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UZ PODRŠKU
EVROPSKOG DRUŠTVA ZA AKVAKULTURU, EAS
I
PANHELENSKOG DRUŠTVA TEHNOLOGA IHTIOLOGA

VI MEĐUNARODNA KONFERENCIJA „VODA I RIBA“

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ISHRANA RIBA: POBOLJŠANJE EFIKASNOSTI HRANLJIVIH MATERIJA I KORIŠĆENJA VODE

Apstrakt

Bilo da je reč o polu intenzivnom ili potpuno intenzivnom sistemu gajenja, proizvodnja vodenih životinja se oslanja na dostupnost esencijalnih hranljivih materija i energije koju dobijamo iz dopunske/dodatne ili kompletne hrane. O kom god sistemu gajenja da je reč, cena ovih hranljivih materija koji se koriste kao dodaci u ishrani ostaje visoka. Odgovarajuća ishrana je neophodna da bi se održale vitalne funkcije sa značajnim uticajem na rani razvoj, rast, zdravlje, kvalitet mesa i reproduktivne performanse. U proteklih nekoliko decenija, istraživanja ishrane su doprinela izvanrednom razvoju intenzivne akvakulture zasnovane na ishrani, unapređenjem kvaliteta hrane i smanjenju cene hrane u ukupnoj proizvodnji. Sada je u potpunosti dokazano da optimizacija kvaliteta ishrane i strategije ishrane mogu veoma da poboljšaju efikasnost proizvodnje ribe, kvalitet životne sredine i nutritivnu vrednost gajene ribe.

Dok je znanje o potrebama za hranljivim materijama za određenu proizvodnju i za svaku vrstu u proizvodnji neophodno, dostupnost tih hranljivih materija prirodno varira u zavisnosti o kom sistemu gajenja je reč. S obzirom da je gajenje u ribnjacima sistem gajenja koji još uvek preovladava u istočnoj Evropi, integrisano znanje o načinu funkcionisanja ekosistema ribnjaka sa preciznom kvantifikacijom toka prirodnih nutrijenta ostaje veliki izazov i za nutricioniste kao i odgajivače riba.

Razvoj akvakulture je blisko povezan sa dostupnim resursima: voda, zemljište, nutritivni izvori u obliku hrane ili đubriva. Iako je napredak ostvaren u određenim sektorima, još dosta treba da se radi na poboljšanju efikasnosti u akvakulturi. Analiza dinamike toka najvažnijih elemenata korišćenjem pouzdanih metoda i modela treba da nam pomogne da shvatimo kako se hranljive materije konvertuju u jestive proizvode koji su značajni za ishranu ljudi. Stehiometrija elemenata je jedan takav integrisani pristup za analizu ne samo protoka hranljivih materija kod jedinki riba već i da bi se dobilo kvantitativno znanje o dinimici nutrijenata u proizvodnim sistemima akvakulture.

Ako se u razmatranje uzme potreba za vodom na globalnom nivou, smanjenje upotrebe vode u proizvodnji vodenih životinja predstavlja pravi izazov za koji treba naći rešenje. Ovaj problem se odnosi i na korišćenje vode na farmi riba kao in a korišćenje vode koje je u vezi sa izvorima hrane. Drugi problem je da li sistemi za proizvodnju u akvakulturi podjednako "koriste vodu" ? Takođe, posedujemo jako malo informacija o prividnom sadržaju vode gajenih riba i rakova, iz tehničkih i konceptualnih razloga.

Pod intenzivnim uslovima gajenja u akvakulturi veliki napredak je učinjen da se manje oslanjamo na sastojke koji proističu iz ribarstva, kao što su riblje brašno i riblje ulje kao izvori proteina i masti iz hrane za ribe. Inkluzija alternativnih izvora proteina i masti takođe treba da uzme u obzir sveukupni uticaj na resurse i korišćenje vode. Povećana efikasnost konverzije hranljivih materija i korišćenje vode su dva glavna problema za koje treba da se nađe rešenje da bi akvakultura bila glavni izvor proteina životinjskog porekla u ishrani ljudi u narednim decenijama.

Abstract

Be it a semi-intensive or a totally intensive culture system, aquatic animal production depends on the availability of essential nutrients and energy supplied in the form of supplementary / complementary or complete feeds. Under any of these circumstances, the cost of these nutrient inputs remains high. Adequate nutrition is essential for the maintenance of vital functions with significant impacts on early development, growth, health, flesh quality and reproductive performance. Over the past decades, nutrition research has greatly contributed towards the remarkable development of feed-based intensive aquaculture around the world, in optimising feed quality and in the reduction of feed cost in the overall production. It is also now fully demonstrated that optimisation of the nutritional quality of the feeds and the feeding strategies can considerably improve the efficiency of fish production, environmental quality and the nutritional value of farmed fish.

While knowledge of the nutritional requirements for a given production and for each of the species involved is indispensable, the supply of such nutrients in an available form will naturally vary depending upon the culture systems. Since pond culture remains by far the predominant form of aquaculture in Eastern Europe, integrated knowledge of the functioning of the pond ecosystem with precise quantification of the natural nutrient flow remains a big challenge for the fish nutritionist and the fish farmer. Development of the aquaculture is closely linked to the availability of resources: water, land, nutrient sources supplied as feeds or fertilisers. Although much progress has been made in selected sectors, there is much room for improvement of efficiency of aquaculture. Analysing the flow dynamics of major elements using reliable methods and models should facilitate our understanding how nutrients are converted into edible products of interest to the human food basket. Elemental stoichiometry is one such integrated approach not only for analysing nutrient fluxes in individual fish but also to gain quantitative knowledge on nutrient dynamics in aquaculture production systems.

Given the global demand for water, reducing water use for aquatic animal production is a real challenge which needs to be addressed. This concerns both on-farm water use and feed-resources associated water use. Whether different aquatic production systems are equally "water-efficient" is another debated issue. We have very little information also as regards the virtual water-content of farmed fish and shrimp, due both to technical and conceptual reasons.

Under intensive aquaculture conditions, much progress has been made to reduce our reliance on fishery-derived ingredients such as fishmeal and fish oil as protein and

fat sources in feeds for fish. Inclusion of alternative protein and fat sources should also take into account the overall impacts in terms of resource and water use. Increasing efficiency in nutrient conversion and water-use are two major issues which need to be addressed to ensure aquaculture as the major supplier of animal protein to humans in the coming decades.

EVROPSKO DRUŠTVO ZA AKVAKULTURU (EAS)

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EAS (www.easonline.org) je nezavisno neprofitno udruženje (osnovano 1976. godine) koje promoviše kontakte i širi informacije među onima koji su uključeni u ili zainteresovani za akvakulturu u Evropi. EAS ima članove u više od 60 zemalja sveta.

Da bi se dostigla ova dva primarna, gore navedena, EAS-ova cilja mi:

- Organizovanjemo godišnje konferencije "Evropska Akvakultura" još od 1981. godine;
- Naši članovi objavljuju radove – u naučnom časopisu sa recenzijom (Međunarodna Akvakultura) i u časopisu i biltenu (Evropska Akvakultura);
- Objavljujemo specijalne i druge radove koji se bave razvojem akvakulture u Evropi;
- Koordiniramo (i učestvujemo) u aktivnostima i inicijativama Evropske Zajednice. Te aktivnosti doprinose širenju informacija o održivoj akvakulturi i podržavaju razvoj iste.
- Učvršćujemo veze sa drugim organizacijama koje se bave akvakulturom, ponajviše sa Svetskim društvom za akvakulturu, Organizacijom za hranu i poljoprivredu (FAO), Međunarodnim savetom za istraživanje mora (ICES), Evropskim udruženjem za proizvodnju životinja, Međjunarodnom organizcijom EUROFISH, Međunarodnim centrom za napredne mediteranske i agronomske studije (CIHEAM), AquaTT, Društvom inžinjering u akvakulturi …

EAS se trudi da premosti jaz između nauke i indistrije, ali takođe i između drugih zainteresovanih strana, naročito onih koji predstavljaju potrošače i društvo.

Od 2007. godine, deo konferencije "Evropska akvakultura" je i trgovinska izložba i drugi, posebni događaji koji se organizuju radi razmene informacija u ovom industrijskom sektoru. Ovakvi najskoriji događaji su privukli skoro 1000 učesnika iz više od 50 zemalja.

Kroz inicijativu Evropske Unije za koordinaciju i za širenje informacija, EAS je omogućio da se zainteresovane strane uključe u aktivnosti i dostigne konsenzus što je rezultiralo sa više od 300 EU projekata iz akvakulture, za industriju i potrošače.

Društvo je jedno od članova koji su osnovali Evropsku Platformu za tehnologiju i inovacije u akvakulturi. Društvo je takođe uključeno u razvoj savetodavne mreže za sektor mekušaca Evrope (EUROSHELL) i harmonizovani sistem za procenu uticaja hrane i pića na životnu sredinu (SENSE).

EAS se trudi da premosti jaz između nauke i industrije, ali potrebno je da:

- Naučnici razmotre načine na koje mere uspeh, a koje su zasnovani na časopisima sa recenzijom i velikim impakt faktorom;
- Proizvođači shvate da je saradnja a ne konkurencija put do uspeha;
- Obe strane imaju zajedničku platformu za promovisanje kontakata i usklađivanje potreba industrije sa istraživačkim programima.

Sam EAS treba da kreira različite vrste članstva – više članova iz industrije; više člnova iz centralne i istočne Evrope i više mladih članova. EAS takođe treba da komunikacijom ojača saradnju sa drugim partnerima u akvakulturi i sa organizacijama potrošača.

THE EUROPEAN AQUACULTURE SOCIETY (EAS)

EAS (www.easonline.org) is an independent non-profit association (created in 1976) that **promotes contacts** and **disseminates information** among all involved or interested in aquaculture in Europe. EAS has members in more than 60 countries.

These two major EAS objectives are achieved by:

- the organisation of an annual aquaculture event "Aquaculture Europe" since 1981;
- our members' publications – the peer-reviewed scientific journal (Aquaculture International) and a magazine and newsletter (Aquaculture Europe);
- special and other publications related to the development of European aquaculture;
- the coordination (and participation in) of European Community actions and initiatives that further the dissemination of information and support the development of sustainable aquaculture;
- the formalisation of ties with other aquaculture organisations, notably, the World Aquaculture Society, FAO, ICES, The European Association for Animal Production, EUROFISH, CIHEAM, AquaTT, Aquacultural Engineering Society…

EAS is therefore trying the bridge the gap between science and industry, but also between other stakeholders, especially those representing consumers and society.

Since 2007, the Aquaculture Europe event has included a Trade Exhibition and other, specific events to maximise the sharing of information with the industry sector. Recent events have attracted close to 1000 participants from more than 50 countries.

Through EU coordination and dissemination initiatives, EAS has facilitated stakeholder involvement and consensus building and summarized more than 300 EU projects in aquaculture, for industry and consumers.

The Society was a founder member of the European Aquaculture Technology and Innovation Platform and is currently involved in the development of an extension network for the European mollusc sector (EUROSHELL) and a harmonized system for the environmental impact assessment of food & drink products (SENSE).

EAS is trying to close the gap between science and industry, but there are challenges ahead:

- Scientists need to review success measurements that are based on peer-reviewed, high-impact journals;
- Producers need to see cooperation rather than competition as pathways to success;
- Both need to have a common forum to promote contacts and align industry needs with research programming.

EAS itself has challenges in further diversifying its membership – with more members from industry; more from CEE countries and more young persons. EAS needs to strengthen further its cooperation with other aquaculture partners and with consumer organisations through communication.

REČ DOBRODOŠLICE EVROPSKOG DRUŠTVA ZA AKVAKULTURU (EAS)

Već godinama EAS pruža podršku na sastancima i događajima za koje smatramo da će pomoći u dostizanju naših glavnih ciljeva u razvoju održive akvakulture u Evropi. Odbor direktora društva pažljivo bira te sastanke. Mi takođe pomažemo u promovisanju događaja: promovišemo ih našim članovima a i šire.

Veoma mi je drago da EAS podržava ovu VI međunarodnu konferenciju “Voda i Riba” u Beogradu kao i desetogodišnjicu zajedničke razmene znanja i iskustva. To su upravo i glavne aktivnosti EAS-a, dok pokušavamo da dostignemo cilj: smanjenje jaza između nauke i industrije.

U martovskom izdanju našeg časopisa “Evropska Akvakultura” nalazi se obiman članak o akvakulturi Srbije čiji su autori Zoran Marković i Vesna Poleksić. Na ovaj način smo našim članovim pružili mnoštvo informacija o istoriji, trenutnom statusu i potencijalu za budućnost akvakulture u vašoj zemlji. Mene je ponajviše interesovalo da čitam o povećanoj proizvodnji šarana, čija proizvodnja stagnira ili pak opada u nekim zemljama.

Naš izabrani predsednik, Sachi Kaushik i izvršni direktor Alistair Lane će biti sa vama ove nedelje. Molim vas obratite im se ukoliko želite da saznate više o našim aktivnostima ili ukoliko želite da postanete naš član.

Želim vam prijatnu konferenciju!

eas
www.easonline.org

Kjell Maroni

Kjell Maroni

Predsednik EAS-a za 2012-2014. godinu

WELCOME FROM THE EUROPEAN AQUACULTURE SOCIETY

Over the years, EAS has lent its support to meetings and events that we consider will help reach our core objectives of developing sustainable aquaculture in Europe. The meetings that we support are therefore carefully selected by the Board of Directors of the society and we try to assist by promoting the event throughout our membership and beyond.

I'm very pleased that EAS is supporting this VI International Water and Fish conference in Belgrade and celebrating your 10th year of mutual exchange of knowledge and experience. This is what EAS is all about, as we continue to pursue our objective of bridging the gap between science and industry.

In the March edition of our "Aquaculture Europe" magazine, we had an extensive article on Serbian aquaculture, authored by Zoran Markovic and Vesna Poleksic, and providing our members with comprehensive information on the history, current status and future potential of aquaculture in your country. I was especially interested to read of the increases in production of carp, which is stagnating or even declining in other countries.

Our President Elect, Sachi Kaushik and Executive Director, Alistair Lane will be with you this week, so please don't hesitate to ask them more about what we do and how you can join us.

Have a wonderful meeting!

Kjell Maroni

EAS President 2012-2014

COMMON CARP AND AQUACULTURE

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ŠARAN I AKVAKULTURA

Apstrakt

Akvakultura je najbrže rastući sektor za proizvodnju hrane od životinja. Njena stopa rasta od 1970. godine je 8.3% godišnje sa 52.5 miliona tona (2008; 68.3 uključujući i vodene biljke) i odgovorna je za skoro polovinu ukupnih zaliha ribe za ishranu (SOFIA, 2010). Riblje ulje i riblje brašno se tradicionalno koriste kao sastojci u ishrani karnivornih ribljih vrsta. Riblje ulje ima visok nivo n-3 polinezasićenih masnih kiselina (n-3 PUFA; 20 i više ugljenikovih atoma i 3 i više dvostrukih veza), naročito eikozapentaenoinske kiseline (EPA; 20:5n-3), dokozapentaenoinske (DPA; 22:5n-3) i dokozaheksaenoinske kiseline (DHA; 22:6n-3), prirodno je i hranljivo za ribe kao i za ljude. Kako se akvakultura razvija, ribljie brašno i ulje postaju skuplji i deficitarni. Zbog toga vremenom počinje da se vrši pritisak na proizvođače hrane za ribe da zamene ove sastojke sa više održivim alternativama (Pickova & Morkore, 2007). Obično se ulje i biljni proteini koriste kao "zamena" u ishrani. Biljna ulja mogu da zamene veliku količinu ribljeg ulja u ishrani mnogih vrsta riba a da pritom to ne utiče na rast ili efikasnost hrane. Ipak, mana ovih alternativa u ishrani je nedostatak n-3 PUFA. To znači da je hranljiva vrednost za ljude ovih gajenih ribljih vrsta smanjena. Nekoliko alternativnih izvora ulja koja se dobijaju iz jednoćelijskih algi, pelaških organizama i beskičmenjka dna koji sadrže visoki nivo n-3 PUFA su indentifikovani i testirani kao hrana za vodene organizme. Njihova cena je ipak veoma visoka da bi se koristili regularno u hrani za vodene organizme.

Šaran (*Cyprinus carpio*) je jedna od najčešće gajenih vrsta na svetu. U 2008. godini, u svetu je proizvedeno 2 987 433 tona, a u Evropi 144 747 tona (FAO, 2011). Šaran je ustanovljena gajena vrsta i konzumira se tradicionalno u Centralnoj Evropi. Šaran je svaštojed koji se hrani planktonom i bentosom kao i detritusom u prirodnim uslovima. U Centralnoj Evropi šaran se gaji u veštačkim zemljanim ribnjacima u kojima je proizvodnja zasnovana na proizvodnji planktona i bentosa, dok se kao dodaci koriste žitarice i ostala dodata hrana. U poredjenju sa karnivornim vrstama, digestivni sistem šarana je adaptiran na ishranu koja sadrži više ugljenih hidrata. U Evropi, ciklus proizvodnje šarana traje 3 do 4 godine.

Postoje dva izvora n-3 PUFA za šarana koji se proizvodi u ribnjacima: i) prirodna hrana, plankton i bentos, koji su bogati n-3 PUFA i ii) n-3 PUFA koju šaran sintetiše iz alfa linoleinske kiseline (ALA). Postoje podaci da, za razliku od morskih riba, šaran može da na prirodan način pretvori ALA u n-3 PUFA (Zheng *et al.*, 2004; Tocher, 2003). Zbog toga je jako važno razumeti i povećati sposobnost šarana da sintetiše n-3 PUFA iz ALA da bi se sačuvao masni kvalitet ribe koja se koristi u ljudskoj ishrani i da bi se na odrziv način koristili izvori hrane za ribu. Stoga, gajenje šarana može da postane neto izvor n-3 PUFA odabirom ribe sa visokim aktivnostima enzima koji učestvuju u elongaciji i desaturaciji masnih kiselina.

Potrebe šaranu za n-3 i n-6 masnim kiselinama su relativno niske (0.5–1%) i mogu se zadovoljiti masnim kiselinama sa 18 C atoma biljnog porekla (Takeuchi 1996). Udeo ribljeg brašna u hrani gajenog šarana je nizak (5%) (Tacon & Metian, 2008), dok se riblje ulje čak ni ne dodaje. Zbog toga je zamena ovih sastojaka znatno lakša u ishrani šarana nego karnivornih vrsta.

Gajenje šarana dakle, može da predstavlja primer dugoročne održive proizvodnje u kojoj se ne oslanjamo na izvore ribljeg ulja i ribljeg obroka. Zahvaljujući prirodnom procesu lanca ishrane u ribnjacima, proces gajenja šarana pretvara 'izgubljene' hranljive materije (naročito azot i fosfor) u vodi u veoma vredno hranljivo meso. U centralnim delovima kontinenta gde ljudi imaju manji pristup morskoj ulovljenoj ribi organizmi koji pripadaju niskom trofičkom nivou su veoma vazan izvor vrednih n-3 PUFA. Takodje, šaran bogat omega 3 masnim kiselinama se može proizvesti zamenom aditiva u hrani koja se unosi u ribnjake pogačom od lana i repe. Ova strategija je zaslužna za proizvodnju šarana sa stabilnim nivoom omega 3 masnih kiselina.

Istraživanja o povoljnim efektima hrane za ribe su najčešće usmerena ka morskim ribama i školjkama. Zbog toga se EPA i DHA na pogrešan način zovu 'morske' masne kiseline ili masne kiseline od 'ribe'. U slatkim i slanim vodama mikroalge u velikoj meri sintetišu ove n-3 PUFA, koje se transportuju putem lanca ishrane kroz sisteme.

Započeta je saradnja sa institutom za eksperimentalnu medicinu IKEM, da bi se istražili efekti koje šaran u ishrani ima na kardio-vaskularne indekse pacijenata koji su podvrgnuti SPA tretmanu. Istraživan je uticaj korišćenja u ishrani šarana (šarana sa povećanim sadržajem omega 3 masnih kiselina) na pacijente posle operacije srčane revaskularizacije koji su podvrgnuti SPA tretmanu. Lipidi plazme, LDL holesterol i TG nivoi su bili znatno poboljšani kod grupe pacijenata koja je konzumirala šarana, za razliku od kontrolne grupe, koja je dobijala piletinu kao izvor proteina životinjskog porekla u ishrani.

Ključne reči: *Šaran, ishrana, DHA, lipidi, proizvodnja u ribnjacima*

Abstract

Aquaculture is the fastest growing animal-food-producing sector with a growth rate from 1970 of around 8.3% per year and with 52.5 million tons (in 2008; 68.3 including aquatic plants) and accounts for almost half of total food fish supply (SOFIA, 2010).

Fish oil and fish meal have been traditionally used as ingredients in aqua-feeds for carnivorous fish culture. Fish oil has a high level of the n-3 highly unsaturated fatty acids (n-3 HUFA; 20 and more carbons and 3 and more double bonds), especially eicosapentaenoic (EPA; 20:5n-3), docosapentaenoic (DPA; 22:5n-3) and docosahexaenoic

acid (DHA; 22:6n-3), being natural and nutritious for fish as well as for humans. As the aquaculture is expanding, fish meal and fish oil become more expensive and scarce. It consequently creates a high pressure on the aqua-feed producers to replace these ingredients with more sustainable alternatives (Pickova & Morkore, 2007). Generally, vegetable sources of oil and protein are used as "The replacement". Vegetable oils can replace substantial amount of fish oil in the diets of many fish species without affecting growth and feed efficiency. However, the drawback of these alternatives is the lack of n-3 HUFA and therefore compromising the nutritive value of farmed fish for consumers. Several alternative oil sources, derived from unicellular algae, pelagic organisms or benthic invertebrates containing high amounts of n-3 HUFA have been identified and tested in aquafeeds. Nevertheless, their prices are still too high to be commonly used in aquafeeds.

Common carp (*Cyprinus carpio*) is one of the most cultured fish species in the world. In 2008, the world and the European production was 2 987 433 tons and 144 747 tons, respectively (FAO, 2011). It is an established cultured species. Carp is consumed as a traditional food in central Europe. Carp is an omnivorous species feeding on plankton and benthos as well as detritus in natural conditions. Typical farming practice in Central Europe is artificial shallow earthen ponds in which the production is based on plankton and benthos production supplemented by cereals or other additional feeds. The digestive system of carp is adapted to a diet including more carbohydrates compared to carnivorous species. The production cycle in Europe usually takes 3-4 years.

There are two sources of n-3 HUFA in carp produced in ponds: i) the natural feed, plankton and benthos, being rich in n-3 HUFA and ii) the n-3 HUFA synthesized by carp from alpha linolenic acid (ALA). It has been reported that carps, in contrast to marine fish, are able to bio-convert ALA to n-3 HUFA (Zheng *et al.*, 2004; Tocher, 2003). It is therefore of interest to understand and maximize the ability of carp to synthesize n-3 HUFA from ALA in order to preserve the lipid quality of the fish as human food and for sustainable utilization of feed resources. Carp culture might therefore be suitable of becoming net producer of n-3 HUFA by selecting fish with high enzyme activities in FA elongation and desaturation.

Carp has also relatively low requirements for both n-3 and n-6 FA (0.5–1%) which can be fulfilled by plant 18 carbon FA (Takeuchi 1996). Inclusion of fish meal in carp culture is low (5%) (Tacon & Metian, 2008) and fish oil being even absent. Thus the substitution of these ingredients will be considerably easier for carp compared to carnivorous aquaculture species.

Carp culture might therefore be an example of long term sustainable production without relying on the supply of fish oil and fish meal. In addition, carp culture turns "lost" nutrients (especially N and P) in water into a highly valuable nutritious flesh by the natural food chain in the ponds. The low trophic levels are especially important source of valuable n-3 HUFA in central parts of continents where the populations have less access to marine fish from capture. In addition, omega-3 carp has been produced by changes in additives into the pond feeds such as rape- and linseed cakes. By this strategy, a production of a stable omega - 3 lipid profile carp has been enabled.

Studies on beneficial effects of fish intake are very often directed towards marine fish and shellfish. EPA and DHA are therefore misleadingly called "marine" fatty acids or "fish" fatty acids. These n-3 HUFA are to a large degree synthesized by microalgae, both in fresh- and saltwater and transported via the food chain in the systems.

Collaboration with the Institute of experimental medicine IKEM, to investigate the effect of carp on cardio-vascular indices of subjects in SPA treatment has been started. Influence of carp (carp with increased content of omega 3 fatty acids) consumption on patients after cardiac revascularization surgery during the follow up SPA treatment was studied. Plasma lipids, LDL cholesterol and TG levels improved significantly in the group of patients receiving carp compared to the control group receiving chicken as the animal protein dietary portion.

