve više konzumira dodatu, što uzrokuje i povećanje dužine crevnih nabora, s obzirom da su pelete, kojom se ribe hrane voluminoznije i većih dimenzija od zooplanktona. Hranjenjem, ribe pune creva i samim tim ih i šire, što omogućava povećanje apsorpcione površine povećanjem dužine crevnih nabora. Ovaj fenomen u stvari predstavlja adaptaciju creva na različite tipove hrane kod riba.

Naše istraživanje je pokazalo da praćenje histologije šarana može da ukaže kako se odvija proces varenja zavisno od dela sezone/razvoja zajednice zooplanktona, ali i vrste dodate hrane u poluintenzivnom sistemu.

Ključne reči: *morfometrija, histologija, crevni nabori, šaran, poluintenzivno gajenje*

INTRODUCTION

In order to enhance sustainability of the semiintensive carp production system in the first decade of the 21st century, replacement of traditionally used cereals occurs in Serbia and the region (Marković et al., 2010). The shift from cereals to pelleted and extruded supplemental feed affect fish nutritional physiology and therefore results in morphological changes in the intestine (Segner et al., 1987, Epler et al., 2009). Our research of intestinal histology has demonstrated that no major pathological changes occur on carp intestine in feeding experiments. The alteration observed couldn't seriously impair the function of digestion. A number of studies use morphometry for evaluation of effects of different feeds on fish intestinal histology (Sanden et al., 2005; Zhou et al., 2007; Liu et al., 2009; Qiyou et al., 2011, Rašković et al., 2011).

The aim of the present study was to evaluate effects of different added feed on intestinal folds (mixture of cereals, pelleted, and extruded feed) using morphometry. Intestinal fold length of carp grown in a semiintensive production system was correlated with carp growth rate.

MATERIAL AND METHODS

The experiment was performed in three earthen ponds, surface of 650 m^2 each, placed side by side at the experimental fish farm of the Center for Fishery and Applied Hydrobiology, Faculty of Agriculture, University of Belgrade, Serbia. 400 common carps (*Cyprinus carpio* L.) were placed in each pond. They were 11 months old with average weight of 150 ± 18 g. The diseases free fish was provided from the commercial fish farm "Neuzina", Serbia. After a period of adaptation of three weeks, fish were grown for one production season (29th April - 26th October 2008) using feed of 3 different treatment level: mixture of grains (wheat, corn and barley mixture in 1:1:1 relation; GF), pelleted feed (PF) and extruded feed (EF). PF and EF were made of the same components (Table 1), but were treated differently – by pelletting and extruding process, respectively. Fish were fed on daily basis, with 3% of their body mass.

Table 1. Chemical composition of the feed.

Feed	GF	EF	PF
Proteins	11.3±0.8	28.5±0.9	26.5±0.8
Lipids	3.3±0.2	7.8±0.1	8.0±0.1
Ash	1.9±0.3	4.7±0.2	4.7±0.3
Fibers	7.4±0.6	3.5±0.1	3.3±0.4
Moisture	9.8±0.7	9.4±0.3	12.0±0.5
Carbohydrates	66.2±2.0	46.1±1.3	45.5±1.7

At the beginning of experiment (29th April 2008) 6 fish were sacrificed and formed the initial group for histology examination. Subsequently, two fishes from each pond were taken every 30 days until 26th October 2008. Specimens were sacrificed with a quick blow to the head, and distal intestine was quickly removed, fixed in 4% formaldehyde, and processed using a standard histological technique: dehydration in an ethanol series, embedding in paraffin, and serially sectioning at 5 µm. Sections were stained with hematoxylin and eosin (H/E) (Humason, 1979). Microphotographs were taken with a Leica DML microscope with the Leica DC 300 camera. Lengths of intestinal folds were measured from the base to the tip of the fold at average of 60 folds per fish. STATISTICA 7.0 (StatSoft, USA) was used in statistical analysis of values. LSD test was used for determination of significant differences between samples. For correlation a Pearson r correlation was used.

RESULTS

Intestinal fold length had a rather regular growth in the three ponds studied (Fig. 1). Fish fed cereals as added feed had the shortest folds. In all three ponds a fall in intestinal fold length occurred in June. In June fold length was even shorter than at experiment start in April and in May. Intestinal folds length constantly increased from June onward in all ponds, regardless of feed used.

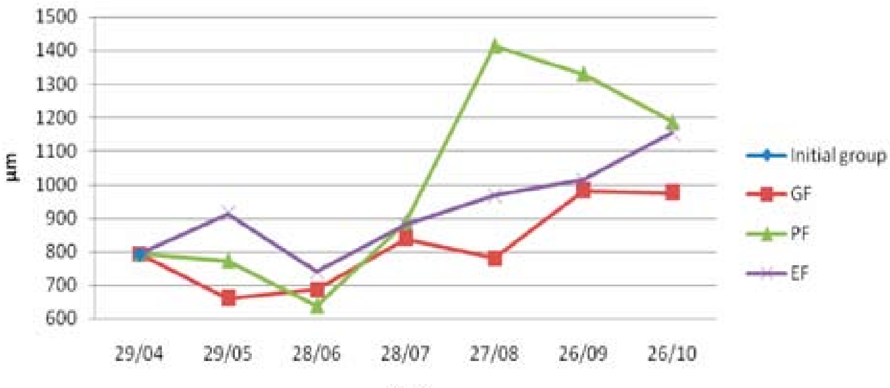

Figure 1. Intestinal fold length (in µm) over a period of experiment duration (29th April-26th October).

The results have shown high correlation level between carp body mass and intestinal fold length in all ponds (Tab. 2). Correlation between fold length and body mass in each pond is given in Tab.2: it was 0.82 in the pond PF, 0.83 in GF, and 0.86 in EF. As it can be seen from the table all correlations obtained were statistically significant.

Table 2. Relationship between length of intestinal folds and body mass of fish in each pond.

Feed	GF	PF	EF
Correlation coefficient	0.83	0.82	0.86
Statistical significance	p=0.02	p=0.02	p=0.01

DISCUSSION

Measurement of the length of intestinal folds is a useful parameter in feeding experiments. It is used in evaluating effects of feed on fish intestine (Ostazevska et al., 2005; Zakes et al., 2008). When intestinal fold length decreases, it can indicate enteritis (Baeverfjord and Krogdahl, 1996) or suboptimal feeding or starvation (Mc Leese and Moon, 1989). Fish were fed 3% of their body mass in our experiment, thus the added feed quantity increased with body mass, and therefore suboptimal feeding couldn't be a reason for fold length decrease.

Our results have shown a positive correlation between body mass and intestinal fold length. They are in accordance with similar findings on other domestic animals such as: pigs (Li et al. 1991), calves (Brooks et al. 1998) broilers (Loh et al., 2010) and turkeys (Ritz et al., 1995).

In our study intestinal folds length has a decreasing trend in carp from all studied ponds until the month of June, although the growth rate increased (Table 3; Dulić et al., 2009). This can be explained by the quantity of natural food gradually reaching its maximum in the pond in this period of the season (Paterson, 1993). According to the analysis of natural food the highest zooplankton biomass is found in months of April, May and June, especially in ponds with PF and EF. Additionally, in this part of the season, the main Cladoceran species was *Bosmina logirostris*, and Copepod Cyclops. sp. However zoobenthos in all ponds investigated during the whole experiment never exceeded 10 g/m^2 (Dulić et al., 2009). Obviously carp consumed natural food during April and even in May, while in June, most probably was a period of adaptation to added feed. After this period of maximum development of natural food, added feed started to be increasingly used. This affected intestinal folds length which increased in size. The reason for this could be explained by a larger volume pellets occupy compared to zooplankton dimensions (for instance, size of *Bosmina longirostris* is 200-400 µm, pellet size is ø3 x 10 mm) and possibly by the high digestibility of natural food, providing additional enzymes (Kibria et al., 1997). The increased absorption area by increased fold length could represent an adaptation to different food type in fish as stressed by Refstie et al. (1997).

Our study has shown that monitoring carp histology during the production season can give insight into the digestion process, its efficiency in the semiintensive system depending on the part of the season /development of natural food, and of the type of added feed.

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CHIRONOMUS PLUMOSUS (DIPTERA, INSECTA) LARVAE AS A SOURCE OF ESSENTIAL FATTY ACIDS IN FEED OF CARP FRY

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LARVE *CHIRONOMUS PLUMOSUS* (DIPTERA, INSECTA) IZVOR ESENCIJALNIH MASNIH KISELINA ZA ISHRANU ŠARANSKE MLAĐI

Abstrakt

U cilju ispitivanja koliko su larve *Chironomus plumosus*-a pogodne za ishranu gajenih slatkovodnih riba, pre svega šarana, ižvršena je analiza hemijskog i masnokiselinskog sastava larvi koje su prikupljene na kraju tromesečnog perioda gajenja šaranske mlađi u dva eksperimentalna ribnjačka bazena u Centru za ribarstvo i primenjenu hidrobiologiju Poljoprivrednog fakulteta Univerziteta u Beogradu.

Udeo sirovih proteina u larvama *Chironomus plumosus*-a iznosio je 6,61 u jezeru L1 odnosno 6,18% u jezeru L2, što predstavlja vrednost adekvatnu za rast svih slatkovodnih vrsta riba. Sadržaj lipida bio je 0,49 odnosno 0,73%, što je energetski povoljno za sve ribe koje žive u toplim vodama. Prirodnu hranu (larve *Chironomus plumosus*) karakteriše i visok sadržaj vode: 88,95 u L1 i 89,62% u L2, a što ih čini pogodnom za ishranu šaranske malađi.

Lipidnu frakciju larvi Chironomidae u jezeru L1 je činilo 45.36% zasićenih i 53.96 nezasićenih masnih kiselina. U lipidnoj frakciji larvi Chironomidae iz jezera L2 nađeno je 53.47% zasićenih i 46.42% nezasićenih masnih kiselina.

Od polinezasićenih (esencijalnih) masnih kiselina nađenih u hironomidama u jezeru L1, najveći deo je pripadao ω-6 linolnoj kiselini (21,37%), zatim ω-3 linolenskoj (3,21%) i eikozopentanskoj ω-3 kiselini (1,27%). Sadržaj linolne kiseline u larvama Chironomidae iz jezera L2 je bio niži i iznosio je 9,78%, eikozopentanska ω-3 kiselina je zastupljena sa 0,45%, a sadržaj linoleinske kiseline je bio viši i iznosio 7,78%.

Nedostatak PUFA sa 22 C atoma je verovatno povezan sa slabom enzimatskom sposobnošću larvi Chironomidae za sintezu ovih kiselna iz njihovih prekursora PUFA sa 18 C atoma.

Izmerena količina ω-3 i ω-6 masnih kiselina u larvama *Chironomus plumosus* je iznosila 0,21 u L1, a u jezeru L2 0,81, zadovoljava nutritivne zahteve šarana.

Kjlučne reči: *Chironomus plumosus, zasićene i nezasićene masne kiseline, ω-6 linolna, ω-3 linolenska i ω-3 eikozopentanska kiselina*.

INTRODUCTION

Chironomidae larvae are widely distributed animal group, inhabiting various types of water basins. In still waters they are dominant component of benthic organisms, since 80% of bottom fauna biomass represents this animal group (Janković, 1966). Chironomidae larvae serve as a rich source of food for many benthophagous fish (larval stage lasts from several weeks to one year). Some Chironomidae species are very important component for fish nutrition because of their suitable biochemical composition (Janković, 1966a).

Nutritional value of farm-raised fish depends on the chemical composition of natural and supplement (industrial) food. Although industrial food for carp fry (Bogut *et al.,* 2007) tends to be similar to natural, containing 10-15% of carbohydrates (without remarkable fiber content) and 12-15 % of lipids (including the necessary essential fatty acids), there are mutiple advantages of nature food. Compared to the industrial food, adventages of natural food are: high digestibility (particulary of proteins), high water content (85-95%), soft and elastic food structure which allows its deformation short after ingestion, and the food movability, allowing fish to react on the „food" motions (Bogut *et al.,* 2007). Further, compared to natural food, unconsumed industrial food, containing high dry matter content, contaminates water manifold. Therefore, it is better to use natural food for fish diet, especially in nutrition of younger fish (Jirasek, 2001).

The aim of this study was to evaluate nutritive value and suitability of *Chironomus plumosus* larvea for carp fry nutrition, by determination of its primary chemical composition and fatty acid content.

MATERIAL AND METHODS

Sampling

Chironomidae larvae were collected from two fish ponds (L1 and L2) of the same area (650 m^2 in bottom base). Ponds are placed side by side, within an experimental fish farm of the Center for Fishery and Applied Hydrobiology, Faculty of Agriculture, University of Belgrade. 1200 carp individuals were placed in each pond (22.07.2010). They are genetically identical (family 27/86), aged two months and individual weight of 5,69±0.02g in the pond L1 and 5,71±0.02g in the pond L2, originated from the breeding program which takes place at the Center for Fishery and Applied Hydrobiology. In the next three months (july-october 2010) carps were fed with extruded (pond L1) and pelleted (L2) feed.

In order to analyze chemical and fatty acid composition of *Chironomus plumosus* larvae, 374 individuals were collected from the pond L1 and 262 individuals from the pond L2 (fourth and fifth larval instar). Until the laboratorical researches, samples were kept on the temperature of -18 ºC.

Chironomus plumosus larvae were sampled with modified Ekman-Birge's grab, addapted for usage in carp farms, grasping area of 87,55cm^2.

Chemical composition analysis of Chironomidae laravae

Crude protein content was determined by Kjeldahl method (N x 6,25). Water content was determined by drying the samples at 103 ± 2ºC, until the constant weight (SRPS ISO 1442/1998). Total lipid content was determined by extraction of fat in Soxhlet apparatus with 200 mL of 30-50°C boiling petroleum ether (SRPS ISO 1443/92). Ash content was determined by dry-ashing in porcelain crucibles in a muffle furnace at 550± 25 ºC (SRPS ISO 936/1999)

Extraction of lipids by ASE

Total lipids for fatty acids determination were extracted from *Chironomus plumosus* larvae tissues by accelerated solvent extraction (ASE 200, Dionex, Sunnyvale, CA). Homogenate of sample mixed with diatomaceous earth was extracted with a mixture of n-hexane and iso-propanol (60:40 v/v) in 33 ml extraction cell at 100°C and nitrogen pressure of 10.3 MPa. The extracts were collected and the solvent was removed under stream of nitrogen in Dionex Solvent Evaporator 500 at 50°C until dryness. The fat extract was further used for fatty acids determination.

FA analysis by capillary gas chromatography (CGC)

Fatty acid methyl esters (FAME) were prepared by transesterification using trimethylsulfonium hydroxide, according to EN ISO 5509:2000 procedure. The GC instrument Shimadzu 2010 (Kyoto, Japan) used for FAME determination was equipped with a split/splitless injector, fused silica cianopropyl HP-88 column (length 100 m, i.d. 0.25 mm, film thickness 0.20 μm, J&W Scientific, USA), and flame ionization detector. The column temperature was programmed. Injector temperature was 250°C and detector temperature was 280°C. The carrier gas was nitrogen at a flow rate of 1.33 ml/min and injector split ratio 1:50. Injected volume was 1 μl and total analysis time 50.5 min. The chromatographic peaks in the samples were identified by comparing relative retention times of FAME peaks with peaks in a Supelco 37 Component FAME mix standard (Supelco, Bellefonte, USA).

RESULTS AND DISCUSSION

Our results of *Chironomus plumosus larvae* chemical composition (table 1) are in accordance with previously published data (Bogut *et al.,* 2007, Steffens, 1986), provided it was noticed slightly lower protein (6,61 pond L1 and 6,18 pond L2) and lipid (0,49 or 0,73%) conntet, higher water content (88.95 or 89,62%), while the ash content in *Chironomus plumosus* larvae was nearly same 1,12% (pond L1) and 1,10% (pond L2). Also, lower lipid content was observed during three-month study period conducted by Mardsen and associates (Marsden *et al*., 1992).

Table 1. Chemical composition of *Chironomidae* larvae in g/100g fresh weight.

Chemical parameter	Pond L1	Pond L2
Protein content, %	6.61	6.18
Water content, %	88.95	89.62
Total lipid content, %	0.49	0.73
Ash content, %	1.12	1.10
Dry matter content, %	11.05	10.38

Most of the essential fatty acids in *Chironomus plumosus* larvae are present in quantities sufficient for the nutrition of most freshwater fish species, including carp. In the pond L1 lipid fraction of Chironomidae larvae was consisted of 45,36% saturated and 53,96 unsaturated fatty acids (Table 2). 53,47% saturarated and 46,42% unsaturated fatty acids were found in the lipid fraction of Chironomidae larvae from the pond L2. These percentage of fatty acids as well as 14 identified fatty acids in *Chironomus plumosus* larvae, fully confirm earlier researches that these organisms are almost ideal food source for carp fry.

Palmitic acid dominated (26,15 i.d. 32,67%), among saturated fatty acids in *Chironomus plumosus* larvae (Table 2), followed by stearic acid (10,75% in L1 i 10,82% in L2).

Oleic acid was most common among monounsaturated fatty acids (12,63% in L1 and 15,96% in L2), followed by palmitoleic acid (6,86 ie. 4,20%, Table 2).

Among polyunsaturated (essential) fatty acids found in Chironomidae larvae in the pond L1 (table 2), the highest concentration was measured for ω-6 linoleic acid (21,37%), followed by ω-3 linolenic acid (3,21%) and ω-3 eicosapentaenoic acid (1,27%). Linoleic acid (9,78%) and ω-3 eicosapentaenoic acid (0,45%) content was lower in Chironomidae larvae collected from the pond L2 (Table 2), while the content of linolenic acid was higher amounted to 7,78% High concentration of linolenic acid is probably associated with *Chironomus plumosus* nutrition, because they consume phytobenthic green algae as a food source.

Ratio of ω-3 i ω-6 fatty acids in *Chironomus plumosus* larvae was 0,21 in the L1, respectively 0,81 in L2 (Table 2).

Comparison of total fatty acids in Chironomidae larvae from the L1 and L2 ponds showed that these larvae do not contain PUFA with 22 carbon atoms. These data are in correspondence with previous results of Sushchik et al. (Sushchik *et al.,* 2003). The lack of PUFA with 22 carbon atoms is probably related to low enzimatic activity of Chironomidae larvae for the synthesis of these acids from their precursor, PUFA with 18 carbon atoms.

Chironomidae larvae in the pond L1 had lower saturated fatty acid content (45,36%) but higher unsaturated content (53,96%) compared to those in L2 pond (Table 2).

In our reseaches amount of saturated fatty acid was higher (45,36 i.d. 53,47%) which differates from the results of Bogut and associates (26,12 %, Bogut *et al.*, 2007).

It should be noticed that in the research mentioned above, *Chironomus plumosus* larvea lacked of stearic acid, while in our research it's amount is 10,75 i.d. 10,82% (Table 2). Content of monostaurated fatty acid in our survey was somewhat lower (28,11% in L1 and 28,04% in L2) in accordance to results published by Bogut and associates

(30,42%, Bogut *et al.*, 2007) as well as the content of polyunsaturated fatty acid 25,85% i.d. 18,38% (34,03%, Bogut *et al.,* 2007).

As it was shown in the research by Bogut and associates (Bogut *et al.,* 2007), n-3-/n-6 ratio was 0,81% which was equal to obtained results from *Chironomus plumosus* larvae in the L2 pond, while that ratio was far lower (0,21%) because of the higher content of n-3 linolenic acid.

Table 2. Fatty acid composition of Chironomidae larvae (% of total fatty acids, given as mean±standard deviation)

Fatty acid	Chironomidae L1	Chironomidae L2
C14:0	2.34±0.09	4.79±0.10
C15:0	1.67±0.08	1.62±0.02
C16:0	26.15±0.41	32.67±0.23
C16:1	6.86±0.38	4.20±0.01
C17:0	2.37±0.04	2.08±0.25
C18:0	10.75±0.33	10.82±0.45
C18:1cis-9	12.63±0.08	15.96±0.29
C18:1 cis-11	7.02±0.23	7.00±0.26
C18:2n-6	21.37±0.93	9.78±0.11
C20:0	2.09±0.03	1.91±0.13
C18:3n-6	-	-
C18:3n-3	3.21±0.12	7.78±1.32
C20:1n-9	1.60±0.06	0.89±0.25
C20:2n-6	-	0.27±0.06
C20:3n-6	-	<0.1
C20:3n-3	-	-
C22:1n-9+C20:4n-6	0.70±0.08	<0.1
C20:5n-3	1.27±0.03	0.45±0.06
C22:5n-3	-	-
C22:6n-3	-	-
SFA	45.36±0.19	53.47±0.64
MUFA	28.11±0.59	28.04±0.78
PUFA	25.85±0.84	18.38±1.25
N-6	21.37±0.93	10.16±0.01
N-3	4.48±0.09	8.22±1.26
N-3/N-6	0.21±0.01	0.81±0.13

Concentration of ω-3 fatty acid (4,48 i.d. 8,22%, Table 2) found in *Chironomus plumosus* is significantly higher compared to other animals and it represents an important source of essential fatty acids not only for fish, but also for humans (Bogut *et al.*, 2007).

What is more important is the fact that larvae of cyprinid fish have higher needs for ω-6 fatty acids (ca. 1% dry feed) than ω-3 (0,05-0,10%, Csengreri, 1993; Takeuchi, 1993; Steffens, 1993; Radünz-Neto etal., 1996). It also important to notice that these larvae are an exceptional source of fatty acids.Our researches have confirmed this fact and showed that *Chironomus plumosus* larvae are enriched with ω-6 fatty acids (21,37 in L1 and 10,16 in L2%) and represents suitable natural food source for carp fry.

Having in mind there are data which show lower ratio value of ω-3 and ω-6 fatty acid in cultured fish compared to fish found in natural habitat (Van Vliet and Katan, 1990), stimulating development of natural feed in carp farms – like *Chironomus plumosus* larvae, which are enriched with not only with essential fatty acids but also in most amino acids – can play very important role in high quality meet production.

CONCLUSIONS

Crude protein content was 6,61% (pond L1) and 6.18% (pond L2) in *Chironomus plumosus* larvae. Fat content was 0,49% and 0,73%. Water content was 88,95% in L1 and 89,62% in L2.

Fat content of the *Chironomidae* larvae from the pond L1 contained 45.36% saturated and 53.96% unsaturated fatty acids, while fat content of larvae from the pond L2 contained 53.47% saturated and 46.42% unsaturated fatty acids.

Among the polyunsaturated (essential) fatty acids isolated from the Chironomidae larvae, the most abundant was ω-6 linoleic acid (21,37%), followed by ω-3 linolenic (3,21%) and ω-3 eikosapentaenoic acid. Linoleic acid content in chironomidae larvae from the pond L2 was lower - 9,78%, ω-3 eicosapentaenoic acid content was 0,45%, and linolenic acid content was higher – 7,78%.

Among saturated fatty acids, plamitic (26,15% i.d. 32,67%) following stearic (10,75% in L1 and 10,82% in L2) acid dominated in *Chironomus plumosus* larvae.

Oleic acid was most common among monounsaturated fatty acids (12,63% in L1 and 15,96% in L2), followed by palmitoleic acid (6,86 ie. 4,20%)

The lack of PUFA with 22 carbon atoms is probably related to low enzimatic activity of Chironomidae larvae for the synthesis of these acids from their precursor, PUFA with 18 carbon atoms.

The measured quantity of ω-3 and ω-6 fatty acids from *Chironomus plumosus* larvae was 0,21 in the pond L1 and 0,81 in the pond L2, which is sufficient for the nutritional requirements of carp.

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EFFECTS OF FEED QUANTITY ON THE WEIGHT GAIN OF CARP JUVENILES REARED IN TANKS

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UTICAJ KOLIČINA HRANE NA PRIRAST ŠARANSKE MLAĐI U TANKOVIMA

Abstrakt

Šaran se gaji u sva tri sistema: ekstenzivni, poluintenzivni i intenzivni. Za razliku od ekstenzivnog gde je prirast ribe isključivo zavistan od prirodne hrane, poluintenzivni i intenzivan sistem gajenja riba su bazirani na delimičnoj, odnosno potpunoj zavisnosti od dodatne hrane.

Kako tokom sezone gajenja, prirodna hrana u ribnjacima sa poluintenzivnim sistemom ima veoma izražen sezonalni karakter, u periodu sa optimalnim temperaturama za rast šarana (od sredine juna pa do kraja avgusta) prirast je u najvećoj meri zavistan od vrste i količine dodatne hrane. Sa ekonomskog, ali i ekološkog aspekta važno je obezbediti hranu koja će rezultirati niskim koeficijentom konverzije, visokim tempom rasta, dobrim zdravstvenim stanjem gajenih riba, visokim kvalitetom finalnog proizvoda, tj. ribljeg mesa i što manjim opterećenjem vodene sredine organskim materijama, fosforom i azotom. Od velike važnosti je i obezbeđivanje adekvatne količine dodatne hrane, odnosno ne dozvoliti da količina hrane bude manja od potreba gajene ribe, ali i ne preterati sa količinom koja od strane riba neće biti racionaln iskorišćena i time smanjiti profitabilnost gajenja riba. U cilju određivanja optimalnog procenta hrane u odnosu na ihtiomasu gajene mlađi šarana urađen je ovaj rad.

Eksperiment je realizovan u Centru za ribarstvo i primenjenu hidrobiologiju, Poljoprivrednog fakulteta, Univerziteta u Beogradu tokom 90 dana. Za prihranu šaranske mlađi korišćena je ekstrudirana hrana sa 38% proteina i 8% masti proizvođača Vetrinarski zavod «Subotica», veličine peleta 2 mm. Eksperiment je realizovan u 4 tretmana sa po 3 ponavljanja. Tretmani su se razlikovali po procentualnom učešću količine hrane u odnosu na ihtiomasu u tanku na početku svakog tridesetodnevnog perioda. U prvom tretmanu je dnevni obrok riba 2%, u drugom 3%, u trećem 4%, dok su ribe u tretmanu četiri hranjene sa 5% hrane u odnosu na ihtiomasu.

U svaki od 12 nezavisnih tankova nakon perioda adaptacije, nasađeno je po 40 jedinki šarana, prosečne mase 6,41 do 6,47 g. Hranjenje riba je obavljano svakodnevno upotrebom hranilica sa trakom.

Na osnovu prosečnih masa riba po periodima i na kraju eksperimenta, statističkom analizom je ustanovljeno da postoje vrlo značajne razlike u prirastu u zavisnosti od dnevnog obroka. Ribe hranjene sa 5% hrane u odnosu na ihtiomasu ostvarivale su statistički značajno veći prirast u odnosu na ribe hranjene sa 2, 3 i 4% hrane. Korišćenjem ovog rezultata se otklanja dilema koja je količina hrane za prihranu gajene šaranske mlađi optimalna tokom gajenja šaranske mlađi u prvoj godini. Pored najboljeg prirasta, šaranska mlađ hranjena sa 5% hrane je imala i najbolji faktor kondicije, specifičnu stopu rasta, kao i termalni koeficijent rasta. U odnosu na ribe hranjene sa 3 i 4% hrane, mlađ hranjena sa 5% je imala nešto veći koeficijent konverzije (za oko 10%), međutim imajući u vidu značajnu razliku u prirastu između svake od 4 hrane, ovo povećanje koeficijenta konverzije je ekonomski opravdano.

Ključne reči: *šaranska mlađ, procenat hrane, prirast*

INTRODUCTION

Carp is cultured in all 3 systems: extensive, semiintensive and intensive. Contrary to extensive rearing where weight gain depends uniquely on natural food, semiintensive and intensive systems are based on partial or complete reliance on added feed.

During the rearing season the natural food in fish ponds with the semiintensive system has a prominent seasonality: in the period of natural food depression with optimal temperatures for carp growth (from mid June to end August) weight gain is primarily dependent from the type and quantity of added feed (Marković, 2010). From the economical, but also ecological point of view it is important to supply feed that will result in low feed conversion coefficient, high growth rate, good health condition, high quality of the final product – fish meat, and as low as possible load of the aquatic environment with organic matter, phosphorus, and nitrogen (Jahan et al., 2003). Of great importance is provision of adequate quantity of added feed, i.e. to prevent insufficient feed quantity, but also not exaggerate with added feed quantity and thus decrease production profitability (Bailey and Alanärä, 2006). In that aim this study was carried out.

MATERIAL AND METHODS

Study of the effects of feed quantity on weight gain of carp fry was performed in the Centre for Fishery and Applied Hydrobiology of the Faculty of Agriculture University of Belgrade. The study lasted 90 days, 2 month old carp (*Cyprinus carpio*) fry was used. Extruded feed with 38 % proteins, 8% lipids, pellet size 2 mm was used in 4 treatments in 3 repetitions. Treatments differed in feed quantity. The added feed was calculated as a percent of body mass of the fish and was recalculated at every 30 days, after the control measurement of the fish. In the first treatment daily diet was 2 %, in the second 3 %, in the third 4%, and in the fourth 5% of the fish total body mass in the tank. Each treatment was different in daily percent of added feed.

Experiment was carried out in plastic tanks 130 l with constant flow through of 0.51 l/min. Tanks were connected to the recirculation aquaculture system (RAS), where chemical, biological and microbiological purification of the used water was done. In the frame of the RAS water heating and aeration provided identical conditions for experiment realization.

In every 12 independent tanks after the adaptation period 40 specimens of two months old carp fry were stocked, average weight 6.41 to 6.47 g. Fish was fed using belt feeder (AGK Kronawitter GmbH). Water quality and environmental conditions (water temperature, electroconductivity, dissolved oxygen, and pH) were measured in each tank every 3rd day using MULTI 340i/SET (WTW, Weilheim, Germany). Feed quantity, depending on treatment (2, 3, 4, 5 %) were measured daily using a digital balance Radwag (THB – 600, accuracy 0.01 g), while fish weight was measured in the 30 days periods.