Keywords: Common carp, nutrition, DHA, lipids, pond production

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CARP PRODUCTION IN SERVICE OF REINFORCEMENT OF SERBIAN AGRICULTURE

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ŠARANSKA PROIZVODNJA – U FUNKCIJI RAZVOJA (POLJO)PRIVREDE U SRBIJI

Apstrakt

Šaranska proizvodnja u svetu poslednjih 60 godina ima trend rasta. U prvoj deceniji 21. veka kreće se između 2,8 i 3,4 miliona tona. Proizvodnja u Srbiji se u poslednjoj decenije kretala od 5 500 tona do preko 12 000 tona (mađi i konzuma). Sa proizvodnjom konzumnog šarana od blizu 1,3 kg (Marković et al. 2012) Srbija zauzima treću poziciju u proizvodnji šarana po glavi stanovnika u svetu, iza Češke Republike i Kine, odnosno na nivou Myanmara (Varadi 2010). Proizvodnja šarana sa pratećim vrstama (belim i sivim tolstolobikom, belim amurom i grabljivicama: somom, smuđom i štukom) se obavlja na oko 11 000 hektara, od ukupne površine od 14 000 hektara. Broj šaranskih ribnjaka je oko 85, od kojih 25 ribnjaka čine 85% ukupnih površina koje su u eksploataciji. Proizvodnja se obavlja u poluintenzivnom sistemu (preko 95% ukupno porizvedenog šarana), zasnovanom na kombinaciji prirodne i dodatne hrane: žitarica i koncentovane hrane (ekstrudirane i peletirane). Nivo proizvodnje poslednjih 3 godine je dvostruko veći od proizvodnje od pre deset godina. Povećanje proizvodnje je najpre usledilo sa privatizacijom ribnjaka (2003. godine) i unapređenjem upravljanja ribnjacima, a potom, od 2005. godine sve češćom zamenom žitarica sa kompletnom – pre svega ekstrudiranom hranom.

Iako akvakultura u Srbiji, sa apsolutnom dominacijom proizvodnje šarana u njoj, danas predstavlja mali segment srpske (poljo)privrede, gde u prodaji i otkupu proizvoda poljoprivrede, šumarstva i akvakulture promet ribom u poslednjoj deceniji čini svega 1 – 1,5% (podaci RZS) potencijal za znatno veći doprinos šaranske proizvodnje u razvoju srpske (poljo)privrede je jako veliki.

Resursi kojima Srbija raspolaže u šaranskoj proizvodnji su:

-Tradicija duža od 100 godina u proizvodnji, a a što podrazumeva nasleđivanje ribarskog zanata radnika kroz generacije zaposlenih na ribnjacima.

-Proizvodne površine (oko 3 000 ha) koji su van funkcije, a čijom rekonstrukcijom bi se proizovodnja šarana mogla povećati za preko 25% (3 000 tona).

-Više desetina hiljada hektara (po nekim procenama i preko 100 000 ha) zaslanjenih, zamočvarenih, pašnjačkih i drugih površina slabe plodnosti koje se ne koriste za druge namene, na kojima bi se mogle izgraditi nove površine pod šaranskim ribnjacima, a čija bi se plodnost značajno unapredila nakon 2 do 3 decenija ribnjačkog korišćanja.

- Raspoloživa građevinska operativa i ljudska radna snaga za izgradnju, a potom za rad na ribnjaku i njegovo održavanje.

- Postojanje domaćih sirovina, prerađivačkih kapaciteta (nekoliko puta većih od trenutno korišćenih) za proizvodnju ekstrudirane hrane za šarana, kao i resursa za unapređivanje kvaliteta hrane za ribe, čime bi sadašnji izvoz žitarica mogao biti delom zamenjen hranom za ribe ili samom ribom.

- Mogućnost proizvodnje daleko veće količine kvalitetne mlađi (larvi) šarana od sadašnje.

- Šaranski ribnjak predstavlja stanište ugroženih i retkih vrsta ptica i sisara, kao i resurs za: proizvodnju zooplanktona, gajenje ukrasnih i korisnih vodenih biljaka, lov, ribolov, ispašu ovaca i goveda, ugostiteljstvo i turizam...

- Ribnjak baziran na iskorišćavanju prirodne produkcije (zooplanktona i faune dna), predstavlja racionalni oblik korišćenja obnovljivih prirodnih resursa u procesu proizvodnje usklađene sa dobrobiti životinja, a čime se stvara ekološki „bio”ili „eko”proizvod - šaran, visoke hranljive vrednosti.

- Šaran je pogodan za unapređenje kvaliteta mesa, sa mogućnošću povećanja nivoa proteina, smanjenja nivoa masti, unapređenja kvaliteta masti u smislu povećanja sadržaja omega 3 masnih kiselina i poboljšavanja odnosa omega 3 i omega 6 masnih kiselina.

- Šaran se tradicionalno koristi u ishrani stanovništva u Srbiji, ali i više evorpskih zemalja.

Da bi se navedeni resursi aktivirali neophodno je:

- Informisanje političara i ekonomista o značaju šaranskih ribnjaka za poboljšanje kvaliteta zemljišta slabe plodnosti i njihovog korišćenja za unapređenje kvaliteta zahvaćenih voda.

- Podsticanje relevantnih institucija za uvođenje stimulativnih i podsticajnih mera za izgradnju ribnjaka, gajenje šarana, njegovu preradu i konzumiranje.

- Izrada strategije razvoja ribarstva (akvakulture) za duži vremenski period.

-Stalno jačanje istraživačkih resursa (materijalnih i ljudskih) i podsticanje istraživačkih programa sa ciljem unapređivanja proizvodnje i kvaliteta ribljeg mesa.

- Podsticanje proizvodnje i izvoza ekstrudirane hrane za ribe, ribe i proizvoda od ribe (šarana) umesto sadašnjeg izvoza žitarica.

- Promovisanje značaja šaranskih ribnjaka kao staništa brojnih zaštićenih biljnih i životinjskih vrsta.

- Stalno unapređenje saradnje i stvaranje mreža između naučnih institucija i proizvođača šarana u zemlji i sa drugim institucijama u Evropi i svetu.

-Promocija šaranskih ribnjaka kao zanimljivih destinacija za odmor od svakodnevnog stresnog života savremenog čoveka nastanjenog u gradovima i rekreaciju (veslanje, ribolov, lov, šetnju, posmatranje ptica...) usklađenu sa prirodnim okruženjem.

- Stvaranje atraktivnih prehrambenih artikala, što bližih tanjiru (kroz jačanje preradjivačke industrije), uz isticanje nutritivnih vrednosti šarana.

- Unapređenje načina plasmana riba, umesto živog šarana ponuda čitavog spektra primamljivih polupreradjevina i preradjevina od šarana.
- Kontinuirana promocija i reklamiranje kvaliteta ribljeg mesa (sa težištem na domaći proizvod – šarana) i njegovog korišćenja u preventivnoj svrsi od niza bolesti (kardiovaskularnih, kancerogenih, dijabetisa) kao i u pravilnom razvoju dece i omladine.
- Organizovan nastup srpskih proizvodjača šarana na sajmovima i u pregovorima sa potencijalnim kupcima (velikim trgovačkim lancima prodajnih objekata)
- Isticanje „bio" i „eko" prednosti mesa šarana u odnosu na brojne konkurentske proizvode

Ključne reči: proizvodnja šarana, akvakultura Srbija
Key words: carp production, aquaculture, Serbia

INTRODUCTION

In the past 60 years carp production in the world has an increasing trend. In the first decade of the 21st century carp production varied from 2.8 and 3.4 millions of tons. The share of European carp production in the total world production also varies: in 1990 it was 402 000 t and represented 35% of the total world production; 7 years later due to political changes in Eastern Europe, 125 274 t carp produced represented only 5% of the total world production (FAO, 2013).

In Serbia carp production in the past decade increased from 5 500 t to over 12 000 tons, 70 % of this production is consumable size carp (Markovic et al. 2012). With the production of 1.6 kg (fry and consumable size) per capita, i.e. 1.3 kg consumable size per capita in the last couple of years, Serbia occupies a relatively high position, after the Czech Republic, China, at the level of Myanmar (Varadi, 2010).

From the total surface area under warmwater fish farms of 14 000 hectares, carp and accompanying species (white bighead and gray bighead, white grass carp and predators: wells/catfish, pikeperch, and northern pike) production is practiced on 11 000 hectares. Biggest part of these farms, approx. 97% is located in the Northern Serbian province Vojvodina. Number of carp farms in Serbia is 85, but 25 of them occupy 85% of the total area under exploitation. Among the 25, 6 farms make use of over 500 ha each.

Serbian carp production technology is predominantly, over 95%, semi-intensive, based on combination of natural food and supplemental one: cereals and compound feed (extruded and pelleted). In the past three years the level of production has doubled compared to the level 10 years ago. Production increase started with farm privatization and farm management improvement in 2003, and after that from 2005 with increased replacement of cereals with compound feed, primarily extruded.

There is an absolute dominance of carp production in Serbian aquaculture, representing a small segment of Serbian agriculture today: in the total turnover (sale and purchase) of agricultural production, forestry and aquaculture, the income from fish culture in the last decade is only 1 – 1.5 % (data of the Statistical Office of the Republic of Serbia). Despite this fact there is a great potential for significantly higher contribution of carp production in Serbian agriculture.

RESOURCES FOR INCREASED CONTRIBUTION OF CARP PRODUCTION IN AGRICULTURE DEVELOPMENT IN SERBIA

Beginnings of carp culture in Serbia are related to White Lake regulation and the year 1894 when „Ečka" fish farm started operating, being, even today, the biggest farm in Serbia. This shows that there is a certain tradition in this species culture, including inheritance of the fishing craft through generations of workers on the farms.

From the total surface area under carp farms – approximately 14 000 ha, some 3 000 ha is out of function. By reconstructing these areas the actual carp and accompanying species production could be increased for over 25% (more than 3000 t).

Carp farms are mostly built on saline, swamp/wetland, grassland and other areas of low fertility. Today there are several tens of thousands hectares (some estimate over 100 000 ha) of such unexploited areas located in the vicinity of water currents: rivers and canals (primarily in Banat). There, new surface areas under carp farms could be built.

In the period of financial crisis lasting in Serbia already for 15 years and the lack of big investments and larger constructions, the existing construction machines (dredge, bulldozer, backhoe…) are mostly out of order. By reconstructing the existing ones and building new farms, the mentioned construction machinery could be put in function, and their operators employed.

According to the data from the National employment service in Serbia 765 000 people are unemployed. Different trade unions estimate this number is over 1 million. The level of unemployment in Banat that has a biggest potential in available space for farm building, according to the National Statistical Office, in 2007 was 78 509, pointing out that there is available work force in these areas. This could create conditions for population retention in rural areas.

Serbia is a big producer of crop cereals that are used directly as added feed for carp or as a component in feed mixtures. As an example in 2011, 6 480 thousand tons of corn, and 2 076 thousand tons of wheat were produced, from Serbia 1 500 thousand tons of corn and over 250 thousand tons of wheat were exported (data from the Statistical Office of the Republic of Serbia). Bearing in mind the fact that a big producer of soy concentrates (that could replace imported fish meal in carp feed) exists in Serbia, this is Sojaprotein from Bečej, its production capacity being about 70 thousand tons per year, as well as the fact that extruded carp feed is produced by 4 animal feed companies (Veterinarski zavod Subotica from Subotice, DTD Ribarstvo – Bački Jarak, Komponenta from Ćuprija, and Eco Feed from Sečanj) with the total capacity of over 40 thousand tons, there is a clear potential in resources of processing industry for carp extruded feed production. To the mentioned production and resources in raw material another one – the research resource existing at the Faculty of Agriculture in Belgrade should be added. In the Laboratory for fish nutrition and in the center for Fishery and Applied Hydrobiology, CEFAH, research is carried out in order to improve feed quality for carp.

In Serbia there are 4 industrial hatcheries, as well as one experimental, with production capacity for carp fry, able to meet the needs of the major part of European cyprinid aquaculture. In addition it should be mentioned that from 6 years ago at the Experimental hatchery of the CEFAH of the University of Belgrade Faculty of Agriculture, a carp breeding program is established (Spasić et al. 2010), and an improved carp production based on quality fry is expected in 5 years.

An important segment of carp production is the fact that carp ponds are sedimentation units for water from surrounding canals and rivers that supply the ponds. By sedimentation of particles (inorganic and bioseston) that are reaching the pond, as well as sedimentation of uneaten feed and feces of the fish that is decomposing, the barren land areas after a certain period of exploitation as carp farms, become fertile and usable for field crops cultivation.

Carp farms are not only objects for warmwater fish cultivation; they are also a habitat for many rare and endangered species of birds and mammals, thus representing a resource and wealth for the whole country.

Semiintensive carp production, based on natural food (zooplankton and bottom fauna) exploitation, supplemented with cereals or concentrated feed represents a rational form of use of the renewable resource and energy source for the production process. Thus an "eco" or "bio" product of high nutritional value is created.

Carp farms are additional production and service resources: for zooplankton production, for ornamental and useful macrophytes, for angling and hunting, for sheep and cow grazing, for catering and tourism.

Carp is a species cultured in its natural environment and both extensive and semiintensive production technologies are in agreement with the principles of animal welfare. As such this type of production represents an advantage compared to other fish species cultured in intensive systems with high stocking density and almost permanent stress conditions, which is reflected on fish flesh quality in human diet.

Carp meat quality can be additionally improved by the increase of protein level, decrease of lipids level, improvement of lipid quality such as increase of omega 3 content and better omega 3 : omega 6 ratio (Marković et al. 2012; Trbović et al. 2012).

Carp is traditionally used in human nutrition in Serbia. Actually Serbia is the unique country where carp is bought in important quantities for family celebrations. Carp is traditionally consumed in several European countries, especially for Christmas Eve, Good Friday or other holidays. European Union has a deficit of fish on its own market of about 1 550 000 tons (Varadi et al, 2010), therefore there is room for carp placement, primarily processed (such as smoked carp).

ACTIVATION OF RESOURCES IN ORDER TO INCREASE CONTRIBUTION TO DEVELOPMENT OF SERBIAN AGRICULTURE

In order to activate mentioned resources it is necessary to:

- Inform politicians and economists about the importance of carp farms for remediation of barren land and their use for improvement of quality of water resources used.
- Encourage relevant institutions to introduce stimulating measures and promote farm building, carp production, its processing and consumption
- Create the Strategy for aquaculture development for a longer period of time
- Permanently reinforce scientific resources (material and human) and encourage research programs aimed to production and fish meat quality improvement
- Incentive for production and export of extruded fish feed, fish and fish products instead of export of cereal crops
- Promote the importance of carp farms as habitat for various protected plant and animal species
- Permanently improve cooperation and networking between research institutions and carp producers in the country as well as with other institutions in Europe and the world.

- Promote carp farms as interesting destinations for retreat from everyday stress of the modern urban populations, and possibilities of recreation (rowing, angling, hunting, walking, bird watching etc…) in harmony with natural environment.
- Create attractive food products through reinforcement of the processing industry, with emphasis of the nutritive value of carp meat.
- Improve placement of fish, instead of live carp, offer attractive semi cooked and processed carp.
- Continuously promote and advertize fish meat quality with emphasis on carp as domestic product and its use in prevention of diseases (cardiovascular, cancer, diabetes) as well as child and youth development
- Organize appearance of Serbian carp producers on fairs and negotiations with potential customers (retail chains)
- Highlight “bio” and “eco” advantages of carp flesh compared to other products

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BIOMANIPULATION MEASURES ON CZECH RESERVOIRS AND THEIR PAST AND CURRENT SUCCESSFULNESS

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MERE BIOMANIPULACIJE NA AKUMULACIJAMA U ČEŠKOJ I NJIHOVA EFIKASNOST U PROŠLOSTI I SADAŠNJOSTI

Apstrakt

Trud da se pozitivno utiče na ekološke procese i kvalitet voda u jezerima i akumulacijama intervencijama u ribljoj zajednici (ribljem nasadu) svrstava se pod ribarsko upravljanje sa specijalnom namenom. Upravljanje ribljim nasadom sa specijalnom namenom, čiji je glavni cilj smanjenje razvoja planktonskih algi, naziva se biomanipulacija. Biomanipulacija se zasniva na eliminaciji planktonofagnih vrsta (bodorke, bucova, deverike, krupatice, crvenperke, šarana, babuške), bilo direktnim otklanjanjem ovih neželjenih vrsta bilo njihovim suzbijanjem nasađivanjem predatorskih vrsta. Smanjenje velike količine planktonofagnih riba dovodi do smanjenja njihove ishrane zooplanktonom, što za posledicu ima razvoj populacije velikih vrsta zooplanktona (Cladocera iz roda *Daphnia*) koji efikasno eliminišu male planktonske alge iz svih nivoa vode, što kasnije dovodi do povećanja bistrine/providnosti vode. Biomanipulacija dakle predstavlja ciljani uticaj na niže karike lanca ishrane korišćenjem riba, s obzirom da su one hijerarhijski viši učesnici u lancu ishrane (Randák et al. 2013). Štaviše, ribe dna se takođe uključuju u proces biomanipulacije. One mogu značajno da ubrzaju proces oslobađanja hranljivih materija sa sedimenata dna. One naime narušavaju „mir“ sedimenata dna (bioturbacija) u potrazi za hranom (Adámek, Maršálek 2013).

Rezultat povećanja biomase predatorskih vrsta omogućava kontrolu razvoja malih ciprinidnih vrsta i naziva se “kontrolisani nasad riba”. On se najčešće postiže održavanjem odnosa biomase predatorskih i ne predatorskih vrsta između 1:6 i 1:10.

Ipak, u Češkoj, “kontrolisani nasad riba” se među rečnim vlastima tumači na pojednostavljen način - kao regularno oslobađanje karnivornih životinjskih vrsta koje se hrane ribom, najčešće kao 0+ riba. Rezultati ovakvog „kontrolisanog nasada“ nisu za-

dovoljavajući. Ipak, činjenica je da je koncentracija fosfora, glavne hranljive materije primarnih proizvođača (npr. cijanobakterija, algi i makrofita) često veoma visoka u Češkim akumulacijama. Zbog stalnog prisustva fosfora, trofički nivo površinskih voda se ubrzano povećava stvarajući nešeljeni razvoj planktonskih algi i cijanobakterija, što za posledicu ima pogoršanje kvaliteta vode.

Neophodno je imati u vidu sledeće: efikasnost mera biomanipulacije ima određena ograničenja. Ona najčešće zavise od opterećenja hranljivim materijama i morfoligije (dubine) vodene površine. Ukoliko količina hranljivih materija u akumulaciji prevazilazi određenu granicu, proces biomanipulacije gubi svoju efikasnost – značajno i dugoročno smanjenje biomase planktonskih algi procesom kontrolisanja ribljeg nasada se ne može očekivati. Stoga, je poznavanje procesa opterećenja hranljivim materijama neophodan uslov za biomanipulacione mere i njihovu održivost u određenim rezervoarima.

Porast trofičkog potencijala vode do eutrofije (ili cak hipertrofije) je najčešće vezan za – bogate naslage fosfora u sedimentima zajedno sa stalnim odsustvom kiseonika u hipolimnionu, i sa viskom prinosom iz spoljnih izvora (dotok). Kada se radi o dubokim akumulacijama sa razlicitim temperaturnim nivoima, spoljni unos ne treba da prelazi vrednost od 0.6 – 0.8g ukupnog fosfora po m^2 akumulacije godišnje (Benndorf et al., 2002), ukoliko je cilj procesa biomanipulacije postizanje značajnog poboljšanja kvaliteta vode. Kada je reč o plitkim akumulacijama i jezerima, maksimalna godišnja vrednost unosa iz spoljnih izvora može da iznosi do 2 g ukupnog fosfora po m^2 površine akumulacije (Jeppesen et al., 1990). Ukoliko je spoljni unos hranljivih materija niži od predložene granične vrednosti, a nivo ukupnog fosfora ipak visok, onda se radi o sistemu sa visokim unutrašnjim opterećenjem koje se ostvaruje oslobađanjem fosfora iz nataloženih sedimenata. Ukoliko proces biomanipulacije u plitkim jezerima (sa prosecnom dubinom od < 3 – 5 m) vodi do značajnog poboljšanja kvaliteta vode, prosečna godišnja koncentracija fosfora ne sme da prelazi 100 - 250 $mg.m^{-3}$ (Jeppesen and Sammalkorpi, 2002). Granicna koncentracija ukupnog fosfora koja je vezana za efikasnost biomanipulacije u dubokim slojevitim jezerima i ribnjacima (prosečne dubine od > 5 – 10 m) još uvek nije jasno definisana direktnim naučnim studijama. Ipak, pretpostavlja se da je granična vrednost koja odgovara prosečnoj godišnjoj koncentraciji ukupnog fosfora između 20 i 50 $mg.m^{-3}$ (Jeppesen and Sammalkorpi, 2002; Mehner et al., 2004). Ukoliko se ova granica koncentracije fosfora prevaziđe, pored biomanipulacije neophodno je uključiti i neke druge, kompleksnije mere koje su deo revitalizirajućeg procesa.

Glavni koraci uspešnog procesa revitalizacije mogu se podeliti u tri kategorije – (i) smanjenje spoljnjeg opterećenja, (ii) smanjenje unutrašnjeg opterećenja i (iii) mere upravljanja.

Smanjenje spoljnjeg opterećenja je verovatno najvažniji korak koji mora da prethodi svim ostalim merama. U suprotnom, ceo proces je uzaludan. Ovaj korak uključuje izgradnju postrojenja za preradu vode – ona otklanjaju fosfor i azot (tercijarni tretman) u slivu akumulacije; zatim preventivne protiv erozivne i protiv poplavne mere i taloženje fosfora na ulazu. Smanjenje unutrašnjeg opterećenja sastoji se od (makar delimične) drenaže vode, zatim nanošenja krečnjaka na suvo dno, (makar ograničenog) otklanjanja sedimenata, i taloženja nutrijenata i cijanobakterija.

Ova druga mera se takođe može tumačiti kao mera upravljanja, koja takođe uključuje aeraciju hipolimniona koji se vezuje za destratifikaciju jezera, smanjenje biomase riba koje se hrane planktonima i vrsta koje se hrane faunom dna (naročito bodorke i deverike). Ovo se najbolje postiže u sezoni mresta. Mera upravljanja takođe uklјušuje mere biomanipulacije (sa vrha ka dnu, top-down) i prikupljanje biomase cijanobakterija sa vodenih površina.

Što je širi spektar primenjenih mera, veće su šanse za uspeh u poboljšanju kvaliteta vode u akumulaciji/vodenoj površini. Ipak, treba imati u vidu da su najefikasnije mere manipulacije lancima ishrane i revitalizacija akumulacije/rezervoara koje se postižu tek posle smanjenja spoljašnjeg opterecenja fosforom ispod određene granice.

Abstract

The effort to positively influence ecological processes and water quality in lakes and reservoirs through interventions in a fish community (fish stock) is the subject of special-purpose fishery management. Special-purpose management of fish stock, whose main objective is to reduce the development of planktonic algae, is called biomanipulation. The biomanipulation principle consists in elimination of populations of planktonophagous species (roach, bleak, common bream, silver bream, rudd, common carp, gibel carp), be it by direct removal of these undesirable fish or by their suppression by means of stocking of predatory fish. Reducing the abundance of planktonophagous fish leads to limitation of their grazing pressure on zooplankton, subsequently, it allows the development of populations of large species of filtering zooplankton (cladocerans of the *Daphnia* genus) that effectively eliminate small planktonic algae from the water column, which is supposed to lead ultimately to increased water transparency. Biomanipulation thus represents targeted influencing of lower components of the food chain through fish, as they represent hierarchically higher component of the food chain (Randák et al. 2013). More appropriately, also benthivorous fish have been also included into subject of biomanipulation efforts as they may contribute considerably to the release of nutrients from bottom sediments due to their disturbance (bioturbation) when searching for food (Adámek, Maršálek 2013).

The result of an effort for increased biomass of predatory fish enabling the control of development of small cyprinid species is denoted as a "controlled fish stock", usually consisting in keeping the ratio between predatory and non-predatory fish biomass ranging between 1:6 and 1:10.

In the Czech Republic, however, the „controlled fish stock" is understood by the river authorities in a quite simplified way, just as a regular release of piscivorous species, mostly as a 0+ fish. Thus, as frequently proved, these measures did not bring results as expected. Nevertheless, as a matter of fact, the concentration of phosphorus as a key nutrient driving the development of the primary producers (i.e. cyanobacteria, algae and macrophytes), is usually very high in all Czech reservoirs. Due to excessive intake of phosphorus, the trophic level of surface waters sharply increases causing an undesirable development of planktonic algae and cyanobacteria, which finally results in deterioration of water quality.

It is necessary to realize that the efficiency of biomanipulation measures has certain limits that are defined mainly by supply of nutrients (or nutrient load) and morphology (depth) of the reservoir. If the nutrient load of a reservoir exceeds a certain limit, the biomanipulation loses its effectiveness – a significant and long-term reduction of planktonic algae biomass through controlled fish stock is hardly to be expected. Knowledge of the nutrient loading is therefore a necessary prerequisite for deciding on the possibility and suitability of conducting biomanipulation efforts in a concrete water reservoir.

An increase of water trophic potential to eutrophy (or even hypertrophy) is usually associated with two main issues - rich deposits of phosphorus in sediments along with

a regular occurrence of anoxic conditions in the hypolimnion, and with high loading from external sources (inflow). With respect to deep, thermally stratified reservoirs, this external load should not exceed the value of 0.6 – 0.8 g of total phosphorus per m^2 of the reservoir surface per year (Benndorf et al., 2002), if the biomanipulation measures are supposed to show significant improvements of water quality. With regard to shallow reservoirs and lakes, the maximum annual value of external load may amount up to 2 g of the total phosphorus per m^2 of the reservoir surface (Jeppesen et al., 1990). If the external nutrient loading is lower than the recommended limit value and yet, the concentration of total phosphorus in the reservoir water is high, then it is a system with a large internal load that is presented by releasing of phosphorus from the accumulated sediments. If biomanipulation effort itself in shallow lakes (average depth of < 3 – 5 m) is to lead to significant improvement in water quality, the average annual concentration of total phosphorus should not exceed 100 - 250 $mg.m^{-3}$ (Jeppesen and Sammalkorpi, 2002). The limit concentration of total phosphorus in relation to the effectiveness of biomanipulation in deep stratified lakes and reservoirs (average depth of > 5 – 10 m) has not been clearly defined by direct scientific studies yet, the limit value corresponding to average annual concentration of total phosphorus of 20 - 50 $mg.m^{-3}$ is assumed (Jeppesen and Sammalkorpi, 2002; Mehner et al., 2004). If this phosphorus limit concentration is exceeded, it is necessary to supplement the biomanipulation measures with other, more complex approaches which are included in revitalisation campaigns.

The principal steps of a successful revitalisation project can be split up into three categories – (i) external loading reduction, (ii) internal loading reduction and (iii) management measures.

The reduction of external loading is probably the most important step which must come before any other measures are applied otherwise the whole effort is useless. It covers above all the construction of efficient water treatment plants preferably with phosphorus and nitrogen removal (tertiary treatment) in the reservoir catchment area, preventive anti-erosive and anti-flooding arrangements, and phosphorus precipitation on the inlet. The internal loading reduction consist in (at least partial) reservoir draining, subsequent liming of dry bottom, (at least limited) sediment removal, and nutrients and cyanobacteria precipitation. The latter can be considered also as a part of management measures which include also the aeration of hypolimnion associated with lake destratification, reduction of planktivorous and benthivorous fish (roach and bream in particular) biomass, most efficiently during the spawning season, biomanipulation (top-down) measures and cyanobacterial biomass harvesting from the water surface.

The wider spectrum of applied measures, the better chance for a success in the improvement of reservoir water quality. However, be in other way, it must be kept in mind, that the best effectiveness of foodweb manipulations and reservoir revitalisation may be obtained after reduction of external P loading below a certain threshold.

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INTENSIFICATION OF COMMON CARP CULTURE IN RURAL AREAS OF NORTHERN VIETNAM BY PLANT-BASED FEEDS WITH PROTEIN SOURCES OF DIFFERENT QUALITIES

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INTENZIVIRANJE PROCESA GAJENJA ŠARANA U RURALNIM OBLASTIMA SEVERNOG VIJETNAMA KORIŠĆENJEM BILJNE HRANE SA IZVOROM PROTEINA RAZLIČITOG KVALITETA

Apstrakt

U planinskom severnom Vijetnamu, ribnjačka akvakultura je važan deo tradicionalnog integrisanog sistema gajenja koji doprinosi proizvodnji proteina kao i finansijskoj dobiti. Akvakultura u Vijetnamu se najčešće zasniva na polikulturi belog amura sa belim tolstolobikom, sivim tolstolobikom, šaranom i nilskom tilapijom kao sekundarnim vrstama. Ova polikultura se odlikuje niskom produktivnošću od 1.5 t ribe po ha^{-1} a^{-1}. Glavni razlozi za nizak stepen proizvodnje ribe u ovom region su: korišćenje hrane za ribe niskog kvaliteta (lišće, trava, nusproizvodi gajenja), nepoznata bolest sa visokom stopom smrtnosti belog amura i nekontrolisani protok vode kroz jezera što prouzrokuje zamućenost vode i gubitak mineralnih hranljivih materija za primarnu produkciju. Polu intenzivan system proizvodnje kao i promena načina nasađivanja u kome bi šaran bio najvažnija vrsta u sistemu polikulture može da doprinese većoj produktivnosti i profitabilnosti akvakulture ribnjaka u planinskom delu Vijetnama. Ove promene koje vode ka polu intenzivnoj polikulturi čija je glavna gajena vrsta šaran zahtevaju promenu u izvorima hrane kao i u proizvodnji hrane. Dok je hrana sa visokom energetskom vrednošću naširoko dostupna u region, izvori hrane bogate proteinima su ograničeni. Zbog toga je obavljeno istraživanje da bi se ustanovilo koji su lokalni izvori bogati proteinom najpogodniji da se koriste kao sastojci u dopunskoj ishrani šarana.

Obavljena su ispitivanja u mrežnim kavezima u ribnjaku koji je bio nasađen sa tradicionalnim ribljim vrstama, sa gustinom nasada od 1.5 ribe po m^2 i šaranom kao glavnom

vrstom. Korišćeno je 16 mrežnih kaveza (veličine 2 x 2 x 2 m). Svaki kavez je nasađen sa po 5 primeraka mlađi šarana. Stajnjak preživara kao tradicionalno đubrivo stimulisala je dostupnost prirodne hrane. U tri mrežna kaveza, kao dodatna hrana za šarana korišćene su četiri izo-azotne hranljive smeše pripremljene sa lokalno dostupnim sastojcima sa visokim nivoom proteina (obrok od ribljeg brašna i soje, komercijalni koncentrat hrane za svinje, komercijalna hrana za mlađ ribe i ostaci od soje/tofu) i energije (obrok od kukuruza i obrok od manioke). Dnevna doza iznosila je 3% telesne mase riba. Tri mrežna kaveza nisu dobijala dodatnu hranu. 3 mrezna kaveza nisu dodatno hranjena i sluzila su kao kontrola. Na svakih 20 dana ribe u mrežnim kavezima su merene i količina hrane je usklađivana sa njihovom težinom; šarani iz šesnaestog mrežnog kaveza su izlovljeni radi analize crevinih sadržaja i zatim su ponovo nasađeni. U istom ritmu su praćeni parametri za merenje kvaliteta vode. Na isti način je praćena abundanca zooplanktona i zoobentosa. Uzorci ribe uzeti pre i posle testiranja su analizirani radi ustanovljavanja hemijskog sastava ribe. Hrana je takođe analizirana radi ustanovljavanja hemijskog sastava i sastava amino kiselina. Tradicionalni način đubrenja je stvarao životnu sredinu sa niskom ali vrlo promenljivom dostpupnošću zooplanktona (83 ± 49 mg m^{-2}) i zoobentosa kao hrane (6.5 ± 7.5 mg m^{-2}). Glavni izvor konzumirane prirodne hrane bio je zooplankton čija je veličina tela bila veća od 1mm. Pošto je gustina zooplanktona bila promenjiva, specifična stopa rasta šarana u određenim vremenskim intervalima u različitim tretmanima se mogla porediti sa dostupnošću prirodne hrane. Pozitivna korelacija između dostupne prirodne hrane i performansi rasta utvrđena je u svim slučajevima. Ipak, dostupnost prirodne hrane pod ovim režimom fertilizacije nije bio dovoljan da omogući neto rast šarana bez dodavanja hrane. Dodatno hranjnje je takođe bilo neophodno da bi se održala proizvodnja.

Kada je reč o različitim vrstama dodavane hrane, ona bazirana na ostacima soje/tofu je bila najmanje efikasna. Sa ekonomske tačke gledišta, komercijalna hrana za životinje sa visokim nivoom proteina (komercijalna hrana za svinje i ribe) poboljšana lokalno proizvedenim sastojcima pokazala je bolju neto dobit nego hrana u čijem su sastavu kombinovani čisti sastojci (obrok od ribljeg brašna i zagrejane soje) s obzirom da su oni manje prisutni na lokalnom tržištu. Ipak, sva dodatna hrana nije imala dovoljnu količinu i kvalitet proteina da bi sama zadovoljila nivo proteina i esencijalnih amino kiselina koje su potrebne za šarana.

Ovi rezultati navode na zaključak da pod dominantnim lokalnim uslovima, dodatna hrana lokalno napravljena od komercijalnih koncentrata hrane za životinja, sastojci bogati energijom mogu da dovedu do povećane proizvodnje ribe ukoliko se kombinuju sa prirodnom hranom koja je stalno dostupna. Ovo će omogućiti da se poveća udeo akvakulture u zaradi kao i u obezbeđenju hrane u domaćinstvima.

Ključne reči: poluintezivna ishrana, šaran, alternativni sastojci hrane, dostupnost prirodne hrane

Keywords: Semi-intensive feeding; Common carp; Alternative feed ingredients; natural food availability

INTRODUCTION

In mountainous Northern Vietnam, pond aquaculture is an important part of the traditional integrated farming system contributing to protein supply and cash income. Typically, this is a polyculture of grass carp with silver carp, bighead carp, common carp and Nile tilapia as secondary species, which has a low productivity of 1.5 t fish ha^{-1} a^{-1} (Steinbronn 2009). The use of low quality feed items (leaves, grass, farming by-products), an unknown disease with high mortalities only of grass carp, and an uncontrolled flow of water through the ponds causing high turbidity and loss of mineral nutrients for primary production are seen as the major reasons for the low fish production of the region. Semi-intensive pond management practices and the adjustment of stocking regime towards the common carp as main species of the polyculture system could result in a higher productivity and profitability of pond aquaculture in the uplands of Vietnam. These modifications towards the common carp based, semi-intensive polyculture necessitate a change in feed resources applied as well as feed production. Whereas energy-rich feed resources are widely available in the region, protein-rich feed resources are limited (Tuan 2010). Therefore it was investigated which of the locally available protein-rich feed resources are most suitable to be used as ingredient in supplemental feeds for common carp.

MATERIALS AND METHODS

A net cage trial was conducted in a farmer's pond stocked with the traditional fish species with 1.5 fish m^{-2} and common carp as main species. Each of the 16 net cages (2x2x2m) were stocked with 5 common carp fingerlings (19.7±4.7 g, 10.7±0.8 cm total body length, N=90). Traditional fertilization with 700 kg ha^{-1} $month^{-1}$ fresh ruminant manure stimulated the natural food availability. In three net cages each, four iso-nitrogenous and iso-energetic feeds (CP: 26 % DM, GE: 19.3 MJ/kg DM) based on locally available high-protein ingredients (fish meal + soybean meal, commercial pig feed concentrate, commercial fish fry feed and tofu residue) and energy ingredients (corn meal and cassava meal) (see Table 1) were fed to the common carp as supplemental feed at a daily rate of 3% of fish body mass. Three net cages were not fed additionally as control. The trial lasted for 82 days. Every 20 days, fish in the net cages were weight and feed amounts were adjusted; common carps from the 16th net cage were harvested for gut content analysis and restocked. In the same rhythm, water quality parameters and abundance of zooplankton and zoobenthos were monitored.

Table 1**.** Composition of trial feeds in percentage. [1]: Pig feed concentrate EH-150S (46% crude protein); [2]: Cargill Aquaxcel; 7414 (40% crude protein)

Feed	Fish meal	Pig feed[1]	Fish feed[2]	Tofu residue	Soy bean	Corn meal	Cassa-va meal	Rice bran	Mine-rals	Vita-mins	Sunf. oil
A	10	-	-	-	43	35	7	3.3	1	0.5	0.2
B	-	41	-	-	-	40.7	7	3.3	1	0.5	6.5
C	-	-	48.6	-	-	33.1	7	3.3	1	0.5	6.5
D	13.5	-	-	79	-	-	-	-	1	0.5	6

Samples of fish carcasses and feeds were analysed for dry matter and crude ash according the AOAC (1990). Crude protein was determined using a C/N analyser (Vario MAX CN, Elementar Analysensysteme GmbH, Germany, N x 6.25). Gross energy (GE) was determined with bomb calorimeter (IKA C 7000, Janke & Kunkel IKA-Analysentechnik, Germany) using a benzoic acid standard. Feeds were analysed for essential amino acid composition. ANOVAs and regressions were used for statistical analysis.

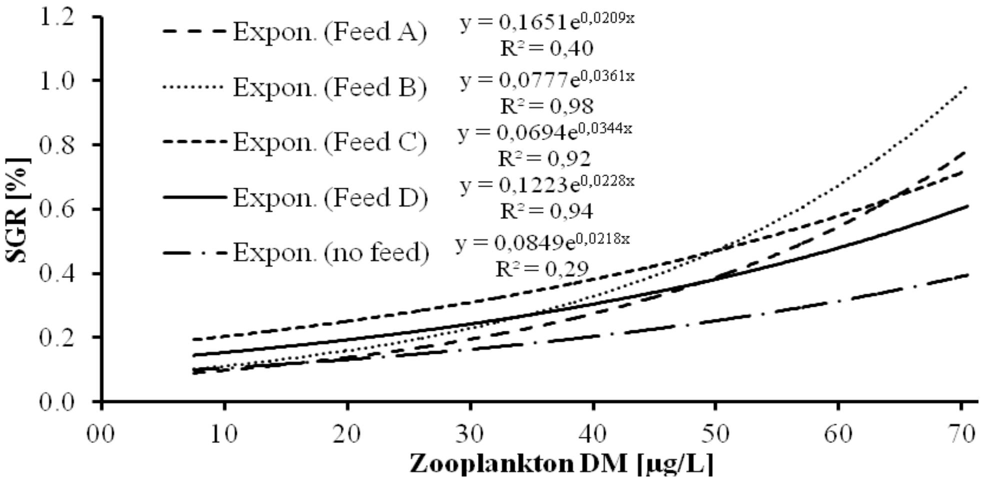

Figure 1. Exponential regression of specific growth rates (SGR) of common carp fingerlings vs. zooplankton DM for the four test feeds and without any additional feeding.

Among the different supplemental feeds, the one based on tofu residue was performing worst by showing the poorest content of essential amino acids (Table 2) as well as proximate common carp compositions similar to the unfed group and significantly different from the three other supplemental feeds (Table 3).

Table 2. Requirements of essential amino acids (EAA) and trial feed content in % of crude protein (CP); grey cells mark lower content than required. Arg: Arginine; His: Histidine; Ile: Isoleucine; Leu: Leucine; Lys: Lysine; Met: Methionine; Phe: Phenylalanine; Thr: Threonine; Trp: Tryptophan; Val: Valine. * (NRC 2011)

EAA	Thr	Val	Met	Ile	Leu	Phe	His	Lys	Arg	Trp
Requirement*	4.7	4.4	2.2	3.1	4.4	4.1	1.6	6.9	5.3	0.9
Feed A	4.1	4.6	1.5	4.2	8.1	5	2.4	3.8	6.1	1.2
Feed B	3.7	4.2	1.7	3.6	3.6	4.1	1.8	4.3	5.5	0.8
Feed C	4.2	5.5	1.8	3.7	8.8	5.1	2.7	4.3	5.9	1.1
Feed D	3.5	3.9	1.4	3.3	3.3	3.8	2	4.1	4.1	0.9

Table 3. Proximate composition of common carp under different feeding treatments.

CA: crude ash; CL: crude lipid; CP: crude protein; DM: dry matter; GE: gross energy.

	No feeding	Feed A	Feed B	Feed C	Feed D
DM [% of FM]	22.4±0.4	28.3±1.8	28.8±2.3	27.5±1.1	25.5±2.1
CA [% of DM]	16.6±0.8 [a]	11.9±0.9[b]	11.7±0.5[b]	11.2±0.4[b]	15.3±1.0 [a]
CP [% of DM]	72.9±1.7[a]	63.6±5.6[b]	62.7±3.1[b]	61.8±0.3[b]	71.4±3.6[a]
CL [% of DM]	7.0±1.0[b]	21.5±5.4[a]	21.8±2.7[a]	22.1±2.6[a]	11.6±4.2[b]
GE [MJ kg-1]	21.0±0.2[b]	24.8±1.2[a]	24.9±0.7[a]	24.9±0.8[a]	22.5±0.9[b]

Feeds A, B and C resulted in similar growth and proximate composition of common carp (Table 3). From an economical point of view, commercial animal feeds with high protein level (commercial pig and fish feeds) stretched with locally produced feed ingredients showed a better net benefit than feeds combining pure feed ingredients (fish meal + heated soybean meal) as they are less established on the local markets and have less controlled quality and higher prices. However, all supplemental feeds were insufficient in protein quantity and quality to solely fulfil the protein and essential amino acid requirements of common carp (Table 2) but were resulting in growth at sufficient availability of natural food resources. Due to the high protein content (Hepher 1988) and nutritional quality of natural food resources (Ventura and Catalan 2010), the CP content as well as content of essential amino acids in supplemental feeds do not need to fulfil all requirements of the cultured fish (De Silva 1995), which increases the profitability due to lower protein ingredient costs.

CONCLUSION

These results lead to the conclusion that under the prevailing local conditions, supplemental feeds made of commercial animal feed concentrates and locally produced, energy-rich ingredients may result in increased fish production if combined with continuously high natural food availability. Further adjustments on interaction of supplemental feed formulation and natural food quantity and quality may be beneficial to increase the production as well as the benefit out aquaculture. This will increase the contribution of aquaculture to household food security and income generation.

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INFLUENCE OF FATTY ACID COMPOSITION OF FISH FEED ON FATTY ACID COMPOSITION OF CARP MEAT

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UTICAJ MASNOKISELINSKOG SASTAVA HRANE ZA RIBE NA SASTAV MASNIH KISELINA MESA ŠARANA

Apstrakt

Riblje meso već dugi niz godina privlači pažnju nutricionista i preporučuje se u ishrani ljudi pre svega zbog visokog sadržaja nezasićenih masnih kiselina, od kojih su naročito značajne polinezasićene omega-3 masne kiseline. Ova jedinjenja smatraju se esencijalnim, jer ih sisari, a samim tim i ljudi, ne mogu sintetisati u organizmu, već ih moraju uneti putem hrane. Dobro je poznato da ove masne kiseline blagotvorno deluju na zdravlje ljudi, smanjuju rizik od pojave oboljenja srčanog mišića i krvnih sudova.

U našoj zemlji, u ishrani se u značajnoj meri koristi šaransko meso. Od ukupnog uzgoja rečnih riba u svetu, šaran zauzima prvo mesto sa 70 % celokupne proizvodnje. Uglavnom se uzgaja u ribnjacima, gde se prirodna ishrana planktonom dopunjava pretežno cerealijama. Kako cerealije sadrže vrlo malo esencijalnih masnih kiselina, i masnokiselinski sastav šaranskog mesa se menja, tako da u mesu tovljenih šarana preovlađuje oleinska kiselina, dok su omega-3 masne kiseline manje zastupljene. Najjednostavniji način da se poboljša sastav masnih kiselina ribljeg mesa jeste da se u hranu za ribe dodaju sirovine sa poželjnim masnokiselinskim sastavom, kao što su laneno, ili riblje ulje.

Cilj ovog rada bio je upravo da se utvrdi kako dodatak lanenog ili ribljeg ulja u eksperimentalnu hranu utiče na promenu masnokiselinskog sastava hrane za ribe, a potom posredno i na njihov sastav u mesu šarana. U eksperimentu je upotrebljavana ekstrudirana hrana za šarane sa naknadno dodatim uljem u vakuum zamašćivaču. Ribe su gajene u plastičnim tankovima sa protokom vode, kako bi se obezbedilo snabdevanje svežim kiseonikom.

Rezulati ovog istraživanja su pokazali da je dodatak obe vrste ulja u značajnoj meri unapredio masnokiselinski sastav hrane za ribe. Dodatak lanenog ulja povećao je relativni udeo α-linolenske kiseline u odnosu na kontrolnu hranu (7,08 % u kontrolnoj hrani u odnosu na 27,57 % u eksperimentalnoj hrani 1), dok je dodatak ribljeg ulja uticao na povećanje sadržaja dokosaheksaenoične (DHA) i eikosapentaenoične (EPA) masne kiseline, koji su u hrani 2 iznosili 4,14 % i 7,32 % (u kontroli su relativni udeli bili 0,36 % i 0,80 %). Kada se govori o ribljem mesu, dodatak obe vrste ulja pozitivno je uticao na masnokiselinski sastav mesa. Tako je odnos n-6/n-3 masnih kiselina u grupi hranjenoj obrokom 1 iznosio 4,64, a u grupi hranjene obrokom 2 bio je 1,87. U oba slučaja odnos je snižen u poređenju sa kontrolnom grupom gde je n-6/n-3 odnos bio 8,97. Prilikom poređenja dve eksperimentalne grupe međusobno, kod mesa šarana hranjenih ekstrudiranom hranom sa dodatkom ribljeg ulja uočava se nešto povoljniji sastav masnih kiselina u odnosu na meso šarana hranjenih obrokom sa dodatkom lanenog ulja, pre svega jer im je značajnije povećan udeo EPA i DHA masnih kiselina. Tako je relativni udeo EPA u ovim uzorcima iznosio 1,0 %, dok je u kontrolnoj grupi bio 0,21 %. U eksperimentalnom uzorku sadržaj DHA je bio 2,47%, a u kontrolnom uzorku 0,67 %. Dakle, sardžaj EPA se povećao skoro 5 puta, a sadržaj DHA oko 3,5 puta u poređenju sa kontrolnim uzorkom.

Može se zaključiti da oba dodata ulja imaju pozitivno dejstvno na masnokiselinski sastav mesa šarana i da ne pokazuju negativan uticaj na rezultate odgoja.

Ključne reči: masnokiselinski sastav, riblja hrana, meso šaran, esencijalne masne kiseline

Keywords: fatty acid composition, fish feed, carp meat, essential fatty acids

INTRODUCTION

For many years, much attention is given to fish meat and its presence in human nutrition. The reason for that lies in significant amount of polyunsaturated fatty acids (PUFA), particularly omega-3 fatty acids (n-3 FAs) which are present in all types of fishes. Long chain n-3 PUFAs are essential because mammals, and therefore humans, cannot synthesize them and must adopt them exogenously from dietary sources (Beare-Rogers et al, 2001; Ivanov et al, 2012). They are derived from α-linolenic acid (ALA), which is the precursor of docosahexaenoic (DHA) and eicosapentaenoic (EPA) acids (Gorjão et al, 2009). These two FAs are known to decrease risk of cardiovascular diseases, especially coronary heart disease (WHO/FAO, 2003).

Carps represent one of the largest groups of cultured fish with around 70% of freshwater aquaculture production. The majority of European carp production is placed in central Europe where it is produced in ponds using traditional semi-intensive techniques.

Fish reflect the lipid pattern of its diet to a high extent. Carp is traditionally reared in earthen ponds and its nutrition is based on natural food with cereal supplementation (Buchtova et al, 2007). Since cereals are rich in carbohydrates and have very low level of n-3 fatty acids, the flesh of the farmed carps generally contains a high level of oleic acid and low level of favourable n-3 PUFA (Chengeri, 1996). An alternative approach to influence muscle lipid composition might be inclusion of rich sources of omega FA in fish feed.

Raw materials which are known as a good source of essential FA are linseed oil and fish oil. Linseed oil is rich in linoleic (LA, 18:2, n-6) and especially α-linolenic acid

(ALA, 18:3, n-3) (Łukaszewicz et al., 2004), while fish oil contains significant amount of DHA and EPA (Jonzo et al, 2000). Fish oil is the main lipid source in feeds of carnivorous farmed fish and aquafeeds use 87% of the global supply of fish oil, of which over 66% is used for salmonids (Tacon et al, 2008).

The aim of this study was to examine how addition of linseed oil and fish oil influences FA composition of fish feed, and to register if there are any changes in fatty acid composition of meat of carps fed with experimental diets.

MATERIALS AND METHODS

In the experiment, carp feeds were formulated according to commercial and physiological consideration. Following ingredients were used for production of basic diet: corn (30 %), wheat (10 %), corn gluten (10 %), fish meal with 70% of protein (10 %), soybean meal (36 %), yeast (3%), and premix (1%). Experimental diets were prepared by addition of 6% of linseed oil (Diet 1) and 6% of fish oil (Diet 2).

Fish feed was produced on twin-screw extruder (Mu Yang MY 90, China) with a screw diameter of 85 mm, length-to-diameter ratio of 20:1, and maximum temperature of 135 °C. Extruder was equipped with diferential diameter conditioner (DDC). Addition of oil was done on laboratory vacuum coater (model F-6-RVC, Forberg International AS, Norway) with capacity of 6 L per batch.

Experimental carps were bred in 2.0 m^3 plastic square-shaped tanks divided into two compartments (about 1.0 m^3 each) by a screen wall positioned along one diagonal of each tank, each with two compartments and with 12 fishes per compartment. Water inflow into the tanks was regulated to ensure oxygen concentration of the outflow water at least 80 % of saturation. During the experimental period natural day-light regime was ensured. Feeding was performed by automatic belt feeders during 10 hours per day.

Fat for fatty acid analyses was extracted from the samples of feed and fish meat using Folch method (Folch, 1957). The preparation of fatty acid methyl esters was done by transmetilation that use 14% wt. boron trifluoride/methanol solution (Sigma Aldrich, MO, USA), as recommended method for this type of substrates. Fatty acid composition analyses were done on a gas chromatograph Agilent 7890A system (Agilent Technologies, Santa Clara, CA, USA) with flame ionization detector (GC-FID), equipped with fused silica capillary column (SP-2560, 100 m x 0.25 mm, I.D., 0.20 µm). Split ratio was 30:1. Carrier gas was helium (purity > 99.9997 vol %, flow rate = 1.5 ml/min). All determinations were done in triplicates.