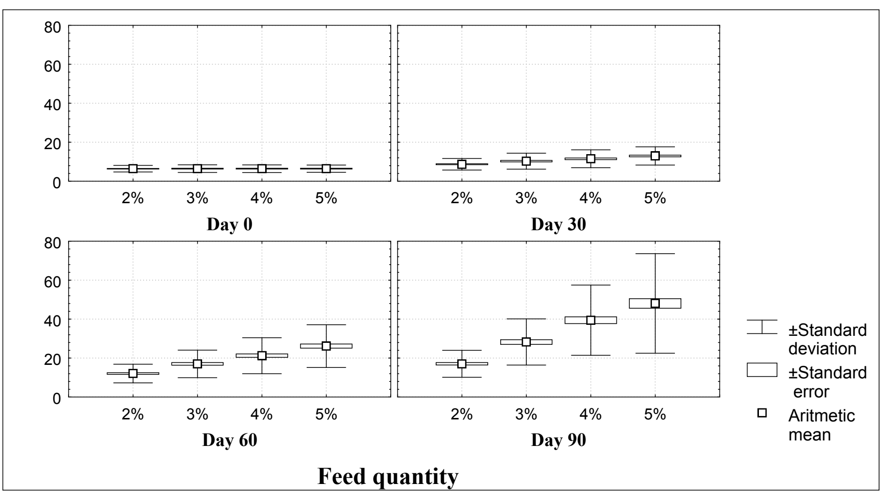
Following parameters for growth performances were calculated using equations: BWG (body weight gain) = final body weight (g) – initial weight (g); SGR (specific growth rate) = (ln final weight – ln initial weights) x (days of trial-1) x 100; FCR (Feed conversion ratio) = (feed intake, kg) x (wet weight gain, kg)$^{-1}$; TGC (Thermal Growth Coefficient) = [(final weight)$^{1/3}$ – (initial weights)$^{1/3}$] x (days x C^{o})$^{-1}$ x 1000; CF (Condition factor) = body weight (g) x (fork length, cm)$^{-3}$ x 100; LH (Relationship between length and body height) = body length / body height.

Statistical testing of the hypothesis was done using parametrical model of analysis of variance in the case of homogeneous data ($c_v \leq 30\%$) and sample variation. For sample with variable values ($c_v > 30\%$) and heterogenous variability a Kruskal Wallis model was employed. Individual comparison of average values were carried out using LSD test ($c_v \leq 30\%$) and Mann-Whitney-ev U test ($c_v > 30\%$).

RESULTS

At the start of the experiment results of Levene's test have shown that variation in tanks was not significantly different for measured parameters pointing out that the experimental setup was correct and that differences in further measurements are effect of different feed quantity.

Figure 1. Fish body mass related to feed quantity per examined period



After a 90 days period average fish body mass in tanks varied from 16.3
After a 90 days period average fish body mass in tanks varied from 16.36 g in tank N° 5 till 68.74 g in tank in tank N°12. Least average body mass had fish from the first treatment 17.08 g, and highest average body mass was reached in treatment 4 – 54.32 g.

Results of the Kruskal-Wallis test ($H = 139.87$; $p < 0.001$) confirm that the average fish body mass was significantly dependent on feed quantity (2, 3, 4, 5 %). According to the results of Mann-Whitney test it can be concluded that increased feed quantity affects average body mass. At experiment end, variances in fish body mass between tanks were significantly different. Kruskal-Wallis test ($H = 181.567$; $p < 0.001$) shows significant differences between average body mass. Mann-Whitney test demonstrates that fish fed 4 and 5 % body weight differ significantly in average body mass ($p = 0.017$). Other groups differ very significantly.

Table 1. Growth parameters, condition factor, and feed conversion ratio in different treatements (feed quantity)

Feed quantity	2%	3%	4%	5%
% weight gain	165.44	370.35	572.53	742.17
Condition factor	1.75	2.00	2.08	2.24
Relationship between length and body height	3.45	3.30	3.27	3.21
BWG- body weight gain	418.73	842.24	1338.21	1723.06
FCR- feed conversion ratio	1.57	1.37	1.39	1.54
SGR- specific growth rate	1.08	1.71	2.11	2.35
TGC- Thermal growth coefficient	0.37	0.65	0.84	0.98

Weight gain of fish during the experiment varied from 418.73 g (treatment 1); 842.24 g (treatment 2), over 1338.21 g (treatment 3), to 1723.06 g (treatment 4). Fish fed 5 % total body weight per tank achieved best weight gain, had best condition factor, and best SGR and TGC. Compared to fish fed 3 % and 4 %, fry fed 5% ichthyomass had slightly higher feed conversion ratio (for approximately 10%).

DISCUSSION

Presence and availability of natural food in the dominant semiintensive culture system affects significantly carp growth (Rahman and Verdegem, 2007). It represents almost irreplaceable set of essential matters for growth and development of cultured fish (Kirbia et al. 1997) because of its excellent protein, free amino acids and oligopeptides, lipids and fatty acids, vitamins, and minerals content. In periods of natural food depression or its insufficient development applying proper quantity and quality of feed is essential for maintenance of good growth rate. In order to assure optimal feeding and management of the semiintensive system, it is important to know nutritional requirements of different age categories of carp (Rahman et al., 2009). In the absence of natural food, in the intensive system growth is entirely dependent on added feed, its quantity and quality.

According to the results obtained it could be concluded that best weight gain was obtained with the treatment 5 % of ichthyomass, and worse with 2 %. Insufficient quantity of available feed in fish causes acute stress (Ruane et al., 2002). Although fish are acclimated to low feed intake, it affects other aspects of fish physiology besides weight gain (Ali and Wootten, 1999). This can be related to the fact that sub optimal feeding cannot recompense all the needs for growth and development, therefore growth is reduced. Compared with fish fed 4 and 5 % body weight, average body mass were very significantly different.

Despite the fact that feed conversion ratio in treatment 4 was 10 % higher than in treatment 2 and 3, the use of 5 % ichthyomass per tank demonstrates economic justification due to the big difference in weigth gain of carp fry. Average fish body mass from treatments 4 and 5 % differ for 25.7 %. By feeding fish with 3 and 5 % body weight, average body mass differed for 78.8 %, while fish fed 2 and 5 % body mass differed in weight gain for even 218 %

Weight gain (BWG) was significantly different in different study periods, as well as treatments. Since fish fed 5 % ichthyomass per tank had highest average body mass, as well as the weight gain. It was very significantly different from weight gain in other treatments, as well as in same tanks in the next study periods (in 30 days intervals). This confirms continuing growth and weight gain in fish fed with higher feed quantity (4 and 5 %). Fish fed smaller quantity (2 and 3 %) had weight gain significantly higher compared to the previous study period, but significantly smaller weight gain compared with treatment 3 and 4 (added feed quantity 4 and 5 %).

CONCLUSIONS

According to the results obtained in different study periods; at the end of the experiment; and by statistical analysis, it can be concluded that there are significant differences in average body mass depending on feed quantity. Fish fed 5 % of their body mass had best average body mass compared to fish fed 2, 3, and 4 % body mass. Besides, they had best weight gain significantly different from previous period (compared to the same tank or treatment). Results obtained using 5 % feed point out proper feed quantity in both the experiment beginning, when average mass was 6.4 g, and also later when average body mass was 54.3 g. This experiment results are solving one important dilemma concerning optimal feed quantity in carp fry nutrition during the first year of production.

ACKNOWLEDGEMENT

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ECTOPARASITES OF THE SEA BASS IN BOKA KOTORSKA BAY

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EKTOPARAZITI BRANCINA U BOKOKOTORSKOM ZALIVU

Abstract

The objective of this work has been to examine the presence, identify ectoparasite species and extensity and intensity of infection of Sea Bass *(Dicentrarchus labrax)* cultivated in cage systems of breeding in Boka Kotorska Bay during a production cycle.

Additionally, pathomorphological and pathohistological changes in infected fish caused by parasites have been examined. The examination covered both juveniles and adult fish.

The examinations were performed during three seasons: in the spring, summer and autumn of 2009. By examining ectoparasitic fauna in cage systems of Cogi in Boka Kotorska Bay, we found the presence of the following parasites: *Diplectanum aequans, Trichodina spp., Amyloodinium ocellatum.*

The most important ectoparasitic species in Sea Bass in our examination was *Diplectanum aequans* – monogenean gill parasite. This parasite in a case of big infestation can cause disease diplectanosis. The extensity of infection caused by identified ectoparasite species varied depending on the season, but the biggest was during the summer, while the intensity was generally low. Therefore, no significant damage was noted in the cage systems. Minor pathomorphological and pathohistological changes were noted in the infected fish.

Due to low intensity of parasitic fauna, no specific therapy administration was required. However, more intensive administration of prophylactic measures and regular veterinary surveillance could result in significant reduction in the presence of parasites and, consequently, more efficient performance.

Key words: *Sea Bass, ectoparasites, cage system*

EFFECTS OF DIFFERENT SALINITIES ON MATURATION PERIOD OF *ACARTIA CLAUSI* COPEPOD OF CASPIAN SEA

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EFEKTI RAZLIČITOG SALINITETA NA SAZREVANJA KOPEPODE *ACARTIA CLAUSI* IZ KASPIJSKOG MORA

Abstract

The present study was conducted to evaluate the effects of salinity on maturation rate of *Acartia clausi* which is a copepod in Caspian Sea. The experiment was done during March to April 2007 and the referred zooplankton was collected from southern coast of Caspian Sea via plankton net. The gathered zooplankton was introduced to culture tanks for mass production. Afterwards *Acartia clausi* nauplius was isolated from tanks and transferred to four different salinities (07, 13, 18, 24psu) with three replicates in separate glass dishes in order to obtain growth and maturation. An equal mixture of *Isochrasis galbana* and *Chaetoceros sp*. alga were used for nauplii daily feeding.

Statistic analysis indicated significant differences among the salinity treatments in the time of maturation ($p<0.05$). However, maturation period in salinities of 7, 13, 18 and 24psu was prolonged to 285.6, 268.8, 288 and 396 hours respectively. In addition, the results of Duncans averages comparison test showed that the 13psu salinity caused the lowest time for the maturation of 50% of the population, and this salinity obtained the first rank (a) among other salinity groups.

Key word: *Acartia clausi, Copepod, nauplius, zooplankton*

INTRODUCTION

Copepods are primary consumers in the oceans which have crucial effect on transferring the carbon of phytoplankton to higher trophic levels (Mckinnon et al., 2003). Also they are the most numerous crustaceans among zooplankton community [(Knueky et al., 2005) and (Schipp et al., 1999)]. Copepods, especially their nauplii, are natural prey items for fish larvae ([Chesney, 2005] and (Chen et al., 2006), typically make up fifty percent or more of their stomach contents (Camus and Zeng, 2008). This makes copepods possible attractive candidates to use as live food for commercial larviculture in hatcheries (Shamsudin et al., 1997) and (Knucky et al., 2005). Three orders of planktonic copepods are important in aquaculture: 1) Calanoida, which have been the copepods that was most successful in colonizing both marine and fresh water environments (Schipp et al., 1991). 2) Cyclopodia and 3) Harpacticoida, which are mostly parasitic (endo or ectoparasites) and due of their bigger size, they have the less importance in aquaculture (Damgaard & Davenport, 1994). Some calanoid species, belonging to the *Acartia* genus are important in aquaculture because of their suitable size for feeding of marine larvae (Mckinnon et al., 2003) and (Toledo et al., 1999).

Life cycle of *Acartia clausi* starts from eggs hatching into nauplius larvae, which consist of a head with a small tail, but no thorax or true abdomen (Almeda et al., 2010). The nauplius moults six times (which lasts for approximately 3.2 days) before emerging as a "copepodid larva"(Calliari et al., 2008). This stage resembles the adult, but has a simple unsegmented abdomen and only three pairs of thoracic limbs (Almeda et al., 2010). After five molting, which lasts around 4.95 days for males and 5.23 days for females, the copepod finally takes on the adult form (Calliari et al., 2008). The difference between male and female is displayed by formation of "fifth leg" which is coarse and circular in males and sharp in females (Mckinnon et al., 2003).

Salinity is one of the most important environmental parameters effecting the seasonal and spatial distribution of the planktonic copepod *Acartia clausi* in the wild (Miller and Marcus, 1994). However, this parameter can also be relatively easily manipulated in aquaculture hatcheries, where mass culture of copepods is desirable in means of providing live prey for culture animals (Milione and Zeng, 2007). *Acartia clausi* is naturally adapted to sudden shift in temperature and salinity, thereby making it suitable for rearing fish species whose larvae occur in either estuarine or oceanic environments (Mckinnon et al., 2003, [Camus and Zeng, 2008). The effects of salinity on productivity of calanoid copepods has shown well, so that it has clear effects on egg production, egg hatching rates ([Hall and Burns, 2002] and [Peck and Holste, 2006]), as well as on nauplii growth for various calanoid copepod species (Chinnery and Williams, 2004). But, to date, no published information is available on the optimum salinity condition to obtain minimum time for maturation of this species which is an important subject in commercial marine larviculture.

MATERIALS AND METHODS

In order to determine the effects of salinity on maturation period of copepod *Acartia clausi*, a random experiment were set up with 4 salinity treatments (7, 13, 18 and 24psu) and 3 replicates. Twelve 800 cc glass dishes were used as experimental plots. In addition, a plankton net (with 100µm mesh size) which was stretched via a boat across Khazaradad port in southern cost of Caspian Sea has been used for collecting copepods. Sampling was done in surface water for 2-3 hours and samples were transferred to laboratory in 1 litter dishes.

In order to purify the mentioned species, the contents of plankton net were diluted in test tubes with filtered sea water. Recognizing and separating the males and females was done by using microscope (Nikon-E-200) and micropipette. Then the adult copepods (one pair of male and female) were presented to individual Petri dishes with a little amount of seawater.

Throughout the experiment period, *A. clausi* was fed with equal mixed *Isochrysis galbana* and *Chaetoceros sp* alga which were cultured purely in Gillard media culture. 25000-30000 alga cellules/cc of media culture was suitable for feeding *A. clausi*. Microscope and Neobar lam were utilized for daily alga counting. The photoperiod was 12h light: 1050 Lx 12 h dark cycle with 1050 Lx light intensity. Salinity and pH were 12-13psu and 7.5-8.5 respectively. During the period, egg production and hatching were accomplished and nauplii were randomly captured by using a fine-tipped pipette for transferring into the 20 litter mass culture tanks.

Various salinity treatments were obtained by either diluting the Caspian Sea water or adding sea salt it. The copepods used in this experiment were gradually acclimated to the various salinities by changing 2.5psu every 12 h until the required salinity levels were reached. 40%-50% of water was exchanged daily using a siphon through out a 38 µm filter.

After appearing the "fifth leg" in copepods, the number of adult females was recorded daily. In addition, the elapsed time for maturation of 50% of copepod communities in each treatment has been determined. Data for maturation rate in different salinity treatments were analyzed by one way ANOVA in order to determine differences. The Duncan's multiple comparison test was used to compare the adults number averages among different treatments. The linear regression model for maturation on the basis of elapsed time was analyzed by utilizing SPSS soft ware, version 11. Results were presented as mean ± standard error (SE).

RESULTS

The elapsed times until maturation in all treatments is shown in table 1. The maturation times for 50% of copepod population in salinity levels 7, 13, 18 and 24psu, resulted in 285.6, 268.8, 288 and 396 hours respectively. Regarding to table 2, the salinity level of 13psu (treatment no.2) caused the lowest maturation time among other salinity treatments.

Table 1. The average number of adult copepods in 4 salinity treatments considering time

Day		**9*th***	**10*th***	**11*th***	**12*th***	**13*th***	**14*th***	**15*th***	**16*th***	**17*th***	**18*th***	**19*th***
Average number of adults in salinity level	***7psu***	0	0	7.5	3	0	0	0.5	0.5	0.5	0.5	0
	13psu	1	1	12	o.5	0	0	0.5	4	0	0	0
	18psu	0	15.67	32.67	19	10.67	16.67	28.33	0	0	0	0
	24psu	0	0	0	0	0	0.3	0.7	1	18.3	2.3	0.3

Table 2. The results of variance analyzes and Duncan's test on maturation time in different salinities

Examined factor	The average in salinity treatments				*fs*
	7psu	*13psu*	*18psu*	*24psu*	
Maturation time of 50% of *A.clausi* communities (hour)	285.6 ^{ab}	268.8 ^{a}	288 ^{ab}	369 ^{ab}	5.487*

*Significant differences in the level of 0.05

As it is seen in table 1, salinity treatments caused significant differences (P<0.05) on the maturation period of 50 percent of *A. clausi* population. Across the wide range of salinity treatments from 7psu to 24psu, the lowest maturation time (268.8 h) was recorded at 13psu and the highest time (369 h) at 24psu. Therefore the salinity of 13psu obtained the first rank among other 4 treatments.

The figures 1 to 4 display the liner regression diagrams between salinity and time of maturation in 50% of copepod population.

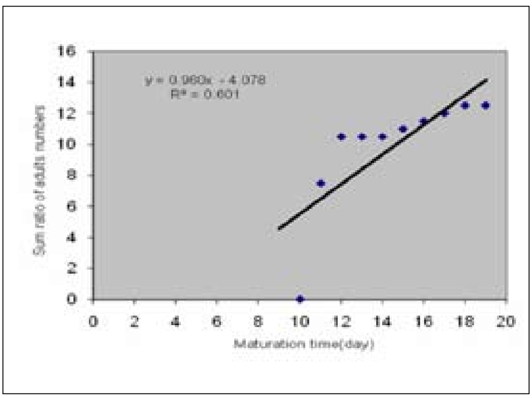

Figure 1. Diagram of maturation for 50% of population in 7psu treatment

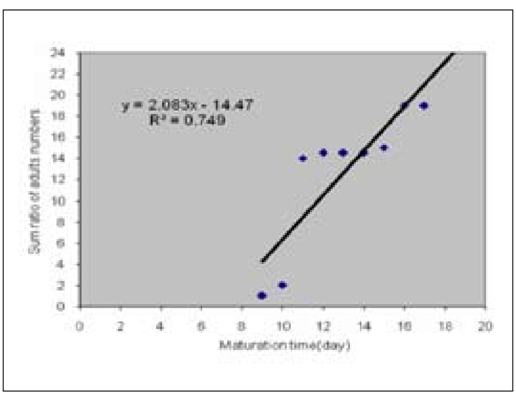

Figure 2. Digram of maturation for 50% of population in 13psu treatment

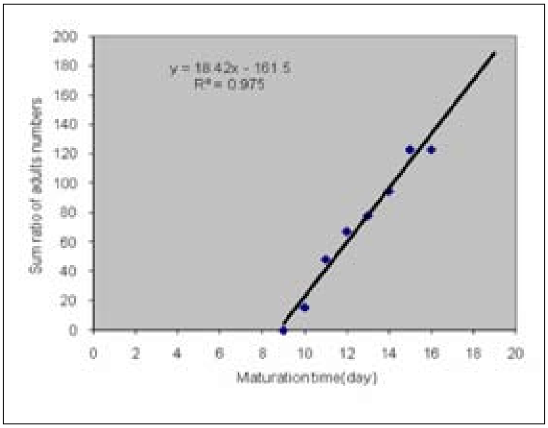

Figure 3. Diagram of maturation for 50% of population in 18psu treatment

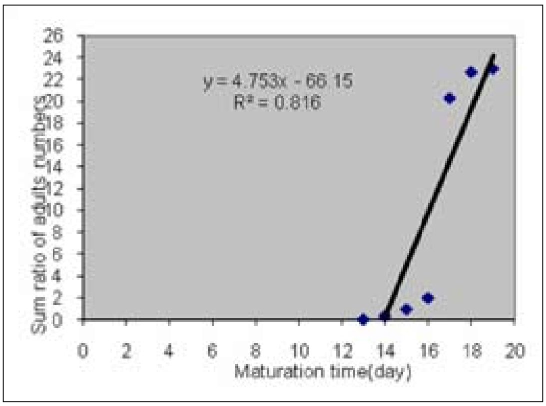

Figure 4. Diagram of maturation for 50% of population in 24psu treatment

As it could be seen from the figures 1 to 4, 13psu salinity has had more appropriate effect on maturation rate of *A.clausi* population

DISCUSSION

Under natural conditions, Calanoida copepods are often well adapted to cope with seasonal salinity fluctuation ([Miller and Marcus, 1994], [Engel and Hirche, 2004] and [Camus and Zeng, 2008]). Species found in estuarine habitats in particular have broad tolerance ranges for this parameter ([Castro and Longoria, 2003] and [Chinnery and Williams, 2007]). This is a useful attribute for aquaculture, as live food for fish larvae will be alive and available in water under various salinity conditions that are suitable for the fish larvae culture (Camus and Zeng, 2008).

For aquaculture purpose, maximizing copepod productivity in the least period is important and salinity conditions used for culturing are likely to provide productivity. For example, previous studies have shown that the highest copepod productivity was observed in the ratio 13- 28psu salinity ([Damgaard et al., 1994] and [Hall et al., 2002]). In addition, it has been stated that copepod survival in 9psu was notably more than 35psu (Cerretto, 1994) and maturation period in18-27 psu was considerably lower than in 9 psu salinity (Calliai et al., 2008). Meanwhile, present study was designed to focus on the salinity which causes the minimum time for maturation and was not reported until now (table2). Nevertheless, in this experiment, cannibalism was not monitored and therefore its effects on maturation time were unknown. We considered this approach (salinity) more pertinent to the ultimate goal of copepod culture for aquaculture.

The findings of this study have indicated that the salinity has had negligible effects on nauplii production and 17.1+-2.6 nauplii were produced in all treatments every day.

Given that *A. clausi* is generally considered a euryhaline species, as it is commonly found in coastal and estuarine waters ([Uye, 1985] and [Mckinnon et al., 2003]), some flexibility is expected in its ability to tolerate salinity changes. The results of this study demonstrated that maturation time decreased significantly at 13psu salinity and most matured copepods were observed at the 11^{th} and 15^{th} days (fig.2). At lower salinities, the most maturation occurred at the 12^{th} and 20^{th} days (fig.1). However, the higher salinity does not determine specific period for the most maturation (fig.3, 4). In summary it seems that in high salinity levels specially 18 and 24psu, the effect of time on maturation is more important than salinity (fig.3, 4). Meanwhile, the maximum maturation in lower salinity levels is more dependent to salinity rather than time. However this is something that needs more study in future. At the same time the results of this study demonstrate that, while *A. clausi* could survive in wide salinity ranges; this factor has strong impacts on the maturation trend of this species. This study clearly was done in order to identify the optimal salinity conditions for *A. clausi* culturing, which seems to be applicable in commercial scales. Based on the finding of this experiment, to achieve maximum productivity of *A. clausi* for aquaculture purposes, the species should be farmed in 13psu salinity.

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SOME BIOLOGICAL PARAMETERS OF SHORTFIN SQUID, *ILLEX COINDETII* (VÉRANY, 1839), IN TRAWL FISHERIES ON THE MONTENEGRIN COAST

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NEKI BIOLOŠKI PARAMETRI VRSTE LIGNJUN MALI ILI TOTANJ (*ILLEX COINDETII* VÉRANY, 1839) UHVAĆENIH POVLAČNOM MREŽOM-KOČOM NA CRNOGORSKOM PRIMORJU

Abstract

U razdoblju od septembra 2009. do septembra 2010. obrađeno je 299 primjeraka vrste lignjun mali ili totanj (*Illex coindetii* Vérany, 1839) uhvaćenih pridnenom povlačnom mrežom-kočom na području Crnogorskog primorja. Uzorci su otkupljivani od ribara po dolasku na mjesto iskrcaja, i potom prenošeni u laboratorij Instituta za biologiju mora u Kotoru.

U laboratoriju je svakom primjerku određena dorzalna dužina plašta pomoću ihtiometra s preciznošću od 1 mm, izmjerena ukupna masa i masa gonada na elektroničkoj vagi (preciznost 0,01 g). Stadiji zrelosti određeni su prema MEDITS–ovoj skali (MEDITS Instruction manual V.5, 1997).

Dužinsko–težinski odnos izražen je preko formule $W = a \cdot L^b$, a parametri a i b određeni su metodom linearne regresije i pretvorbom u prirodne logaritme ($\ln W = \ln a + b \cdot \ln L$) (Huxley, 1924; Jensen, 1976).

Gonadosomatski indeks izražen je preko formule:

$$GSI = \frac{W_{gonad}}{W}$$

Odnos spolova izražen je kao odnos broja ženki u ukupnom broju spolno određenih primjeraka:

$$SR = \frac{N_f}{N_f + N_m} \cdot 100$$

U ukupnom uzorku bilo je 68 (23%) ženki, 143 (48%) mužjaka i 88 (29%) jedinki neodređena spola. Odnos spolova bio je 32,27 u korist mužjaka. Prosječna dužina plašta

iznosila je 11,04 cm sa mužjacima u rasponu od 7,4 do 15,9 cm, a ženke od 7,5 do 20,7 cm. Najviše jedinki pripadalo je II. stupnju zrelosti.

Dužina prve spolne zrelosti ($DML_{50\%}$) izračunata je za ženke (150 mm), mužjake (133 mm) i oba spola zajedno (90 mm). Također su izračunate dužine pri kojima 25%, odnosno 75% populacije dostiže spolnu zrelost.

Gonadosomatski indeks izračunat je za mužjake i ženke. Kod mužjaka vrhunac dostiže u junu i poslije toga je u stalnome padu, dok kod ženki vrhunac dostiže u julu.

Dužinsko–težinski odnos pokazao je alometrijski negativan rast na cijelom uzorku, ali i odvojeno za mužjake i ženke. Kako je *I. coindetii* brzorastuća, kratkoživuća vrsta, izračunati su i sezonski parametri za jesen, zimu i ljeto (u proljeće 2010. nije bilo uzorkovanja) kako bi se dobio točniji uvid u rast lignjuna. Ipak, i zimski i ljetni period pokazuju negativan alometrijski rast (s iznimkom ženki u ljetnom razdoblju), dok je rast alometrijski pozitivan za oba spola i ukupni uzorak u jesenjem periodu.

Usporedba sa ranijim istraživanjima vrste *I. coindetii* u Jadranu pokazuje nešto drugačije rezultate od onih dobivenih u ovome istraživanju, što se djelomice može objasniti relativno malim brojem uzoraka, nedostatkom uzoraka iz proljetnog razdoblja i neravnomjernog odnosa spolova.

Ključne reči: *Lignjun, Crnogorsko primorje, dužinsko–težinski odnos, GSI*

INTRODUCTION

Shortfin squid, *Illex coindetii* Vérany, 1839 is one of the economically most important species of cephalopods in the Atlantic, Mediterranean and Adriatic Sea. In the Adriatic it constituted about 95% of the total cephalopod catch until 2003 (FAO, 2006; Ceriola, 2006).

Despite its importance for fisheries purpose, the current information on the *I. coindetii* biology in the Southern Adriatic (especially its eastern part) is rather poor and published data focus mainly on the distribution of the species. This paper aims to provide information on some important biological parameters (length–weight relationship, size at first maturity, gonadosomatic index) of *I. coindetii* from the eastern part of South Adriatic, which can complement information on this species available from other sources.

MATERIALS AND METHODS

The samples were taken in period from September 2009 to August 2010, obtained from fishermen upon landing, and further processed in laboratory. The length was measured to the nearest mm, and the total body weight and weight of gonads (using a precise electronic balance) to the nearest 0.01 g.

Length–weight relationship was determined for the entire sample and seasonally, according to the formula $W = a \cdot L^b$. Parameters a i b were estimated using ordinary least–square regression after transforming the data in natural logarithms ($\ln W = \ln a + b \cdot \ln L$) (Huxley, 1924; Jensen, 1976).

The gonadosomatic index was calculated as a proportion of the gonad mass to the total body mass (Barber & Blake, 2006):

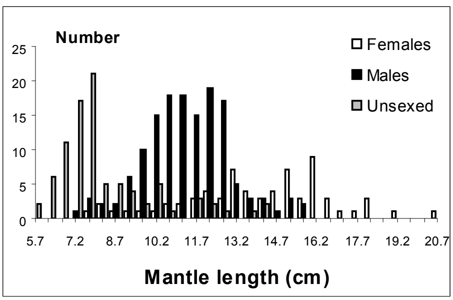
$$GSI = \frac{W_{gonad}}{W}$$

Maturity stages were determined according to the MEDITS Instruction manual V.5 (1997). Sex ratio was given as a proportion of females in the sample, according to the formula:

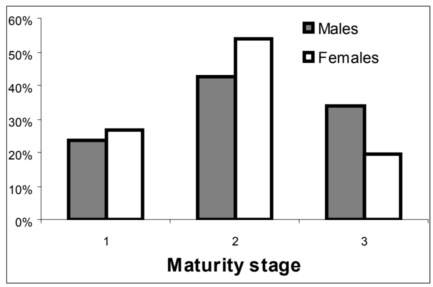
$$SR = \frac{N_f}{N_f + N_m} \cdot 100$$

RESULTS AND DISCUSSION

During the sampling period, a total of 299 *Illex coindetii* specimens were sampled in 7 samplings. The dorsal mantle length (DML) for the sampled specimens ranged from 5.5 to 20.7 cm, with an average length of 11.04 cm. Length frequency distribution (LFD) of males ranged from 7.4 to 15.9 cm, while that of females was from 7.5 to 20.7 cm. The majority of the sample consisted of individuals with DML in the 10.0 to 12.5 cm range (Figure 1.A).



A



B

Figure 1. Length frequency distribution by sex of *Illex coindetii* (A) and Gonad maturity stages proportion by sex of *Illex coindetii* (B)

Of the 299 specimens, 88 (29%) were unsexed or immature, 143 (48%) were males, and only 68 (23%) were females (Table 1). Sex ratio for the whole sample, expressed as the proportion of females in the subsample composed of sexed individuals was determined at 32,38 in favour of males. The vast majority of males were between 9.0 and 13, while females were mostly between 13 and 16.5 cm DML (Figure 1.A). The samplings had individuals of maturity stage 2 as the most abundant (Figures 1.B).

Table 1. Gonad maturity stages by sex of *Illex coindetii*

Maturity stage	M				F			
	No	%	DML min	DML max	No	%	DML min	DML max
1	38	26.57	7.4	12.6	16	23.53	7.5	15.2
2	77	53.85	9.6	15.9	29	42.65	9.0	20.7
3	28	19.58	11.1	15.2	23	33.82	11.6	18.2
Total	143	100.00			68	100.00		

Table 2. Length at first maturity and logistic curve parameters

Sex	Logistic curve parameters		Length at maturity (mm)		
	a	*b*	$ML_{25\%}$	$ML_{50\%}$	$ML_{75\%}$
♂	7.768003	0.580994	115	133	153
♀	5.158364	0.343528	118	150	182
♂+♀	5.331835	0.591038	72	90	110

The size at first maturity ($ML_{50\%}$) for the entire sample (males and females) was estimated at 90 mm (2.C), or at 134 for males and 159 mm for females (Table 2) The estimated values of $ML_{25\%}$ and $ML_{75\%}$ are given in Table 2.

Gonadosomatic index (GSI) for males ranged from 2 to 5.5, with a peak in June and a steady drop afterwards. The female GSI was in the 5.4 to 6.7 range, with a peak in July (2.A.).