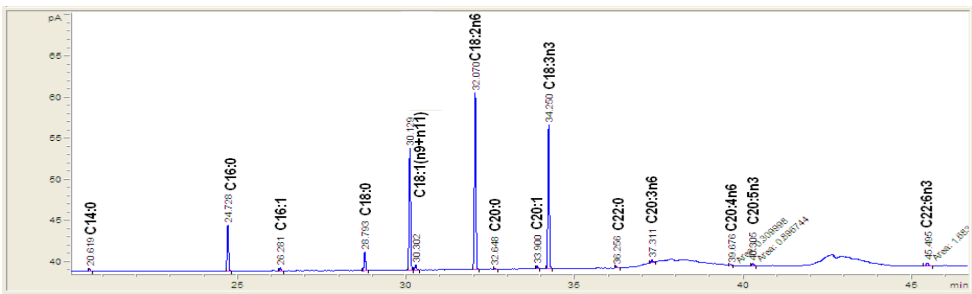
RESULTS AND DISCUSSION

Results of FA composition of fish feed and carp meat from the control and experimental groups are presented in Table 1. As it can be seen, both experimental diets had significantly ($p \leq 0.05$) higher relative content of n-3 FA, while content of n-6 FA decreased. These changes resulted in more favourable n-6/n-3 ratio in comparison with control diet (n-6/n-3 ratio of 5.85), which was 1.14 in Diet 1 and 2.25 in Diet 2. According to WHO and FAO, this ratio should be less than 4 (FAO/WHO, 2003). It can be concluded that only control diet did not fulfil this recommendation.

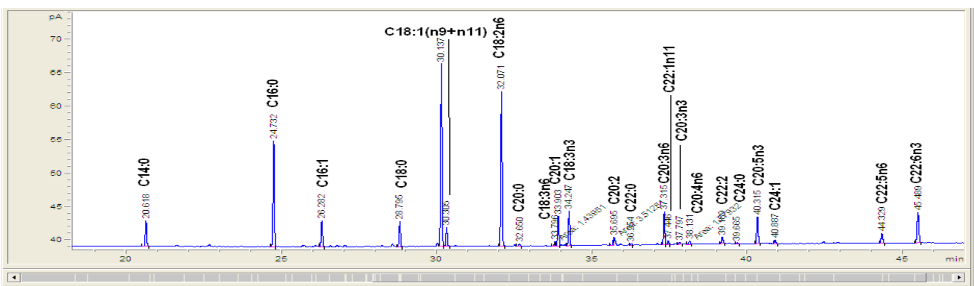
PUFA/SFA ratio was the highest in Diet 1 (5.02), mostly due to the very high content of ALA, which was 27.57 % in this sample. The lowest level of PUFA/SFA ratio recommended by WHO and FAO is 0.4 (FAO/WHO, 2003). Diet 2 showed significant increase in EPA (%) and DHA (%) in comparison with control diet (4.14 % and 7.32 %, respectively). Figure 1 presents GC-chromatograms of Diet 1 (a) and Diet 2 (b). Mentioned differences are even more obvious in fatty acid composition of carp meat. Both groups fed with experimental diets had better n-6/n-3 ratio than control group, and carps fed with Diet 2 showed the best results with n-6/n-3 ratio of 1.87.

In this sample, EPA content increased almost five times in comparison with control group (1.01 % vs. 0.21 %), while DHA content increased more than 3.5 times (2.47 % vs. 0.67 %). EPA content in meat of carp fed with Diet 1 increased to 0.52 % and DHA content increased to 1.2 %. It can be concluded that addition of fish oil in fish feed gave more favourable fatty acid composition of carp meat than addition of linseed oil.

Looking at PUFA/SFA ratio of carp meat samples, it can be seen that all samples had ratio higher than 0.4. Although addition of linseed oil and fish oil decreased, this level in comparison with control carp meat samples, it is still above recommended, so it cannot be considered as undesirable effect.



(a)



(b)

GC chromatograms of Diet 1 (a) and Diet 2 (b)

Table 1: Fatty acid compositions of control diet, Diet 1, Diet 2 and meat of carp fed with the different diets

	% of fatty acid in total fatty acid content					
Fatty acid	C diet	D 1	D 2	C Carp	D1 carp	D2 carp
C12:0	ND	ND	ND	0.02	0.02	0.03
C14:0	0.43	0.40	2.96	0.77	0.03	1.40
C14:1	ND	ND	ND	0.44	0.05	0.06
C16:0	11.46	8.06	12.72	16.04	16.38	16.85

C16:1	0.59	0.62	3.32	0.35	0.1	6.47
C17:0	ND	ND	ND	0.21	0.21	5.94
C17:1	ND	ND	ND	4.77	0.15	0.05
C18:0	4.70	3.43	3.08	5.56	5.53	4.71
C18:1 *n-9c* + *n-11c*	24.85	23.78	26.42	42.79	45.11	40.21
C18:2 *n-6c*	47.42	32.83	19.85	21.32	16.48	11.43
C20:0	0.43	0.27	0.33	0.14	0.12	0.05
C18:3 *n-6*	ND	ND	0.56	0.49	0.27	0.18
C20:1	0.71	0.63	4.19	2.05	0.46	0.8
C18:3 *n-3* (ALA)	7.08	27.57	4.52	1.87	1.98	2.87
C21:0	ND	ND	ND	0.02	8.26	1.38
C20:2	ND	ND	1.57	0.12	0.26	ND
C22:0	0.41	0.30	0.16	ND	0.34	0.23
C20:3 *n-6*	0.66	0.56	4.41	0.57	0.47	0.35
C22:1 *n-9*	ND	ND	0.54	0.34	0.37	1.04
C20:3 *n-3*	ND	ND	0.24	0.06	0.07	0.11
C20:4 *n-6*	0.11	0.16	0.47	0.04	0.26	0.11
C22:2	ND	ND	0.96	0.11	0.22	1.12
C24:0	ND	ND	0.12	0.88	0.87	0.71
C20:5 *n-3* (EPA)	0.36	0.43	4.14	0.21	0.52	1.01
C24:1	ND	ND	0.49	ND	ND	0.3
C22:5 *n-6*	ND	ND	1.62	ND	ND	ND
C22:6 *n-3* (DHA)	0.80	0.97	7.32	0.67	1.2	2.47
n-3	8.24	28.97	16.22	2.81	3.77	6.46
n-6	48.19	32.99	36.52	25.23	17.48	12.07
n-6/n-3	5.85	1.14	2.25	8.97	4.64	1.87
SFA	17.43	12.46	19.37	7.98	46.3	54.82
MUFA	26.15	25.02	34.96	46.18	20.27	19.65
PUFA	56.42	62.52	45.67	24.67	33.43	25.53
PUFA/SFA	3.24	5.02	2.36	3.09	0.61	0.77

ND – not detected. results in the table are presented as mean, n =3; SFA - saturated fatty acids, MUFA - monounsaturated fatty acids, PUFA - polyunsaturated fatty acids; C diet – control diet, D1 – diet 1, D2 – diet 2

CONCLUSIONS

Based on experimental result, it can be concluded that fish feed significantly influenced fatty acid composition of carp meat. By addition of linseed oil and fish oil in the diet, n-3 fatty acid content of carp meat increased for 0.96 % and 3.65 %, respectively. Ratio n-6/n-3 was the most favourable in meat of carp fed with Diet 2, and it was 1.87. It is obvious that both linseed oil and fish oil were proven as a good source of omega fatty acids in carp feed, but addition of fish oil gave slightly better results.

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MULTIVARIATE ANALYSIS OF FATTY ACID PROFILES OF CARP MEAT DURING SEMI – INTENSIVE FARMING

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MULTIVARIJANTNA ANALIZA SASTAVA MASNIH KISELINA MESA ŠARANA U TOKU POLUINTENZIVNOG GAJENJA

Apstrakt

Poređenje masnokiselinskog sastava mesa šarana pomoću multivarijantnih metoda, kao što su analiza glavnih komponenti (PCA) i linearna diskriminaciona analiza (LDA), omogućava razdvajanje riba prema načinu ishrane i bolje razumevanje promena u sastavu masnih kiselina tokom uzgoja. U periodu od aprila do oktobra, sa šaranskog ribnjaka u kojem je riba bila prihranjivana ekstrudiranom hranom, ispitano je dvadeset osam šarana. Mase riba su se značajno povećale između juna i septembra ($P < 0,001$) i septembra i oktobra ($P < 0,001$), zbog intenzivnog prihranjivanja šarana tokom letnjeg perioda, a sadržaj ukupnih lipida se značajno povećao između septembra i oktobra ($P < 0.001$). PCA je pokazala da je postojala visoka pozitivna korelacija između mase šarana, sadržaja lipida i oleinske kiseline ($r > 0,6$; $P < 0,0001$). Zbog veće dostupnosti prirodne hrane, u aprilu i junu, došlo je do povećanja sadržaja n-3 polinezasićenih masnih kiselina u mesu šarana, što je doprinelo boljem kvalitetu ribe. Prihrana šarana sa ekstrudiranom hranom uticala je na povećanje sadržaja n-6 polinezasićenih masnih kiselina, koje su u septembru bile značajno veće u odnosu na juni ($P < 0,01$), ali i na smanjenje nutritivno važnih n-3 polinezasićenih masnih kiselina ($P < 0,01$). Odnos n-3/n-6 je bio najveći u junu (0,30), a najmanji u oktobru (0,16). LDA analizom postignuto je razdvajanje šarana prema periodu uzorkovanja, što je u korelaciji sa vrstom unete hrane u ovim periodima. Najveća sličnost u masnokiselinskom sastavu ustanovljena je između šarana u septembru i oktobru, kao posledica smanjenja količine dostupne prirodne hrane i većeg unosa ekstrudirane hrane. PCA i LDA su pokazale da je došlo do značajnih promena u sastavu masnih kiselina šarana tokom uzgoja ribe. Rezultati koji su dobijeni u ovom

radu o uticaju ishrane na sastav masnih kiselina i sadržaj lipida u mesu šarana doprineće poboljšanju načina ishrane i konsekventno kvalitetu mesa gajenog šarana.

Ključne reči: masne kiseline, šaran, analiza glavnih komponenti, linearna diskriminaciona analiza

Keywords: fatty acids, common carp, principal component analysis, linear discrimination analysis

INTRODUCTION

Fresh water fish belonging to the Cyprinid family is economically important to human nutrition in Serbia. The geo-hydrological conditions in the country are very convenient for carp (*Cyprinus carpio*) breeding which is one of the most cultivated fish species due to rapid growth and easy cultivation.

Fatty acid composition of farmed fish differs from the fatty acid composition of the fish from open waters because of the diets. Other factors, such as the size or age of the fish, reproductive status, geographic location, season, and temperature may influence the fatty acid composition of the fish muscle (Alasalvar et al., 2002, Rasoarahona et al., 2004, Jensen et al., 2007).

Comparison of the fatty acid composition by using multivariate data analysis indicates that the fatty acid composition of the muscle tissue of the fish fed different foods differs and that it is similar to the fatty acids of the food (Barrado et al., 2003). The use of multivariate methods, such as principal component analysis (PCA) and linear discrimination analysis (LDA) affords a better understanding of the fatty acid composition of the carp meat according to the fish diet and summarizes the statistical correlation among fatty acids.

The aim of this study was to determine and compare the fatty acid profiles of carp meat during growth, reared in semi-intensive farming conditions and fed extruded feed as supplementary diet.

MATERIALS AND METHODS

Fish samples

Twenty-eight fish were collected in April, June, September and October 2009 from the fish farm ‘’Ečka”AD, with a semi-intensive carp breeding system. Fish of one-year age was submitted to trial from spring to autumn. Except naturally occurring food, fish was additionally fed extruded feed consisting of maize, soybean meal and fish meal (23.81 % proteins, 6.97 % lipids), according to the breeding season and to the fish farm productivity. Feed provided to the fish was as follows: in April 0.1 % to 0.3 %, in May 0.3 % to 1 %, in June 1 % to 2 %, in July and August 3 %, in September 2 % to 3 %, with respect to fish biomass and depending on the water temperature, its saturation with oxygen and on the amount of accessible natural food. The weight of each fish was determined upon arrival to the laboratory. Before analysis, the skin, heads, tails, fins, and intestines were removed and fish was filleted. The obtained fillets were homogenized in a laboratory blender.

Fatty acid analysis by GC

Lipids for the fatty acid determination were extracted with hexane/isopropanol mixture by accelerated solvent extraction (ASE 200, Dionex, Sunnyvale, CA), (Spirić et al., 2010). Total lipids were further converted to fatty acid methyl esters (FAMEs) by trimethylsulfonium hydroxide (EN ISO 5509:2000). FAMEs were determined by gas-liquid chromatography (GLC, Shimadzu 2010) equipped with flame ionization detector and capillary HP-88 column (Spirić et al., 2010). Results were expressed as the mass of the fatty acid (g) in 100 g of fatty acids.

Statistical analysis

Analysis of variance (ANOVA) with Tukey - Kramer test was used to analyze the data at P = 0.05 level. Principal component analysis (PCA) and linear discrimination analysis (LDA) was performed using JMP 8.0.1 software (SAS Institute Inc. NC, USA).

RESULTS AND DISCUSSION

The temperature of water in the farm, the average carp weight and lipid content during rearing are given in Table 1. A significant increase in weight of the fish between June and September ($P < 0.001$), and September and October ($P < 0.001$) was established due to the intensive feeding during summer when carp consumed large quantities of supplementary feed. The favourable environmental conditions in the aquatic environment contributed to the increase of fish biomass as well. The obtained data indicate that from April to September total lipids slightly increased with the size of fish, but a significant increase occurred between September and October ($P < 0.001$). The amount of feed and consequently the intake of energy influenced the increase in the content of lipids in fish muscle. However, the quality of feed is the most important (Haard, 1992; Rasmussen, 2001).

Table1. Water temperature, carp weight and total lipids during rearing

	April (n=6)	June (n=7)	September (n=7)	October (n=8)
Water temperature, °C	14	22	20	6
Fish weight, g	598 ± 162^{C}	874 ± 142^{C}	1439 ± 173^{B}	1984 ± 322^{A}
Total lipids, %	2.25 ± 0.71^{B}	2.37 ± 0.29^{B}	3.02 ± 1.03^{B}	4.72 ± 0.71^{A}

n - number of samples; $^{A, B, C}$ - Values in the same row followed by the same letters do not differ significantly ($P>0.05$)

Data for the content SFA, MUFA and PUFA, n-3 and n-6 PUFA and n-3/n-6 ratio in carp muscle are presented in Table 2.

Table 2. Fatty acid compositions of carp fed extruded feed (% of total fatty acids) during growth

Fatty acids	April (n=6)	June (n=7)	September (n=7)	October (n=8)
SFA	28.68 ± 5.03^{A}	28.97 ± 1.25^{A}	24.87 ± 1.01^{B}	23.66 ± 0.80^{B}
MUFA	38.77 ± 2.57^{B}	40.52 ± 2.48^{AB}	41.64 ± 2.60^{AB}	42.43 ± 2.93^{A}
PUFA	32.52 ± 4.33^{A}	30.49 ± 3.12^{A}	31.53 ± 1.92^{A}	32.56 ± 2.37^{A}
n-6	24.71 ± 4.88^{AB}	22.86 ± 3.24^{B}	26.96 ± 1.75^{A}	27.99 ± 1.91^{A}
n-3	5.37 ± 1.06^{B}	6.59 ± 0.89^{A}	4.57 ± 0.59^{B}	4.57 ± 0.66^{B}
n-3/n-6	0.23 ± 0.07^{B}	0.30 ± 0.07^{A}	0.17 ± 0.02^{BC}	0.16 ± 0.02^{C}

n - number of samples; $^{A, B, C}$ - Values in the same row followed by the same letters do not differ significantly ($P>0.05$)

From the presented data, it might be seen that the levels of MUFA significantly increased during fish grow while the levels of SFA decreased. The share of total PUFA did not change significantly ($P > 0.05$).

ANOVA test indicate that the content of n-6 PUFA increased significantly between June and September ($P < 0.01$), what is associated with increased feed intake during summer period, while the levels of n-3 PUFA decreased ($P < 0.01$). The increase in n-6 PUFA led to a reduction of the n-3/n-6 ratio, and, thus, in the reduction of the quality of fish. Henderson and Tocher (1987) reported n-3/n-6 values of 0.5 - 3.8 for freshwater fish. The n-3/n-6 ratio in this study was the highest in June (0.30) and the lowest in October (0.16), what indicates that carp feed was rich in n-6 and poor in n-3 PUFA.

Changes in the fatty acid profile in carp during rearing can be better visualized by PCA. Considering groups of FA and the most important FA, such as oleic, 18:1n-9; linoleic, 18:2n-6; linolenic acid, 18:3n-3; EPA, 20:5n-3; DPA, 22:5n-3 and DHA, 22:6n-3 (which are not presented in the Table 2), PCA clearly differentiate carp according to the investigated period of sampling (Figure 1 and 2). PCA of the fatty acid profiles, taking carp weight and lipid content as variables, resulted in two principal components model describing 74.6 % of the total data variability.

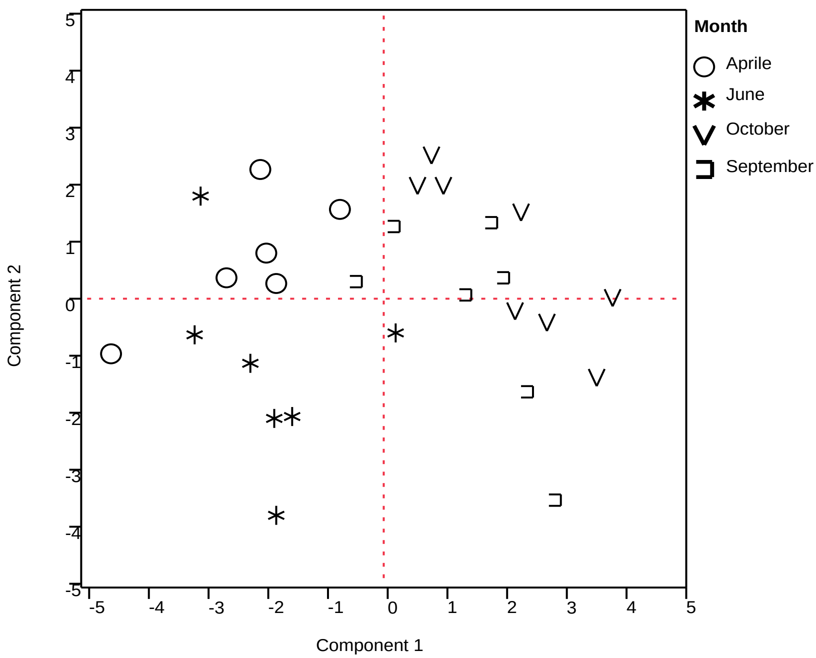

Figure 1. Principal component analysis score plot of the fatty acid profile of carp fed extruded feed

The score plot of the first two principal components (Figure 1) indicates grouping of carps during growth according to months of sampling.

Oleic acid mostly contributed to the variability on the positive part of the PC1. High positive correlation of oleic acid with carp weight and total lipids ($r > 0.6$; $P < 0.0001$) indicates that with the increase of carp weight the total lipids and the content of oleic acid increased as well. Linoleic acid that contributes to the positive part of the PC2 enables to distinguish carp in September and October with higher amounts of this FA.

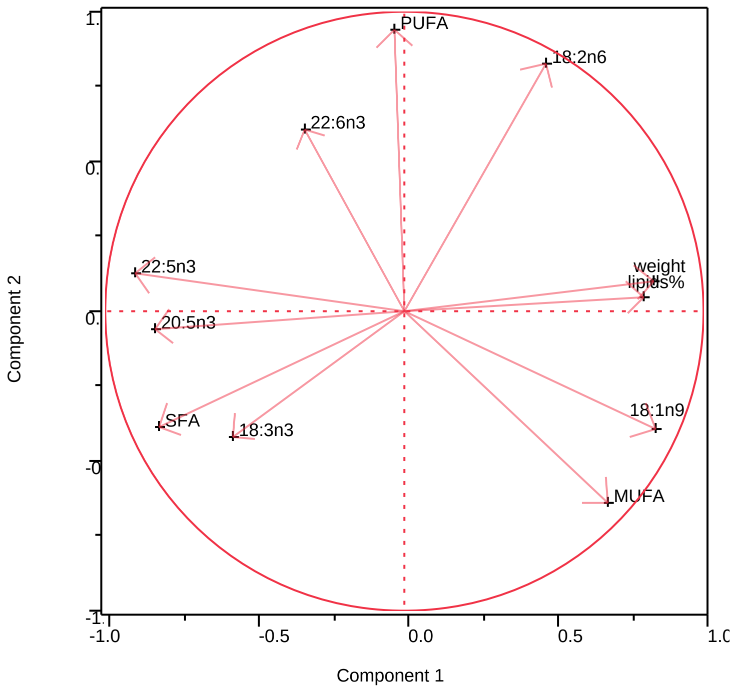

Figure 2. Principal component analysis loading plot of the fatty acid profile of carp fed extruded feed

On the negative part of the PC1, DPA, EPA and linolenic acid correspond to carp which contained higher quantities of these fatty acids in April and June. Carp can adjust the fatty acid metabolism to the prevailing temperature in such a way that increase in the temperature give rise to the creation of saturated fatty acids (Tocher, 2003), what probably is the reason why SFA were presented in higher quantities in June when temperature was the highest. The presence of linolenic acid and long-chain PUFA (EPA, DPA) in carps in April and June could be due to the higher intake of natural food. The natural food, represented by zooplankton and benthos, is a source rich in linolenic acid and EPA (Domaizon et al., 2000; Bogut et al., 2007; Živic et al., 2011), but poor in DHA. Bell et al. (1994) reported that DHA in freshwater invertebrates was present in small amounts. The availability of natural food in April and June probably caused the increase in the content of n-3 fatty acids in carp, what consequently resulted in a better fish quality (Table 2).

By the LDA, the separation between carp samples might be improved. LDA, as shown in Figure 3, clearly differentiates carps in four groups. The results of the classification are very satisfactory and allow 96 % of fish to be correctly grouped. Out of 28, 27 carps were classified according to the months of sampling.

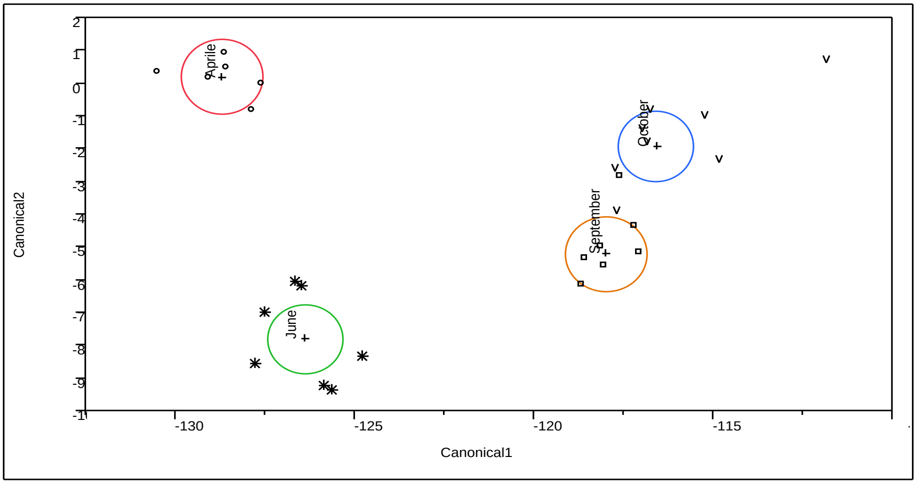

Figure 3. Canonical plot of the fatty acid profiles of carp fed extruded feed

The shortest distance between the points on the canonical plot in Figure 3 represents the smallest differences in the FA profiles of the samples. Fish in April and June were very distant one from the other, and far from September and October, what correlates to the type of ingested food in this period. The greatest similarity was observed between carps in September and October because of the high ingestion of supplementary feed.

CONCLUSION

Based on PCA and LDA it might be concluded that there were significant changes in the fatty acid composition of carp during growth. The presence of natural food in the carp farm influenced the fatty acids composition of carp in different months of sampling. However, the feeding of carp with extruded feed influenced the increase in quantities of MUFA and n-6 PUFA and the decrease in quantities of nutritionally important n-3 PUFA. The highest n-3/n-6 ratio was obtained in June (0.30) and the lowest in October (0.16), what indicates that the applied extruded feed was rich in n-6 and poor in n-3 PUFA. Analysis of the fatty acid composition in combination with multivariate analysis is a useful tool for differentiation of carp during rearing according to the available food in the farm and supplementary feed as well. Data on the effect of diet on the lipid content and fatty acid composition of carp would contribute to improving nutrition and consequently the quality of carp meat.

ACKNOWLEDGEMENT
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EDUCATION AND TRAINING IN AQUACULTURE AND WATER PROTECTION AT FACULTY OF FISHERIES AND PROTECTION OF WATERS, UNIVERSITY OF SOUTH BOHEMIA IN ČESKÉ BUDĚJOVICE, THE CZECH REPUBLIC

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OBRAZOVANJE I USAVRSAVANJE U AKVAKULTURI I ZAŠTITI VODA NA FAKULTETU ZA RIBARSTVO I ZAŠTITU VODA, UNIVERZITET JUŽNE BOHEMIJE U ČEŠKIM BUDEJOVICAMA, ČEŠKA REPUBLIKA

Apstrakt

Fakultet za Ribarstvo i Zaštitu Voda, Univerziteta Južne Bohemije u Češkim Budejovicama, Češka Republika, jedan je od najkompleksnijih radnih mesta u Evropi sa osnovnim, master i doktorskim studijama, naucnim i primenjenim istraživanjima, inoguralnim i profesionalnim pravima u oblasti ribarstva i zastite voda. Fakultet poseduje opremu i objekte za bavljenje naukom i istrazivanjima u oblasti akvakulture, hidrobilogije, toksikologije, bolesti, riba, reprodukcije, genetike, gajenja riba i rakova. Fakultet takođe poseduje veliki broj izdanja odnosno jedinstvenu biblioteku iz oblasti ribarstva.

Studentima iz inostranstva Fakultet nudi razne programme:

1) **kratkoročne stipendije za razmnenu studenata (http://www.frov.jcu.cz/cs/exchange-students/exchange-students),**
2) **internacionalna letnja škola (http://www.frov.jcu.cz/en/mezinarodni-letni-skoly/summer-fishery-school-2013),**
3) **master studije (http://www.frov.jcu.cz/en/co-lze-studovat-jake-obory/m-f-aquaculture),**
4) **doktorske studije (http://www.frov.jcu.cz/en/co-lze-studovat-jake-obory/d-f-fishery).**

1) Kratkoročne stipendije za razmenu studenata

Studenti koji imaju želju da posete Fakultet za Ribarstvo i Zaštitu Voda su više nego dobrodošli. Ukoliko želite da nas posetite putem ERASMUS programa ili na bilo koji drugi način (kratkoročna poseta ili rad na tezi) molimo stupite u kontakt sa nama (vkaspar@frov.jcu.cz, lkacerova@frov.jcu.cz) da bismo razmotrili mogućnosti vaše posete.

2) Internacionalna letnja škola

Letnja škola je četvoro-nedeljni kurs za studente osnovnih i master studija kao i za PhD studente. Održava se u Julu. Tokom kursa, imaćete priliku da radite na manjim istraživačkim projektima u veoma dobro opremljenim laboratorijama i prostorijama za izvođenje eksperimenata. Za sve ovo vreme imaćete podršku naših istraživača sa iskustvom i/ili PhD studenata sa Fakulteta za Ribarstvo i Zaštitu Voda. Upravo zbog toga, očekujemo da kandidati imaju osnovno znanje engleskog jezika. Pored rada u laboratorijama, posetićete ribnjake i druga interesantna mesta, kao sto su Český Krumlov, Ribnjak Třeboň Hld. Inc., Třeboň, Nové Hrady Fishery i tako dalje. Omanji završni simpozijum je sastavni deo ovoga kursa. U okviru simpozijuma, pred kolegama i istraživačima sa Fakulteta za Ribarstvo i Zaštitu Voda aktivno ćete prezentovati svoj rad i rezultate istoga. Ovakvo jedinstveno iskustvo obogatiće nadalje vaše studije i privatni život.

3) Master studije

Master studije iz oblasti akvakulture pripremaju profesionalce i stručnjake za srednje i više menadžerske funkcije u preduzećima i farmama za gajenje riba, za funkcije u javnom sektoru, kao i istrazivačke i obrazovne funkcije. Ove studije ih takođe kvalifikuju za pripremanje i upravljanje razvojnim i istrazivačkim projektima u oblasti ribarstva i vodene sredine.

Studenti master studija stiču znanje iz raznih oblasti akvakulture i ribarstva uključujući toksikologiju vode, hemijske procese u vodenoj sredini i hemiju materija koje zagađuju vode.

Studenti master studija mogu da se specijalizuju u oblasti reprodukcije riba i genetike riba ili u oblasti sistema u akvakulturi. Studenti su u mogućnosti da primene svoje znanje u drugačijim klimatskim uslovima i/ili na drugim vrstama riba, vodenim organizmima ili sistemima proizvodnje. Plan rada je pogodan i za studente osnovnih studija iz drugih oblasti.

Nastava je bazirana na tradicionalnoj Češkoj kulturi gajenja riba, čiji se centar nalazi u ovom regionu kao i na kvalitetnoj naučnoj bazi Fakulteta a Ribarstvo i Zaštitu Voda.

Nastavni sistem: Primenjuje se sistem kredita - ECTS. Studenti moraju da ostvare minimum 120 kredita – od kojih 105 moraju biti ostvareni iz obaveznih predmeta i 15, koji moraju biti ostvareni kroz obavezno-izborne predmete.

Mogućnosti za dalje školovanje: Studenti master studija imaju mogućnost da nastave sa doktorskim studijama u oblasti Ribarstva na Fakultetu za Ribarstvo i Zaštitu Voda. Ove studije su akreditovane i na Češkom i na Engleskom jeziku.

Školarina: €20 po semestru.

Stipendije: Fakultet za Ribarstvo i Zaštitu Voda pruža smeštaj, srednje i visoke stipendije prema regulativama za stipendije Univerziteta Južne Bohemije i Fakulteta za Ribarstvo i Zaštitu Voda. Fakultet takođe pruža specijalne stipendije za talentovane studente sa odličnim rezultatima i postignućima. Ukoliko ste talentovani imaćete dovoljno novca za život u Češkoj Republici u toku druge godine studija.

Prijem studenata: Standardna aplikacija na internet stranici fakulteta **http://www.frov.jcu.cz/en/co-lze-studovat-jake-obory/m-f-aquaculture**

Doktorske studije (Ph.D.)

Akreditovane doktorske studije u oblasti Ribarstva su interesantna mogućnost za nastavak sticanja novog naučnog znanja na osnovu individualnog plana. Ove studije produbljuju znanje i mogućnosti stečene na master studijama. Stići ćete detaljnu profesionalnu strucnost, najčešće u kontekstu trenutnih naučnih i istraživačkih trendova u akvakulturi, genetici i reprodukciji riba, ribarstva, zaštite voda, hidrobiologije, toksikologije voda, itd.

Studenti su deo radnih grupa u naučnim laboratorijama našeg fakulteta. Grupe se razlikuju prema istrazivačkim temama. Osim timskog rada, od studenata se traži kreativnost i samostalan rad kao i da objave rezultate istraživanja u naučnoj publikaciji.

Cilj studija je da pripremi studente za njihova buduća istraživanja, naučnu i pedagošku karijeru na Univerzitetima ili istraživačkim institucijama, kao i za upravljačke funkcije u kompanijama.

Nastavni sistem: Primenjuje se sistem kredita. Studije se zasnivaju na individualnom nastavnom planu koji se sastoji od naucnog i istrazivačkod dela. Student pravi individualni nastavni plan posle konsultacija sa njegovim/njenim mentorom. Odbor odseka mora da odobri nastavni plan. Individualni nastavni plan se pravi na osnovu teme i istrazivačkih aktivnosti, teze i prethodnog obrazovanja.

Mogućnosti za budućnost: Doktoranti sa odličnim rezultatima obično postaju članovi istraživačkog tima fakulteta ili apliciraju za post doktorske pozicije

Školarina: €20 po semestru.

Stipendije: Studenti dobijaju stipendiju koja pokriva troškove života u Češkoj Republici. Osim stipendije, studenti imaju mogućnost da rade na našem fakultetu dok studiraju.

Prijem studenata: Pogledajte ponuđene teme za disertacije za narednu akademsku godinu i izaberite jednu. Kontaktirajte mentora koji je predložio temu. Ukoliko dođe do obostranog sporazuma, popunite aplikacioni formular za doktorske studije

http://www.frov.jcu.cz/en/co-lze-studovat-jake-obory/d-f-fishery

Abstract

The Faculty of Fisheries and Protection of Waters (FFPW) of the University of South Bohemia (USB) in České Budějovice, Czech Republic, is one of the most complex working places within Europe with bachelor, master and doctoral studies, scientific and applied research, inaugural and professorial rights in the field of fisheries and protection of waters. The faculty has experimental background for study and research of aquaculture, hydrobiology, toxicology, fish diseases, reproduction, genetics, fish and crayfish culture and a unique fishery library.

The Faculty offers to students from abroad various programs:

1) **short term fellowships (http://www.frov.jcu.cz/cs/exchange-students/exchange-students),**
2) **international summer school (http://www.frov.jcu.cz/en/mezinarodni-letni-skoly/summer-fishery-school-2013),**
3) **master study (http://www.frov.jcu.cz/en/co-lze-studovat-jake-obory/m-f-aquaculture),**

4) doctoral study (http://www.frov.jcu.cz/en/co-lze-studovat-jake-obory/d-f-fishery).

Short term fellowships

Students having an interest to visit Faculty of Fisheries and Protection of Waters are warmly welcome. In case of your interest to visit us through ERASMUS program or another type of visit (short term visit or work on diploma thesis) do not hesitate to contact us (vkaspar@frov.jcu.cz, lkacerova@frov.jcu.cz) to discuss possibilities.

International summer school

The Summer School is a four-week course for bachelor, master and PhD. students organised in July. During the course you will be working on a small research project in very well-equipped laboratories and experimental facilities under the guidance of experienced researchers and/or Ph.D students of the FFPW. That is why we expect candidates with at least partial knowledge of the English language. The work in laboratories is completed with excursions to fish farms and visits to other interesting places, such as the city of Český Krumlov, Fisheries Třeboň Hld. Inc., the city of Třeboň, Nové Hrady Fishery and so on. A small final symposium makes an integral part of this course. Within the symposium, you will actively present in front of colleagues and researchers of the FFPW USB a short contribution concerning your work and results obtained Such unique experience enriches you for your further study and personal life.

3) Master study

Master study of the Aquaculture field prepares professionals and specialists for middle and higher management in enterprises and farms focused on fish culture, for positions in public service, research, education, developmental and research project preparation and management concerning fisheries and water environment. Graduates obtain knowledge from various fields of aquaculture and fishery including aquatic toxicology, chemical processes in aquatic environment and chemistry of compounds polluting waters.

Students can specialize in the field of fish reproduction and genetics or special aquaculture systems. Graduates will be able to apply their knowledge in other climatic conditions and/or in other fish species and water organisms or production systems. Study plan was constructed to enable education also to bachelors' graduates of other fields. Teaching activities have a pillar in traditional Czech fish pond culture whose centre is located in this region and in a quality of scientific base at Faculty of Fisheries and Protection of Waters.

Study system: Credit system implemented. Students have to get a minimum of 120 credits – of this number, 105 credits must be acquired in compulsory subjects and 15 credits must be acquired in compulsory-selectable subjects.

Further education possibilities: The graduates can continue studying the doctoral field of study „Fishery" directly on the Faculty of Fisheries and Protection of Waters that is accredited both in the Czech and in the English language.

Tuition fees: Tuition fee is €20/semester.

Scholarships: FFPW provides accommodation, merit and premium scholarships according to scholarship regulations of USB and FFPW and special scholarships for gifted students with excellent results and extraordinary activity. If you are gifted you would have enough money to cover living costs in the Czech Republic during 2nd year of study.

Admission procedure: Use standard application form to study at a faculty **http://www.frov.jcu.cz/en/co-lze-studovat-jake-obory/m-f-aquaculture**

Doctoral study (Ph.D.)

Accredited doctoral study program in field Fishery represents an interesting possibility to continue with acquiring new scientific knowledge according to an individual study plan. This study deepens knowledge and abilities acquired in the master study in relation to detailed professional specialization usually in context with current scientific and research issues in aquaculture, fish genetics and reproduction, fisheries, water protection, hydrobiology, aquatic toxicology etc. Students will be involved in working groups in scientific laboratories of our faculty based on their research topics. Aside ability to work in team, students will be requested to show creativity and self-activity and ability to publish results in scientific literature. Aim is to prepare students for their future research, scientific or pedagogical career at universities or research institutions and management of companies.

Study system: Credit system implemented. Study is determined by an individual study plan that consists of an educational section and a research section. Individual study plan is drawn up by the student after consultation with his or her supervisor and has to be authorized by Departmental committee. Individual study plan is chosen with respect to the topic or research activities and thesis and previous education.

Further possibilities: The graduates with excellent results usually become members of research teams of faculty or apply for postdoctoral internship.

Tuition fees: Tuition fee is €20/semester.

Scholarships: Students will get scholarships to be able to cover their living costs in the Czech Republic. Besides scholarships, students will get a minimal work load at our faculty.

Admission procedure: Look at the offered themes of Ph.D. thesis for forthcoming academic year and select one of the **proposed topics**. Contact supervisor of selected topic. In case of a mutual deal, complete the application form for the doctoral study **http://www.frov.jcu.cz/en/co-lze-studovat-jake-obory/d-f-fishery.**

HIGHER EDUCATION FOR AQUACULTURE/FISHERY AT THE FACULTY OF AGRICULTURE UNIVERSITY OF BELGRADE: IMPLEMENTATION OF THE FIRST BOLOGNA REFORM – LINKING THEORY AND PRACTICE

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VISOKO OBRAZOVANJE ZA AKVAKULTURU/RIBARSTVO NA POLJOPRIVREDNOM FAKULTETU UNIVERZITETA U BEOGRADU: IMPLEMENTACIJA PRVE "BOLONJSKE" REFORME – POVEZIVANJE TEORIJE I PRAKSE

Apstrakt
U radu je opisana implementacija reforme visokog obrazovanja za Ribarstvo/Akvakulturu na Poljoprivrednom fakultetu Univerziteta u Beogradu. Proces stvaranja novih kurseva vezanih za vodene ekosisteme, njihovu zaštitu i eksploataciju i definisanje kompetencija studenata za akvakulturu na svim nivoima visokog obrazovanja (osnovne, diplomske, specijalističke i doktorske studije) omogućen je unapređenjem obrazovnih i istraživačkih objekata i sredstava, kao i bliskom komunikacijom sa proizvođačima ribe i ostalim interesnim grupama vezanim za gajenje riba.

Ključne reči: visoko obrazovanje, akvakultura, ribarstvo, reforma, Poljoprivredni fakultet, Univerzitet u Beogradu
Keywords: higher education, aquaculture, fishery, reform, Faculty of Agriculture, University of Belgrade

INTRODUCTION

Despite the fact that fish farming in Serbia is practiced for more than 150 years, modernized semi intensive carp culture is an emerging agricultural practice in Serbia in the past decade (Markovic et al., 2011). The use of extruded feed adapted to requirements of different age categories of fish, season and pond productivity, and the establishment of the first carp and trout selective breeding program, together with activities on aquatic resources preservation and remediation, required rethinking of the learning outcomes (LO) needed for modern aquaculture practice, and therefore a reform of the higher education (HE) for aquaculture/fishery. The fact that the teaching staff in Fishery of the Faculty maintained excellent relations with alumni, fish producers and other aquaculture practitioners, as well as the fact that the teaching and research staff managed to obtain several research and education projects to improve education and research facilities, made LO reconsideration a feasible task.

HISTORY

Fishery was the course that was first introduced at the Faculty of Agriculture University of Belgrade as an elective subject from 1969. It emerged from the course Zoology. In 1987 Fishery became a compulsory one-semester course in Animal Sciences. It was thought by our late professor Vera Mitrović Tutundžić, doyen of Serbian aquaculture teaching. The pre- Bologna system consisted basically of: Diploma level - 4 years, and postgraduate levels: master (2 years study and master thesis) and a doctoral thesis. Fishery and Zoology courses were the unique ones dealing with aquatic ecosystems, their protection and exploitation at the undergraduate level. Postgraduate studies were also possible in Fishery, and were actually very accepted amongst students.

THE HE REFORM

With the adoption of the Bologna Declaration in 2003 the reform process of the whole HE system in Serbia started. Courses modularization; ECTS; introduction of student mobility; 3 study levels – bachelor, Master, and PhD; definition of LO, are amongst the processes that enabled harmonization of Serbian HE with the European Higher Education Area (EHEA).

The reform process at the Faculty of Agriculture consisted of rather long discussions and critical reflections about reorganization, improvement, and need of the Serbian agricultural education to adapt to EHEA. With the assistance of the University of Hohenheim, Germany, and University of Thessaloniki, Greece, through the TEMPUS JEP project the Faculty started, and with the adoption of the Law on Higher Education in 2005, the Faculty completed its reform (Poleksic et al., 2006). The acceptance of courses modularization, introduction and allocation of ECTS, and different study levels: BSc, MSc, PhD, and Specialisation offered the opportunity to introduce courses related to aquatic biology and aquatic animals' protection and production. In this period a research area was defined as Applied Zoology and Fishery (AZA) thus profiling the research staff recruited mainly from the Faculty of Biology, from the Zootechnics Department of the Faculty of Agriculture, as well as from other faculties.

INTERNATIONALIZATION – BUILDING FACILITIES FOR AQUACULTURE PRODUCTION AND TRAINING

The renewal of the international relations with leading European institutions made possible investigation and experiments on carp nutrition and the first Serbian selective breeding program, established within the cooperation with Norwegian colleagues from

NOFIMA MARIN, former AKVAFORSK. This cooperation led to the formation of the first Fish feeding Laboratory (in Zemun within the Faculty) and a Hatchery (in Radmilovac – faculty’s school estate). Followed participation of the AZA group in one FP6 project: Water Resources Strategies and Drought Alleviation in Western Balkan Agriculture (WATERWEB) that studied Radmilovac experimental school estate aquatic resources and contributed to building of the first outdoor ponds in Radmilovac. Coordination of the FP7 REGPOT framework project “Reinforcement of sustainable aquaculture” (ROSA) with NOFIMA MARIN, Norway and HAKI, Hungary as partners, practically established the Centre for Fishery and Applied Hydrobiology, CEFAH (Markovic and Poleksic, 2009). The project brought the improved hatchery and laboratory equipment, established a Laboratory for waters quality analysis, and enabled building of the experimental carp farm at the Faculty of Agriculture University of Belgrade Experimental School Estate Radmilovac.

Recently, a new project “Little Danube” has started: a remediation of the polluted stream Sugavac and its transformation into a model of the Danube River flowing across Radmilovac. The project permitted construction of the Aquarium building for freshwater fish from the Danube region, and a whole polygon representing, to date, the lower course of the river and its ecosystems.

In addition to facilities built in Radmilovac, education projects (TEMPUS - EC and WUS - Austria) allowed foundation of the Histology laboratory and a research Microscopy laboratory as well as a Central Teaching Laboratory equipped with microscopes and computers.

Finally, modernization of equipment, laboratories foundation, and building facilities was amended with teaching staff additional education in modern teaching methodology (Poleksic et al., 2004); actually part of the teaching staff of AZA were trained in interactive teaching/learning (project with Education forum in 2003), and few years later, a group of teaching staff undergone training in e-learning methodology (WUS project, EPA Master in 2008/09). As a result, the principles of active teaching/learning methodology, including practical training are widely used by the AZA teaching staff, and up to now, there are 5 courses that are thought in the “blended” form (combining face to face and online teaching) and others that are in development, serving “at least” as a repository of the teaching material and information.

THE REFORMED CURRICULUM

Accredited in 2008 in the first round of accreditation in Serbia, together with other study programs and the Faculty as institution, the Study Program Zootechnics contains compulsory and elective courses dedicated to aquaculture and environmental, particularly aquatic ecosystems protection and exploitation.

The following tables summarize the list of courses offered at all study levels by the group from the AZA scientific area:

Bachelor level – 8 semesters 240 ECTS

subject	semester	ECTS	Status*:
Zoology	1	7	C
Environmental protection	2	5	E
Earthworm and snail farming	4	6	E
Culture of ornamental aquatic plants, invertebrates and fish	5	6	E
Fishery / Aquaculture	6	6	C
Fishery and fishery waters management	6	6	E
Technical and technological aspect in construction and equipment of aquaculture facilities	6	6	E

* C - compulsory, E - elective

Besides these, Bachelor in Zootechnics consists of all other courses needed for a BSc level in this discipline: Mathematics, Chemistry, Anatomy, Physiology, Genetics, Population genetics, Animal health protection, Agricultural mechanization, Statistics. Sociology, Economics and management group of subjects, etc…, thus providing student’s competences defined for Bachelor in Zootechnics. Concerning aquaculture related subjects: after these subjects completion the student will be able to monitor aquatic environment (by monitoring abiotic and biotic environmental factors), describe diversity, distinguish and classify ichthyofauna, fish and aquatic organisms morphology, anatomy and physiology, customarily examine the fish, plan fish farm facilities, and participate in managing the farm technological process. It should be mentioned that the course Aquaculture is offered as elective subject in the Horticulture curriculum of the Faculty.

Master level – 2 semesters 60 ECTS

subject	semester	ECTS	Status*:
Nutrition of fish and other aquatic organisms	1	7	E
Reproduction of fish and other aquatic organisms	1	7	E
Health protection of fish and other aquatic organisms	2	7	E
Production technology in aquaculture	2	7	E
Animal histology	2	7	E

* C - compulsory, E - elective

For this study level a Diploma work (15 ECTS in 2 semesters) is planned, as well as compulsory Planning of experiments and data analysis in Zootechnics (9 ECTS). Each Master specialization requires Professional practice – 3 ECTS and Study research work - 5 ECTS. Both are offered in the frame of CEFAH activities.

Specialization level – 2 semesters 60 ECTS

subject	semester	ECTS	Status*:
Fish nutrition	1	8	E
Fish reproduction	1	8	E
Fish culture	2	10	E

* C - compulsory, E - elective

Specialization work (18 ECTS in 2 semesters) is obligatory for all students together with Management of production processes in Zootechnics (8 ECTS) and Study research work (8 ECTS).

In the frame of the Master studies “Environmental protection in agriculture (EPA)” offered by the Faculty, following courses are organized by AZA staff:

Master studies Environmental protection in agriculture EPA

subject	semester	ECTS	Status*:
Water pollution and monitoring	2	4	C
Ecological aquaculture	2	4	E
Ecological farming of non domestic animals	2	4	E

* C - compulsory, E - elective

EPA teaches principles of sustainable and responsible agriculture including all aspects of resources protection and modern agricultural practice and legislation.

Doctoral study level – 6 semesters

subject	semester	ECTS	Status*:
Molecular genetics and histology of domestic and cultured animals	1	10	E
Animal Ecology	2	8	E
Fish Embryology	2	8	E
Production systems in aquaculture	3	6	E
Breeding technology in aquaculture	3	10	E
Monitoring of aquaculture systems and recipient protection	4	6	E
Fish processing	4	6	E

* C - compulsory, E - elective

In addition to the subject listed, following make a profile of a Doctoral degree in Aquaculture: Experimental statistics (7 ECTS), Sampling and data analysis in Zootechnics (10 ECTS), Animal Genetics, Selection and Breeding (8 ECTS), Research methodology (10 ECTS), Study research work (40 ECTS), and Doctoral thesis (70 ECTS).

NUMBER OF STUDENTS

The reformed curriculum in Zootechnics enrolls 70 to 90 students each year. Among them, depending of the generation, approximately 30 to 40 reach the third year in Zootechnics and learn Fishery. In addition 20 to 30 students in Horticulture take each year Aquaculture as elective course. The MSc as well as Specialization level started enrollment only last year. Up to now 9 PhD students have enrolled the reformed Doctoral curriculum. First among these students defend their theses these days, and the AZA staff is looking forward to reinforcement of our group and improvement of the teaching/learning.

HE IN AQUACULTURE - FUTURE PLANS

For the new accreditation cycle in Serbia the Faculty of Agriculture has prepared and recently submitted a revised curriculum including revised LO. After the self evaluation, analysis of students' achievements, and reconsideration of the LO/qualifications needed for responsible aquaculture practice, and counting on the mentioned staff qualifications reinforcement the AZA group will be able to offer some new courses and improve/modify the existing ones.

Also, possibilities of vocational life long learning (LLL) trainings are envisaged through one submitted projects and existing programs in teachers' education will be continued.

CONCLUSIONS

The reform process of Serbian higher education in the area of Agricultural Sciences allows studies flexibility and profiling from the BSc level, and particularly at MSc, Specialization, and PhD level. The specialization towards aquaculture was made possible with the development of a series of courses dedicated to aquatic environment, its protection and sustainable use that were incorporated in the curriculum for Animal sciences. For the new round of accreditation the AZA group has suggested some renewed courses and some new ones. Learning outcomes have been rethought and redefined.

ACKNOWLEDGMENT

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BEYOND THE LEVELS OF DOMESTICATION IN FISH: MUST ALL FARMED SPECIES BE DOMESTICATED?

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DOMESTIFIKACIJA RIBA: MORAJU LI SVE GAJENE VRSTE BITI DOMESTIFIKOVANE?

UVOD

Danas u poljoprivredi dominira nekoliko vrsta sisara koje su domestifikovane pre više od 12 000 godina (Diamond, 2002). Pet najprisutnijih vrsta (goveda, svinje, ovce, koze i konji) danas predstavljaju skoro 94% stoke sisara. Kao posledica toga, danas postoji jasna razlika između brojnih divljih (iz lova) i nekoliko domestifikovanih vrsta (proizvedenih na farmama) kojima se čovek hrani. Slično poljoprivredi, na akvakulturu se često gleda kao na jedino rešenje koje može da obezbedi više hrane iz mora obzirom da je ulov marinskih vrsta ili stabilizovan ili u opadanju od kasnih osamdesetih godina prošlog veka (Watson and Pauly, 2001; FAO, 2012). I zaista, malo je verovatno da će ribarstvo biti u mogućnosti da proizvede vise morskih prehrambenih proizvoda nego danas, *ca*. 90 miliona tona godisnje, uključujući i mora i kopnene vode (FAO, 2012). Ipak, u poređenju sa poljoprivredom, akvakultura je značajno mlađi sektor koji se veoma oslanja na prirodne izvore u proizvodnji brojnih vrsta sa promenljivim nivoom proizvodnje (Bostock et al., 2010; Jobling, 2010). Od 313 vrsta ili grupa vrsta koje su zabeležene u bazi podataka Organizacije za hranu i poljoprivredu Ujedinjenih Nacija (FAO) iz 2009-te godine a koje su gajene od 1950te, 28.4% se više nije proizvodilo u 2009. godini a 17.9% imalo je proizvodnju nižu od 100 tona. Samo 12.1% vrsta ima proizvodnju višu od 100 000 tona (Teletchea and Fontaine, 2013). Staviše, trajanje konsekutivne proizvodnje zabeležene u FAO-voj bazi podataka bilo je veoma kratko za većinu vrsta ili grupa vrsta: samo jedna godina za 10.2% i od dve do pet godina za 15.3%. Samo je 18.8% bilo gajeno više od četrdeset godina (Teletchea and Fontaine, 2013).