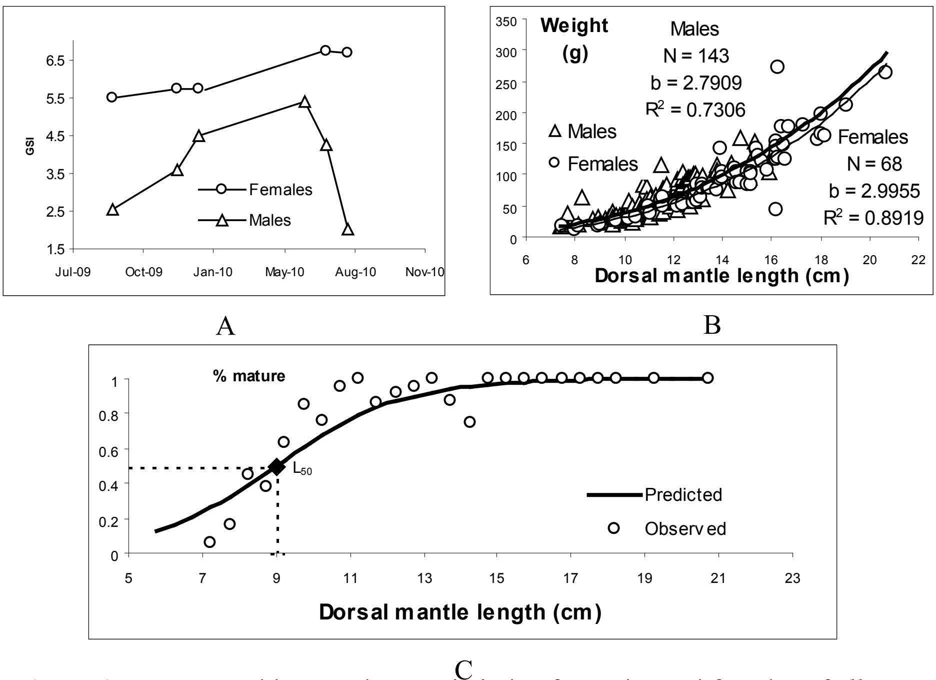


Figure 2. Mean monthly gonadosomatic index for males and females of *Illex coindetii* (A), length–weight relationship for males and females (B) and length at first maturity (50%) for both sexes (C)

The power coefficient of the LW relationship for the total sample was an allometric negative for both sexes ($b_♂$ = 2.7909, $b_♀$ = 2.9955) (Table 3, Figure 2.B). Due to short life–span and fast growth of the species in question, seasonal power coefficients for autumn, winter and summer were also calculated (there were no sampled specimens in the spring period). The autumn sample showed a positive allometric growth for males ($b_♂$ = 3.7970), females ($b_♀$ = 3.0691), and the total sample (b_{AUT} = 3.0369). The winter and summer samples showed negative allometry, with the exception of females in summer sampling ($b_♀$ = 3.34909) (Table 4).

Table 3. Length–weight relationship parameters

Sex	Mantle length Min.	Mantle length Max.	n	LWR parameters *a*	LWR parameters *b*	LWR parameters r^2
♂	7.4	15.9	143	0.0626	2.7909	0.7306
♀	7.5	20.7	68	0.0318	2.9955	0.8919
♂+♀+0	5.5	20.7	298	0.0709	2.7160	0.8661

Table 4. Length –weight relationship parameters by season

Season	Sex	Mantle length Min.	Mantle length Max.	n	LWR parameters *a*	LWR parameters *b*	LWR parameters r^2
Autumn	♂	9.0	12.5	23	0.0049	3.7970	0.9650
	♀	8.0	15.8	11	0.0250	3.0691	0.7040
	♂+♀+0	6.0	15.8	17	0.0288	3.0369	0.9405
Winter	♂	7.4	15.9	34	0.0940	2.6482	0.8578
	♀	7.5	20.7	28	0.0810	2.6358	0.8147
	♂+♀+0	6.7	20.7	6	0.1255	2.5084	0.8869
Summer	♂	8.3	15.2	85	0.0734	2.7324	0.6563
	♀	8.9	18.0	31	0.0133	3.3490	0.9284
	♂+♀+0	5.5	18.0	64	0.0847	2.6549	0.8352

CONCLUSION

The data presented in this paper differs somewhat from the data reported in previous research in the Adriatic Sea (Ceriola *et al.*, 2006, Petrić *et al.*, 2010) (Tables 5–6). While the general lack of data regarding *I. coindetii* (and practically all other cephalopod species) in Montenegrin waters since the 1980s (Mandić, 1984.) makes any kind of new information valuable, it is still necessary to further research biological parameters of this species due to its significant economical value and its abundance in the catch.

Table 5. Comparison of data from previous research on *I. coindetii* in the Adriatic

Author	Sex	n	LWR parameters *a*	LWR parameters *b*	LWR parameters r^2
Petrić *et al.*	♂	265	0.000040	3.4496	0.92
	♀	249	0.000200	3.0181	0.97
Ceriola *et al.*	♂	139	0.000003	3.5800	0.94
	♀	148	0.000030	3.0000	0.98
	♂+♀+0	492	0.000041	2.9500	0.97

Table 6. Length at maturity and the logistic curve parameters as per Ceriola *et al.*, 2006.

Sex	Logistic curve parameters		Length at maturity (mm)		
	a	*b*	$ML_{25\%}$	$ML_{50\%}$	$ML_{75\%}$
♂	12.690	0.9300	125	137	149
♀	15.147	0.1040	135	146	156

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HEALTH RISK ASSESSMENT VIA THE CONSUMPTION OF MUSSELS (*MYTILUS GALLOPROVINCIALIS)* FROM THE BOKA KOTORSKA BAY, MONTENEGRO

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PROCENA ZDRAVSTVENOG RIZIKA PUTEM KONZUMIRANJA DAGNJE (*MYTILUS GALLOPROVINCIALIS*) IZ BOKOKOTORSKOG ZALIVA, CRNA GORA

Abstrakt

Sa povećanjem korišćenja morskih plodova u ishrani u poslednjih nekoliko godina, školjke su postale komercijalno važna vrsta u svetu. Zahvaljujući sadržaju proteina, kalcijuma i gvožđa, vitamina, omega-3 masnih kiselina, selena i joda, školjka predstavlja značajnu namirnicu u ishrani ljudi. Međutim s obzirom na veliki kapacitet akumulacije kontaminanata mogu predstavljati i potencijalnu opasnost za ljude. Istovremeno, školjka *M. galloprovincialis* se koristi kao bioindikator zagađenja morske sredine, jer toleriše velike koncentracije zagađivača iz okoline.

Školjka *M. galloprovincialis* je široko rasprostranjena u priobalnim vodama Crne Gore. Uzgaja se na farmama i prvenstveno je namenjena za tržište, ali se i dalje divlje školjke ove vrste prikupljaju za ličnu potrošnju od strane lokalnog stanovništva i turista. U Crnoj Gori trenutno postoji oko 16 farmi školjki i svaka od njih proizvodi od 10 do 50 tona godišnje. Sve farme se nalaze u Bokokotorskom zalivu, gde su okeanografski, fizički, hemijski i biološki uslovi pogodni za uzgoj ove vrste. Iako se uzgoj ove školjke povećao u odnosu na predhodnu deceniju ova grana industrije je i dalje nedovoljno razvijena.

Cilj ovoga rada bio je da se odredi kvalitet školjke *Mytilus galloprovincialis*, odnosno da se proceni eventualni zdravstveni rizik putem konzumiranja ove morske vrste sa više lokacija iz Bokokotorskog zaliva.

Uzorkovanje školjki vršeno je sezonski (zima, proleće i jesen) u 2008. godini. Odabrano je sedam lokacije unutar samog zaliva: Herceg Novi, Perast, Sv Stasija, Opatovo i Tivat, lokacije sa kojih su uzorkovane divlje školjke, i lokacije Kukuljina i Krašići sa kojih su uzorkovane uzgajane školjke. U svim uzorcima školjki određivane su koncentracije kadmijuma i olova.

Izmerene koncentracije kadmijuma i olova upoređene su sa maksimalno dozvoljenim koncentracijama regulisanim evropskim i crnogorskim regulativama. Koncentracija Cd u svim ispitivanim uzorcima je manja od maksimalne dozvoljene koncentracije propisane evropskom i crnogorskom regulativom za kvalitet školjki. U slučaju olova, lokacija Tivat je jedina lokacija gde srednja vrednost za ispitivane tri sezone prelazi maksimalno dozvoljenu koncentraciju propisanu od strane Crne Gore.

Na osnovu koncentracije za ova dva ispitivana elementa i prosečne nedeljne potrošnje izračunat je nedeljni unos Cd i Pb u ljudski organizam konzumiranjem školjki.

Nedeljni unos kadmijuma i olova ispod je preporučenog maksimalnog limita Svetske zdravstvene organizacije. U najgorem slučaju, uzimajući u obzir maksimalno izmerene vrednosti ispitivanih elemenata, nedeljna količina školjki koju je neophodno uneti u organizam, a koja bi mogla da izazove zdravstvene probleme kod čoveka je 750 g u slučaju kadmijuma i 940 g u slučaju olova.

Ključne reči: *školjka, kadmijum, olovo, nedeljni unos*

INTRODUCTION

With the increase in the consumption of seafood in recent years marine mussels have become commercially more important seafood species worldwide (Stankovic et al., 2011). The mussel *M. galloprovincialis* is widely distributed in the coastal waters of Montenegro, where mussels are cultivated on farms for the market, while wild ones are still hand-collected for personal consumption. Consumption patterns for mussels and aquaculture products have increased in recent years in the Mediterranean region and also in Montenegro. Cultivation of mussels along the Montenegrin coast has been increasing, 150 tones/per year in last decade (FAO, 2007), particularly in the Boka Kotorska bay due to the good natural conditions, but it is still underdeveloped.

Mussels are important species, both economically and ecologically. This type of shellfish is commercially important seafood since it provides a good source of proteins, Ca and Fe, some vitamins, omega-3 fatty acids, selenium and iodine for human consumption (Dahl et al., 2010). Also they are available throughout the year, reasonably tolerant to environmental change and pollution; they have good net accumulation capacities making them an excellent metal biomonitoring agent (Stankovic et al., 2011).

Pollutant trace elements have the ability to bioconcentrate in mussels directly from the water, bioaccumulate and biomagnify in the food chain, causing higher trophic organisms to become contaminated with high concentrations of chemical contaminants (Suseno et al., 2010). The bioaccumulation of these metal concentrations in marine organisms depends on many factors, either environmental, such as metal concentrations in sea water, temperature, salinity, dissolved oxygen, pH or purely biological *i.e.,* species, tissues, organs, feeding conditions (Sunlu, 2006).

Trace elements, such as Cd and Pb can be present in food either naturally or as a result of human activities, such as mining, irrigation, energy extraction, agricultural practices, incineration, industrial emissions and car exhausts. These metals are very toxic and their absorption and toxicity depends on dose and, among other diet constituents, on the intake of essential metals through diet. These elements have no known bio-importance in human biochemistry and physiology and their intake, even at very low concentrations, can cause toxic effects, because they tend to accumulate in the human body over time.

Cd, a metal with high toxic effects, which is strongly bioaccumulated in mussels, has an elimination half-life of 10–30 y and accumulates in the human body, particularly the kidney. Cd may act as an acute and chronic type of poison. Over time, Cd can accelerate osteoporotic process, since a high calcium dose can inhibit Cd absorption. The reverse situation - the inhibition of calcium absorption by Cd - has also been reported. This interaction is of special importance because of the suggested role of Cd in the development of bone softening due to decalcification, a characteristic of ltai-itai disease (Han et al., 2000).

Absorbed Pb is bound to erythrocytes in the blood and initially distributed to the liver, kidney and heart, where it preferentially binds to cell membranes and mitochondria (Widmeyer et al., 2004). Most forms of Pb are then distributed and stored in the bones. Pb is known to cause both acute and chronic adverse effects in the hematopoietic, nervous, gastrointestinal and renal systems (Widmeyer et al., 2004). Acute poisoning causes gastrointestinal colic, often resulting in mortality, while chronic poisoning causes anemia due to a decrease in the hemoglobin levels leading to organ damage in one or all of the four above-mentioned systems (Stankovic et al., 2011).

The aims of this study were to evaluate levels of Cd and Pb in relation to the maximum limits prescribed by national / international regulations, *i.e.*, to investigate whether the concentrations of these metals were within the permissible limits, thus rendering these mussels acceptable for human consumption.

MATERIALS AND METHODS

The mussel samples were collected seasonally (spring, fall and winter) from seven sites in Boka Kotorska bay, Montenegro: Herceg Novi, Perast, Sveta Stasija, Opatovo, Tivat, Kukuljina and Krasici during the year 2008. From each site in each season, pooled samples of 25–30 mussels, similar in length were selected, cleaned and rinsed with deionized water, dissected fresh and the soft tissue was rinsed with Milli Q water. All samples were measured before freezing at –10 ºC. The freeze-dried samples at – 40 ºC for 48 hours were weighed, homogenized to a fine powder and stored until analyses. The average water content of the soft mussels tissues were: 87.7 % (winter), 81.8 % (spring) and 79.8 % (fall).

The results from the determinations of Cd and Pb are presented as mean values of one pooled sample with 5 replicates of approximately 0.5 g from each station in each season. Approximately 0.5 g of the soft tissue was digested with a mixture of concentrated HNO_3 and H_2O_2 in a High Microwave Digestion System (CEM CORPORATION, MDS-2100). The digested samples were diluted to 25 ml with Milli Q water containing 1.0 % HNO_3. The analyses for Pb and Cd were performed using Graphite Furnace Atomic Absorption Spectrometry (Perkin-Elmer, 4100ZL, with Zeeman background correction). The accuracy of the applied analytical procedure for the determination of heavy metals in mussels was tested using SRM 2976 (Mussel homogenate; NIST) certified reference material.

RESULTS AND DISCUSSION

The mean concentrations of Cd and Pb in the mussel *Mytilus galloprovincialis* from the year 2008 are shown in Table 1.

Mean Cd concentrations in mussels from the Boka Kotorska bay ranged from 0.28 to 0.40 mg kg^{-1}. The highest mean value of Cd concentration in mussel samples (0.40 mg kg^{-1}) was obtained from Perast site (Table 1). All samples contained cadmium below the maximum level fixed by the European Commission Decision (Commission regulation (EC), 2006) and Montenegrin regulation (Montenegrin Food Regulation, 2002), Table 1.

The highest mean lead concentration was found in mussels from Tivat site (1.20 mg kg^{-1}) which is above the maximum level fixed by the Montenegro (Montenegrin Food Regulation, 2002), but below the maximum level fixed by the European Commission Decision (Commission regulation (EC), 2006). The same case is with the maximum measured Pb concentrations for sites Perast, Opatovo and Kukuljina. The maximum Pb concentration was measured in mussels from site Tivat, and it was the only sample that had level of Pb above the maximum level fixed by the European Commission Decision (Commission regulation (EC), 2006).

Table 1. The mean and range concentrations of Pb and Cd (mg/kg wet weight) in the soft tissue of mussels collected from seven sites in the year 2008

Location	Nature	WB	Cd (mg/kg)	Pb (mg/kg)
Herceg Novi	wild	wet	0.31 (0.18-0.37)	0.55 (0.46-0.67)
Perast	wild	wet	0.40 (0.22-0.65)	0.76 (0.32-1.31)
Sv Stasija	wild	wet	0.33 (0.23-0.42)	0.39 (0.29-0.56)
Opatovo	wild	wet	0.35 (0.27-0.44)	0.86 (0.49-1.39)
Tivat	wild	wet	0.36 (0.26-0.51)	1.20 (0.77-1.86)
Kukuljina	cultivated	wet	0.29 (0.24-0.37)	0.58 (0.29-1.10)
Krasici	cultivated	wet	0.28 (0.21-0.32)	0.42 (0.23-0.68)
Permissible limits by Montenegrin Food regulation (2002)		wet	1.0	1.0
EU (2006) Comm. Regulation (EC) No. 188/2006		wet	1.0	1.5

In this study the Provisional Tolerable Weekly Intake (*PTWI*) was used for the calculation of the metal concentration levels of concern associated with mussel consumption in the study area. The weekly intake of Cd and Pb through mussels consumption has been calculated by using average values for consumption of 46.8 grams per person per day (GEMS/FOOD Regional Diets, 2003) and Cd and Pb concentrations, Table 2.

Based on the mean, maximum and minimum concentrations of Cd and Pb, the consumption of 46.8 g of mussels on a daily basis or 327, 6 g/person per week from all sites are below the *PTWI* value for Cd (7 μg kg^{-1} bw $week^{-1}$) and Pb (25 μg kg^{-1} bw $week^{-1}$), (FAO/WHO, 2004),Table 2. In the worst case, taking into account the maximum measured concentration, weekly intake of mussels must be larger than 0.75 kg and 0.94 kg to exceed the *PTWI* values for the Cd and Pb, respectively (Table 2).

Table 2. Weekly intake of Cd and Pb based on max, min and mean obtained value

		Cd	Pb
Provisional tolerable weekly intake (μg kg^{-1} bw $week^{-1}$)		7	25
	Location	Average consumption (327, 6 g/person per week)	
Weekly intake based on maximum value (μg kg^{-1} bw $week^{-1}$)	H.Novi	1.73	3.14
	Perast	3.04	6.13
	SvStasija	1.97	2.62
	Opatovo	2.06	6.51
	Tivat	2.39	8.70
	Kukuljina	1.73	5.15
	Krasici	1.50	3.18
Weekly intake based on minimum value (μg kg^{-1} bw $week^{-1}$)	H.Novi	0.84	2.15
	Perast	1.03	1.50
	SvStasija	1.08	1.36
	Opatovo	1.26	2.29
	Tivat	1.22	3.60
	Kukuljina	1.12	1.36
	Krasici	0.98	1.08
Weekly intake based on mean value (μg kg^{-1} bw $week^{-1}$)	H.Novi	1.45	2.57
	Perast	1.87	3.56
	SvStasija	1.54	1.83
	Opatovo	1.64	4.02
	Tivat	1.68	5.62
	Kukuljina	1.36	2.71
	Krasici	1.31	1.97
Amount of mussel per week required to exceed limit (kg)	Based on minimum	2.72	7.61
	Based on maximum	0.75	0.94

CONCLUSION

Concentrations found for Cd and Pb indicate that the investigated mussels from Boka Kotorska bay pose no health risk to seafood consumers. Based on FAO/WHO recommended safe limit, Cd and Pb contents were within the permissible range established for safe human consumption.

ACKNOWLEDGEMENTS

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A COMPARATIVE STUDY ON FISHERY AND BIOLOGY OF *PARAPENAEUS LONGIROSTRIS* IN E. IONIAN AND S. ADRIATIC SEAS

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KOMPARATIVNA STUDIJA ULOVA I BIOLOGIJE *PARAPENAEUS LONGIROSTRIS* U ISTOČNOM JONSKOM I JUŽNOM JADRANSKOM MORU

INTRODUCTION

The deep water rose shrimp, *Parapenaeus longirostris* (Lucas, 1846), shows a wide geographic distribution in the E. Atlantic, as well as in the Mediterranean and its adjacent seas and its bathymetric distribution ranges between 20 and 750 m. In the above area, many studies carried out in the last years allowing collecting detailed information on its ecology and biology (e.g. Sobrino *et al.*, 2005). This is the first attempt to describe and compare the actual information available concerning fishery and biology of this species in Greek (E. Ionian) and S. Adriatic Sea (Montenegrin) waters, in the period 2006-2008.

MATERIAL AND METHODS

The samples were caught in the frame of the MEDITS Project and the National Project of Fishery Collection Data in Greece and from National Project of Monitoring of Demersal resources and FAO-ADRIAMED Project from Montenegro. All samples were caught using an experimental (MEDITS, 20 mm cod end) or commercial trawler (National and FAO-ADRIAMED, 40 mm cod end stretched mesh size). The sampling depth was between 71-586 m in Greek and 50-200 m in S. Adriatic waters. A total of 3059 ind. (1802 females, 1257 males) were caught in the Ionian and 2167 ind. (1539 females, 628 males) from the Montenegrin waters.

RESULTS

This shrimp is one of the most important species of the crustacean landings in Aegean and Ionian Greek seas (Kapiris *et al.*, 2007). The used fishing gears are trawls, seine nets, ring nets and other (Hellenic Statistical Agency data, ELSTAT data). Its annual catches showed a weak negative trend with time (2000-2008) and the annual economic value ranged from 22%-50% of the total crustacean value. An evaluation of the exploitation state of this resource indicates a general over- or full exploitation in Greek waters (Kapiris *et al*., 2007).

The last decade this shrimp represents a very high CPUE consisting one of the most important commercial species in Montenegrin trawl fishery (Kasalica et al., 2007). An estimation of the exploitation state of this resource indicates that this population is not threatened by high rate of the trawler fishing (Kasalica, 2005).

The carapace length (CL) of females in both areas (7.08-36.86 mm, in the Greek waters and 14-37 mm in Montenegrin waters) was statistically bigger than males (11-32.48 mm and 14-32 mm in the Greek and Montenegrin waters, respectively) (Mann-Whitney test, P>0.05). In general, the mean value of females' CL caught in Greek waters (21.94 mm±5.79) was statistically bigger than that of the S. Adriatic (19.93 mm±11.20), while the mean value of the males' CL caught in the Ionian (18.90 mm±3.87) was significantly smaller than that of the Montenegrin one (21.08 mm±2.56) (Kolmogorov-Smirnov test, P=0.00, in both cases).

Accordingly, females were statistically heavier than males, in both areas. In both areas, the younger individuals (CL: 7-12 mm) were caught in summer. The sex ratio (M/M+F) indicated that females were more abundant in both areas (0.408 and 0.286, for Greek and Adriatic fishery, respectively). This size dimorphism for the species has been shown in many other studies carried out in the Mediterranean (e.g. Sobrino et al., 2005).

A clear CL-depth distribution was not found in both areas, for both sexes. The correlation coefficients of the linear model (Depth=a+b*carapace length) were low, especially in Montenegro. The results for the CL-weight relationships by sex showed a negative allometry in growth, giving b values significantly less than 3, especially for males. The estimated slopes (b values) were higher, as expected, for the Greek specimens (2.620 and 2.389, for Greek ♀ and ♂, 2.309 and 2.032 for Montenegrin ♀ and ♂).

Bhattacharya's method (FiSAT package), was used to identify and isolate the different size groups, for both sexes. The general pattern showed that the males exhibited 2 (Ionian) and 1-year (Montenegro), while females showed more (3 and 2-year modes for Ionian and Adriatic, respectively). The estimated mean values were lower than those of the rest Mediterranean (Sobrino et al., 2005) reflecting possibly the different fishing exploitation of this resource.

The Montenegrin females presented a higher mean CL in each maturity stages (Stage I: immature, Stage II: maturing, Stage III: mature, Stage IV: spent) (t test, P=0.00). Although, the minimum mature females' CL was much lower in the Ionian (10 mm) than the S. Adriatic (16 mm). In the Ionian Sea mature female presented two peaks: one in spring and the other in autumn, while the spawning season is much more protracted in Montenegrin waters. In this area, mature females (stage III) found all the sapling months, with a slight peak in autumn-beginning of winter, reinforcing the view that the reproductive activity is continuous year-round.

DISCUSSION

The observed differences in biology and ecology of *P. longirostris* could be attributed to the different mesh size of the trawler, the sampling depth, and the different hydrological conditions and, obviously, to the different fishing pressure prevailed in both areas.

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SOME ASPECTS OF THE BIOLOGY OF THE MEDITERRANEAN HORSE MACKEREL, *TRACHURUS MEDITERRANEUS* (STEINDACHNER, 1868) IN MONTENEGRIN WATERS (SOUTH ADRIATIC SEA)

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NEKI ASPEKTI BIOLOGIJE ŠNJURA PUČINARA, *TRACHURUS MEDITERRANEUS* (STEINDACHNER, 1868) U VODAMA CRNOGORSKOG PRIMORJA (JUŽNI JADRAN)

Abstrakt

Šnjur pučinar, *Trachurus mediterraneus* (Steindachner, 1868) je semi-pelagična riba koja se u Jadranu najčešće lovi na dubinama od 20 do 200 m. U Jadranskom moru, ova vrsta je važan objekat i demerzalnog i pelagičnog ribolova. Iako su biloške karakteristike ove vrste dokumentovane za Jadran, ipak za Crnogorsko primorje nema takvih podataka. U radu se iznose rezultati analize nekih bioloških aspekata (raspodjela dužinskih frekvencija, dužinsko-težinski odnos, odnos polova, dužina pri kojoj 50% jedinki doživi polnu zrelost) šnjura pučinara, u vodama Crnogorskog primorja u okviru Pilot Studije FAO AdriaMed projekta. Uzorci su uzimani mjesečno sa komercijalnih brodova u periodu od jedne godine tj. od septembra 2007 do septembra 2008. Ukupno je analizirano 429 jedinki, odnosno 287 ženki i 162 mužjaka. Totalna dužina uzorkovanih jedinki je bila od 11.0 do 31.0 cm sa srednjom vrijednošću od 17.66 cm TL. Dužinski raspon kod ženki je bio od 11.0 do 31.0 cm, a kod mužjaka od 11.5 do 28.6 cm. Kod ženki, u ulovu su najzastupljenije bile jedinke od 15.5 do 18.0 cm dok su kod mužjaka bile od 15.5 do 19.0 cm. Odnos polova je bio 0.38. Ženke su procentualno zastupljenije skoro u svim dužinskim klasama. Zrele jedinke šnjura (stadijumi 2-sazrijevanje, 3-zrele i 4-izmriješćene) se nalaze u dužinskom rasponu od 14.4 do 28.6 cm kod mužjaka i od 13.9 do 29.4 cm kod ženki. Nezrele jedinke (stadijum 1) sačinjavaju skoro 23% ukupnog uzorka, dok je najveći dio uzorkovanih jedinki bio u drugom stadijumu zrelosti. Dužina pri kojoj 50% jedinki dostigne prvu polnu zrelost je procijenjena na 13.38 cm TL, odnosno 16.24 cm kod mužjaka i 14.32 cm kod ženki. Koeficijent b dužinsko-težinskog odnosa je kod ženki iznosio 2.8546, a kod mužjaka 3.0413.

Ključne riječi: šnjur pučinar, biološki aspekti, Crnogorsko primorje

INTRODUCTION

Trachurus mediterraneus is a semi-pelagic carnivorous fish, living in both the Mediterranean and Black Seas as well as along the eastern coasts of the Atlantic from the English Channel to Morocco (Viette *et al*., 1997). In the Adriatic Sea, this species is important object of pelagic and demersal fishing (Šantić *et al*., 2002). According to Jardas (1996) this species is most commonly found at about 20-200 m depth. Despite fact that the biology of this species has been well documented for the Adriatic (Arneri, 1984; Viette *et al*, 1997; Šantić *et al*., 2002), biological studies of this species in Montenegrin coast are very limited.

So, the aim of this paper is to investigate some biological aspects of *T. mediterraneus* such as length-weight relationship, length frequency distribution, sex ratio, size at first maturity in Montenegrin territorial waters.

MATERIALS AND METHODS

Specimens of Mediterranean horse mackerel were caught monthly from September 2007 to September 2008 on Montenegrin shelf (Fig. 1), using commercial bottom trawls. Hauls were performed during the day at depths ranging from 50 to 200 m. Immediately after capture, specimens were processed. Total length (TL) of fish examined was measured to the nearest 0.1 cm and weight to the nearest 0.01 g. The sex ratio was expressed as a fraction of males over the total of males and females combined. According to MEDITS protocol, four different maturity stages were established for females and males, based on the macroscopic observations of gonads: immature (1), in maturation (2), spawning (3) and post-spawning (4). Length at first maturity was estimated by sexes and for the entire population, based on the length and maturity stage data.

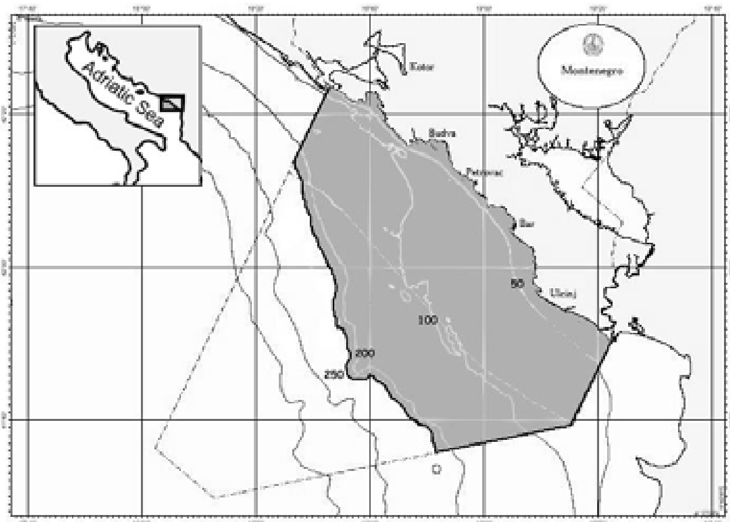
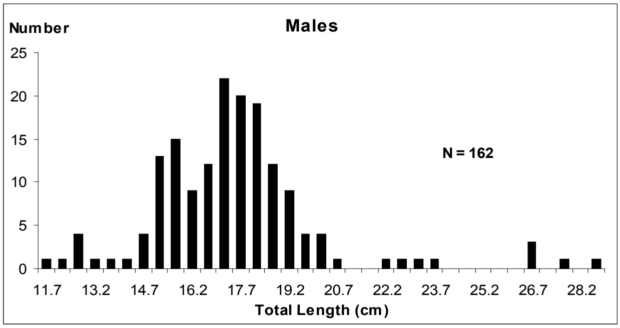


Figure 1. Map of the investigated area

Maturity stages 2, 3 and 4 were included in the mature category while maturity stage 1 was included in the immature category. Length-weight relationship of horse-mackerel was estimated by equation: $W = aL^b$ (Ricker, 1975), where W is the total body weight (in g) and L is the total length (in cm), a is coefficient related to the body form and b is coefficient of allometry.

RESULTS AND DISCUSSION

During the time of investigation, 429 specimens of Mediterranean horse mackerel were analyzed. The total length ranged from 11.0 to 31.0 cm with an overall average length of 17.66 cm. This length range does not correspond with the maximum limit of 10-50 cm proposed by Fisher *et al*. (1987) for the size range of *T. meditteraneus*. Length frequency distribution of males shows that the size range extended from 11.5 to 28.6 cm with size groups of 15.0 and 19.0 cm dominating in the catch while the length frequency distribution of females ranged from 11.0 to 31.0 cm with size groups 15.5 to 18.0 cm dominating the catch (Figure 2). Females showed a higher median length than males (17.82 versus 17.41 mm). The LFD of the whole sample (pooled data) showed that the majority of catches consist of individuals ranging in length from 15.0 to 18.5 cm TL (Figure 3).



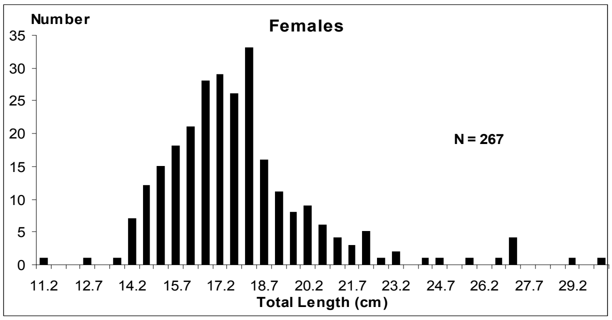


Figure 2. Length frequency distribution by sexes of *Trachurus mediterraneus* caught in national waters of Montenegro during 2007-2008 sampling period.

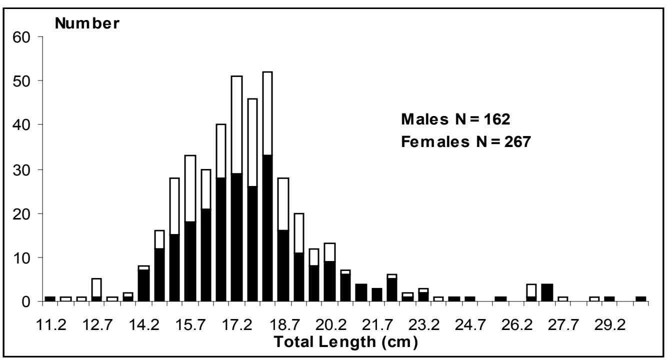


Figure 3. Length frequency distribution for the whole sample of *Trachurus mediterraneus.* Black bars = females; white bars = males

The sample was composed of 267 females (62.2%) and 162 males (37.8%). The overall sex ratio during the period of investigation was in favour of females (0.38). The sex ratio by size class showed female predominance in almost all length classes (Figure 3). Mature specimens of *T. mediterraneus* were found at wide size range, TL varying between 14.4 and 28.6 cm TL in males and 13.9 and 29.4 cm TL in females (Table 1).

Table 1. Gonad maturity stages by sex of *Trachurus mediterraneus* during the 2007-2008 sampling period. F = female; M = male; TL = total length in cm; 1-4 = gonad maturity stage.

Trachurus mediterraneus	M				F			
Maturity stage	No	%	TL		No	%	TL	
			min	max			min	max
1	50	30.86	11.5	26.5	40	14.98	11.0	31.0
2	91	56.17	14.4	27.7	163	61.05	13.9	25.6
3	17	10.49	15.2	26.8	18	6.74	16.2	24.6
4	4	2.47	17.2	28.6	46	17.23	16.7	29.4
Total	162	100.00			267	100.00		

During the whole sampling period very small number of mature specimens (in maturity stage 3) was found, and only 4 post-spawning males were recorded. The largest fraction of caught specimens was in the maturity stage 2 (Figure 4). In the Gulf of Trieste, the smallest mature male was 15.6 cm long and the smallest mature female 16.0 cm long (Viette *et al*., 1997). Different results were also reported by Demirel (2010) in the Marmara sea, where the smallest size of mature individuals was found as 11.5 cm for both sexes.

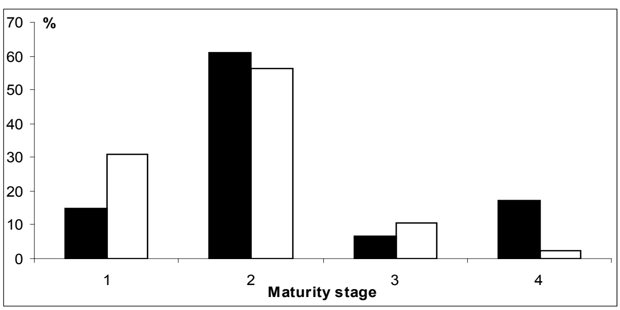


Figure 4. Gonad maturity stages proportion by sex of *Trachurus mediterraneus* during the 2007-2008 sampling period. Black bars = females; white bars = males.

The size at first maturity ($L_{50\%}$) estimated for both sexes combined was 13.38 cm TL (Figure 5) while the size at first maturity is reached at 16.24 cm for males and 14.32 cm for females. Our results do not match with Demirel (2010) who was reported that the first maturity length was calculated to be 12.2 cm for females and 12.5 cm for males in the Marmara Sea.

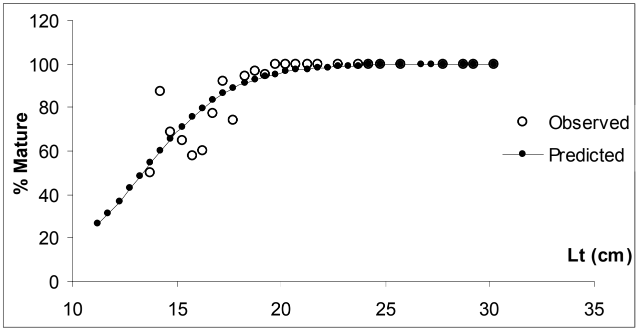


Figure 5. Length at first maturity (50%) for both sexes of *Trachurus meditteraneus*

The length-weight relationships of horse-mackerel in Montenegrin territorial waters were:for females $W=0.0072*L^{3.0413}$ and for males $W=0.0119*L^{2.8546}$.(Fig. 6). A similar result for females was reported by Šantić *et al.*, (2002) for the eastern central Adriatic. These authors reported that the L-W relationship, which differs between sexes, was estimated for males b = 2.9929, a = 0.0087 and for females b = 3.0598, a = 0.0069, indicating negative deviation from ideal allometric growth($b < 3$) for males, and positive deviation ($b > 3$) for females.

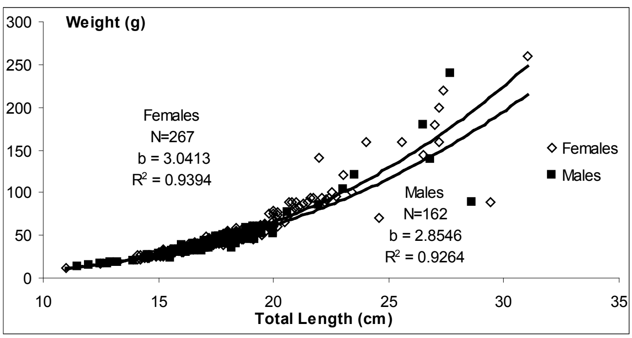


Figure 6. Length-weight relationship for males and females, and their respective estimated slopes (b) for *Trachurus meditteraneus* caught in Montenegrin national waters during the 2007–2008 sampling period. N = number of specimens.

CONCLUSIONS

Some biological aspects of *T. mediterraneus* has been studied for the first time in the Montenegrin coast (South Adriatic Sea). These results will provide the basis for future stock assessment and management studies on this species.

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INFLUENCE OF *AEROMONAS SALMONICIDA* ON BIOFOULING AND MICROBIAL CORROSION OF NICKEL COPPER ALLOY COATING OF SEA CAGE

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UTICAJ *AEROMONAS SALMONICIDA* NA OBRASTANJE I MIKROBIJALNU KOROZIJU PREVLAKE OD LEGURE NIKLA I BAKRA NA KAVEZNE SISTEME U MORU

Extended abstract

Biofouling acts one of the most serious problems to marine industry and aquaculture development. In the marine environment, surfaces immersed in seawater are colonized by micro-biofoulers such as marine bacteria, algae, and protozoa (Callow and Callow, 2002; Dobretsov et al., 2006). Biofouling of sea cages is a great concern for the salmon growers. When a sea cage is first immersed, there will be a succession of organisms that colonize the cage (Hodson et al., 1997). Bacteria produce an exo-polysaccharide layer (EPS), which act as a protective barrier from the treated surface but also harmful to the lower substratum, in case of the physical degradation or bio-deterioration of the metal surface. This situation is called Microbial corrosion (Yuan and Pehkonen, 2009). In addition, there is a possibility that cage can act as a reservoirs for some disease-causing organisms so one of these bacteria is *Aeromonas salmonicida*. This bacterium is an aquatic Gram-negative bacterium causing lethal disease furunculosis in salmonid fish. Along with other members of the family Vibrionaceae (*V. anguillarum, V. ordalii, A. hydrophila*), diseases caused by these organisms can rapidly decimate populations of farmed marine or freshwater fish (Garduna et al., 1994). To reduce the amount of fouling, increase the water quality and decrease the chance of disease occurrence, cage can be coated with antifouling copper alloys and paints (Hodson and Burke, 1994). On the other hand, the tremendous applications of Ni-Cu alloys in different industries, especially in ships where chloride containing waters are always used, making the corrosion processes under the influence of chloride ions understandable and the control of these processes important subject of intensive investigations (Badawy et al., 2005). Therefore

this study examines the effects of *A. salmonicida* in biofouling on Ni-Cu cage coating and its microbial corrosion influence.

This study was performed using pure cultures of the *A. salmonicida,* isolated from water samples. The isolated bacterium was characterized on the basis of 16S rRNA sequences and submitted to NCBI under Accession No. GU907676 (San et al., 2010). The bacterium was cultured then centrifuged and used for bio-corrosion experiments. Electrochemical measurements were carried out in a conventional three-electrode cell. The polarization curves were measured using CompactStat Potentiostat (IviumStat, The Netherlands). Mild steel was used as a working electrode in electrochemical studies. The Ag/AgCl (sat. KCl) electrode (CHI111, CH Instrument, USA) was used as the reference electrode and a platinum wire (CHI115, CH Instrument, USA) was used as counter electrode for all experiments. Ni-Cu alloy was electrodeposited from a nickel-copper bath.

The corrosion potential of Ni-Cu electrodes was -0.08 V (vs. Ag/AgCl) but, the corrosion potential of Ni-Cu electrodes in the medium inoculated with the bacterium shifted to cathodic site, -0.63 V (vs. Ag/AgCl) means the increased rate of corrosion. In addition, increase of corrosion current and corrosion current densities with an increase in presence of bacterium means the increased rate of corrosion. Besides, pH values of the medium did not change throughout the experiment (6.8) but after immersion, the pH value decreased to acidic value, 5.5 Furthermore, SEM micrographs show influence of biofouling by bacterium to Ni-Cu alloy. As a consequence, *A. salmonicida* colonize, strongly adhere and biofouling on alloy surfaces. This situation results in causing the lethal disease in salmon fish and microbial corrosion of cage material.

Key words*: *Aeromonas salmonicida*; *Biofouling; Microbial corrosion; Nickel-Copper Alloy; Sea cage

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TERRESTRIAL NATURAL AND ANTROPOGENIC DISTRUBITION OF HEAVY METAL (CU) IN SEDIMENTS OF THE TRABZON REGION IN THE BLACK SEA

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PRIRODNA I ANTROPOGENA DISTRIBUCIJA TEŠKOG METALA(CU) U SEDIMENETIMA TRABZONSKOG REGION CRNOG MORA

Abstract

In this study, dispersion of Cu element was examined in the coastline of Trabzon, which is the most populated and industrialized part of the Eastern Black Sea region. Sampling studies were carried out in 3 distinct areas on the coast of Trabzon, Değirmendere region,Yanbolu region and Solaklı region. In each region, 8 stations were determined to be studied having various distances, from 0 to 250 meters, from the coast within the boundiries of the studied areas. During the study, the bottom sediment layer (0-5 cm) was taken from 3 different regions and a total 24 stations for sediment analysis were established.

We found that heavy metal concentration in the Yanbolu region had a minimum concentration value (52,06 ± 14,42 ppm), Solaklı region had a maximum concentration value (78,57 ± 15,84 ppm) and Değirmendere region had a concentration value (63,97 ± 6,92 ppm) in the spring season. Yanbolu region had a minimum concentration value (53,97 ± 9,40 ppm), Solaklı region had a maximum concentration value (90,45 ± 38,46 ppm) and Değirmendere region had a concentration value (61,26 ± 12,78 ppm) in the summer season. Yanbolu region had a minimum concentration value (64,55 ± 7,17 ppm), Solaklı region had a maximum concentration value (107,06 ± 26,98 ppm) and Değirmendere region had a concentration value (86,05 ± 3,19 ppm) in the autumn season. Yanbolu region had a minimum concentration value (56,87 ± 7,71 ppm), Solaklı region had a maximum concentration value (76,21 ± 15,65 ppm) and Değirmendere region had a concentration value (68,70 ± 8,89 ppm) in the winter season. We found that heavy metal (Cu) concentration in the Yanbolu region had the lowest concentration value and Solaklı region had the highest concentration value in the study area in all seasons. According to all seasons, the spring season had the lowest concentration value and the autumn season had the highest concentration value.

When the sediment enrichment factor (SEF) is present in all regions, because the SEF values was higher than 1 for the spring reason (Değirmendere region: 1,64, Yanbolu region: 1,39, Solaklı region: 1,79), the summer reason (Değirmendere region: 1,68, Yanbolu region: 1,51, Solaklı region: 2,14), the autumn season (Değirmendere region: 2,02, Yanbolu region: 1,66, Solaklı region: 2,72), the winter (Değirmendere region: 1,87, Yanbolu region: 1,60, Solaklı region: 1,91) of this element, it can be concluded that the sediment layer in Trabzon is rich in this metal.

According to Pollution Load Index (PLI), the spring reason (Değirmendere region: 1,15, Yanbolu region: 1,71, Solaklı region: 1,09), the summer reason (Değirmendere region: 1,33, Yanbolu region: 1,18, Solaklı region: 2,87), the autumn season (Değirmendere region: 1,90 Yanbolu region: 1,42, Solaklı region: 2,49), the winter (Değirmendere region: 1,51, Yanbolu region: 1,24, Solaklı region: 1,66)) of this element have also been calculated.

The examination of sediment enrichment factor (SEF) and Pollution Load Index (PLI) reveal the presence of Cu metal which is indicative of heavy metal pollution in the sampling area.

Key words: *Black sea, Sediment, Heavy metal, Sediment enrichment factor (SEF), Pollution Load Index (PLI)*

THE MODELING GASTRIC EMPTYING IN EUROPEAN SEA BASS *DICENTRARACHUS LABRAX L.*

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MODELOVANJE PRAŽNJENJA CREVA EVROPSKOG BRANCINA *DICENTRARACHUS LABRAX L.*

Abstract

Digestion physiology of an animal is important since it is essential source of information for aquaculture and also it helps to outline the rule of such animal in the aquatic food web. Especially it is vital for the management issues of the living resources concerning multispecies VPA. Bearing in mind, sea bass is a one of the most important sea fish having high salinity and temperature tolerance and commercial value not only in Turkey, but in the European countries as well. It has growing culturing potential in Turkey and Europe, but not much detailed studies concerned feeding and digestion physiology have been performed yet.

In this study, digestion physiology; gastric emptying in *Dicentrarchus labrax* force fed on artificial (formulated) food will be investigated. Factors affecting gastric emptying rate (GER) and time (GET) in sea bass will be studied. An attempt will also be made to model GER and GET in sea bass. Food consumption and feeding periodicity and return of appetite of sea bass will also be worked out.

THE RELATIONSHIP BETWEEN OTOLITH AND SIZE OF MEDITERRANEAN HORSE MACKEREL (TRACHURUS MEDITERRANEUS, STEINDACHNER, 1868) IN THE SOUTH-EASTERN BLACK SEA

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ODNOS OTOLITA I VELIČINE MEDITERANSKE SKUŠE (TRACHURUS MEDITERRANEUS, STEINDACHNER, 1868) U SEVERO-ISTOČNOM DELU CRNOG MORA

Abstract

Mediterranean horse mackerel has an important commercial value for the Turkish fisheries. It was the second important species after anchovy in Turkey with a 115000 t production in 1986. Due to collapse of anchovy stocks since the early 1990's, amount of catch has gradually decreased and production of horse mackerel in 2004 and 2005 decreased to 6301 t and 8287 t, respectively. It was 2651 t and 5982 t in the Southeastern Black Sea in respective years.

Knowing the relationship between otolith length and fish length provides important information in determining the length of fish from the otoliths found in archaeological areas and stomach of the predators, validate ageing studies and mainly for the back calculation for the length of the rare species (Echeverria, 1987; Panfili and Tomás, 2001). Moreover, in some fish species the fish age can be determined by the otolith weight due to a high correlation existing between these two parameters (Pawson, 1990; Mardinale *et al*. 2000). There are no studies on ageing of Mediterranean horse mackerel in the Black Sea. There is only one comprehensive research on the relationships between otolith dimensions and fish length which has been carried out on *Trachurus mediterraneus* in the Sea of Marmara (Bostanci, 2009). Polat and Kukul (1990) studied ageing methods of Atlantic horse mackerel *Trachurus trachurus* in the Black Sea.

In the present study it was aimed to derive all possible relationships between otolith dimensions and fish size using samples of Mediterranean horse mackerel caught in the South-Eastern Black Sea. These models can be used to estimate fish age from selected independent parameters especially for the routine monitoring studies for fisheries management.

As a result, mean otolith length, width and weight of all samples was derived as 4.321 mm, 2.507 mm and 0.0109 g, respectively. Otolith length (OL)-otolith weight (OW), otolith length (OL)-otolith width (OW_i), fish total weight (TW)-otolith weight (OW), fish length (TL)-otolith length (OL), otolith length (OL)-age (A), otolith weight (OW)-Age (A), otolith length (OL)-fish total weight (TW), otolith weight (OW)- fish length (TL), otolith width (OW_i)- fish length (TL), otolith width (OW_i)- otolith weight (OW) relationships were derived as $OW=0.0006OL^{1.8971}$(n=394, r=0.853), OW_i=0.923+0.367OL (n=551,r=0.814), OW=0.0039+0.0003TW (n=455,r=0.868), OL=1.0035+0.2458TL (n=557,r=0.811), A= 3.3398+0.4554OL (n=531, r=0.711), A=0.0051+0.0021OW (n=453, r=791), $TW=0.5218OL^{2.473}$ (n=531, r=0.774), TL=8.1884+598.31OW (n=453, r=869), TL= 0.5874+5.635, OW (n=551, r=0,793), OW=0.031+5.635OW_i (n=551, r=0.793), respectively.

Regarding the relationships between fish and otolith size the highest correlation was found between fish length and otolith weight (r=0.869).

It was also observed that there are significant differences between same relationships derived from the two research studies due to work on the samples obtained from different habitats as Sea of Marmara and the Eastern Black Sea.

Key words: *Age determination, otolith size, fish size, Trachurus mediterraneus, South-eastern Black Sea*

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PROXIMATE ANALYSIS OF SOME RAY SPECIES CAUGHT BY TRAWLING IN MEDITERRENEAN GULF OF ANTALYA (TURKEY)

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HEMIJSKI SASTAV NEKIH VRSTA RAŽA ULOVLJENIH KOČARENJEM U MEDITERANSKOM ZALIVU ANTALIJA (TURSKA)

Abstract

The objective of this study is to determine the proximate analysis of some ray species caught by trawling in the Gulf of Antalya. Proximate analyses revealed non significant ($P>0.05$), differences in moisture among brown ray, thornback ray, common stingray and spiny butterfly ray. Of all the species analyses spiny butterfly ray had the highest protein content (22.46±2.18%). Common stingray and spiny butterfly ray had significantly different ($P<0.05$) levels of lipids compared to the others. Common stingray also had the highest ash content (2.96±0.02%) compared to the others. Therefore, it was concluded that discard fish species such as brown ray, thornback ray, common stingray and spiny butterfly ray would be use as a source of protein for human diet.

Key words: *Rajidae, proximate analysis, trawl, Gulf of Antalya, protein*

INTRODUCTION

Fishery products, like many other animal products, contain water, proteins and other nitrogenous compounds, lipids, carbohydrates, minerals and vitamins. Proteins and lipids are the major components of fish (Murray and Burt, 2001). The chemical composition of fish varies greatly from one species and one individual to another depending on sex, age, environment and season. Therefore, a substantial normal variation is observed for the constituents of fish muscle. The knowledge of proximate composition of fishery species has fundamental importance in the application of different technological processes (Connell, 1975). Proximate composition is also important as an aspect of quality of raw material, sensory attributes and storage stability (Sikorski, 1994).

All rays has a flattened body with broad, wing like pectoral fins. The body is kite-shaped with a long, thorny tail. The back is covered in numerous thorny spines. The rays are usually found on sediment type seabeds such as mud, sand or gravel at depths between 10-60 m (Anonymous, 2011).

In this study, rays; Brown ray (*Raja miraletus*), Thornback ray (*Raja clavata*), Common stingray (*Dasyatis pastinaca*) and Spiny butterfly ray (*Gymnura altavela*) are locally commercially important in the Mediterranean; bycatch of the demersal fishery (FAO, 2005). There is a little information on proximate composition of these rays from Turkey. Therefore, the aim of this study was to investigate proximate composition of the four rays caught in the Gulf of Antalya.

MATERIALS AND METHODS

In the Gulf of Antalya several trawls from different depths were performed using the research vessel of Akdeniz University, and this resulted in the selection of four ray species: Brown ray (*Raja miraletus*), Thornback ray (*Raja clavata*), Common stingray (*Dasyatis pastinaca*) and Spiny butterfly ray (*Gymnura altavela*) have been obtained. In the Gulf of Antalya two different trawl regions were selected. In this regions shallow trawls (25-50 m) and mid depht trawls (150-200 m) were performed. 11 trawls occurred during the day and 10 during the night, resulting in a total of 21 trawls (Table 1). The fish were then transported to our laboratory in polystyrene boxes in crushed ice. The viscera of the fish were removed and the leftovers such as blood, mucus and tissue pieces were washed with large amount of water.

The chemical contents of four discard fish species were determined according to the Official Methods of Analysis. Moisture content was determined according to the Official Method 950.46 (2002a). Crude protein content (Nx6.25) was calculated using the Kjeldahl method 928.08 (2002b). Lipid (fat) content was determined according to the Soxhlet method 960.39 (2002c). Crude ash (inorganic matter) was determined according to method 920.153 (2002d).

Every parameter was measured in triplicate for each sample. Statistical analyses were performed using SPSS 9.0 for Window software (SPSS INC. Chicago, IL, USA). Analysis of variance (ANOVA) were used for statistical significance was at $P<0.05$.

Table 1. The catching some ray species and their amounts from shallow (25-50 m) and mid depth (150-200 m) water trawling.

Some Ray Species	Shallow trawls (Kg)	Mid depth trawls (Kg)	Total caught amount (Kg)
Brown ray (*Raja miraletus*)	4.20	19.60	23.80
Thornback ray (*Raja clavata)*	0.43	76.22	76.65
Common stingray (*Dasyatis pastinaca)*	-	11.60	11.60
Spiny butterfly ray (*Gymnura altavela)*	-	36.30	36.30

RESULTS AND DISCUSSION

Table 2 shows the result of biometric measurements of the four fish species. The weight of spiny butterfly ray is higher than other three rays.

Proximate composition of four rays was presented in Table 2. The average moisture content was determined as 76.76±0.07% for brown ray; 77.33±0.01% for thornback ray; 76.59±1.87% for common stingray and 75.38±0.07% for spiny butterfly ray. The water proportion was higher in thornback ray than other species and this differences were found statistically insignificant (P>0.05) (Table 1). There were no statistically significant differences (P>0.05) in moisture content among all species. The chemical composition of fish muscle varies greatly from one species and one individual to another depending on age, sex, environment and season (Turan et al. 2006). Actually, the variation in the chemical composition of fish is closely related to feed intake, migratory swimming and sexual changes in connection with spawning (Bandarra et al. 2001; Tzikas et al. 2007). For this reason it was difficult to compare our findings with those of other researchers.

The lipid content was found the most highest in spiny butterfly ray, whereas the lowest was in the brown ray (See Table 2). All of the rays can be classified as a lean fish with its low fat content.

The crude ash content of brown ray, thornback ray, common stingray, and spiny butterfly ray were determined as 1.30%, 1.36%, 2.96%, and 1.33%, respectively. The ash content of common stingray was determined to be the best high among all species.

The protein content of spiny butterfly ray is higher than three rays, whereas the lowest was in brown ray. This difference was statistically significant (*P*<0.05). In addition, protein proportion in the thornback ray was determined as 18.76±0.10%. In a study concerning thornback ray, protein proportion was found as 20.02±0.00% (Turan et al. 2007). This value was higher than our. It was thought that these differences were from different environment or age. Yılmaz and Akpınar (2003) determined the protein content of thornback ray (*Raja clavata*) as 19.46%. In this research, it was determined that the thornback ray has high protein content as well as other species.

Table 2. Biometric measurement of some ray species.

Species	Total Weight (g)	Total Lenght (cm)
Brown ray	321.07±50.92	37.37±1.11
Thornback ray	335.65±21.60	36.60±11.05
Common stingray	475.50±40.08	39.20±0.89
Spiny butterfly ray	748.60±32.00	30.20±0.74

Table 3. Chemical composition of some ray species.

Species	Moisture	Protein	Lipid	Ash
Brown ray	76.76±0.07[a]	17.77±1.26[b]	0.25±0.02[c]	1.30±0.02[b]
Thornback ray	77.33±0.01[a]	18.76±0.10[b]	0.47±0.01[b]	1.36±0.11[b]
Common stingray	76.59±1.87[a]	19.35±0.56[b]	0.75±0.00[a]	2.96±0.02[a]
Spiny butterfly ray	75.38±0.07[a]	22.46±2.18[a]	0.77±0.01[a]	1.33±0.09[b]

Values are shown as mean ± standard deviation of triplicate measurements. Different superscript letters in the same row indicate significant differences among groups ($P<0.05$).

CONCLUSIONS

In the study, whereas some differences were found among species, chemical compositions of ray species were very similar. Lipid contents of all the species were found as poor. However, protein content were found to be high in all the species. A great portion of these rays caught in Turkey is discarded. By-catches can be evaluated as a valuable source for fish processors. Besides, all of the rays can be used as a good protein source for human diet.

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DETERMINATION OF HEAVY METALS LEVELS IN AQUATIC ORGANISMS FROM TRABZON COAST OF THE EASTERN BLACK SEA

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ODREĐIVANJE NIVOA TEŠKIH METALA U VODENIM ORGANIZMIMA SA OBALE TRABZON U ISTOČNOM CRNOM MORU

Abstract

Heavy metals are very important pollutants for aquatic organisms because of the bioaccumulation. The effect of heavy metal pollution in enclosed seas is bigger than in open seas. Pollution intensity on living organisms depends on industrial activities and their nutrition types. The eastern Turkish Black Sea coast is not an industrialized area but agricultural activities are greater than in western Black Sea coast.

The pollution of the aquatic environment with heavy metals has become a worldwide problem during recent years, because they are destructible and most of them have toxic effects on organisms. Heavy metals, even in small quantities, are very toxic for life. The gravity of toxic effect depends on nature, metal concentration, body resistance and presence of other contaminants. Plankton is a key component in the cycling of bio elements in coastal ecosystems. Contaminated fish with heavy metals are potential risk for humans who consume them.

The study has been performed at 41° 00' 44" N, 40° 07' 40" E coordinate in eastern Black Sea. Sampling was done twice, in July and October 2010. Concentration of heavy metals (Cu, Cd, Fe, Mn, Zn) were determined in the two different demersal (benthic) fish species of red mullet (*Mullus barbatus ponticus*) and whiting (*Merlangius merlangus euxinus*). Plankton sampling was done by using plankton scoop. GBC 905 model AAS was used for metal determination. Cu and Cd were measured by GFAAS while Zn, Fe, and Mn determined by FAAS. In this study some physical (temperature, sigma-t, electrical conductivity) and chemical parameters (dissolved oxygen, hydrogen sulfide) were measured at 12 different depths of Black sea water column depending on different layer characteristics.

Metal concentration determined in whiting, red mullet and plankton respectively as mg/kg of dry weight; Cu 0,329±0,131; 0,362±0,260; 18,4±8,1 and Zn 17,3±1,8; 24,0±8,6; 1218±959 and Fe 10,1±4,2; 28,6±12,3; 4366±526 and Mn 0,5±0,1; 1,0±0,3; 6299±3761 and Cd 0,207±0,034; 0,208±0,070; 50,1±18,9. The results showed that metal concentrations in plankton are higher than in fish species.

Key words: *heavy metals, aquatic organisms, Cu, Cd, Zn, Fe, Mn*

ESTIMATE OF ANCHOVY (*ENGRAULIS ENCRASICOLUS*, L.) BIOMASS IN THE SOUTHERN ADRIATIC SEA BY DEP (DAILY EGG PRODUCTION) METHOD (2005-2010)

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PROCJENA BIOMASE INĆUNA (*ENGRAULIS ENCRASICOLUS*, L.) PRIMJENOM DEP (DAILY EGG PRODUCTION) METODE NA PODRUČJU JUŽNOG JADRANA (2005-2010.)

Abstrakt

DEP metoda (daily egg production method, DEPM) je zasnovana na istraživanju ihtioplanktona (jaja i larve riba) i koristi se širom svijeta za procjenu biomase male plave ribe (Hunter and Lo, 1993; Lasker, 1985; Somarakis et al, 2004). Ova metoda se uspješno primjenjuje već 25 godina u čitavom svijetu i prvenstveno je dizajnirana za ribe koje imaju neodređen fekunditet, kakva je većina vrsta iz reda Clupeiformes. Zbog toga se najčešće upotrebljava za procjenu biomase male plave ribe, u prvom redu inćuna (Engraulidae) i srdela (Cupeidae). Pored procjene biomase ova metoda daje značajne podatke o karakteristikama i načinu mrijesta, kao i bitne reproduktivne parametre ribljih populacija. Podaci dobijeni DEP metodom mogu pomoći u razumijevanju mehanizama kojim prirodne promjene utiču na reproduktivnu biologiju i stepen preživljavanja ranih razvojnih stadijuma male plave ribe (Regner, 1985; Somarakis et al, 2004).

Inćun (*Engraulis encrasicolus*, L.) je jedini predstavnik roda Engraulis u Mediteranu. Inćun je, uz srdelu, ekonomski najznačajnija vrsta riba u Jadranu i Mediteranu. Dostiže dužinu do 20 cm, migratorna je i planktofag.

Mrijesti se od početka proljeća, obično od aprila do oktobra, a nekada od marta do novembra (Zavodnik, 1970; Merker and Vujošević, 1972; Regner, 1972, 1985, Piccinetti et al.., 1979). Mrijesti se više puta tokom sezone mriješćenja („multiple spawner“ Blaxter and Hunter, 1982).

U okviru FAO AdriaMed Projekta (koji se realizuje već sedam godina između Crne Gore, Italije, Albanije, Hrvatske i Slovenije) u vodama crnogorskog primorja DEP metoda je prvi put primjenjena 2005 godine za procjenu biomase inćuna. Nakon toga, istraživanje je prošireno na albanske teritorijalne i susjedne međunarodne vode u julu 2008 godine.

U julu 2010 godine procjena biomase inćuna primjenom DEP metode u okviru istog projekta proširena je na područje cijelog južnog Jadrana (GFCM geografska subregija 18). Procjenjena biomasa inćuna u avgustu 2005 godine iznosila je 3842 t., u julu 2008 godine 52 273.2 t dok je u julu 2010 godine iznosila 100 352.07 t.