Da bi se bolje opisale različite strategije proizvodnje ribe (Ottolenghi et al., 2004; Welcomme et al., 2010; Klinger et al., 2013), nedavno je predložena nova klasifikacija koja se sastoji od pet nivoa “domestifikacije”, gde je 1 oznaka za najmanje a 5 oznaka za najviše domestifikovane vrste (Teletchea and Fontaine, 2013). Od 250 vrsta zabeleženih u FAO-voj bazi podataka iz 2009-te (osim isključenih vrsta), 39 pripada nivou 1 (prvi pokušaji privikavanja na uzgojnu sredinu, npr. *Anguilla rostrata*; *Scardinius erythrophthalmus)*, 75 pripada nivou 2 (ovaj nivo se takođe naziva ‘akvakultura bazirana na ulovu’, npr. *Thunnus thynnus, Aspius aspius*), 61 vrsta pripada nivou 3 (one vrste koje su ceo zivot zatočene, sa novim vrstama iz divljine, npr *Solea senegalensis, Rutilus rutilus*), 45 pripada nivou 4 (one vrste koje su ceo život zatočene, bez novih vrsta iz divljine, npr. *Pagrus pagrus, Sander lucioperca*), i 30 pripada nivou 5 (program selektivnog gajenja koji ima određen cilj, npr. *Salmo salar, Cyprinus carpio*). Od danas, 70% od 250 vrsta riba koje se nalaze na FAO-voj listi spadaju u prva tri nivoa domestifikacije koja zavise od dostupnosti divljih resursa (Ottolenghi et al., 2004). Nasuprot tome, samo nekoliko vrsta, ili tačnije populacija, se mogu smatrati potpuno domestifikovanim (Balon, 2004; Bilio, 2008), npr. životinja selektivno gajena u zatočeništvu i izmenjena u odnosu na svoje pretke je veoma značajna za ljude koji kontrolišu gajenje životinja i njihovu ishranu (Clutton-Brock, 1999), slično govedima i ovcama.

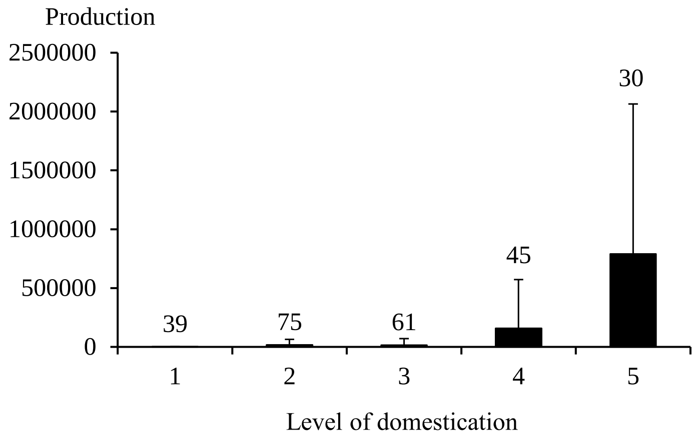
Zasnovano na ovoj novoj klasifikaciji, možemo se zapitati da li gajene vrste moraju biti domestifikovane da bi dostigle značajni nivo proizvodnje? Ili, drugim rečima, da li su nivoi proizvodnje i domestifikacija u pozitivnoj korelaciji?

MATERIJALI I METODE

Ovakve analize zahtevaju postojanje preciznih podataka o: (i) nivou domestifikacije svake vrste, (ii) globalnoj proizvodnji po vrsti, i (iii) procentualnom iznosu proizvodnje po nivou domestifikacije za svaku vrstu. Iz 2 baze podataka: ‘Teletchea and Fontaine’ iz 2013-te i FAO (http://www.fao.org/fishery/en), mogu se dobiti prve dve vrste podataka. Ipak, s obzirom na prirodu podataka koje su FAO-vi članovi i pridružene nacije dale FAO-u (Klinger et al., 2013), trenutno je nemoguće znati koliko jedna vrsta proizvodi u vezi sa njenim nivoom domestifikacije. I zaista, kao što je ranije primećeno, dostizanje određenog nivoa nužno ne znači da će cela proizvodnja biti bazirana na tom nivou (Teletchea and Fontaine, 2013). Uzmimo za primer nivo 5, osim Atlantskog lososa (*Salmo salar*) kod koga vise od 95% gajenih jedinki širom sveta dolazi iz genetski unapređenih nasada, za druge vrste procenat je dosta niži, 22% kod kalifornijske pastrmke (*Oncorhynchus mykiss*) (Gjedrem and Baranski 2009; Gjedrem, 2010). Uprkos ovim ograničenjima, mi smo privremeno ocenili moguću povezanost između nivoa domestifikacije i proizvodnje, pod pretpostavkom da je sva proizvodnja zasnovana na nivou domestifikacije za svaku vrstu (Teletchea and Fontaine, 2013).

REZULTATI

Na prvi pogled se cinilo da postoji pozitivna veza izmenju nivoa domestifikacije i nivoa proizvodnje u 2009 (Slika 1). Ipak, kada je rađena dublja analiza varijabilnosti po nivou, pronađene su velike razlike, od minimalnih do maksimalnih [0, 6972] za nivo 1, [0, 237084] za nivo 2, [0, 329972] za nivo 3, [0, 2 418821] za nivo 4 i [0, 4 159919] za nivo 5. Na primer, uprkos tome sto su klasifikovane kao nivo 2, 6 vrsta (8% vrste na ovom nivou) proizvelo je vise od 150000 tona 2009., među kojima su i vrste *Seriola* *quinqueradiata* i *Misgurnus anguillicaudatus.* Nasuprot tome, sedam vrsta (23%) klasifikovanih na najvisem nivou, proizvelo je manje od 1000 tona, među kojima je bilo 4 vrste jestera (*Acipenser* spp).



Slika 1. Poređenje nivoa proizvodnje (FAO baza podataka, vrednosti iz 2009. godine) po nivou domestifikacije (Teletchea and Fontaine, 2013) za 250 vrsta riba koje se nalaze na FAO-voj listi. Da pojasnimo, naznačene su samo pozitivne standardne devijacije. Broj iznad svakog histograma pokazuje broj vrsta po nivou.

DISKUSIJA

Ako uzmemo u obzir prirodu podataka u FAO-voj bazi podataka (Klinger et al., 2013), danas je nemoguće sigurno zaključiti da li su nivoi domestifikacije i proizvodnje pozitivno povezani, kao sto je predloženo na Slici 1. Ipak, čini se da je ceo životni ciklus u zatočeništvu pozitivno povezan sa značajnom proizvodnjom za nekoliko vrsta (15 najviše proizvođenih vrsta u 2009. su dostigle nivo 4 i 5), iako to nije uvek slučaj, najverovatnije zato što su postojale jedinke iz divljine koje su korišćene za dobijanje gameta (Ottolenghi et al., 2004; Welcomme et al., 2010). Pored same domestifikacije, razni faktori, među kojima kapital, tehnologija, regulatorna ograničenja, marketing, pozitivan ili negativan uticaj na životnu sredinu, i dostupnost odgovarajućeg prostora u vodenim i kopnenim sredinama, su takođe razlog što određene vrste mogu ili ne mogu da dostignu visoke nivoe proizvodnje (Le François et al., 2010; Suquet 2010; Klinger et al., 2013).

Analiza je ukazala na to da iako su brojne vrste daleko od procesa domestifikacije (Balon, 2004; Bilio, 2008; Teletchea and Fontaine, 2013), samo je oko 7% globalne proizvodnje riba (33 miliona tona u 2009. godini) zahtevalo unos riba iz divljine (kombinovana proizvodnja vrsta nivoa 1, 2 i 3). Ipak, ovaj procentualni iznos je verovatno dosta viši obzirom da je delić (nepoznat) proizvodnje vrsta klasifikovanih nivoima 4 i 5 takođe zahtevao unos riba iz divljine (Bartley et al., 2009; Gjedrem and Baranski, 2009). Potrebno je bolje poznavanje procenata globalne proizvodnje u akvakulturi koja je zasnovana na unosu iz divljine da bi se bolje razumeo spektar trenutne proizvodnje ribe (Klinger et al., 2013), I da bi se ocenila održivost različitih načina gajenja riba (Ottolenghi et al., 2004; Bilio, 2008).

Sve u svemu, ovi rezulati ukazuju da je u prošlih deset godina akvakultura prošla kroz sve stupnjeve kao i poljoprivreda sa jako velikom proizvodnjom u 2009. godini – tada je gajenje bilo zasnovano na nekoliko domestifikovanih vrsta. U budućnosti, industrija može da nastavi da se fokusira na nekoliko domestifikovanih ribljih vrsta, koje se uvoze u mnogim zemljama širom sveta. Industrija takođe može da se okrene diverzifikaciji među različitim vrstama primarno se fokusirajući na domestifikaciju domaćih vrsta. Prednosti i mane ova dva glavna scenarija su posebno razmatrane u radovima De Silva et al. (2009), Diana (2009), and Teletchea and Fontaine (2013).

INTRODUCTION

The agricultural world today is dominated by a few mammal species that were domesticated over the past 12 000 years (Diamond, 2002). In particular, the five major species (cattle, pig, sheep, goat and horse) represent nearly 94% of mammalian livestock today. As a result, a clear dichotomy now exists between the numerous wild (from hunting) and the few domesticated mammals (produced in farms) used for human consumption. Similar to agriculture, aquaculture is often viewed as the only solution that can provide more seafood given that harvesting wild marine stocks are either stabilizing or more probably declining since the late 1980s (Watson and Pauly, 2001; FAO, 2012). Indeed, it is unlikely that fisheries will be able to supply more aquatic food products than today, *ca.* 90 million tones per year, including both marine and inland captures (FAO, 2012). Yet, compared to agriculture, aquaculture is a considerably younger sector that relies strongly on natural sources to farm numerous species with various production levels (Bostock et al., 2010; Jobling, 2010).Among the 313 species or group of species recorded in the United Nation's Food and Agriculture Organization (FAO) database in 2009, which have been farmed at one time since 1950, 28.4% were no longer being produced in 2009 and 17.9% produced less than 100 t. Only 12.1% produced more than 100 000 t (Teletchea and Fontaine, 2013). In addition, and more importantly, the duration of consecutive production recorded in the FAO database was very short for most species or groups of species, only one year for 10.2% and from two to five years for 15.3%; only 18.8% have been farmed for more than 40 years (Teletchea and Fontaine, 2013).

To better describe the various strategies for fish production (Ottolenghi et al., 2004; Welcomme et al., 2010; Klinger et al., 2013), a new classification comprising five levels of "domestication" with 1 being the least to 5 being the most domesticated was recently proposed (Teletchea and Fontaine, 2013). Among the 250 species recorded in the FAO database in 2009 (*i.e.*, excluding group of species), 39 belong to the level 1 (first trials of acclimatization to the culture environment, *e.g., Anguilla rostrata, Scardinius erythrophthalmus*, 75 to the level 2 (also known as capture-based aquaculture, *e.g., Thunnus thynnus, Aspius aspius*), 61 to the level 3 (entire life cycle closed in captivity with wild inputs, *e.g., Solea senegalensis, Rutilus rutilus*), 45 to the level 4 (entire life cycle closed in captivity without wild inputs, *e.g., Pagrus pagrus, Sander lucioperca*), and 30 to the level 5 (selective breeding programme is used focusing on specific goal, *e.g., Salmo salar, Cyprinus carpio*). As of now, 70% of the 250 species listed in the FAO belong to the first three levels of domestication thatdepend on the availability of wild resource (Ottolenghi et al., 2004). In contrast, only a few species, or more accurately populations, can be considered truly domesticated (Balon, 2004;Bilio, 2008), *i.e.*, an animal selectively bred in captivity and modified from its wild ancestors making it more useful to humans who control the animal's breeding and food supply (Clutton-Brock, 1999), similar to cattle or sheep.

Based on this new classification, one may ask if farmed species must be domesticated to reach a significant production, or in other terms, whether the levels of production and domestication are positively correlated.

MATERIALS AND METHODS

Such analyses require having accurate data on: (i) the level of domestication for each species, (ii) the global production per species, and (iii) the percentage of the production per domestication level for each species. Based on the databases of Teletchea and Fontaine (2013) and FAO (http://www.fao.org/fishery/en) respectively, the first two kinds of data are available. Yet, given the actual nature of data provided to the FAO by its members and associated nations Klinger et al., 2013), it is currently impossible to know how much of the production of a species is based on each domestication level. Indeed, as already noticed, reaching a particular level does not necessary imply that the entire production is based on this level (Teletchea and Fontaine, 2013). For instance for the level 5, except for the Atlantic salmon (*Salmo salar*) for which more than 95% of farmed individuals worldwide is coming from genetically improved stocks, for other species the percentage is generally much lower, such as 22% in rainbow trout (*Oncorhynchus mykiss*) (Gjedrem and Baranski 2009; Gjedrem, 2010). Despite these limits, we tentatively estimated the possible relation between the levels of domestication and production, assuming that all production is based on the level of domestication for each species (Teletchea and Fontaine, 2013).

RESULTS

At first sight, it seemed that there was a positive link between the levels of domestication and the levels of production in 2009 (Figure 1). Yet, when analyzing more deeply the variability per level, large differences were found, ranging from a min and a max of [0, 6972] for the level 1, [0, 237084] for the level 2, [0, 329972] for the level 3, [0, 2 418821] for the level 4 and [0, 4 159919] for the level 5. For instance, despite being classified at the level 2, six species (8% of the species at this level) produced more than 150000 tonnes in 2009, among which Japanese amberjack (*Seriola quinqueradiata*) and pond loach (*Misgurnus anguillicaudatus*). Conversely, seven species (23%) classified at the highest level produced less than 1000 tonnes, among which four species of sturgeons (*Acipenser* spp).

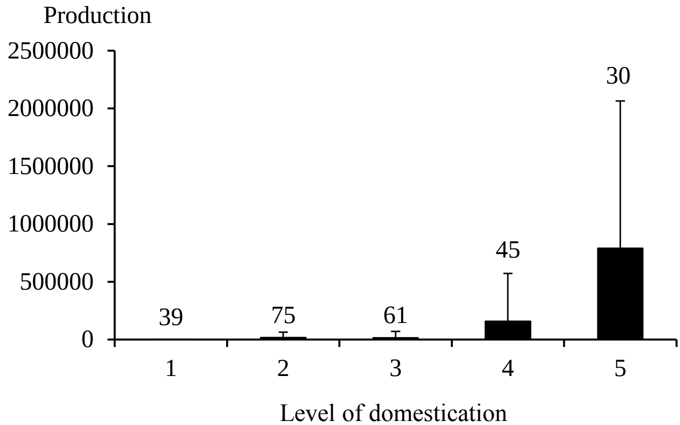


Figure 1. Comparison of the level of production (FAO database, 2009 values) per level of domestication (Teletchea and Fontaine, 2013) for the 250 finfish species listed in the FAO. For clarity, only positive standard deviations are indicated. The number over each histogram indicates the number of species per level.

DISCUSSION

Given the actual nature of the data in the FAO database (Klinger et al., 2013), it is today impossible to definitely conclude whether the levels of domestication and production are positively linked, as suggested in the Figure 1. Nevertheless, it seems that fully closing the life cycle in captivity was positively related with a significant production forseveral species (the top 15 most produced species in 2009 all have reached levels 4 or 5), albeitit was not always the case, more probably because wild resource was available for seeding (Ottolenghi et al., 2004; Welcomme et al., 2010). Besides domestication *per se*, various factors, among which capital, technology, regulatory constraints, marketing, environmental externalities, and the availability of suitable space in aquatic and terrestrial environments, also explain why a given species may or may not reach a significant production (Le François et al., 2010; Suquet 2010; Klinger et al., 2013).

The present analysis also allowed highlighting that even though numerous species are far from being domesticated (Balon, 2004; Bilio, 2008; Teletchea and Fontaine, 2013), only about 7% of the global finfish production (33 million tons in 2009) seemed to require wild inputs (production of levels 1, 2 and 3 combined). Yet, this percentage is most likely much higher given that a fraction (unknown) of the production of the species classified at levels 4 and 5 also relied on wild inputs (Bartley et al., 2009; Gjedrem and Baranski, 2009). A better understanding of the percentage of the global aquaculture production that is based on wild inputs is much needed in order to better traduce the current spectrum of finfish production (Klinger et al., 2013), and evaluate the sustainability of different farming practices (Ottolenghi et al., 2004; Bilio, 2008).

Altogether, these results imply that aquaculture has followed nearly the same path as agriculture in the past decades with the bulk of the production in 2009based on the farming of a few domesticated finfish species. In the future, the industry might continue to focus on few truly domesticated species, which have generally been imported in numerous countries worldwide, or really proceeds with inter-specific diversification by focusing primarily on the domestication of native species. The advantages and drawbacks of these two main scenarios are discussed notably in De Silva et al. (2009), Diana (2009), and Teletchea and Fontaine (2013).

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CONTROLLED REPRODUCTION OF PIKEPERCH, SANDER LUCIOPERCA (L.) – ACHIEVEMENTS AND PROSPECTS

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KONTROLISANA REPRODUKCIJA SMUĐA, *SANDER LUCIOPERCA* (L.) – DOSTIGNUĆA I IZGLEDI ZA BUDUĆNOST

Apstrakt

Smuđ, *Sander lucioperca*, je vrsta ribe koja ima visoku komercijalnu vrednost na Evropskom tržištu i jedan je od najboljih kandidata za intenzivnu proizvodnju u slatkim vodama. Do sada su se mnogobrojne studije bavile različitim aspektima razmnožavanja smuđa. Ipak, još uvek postoje mnogi aspekti koji nisu proučeni, koji su nejasni i veoma često nepredvidivi. To je ono što čini ovaj deo akvakulture veoma teškim za mnoge odgajivače riba i naučnike. Stoga je i cilj ovog članka da da precizan osvrt na trenutna dostignuća u kontrolisanoj reprodukciji smuđa kao i da ukaže na nove načine istraživanja u ovom polju. Da bi se dao opis trenutnog statusa veštačke reprodukcije smuđa opisani su: trenutno stanje hormnonske stimulacije ovulacije i spermijacije, nova klasifikacija stupnjeva sazrevanja, metod za procenu kvaliteta jaja kao i protokoli za tretman jaja.

Još uvek jako malo znamo o nekim aspektima kontrolisane reprodukcije smuđa uprkos veoma intenzivnim istraživanjima. Rezultati objavljeni u poslednje tri godine pokazuju da još mnogo treba da se radi na razmatranju ove faze proizvodnje smuđa u akvakulturi.

Jedan od glavnih problema je, još uvek, optimizacija hormonalne stimulacije i mužjaka i ženki. Razvoj protokola za hormnonsku indukciju ovulacije može da prouzrokuje ne samo usklađivanje i predviđanje trenutka ovulacije već i kontrolu nad kvalitetom jaja. Prema najnovijim nalazima efekta hormonske stimulacije i temperature na period latencije može se zaključiti da kombinovana stimulacija temperaturom i hormonskim agentima može da postane dobra metoda u kontroli ovulacije ove vrste. Ipak, potrebno je detaljnije istražiti finalno sazrevanje jajnih ćelija (process koji između ostalog uključuje migraciju vezikula gaminativnih ćelija i njihovu razgradnju [GVBD]) i efekate različitih doza i različitih preparata na datoj temperaturi i uslovima gajenja. Ovaj

aspekat je još važniji u gajenim nasadima koji su u potpunosti zavisni od uslova koje obezbeđuju farmeri.

Što se tiče daljih istraživanja gajenih nasada potrebno je razviti posebne protokole reprodukcije za sve uslove gajenja. Pored stimulacije ovulacije, pažnju treba posvetiti i stimulaciji spermijacije koja predstavlja veliki problem kod ove vrste (lični podatak).

Opšti rezultat reprodukcije smuđa se takođe može poboljšati primenom različitih metoda za in vitro oplođenje. Protokoli za tretman jaja posle oplođenja takođe treba da se unaprede. Iskustvo nekih naučnika ukazuje na to da su protokoli razvijeni u laboratorijama nedovoljno precizni i manje efekasni u određenim komercijalnim uslovima. Zbog toga je potrebno posebno ispitati transfer metoda razvijenih u laboratorijama na uslove komercijalne proizvodnje.

Ključne reči: Sander lucioperca, reprodukcija, hormonalni stimulacija
Keywords: Sander lucioperca, reproduction, hormonal stimulation

INTRODUCTION

The pikeperch, *Sander lucioperca,* is a species with a very high commercial value on the European market and it is one of the most promising candidates for intensive freshwater aquaculture production. It resulted in recent years in increased scientific activity aiming at the artificial reproduction, egg treatment, larvae and juveniles intensive rearing (e.g. Kestemont and Mélard 2000, Kestemont et al. 2007, Żarski et al. 2012a, 2012b, 2013). Among the production steps, one of the main bottleneck in this species is the control over the reproduction which directly affect effectiveness of further culture procedures (e.g., Kucharczyk et al. 2007, Zakęś and Demska-Zakęś 2009, Żarski et al. 2012a).

To this date numerous studies aimed at different aspects of reproduction of pikeperch. However, as considering controlled reproduction there is still many aspects which were not studied or which remains unclear and very often unpredictable what makes this step of aquaculture quite difficult for many fish farmers and scientists. Therefore, the aim of this article is to concisely review the current achievements in controlled reproduction of pikeperch as well as to point out the probable ways of further research in this field.

CURRENT ACHIEVEMENTS IN CONTROLLED REPRODUCTION OF PIKEPERCH

Hormonal stimulation

One of the biggest problem in controlled reproduction of pikeperch is to obtain high quality 'dry' eggs which are necessary for further procedures of *in vitro* fertilization. To this end, initially various kinds and doses of hormonal preparations in stimulation of ovulation in wild or pond reared females were tested (e.g., Kucharczyk et al. 2007, Zakęś and Demska-Zakęś 2009). This resulted in designation of the most suitable hormonal preparations for induction of ovulation in this species. According to the literature review provided by Zakęś and Demska-Zakęś (2009) the most suitable for ovulation induction was the hCG (human chorionic gonadotropin) and the gonadoliberine analogues (GnRHa), which are commonly used in aquaculture of cyprinids (e.g. Kucharczyk et al. 2008), were less efficient. However, in a recent study Żarski et al. (2012c) suggested that effectiveness of hormonal preparation (mostly GnRHa) was dependent on the maturity stage of the fish. The fish more advanced in maturation responded for hormonal treatment (with GnRHa) more efficiently. Nevertheless, the hormonal injection with hCG seems to be still the most suitable for controlled reproduction in pikeperch while the application of GnRHa was characterized by a lower efficiency in stimulation of ovulation as comparing to hCG (Kristan et al. 2012a, Żarski et al. 2012c).

One of the less studied aspect as considering hormonal treatment in aquaculture is the stimulation of spermiation with application the hormonal agents. In recent years, few studies have been made to improve the sperm quantity and quality in freshwater fishes by hormonal therapy (e.g. Cejko et al. 2010). In the case of pikeperch it was reported that males usually spermiate without the need of hormonal treatment (Kucharczyk et al. 2007). However, Żarski et al. (2012c) reported that hormonal stimulation generally improved the sperm motility regardless the hormonal preparation used (hCGor GnRHa).

Synchronization of ovulation

The other obstacle in controlled reproduction of pikeperch is prediction of moment of ovulation (Żarski et al. 2012a). Especially, when pikeperch females are able to release the eggs in the tank even without the presence of the males (e.g., Kucharczyk et al. 2007, Żarski et al. 2012a). It was obvious that the moment of ovulation was strictly related with the maturation stage of the females, which was always determined before the hormonal injection, analogously to the protocols widely applied for cyprinids (see e.g. Brzuska 1979, Kucharczyk et al. 2007, Zakęś and Demska-Zakęś 2009). However, it still did not allow to predict moment of ovulation accurately. Therefore, Żarski et al. (2012a), after the extensive studies on Eurasian perch, *Perca fluviatilis* L., which allowed the development of new classification of maturation stages for freshwater percids (Żarski et al. 2011), implicated (with minor changes) the new system of maturational stages for pikeperch (see Żarski et al. 2012a). This ‘new system’ allowed considerable better synchronization of ovulation and much more precise prediction of ovulation in pikeperch. Also, the results obtained by those authors suggested that even effectiveness of hCG was strictly dependent on the maturation stage of females, where hCG treatment of less advanced fish (stage I and II of classification given by Żarski et al. 2012a) affected lower survival rate of embryos. Such observation explained in some extent variable embryonic survival of eggs obtained from hormonally stimulated pikeperch. However, the variable egg quality still widely observed during controlled reproduction of this species indicates that there is much more work needed to develop efficient hormonal treatment protocols.

Egg quality evaluation

Possibility of evaluation of the egg quality in aquaculture is of obvious importance (e.g. Bobe and Labbe 2010). It allows for evaluation of the effectiveness of culture (e.g. photothermal regime, feeding) and reproductive (e.g. hormonal treatment) protocols. On the other hand, fast, easy and objective egg quality indicator applicable before the *in vitro* fertilization may allow to choose only the highest quality eggs for further procedures. Additionally, such egg quality indicator would be very useful for scientists who working on the improvement of reproductive protocols and strict breeding procedures (e.g. gene banking, selective breeding). Application of high quality eggs for fertilization trials may save time and very often precious material (e.g. cryopreserved sperm). To date, the embryonic survival was the most widely applied egg quality indicator in pikeperch (e.g. Kucharczyk et al. 2007, Zakęś and Demska-Zakęś 2009, Żarski et al.