Ključne reči: *DEP metoda, Engraulis encrasicolus, biomasa, južni Jadran*

INTRODUCTION

Anchovy (*Engraulis encrasicolus*, L.) is one of the economically most important Mediterranean small pelagic species with very wide distribution all over the Adriatic Sea. It is found all over the Mediterranean and the Black sea. Anchovy approaches the coast in spring when the temperature rises, and reaches sexual maturity at the end of the first year of life. Reproductive period of anchovy in the Adriatic Sea lasts from spring to autumn, usually from April to October, and sometimes from March to November (Zavodnik, 1970; Merker and Vujošević, 1972; Regner, 1972, 1985, Piccinetti *et al.*, 1979).

Main spawning area of anchovy is in the eutrophic waters of the western part of the shallow northern Adriatic and along the Italian coast to the peninsula of Gargano (Regner, 1996).

DEPM survey is part of the AdriaMed project since 2005, when it was conducted for the first time in southeast Adriatic Sea (Montenegrin continental shelf) for estimation of spawning stock biomass of anchovy (*Engraulis encrasicolus*). In July 2008 the survey was extended to Albanian territorial and adjacent international waters – from Boka Kotorska to Valona. Latest survey was performed in July of 2010 and it was extended to entire southern Adriatic Sea (GSA 18).

MATERIAL AND METHODS

DEPM (Daily Egg production Method) is the method developed by Coastal research division of the Southwest Fisheries Center, La Jolla (SWFC, California). Method is based on ichthyoplankton investigation for estimating spawning stock biomass (SSB) of batch spawners with indeterminate annual fecundity, in particular Clupeids and Engraulids. It was developed in the late 1970s and since then, it has been applied to a variety of small pelagic stocks. Application of this method requires knowledge of the boundaries of the spawning area of target species.

The spawning stock biomass estimation is based on the model described by Parker (1980):

$$B = \frac{E}{k * Frb * f * R}$$

Where: B = spawning biomass in metric tons; E = number of eggs produced per day over the surveyed area; k = conversion factor from grams to metric tons; Frb = relative batch fecundity; f = spawning frequency; R = sex ratio (fraction of mature females by weight).

Spawning frequency (fraction of mature females spawning per night) was estimated using postovulatory follicles (POF's) method (Hunter and Macewicz 1985) and by hydrated oocyte method (this method is based on the number of hydrated females compared to the total number of treated females).

Ralative batch fecundity was calculated as a number of eggs produced per unit of female weight (ovary-free weight).

RESULTS AND DISSCUSSION

Results of biomass assessment applying DEPM in southern Adriatic Sea are presented in Table 1. The DEPM assumes that the estimated parameters are constant over the area and duration of the survey (Somarakis *et al*., 2002). The distribution and abundance of anchovy eggs are presented in Figure 1. The data were processed in Surfer Golden Software 8 applying the kriging method. Anchovy spawning areas and number of anchovy eggs per square meter per day are presented too.

Table 1. Summarized biomass estimates of anchovy stocks in southern Adriatic Sea (in metric tons).

Year	Region	Surveyed area (km^2)	DEPM (Spawning biomass)
2005	Montenegrin continental shelf	5401	3842
2008	Montenegrin and Albanian continental shelf	14446.16	52 273.2
2010	Southern Adriatic sea (GSA 18)	17516	110 352.07

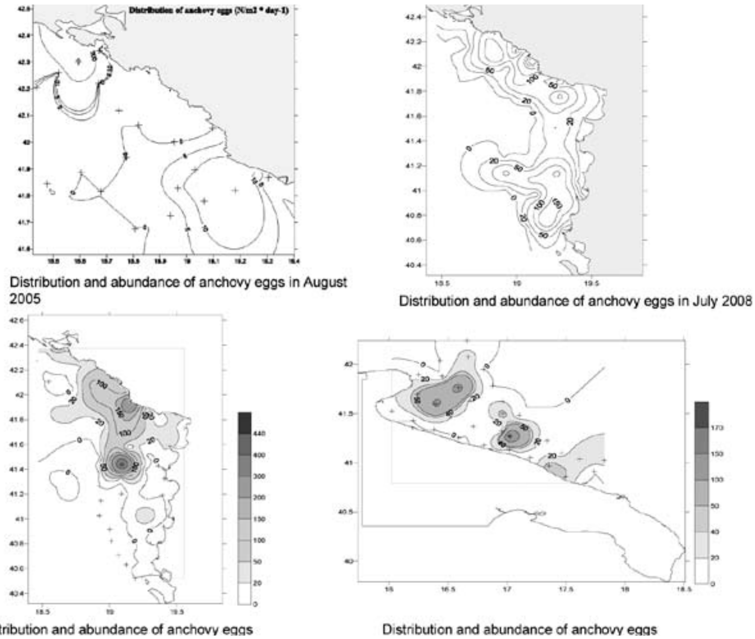

Figure 1. Distribution and abundance of anchovy eggs in southern Adriatic Sea. Numbers of eggs were adjusted as a number of individuals per m^2 of the surface.

Previous estimations of anchovy spawning stock biomass by DEP Method in southern Adriatic Sea indicate to already well known fluctuations in anchovy biomass.

Large annual oscillations in anchovy biomass can be explained by fluctuations in abiotic and biotic factors in the Adriatic Sea (Regner 1985, 1996). In view of the scarce information on the spawning ecology of *Engraulis encrasicolus* in the Southern Adriatic, and the importance of anchovy in fisheries, the aim of DEPM estimations was to investigate distribution of spawning in relation to environmental conditions, and to estimate anchovy biomass applying this method.

On the Montenegrin coast, in contrast to other Adriatic countries, commercial fishery of small pelagic species at the open sea is still undeveloped, and almost exclusively small scale fishery gears are in use. At present, there is only one active vessel (purse seiner) which exploits those resources in Montenegro, but the catches are poor, probably because of unskillful crew and some technical problems.

Based on estimated biomass by DEP method, MSY (Maximum sustainable yield) can be determined, that is, maximum amount of fish that may be caught without compromising the dynamics of natural populations

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IMPACT OF TRAWLING ON BENTHIC BIOCENOSES

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UTICAJ KOČARENJA NA BIOCENOZE BENTOSA

Abstract

Kočarenje predstavlja način ribolova gdje se mrežom povlači po morskom dnu. Ovaj način je u velikoj mjeri zastupljen u otvorenim vodama Crne Gore. Proces kočarenja uzrokuje brojne negativne efekte na morskom dnu kao što su degradacija bentosnih biocenoza i narušavanje strukture supstrata. Korišćenje mreže, koja je obično neselektivna sa morskog dna bivaju zahvaćeni i organizmi koji nemaju komercijanu vrijednost. Značajan dio ulova pripada različitim vrstama beskičmenjaka i ribljoj mlađi, čija veličina ne zadovoljava standarde tržišta, i sve ovo skupa čini nejestivi dio. Ovaj materijal poslije selekcije biva vraćen u more. To doprinosi narušavanju ravnoteže unutar životnih zajednica na morskom dnu. Kočarenje može da dovede do favorizovanja određenih vrsta dvojako. Jedan od načina je eliminisanje prirodnih predatora iz okruženja a drugi način je povećavanjem dostupne količine hrane putem vraćanja u more nejestivog dijela ulova. Kočarenje može dovesti do regresija u livadama *Posidonia oceanic*. Podizanjem mulja sa morskog dna stvaraju se veliki oblaci suspendovanih čestica u vodenom stubu, dovode do zamućenja vode tako da količina svjetlosti koja dopire do dna biva značajno smanjena. Ove čestice se talože na listovima morskih cvjetnica i usporavaju njihovu produkciju. Jedan od načina ugrožavanja morskih cvjetnica putem kočarenja je i čupanje rizoma iz podloge koje se dečava prilikom povlačenja mreže po morskom dnu.

Stepen uticaja zavisi u jednu ruku od fizičkih karakteristika opreme (material i težina) i uslova pod kojima se koristi (brzina kretanja i vrijeme trajnja) i u drugu ruku od vrste sedimenta i bentosne zajednice koja je na njemu razvijena.

Rezultati prikazani u radu su nastali kao dio projekta "Biološki resursi, jestivi i nejestivi, u kočarskom ribolovu na crnogorskom primorju" zbog toga što na datom prostoru ne postoji ni jedan projekat koji tretira ovaj problem zasebno.

Podaci su dobijeni na osnovu deset poteze (rastojanje između dvije tačke gdje počinje i završava vučenje mreže), na kojima je dubina bila u rasponu od 42 m do 350 m.

Dobijeni podaci pokazuju da je količina nejestivog dijela ulova bila u rangu od 1% do 36%. Ovo ukazuje da velika količina morskih organizama biva zahvaćena mrežom sa morskog dna i ponovo vraćena nazad jer nema upotrebnu vrijednost.

Ključne reči: *kočarenje, bentosne biocenoze, nejestivi ulov*

INTRODUCTION

Trawling is fishing mode where the mash is pulled over the seabed. This fishing method is greatly present in the open sea of the Montenegro. Process of trawling generates numerous negative impacts on the seabed such as degradation of composition of benthic biocenoses and the structure of substrate (Tudela & Sacchi, 2003). Now days this problem has higher significance than before and the number of project who treat the problem is in progress. Trawling has strong negative influence on the sea bottom life for two reasons. One effect is manifested through unselective collection of the living organisms that is essentially useless for commercial purposes, either because of their inedibility either because of its size that does not meet commercial standards (Charbonnier, 1990; Machias et al. 1999). Reducing the number of certain species of animals disturbs the equilibrium of the bottom communities. Another negative effect realized by trawling is raising large amounts of suspended particles in the water column and thus reduces the amount of light that reaches the seafloor, slowing down production in the first place seagrasses (Tudela & Sacchi, 2003).

The level of this impact depends on one hand, on the physical characteristics of the gear (materials and weights) and the conditions of its utilization (speed and duration) and, on the other, on the type of sediment and the benthic biocenoses on it (Hall, 1999).

MATERIAL AND METHODS

Research on the impact of trawling on demersal biocenoses is part of the project “Biological resources, edible and inedible, in trawling fisheries at Montenegrin coast”. Data collection was conducted during the summer 2009, on ten stations along the open part of Montenegrin coast. The material was collected in the depth range of 42 m to 350 m. Withdrawal of the network is carried out in positions where were present different types of biocenose on different substrates. The analysis included measurements of inedible catch – discard (various groups of invertebrates and juvenile fish). The collected material is measured by the trade balance and the percentage was determined by its representation of the total catch. The inorganic content of the mesh has been subjected to measurement too (mostly waste of anthropogenic origin).

RESULTS AND DISCUSION

The study comprised ten positions with various types of substrates as movable as unmovable. The muddy was dominated surface but the fishnet subsided over the sandy bottom as well as hard surfaces. Analysis of net content based on the percentage content of certain groups is shown in Figure 1. Participation of inorganic part of the catch ranged from 0% to a maximum of 50%. The inorganic part was mainly of anthropogenic origin and it was dominated by car tires and plastic bottles. As for inedible part, its share in the total catch was within the range of 1% (position 4) to 36% (position 1). Within inedible material was prevailing invertebrate group echinoderms. Within this group according to the number of individuals stood out sea lilies (*Antedon mediteranea*) while the related to biomass sea cucumber (*Parastichopus regalis*) was dominated. Representatives of other invertebrates present on the investigated sites were Ascidiae, Mollusca, Cnidaria. As for the fish that really belong to the inedible part (discard) it was hard to determine their quantity, because the fishermen offer for sale on the market samples which size do not meet commercial standards.

One of the problems that occur in the fishing industry is that the whole non-edible (by-catch) portion returns back into the sea. In this way, discarded biomass can lead to changes in ecosystem structure favouring certain species that feed on animals recovered (Morant et al. 2000). Fishing may favour individual species in two ways by removed their predators from environment or increases the amount of available food by returning discard into the sea.

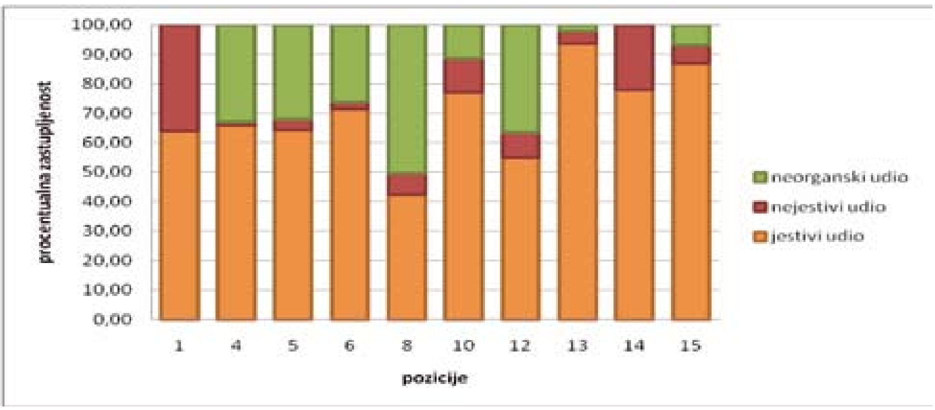

Figure 1. The percentage share of edible, inedible and inorganic part of the catch in trawl fishnet.

The level of this impact depends on one hand, on the physical characteristics of the gear (materials and weights) and the conditions of its utilization (speed and duration) and, on the other, on the type of sediment and the benthic biocenoses on it (Hall, 1999).

Indirect effects on the seabed is reflected by size of the stress that benthos is exposed (Jones, 1992). Trawling is responsible for raising large amounts of sediment and its suspension in the water column. These clouds of mud have a negative effect on fish but also negatively affect production of benthic communities. However, the exact consequences of this phenomenon are not known.

Besides leading to disproportion in animal components of biocenoses trawling is the main reason of regression that occur in the meadows of *Posidonia oceanica* (Martin et al., 1997). In this way disturbed community, which used to be a shelter for large numbers of fish and their spawning place, cease to be (Sanchez-Jerez & Ramos-Espla 1996). Fish lose their natural habitat, and gradually comes to reducing their populations. Unfortunately we have no data that would detail out this problem but few studies are conducted in France and Italy, where it was done comparing the situation between

Posidonia meadows in areas where fishing is allowed and protected areas where fishing is prohibited (Buia et al . 1999; Harmelin-Vivien 2000; Francour 1999) and obtained results show decreasing mean weight, density and biomass of fish in exploited areas.

CONCLUSION

The use of networks in demersal fisheries has multiple negative effects on wildlife of the sea floor. The exact effects of this process are not fully tested because in our country has not conducted any detailed research, and an insufficient number of projects dealing with these issues in the region.

It is known however that non-selective fishing net from the ocean floor collect organisms that are builders of benthic biocenose and have no commercial value. They include representatives of numerous groups of invertebrates and juvenile fish, which did not reach commercial size. Besides animal components trawl is in violation of the vegetable component. This primarily refers to the area where the present *Posidonia oceanica*, whose development are prevented by particles of suspended sediments.

Own studies have shown that the proportion of the by-catch move up to 36%, which means that one third of the fishnet content is unusable for food and being separated from their natural habitat.

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SOME BIOLOGICAL PARAMETERS OF SARDINE, *SARDINA PILCHARDUS* WALB. 1792, IN MONTENEGRIN WATERS

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NEKI BIOLOŠKI PARAMETRI SRDELE, *SARDINA PILCHARDUS* Walb. 1792, U CRNOGORSKIM VODAMA

Abstrakt

Srdela, Sardina pilchardus, je jedna od najrasprostranjenijih i komercijalno najvažnijih vrsta riba u Jadranskom moru (FishStat Plus, FAO). Industrijski ribolov srdele i inćuna u Crnoj Gori je još uvek nerazvijen, pa se ove vrste uglavnom love alatima malog obalnog ribolova, tj. mrežama potegačama male veličine oka (5-6 mm) u Bokokotorskom zalivu. Podaci predstavljeni u ovom radu rezultat su istraživanja sprovedenog u okviru Pilot studije AdriaMed projekta u periodu septembar 2007. – septembar 2008. godine. Tokom Pilot studije putem intervjua sa ribarima prikupljane su informacije o ulovu i ribolovnom naporu svih aktivnih tipova brodova, a takođe su uzimani i biološki uzorci komercijalno važnih vrsta kako bi se proučavale njihove biološke karakteristike. Uzorci srdele prikupljeni su u području Bokokotorskog zaliva (koji predstavlja jedno od najproduktivnijih područja na crnogorskom primorju, pa je mrestilište i hranilište mnogih ribljih vrsta. Uzorci su prikupljani mesečnom dinamikom. Prikupljeni su sledeći podaci: totalna dužina tela sa preciznošću 0.1 cm, totalna težina sa preciznošću 0.01 g, pol i stadijum zrelosti gonada (upotrebljena je skala sa četiri stadijuma zrelosti, 1 – nezrele, 2 – sazrevanje, 3 – zrele i 4 – izmrešćene jedinke). Na osnovu ovih podataka određen je dužinski raspon, distribucija dužinskih frekvenci, odnos polova, dužina dostizanja polne zrelosti ($L_{50\%}$ dužina pri kojoj je 50% populacije polno zrelo, kao i $L_{25\%}$ $L_{75\%}$), kao i dužinsko-težinski odnos prema formuli $\log W = \log a + b \log L_T$. Srdela je bila najzastupljenija u ulovu u periodu april – jul. Dužinski raspon iznosio je od 7.3 do 16.6 cm, sa srednjom vrednošću od 13.1 cm. Kod oba pola prisutne su dve grupe individua (Sl. 2). Kod ženki prvu grupu čine individue dužinskog rapona 7.5 – 12 cm, a druga grupa obuhvata adultne jedinke dužine 12.5 – 16.5 cm. Kod mužjaka prvu grupu čine mlade jedinke dužine manje od 12.5 am, dok drugu, mnogoizraženiju grupu, čine jedin-

ke dužinskog raspona 13 – 16 cm. Od ukupnog broja jedinki (613) ženke čine 51.4% a mužjaci 48.6% (Tab. 1). U većim dužinskim klasama (>15 cm) ženke su zastupljenije od mužjaka (78%) (Sl. 3, 4). Zrele jedinke srdele obuhvataju dužinu 7.3 – 15 cm kod mužjaka, a kod ženki 8 – 16.6 cm. Nezrele jedinke čine oko 21% ukupnog uzorka, dok je izmrešćenih jedinki pronađeno 26 tokom čitavog istraživanja. Najveći broj uzorkovanih jedinki bio je u drugom stadijumu zrelosti. Jedinke u fazi mresta najzastupljenije su bile u periodu februar – mart. Procenjeno je da je dužina pri kojoj 50% populacije srdele dostigne polnu zrelost 12. 29 cm, dok su $L_{25\%}$ i $L_{75\%}$ procenjeni na 11.59 i 12.98 cm (Sl. 5). Koeficijent b dužinsko-težinskog odnosa kod mužjaka iznosio je 3.138, a kod ženki 2.973.

Ključne reči: *srdela, biološke karakteristike, Bokokotorski zaliv*

INTRODUCTION

The FAO AdriaMed Project provides support to the Adriatic countries in developing the necessary expertise and tools for the appraisal of the fisheries resources and of the main socio economic aspects related to the fisheries. The Montenegro joined AdriaMed in 2004 and since then the Project assisted the country in the establishment of a system for the fisheries resources evaluation and management. A Pilot study on biological and socio-economic fishery data collection was scheduled and implemented in Montenegro by the Institute of Marine Biology of Kotor with the support of the AdriaMed Project in the period September 2007 - September 2008. The information on catch and effort of all the active fleet segments in the sampling ports were gathered by interviewing the fisherman, also the biological samples of the main target species were taken to study their biological characteristics.

MATERIAL AND METHODS

Sardine samples were collected in the Boka Kotorska Bay (Fig. 1), a closed marine bay with many freshwater springs and runoffs, and is prone to rather great temperature and salinity variations. It is one of the most productive areas of the Montenegrin coast and it seems to be a nursery ground for sardine and other small pelagic fish species.

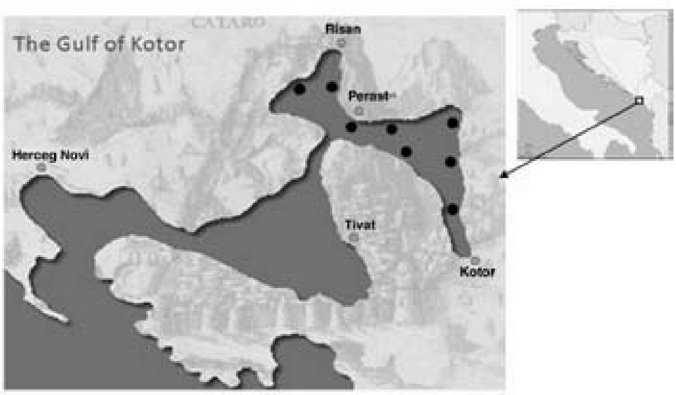

Figure 1. Map of the study area

The beach seine samples were collected monthly from September 2007 to September 2008, except in January when there were no catch due to a bad weather conditions. The following data was collected: total length to the nearest 0.1 cm, total weight to the nearest 0.01 g, sex and sexual maturity. For fishes a four maturity stages scale was considered (immature, maturing, mature and spent – resting; respectively, stages 1, 2, 3 and 4).

These data allowed to estimate the size range and length frequency distribution, the sex-ratio as proportion of males over the combined number of males and females, the size at first maturity (size at 50 percent of maturity, $L_{50\%}$) and size at 25 and 75 percent of maturity ($L_{25\%}$ and $L_{75\%}$) according to the classical logistic model and the length-weight relationship parameters using a power function. The length–weight relationship was determined according to the logarithmic form of the original exponential equation: $\log W = \log a + b \log L_T$, where a is the proportionality constant, b the allometry coefficient, W is fish weight in grams, and L_T is total length in centimetres.

RESULTS AND DISSCUSSION

Sardine, *Sardina pilchardus*, was more abundant in the catch in April-July period. Size range of the collected specimens was from 7.3 to 16.6 cm, with an overall average length of 13.1 cm. The LFD by sex showed a continuous pattern between 8 and 16.5 cm TL, even if two groups of individuals were apparent in both sexes (Figure 2). In females there was a first group of individuals included between 7.5 and 12.0 cm TL, and a second group describing the adults with size included between 12.5 and 16.5 cm TL. In males the first group comprised specimens < 12.5 cm TL, while the second, more evident, group was included between 13 and 16.0 cm TL (mode about 13.5 cm TL).

On the whole sample (613 individuals), females and males components were 51.4 and 48.6 percent respectively and no unsexed individuals were recorded (Tab. 1). The sex ratio value of the whole data set was close to 0.5 (0.48). More females than males (78%) in the higher TL classes (from 15 cm) were recorded (Fig. 3, 4). Similar results are reported for the same area for the period 2006-07 (Pesic *et. al.*, 2010). Mature specimens of *S. pilchardus* were found at wide size range, TL varying between 7.3 and 15.7 cm in males and 8.0 and 16.6 cm TL in females. According to Sinovcic *et. al.* (2008) sardine at the end of the first year of life, the smallest mature female are at 7.1 cm and male at 7.3 cm. A large fraction of immature individuals (more than 21%) was found, but only 26 post-spawning specimens were sampled during the whole study period. The largest fraction of the sampled specimens was in the 2 maturation stage. Period of highest occurrence of spawning specimens was during February and March. Spawning of this species is mainly during the October – April period (Muzinic, 1954; Regner *et. al.*, 1981, 1983, Nejedli *et. al.*, 2004, Pesic *et. al.*, 2010). The peak of spawning based on the investigations of GSI and oocyte composition in sardine ovaries in Bokaotorska Bay is in February (Pesic *et. al.*, 2010).

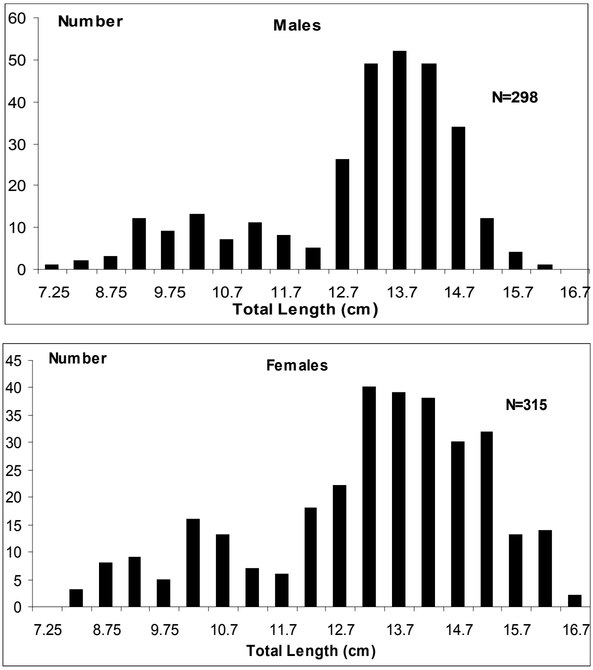

Figure 2. Length frequency distribution of *Sardina pilchardus* caught in national waters of Montenegro during 2007/2008 by sex. N = number of specimens

Table 1. Sex and gonad maturity stages of *Sardina pilchardus*. F = female; M = male; TL = total length in cm; 1-4 = gonad maturity stages

Sardina pilchardus	M				F			
Maturity stage	No	%	TL min	TL max	No	%	TL min	TL max
1	68	22.82	8.7	14.2	63	20.00	8.3	16
2	200	67.11	7.3	16.2	174	55.24	8.1	16.3
3	23	7.72	13.1	15.2	59	18.73	12.1	16.6
4	7	2.35	13.8	15.2	19	6.03	12	15.2
Total	298	100.00			315	100.00		

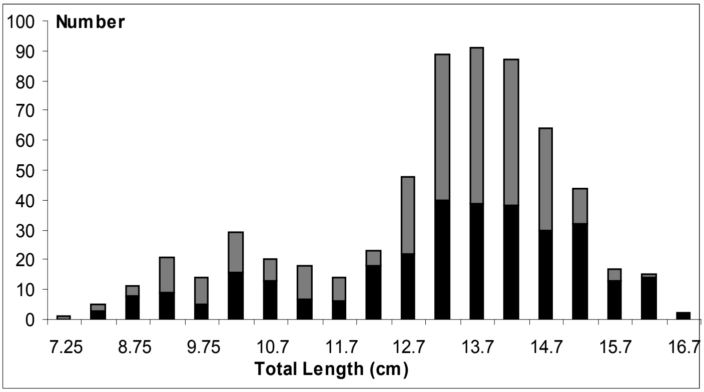

Figure 3. Length frequency distribution by sex. Black bars = females; stripped bars = males.

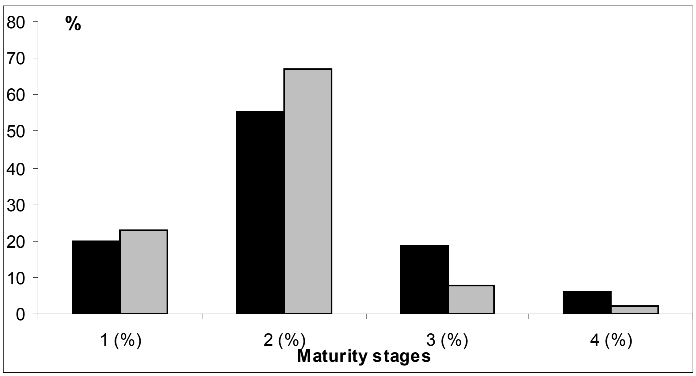

Figure 4. Sex and gonad maturity stages proportion. Black bars = females; stripped bars = males.

The proportion of mature specimens as a function of size (TL) fitted the logistic model and the computation of the ogive parameters provided an estimate of $L_{50\%}$ of 12.29 cm (Fig. 5); the value of $L_{25\%}$ was 11.59 cm, whereas that of $L_{75\%}$ was 12.98 cm. The power coefficient (*b*) of the LW relationship was 3.138 for males and 2.973 for females. For the same area in the period 2006-07 Pesic *et. al.* (2010) reported the power coefficient *b* for male 3.113 and for females *b*=3.077.

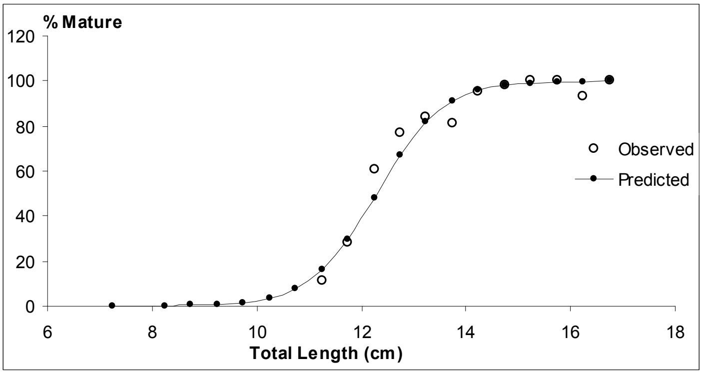

Figure 5. Proportion of mature specimens by size compared with estimated logistic curve.

CONCLUSIONS

The results of this study pointed out that a large fraction of immature individuals, more than 21%, are caught through small-scale fishery in the Boka Kotorska Bay. Some important management decisions need to be recommended for this area. On the other side, this type of fishery has a long tradition in Boka Kotorska Bay and must be preserved.

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CHANGE NUMBER OF ENTEROBACTERIA DURING STORAGE OF COLD SMOKED TROUT PACKED IN VACUUM AND MODIFIED ATMOSPHERE

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PROMENA BROJA ENTEROBAKTERIJA U TOKU SKLADIŠTENJA HLADNO DIMLJENE PASTRMKE PAKOVANE U VAKUUMU I MODIFIKOVANOJ ATMOSFERI

Abstrakt

Danas je hladno dimljena riba sastavni deo naše ishrane. Razlog tome treba tražiti kako u povećanju proizvodnje ribe u akvakulturi, koja je samim tim dostupna u većoj količini za preradu, tj. dimljenje, tako i u sve većoj potrebi čoveka da se okrene zdravoj ishrani. Pri tome, hladno dimljena riba, pored toga što zadovoljava visoke nutritivne kriterijume, ona je i atraktivna za potrošača zbog svojih specifičnih senzornih karakteristika. Međutim, i pored toga što je dimljena riba, proizvod poznat vekovima, postojeći problem u proizvodnji dimljene ribe u zemljama Evropske Unije, ali i kod nas, predstavlja nepostojanje unapred utvrđenih kriterijuma koji treba da zadovolje dimljeni proizvodi od ribe. Pri tome se misli na definisanje jedinstvenih kriterijuma kvaliteta dimljene ribe i usaglašavanje pojedinih faktora proizvodnje (soljenje, dimljenje, izbor sirovine, pakovanje), kao i na određivanje održivosti tj. roka trajanja dimljenih proizvoda od ribe, koji se razlikuje od proizvođača do proizvođača. Održivost dimljene ribe, odnosna nastanak kvara, zavisi,od inicijalne kontaminacije, uslova proizvodnje, rukovanja sa proizvodom nakon proizvodnog procesa i temperature skladištenja i načina pakovanja. Iako kvar, hladno dimljenih proizvoda od ribe može nastati delovanjem različitih mehanizama, najvažniji razlog kvara mesa ribe i proizvoda od ribe je rast mikroorganizama i stvaranje produkata, rezultata njihove metaboličke aktivnosti koji dovode do pojave nepoželjnog mirisa i ukusa.

Pakovanje dimljenih proizvoda od mesa ribe u vakuumu, odnosno modifikovanoj atmosferi, može u velikoj meri uticati na održivost proizvoda, ali i udovoljiti zahte-

vima savremenog potrošača koji traži hranu visokog kvaliteta, koja je zadržala senzorne karakteristike i nutritivnu vrednost sirovine od koje je proizvedena, a da je uz to i bezbedna po njegovo zdravlje. Osnovni cilj pakovanja hrane u vakuumu jeste uklanjanje kiseonika iz hrane, s obzirom da on omogućava rast aerobnih mikroorganizama (bakterija, kvasaca i plesni) koji najčešće dovode do kvara mesa ribe i proizvoda, dok se pakovanjem hrane u modifikovanoj atmosferi, konzervišućim delovanjem primenjenih gasova, onemogućava ili usporava rast i razmnožavanja mikroorganizama, odgovornih za nastanak kvara. Zato se prisustvo i promena broja određenih grupa mikroorganizama često uzima kao parametar održivosti ribe i proizvoda od ribe. U literaturi se često kao mikroflora koja je odgovorna za nastanak kvara hladno dimljenih proizvoda, pored ostalih spominju i bakterije iz familije Enterobacteriaceae. Pored značajne uloge ovih bakterija u nastanku kvara hladno dimljenih proizvoda od ribe, postojanje mogućnosti trovanja ljudi, sa pojedinim vrstama bakterija iz ove familije, opredelila nas je da nam cilj istraživanja bude utvrđivanje prisustva i broja bakterije iz familije Enterobacteriaceae u gotovim proizvodima hladno dimljene pastrmke pakovane u vakuumu i modifikovanoj atmosferi, u toku šest nedelja skladištenja pri +3 °C. Za eksperiment su formirane četiri grupe hladno dimljenih fileta pastrki. Prva grupa (I) uzoraka je vakuumirana, a ostale tri su pakovane u tri različite modifikovane atmosfere: druga grupa (II) – 50% CO_2 + 50% N_2, treća grupa (III) - 60% CO_2 + 40% N_2 i četvrta grupa (IV) - 90% CO_2 + 10% N_2, a nultog, a zatim svakih sedam dana, šest nedelja utvrđivan je ukupan broj enterobakterija. Rezultati naših ispitivanja pokazuju da je u uzorcima sve četiri grupe u toku skladištenja došlo do statistički značajnog porasta ukupnog broja enterobakterija. Takođe, rezultati su potvrdili činjenicu da CO_2 deluje inhibitorno pre svega na gram-negativne bakterije, kakvi i jesu mikroorganizmi iz familije Enterobacteriacea, s obzirom da je ukupan broj enterobakterija tokom celog perioda skladištenja bio statistički značajno niži u uzorcima fileta hladno dimljene pastrmke pakovane u smeši gasova. Ujedno, najslabija stopa rasta enterobakterije utvrđena je u filetima hladno dimljene pastrmke IV grupe, tj. grupe u kojoj je procentulno ugljen dioksid bio najzustupljeniiji, tj.da je u njima najviše izraženo antimikrobno dejstvo ugljen dioksida.

S obzirom da kod proizvođača ribe u našoj zemlji postoji interes da prošire asortiman proizvodnje, a u toj mogućnosti proširenja asortimana najinteresantnija je proizvodnja dimljene ribe. Implementacija savremenih načina pakovanja u proizvodnji hladno dimljene pastrmke, ovakav proizvod treba da učine što pristupačnijim i za potrošača. Otuda i u našoj zemlji postoji interes za ispitivanje različitih načina pakovanja koji utiču na kvalitet dimljene pastrmke. A praćenje promena ukupnog broja enterobakterija, kao jednog od najznačajnih indikatora kvaliteta hladno dimljene ribe, u ispitivanim uzorcima pakovanim u vakuumu i modifikovanoj atmosferi, predstavlja samo jedan korak u ka uspostavljanju objektivnih kriterijuma za ocenu kvaliteta, ovog nutritivno vrednog proizvoda od ribe.

Ključne reči: *dimljena riba, vakuum, modifikovana atmosfera, Enterobacteriaceae, kvalitet*

INTRODUCTION

Smoked fish is an integral part of our diet. Smoked fish have lost its image of luxury products, the reason being the steady growth of fish farming in aquaculture in recent years, which contributes to increasing the quantity of fish in the market, and consequently, the amount of fish available for processing, or smoking. This situation, contributes that the world market is regularly supplied by smoked fish, and such uniformity of the market contributes to the price of this product are held for years at the same level, leading to increased demand, consumption, and consequently increasing the production of smoked fish.

The definition of cold-smoked fish, which has been given by the Codex Alimentarius Commission (1979), and The Association of Food and Drug Officials (AFDO 1991) reads: "Cold smoked fish is smoked fish that is produced by exposure to smoke fish and temperature, where there is only a partial coagulation of protein as effect of temperature. Accordingly, such a meager definition of fish products, it is expected that the production of cold-smoked fish, using various parameters of the technological procedure of processing fish. It is therefore to be expected that the quality of cold smoked fish products on the market vary from manufacturer to manufacturer, and is an emerging problem in the production of smoked fish in the European Union and in our country, luck of predetermined criteria that should satisfy the smoked fish products. Defining of uniform criteria for the quality of smoked fish and alignment of certain factors of production (salting, smoking, choice of raw materials, packaging) would contribute to the production of safe products, consistent quality. Second, no exist of uniform, objective criteria for assessing the quality, the producers of smoked fish can have difficulty in monitoring changes in quality and determine the shelf life of products.

The quality of smoked fish is dependent on many factors, those related to fish production (nutrition, environmental conditions, genetic factors, gender and sexual maturity, life cycle) (Hovde et al., 2007) and those that relate the processing and fish processing (slaughter and processing, curing, smoking, packaging) (Sikorski and Kolodziejska, 2002). Special attention was paid to the sustainability of smoked fish that is usually determined with bacteriological status, physical and physic-chemical properties and sensory properties (Ibrahim et al., 2008; Siskos et al., 2007, Cardinal et al., 2004, Rorabaugh et al., 1999).

Shelf life of food, and thus a smoked fish products, can be defined as the time between the packages of food and time in which the product is particularly safe to the health of consumers and in which its sensory characteristics (smell, taste, appearance, texture) and nutritional value unchanged and acceptable to consumers (McMillin, 2008; Sørheim et al. 1997). Shelf life is the time period in which there is no appearance of signs of spoilage. Spoilage of fish and fish products can be defined as any change in fish, which makes the product unusable (unacceptable) for human consumption (Arashisar et al., 2004). The most common reason for spoilage of smoked fish products is microbial activity. Microbial growth and the creation of products, found their metabolic activity (the creation of amines, sulfides, alcohols, primarily ethanol, aldehydes, ketones, organic acids) lead to the undesirable odor and taste and appearance of discoloration (Leroi et al., 2001). What part of the micro flora will grow in the product is determined by the parameters that are related to the production process itself, by storage conditions and packaging, and the presence and change of certain groups of microorganisms is often taken as a parameter for the shelf life of fish and fish products (Muratore and Licciardello , 2005; Siverstvik et al., 2002, Paludan-Müller et al., 1998). For this reason, the shelf life of fish products depends primarily on the initial contamination, the conditions of production, handling the product after the manufacturing process and storage temperature and the type of packaging (Caglak et al., 2008, Goulas, and Kontominas, 2007; Stamatis and Arkoudelos, 2007; Siverstvik et al., 2002).

Modern consumers demand high quality food that has retained sensory characteristics and nutritive value of raw material from which it is produced, and that it is also safe and on their health. This demand is largely achieved by packaging the products in vacuum or modified atmosphere. In this way the demands of consumers are met, and also manufacturers have everything to gain - not only do they keep, but this way the possibilities to expand the market. Despite the constant evolution in materials and methods of packaging, the basic principle of packaging remained the same. This is to avoid contamination, delay spoilage, permit an enzyme reaction that could improve the softness, reduce the loss of weight and where possible making sure to retain the sensory characteristics of products (Dainelli et al., 2008, Kerry et al., 2006; Hill, 2003, Murcia et al., 2003; Vermeiren et al., 1999). The main objective of food packaging in vacuum and gas mixture is to remove oxygen from the food, since it allows the growth of aerobic microorganisms (bacteria, yeasts and molds) which leads to spoilage of fish and fish products, and to prevent oxidative valence, which deplete vitamins, pigments, lipid components, reducing the quality and nutritive value of the product (Hill, 2003).

Packaging of smoked fish meat products in vacuum are perfect for keeping the product up to three weeks. When packing in a vacuum, eliminating air in packing impermeable to oxygen, forming anaerobic/microaerophilic conditions, increasing the CO_2 content and decreasing pH products thereby inhibiting the growth of aerobic gram-negative bacteria and providing a better shelf life of fish (Soccol and Oetterer, 2003). These changed conditions inhibit the growth of aerobic bacteria and facultative anaerobes allow growth and developing microflora dominated lactic acid bacteria, and a smaller number are present and *Brochotrix thermosphacta, Enterobacteriaceae,* other gram-negative bacteria, micrococcus and yeasts (Leroi et al., 1998; Lyhs et al., 1998, Truelstrup Hansen and Huss, 1998; Truelstrup Hansen et al., 1995).

Packaging of food in the mixture of gases, i.e. modified atmosphere, or MAP (Modified Atmosphere Packaging) packaging technology is leading 21st century, which basically acts as a vacuum packaging, only difference is that the vacuum packing internal conditions that inhibits microorganisms are developed in the package, while in the MAP gas mixtures initiated in order to create the same conditions (Radetić et al. 2007; Goktepe and Moody, 1998). In the last decade of the 21st century, packaging in modified atmosphere gained considerable popularity as a modern, non-thermal method of food preservation (Patsias et al., 2006). There is evidence that the packing of fish and fish products in the mixture of gases to increase their shelf life from 0% to 280% of the fish and fishery products stored in air (Özogul et al., 2004), or about 1.5 to 2 times longer (Erkan et al., 2006; Ward, 2001).

The modified atmosphere includes replacement of air in the packing with a mixture of gases. The most common combination of gases is carbon dioxide, nitrogen and oxygen and their optimal ratio may vary depending on the type of fish (Mullan, 2002). Preserving action of gases, primarily carbon dioxide, used in the packaging of foods based on their ability to preventing or slowing growth and reproduction of microorganisms, influence on stopping or slowing down the degradation caused by microorganisms or natural chemical agents that deeply modify the product making it unfit for consumption

(Masniyom et al., 2002). It was found that the degree of inhibition of microorganisms mainly psychrotrophic, aerobic gram-negative bacteria is proportional to the content of CO_2 (Pantazi et al., 2008). The mechanism by which carbon dioxide slows the growth of bacteria and fungi is complex. It is known that carbon dioxide is causing damage to cell membranes and causes changes in the function of the cell membrane, then reacts with the lipid of cell membrane, thus changing the ability of the membrane transport of certain ions, causing a direct inhibition of the synthesis of certain enzymes or decrease the speed of enzymatic reaction, penetrate the bacterial membranes leading to changes in intracellular pH value (acidification) (Cornforth and Hunt, 2008) and causes direct changes in the physic-chemical properties of proteins (Goulas, 2008; Siverstvik et al., 2002).

There is no doubt that composition of dominant microflora in cold smoked fish products packed in the mixture of gases depends on the mixture of gases used in packaging, and the predominant microflora in cold-smoked fish products packed in modified atmosphere, is the one that is resistant to CO_2 (Siverstvik et al., 2002). In general, gram-negative bacteria are much sensitive to the effect of CO_2, and are also the most inhibited microorganisms. The most sensitive microorganism are *Pseudomonas spp*., then *Enterobacetriacea*, *Acinetobacter spp*., *Achromobacter*, *Flavobacterium*, and *Moraxella spp*. and psychrotrophic bacteria that lead to spoilage of fish. As mentioned most resistant is *Clostridium spp*. (Jay et al., 2005; Devliegher and Debever, 2000). Gram positive bacteria, such as lactic acid bacteria, mainly *Lactobacillus spp.* and *Leuconostoc spp.* and then *Brochothrix thermosphacta*, are not sensitive to the effects of carbon dioxide, and in fish products packed in the mixture of gases they become the dominant flora *(Limbo i sar., 2010*; *McMillin, 2008; Erkan i sar., 2006; Sanjeev i Ramesh, 2006; Siverstvik i sar*. *2003; Mokhele i sar., 1983)*.

As the *Enterobacteriaceae* family of bacteria that is often mentioned in literature as an indicator of spoilage cold smoked fish (Ólafsdóttir et al., 2005), and some of them are pathogenic to humans, the aim of our research was to determine the presence and examine the change in total number of enterobacteria in cold smoked trout packed in two ways, in vacuum and modified atmosphere because there is the lack of such data in this area of obstacles for the establishment of unified criteria for quality and sustainability of cold-smoked fish packed in vacuum and modified atmosphere.

MATERIAL AND METHODS

Salmon trout *(Oncorhynchus mykiss*), used for experiments weighing about 1 kg. After primary treatment, trout were washed and soaked in vats for curing (wet salting) for 24h and then were pressed, laid on the grid in chambers for over an hour at 20°C. Smoking was performed on the automated smokehouse at a temperature of 28°C for eight hours. The smoking product was used beech sawdust and the smoke was later developed its combustion in the generator, separate from the smoking chamber. Upon completion of the process of smoking, the fish was cooled at 2 ° C for 10 hours. So cold, the fish was threaded with machine to burn ("slicing"), into thin fillets thickness to 0.5 cm. Fillets were then packaged, in about 75 grams. Upon completion of the manufacturing process there were four groups of samples. The first group (I) sample was vacuum packaged, and the other three were packaged in three different modified atmospheres: the second group (II) - 50% CO_2 + 50% N_2, the third group (III) - 60% CO_2 + 40% N_2, and the fourth group (IV) - 90% CO_2 + 10% N_2. Packaging of smoked trout fillets in modified atmosphere was used Multivac device (Multivac C350, D-87787 Wolfertschwenden, Germany). Packing material was foil OPA / EVOH / PE (oriented polyamide / ethylene vinyl alcohol / polyethylene, UPM - Kymene, Walki Films, Finland) with low gas permeability (permeability of O_2 5 cm^3/m^2/day at 23°C, of N_2 1 cm^3/m^2/day at 23°C, of CO_2 23 cm^3/m^2/day at 23 C and of water vapor 15 g/m^2/day at 38°C). Packages were filled with mixture of gas producer Messer Tehnogas. Ratio of gas/sample in the package was 2:1. After packing the samples of all four groups of trout were kept for six weeks, at a temperature of +3°C. The samples were analyzed by zero, seventh, fourteenth, twenty, twenty-, thirty-fifth and forty-second days of storage in order to determine the number of enterobacteria. Determination of the total number of enterobacteria n cold smoked trout fillets from all four groups was carried out according to the method ISO 21528-2: 2004 (E) - Microbiology of food and animal feeding stuffs - Horizontal Methods for the detection and enumeration of *Enterobacteriaceae* - Part 2: Colony-count method.

RESULTS AND DISCUSSION

As already stated, Gram negative bacteria are more sensitive to the effects of carbon dioxide. Our results confirm an existing fact. The total number of *Enterobacteriaceae* on the first test in group I was the log CFU / g 1.65 ± 0.20 and was statistically significantly lower than the total number of *Enterobacteriaceae* determined in samples of group II (log CFU / g 2:51 ± 0.24), and samples in Group III (log CFU / y 2:59 ± 0.72), while no statistically significant difference from the total number of *Enterobacteriaceae* in samples of group IV (log CFU / g of 2:03 ± 0.29) (Figure 1 and Figure 2). Statistically significant difference was found between the total number of *Enterobacteriaceae* samples III and IV group ($p < 0.05$), and there were no significant differences between the number of *Enterobacteriaceae* in samples II and III groups. In all groups during storage there was a statistically significant increase in the total number of *Enterobacteriaceae*, which can be seen from the so-called feature. best suited for real, and based on regression equations for each group. Specifically, the positive value of coefficient "b" in the regression equation for all four groups shows a tendency to decrease the total number of *Enterobacteriaceae* in all groups, with what coefficient "b" has the highest value, the regression equation, the fillets of the first group and the lowest fillets in group IV, which indicates that the weakest rate of growth had *Enterobacteriaceae* in frozen group IV, ie. that in their most pronounced antimicrobial effect of carbon dioxide, which is also the highest percentage in the mixture of gases which are packaged fillets Group IV (Figure 2).

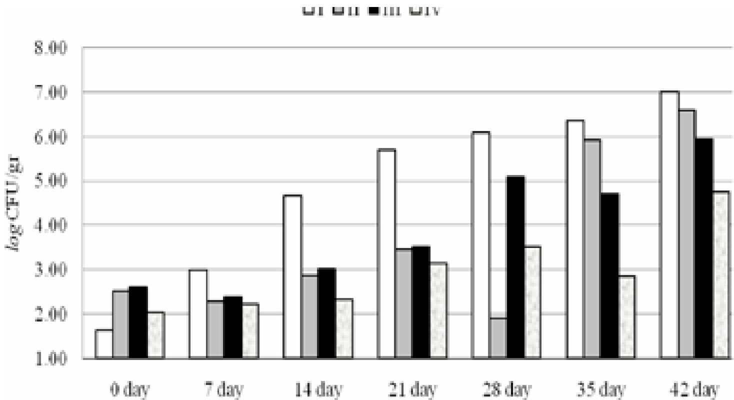

Figure 1. Comparative review the total number of *Enterobacteriaceae* in all four groups of cold smoked trout fillets during six weeks of storage

After six weeks of investigation (after six weeks of storage), it was found that the total number of *Enterobacteriaceae* group IV samples (log CFU/g 4.75 ± 0.16) was lowest and was significantly different from the total number of *Enterobacteriaceae* found in samples of group (log CFU/g 7:01 ± 0:53), Group II (log CFU/g 6.60 ± 0.47) and Group III (log CFU/g 5.94 ± 0.34) (Figure 1 and Figure 2). The total number *Enterobacteriaceae* of I group and II group did not differ significantly, but the number of *Enterobacteriaceae* determined in the two groups was statistically significantly higher than the total number of *Enterobacteriaceae* samples of group III. Our results confirm the fact that CO2 has inhibitory effect mainly on gram-negative bacteria, and what are the microorganisms from the family *Enterobacteriacea*. Comparing the results from the literature we can accept claim of Paludan-Müller and et al. (1998) that carbon dioxide has dramatically reduced the growth of gram-negative bacteria, or in such a finding can be restricted, to be more specific and apply only to Group IV samples are packed in the compound where the percentage of CO_2 was the most common (90%), and which, indeed, the total number *Enterobacteriacea* was significantly smaller and the statistical significance level of $p < 0.001$, compared to all groups that were the subject of investigation.

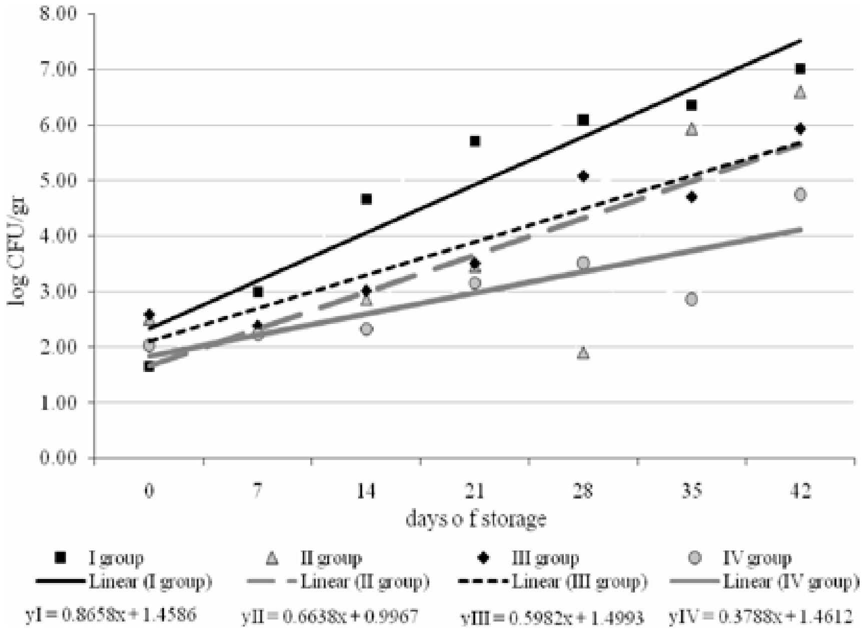

Figure 2. Best custom made and regression equations for the total number of *Enterobacteriacea* in all four groups of cold smoked trout fillets during six weeks of storage.

CONCLUSION

We confirmed in our research that the most sensitive *Enterobacteriaceae* family of bacteria when it comes to food packaging in the mixture of gases containing carbon dioxide. Given that the application of CO_2 successfully reduced the rate of growth of enterobacteria, our results suggest that consumption of cold-smoked fish products packed in the mixture of gases, there is little risk of poisoning people enterobacteria and so it is with food safety, particularly desirable to use food packaging in modified atmosphere. We believe that the perceived benefits of a sound basis for setting a new hypothesis to define the unique quality criteria as cold smoked trout packaged in a mixture of gases as well as those packaged in a vacuum, where it is necessary that special attention be paid to study this type of product safety for human health. Given the very small number of data in the literature pertaining to cold smoked trout packaging in modified atmosphere, further research (with more difference atmosphere) should examine the possibilities of other combinations and relationships of gases in the atmosphere packaging, and find the ideal combination of packaging cold smoked trout fillets in the atmosphere.

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VARIATION IN *ENTEROBACTERIACEAE* COUNT DETERMINED IN RAINBOW TROUT (*ONCORHYNCHUS MYKISS*) AND CARP (*CYPRINUS CARPIO*) STEAKS PACKED IN VACUUM AND MODIFIED ATMOSPHERE

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PRAĆENJE PROMENE BROJA BAKTERIJA FAMILIJE *ENTEROBACTERIACEAE* U PASTRMCI I ODRESCIMA ŠARANA UPAKOVANIM U VAKUUM I MODIFIKOVANU ATMOSFERU

Abstrakt

Kvar ribe može se definisati kao bilo koja promena u mesu ribe koja proizvod čini neprihvatljivim za ljudsku ishranu i najčešće nastaje kao rezultat rasta mikroorganizama. Od toga koji su mikroorganizmi najzastupljeniji u ribi u trenutku ulova u velikoj meri zavisi i kolika će biti njena održivost tokom skladištenja. Bakterije koje pripadaju familiji Enterobacteriaceae su fakultativni anaerobi, gram negativni i katalaza pozitivni mikroorganizmi. Ovoj familiji pripadaju sledeći rodovi: Salmonelle, Escherichia, Proteus, Shigella, Serratia, Citrobacter, Klebsiella, Enterobacter i Erwinia, a neki od njih su izraziti patogeni i izazivači velikog broja različitih oboljenja kod ljudi, kao što su septikemija, pneumonija, meningitis, infekcije urinarnog trakta, bolesti organa za varenje i mnogih drugih bolesti. Široko su rasprostranjeni u spoljašnjoj sredini, a neki su saprofitski stanovnici digestivnog trakta.

Cilj ovog eksperimenta je bio da se ispita rast bakterija familije Enterobacteriaceae u svežoj pastrmci i odrescima šarana upakovanim u vakuum i modifikovanu atmosferu. Uzorci su podeljeni u tri grupe. Grupa I je upakovana u modifikovanu atmosferu sa odnosom gasova 60%CO_2 i 40%N_2, grupa II je upakovana u modifikovanu atmosferu sa 40%CO_2 i 60%N_2 dok je III grupa upakovana u vakuum. Odnos gas/uzorak u pakovanju bio je 2:1. Svi uzorci su skladišteni pri istovetnim uslovima na temperaturi od +3˚C. Određivanje broja bakterija familije Enterobacteriaceae prema ISO 21528 -2:2004 obavljeno je 0, 7. i 14. dana skladištenja. U pastrmci i odrescima šarana upakovanim u modifikovanu atmosferu sa 60%CO_2 i 40% N_2 (I grupa), rast ukupnog broja enterobakterija bio je sporiji nego u pastrmci i odrescima šarana upakovanim u modifikovanu atmosferu sa 40%CO_2 i 60%N_2 (II grupa) ili pak u vakuum (III grupa). Ovo se može objasniti antimikrobnim dejstvom ugljen-dioksida, koji je procentualno najzastupljeniji u smeši gasova u koju su upakovani uzorci I grupe kao i činjenicom da ugljen-dioksid deluje inhibitorno pre svega na gram-negativne bakterije, kakvi i jesu mikroorganizmi iz familije Enterobacteriaceae. Najveću stopu rasta imale su enterobakterije u pastrmci i odrescima šarana III grupe tj. u uzorcima upakovanim u vakuum. Ovi rezultati ukazuju na sposobnost Enterobacteriaceae da rastu u anaerobnim uslovima, na temperaturi frižidera u proizvodima upakovanim u vakuum.

Modifikovana atmosfera smanjuje ukupan broj enterobakterija, a najniža vrednost je dobijena kod odrezaka šarana upakovanih u modifikovanu atmosferu sa 60%CO_2 i 40%N_2.

Ključne reči: *Enterobacteriaceae, pastrmka, odresci šarana, modifikovana atmosfera, vakuum*

INTRODUCTION

Spoilage of fish can be defined as any change in fish meat rendering the product unfit for human consumption, and often is result of bacterial growth. Initial contamination by microorganisms depends on numerous factors: type of fish habitat (sea or fresh water, tropical or cold water, open water fish, or fish from coastal area or seabed fish), and on level of water contamination. Type of microflora growing in the product is determined by intrinsic (poikilotherm nature of the fish, pH post mortem, presence of trimetilamine oxyde and other non-protein nitrogen compounds, content of fat) and anthropogenic parameters (storage temperature, procedures during production, packaging, etc.) (Siverstvik et al., 2002).

Bacterial flora of fish that has just been caught is very diverse. The following Gram negative species are present: *Pseudomonas*, *Moraxella*, *Actinetobacter*, *Shewanella*, *Flavobacterium*, *Vibrio, Aeromonas*. The major Gram-positive species are *Micrococcus* and coryneform bacteria.

Bacteria belonging to the family *Enterobacteriaceae* are facultative anaerobes, Gram negative and catalase positive microorganisms. The following genera belong to this family: *Salmonella, Escherichia, Proteus, Shigella, Serratia, Citrobacter, Klebsiella, Enterobacter and Erwinia*, and some of them are major pathogens and causes of numerous diseases in humans, such as septicemia, pneumonia, meningitis, urinary tract infections, diseases of the digestive organs and many other diseases (Govedarica, 1995). They are widespread in the environment, and some are saprophyte inhabitants of the digestive tract.

In 31% of tested samples of fresh water fish, stored at +7˚C in retail stores Lindberg et al. (1998) proved the presence of *Enterobacteriaceae*. Addition of the conservants, vacuum packaging and packaging in modified atmosphere influence the microbial population of foodstuffs (Vasilopoulos et al., 2010). Vacuum packaging supresses the growth of *Psudomonas-Actinetobacter-Moraxella* association, while in contrast, microaerophillic *Enterobacteriaceae* are growing (Radetić et al., 2007). Gram negative bacteria are more sensitive to the effect of CO_2 mainly used in concentrations of 40-60%

for packaging of foodstuffs in modified atmosphere. CO_2 inhibits the growth of microorganisms of spoilage, especially *Pseudomonas spp*, *Enterobacteriacea*, *Acinetobacter spp*., *Achromobacter*, *Flavobacterium* and *Moraxella spp*., as well as psychotrophic bacteria which cause meat spoilage. Arashisar et al. (2004) established that the growth rate of aerobic mesophilic and psychotrophic bacteria, as well as enterobacteria during whole storage period was the lowest in trout fillets packaged in atmosphere with 100%CO_2. Results obtained by Milijašević et al. (2010) show antimicrobial effect of CO_2, where significantly lower count of aerobic mesophilic bacteria and enterobacteria was determined in carp steaks packaged in atmosphere with 100%CO_2 compared to steaks packaged in the gas mixture of 40%CO_2 and 60%N_2.

MATERIAL AND METHODS

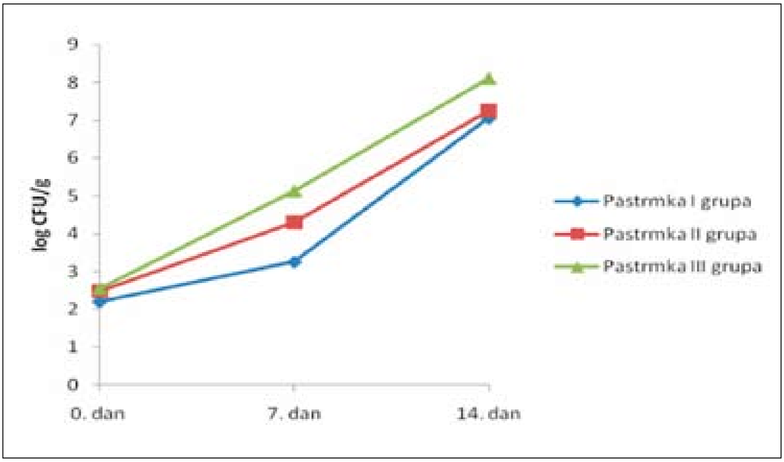
Rainbow trout (*Oncorhynchus mykiss*) used in the experiment was farmed in the same conditions and comes from trout pool located on the slopes of mountain Zlatibor. Fish was transported live from the fish farm to the slaughtering and processing facility, where it was placed in reception pool, and subsequently stunned by using electricity. Slaughtering and evisceration of fish was performed on an automatic apparatus, and carcasses were washed manually, under the stream of water. Marketable carp (*Cyprinus carpio*) originated from the fishpond located in the low land region of Serbia, where semi intensive farming was used. In this experiment, two year old carps of average body weight of 2,5 kg were used. Carps were transported live to the fish slaughtering and processing facility, where they were stunned, slaughtered, scale cleared, and carcass was cut in steaks 2 cm thick. Three sample groups of cleaned trout and carp steaks were formed. First two groups were packaged in modified atmosphere with different gas ratios: 60%CO_2+40%N_2 (I group) and 40%CO_2+60%N_2(II group), whereas the third group of samples were vacuum packaged. The machine used for packaging of samples was Variovac (Variovac Primus, Zarrentin, Germany), and material used for packaging was foil OPA/EVOH/PE (oriented polyamide/etilene vinyl alcohol/polyetilene, Dynopack, Polimoon, Kristiansand, Norway) with low gas permeability (degree of permeability for O_2 – 3,2 cm^3/m^2/day at 23˚C, for N_2 - 1 cm^3/m^2/day at 23˚C, for CO_2 – 14 cm^3/m^2/day at 23˚C and for steam 15 g/m^2/day at 38˚C). Ratio gas : sample in the package was 2:1. All samples were stored in the same conditions at the temperature of +3˚C and on 0, 7. and 14. day of storage, microbiological testing was performed.