2012a). However, until recently the earliest possible time allowing determination of egg quality was 72 h post fertilization (Żarski et al. 2012a). The most recently Żarski et al. (2012b), during observation of the egg swelling process, recorded that cortical reaction intensity (observed between 3 and 5 min post activation) was an objective and reliable egg quality indicator. It allowed for considerably precise evaluation of egg quality before fertilization of particular egg batches and facilitated hatchery work with pikeperch eggs (for details see: Żarski et al. 2012b).

Eggs fertilization and adhesiveness elimination

The effectiveness of *in vitro* fertilization protocols depends, among others, on the activating medium used and time of contact sperm with water (e.g. Żarski et al. 2012d) as well as sperm-to-egg ratio (e.g., Kristan et al. 2012b). In the case of pikeperch only the latter variable was studied to date. It was found that during fertilization protocol 100,000 of spermatozoa should be used per each egg (Kristan et al. 2012b). There is still missing many data considering different activating medium as well as necessary time of contact of gametes with each other. Until now, only 'hatchery water' was used for *in vitro* fertilization and no data on the egg activation period (what was found to be crucial for fertilization effectiveness in perch by Żarski et al. 2012d) or other fertilization protocols (e.g. different activating solutions) are available.

In the case of pikeperch eggs, before the incubation in commonly used incubating devices (commonly known as 'Weiss' or 'Zug' jars), it is necessary to remove the adhesive layer of the eggs. For that purpose few methods were developed, where the most commonly applied were tannic acid (sometimes preceded by immersion in Woynarovich solution), talc with salt or milk, milk or clay (see e.g. Zakęś and Demska-Zakęś et al. 2005, Bokor et al. 2008, Żarski et al. 2013). All the methods were based on the treatment of the eggs few minutes following fertilization. However, Żarski et al. (2013) reported, that the effectiveness of the eggs unsticking procedures depends on the time following egg activation. The authors has proven that the tannic acid was the most effective after the egg finished their swelling period (water-hardening period), i.e. 30 minutes following activation. Application of the tannic acid for the elimination of the adhesion in pikeperch eggs 30minutes following egg activation allowed to shorten the period of eggs immersion (down to 1 min) with the average concentration (0.75 g L^{-1}) of tannin. Until that time the usual immersion time (allowing moderate effectiveness of eggs unsticking) was 5min with concentration of 0.5 to 10 g L^{-1} (Demska-Zakęś et al. 2005) what was reported to affect hatching rate (due to the chorion hardening caused by the tannic acid). However, the most recently the enzyme (Alcalase) treatment was successfully applied in laboratory condition (Kristan et al. 2012c). This proofs that the methods of eggs unsticking is still developing. Existence of few effective methods will create very convenient situation in the future where farmers will be having choice to use the most preferable by them protocol.

SOME PROSPECTS OF THE RESEARCH AND DEVELOPMENT IN CONTROLLED REPRODUCTION OF PIKEPERCH

Despite quite intensive research activities at improvement of the pikeperch controlled reproduction it is still very little known about some aspects. The results published within last three years has shown that there is still many work to be done as considering this phase of aquacultural production of this species.

One of the main problem is still the optimization of hormonal stimulation of both, males and females. The development of the protocols for hormonal induction of ovulation may allow not only the higher synchronization and prediction of moment of ovulation but also the control over the egg quality. According to the latest findings of hormonal stimulation (Kristan et al. 2012a) and temperature effect on latency time (Żarski et al. 2012c) it may be suggested that combined stimulation with temperature and hormonal agents may become a valuable tool in control of ovulation in this species. However, it require more detailed investigation of final oocyte maturation (process involving among others germinal vesicle migration and its break-down [GVBD]; Nagahama and Yamashita 2008) and the effect of different doses and different preparations at a given temperature and culture conditions. This aspect is even more important in cultured stocks which are totally subjected to the conditions provided by the farmers.

As considering the cultured stocks it has to be pointed out, that in the future research a specific reproductive protocols should be developed for each culture conditions. Despite the importance of stimulation of ovulation a special attention should be paid to the stimulation of spermiation, since the sperm of cultured fish is very often characterized by low motility rate (20-30%) (unpublished data).

The general reproduction outcome of pikeperch may be also improved by the application of different activating solutions for *in vitro* fertilization. This step of reproduction was not studied to date in this species. Additionally, the egg treatment protocols following fertilization needs improvements as well. In some specific cases, during semi-commercial conditions even 1 min immersion in tannic acid (as described for laboratory conditions by Żarski et al. 2013) was reported to have negative effect on the hatching rate (U. Ljubobratovic, personal communication). On the other hand, the trials under semi commercial conditions of the Alcalase treatment proved also to be less efficient as compared to that reported by Kristan et al. (2012c) for laboratory conditions (personal observation). It is therefore needed to make the critical trials on the transfer of the methods developed under laboratory conditions to the commercial scale.

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COMBINATION OF INTENSIVE (RAS) AND EXTENSIVE (POND) AQUACULTURE FOR JUVENILE PRODUCTION IN PIKEPERCH (*SANDER LUCIOPERCA*)

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KOMBINOVANJE INTENZIVNE (RAS) I EKSTEZIVNE (RIBNJAK) AKVAKULTURE ZA PROIZVODNJU MLAĐI SMUĐA (*SANDER LUCIOPERCA*)

Apstrakt

Ovaj prilog daje kratak osvrt na naše rezultate u oblasti gajenja mlađi smuđa. Opisuju se sve faze gajenja, do dostizanja krajnje telesne težine ribe od 75g: 1) gajenje larvi i mlađi u ribnjaku, 2) izlov mlađi iz ribnjaka, 3) privikavanje mlađi na recirkulacioni sistem (RAS), 4) gajenje mlađi u RAS-u u trajanju od 90 dana, 5) nasađivanje mlađi iz RAS-a u ribnjake, 6) gajenje mlađi u jezerima u toku zime i proleća, 7) izlov mlađi iz ribnjaka i privikavanje na uslove RAS-a i 8) gajenje u RAS-u. Svaka faza gajenja je ocenjena istim parametrima proizvodnje: krajnjim TL (mm) i težina W (g), specifičnom stopom rasta (SGR_W in $\%.d^{-1}$), stopom preživljavanja (S u %), stopom kanibalizma (C u %) i koeficijentom konverzije hrane (FCR) ukoliko se primenjuje veštačka hrana.

Iniciajalno gajenje u ribnjaku je dalo veoma dobre i obećavajuće rezultate kada je reč o specifičnoj stopi rasta (SGR_W= 20.3 $\%.d^{-1}$) i preživljavanja (27.4%). Veoma velika efikasnost kao što je visoka stopa preživljavanja (78.5%) se pokazala kod mlađi koja je posle ribnjaka privikavana na RAS. Kombinacija gajenja u ribnjaku i RAS sistemu obezbeđuje mlađ za stabilno i efikasno gajenje u RAS sistemu. Njega karakteriše visoka stopa preživljavanja (S = 88.7 – 97.5 %) i visoka stopa rasta (SGR = 1.8 - 4.2 $\%.d^{-1}$). Iako je naredna grupa mlađi gajena u uslovima ribnjaka u toku zimske sezone imala nižu stopu preživljavanja (65%), ovaj sistem može da omogući regulisanje proizvodnje mlađi u vremenu.

Rezultati ove studije su pokazali da kombinacija gajenja mlađi smuđa u ribnjacima i RAS sistemima može da proizvede visoko kvalitetnu mlađ sa mogućnošcu da se proizvodnja vremenski reguliše.

Ključne reči: Sander lucioperca, akvakultura, efikasnost, ribnjak, RAS.
Key words: Sander lucioperca, aquaculture, efficiency, pond, RAS

INTRODUCTION

The pikeperch with delicate flesh and attractiveness as a game fish is among the most valuable freshwater fish in Europe and highly sought by the European market. The major supply of marketable pikeperch is provided by natural fisheries. Unfortunately, pikeperch catches are drastically decreasing in Europe due to overfishing and the decline of wild stock. Current pikeperch market is undersupplied in most European countries leading to the double or triple price of marketable fish. Increasing interest and expansion of aquaculture activity in pikeperch can be recorded from 1990's. Therefore, extensive and intensive pikeperch aquaculture has been developed during the past 20 years in Europe. However, the most of pikeperch farms has still a lot of technology problems with optimization of broodstock husbandry, reproduction and production of high-quality larvae and juveniles. The aim of our study was to establish and evaluate the complete system of juvenile pikeperch production mainly for intensive aquaculture with diversification of production in the time.

MATERIAL AND METHODS

This abstract summarizes our results related to pikeperch juvenile culture included following phases up to final fish body 75g: 1) larval and juvenile pond culture, 2) harvesting of juveniles from ponds, 3) weaning of juveniles in RAS, 4) 90 days juvenile culture under RAS, 5) stocking of juveniles from RAS to pond conditions, 6) pond juvenile culture during winter and spring, 7) harvesting and weaning of juveniles from pond into RAS conditions and 8) ongrowing culture under RAS. Each phase culture was evaluated by same production parameters: final TL (mm) and weight W (g), specific growth rate (SGR_W in $\%.d^{-1}$), survival rate (S in %), cannibalism rate (C in %) and food conversation rate (FCR) if artificial food was applied.

1) Larval and juvenile pond culture was performed in 8 ponds (with area 0.2 – 1.54 ha and initial density 200 000 – 400 000 larvae.ha^{-1}). The feeding and growth of fish and food supply were studied in all used ponds at one week interval. According actual fish size and food supply, the harvesting term was established in each pond during last inspection, when food supply was decreasing. Average duration of this culture phase was 35 days when harvesting of ponds was done 7-5 days after last inspection.

2) All fish were gently caught by nets in the outlet channel during **the harvesting of juveniles from each pond**.

3) Weaning of juveniles in RAS was done according to the study Policar et al. (2013) when pikeperch were weaned with frozen chironomid larvae (*Chironomus plumosus*), its combination with artificial food (INICIO Plus 0.8 – 1.1mm) and then exclusively with artificial food. All survival fish were weaned after 12-days adaptation.

4) In total, 100 800 weaned fish were stocked with initial stocking density 8 fish.L^{-1} into 18 rearing tanks (volume 700 L) for **90days juvenile culture under RAS**. Fish were fed with INICIO food (1.1, 1.5, 2 mm with feeding rate 10, 7.5, 5%) and kept un-

der optimal temperature (22 – 24 °C) and oxygen saturation (100 %). Fish grading was applied each 10 days for the elimination of cannibalism rate.

5) **Stocking of juveniles from RAS to pond conditions** was performed for time diversification of pikeperch production in the time. In total, 3 000 pikeperch were stocked into three ponds with identical area 0.15 ha (1 000 fish per each pond). Forge fish (*Pseudorasbora parva*, TL= 36.6±9.4 mm and W= 0.5±0.1 g) was stocked in all ponds with initial density 200 000 fish per each pond such as prey fish for pikeperch.

6) **Pond juvenile culture** took place **during winter and spring** from Sept.15, 2011 till April 20, 2012 (208 days) under natural conditions (average temperature= 5.6 ± 4.2 °C and oxygen saturation= 87 ± 25 %).

7) **Harvesting and weaning of juveniles from pond into RAS conditions** was performed after successful pond culture during winter and spring. All surviving juveniles were harvested by nets, counted and disinfected with formaldehyde (dose: 15 $ml.100\ L^{-1}$ and exposure time: 20 min.). In total, 1800 juveniles were stocked into three tanks (700 L) within RAS under identical initial fish density 0.86 $fish.L^{-1}$. Fish were exclusively fed with artificial food (INICO 2 mm with feeding rate 2%).

8) **Ongrowing culture under RAS** was done in two ways such as: continued ongrowing culture immediately after 90days juvenile culture under RAS (45 days) and ongrowing culture after pond culture (42 days). Both these rearing were performed up to 75g fish, when fish were produced in two different time – the first at the end of October 2011 and the second at the beginning of July 2012. These cultures were performed under closed same optimal controlled conditions for pikeperch.

RESULTS

Production parameters from all tested culture phases are presented in the following Table. Initial pond culture showed very good and promising results related to growth (SGR_W= 20.3$\%.d^{-1}$) and survival (27.4%) rates. Very good efficiency such as high survival rate (78.5%) was found during of the weaning of pond-cultured juveniles under RAS. The combination of pond and RAS culture produces juveniles for stable and effective RAS culture characterized by high survival (S = 88.7 – 97.5 %) and growth rate (SGR = 1.8 - 4.2 $\%.d^{-1}$). Next juvenile culture under pond conditions during cold season caused lower fish survival rate (65%), however this system can divide juvenile production in the time.

Culture phase	Initial TL (mm)	Initial W (g)	Final TL (mm)	Final W (g)	SGR_W ($\%.d^{-1}$)	C (%)	S (%)	FCR
Larval and juvenile pond culture (35 days)	5.2 ±0.44	0.00085 ± 0.00005	44.3 ± 0.3	0.55 ± 0.05	20.3 ± 2.4	1.0 ± 0.5	27.4 ± 10.5	——
Weaning of pond juveniles (12 days)	44.3 ± 2.1	0.55 ± 0.1	46.5 ± 2.5	0.60 ± 0.1	0.73 ± 0.2	5.0 ± 2.0	78.5 ± 8.5	1.6 ± 0.3
Juvenile culture under RAS (90 days)	46.5 ± 2.5	0.60 ± 0.1	141.4 ± 10.0	24.6 ± 5.6	4.2 ± 1.1	10.5 ± 2.5	88.7 ± 5.3	1.7 ± 0.3
Continued on growing phase under RAS (45 days)	141.4 ± 10.0	24.6 ± 5.6	220.5 ± 22.3	78.6 ± 25.2	2.6 ± 0.4	2.0 ± 0.5	95.5 ± 1.2	1.8 ± 0.3
Pond juvenile culture during winter and spring (181 days)	141.4 ± 10.0	24.6 ± 5.6	178.7 ± 15.89	46.1 ± 14.2	0.3 ± 0.05	——	65.0 ± 5.0	——
On growing culture under RAS after pond culture (28 days)	178.7 ± 15.9	46.1 ± 14.2	213.2 ± 19.9	76.2 ± 24.3	1.8 ± 0.6	1.8 ± 0.5	97.5 ± 1.0	2.0 ± 0.5

CONCLUSIONS

Results of our study showed that the combination of pond and RAS culture of pike-perch can provide high-quality juvenile production with possibility to divide production in the time.

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FISH SPERMATOZOA, PHYSICAL AND BIO-ENERGETIC INTERACTIONS WITH THEIR SURROUNDING MEDIA

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SPERMATOZOIDI RIBA, FIZIČKA I BIOENERGETSKE INTERAKCIJE SA OKOLNOM SREDINOM

Apstrakt

Kod riba sa spoljašnjim oplođenjem, spermatozoidi se prilikom mresta isporučuju rastvoreni u pratećoj semenoj tečnosti u neposrednu okolinu gde se trenutno aktiviraju pokretima bičeva i na taj način stupaju u različite fizičke interakcije. Prvo se javlja osmotski signal na membrani spermatozoida i odmah zatim se talasi prenose sa vrha ka kraju duž biča oblika pantljike/trake (umesto cilindričnog oblika kod većine vrsta), što poboljšava efikasnost kretanje unapred. Zatim, zbog prisustva ovih "peraja" na bičevima, odnos površine i zapremine ribljih spermatozoida je mnogo veći nego kod većine drugih vrsta. Ovo vodi do bolje i brže trans membranske razmene, bilo da se radi o osmotskom, jonskom, gasovitom (CO_2) ili vodenom transferu. Treće, fizička veza između bičeva i površina sa kojim oni reaguju (na primer staklo za slajdove mikroskopa) dovodi do značajnog poboljšanja njihovih pokreta dok plivaju u blizini ovakvih povrsina (kao sto je ljuska jajeta).

Što se tiče bioenergetskih aspekata, spermatozoidi riba brzo plivaju dostižući veoma visoku frekvenciju udarca biča (do 70 - 100 Hz), što podrazumeva veliku potrošnju zaliha ATP-a. Njihova stopa respiracije i proizvodnje ATP-a u mitohondrijama je previše niska u poređenju sa potrošnjom ATP-a neophodnom za pokrete bičeva, odnosno njihovih dineinskih molekula u sklopu aksoneme bičeva. Kao posledica ovoga, intraćelijski nivo ATP-a se smanjuje u toku kretanja što prevremeno narušava pokretljivost ali takođe i druge funkcije kao sto je rad jonske i vodene pumpe. Ubrzo posle (od jednog do nekoliko minuta) aktivacije, nedostatak ATP-a je toliki da pokretljivost bičeva prestaje u potpunosti. Tok ovakvog procesa se moze preokrenuti, pošto se pokazalo da se zalihe ATP-a mogu nadoknaditi u uslovimu u kojima se sprečava pokretljivost spermatozoida, što otvara mogućnost za drugu rundu pokretljivosti.

U ovoj prezentaciji prikazane su specifične odlike ribljih spermatozoide uključujući i efekte temperature na metabolizam ATP-a.

Ključne reči: spermatozoidi riba, pokretljivost, osmotski stres, sadržaj ATP
Keywords: fish spermatozoa, motility, osmotic stress, ATP content

INTRODUCTION

In fish species with external fertilization, spermatozoa, which are immotile in the male tracts, are delivered at spawning by dilution of milt (spermatozoa and their accompanying seminal fluid) into the surrounding medium where they immediately activate the movement of their flagella and therefore are submitted to various physical interactions, which regulate their motility period.

MATERIAL AND METHODS

Details on video microscopy, stroboscopy, high-speed camera recordings, measurement of high-energy compound (ATP and Creatine-Phosphate) can be found in previous publications (Cosson, 2008; Dreanno et al., 2000).

RESULTS AND DISCUSSION

Any efficient forward movement of a spermatozoon needs for its flagellum to generate waves, which should propagate from head to tip. Fish sperm are inactive in testis and seminal fluid but activate at contact with low osmotic conditions in case of fresh water fishes while in marine fishes, the hyper-osmolality (high salt concentration) of sea-water is the main activation signal. Several steps in the activation process should be distinguished temporally. As a first step, due to difference of osmolality between the seminal fluid and the surrounding medium (fresh or sea water), an osmotic (in most fish species) and ionic (K^+ ions in salmonids and chonstrosteans) signal (Morisawa & Suzuki, 1980; Alavi & Cosson, 2006; Cosson et al., 1999; Perchec-Poupard et al., 1997) is perceived at the sperm membrane level: immediately after, waves consequently start to be propagated at high beat frequency from head to tip along the ribbon shaped flagella (instead of cylindrical shape in most other species), this feature leading to affect the forward displacement efficiency of spermatozoa for hydrodynamic reasons (Cosson, 2007 ; Boryshpolets al., 2012).

At motility activation, the main signals responsible for the transfer of information from the membrane to the axoneme are involving membrane polarization, Ca^{2+} entry in the sperm cell, intracellular cAMP rise and phosphorylation of some specific protein components of the axoneme, depending on species (Cosson, 2007b). The osmolality activating signal is observed to be reversible after reversal of exposure back to initial osmolality environment (Cosson et al., 1999; Bondarenko et al., 2013). A second period of motile activity was observed in marine species as well in fresh water fish species (Cosson, 2010). In some of these species, it was shown that ability for spermatozoa to fertilize egg is saved through this experimental procedure (Linhart et al., 2010).

As a second event in the motility period, linked to the presence of some lateral extensions of the flagellar membrane so-called "fins" (see below), the surface to volume ratio of fish spermatozoa appears to be much larger than usually observed in most other species, leading to better and faster trans-membrane exchanges, thus allowing either ions, gas (CO_2) (Inaba et al., 2013) or water transfer (Cosson et al., 1999). In this respect, fish sperm cells will adapt rapidly to extreme osmotic conditions because of this large membrane surface to volume ratio when reacting as an osmometer (Cosson et al., 1999; Bondarenko et al., 2013). A third feature observed during the motility period deals with the physical relationship between flagella and interacting surfaces (glass slide or egg surface), which leads to significant changes of their swimming behavior when swimming in the vicinity of specific surfaces (Boryshpolets et al., 2012). The presence of longitudinal extensions of the flagellar membrane, so-called “fins” is specific to fish sperm and were shown to improve swimming efficiency (Gillies et al., 2012). Nevertheless, the flagellar membrane appears partly damaged during and at the end of the motility period, due to osmolarity stress imposed at activation step: some looping effect appears at the distal tip of flagellum while blebs are frequently observed along the flagellar membrane (Perchec et al., 1996). Obviously these defects contribute to lower the swimming efficiency of the flagellum and reduce the duration of the motility period.

As a general rule and for additional reasons, duration of the motility period lasts for short in case of fish spermatozoa as compared to other species (Cosson, 2010). Right after activation, fish sperm flagella beat at frequency up to 70-100 Hz, leading to a translating movement at high velocity; regarding bio-energetic aspects, these features imply a fast consumption of the sperm ATP stores and motility of fish sperm mostly relies on this ATP store (Cosson, 2010 & 2013). Fish sperm structure belongs to sperm with “simple” structure called “aquasperm”, which mostly consists of nucleus and mitochondrion localized at the proximal base of the flagellum (Cosson, 2007b). Also in fish sperm, the respiration rate and consequently, the rate of ATP production by mitochondria are too low as compared to their high ATP consumption rate by flagellar dynein-ATPase motors (Cosson, 2010). As a consequence, intracellular ATP level decreases rapidly during the motility period, which precociously impairs motility, as well as other functions such as ionic or water pump. Within a short period after activation (one to several minutes, depending on species), the lack of ATP and intracellular ionic concentration both lead to conditions where flagellar motility fully stops (Cosson, 2010).

Transiently after motility activation, a gradient of ATP concentration (and other energetic compounds such as Creatine-phosphate) gradually becomes established from the base of flagella, where mitochondria producing ATP are located, to the distal tip. Consequently, such unequal distribution of ATP gives rise to a situation where waves develop with normal amplitude in the proximal flagellum but become more and more dampened in the distal part, prior to full stop.

Such decrease of internal ATP concentration will lead to a decrease in value of most swimming parameters describing the motility: the wave amplitude gradually decreases (see fig. 1), the beat frequency also decreases but also the portion of flagella where waves develop become more and more restricted to the proximal part (in the vicinity of the head) and consequently the number of waves along the flagellum is more and more restricted. Altogether, such decrease of all parameters will combine in such a way that global velocity of forward displacement will gradually and significantly decrease during the motility period.

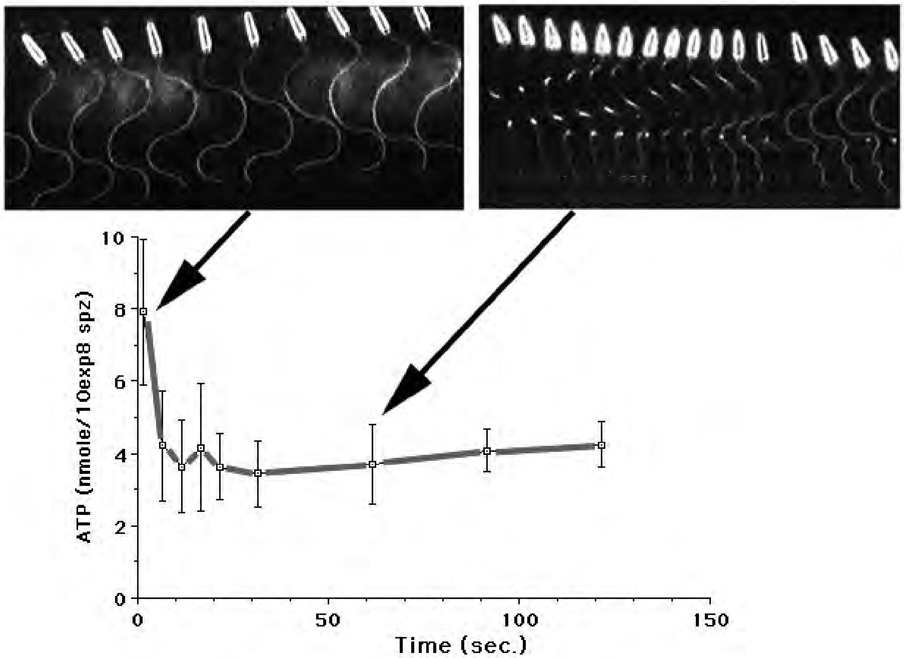

Figure 1. Changes of the flagellar waves pattern in relation to the ATP content during the motility period of sturgeon spermatozoa. Right after initiation of motility (5 sec.), intracellular ATP concentration allows propagation of waves with large amplitude and high beat frequency (left series in upper panel); later in the motility period (60 sec.), ATP concentration is lower and waves propagate mostly in the part of flagellum proximal to the head (right series in upper panel).

Consequently velocity of forward displacement becomes lower and lower as a function of the time elapsed since motility activation. (modified from Billard et al., 1999). In addition, during this period, the integrity of the flagellar structure is affected by side effects due to exposure to drastic osmolality imposed to sperm cells by the harsh conditions of activation. ATP depletion during the motility period contributes to decrease the activity of ion re-equilibrating membrane pumps, but such phenomenon can be reversed, provided ATP stock be replenished.