Microbiological testing

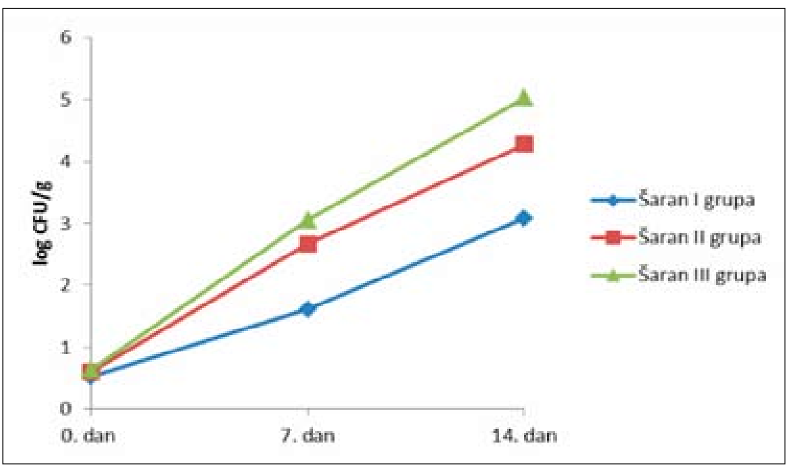
Number of bacteria of the family *Enterobacteriaceae* was determined according to ISO 21528 -2:2004. Medium used for detection and enumeration of bacteria of the family *Enterobacteriaceae* was VRBD (violet red bile glucose agar).

RESULTS AND DISCUSSION

During storing of packaged carp steaks and trout at the temperature of +3˚C, in duration of fourteen days, there was a statistically significant increase of total enterobacteriaceae count in all tested sample groups. In trout and carp steaks packaged in modified atmosphere with 60%CO_2 and 40% N_2 (I group), growth of total enterobacteriaceae count was slower than in trout and carp steaks packaged in the modified atmosphere with 40%CO_2 and 60%N_2 (II group) or vacuum packaged samples (III group) (Graph 1. and 2.). This can be explained by antimicrobial effect of carbon dioxide which was most present in percentages in gas mixture used for samples of group I, as well as the fact that carbon dioxide has inhibitory effect primarily on Gram negative bacteria, such as the microorganisms of the family *Enterobacteriaceae*. The highest growth rate was established for enterobacteriaceae in trout and carp steaks of group III, i.e. vacuum packaged samples. These results show the ability of *Enterobacteriaceae* to grow in anaerobic conditions, at fridge temperature in the vacuum packaged products. Researches by other authors relating to the testing of the enterobacteriaceae count in fresh fish packaged in the modified atmosphere (Arashisar et al., 2004; Milijašević et al., 2010; Torrieri et al., 2006.) are in accordance with our results.



Graph 1. Change of the total enterobacteriaceae count I, II and III group of trout samples



Graph 2. Change of the total enterobacteriaceae count I, II and III group of carp steak samples

CONCLUSION

Based on research results it was concluded that total enterobacteriaceae count in all three tested groups of trout was statistically significantly higher compared to enterobacteriaceae count recorded in carp steaks packaged and stored in same conditions. The lowest growth rate was established in case of enterobacteriaceae in trout and carp steaks of the group I, i.e. samples packaged in the modified atmosphere with 60% CO_2 and 40% N_2.

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THE EFFECT OF ADDING ZEOLITE ON TROUT MEAT QUALITY

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UTICAJ DODAVANJA ZEOLITA NA KVALITET MESA PASTRMKE

Abstrakt

U radu je ispitivan uticaj zeolita tipa “Minazel” kao aditiva hrane za pastrmke primenjenog u koncentraciji od 1% i 2% na sledeće proizvodne parametre: osnovni hemijski sastav mesa, mikotoksikološku ispravnost, senzorne osobine, randman i koncentraciju pojedinih makro i mikroelemenata.

Primenjene koncentracije zeolita, kao aditiva hrane nisu uticale na hemijski sastav pastrmskog mesa, koncentraciju minerala (Ca, Cu, Zn, Pb i Mn) i njegove senzorne osobine. Izvesni stepen pozitivnog uticaja ispoljen je u pogledu randmana mesa i prosečne telesne mase pastrmki posle ezenteracije.

Ključne reči: *zeolit, kalifornijska pastrmka, meso, kvalitet, minerali*

INTRODUCTION

Meat fish, especially salmonids is a very respected nutritional food which is characterized by a favorable biologically valuable protein, low fat content and high content of vitamins and minerals.The above nutritional characteristics make it highly valuable nutritional food product that is used not only in the diet of healthy individuals, but as in the diet convalescents purposes. Meat quality is a set of several features of which the most important are: organoleptic properties (appearance, texture, color, smell and taste), chemical properties and hygienic-toxicological safety (Baltić et al. 1997). Meat quality of trout and other fish species are influenced by numerous factors specific to each pond or a natural ecosystem (Spinelli,1979). The main differences between the meat quality of fish and domestic animals are the percentage of edible parts in pigs is 71%, 55% broiler and 52% of carp and trout 61%. The protein content in meat of pigs is about 9%, 11% of broiler chickens, carp 16% and 19% of trout. Also, fish meat is one of the richest sources of minerals, and especially phosphorus, resulting its biological value is considerably higher than the meat of warm-blooded animals. Significantly, the fish oil containing over 50% unsaturated fatty acids, and carbohydrate content in meat of fish is negligible, and represents an indispensable dietary food (Huisman,1979; Steffens, 1980; Rašeta et al.,1984, Milinković,1986; Baltić et al.,1997). Modern concepts of nutrition in intensive aquacultural production are based on the use of various additives to achieve maximum performance results. Proceeding from this,it is accessed in this paper studies the influence of zeolite type Minazel, in addition to food on the main parameters of meat quality rainbow trout.

MATERIAL AND METHODS

The study was conducted at the trout pond in Gornja Trešnjica during the period of 150 days on 24.540 fish which were divided into five groups: one control and four experimental groups of 4908 fish in each pool. Initial density of plantation was 98 fish/m^3, or 86 fish per m^2 of water surface. Formed groups of fish were fed with dry pelleted food of domestic origin, and the pellet size and number of meals during the test was determined by previously given to food tables (Phillips, 1970).

Co-group of fish was fed with pellets without the addition of zeolite, while the experimental group of fish O-I and O-II offered feed supplemented with 1% zeolite, and the experimental groups O-III and O-IV were fed with pellets supplemented with 2% zeolite. Determination of the meat quality of fish was carried out at the end of the experiment, and applied to the determination of sensory properties (appearance, color, smell and taste), chemical composition (protein, fat, ash, water and minerals), and setting mucotoxicological safety of meat, at the end experiment. All tests were performed on samples from 20 fasted fish per treatment, after 24-hour cooling meat at 4^{0}C. The chemical composition of meat was analyzed using standard methods of testing (SRPS ISO). Total water content was determined by drying samples to constant weight, crude ash by incineration and annealing the sample at 500^{0}C to 600^{0}C, total protein by Kjeldahl method in the basis of nitrogen content, total fat by extraction by Soxlett in the pre-drying the sample, microminerals (Cu , Mn, Zn and Pb) by atomic absorption spectrophotometry AAS, and Ca spectrophotometrically. For evaluation of sensory properties of meat,thus acceptability of meat fish, was used method called Rang test (Baltić, 1994). Determination of residues of mycotoxins in fish meat was carried out by the method of thin-layer chromatography (Balzer et al., 1978).

The results of the experiment are grouped into appropriate series and statistically analyzed on a computer using the usual mathematical - statistical procedures that include variance analysis and evaluation of the significance of the results obtained (the difference) using the test called Tukey honest significant difference test.

RESULTS AND DISCUSSION

The data listed in Table 1 indicate closer to the chemical composition of meat of fish examined. Based on the chemical composition shown it can be concluded that the average water content of the meat was very constant and ranged from 76,71% to 76,91% in the experimental group of fish whose food is treated with zeolite and the Co-group which was not used zeolite as a feed supplement to 76,74%.

A similar trend of relative uniformity between the groups was found regarding the concentrations of protein in meat, whose average content ranged from 19,00% (0-I group) to 19,26% (0-IV group). The average body fat content of trout was also uniform and ranged from 2,58% (0-IV group) to 2,70% (0-I group). The average ash content in the tested meat samples, was also constant and varied from 1,8% (0-II group) to 1,43% (0-III group).

The results of the basic chemical composition of meat of fish in this experiment are consistent with the findings of most other authors. According to their research, the water content in meat of trout ranges from 74,18% to 79%, then the protein content of 19,20% to 21,31%, fat content of 0,50% to 4,00% and ash content of 0,40% to 1,80% (Francetić, 1967; Peters, 1980; Vukašinović et al., 1989; Rašeta et al., 1994; Veljković et al.,1995; Hristić et al., 1996; Baltić et al., 1997).

Table 1. The average chemical composition of meat of trout at the end of the experiment, (%)

PARAMETER %	GROUP				
	Co	**0-I** (1% zeolite)	**0-II** (1% zeolite)	**0-III** (2% zeolite)	**0-IV** (2% zeolite)
Total protein	19,20	19,00	19,10	19,26	19,18
Total Fat	2,67	2,70	2,63	2,60	2,58
Ash	1,39	1,39	1,38	1,43	1,41
Water	76,74	76,91	76,89	76,71	76,83
Dry matter	23,26	23,09	23,11	23,29	23,17

Analyzing the chemical indicators of meat quality studied fish, it can be concluded that, despite the existence of minimal differences in the numerical values set forth by the difference from the standpoint of statistical analysis were not significant ($p>0,05$). Different dietary treatments did not affect the zeolite water content, protein, fat and ash in meat trout, which is consistent with research by Veljović et.al., (1998). By mycotoxicological analysis of meat there was not found the residues presence of mycotoxins of the meat sample.

Table 2. Average values of mineral content in meat trout, (mg/kg)

PARAMETER mg/kg	GROUP				
	Co	**0-I** (1% zeolite)	**0-II** (1% zeolite)	**0-III** (2% zeolite)	**0-IV** (2% zeolite)
Copper,Cu	21,20	23,00	22,50	20,90	2180
Manganese,Mn	56,20	63,50	59,0	54,6	61,0
Zinc,Zn	112,0	108,5	121,0	116,0	110,0
Lead,Pb	0,025	0,010	0,045	0,021	0,039
Calcium,Ca	28,0	19,5	32,0	24,0	26,5

Mean values of certain minerals in the meat of the trout examined are shown in Table 2. Based on these data, we can see considerable uniformity in terms of calcium content in meat and microminerals of rainbow trout. Cu content ranged from 20,90 (O-III) to 23,0 mg/kg (O-I), the concentration of Mn ranged from 54,60 (O-III) to 61,0 m /kg (O-IV), then Zn from 110,0 (O-IV) to 121,0 mg / kg (O-II), then Pb from 0.010 (O-I) to 0,045 mg/kg (O-II) and at the end Ca from 19,5 (O-I) to 32,0 mg / kg (O-II).

The results obtained in terms of copper, manganese, zinc, lead and calcium in the meat of the trout were in the normal reference framework for this type of fish (Brown et al., 1977; Vukašinović et al., 1989; NRC, 1991; Baltić et al.,1997; Vukićević,1998). Statistical analysis revealed no significant differences, both between treated groups and between control groups and other groups of fish ($p>0,05$).

A very important indicator of the quality of meat fish, other than the chemical composition and its sensory properties were investigated in this experiment by method Rang test or meat acceptability by consumers. The fish meat samples in all experimental groups assessed the acceptability, and the results are shown in Table 3.

Table 3. "Rang testa" results acceptability of meat trout at the end of experiment

GROUP		GROUP				
		Co	**0-I** (1% zeolite)	**0-II** (1% zeolite)	**0-III** (2% zeolite)	**0-IV** (2% zeolite)
TOTAL		**109**	**109**	**107**	**105**	**106**
DIFFERENCE	**Ko**	-	-	2	4	3
	0-I	-	-	2	4	3
	0-II	-	-	-	2	1
	0-II	-	-	-	-	1
	0-IV	-	-	-	-	-

From the results (Table 3) we can see a great match acceptability sensory properties of fish meat between two groups. According to Veljovic et al. (1998), the addition of zeolite to trout food at a concentration of 0,5% had no effect on sensory properties and chemical composition of meat trout. Slightly higher values of Rank test, made in Co-group and 0-I group could be attributed to slightly higher fat content in meat of fish of these groups, as some researchers reported a higher fat content in the body of fish, conditions, and better sensory properties acceptability of meat fish, or better values of Rank test (Spinelli, 1979; Nose,1979; Hebber et al., 1979; Plavša et al. ,2000). Based on the results of Rank test, obtained in this research, can be concluded that the application of zeolite as a food additive had no effect on the sensory properties of meat of fish examined.

Table 4. The average body weight of trout (g) with the dressing percentage (%)

PARAMETER	GROUP				
	Co	0-I	0-II	0-III	0-IV
Body weight of uncleaned fish, g	239,51	260,15	266,24	246,94	250,86
Body weight of the cleaned fish, g	208,00	226,20	231,99	214,59	218,10
Weight womb,%	13,15	13,05	12,86	13,10	13,06
Dressing percentage (%)	86,84	86,95	87,13	86,90	86,94

Data on average body weight of trout before and after the actual ezenteracion dressing percentage, are shown in Table 4. Based on the established results can be seen that the highest body mass of cleaned fish had 0-II group (231,99 g), which is understandable, considering that this group had the highest average body weight before ezenteracion (266,24 g). The lowest final body weight had the fish Co-group (239,51 g), in line with the lowest yield and body weight after ezenteracion (208,00 g). Analysis of data (Table 4) showed that fish 0-II group, made the best dressing percentage of 87,13%, followed by 0-I group with dressing percentage of 86,95%, then 0-IV group with 86,94 %, 0-III group with 86,90% and at the end the Co-group which has achieved the lowest dressing percentage of 86,84%.

Based on test results yield, as a very important factor of meat quality trout conclusions can be drawn that the fish whose food containing zeolite scored better yield compared to the fish co-group. Considering that the applied zeolite content in food of 1% results in achieving the best value meat yield of fish, the same concentration of zeolite is shown in this study as the most optimal dose for improving yield of meat trout.

CONCLUSION

Analyzed the quality of meat for consumption of trout in this experiment indicated that the applied zeolite concentration (1% or 2%) as a food additive, did not affect the basic chemical composition and content of the studied minerals in meat of fish. The numerical values of moisture, protein, fat, ash and minerals in meat of fish of all experimental groups were very equal in the absence of significant differences ($p>0,05$). The recorded values of measured parameters of the chemical and mineral composition of meat trout ranged in normal physiological limits for these indicators. Mycotoxicological analysis examined samples of fish meat, was not found the presence residues of mycotoxins. Zeolite as a food additive, used in different concentrations did not affect the sensory properties of meat trout.

Addition of the tested food additives contribute to the achievement of greater body mass of 231,99 g of fish 0-II groups which were given the zeolite pellets at a concentration of 1%, compared to fish fed the Co-group without zeolite, with an average body weight of fish was 208,00 g. The average yield of treated and cold carcasses ready to roast was the best at fish 0-II group (with the addition of 1% zeolite in feed) 87,13%, and the worst in fish co-group (without addition of zeolite in feed) 86,84%.

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COULD COMMERCIAL FISHERMEN INFLUENCE STATE DECISION IN THE REPUBLIC OF SERBIA?

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MOGU LI KOMERCIJALNI RIBARI DA UTIČU NA DONOSIOCE ODLUKA U REPUBLICI SRBIJI?

Abstrakt

Formiranje cena primarnih proizvoda po pravilu nije potpuno slobodno već država u većoj ili manjoj meri vrši uticaj, što je praksa u većini zemalja u svetu. Svako mešanje države u slobodno formiranje cena po pravilu donosi gubitak blagostanja. Postavlja se pitanje razloga za ovakvo delovanje države. Navedenim pristupom država u povoljniji odnosno nepovoljniji položaj stavlja pojedince i određene grupe. Očigledno je da se države svesno odriču dela blagostanja da bi postigle neke druge ciljeve. Posebno je važan način na koji se određuje koja će grupa biti privilegovana a koja oštećena.

Osnovno pitanje koje se pri tome postavlja se odnosi na način donošenja odluka. U objašnjenju načina donošenja odluka koriste se dva pristupa, i to: normativni pristup i pristup Nove političke ekonomije.

Normativni pristup polazi od pretpostavke da se svaka odluka orijentiše prema datoj ciljnoj funkciji i ciljnim ograničenjima. Normativna analiza se bavi temom formiranja cena proizvoda primarnog sektora u uslovima postojanja opšteg cilja društva, kao i efektima pojedinih državnih mera na ukupnu privredu. Prema ovom pristupu političar niti mora da se pridržava svojih obećanja od juče, niti mora da vodi računa o vremenskoj konzistentnosti svojih odluka. Umesto toga, političar u skladu sa normativnim pristupom pokušava da u svakom momentu maksimira društveno blagostanje.

Osnovna hipoteza od koje se polazi glasi da svi koji utiču na donošenje političkih odluka pokušavaju da realizuju lične ciljeve u okviru svojih mogućnosti. Donošenje političkih odluka se može uporediti sa igrom. Tok i rezultat igre zavise sa jedne strane od igrača, a sa druge strane od pravila igre. Igrači su politički akteri koji mogu biti nosioci politike prema tradicionalnoj definiciji, ali takođe mogu biti i organizacije i osobe koje utiču na tok i rezultat politike. Pravila igre su jednim delom utvrđena zakonima, a delimično proizilaze i iz nepisanih pravila ponašanja aktera.

Za političare se pretpostavlja da se oni moraju podvrgavati ponovnom izboru u redovnim vremenskim razmacima. Time se objašnjava proces donošenja odluka u demokratskom sistemu. Pretpostavlja se da je cilj političara da maksimiraju dohodak, lični ugled i moć i da oni ove ciljeve najbolje mogu da dostignu obavljanjem neke političke funkcije. Da li bi političari nekim alternativnim zaposlenjem mogli da ostvare veći dohodak, zavisi od uređenja političkog sistema. Ako se, međutim, pođe od nealtruističkog načina ponašanja, političari su preuzimanjem funkcije obelodanili svoje preferencije. Zbog toga se može pretpostaviti da se dohodak, moć, lični ugled i druge varijable koje mogu da ulaze u njihove ciljne funkcije bolje mogu realizovati obavljanjem političke funkcije nego neke alternativne delatnosti.

Moć neke grupe određena je između ostalog i njenom veličinom i organizovanošću. Po pravilu veličina grupe i njena organizovanost su negativno korelirane. Primarni proizvođači u Srbiji zbog svoje brojnosti potencijalno predstavljaju značajnu interesnu grupu koju bi političari trebalo da uvažavaju. Ribari u Republici Srbiji su mala grupa koja bi mogla uticati na donošenje odluka koje su vezane za sektor ribarstva. Ovaj rad ima za cilj da objasni odnose političara prema pojedinim interesnim grupama, načine na koje grupe mogu ostvariti uticaj na političare i donosioce odluka u pogledu formiranja cena, kao i položaj ribara kao male grupe birača.

Ključne reči: *ribarstvo, formiranje cena, interesne grupe, donošenje odluka*

INTRODUCTION

In principle price setting of primary products in Serbia is free, but there are state regulations that influence price level, too. The similar situation exists in most countries in the world. Economists point out that this practice of price setting has negative welfare effects. Even though the fact that there are negative effects of state measures on price setting of primary products, society and politicians accept such measures.

If we assume that society and politicians do not consider welfare differently from economists, then it can be assumed that the politicians by decision making are not oriented towards the general economic effects on welfare.

The main issue refers to the question on decisions making. In explaining this phenomenon two approaches are used, namely: a normative approach and new political economy approach.

The normative approach assumes that every decision is oriented towards a given goal and that there are limitations of target function. Normative analysis is focused on price setting of products assuming there is a general goal of society, as well as on the effects of certain government measures on the overall economy. According to this approach, nor a politician has to comply with its promises, or has to care about the time consistency of their decisions. Instead, the politician in accordance with the normative approach every moment is trying to maximize the social welfare.

From the new political economy method it could be concluded that there are no general rules applicable to price setting for all products of primary sector, including fishery. Both, stakeholders and institutions are specific for every country and for the given time. Therefore, stakeholders and the rules for each particular country have to be defined. In addition, one should keep in mind that decisions depend on the time. Decisions in the past have had created a situation that have consequences for decision making in the present. This approach of the new political economy differs from the traditional normative policy analysis.

The new political economy approach use tools of economic theory in analysis of political decisions. This method is used to explain the principle of price setting in the primary sectors in the Republic of Serbia. The primary sector in this paper includes agriculture, hunting, forestry, water management and fishery.

MATERIAL AND METHODS

The basic hypothesis is that all those who influence decision making are trying to achieve their personal goals in given situation and with given personal abilities. Political decisions can be compared to the game. The course and outcome of a game depends on one hand of the player, and on the other hand of the rules of the game.

According to the traditional definition players are politicians. However, players could be organizations and individuals who influence the course and result of the policy making.

Game rules are in part determined by the laws, and partially resulting from the unwritten participants' behaviour rules. Each rule, written or unwritten, limits the freedom of the participants' behaviour.

According to the new political economy method individual participant behaviour is entirely comparable to the entrepreneur who maximizes its profits with existing restrictions or to the household that maximizes its benefits in terms of the given goal and limits. This comparison of individual and entrepreneur or households appears completely rational. It is important to emphasize that there are entrepreneurs who renounce profit in order to achieve social goals. However, this research used the basic hypothesis that the politicians want to maximize their own personal benefit.

RESULTS AND DISCUSSION

According to Tyers and Anderson (Tyers & Anderson, 1992) in the price setting of primary products, key players are these three groups, politicians, interested parties and consumers. The main hypothesis of those authors is that all groups behave rationally and try to maximize their goal functions. However, in achieving the goals there are several restrictions, of which the main are asymmetric information of the groups in the game.

For politicians it is assumed that they have to be re-elected at regular time periods. This explains the decision making process within a democratic system. It is assumed that the aim of politicians is to maximize profit, personal reputation and power. These goals they can achieve through certain political function. Whether politicians with certain alternative employment are able to achieve higher profit depends of the political system. If, however, we start from the not altruistic behaviour, politicians through taking over their functions make public their preferences. Therefore, it is assumed that profit, power, personal prestige and other variables that may go into their objective functions can be better realized by performing political functions rather than some alternative activity. In the Republic of Serbia many examples for this could be found. For example a politician due to his position has specific information and shares it with some companies for any kind of compensation.

From the target function and limitations it can be concluded that politicians will try to maximize the number of votes at the next election. If all voters would be perfectly informed and if they would behave rationally each election would shows their preferences. In this situation the politician that increases welfare of the most voters would be elected. However, politicians as candidates on elections know that voters do not have perfect information. Therefore, politicians by favouring particular groups could turn the election result to their favour.

According to Downs it could be noticed that a democratically elected government takes stronger into account the interests of consumers rather than producers, because producers are better informed about the benefit done by politicians than consumers of negative effects.

In developing countries there is a trend of evident taxation of primary producers to benefit of consumers. Therefore, only the basic Downs statement can be taken that protection or taxation of primary producers depends on power of key players on the **political market**. This revised view is fully applicable for the Republic of Serbia.

As a rule politicians do offer protection to some stakeholders. They, however, have to take into account that with the realization of certain level of protection will have to accept certain losses. For example, consumers could punish politicians by nonparticipation in voting or choosing another party. The relative index of protection is defined as the effective coefficient of protection for primary relative to a given effective coefficient of protection of industrial sector. Index higher than one, indicates that government measures are effectively subsidizing primary in relation to the industrial.

Potential beneficiaries of protection of primary sector in industrialized countries are producers, while potential beneficiaries of taxation of primary sectors in developing countries are consumers. How we could explain this situation?

In developing countries primary producers are neither well informed nor well-organized. Therefore it seems obvious that in these countries self-interested politicians are less occupied with granting privileges to this group. More important is group of consumers, who are better informed and better organized. Therefore, the politicians according to this hypothesis tend to taxing producers for the benefit of consumers. The protection is negative, i.e. the ratio of protection is less than one.

In most industrial countries primary producers are relatively homogeneous group of voters. Political parties are therefore often attempting to address this group of voters. However, surprisingly the parties, which usually receive a few votes from this group, take into account this group of voters during designing their politics or their programs. This is because of fact that parties which primary producers do not vote hope that this group could change opinion or the group could boycott election what would be crucial for election results. History of the European Union shows that primary producers, especially in election years enjoy special privileges. On the contrary, in developing countries primary producers have less importance as a voting group. Although in these countries, more people work in primary sector and most of them are poor and inadequately organized. Therefore, voters in these countries could impose less in politics than in industrial countries.

According to Olson's voter importance depend on two factors: the size of the group and its organizational strength and there is a negative correlation between these two factors. The bigger the group is, more difficult is to organize. The organizational strength of a group depends on the benefits that individual member could expect from membership and perhaps depends on active participation. If individual group member benefits from group activities even when he is not a member, his affinity to act as a "**free rider**" is high. Although the activities of the group are appreciated, there is no willingness to pay for it. Membership in a group can be more attractive if the members receive **selective stimulants** or if there is direct connection between membership and benefit.

Selective stimulants could exist for example by providing important information only to members of the group or with special extension services.

In our case a union of fisherman for example can be strengthened by non-transparent, protectionist state policy. The great need for information of individual member cannot be satisfied in any other way than through membership in union.

The principle of "free rider" is evident for Serbian larger group of primary producers, as the right to receive state subsidies has each member regardless of membership in associations.

From Olson statements it cannot be concluded that the political significance of a particular group increases with decreasing size of the group. Group size and organizational strength play a major role. In the Republic of Serbia this rule applies and hypothesis that primary producers are not organized as a voting group is realistic (Koester & Zaric, 2009).

Typically primary producers accomplish their own interests rather through co-operation with interest groups outside primary sectors than with other primary producers. In elections primary producers in Serbia usually vote for politicians who do not necessarily take into account the primary producers welfare.

Anderson and Hayami have analysed the correlation between group size and its political importance for several countries. They came to the conclusion that the political ability of group to impose their interests is falling when their share in total employment in economy falls below 5 %. However, these authors pointed out, that this rule is not applicable for every country and every time. The power of the group depends of the existing state policy. In the case the policy is not in favour of the group it could lead, to an increase of group members. Type of organization is important as well, while one central association will, for example, have more influence than the series of different associations. From this finding we could conclude that Serbian fishermen have a chance to influence government decisions.

CONCLUSION

In the Republic of Serbia the state regulations influence price level of primary products. The basic quotations are welfare effects. In the analysis of effects basically two methods are applicable, normative and new political economy approach. According to the new political economy approach political decisions can be compared to the game. The outcome of a game depends of the player on one hand and of the rules of the game on the other.

Fishermen in Serbia are small group which could be well organised and in which each member would have a benefit. However, in the past as voters primary producers did not take decisions which would lead to the positive welfare effects for them. Therefore by creating regulation that are related to the fishery fishermen' opinion was usually not taken into account.

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EFFECT OF AGREEMENT ON STABILIZATION AND ASSOCIATION OF IMPORT AND EXPORT OF FISH AND FISH PRODUCTS

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UTICAJ SPORAZUMA O STABILIZACIJI I PRIDRUŽIVANJU NA UVOZ I IZVOZ RIBE I PROIZVODA OD RIBE

Abstrakt

Sporazum o stabilizaciji i pridruživanju (SSP) je međunarodni ugovor, potpisan 29. aprila 2008. godine između Republike Srbije i Evropske unije (EU). Dve najvažnije obaveze koje Republika Srbija preuzimaovim sporazumom biće uspostavljanje zone slobodne trgovine i usklađivanje zakonodavstva Republike Srbije sa pravom EU. Sporazumom se stvara zona slobodne trgovine između Srbije i EU u prelaznom periodu od šest godina. Rok za liberalizaciju trgovine je određen u skladu sa sposobnošću srpske industrije i poljoprivrede da se prilagode slobodnoj trgovini ali i sa željom Srbije za što bržim završetkom reformi i pristupanjem Evropskoj uniji. Obaveza Srbije se sastoji u postepenom ukidanju carina na uvoz robe poreklom iz Evropske unije u prelaznom periodu. Sa druge strane, Evropska unija ovim ugovorom potvrđuje slobodan pristup robi iz Srbije tržištu Evropske unije. Tempo liberalizacije i stepen zaštite zavisi od stepena osetljivosti proizvoda za industriju Srbije. Definisane su tri grupe indistrijskih proizvoda, prema osetljivosti, za koje će liberalizacija biti ostvarena nakon perioda od dve, pet odnosno šest godina. Za proizvode koji se ne nalaze na ovim listama carine će biti ukinute momentom stupanja na snagu sporazuma. Sporazumom o stabilizaciji i pridruživanju predviđa se i obaveza Republike Srbije da u dogovorenim rokovima uskladi domaće zakonodavstvo sa propisima koji spadaju u pravne tekovine EU. S obzirom na obim pravnih tekovina EU određena su prioritetna područja koja imaju direktan uticaj na stvaranje zone slobodne trgovine između EU i Srbije: zaštita konkurencije i kontrola dodele državnih pomoći (subvencija), pravo intelektualne svojine, javne nabavke, standardizacija i zaštita potrošača.

Narodna skupština Republike Srbije 9. septembra 2008. godine ratifikovala je Sporazum o stabilizaciji i pridruživanju (SSP) i **Prelazni trgovinski sporazum**. SSP i Prelazni sporazum potpisani su 29. aprila 2008.godine u Briselu. Od 1. februara 2009. Srbija jednostrano primenjuje prelazni trgovinski sporazum.

Zona slobodne trgovine

Zona slobodne trgovine podrazumeva trgovinu bez carina i količinskih ograničenja osim u slučaju koji su odobreni ovim Sporazumom. Sporazum predviđa da se sva ograničenja izvoza u EU ukinu odmah (uz pojedine izuzetke), dok se uvoz liberalizuje u roku od 5 godine. U tom roku će se fazno smanjivati carine industrijskih proizvoda, tako da u šestoj godini budu nula, dok će neki poljoprivredni proizvodi ostati sa određenom, ali znatno manjom carinskom zaštitom.

Svi industrijski proizvodi su podeljeni u 4 grupe: neosetljivi, osetljivi, veoma osetljivi i najosetljiviji proizvodi i svaka grupa ima svoju dinamiku liberalizacije. Grupisanje je vršeno prema stepenu dosadašnje carinske zaštite, ekonomskim, fiskalnim i socijalnim efektima liberalizacije, značaju sektora i sl. S druge strane za svaku tarifnu liniju poljoprivrednih proizvoda važi sopstvena dinamika i „pod" liberalizacije.

Damping i subvencije

Nijedna odredba ovog sporazuma ne sprečava bilo koju od strana da preduzme odbrambene trgovinske mere u skladu sa stavom 2. ovog člana i članom 41.

Ukupna vrednost izvoza ribe i proizvoda od ribe u poslednjih nekoliko godina je u porastu. U 2006 godini iznosila je 863.000 dolara, a 2010. godine 1.053.000 dolara. Izvoz ribe potencira proizvodnju i neophodno je stvoriti uslove da se dosadašnji minorni izvoz poveća. U vezi sa ovim posebno je važno određenim sistemskim merama ekonomske politike stvoriti uslove za aktiviranje izvoza ribe i proizvoda od ribe. Izvoz je skup posao i neophodna je pomoć držve. Potencijalni izvoznici suočavaju se sa nizom problema od kojih je i to da Vlada Republike Srbije prema Uredbi o korišćenju sredstava za podsticaj izvoza poljoprivrednih i prehrambenih proizvoda nije obuhvatila ribu i prerađevine od ribe.

Jednostranom primenom SSPa u poslednje četiri godine došlo je do povećanja zastupljenosti izvoza u zemlje EU sa 9% na 29%.

Uvoz iz zemalja EU za sada nema drastičnih promena u odnosu na ukupni uvoz i nalazi se u intervalu od 18-20%. Proizilazi da jednostrana primena SSP-a nije donela očekivane promene.

Sporazum o stabilizaciji i pridruživanju će svoje prave efekte doneti tek ratifikovanjem svih država članica. Oblast ribarstva je svrstana u osetljivu oblast i zaštita domaće proizvodnje je na očekivanom nivou. Međutim i dalje će biti prisutan problem konkurentnosti naših proizvoda na tržištu EU jer ribarstvo Srbije nije potpomognuto od srane države. Srbiji je neophodna uprava za ribarstvo i pripadajući fond za razvoj ribarstva kao i povoljni krediti koji će pomoći proizvođače ribe da ravnopravno nastupaju na tržištu EU. Mogućnosti za izgradnju novih toplovodnih ribnjaka su veoma velike jer postoje dovoljne količine vode prihvatljivog kvaliteta, a takođe i zemljište koje se ne koristi za ratarsku proizvodnju i može se kupiti reltivno povoljno

Ključne reči: *Sporazum o stabilizaciji i pridruživanju, riba, uvoz, izvoz*

INTRODUCTION

The Stabilisation and Association Agreement (SAA) is an international treaty, signed on the 29th April 2008 between the Republic of Serbia and the European Union (EU). Two most important obligations for the Republic of Serbia will be establishment of free trade and harmonization of legislation of the Republic of Serbia with the EU. The agreement creates a free trade between Serbia and the EU for a transitional period of six years. The deadline for the liberalization of trade is determined in accordance with the capacity of Serbian industry and agriculture to adapt to free trade, but also with Serbia's desire for faster completion of reforms and accession to the European Union. Serbia's obligation consists in the gradual abolition of tariffs on imported goods originating in the EU for a transitional period. On the other hand, the European Union confirms with this agreement the free access of goods from Serbia to the EU market. The pace of liberalization and the degree of protection depends on the degree of sensitivity of products for industry of Serbia. Three groups of industrial products were defined based on sensitivity, for which liberalization will be achieved after a period of two, five or six years. For products that are not on the lists duty will be abolished at the moment of entry into force of agreement. SAA also foresees the obligation of the Republic of Serbia to harmonize domestic legislation according to the agreed timetable with the regulations that fall under the EU acquis. The priority areas that have direct impact on creating free trade zone between the EU and Serbia were determinated in relation to the scope of EU legislation: protection of competition and control allocation of state subsidies, intellectual property rights, public procurement, standardization and consumer protection.

Serbian parliament ratified on 9th September 2008 the Stabilization and Association Agreement (SAA) and **Interim Agreement**. SAA and the Interim Agreement were signed on 29th April 2008 in Brussels. From the 1st of February 2009 Serbia is unilaterally implementing the Interim Trade Agreement.

With ratification of the Stabilization and Association Agreement (SAA) Serbia became an associate member of the European Union (not an EU member state!). So far, the SAA has been ratified by 17 EU member states and the European Parliament and it is expected that further 10 EU member states will ratify this Agreement. Although the content of this agreement is mixed, it is essentially a trade agreement because the greatest effects are made by creating free trade zone, that is being created through gradual elimination of all tariffs on industrial goods and almost all tariffs on agricultural products, and gradual removal of non-tariff barriers to trade. In this sense, it is important that **the agreement is indefinite**.

Free trade agreement

The free trade means trade without custom duties and quantitative restrictions except in cases authorized by this agreement. The agreement provides that all restrictions on exports to the EU are immediately abolished (with some exceptions), while imports are to be liberalized within 5 years. Within this period tariffs of industrial products will be reduced in stages, so that in the sixth year they are zero, while some agricultural products will remain with a certain tariff protection, but significantly smaller.

All industrial products are divided into four groups: insensitive, sensitive, very sensitive and the most sensitive products, and each group has its own dynamics of liberalization. The grouping was done according to the current level of tariff protection, economic, fiscal and social effects of liberalization, the importance of the sector, etc.

On the other hand, to each tariff line of agricultural products its own dynamics and the bottom liberalization are applied.

Article 33

Protection of geographical indications for agricultural, fish and food products, with the exception of wine and spirits

1. Serbia shall ensure the protection of appellations of origin community registered in the Community by Council Regulation (EC) No. 510/2006 from 20^{th} March 2006 on the protection of geographical indications and designation of origin for agricultural products and food in accordance with the provisions of this article. Geographical indications from Serbia will be able to get registered in the Community under the terms of the above-mentioned regulation.

Dumping and Subsidies

1. Nothing in this Agreement shall prevent any party to take defensive trade measures in accordance with paragraph 2 of this article and article 41.
2. If one of the parties establishes that the trade with the other side implements dumping and/or subsidies on the basis of which it is possible to introduce compensatory measures, that party may take appropriate measures against this practice in accordance with the WTO Agreement on Implementation of Article VI of GATT 1994.

EU CONCESSIONS THE SERBIAN FISH PRODUCTS

Export the following products originating in Serbia to the EU will be subject to these concessions.

Tariff code	Nomination	Since the Agreement entry into force to 31 December of the same year (n)	From 1 January to 31 December (n +1)	For each subsequent year from 01 January to 31 December
0301 91 10 0301 91 90 0302 11 10 0302 11 20 0302 11 80 ex 0304 19 911 0304 29 15	Trout (*Salmo trutta, Oncorhynchus mykiss, Oncorhynchus clarki,* alive, fresh or chilled;	Quota: 15 tons at a rate 0% Over the Q: 90% MFN rates	Quota: 15 tons at a rate 0% Over the Q: 80% MFN rates	Quota: 15 tons at a rate 0% Over the Q: 70% MFN rates
0301 93 00 0302 69 11 0303 79 11 ex 0304 19 19 ex 0304 19 91	Carp: live, fresh or chilled, frozen, dried, salted , smoked, fillets and other fish	Quota: 60 tons at a rate 0% Over the Q: 90% MFN rates	Quota: 60 tons at a rate 0% Over the Q: 80% MFN rates	Quota:60 tons at a rate 0% Over the Q: 70% MFN rates

CONCESSION ON SERBIAN FISH PRODUCTS ORIGINATING IN THE EU

Imports of the following products to Serbia originating in the Community is subject to the following concessions.

It is characteristic that the fishery has a share of below 10 % in the GDP of agriculture and fisheries activities (Marković and Jovanović, 2010). There are large possibilities for our country to develop its fisheries. Domestic production of fish is inadequate, despite the favorable bio-ecological characteristics of our region (Miščević, 2006).

Serbia and Montenegro participate with 0.05% in the European production of fishery products. They are at the bottom of the European scale (28th place in Europe) with more production than Belarus and less than Romania (Vlahović, 2003). For a period of ten years (1993-2003) the production recorded an increase of 9.2%.

Tariff code	Nomination	Tariff rate (% of MFN)					
		2008	2009	2010	2011	2012	2013th and years to come
0301	Fish, alive:						
	Other live fish:						
0301 91	Trout (*Salmo trutta, Oncorhynchus mykiss,*						
0301 91 90	Other	90	75	60	40	20	0
0301 92 00	Eel (*Anguilla spp.*)	90	75	60	40	20	0
0301 93 00	Carp	90	85	80	75	65	60
0301 99	Other:						
	Freshwater fish :						
0301 99 19	Other	90	75	60	40	20	0
0302	Fish, fresh or chilled, excluding fish fillets and other fish meat of tariff code 0304:						
	Salmonidae, excluding livers and roes:						
0302 11	Trout (*Salmo trutta, Oncorhynchus mykiss,*	90	75	60	40	20	0
0302 69	Other:						
	Freshwater fish:						
0302 69 11	Carp	90	75	60	40	20	0
0302 69 19	Other	90	75	60	40	20	0
0302 70 00	Livers and roes	90	75	60	40	20	0
0303	Fish, frozen, excluding fish fillets and other fish meat of fariff code 0304:						
0303 21	Trout (*Salmo trutta, Oncorhynchus mykiss,*	90	75	60	40	20	0
0305	Fish, dried, salted or in brine;	90	75	60	40	20	0
1604	Prepared or preserved fish, caviar and caviar substitutes	90	75	60	40	20	0

Exports of fish and fish products

Table 2. Exports of fish and fish products

(at 000 US dollars)

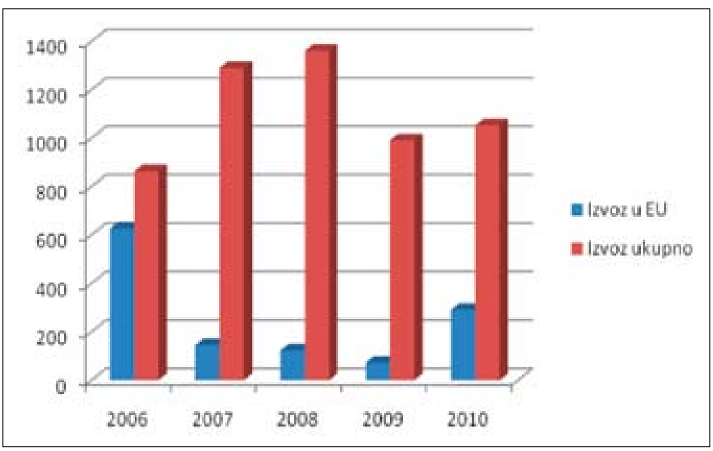
Year	Exports to EU	Index	Exports total	Index	Share of exports to EU in total exports
2006	626	100	863	100	72 %
2007	145	23	1289	149	11 %
2008	123	20	1361	158	9 %
2009	72	11	991	115	7 %
2010	291	47	1053	122	28 %

Source of data: Statistical Office of Serbia

Exports of fish emphasizes production of fish and it is necessary to create conditions to increase the previous minor export. In realtion to this it is especially important with certain systemic measures of economic policies to create the conditions for activating the export of fish and fish products. Export is an expensive affair and it is necessary that the state supports it (Miščević, 2009). It is also necessary to conduct market research as a necessary precondition for making timely and rational marketing decisions in the market economy. It is not limited exclusively to the problems of placement of manufactured goods. It is also important in terms of the long-term orientation of producers on those products that can bring the greatest profits. It is also necessary to adapt to the consumer demand (Vlahović, 2004).

The total export value of fish and fish products has increased in recent years. In 2006 it was 863,000 US dollars and in 2010 1.053 million US dollars. Potential exporters are facing many problems. One of them is that the Serbian Government did not include fish and fish products in the Decree on the use of funds to encourage exports of agricultural and food products.

With the unilateral implementation of SAA in the last four years it came to an increased presence in exports to the EU from 9% to 29%.



Graf 1. Exports of fish and fish products

Imports of fish and fish products

Table 2. Imports of fish and fish products

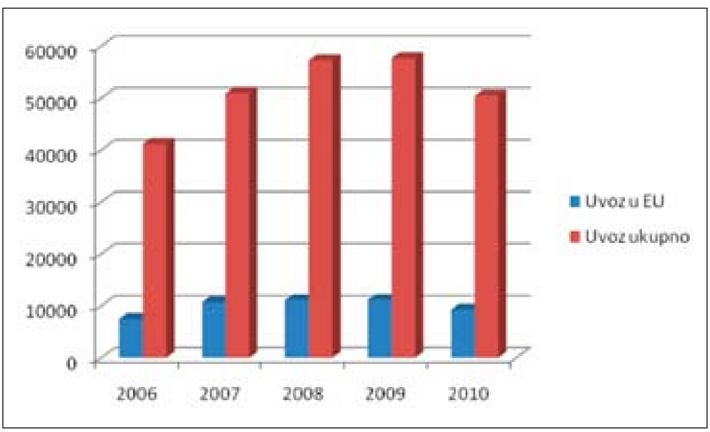
(at 000 US dollars)

Year	Imports from EU	Index	Imports total	Index	Share of imports from EU in total imports
2006	7486	100	41210	100	18 %
2007	10715	143	51024	124	21 %
2008	11133	149	57290	139	19 %
2009	11172	149	57755	140	19 %
2010	9278	124	50518	123	18 %

Source of data: Statistical Office of Serbia

The total value of imports of fresh fish and fish products in recent years is characterized by enormous growth. The total imports in 2006 were 41 million US dollars while in 2010 it was increased and was 50 million US dollars.

Almost half of the imported fish in Serbia was hake which are usually imported from Argentina and Norway (Miščević, 2004). However, the situation has drastically changed in 2008 when our country began to intensively import fish from Vietnam. In 2010 the import of fish from Vietnam twice surpassed imports from Argentina. There are no drastic changes in imports from EU countries in relation to total imports and it is in the range of 18-20%. It follows that the unilateral application of the SAA has not brought the expected changes.



Graf 2. Imports of fish and fish products

CONCLUSION

The Stabilisation and Association Agreement will bring its real effects only when ratified by all EU member states. Fisheries is classified as a sensitive area and protection of domestic production is as expected. But the problem of competitiveness of our products on the EU market will still exist, because the fishery in Serbia is not supported by the state. Serbia needs a Department for fisheries and an associated fund for fishery development as well as favorable loans to help fish producers to perform equally in the EU market. There are very large opportunities to build new carp ponds because there are sufficient waters of an acceptable quality, as well as land that is not used for crop production and can be bought relatively favorable (Miščević, 2008).

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SURVEY OF SOME CHEMICAL COMPOSITIONS AND FATTY ACIDS IN MEET OF CULTURED COMMON CARP (CYPRINUS CARPIO) AND GRASS CARP (CTENOPHARYNGODON IDELLA)

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PREGLED NEKIH HEMIJSKIH JEDINJENJA I MASNIH KISELINA U MESU GAJENOG ŠARANA (*CYPRINUS CARPIO*) I BELOG AMURA (*CTENOPHARYNGODON IDELLA*)

Abstract

This study was conducted to determine of some chemical compounds (proteins, lipids, moisture and ash) and fatty acids in cultured two species of common carp (*Cyprinus carpio*) and grass carp (*Ctenopharyngodon idella*). Results of this study showed that the amount of saturated fatty acids (SFA) in common carp and grass carp were 35.21 ± 2.19% and 27.18 ± 2.63%, respectively and saturated fatty acids (SFA) in common carp was higher compared to grass carp ($p<0.05$). Levels of polyunsaturated fatty acids (PUFA) in the common carp and Grass carp were 23.5±2.59 and 31.55±1.38 %, respectively and there was significant difference between the two species ($p<0.05$). Mono unsaturated fatty acids (MUFA) in the common carp and *Grass carp* were 31.41±2.06 and 35.12±1.78 %, respectively. There was no significant difference in MUFA between the two species ($p>0.05$). This study showed that PUFA was higher than SFA in Grass carp while SFA was higher than PUFA in common carp. There were significant differences in protein, lipid and moisture in two species ($p<0.05$) but there was no significant difference the amount of ash in two fish species ($p>0.05$).

Key words: *Chemical composition, Fatty acid, Common Carp (Cyprinus carpio), Grass carp (Ctenopharyngodon idella)*

INTRODUCTION

Fish and other sea food consumption increased in recent years and demand for sea products is growing with the increasing of population, income and also priority of sea products toward other food. Fishes have essential unsaturated fatty acids, protein with high biological value, minerals and vitamins that makes them distinguished from other creatures (Stolyhwo et al ,2006). Also fishes have large amounts of essential amino acids including Lysine that the level of this amino acid in fishes is more than plants (plant food). The most important and noticeable properties in fish oil, is the presence of abundant unsaturated fatty acids. Fish oil have a large amounts of essential fatty acids like (C_{2o}:5n-3,EPA), (C_{22}:6n-3,DHA) and (C_{20}:4n-6,ARA) that couldn't be recognized by human's body, so their existence in human's food is necessary. So many researches showed that fatty acids have a vital role in the correct functioning of cardiovascular system, nervous system, reproduction and immune system. And also involved in many processes of cell membrane and biochemical of body (palmeri et al ,2007). Cell membrane structure mainly formed by fatty acids in body. Cell membrane permit the level of essential amount nutrients to enter into cells and the wastes to get out rapidly from cells. For cells to be able to exchange the nutrients and the wastes, fluidity cell membrane should maintain its stability and integrity. Cells lacking healthy membrane lose the ability for holding water and nutrients and also lose the ability to communicate with other cells. Since the cell membrane consists of fat so their fluidity and flexibility depends on the type of fat consumed. Consumption of saturated (hydrogenated) makes the cell membrane hard and rigid and cause cell membrane fluidity against consumption of unsaturated fatty acids.

Fish body like other animals have water, protein, non-protein nitrogen compounds, fat, minerals, vitamins, and low amount of hydrocarbon. The amount of these ingredients and their changes in fish body can be used as an indicator for physiological conditions. Chemicals in fish muscle is different and is based on the (type) species, ration and diet composition, farming operation and environmental conditions (Sidhu, 2003), size, age, reproductive cycle, salinity, temperature, geographical location and fishing season(Inhamuns et al., 2008) and genetic factors in muscle (Bayir et al., 2006) (Inhamuns et al., 2008; Bayir et al., 2006).

Due to fishing restriction in water resources, aquaculture is the only way which is answerable to the increasing demand for fish and sea food (Cahu et al., 2004). *Cyprinus carpio* and *Ctenopharyngodon idella* are two species of fishes that could be seen in most environment and due to rapid growth, easiness of breeding and high food efficiency they are cultured in almost all places in the world (Tokur et al., 2006). These two species are counted as the important farming species in Iran , as now are included about 50% of fish in warm water fish ponds. Accordingly, considering abundance of producing these two species, surveying their body composition is very important. According to the subjects said about the nutritional value of fishes, this study has been done for investigating the level of fatty acids and some chemicals (protein, lipid, moisture and ash) of these two species muscle tissue.

MATERIALS AND METHODS

Sampling

Fishes *Cyprinus carpio* and *Ctenopharyngodon idella* were obtained from one of the fish ponds of Noshahr on July 2009. They were all kept in iced boxes and transported to the laboratory where they were washed with cold water, weighed and measured. The average length and weight were 37±1cm and 860±72 g for the cultured *Cyprinus carpio* and 50±1 cm and 900±50 g for *Ctenopharyngodon idella*. Total protein was determined by the Kjeldahl method (Ritzmann and Daniels, 1975) by which the concentration of nitrogen is measured. A conversion factor of 6.25 was used to convert total nitrogen to crude protein for all varieties of fish. The muscle was homogenized in a food processor (Braun Combimax 600), and moisture content of 5 g of homogenized sample was determined by drying the sample in an oven at 105˚ C until a constant mass was obtained (AOAC,1990). Ash was determined by using the basic AOAC method (1990) heating the samples in the furnace at 550 ˚C for 8–12 h. The analyses were repeated three times, and the results are presented as mean S.D. of determinations for triplicate samples. The lipids were extracted using a modified method of Bligh and Dyer (1959). Accordingly after homogenizing tissue samples, on (1gram) of uniformed tissue, 15 milliliter of mixed chloroform/methanol added for extracting lipid. Then by using distilled water to put the mixture into three phases and its lipid separated like solution in chloroform. Then by using thermal (about 50˚c to 70° c and very low flow of nitrogen gas) , solvent firing happened and its approximate weight calculated. For this reason the desired lipid in all cases was about 0/05 gram (Folch et al., 1957). Then with the methylation process, fatty acid methyl esters dissolved in hexane were obtained. Finally 1 microlitre of this solution injected to Gas Chromatography (GC) model HP-6820 for identifying fatty acids (AOAC, 1990). To identify fatty acids in each sample, 37pieces mixed fatty acids of Supelco Company was used.

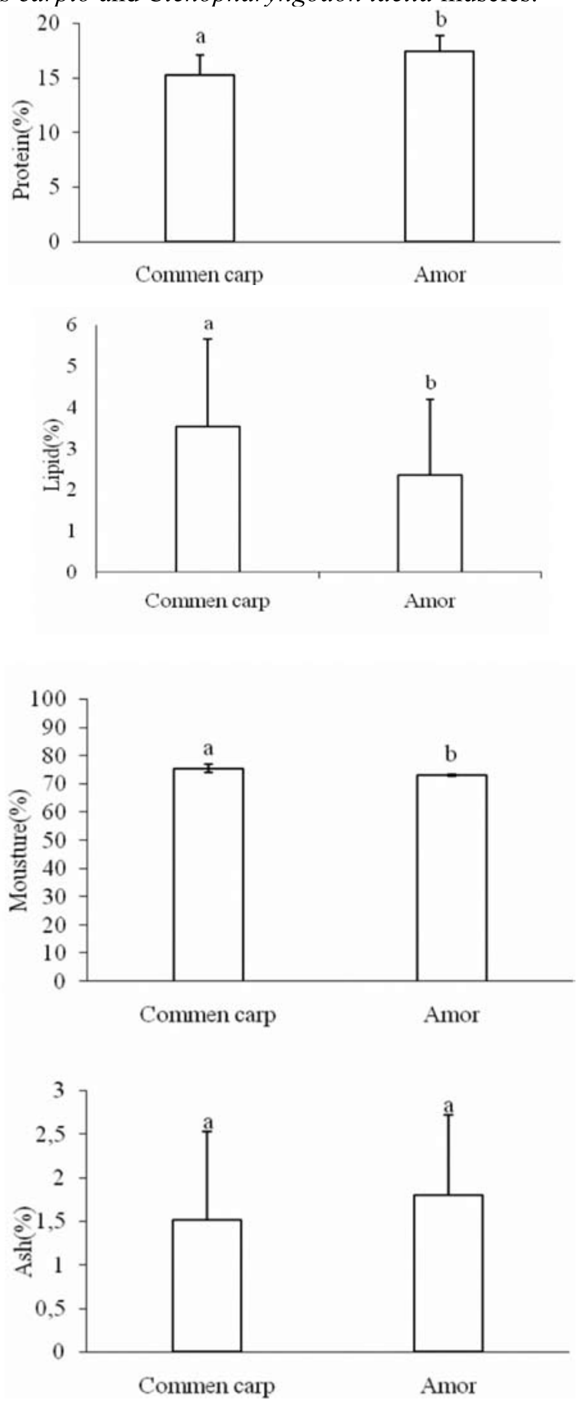
Statistical analysis

After gathering and data entry at first their normality was investigated by kolmogorov-smirnof test. Significant difference among chemicals between two fishes was determined by using T test about 5% probabilities. All statistical analysis was done by SPSS(ver. 19.5) software.

RESULTS

Results of chemical compounds (protein, lipid, ash and moisture) reported in muscle tissue of both *Cyprinus carpio* and *Ctenopharyngodon idella* in diagram 1. Considering the results, amount of protein in *Ctenopharyngodon idella* (17/41 ± 1/47 percent) was more than *Cyprinus carpio* (15/2± 1/9).(p<0/05). Amount of lipid in *Ctenopharyngodon idella* 2/35± 1/83 percent and in *Cyprinus carpio* was 3/53 ± 2/12 percent (p<0/05). Amount of ash in grass carp was 1/8± 0/92 and in common carp 1/5±1.02 and there was no significant differences in muscle tissue of both fishes. Also results showed that there was a significant difference in muscle tissue of both fishes and in the level of moisture, as the amount of moisture in *Ctenopharyngodon idella* (73/1±10/38 percent) was less than *Cyprinus carpio* (75/48±1/58 percent) (p<0/05) (Graf 1).

Graf 1. *Cyprinus carpio* and *Ctenopharyngodon idella* muscles.



Amount of saturated fatty acids (SFA) and Mono unsaturated fatty acids (MUFA) in *Cyprinus carpio* muscles was orderly 35/21±2/19 percent and 31/24±2/06 percent. Also the amount of poly unsaturated fatty acids (PUFA) in *Cyprinus carpio* was 23/5±2/59. In *Ctenopharyngodon idella* the level of saturated fatty acids (SFA) and Mono unsaturated fatty acids (MUFA) was orderly 27/18±2/63 percent and 35/12±1/78. Also amount of poly unsaturated fatty acids (PUFA) in *Ctenopharyngodon idella* was 31/55±1/38 percent. Saturated fatty acids in *Cyprinus carpio* muscles is more than *Ctenopharyngodon idella* muscles (p<0/05)(diagram 1). The results of statistical analysis showed the total amount of Mono unsaturated fatty acids (MUFA) in *Cyprinus carpio* does not have any significant difference with its amount in *Ctenopharyngodon idella*(p<0/05). Also the amount of poly unsaturated fatty acids (PUFA) in *Cyprinus carpio* was less than *Ctenopharyngodon idella* (P<0/05). In *Ctenopharyngodon idella* poly unsaturated fatty acids was more than saturated fatty acids while in *Ctenopharyngodon idella* poly saturated fatty acids was more than poly unsaturated fatty acids. Amount of n-3/n-6 in *Ctenopharyngodon idella* was 0/52±0/03 and in *Cyprinus carpio* was 0/82±0/13 and there was no significant difference between two species (p<0/05). Also PUFA/SFA ratio in *Ctenopharyngodon idella* (1/04±0/10) was more than *Cyprinus carpio* (0/71±0/03) (p<0/05) (table1).

Table 1. Comparison of amount of fatty acids in cultured *Cyprinus carpio* and *Ctenopharyngodon idella* muscles (N=10)

*Our data is expressed based on average ± standard deviation. Different letters in each column show the signicant difference(P<0/05).

spices	PUFA/SFA	n-3/n-6	EPA+DHA	PUFA	MUFA	SFA
Common carp	0/71±0/04 [a]	0/82 ± 0/18 [a]	7/81 ± 0/19 [a]	23/5±0/2 [a]	37/41 ± 1/2 [a]	35/21± 3/32 [a]
Grass carp	1/04 ±06 [b]	0/52 ± 0/35 [a]	6/68 ±2/14 [a]	31/55±3/12 [b]	35/12 ±0/9 [a]	27/18± 0/54 [b]

DISCUSSION

In this survey investigation was done on two species of *Ctenopharyngodon idella* and *Cyprinus carpio*. Investigating chemicals of freshwater fishes is very important because useful information for experts related to food resources having low fat, high protein, and being easily accessible. In this survey level of moisture in *Cyprinus carpio* was 5/48±1/57 percent and in *Ctenopharyngodon idella* was 73/11±0/38 that was according to the findings of others in this background (Islam& Jaadder, 2005; Abii et al., 2007). Fishes based on their body fat are divided into three categories lean(fat lower than 5 percent) fishes with average fat (fat between 5-10 percent) and fatty fishes (fat more than 10 percent) (Suriah et al., 1995). In this survey the amount of fat in *Cyprinus carpio* and *Ctenopharyngodon idella* was orderly 3/53±2/12 and 2/53 ±1/83percent that by said categorization, the investigated fishes was of lean fishes which was different in this background by Jabben & Chaudhry’s findings (2011) which was likely because of sampling location, sampling season, nutrition condition of fish , environmental condition, size and age and etc. the amount of protein in *Ctenopharyngodon idella* was 17/41 ±1/47percent and in *Cyprinus carpio* was 15/2±1/9 percent which was according to Khorramgah et al., (2007) findings.also the amount of ash in this survey was more than Khorramgah et., al., (2007). So many surveys showed that chemical compounds in fish muscles in different species or even in one species has difference depends on gender, age, environmental condition and season. According to the shown results, chemical compounds of two cultured fishes *Ctenopharyngodon idella* and *Cyprinus carpio* considering being in the same environmental condition, having differences with each other and with other done study in this bachground.

Cultured *Cyprinus carpio* have more saturated fatty acids (SFA) than cultured *Ctenopharyngodon idella*, while the amount of poly unsaturated fatty acids in *Cyprinus carpio* is less than its amount in *Ctenopharyngodon idella*. This difference could be related to the type of feeding and culture system. *Cyprinus carpio* that cultured as a semi-extensive in the earth ponds, mainly depends on natural foods and benthic communities in the ponds, while *Ctenopharyngodon idella* has herbivore diet and in addition to feeding from reed around the earth pond, fed manually by forage and hay (Khorramgah et al 2007).

Transferring of PUFA and particularly EPA and DHA in fish food chain shows which normally plankton feeders has the highest PUFA and benthic carnivores feeds from invertebrates, has the lowest amount of PUFA(Arrayed et al.,1999). Considering type of food diet in *Cyprinus carpio* toward herbivora Ctenopharyngodon idella, and that the benthic invertebrates form much of the foods of *Cyprinus carpio*, in this survey also the amount of PUFA in *Cyprinus carpio* and *Ctenopharyngodon idella* is less than the amount of MUFA. The lowest amount suggested for PUFA/SFA ratio, is 0/45 (HMSO,1994) which in this study for *Cyprinus carpio* calculated 0/71and for *Ctenopharyngodon idella* calculated 1/04.

n-3/n-6 ratio, is the appropriate indicator for relative comparison of nutritional value of fish fat (Tokur et al.,2006). Generally amount of n-6 among freshwater fishes is more than n-3 (Jabeen & Chaudhry 2011). Also in present study like cultured *Cyprinus carpio* or wild one (Khorramgah et al., 2007) and cultured channel catfish (Tokur et al.,2006), and higher amount of omega-6 than omega-3 was observed in cultured *Cyprinus carpio* and *Ctenopharyngodon idella*. Lots of surveys showed that the amount of saturated fatty acids in freshwater fishes is more than unsaturated fatty acids. In this survey the amount of saturated fatty acids in *Cyprinus carpio* was more than unsaturated fatty acids but in *Ctenopharyngodon idella* unsaturated fatty acids was more than saturated fatty acids that it likely because of the type of feeding of *Ctenopharyngodon idella* that feeds from plants.

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