Such ATP homeostasis and consequently motility parameters are also subjected to temperature effects: the lower the temperature, the longer the motility period.

CONCLUSION

So far, mechanisms of fish sperm motility were deeply explored only in a restricted number of species, mostly belonging to fresh water species such as salmonids (trout or salmon), cyprinids (carp) and chondrosteans (sturgeons) or belonging to marine species (sea bass, turbot, cod as examples). Among such diversity of situations, general rules are emerging which emphasize the major role of osmolality, ions and ATP content at the activation step as well as during the motility period until its achievement.

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ARTIFICIAL PROPAGATION AND REVEALED REPRODUCTION FEATURES OF WEATHERFISH (*MISGURNUS FOSSILIS*)

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VEŠTAŠKO RAZMNOŽAVANJE I REPRODUKTIVNE OSOBINE ČIKOVA (*MISGURNUS FOSSILIS*)

Apstrakt

U našem istraživanju je dvanaest ženki i osam mužjaka čikova veštački reprodukovano pre sezone mresta. Riba je uneta u laboratorijske tankove u rano proleće i ženke su tretirane sa 10 mg/kg telesne težine sa CP (ekstrakt hipofize šarana), dok su mužjaci hipofizirani sa 5mg/kg telesne težine radi izazivanja ovulacije i spermijacije. Ženke su ovulirale u narednih 18 do 24 časa i posle istiskivanja jaja su bila oplođena. Vrednosti pseudogonadosomatskog indeksa PSGI kod 4 ženke veoma su varirale (3.6 – 22.2%), stopa oplođenja je varirala od 30.34 do 93.81 % posle 24 časa od oplođenja. Tri dana po oplođenju larve su se izvalile (14.84-91.8%) i započele sa prvom egzogenom hranom šestog dana. Čikov se može razmnožavati kao i šaranske vrste u mrestilištima, jedina poteškoća je mala količina gameta. Veštačko razmnožavanje i uzgoj larvi može da pomogne u značajnom jačanju populacija, tako da bi bila moguća repopulacija već redukovanih populacija i stvaranje novih staništa koja odgovaraju ovoj vrsti. Vijabilne larve iz interspecijske hibridizacije su se izlegle i na osnovu njihove morfologije mlađ nije ličila na hibride, što bi moglo da ukaže na sposobnost aseksualnog razmnožavanja. Genetička analiza nije pokazala genom mužjaka kod mlađi. F1R1 potomci su bili 50% tetraploidi (4n=100)i 50 % heksaploidi (6n=150). Ovo je prvi rezultat stvaranja heksaploida (broj hromozoma 150) čikova u laboratorijskim uslovima.

Ključne reči: interspecijska hibridizacija, Misgurnus fossilis, Carassius carassius, ploidija
Keywords: inter-specific hybridisation, Misgurnus fossilis, Carassius carassius, ploidy

INTRODUCTION

Abundance of weatherfish (*Misgurnus fossilis*) has been decreasing from the last century mainly due to habitat destruction. It is categorized as „vulnerable“ on the IUCN Red List. Systematic stockings of weatherfish into adjacent streams, canals and still waters might help to develop self-sustaining populations of *M. fossilis* in places where the species disappeared or occurs only sparsely. Artificial propagation and rearing of the larvae may help in strengthening population considerably, thus re-population of decreased stocks and creating new habitats – suitable for demand of the species – shall be possible. Our aim was to reveal its reproduction biology and improve effectiveness of its rearing under laboratory conditions.

MATERIAL AND METHODS

Spawning was induced by gradual increase of temperature in the tank from 10 to 20 °C for 6 days and females were injected with a single dose of 10 mg per kg of body weight of dry carp pituitary extract (CP) 18-24 hours before stripping, males were injected with a single dose of 5 mg per kg of body weight of CP. Females were anaesthetized in a solution of clove oil *(Syzygium aromaticum,* L. 1 aliquot per 1 L of water), then they were removed from the water onto wet towels and their genital aperture was dried with dry paper towels. Fertilisation tests of *M. fossilis* were carried out by using the dry fertilisation method. The testes were surgically removed and sperm was obtained by squeezing the organs through cheesecloth. This required sacrificing the males but enhanced sperm yield. The freshly stripped eggs were mixed with testicular sperm before freshwater was added to them. Eggs were incubated in 2 L plastic tanks and the larvae were reared in recirculation systems and grown exclusively on live food (*Artemia, Chironomus, Tubifex*) for 3 months.

In order to uncover the polyploidization and special asexual reproduction abilities of the species, stripped egg batches of weatherfish were fertilized by Crucian carp sperm (*Carassius carassius*) under laboratory conditions. Two females from this crossing were reared until mature stage (one year old fish) and propagated with *M. fossilis* males. After the propagation the females and males including *C. carassius* were investigated by using SCoT genetic analysis. *C. carassius* (male) genom were looked for offspring. The ploidy of embryos was determined using chromosome preparation and staining.

RESULTS AND DISCUSSION

The summarised result in connection with propagation in Table 1. can be seen.

Table 1. Summarised results of M. fossilis propagation * female originated intraspecific progation (M. fossilis × C. carassius).

No of females	Females body weight (g)	Stripped egg (g)	PGSI (%)	Fertilisation (%)	Hatching (%)
1	20.38	3.71	18.2	66.38	84.1
2	13.66	1.48	10.8	51.09	15.2
3	11.07	1	9.0	50.13	91.8
4*	11.57	0.42	3.6	30.34	14.81
5	66.99	12.59	18.8	93.13	72.8
6	61.39	11.36	18.5	93.81	71.5
7	70.45	3.21	4.6	52.94	51.5
8	28.1	6.228	22.2	81.02	70.4
9	25.77	2.485	9.6	53.61	53.1
10	25.30	1.804	7.1	64.19	51.7
11	20.29	1.164	5.7	90.62	48.8
12	19.45	2.116	10.9	92.15	33.3
mean±SD	**31.2±21.9**	**4.0±4**	**11.6±6.3**	**68.3±21.4**	**54.9±24.8**

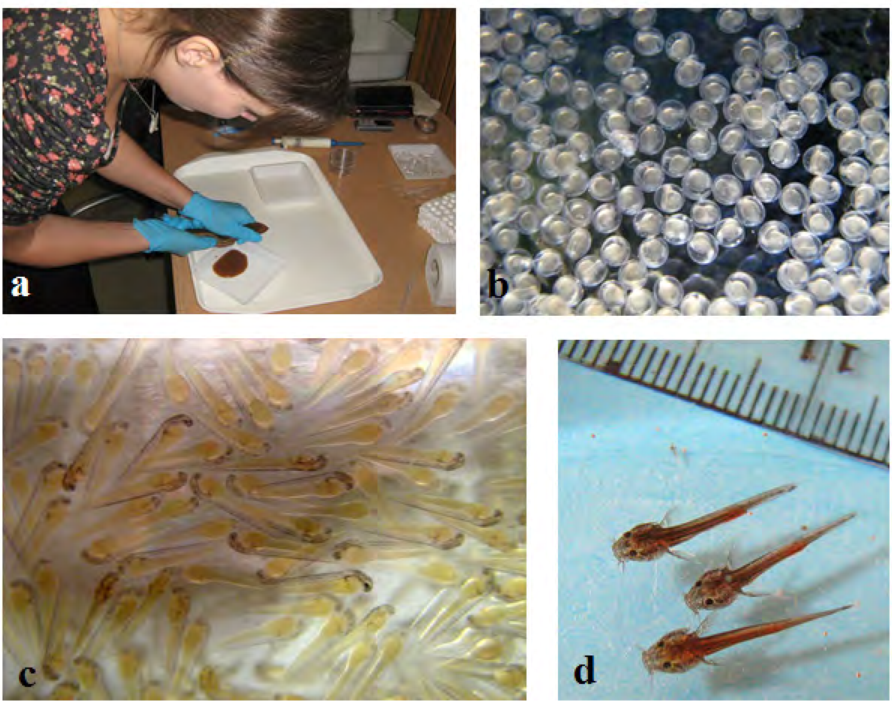

Figure 1. a: Egg stripping, b: Developing egg on the 2nd day, c: Embryos on the 1st day, d: 14 days old weatherfish.

Several reproduction parameters (latency time, fertilisation rate, hatching rate, etc.) and developmental factors were investigated and our results were similar which was described in literature (Drozd et al. 2009, 2010, 2011; Demény et al. 2009).

Reared, 3 month old weatherfish offspring provide a good stock for introduction to their original habitat or nature protected areas under optimal conditions (Table 2).

Table 2. Summarised data about the introduction (*Model Area for Hungarian protected and threatened marsh fish species).

Site	Date	Number of individuals	Spawning time	Introduced fish
Pusztaszer	04.09.2011	150	03. 2011	7-8 cm, 2-4 g
Szada, Illés-pond*, no. VI.	10.14.2011	20	03. 2011	8-10 cm, 3-5 g
Szada, Illés-pond*, no. II.	06.06.2012	200	05. 2012	1-2 cm
Szada, Illés-pond*, no. III.	06.06.2012	200	05. 2012	1-2 cm

From inter-specific hybridisation three larvae hatched, one of them died after two days, but two females were managed to rear to maturation phase. According to their morphology the weatherfish did not seem to be a hybrid that may refer to its ability to propagate asexually. The genetic analysis did not show male genom in the juveniles. One of them were managed to propagate and was fertilised with *M. fossilis* sperm. The results of chromosome preparation suggested F1R1 offspring were 50 % tetraploid (4n=100) and 50 % hexaploid (6n=150). According to result of Drozd et al. (2010), who investigated the ploidy level of wild *M. fossilis* in total, 19 triploids, 20 intermediate aneuploids and 77 tetraploids were recognized in a 1:1:4 ratio regardless of sex. The sex ratio of triploids and tetraploids was 1:1 and differed from that of intermediate aneuploids (3:1 for males). Our results showed to create viable hexaploid (150 chromosome number) *M. fossilis* under laboratory conditions.

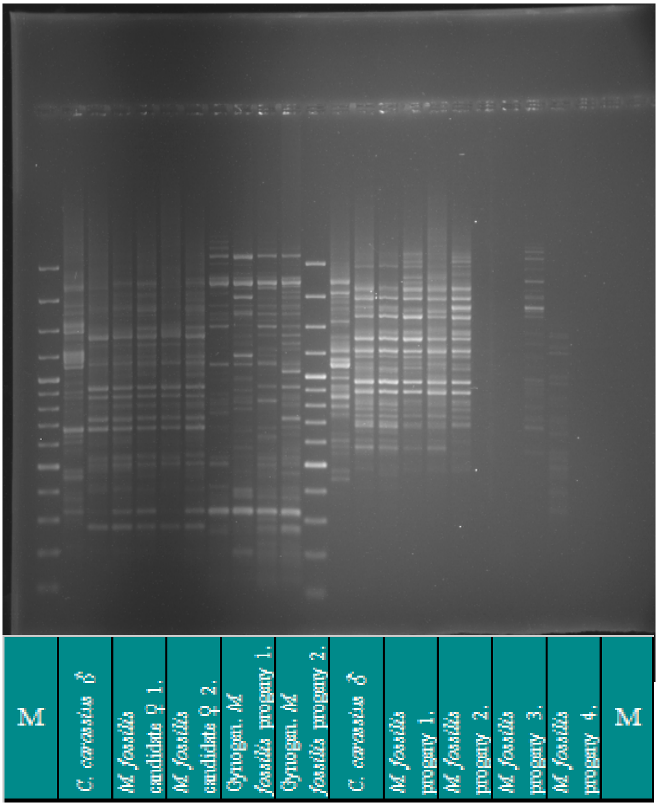

Figure 2. DNA pattern of broodstock (M. fossilis and C. carassius) and their offspring applying primer „SCoT 12”.

CONCLUSIONS

Weatherfish can be propagated with the same method as Cyprinus-like species in hatchery, the only difficulty is the small amount of sperm.

In contrast to literature data about the ploidy level of wild *M. fossilis* viable hexaploid (n=150) fish could be created by using intra- and after interspecific propagation. Our final aim is to determine the ploidy and capability of hybridisation of both the maternal fish individuals and their offspring gained via sexual and asexual propagation. Flow cytometric investigations are in progress, that may make possible to reveal the chromosome number of all specimens in rearing including the grown offspring.

ACKNOWLEDGEMENTS

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EVALUATION OF BIOLOGICAL INDICATORS OF YOUNG PERSIAN STURGEON (*ACIPENSER PERSICUS*) GROWN AT POND METHOD WITH DIFFERENT ARTIFICIAL FEEDS

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EVALUACIJA BIOLOŠKIH INDIKATORA MLAĐI PERSIJSKE JESETRE (*ACIPENSER PERSICUS*) GAJENE U RIBNJACIMA SA RAZLIČITOM VEŠTAČKOM HRANOM

Apstrakt
Cilj ovih istraživanja je bio da se pomoću bioloških i hematoloških parametara, kao i hemijskog sastava tela proceni rast mlađi persijske jesetre gajene u ribnjacima sa različitom veštačkom hranom.

Istraživanja su obavljena u toku 2102. godine na mretsilištu za jesetre Khylly, Azerbejdžan. Istraživane su larve i mlađ persijske (Kura) jesetre (*Acipenser persicus*), gajene u ribnjacima. U 4 bazena ishrana mlađi je obavljena sa veštačkim starterima za jesetre marke TB strane proizvodnje (opcija I), dok je u 4 preostala bazena mlađ hranjena veštačkom hranom proizvedenom na ribnjaku (opcija II). Persijksa jesetra je gajena u ribnjaku u toku 45 dana.

Količina proteina, masti, pepela i ugljenih hidrata u telu mlađi jesetre koja je hranjena sa različitom hranom (7 individua) je određivana standardnim metodama. Kao indikatori za određivanje fiziološkog statusa mlađi ribe su korišćeni sadržaj hemoglobina i ukupnih proteina u krvi, kao i sedimentacija eritrocita, broj eritrocita i broj leukocita u 1mm^3 krvi.

Ranija istraživanja uzgoja u ribnjačkim bazenima su pokazala da je omogućavanje ishrane organizmima imalo pozitivan efekat na celokupno preživljvanje larvi u toku i posle prelaska na egzogenu ishranu. U toku prelaska na egzogenu ishranu broj uginulih

larvi u svim varijantama eksperimenta nije prevazilazio 4-6%. I 5-6 dana posle prelaska na egzogenu ishranu broj uginulih larvi je bio mali.

Praćenje rasta i preživljavanja larvi persijske jesetre u bazenima su pokazala da je korišćenje naupliusa *A. salina* kao polazne ishrane žive hrane u prvim fazama ishrane veoma povoljno za rast jesetri u bazenima.

Mlađ riba koje su hranjene sa veštačkom hranom stranog proizvođača (opcija I) je pokazivala relativno bolji rast, kao i bolje biološke i hematološke parametre, što je, po našem mišljenju rezultat povoljnijeg hemijskog sastava i sadržaja ove hrane. Analize hemijskog sastava tela persijskih jesetri u čijoj su ishrani korišćene različite hrane takođe pokazuju da kvalitet gajene mlađi riba zavisi od kvalteta korišćene hrane. Uslovi skladištenja, ishrana i koeficijent ishrane takođe imaju veliki uticaj na kvalitet, kao i na vrednosti parametara krvi i hemoglobina kod mlađi riba.

Pozitivna strana uzgoja u ribnjačkim bazenima leži u tome da mlađ ribe raste u potpuno kontrolisanim uslovima i željeni rast eksperimentalnih riba u gustim nasadima i kratkom vremenskom roku može da se ostvari tačnim pridržavanjem pravila za optimalni biotehnološki rast jesetri korišćenjem efektivnih i visoko dostupnih po veličini organizama za ishranu (Mamedov et al., 2009). U tom slučaju, kvalitet mlađi ribe u uzgoju i njen fiziološki status zavise od uslova skladištenja i kvaliteta korišćene hrane.

Ključne reči: persijska (Kura) jesetra, larve, eritrociti, ribnjački metod, biohemijski parametri

Keywords: Persian (Kura) sturgeon, larvae, erythrocyte, pond method, biochemical parameters.

INTRODUCTION

Given the technical capabilities of Khylly Sturgeon Fish Farm at growing of young sturgeons at pond method the feeding of larvae and young beluga (*Huso huso*), Persian (Kura) sturgeon *(Acipenser persicus*) and ship sturgeon (*Acipenser nudiventris)* until they reach 170 mg of sample, and stellate sturgeon (*Acipenser stellatus*) up to 100 mg of sample is exclusively done with live feed. When they reach the above sample (100-170 mg) gradually they are transferred to feeding with artificial feed of factory or foreign production.

The objective of our research was to examine the fish breeding and biological and haematological parameters, as well as the chemical composition of the body of young sturgeon at their growing in different artificial feeds by pool method.

MATERIAL AND METODS

The study was conducted in 2012 at Khylly Sturgeon Fish Farm of Azerbaijan. The larvae and young Persian (Kura) sturgeon *(Acipenser persicus)*, grown by pond method were studied. Persian sturgeon larvae received from one female originally was placed in 4 plastic ponds with an area of 3.14 m^2. The initial placing density of the experimental fish in all ponds was 2.5 thousand individuals /m^2.

Feeding of larvae of Persian sturgeon in all ponds was performed 2-3 days prior to their transition to exogenous feeding. Thus as the live feeds were used only nauplius of *Artemia salina*. Five days after the transfer of larvae to exogenous feeding as live feeds different size forms of *Daphnia magna,* and chopped and whole *Enchytraeus albidus* were also used. Growing conditions and feeding rate with live feeds of all experimental fish until larvae reaches 170 mg of sample (20 days after hatching) were similar. Then the experimental fish placing density on each pond is halved, resulting in the total number of pond with fish as 8 ponds. From this point the experimental fish gradually are transferred to the feeding with artificial feed. On 4 ponds feeding of fry is performed with artificial starter feed for sturgeon brand of "Starter feed for sturgeon fish", produced by "Raanan Fish Feed Ltd., Israel" (option - I), while the remaining 4 ponds with artificial food of fish farm production (option - II). Daily rate of feeding of the experimental fish in the ponds during growth depending on the growth conditions (temperature, density of placing, age of farmed fish, the type of feed fed) varied from 15-20 to 5-7% of the weight of farmed fish. The period of growth of young Persian sturgeon in the ponds as a whole was 45 days.

Grown young fishes were evaluated on a number of fish breeding and biological, physiological and biochemical parameters. Amount of protein, fat, ash and carbohydrates in the body of the young sturgeon fish grown with different feeds (7 pcs.) was determined by the standard method (Ivanov, 1963; Sklyarov et al., 1984). The content of haemoglobin, total protein and erythrocyte sedimentation rate of blood, red blood cells and white blood cells in 1 mm^3 of blood were taken from the haematological indicators characterizing the physiological status of young fishes.

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 determinate, 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. Obtained data were processed by the standard methods of the statistical analysis and software package Stadia is used.

RESULTS AND DISCUSSION

It was found that in a ponds method of growing an earlier acquaintance with feed organisms beneficially effects on overall survival of larvae during and after the transition to exogenous feeding. In the period of transition to exogenous feeding the number of dead larvae on all variants of the experiment did not exceed 4-6%. And 5-6 days after the transition to exogenous feeding the number of dead larvae was few.

Monitoring the growth and survival of the Persian sturgeon larvae in the ponds showed that the use of nauplius *A.salina* as starting live feed organisms in the early stages of feeding is a very good element for growing sturgeon in pond. This is explained by the following circumstances: -

- Nauplius of *A.salina* contains 50-55% of protein, and various irreplaceable amino acids, semi unsaturated fatty acids and bioactive compounds (Sorgeloos and Leger, 1992; Van Stappen, 1996; Mikhailova, 2000; Mamedov et al., 2009).
- Nauplius of *A.salina* is small and accessible to prelarvae and larval sturgeon;
- Nauplius of *A.salina* have negative buoyancy when released into the freshwater

conditions and are readily available for actively feeding larvae in the bottom layers and the bottom of the pond without much energetic efforts by them for searching of food (Gershanovich et al., 1987).

In the early stages of development, in the transition from the yolk to the active feeding, deep changes occur in the body of sturgeon larvae related to the preparation for capture, digestion and assimilation of food. This stage is characterized by significant morphophysiological shifts in organs and tissues of farmed fish (Gerbilsky, 1957; Korzhuyev and Sharkova, 1967; Krasnodembskaya, 1990). Inconsistency between the used food and larvae needs at this time leads to starvation, which significantly reduces the growth of young fish and increases the percentage of waste.

After the transition to artificial feeding with different foods some differences were found in the development of the experimental fish. Results of growing of fry and young Persian sturgeon using different artificial feeds (Table 1, 2) are presented in table 3.

Table 1. The composition of foreign starter feed for sturgeon (option - I).

№	Description	Contents
1	Protein	60 %
2	Fat	9,0 %
3	Ash	9,5 %
4	Ca	2,7 %
5	Fibre	0,3 %
6	Vitamin A	30000 i.u /kg
7	Vitamin D_3	3000 i.u /kg
8	Vitamin E	400 i.u /kg
9	Vitamin C	300 i.u /kg
10	P	1,7 %
11	Lysine	4,4 %
12	Methionine + Cysteine	2,5 %
13	Copper	5 mg//kg

Table 2. Artificial food for larvae and young sturgeon of Farm production (option - II).

№	Description	Content, %
1	Fish meal	45
2	Wheat flour	5
3	Fodder yeast (hydrolysis)	20
4	Milk powder	12
5	Oil cake	8
6	Premix	2
7	Fish oil (before pressing + after cooling)	2+6
8	Crude protein,%	48
9	Crude Fat, %	13
10	Ash, %	13
11	Crude fibre, %	0,8
12	Moisture, %	7,7

Table 3. Fish growing and biological indicators of the young Persian sturgeon grown on different artificial feeds

№	Indicators	Option - I	Option - II
1	Weight at start, mg	170,0	170,0
2	Final weight, mg	2050,0	1830,0
3	Period of growing with artificial feed, days	25,0	25,0
4	Survival rate, %	93,0	88,0
5	Fulton condition factor	0,57	0,53
6	Specific growth rate, % per day	10,0	9,9
7	Feeding ratio	1,0	1,2

Young fish used artificial feed of foreign production (option I) was characterized by relatively better fish growing and biological and haematological parameters (table 4, 5), which, in our opinion, due to more rational chemical composition and content of the feed.

The results of analysis of the chemical composition of the body of young Persian sturgeon grown in pond for 45 days with the use of live and artificial feeds are shown in table 4. Analysis of the chemical composition of the body grown at different feeds of young Persian sturgeon also showed that the quality of the farmed young fishes depends on quality of the received feed. Conditions of storing, feeding and ratio of feeding also has a great impact on the quality, the amount of blood and haemoglobin (table 3, 5) of the grown young fish.

Table 4. Comparative characteristics of the chemical composition of the body of young Persian sturgeon grown with different artificial feeds (in % of raw material).

№	Number of fish, pcs	Weight of young fish, mg M ± m	Dry Substance, % M ± m	Protein, % M ± m	Fat,% M ± m	Ash, % M ± m
I	7	2050±150	17,5±0,6	14,5±1,2	0,5±0,03	2,5±0,2
II	7	1830±110	16,4±0,8	13,6±1,3	1,1±0,2	1,7±0,2

Table 5. Fish growing, biological and haematological parameters of young Persian sturgeon grown in pool using different artificial feeds.

Indicators	Options of artificial feeds	
	I	II
Growing period, days (from the time of hatching)	45	45
Body length, mm	71,1	70,1
Body weight, mg	2050±150	1830±110
Haemoglobin, g / l	40,4±2,5	35,9±2,3
Total protein, g / l	19,2±0,5	15,9±0,9
ESR, mm / h	2,4±0,04	2,0±0,04
Erythrocyte, thousand pcs / ml	455,5±39,6	437,5±42,6
Leukocytes, thousand pcs / ml	8,6±0,8	7,4±1,2

Comparative analysis of the obtained results with the combined and pool methods of growing of sturgeon (Golovonenko, 1964; Daudova et al., 2008; Jabarov et al., 1987; Zagrebina and Klimov, 2007) showed that at the observation of above technologies of feeding the young pond grown Persian sturgeon corresponds to the accepted norms in their physiological parameters. However, the best results were obtained with the use of artificial feed produced abroad.

CONCLUSIONS

Our results for growing of young sturgeon using different artificial feeds are important in growing of young fish by pond method. It is the right choice of feed combined with favourable environmental factors ultimately determines the physiological usefulness of young station fish grown in pond.

Positive element in pond growing is that young fishes are grown in fully manned conditions, and the desired growth of the experimental fish at high density placing them in a relatively short period can be achieved by strict observance of the rules of optimal biotechnological growing of sturgeon using effective and highly accessible food organisms in size (Mamedov et al., 2009). In that case, the quality of grown young fish and its physiological usefulness depends on conditions of storing and quality of received feed.

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THE GROWTH OF WEIGHT AND BODY LENGTH OF YOUNG RAINBOW TROUT (*ONCORHYNCHUS MYKISS WAL.*) ORIGINATING FROM DIFFERENT BROODSTOCK

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RAST MASE I DUŽINE TIJELA MLAĐI DUŽIČASTE PASTRMKE (*ONCORHYNCHUS MYKISS* WAL.) PORIJEKLOM OD RAZLIČITIH MATIČNIH JATA

Apstrakt

Eksperiment analize karakteristika rasta mase i dužine tijela mlađi dužičaste pastrmke (*Oncorhynchus mykiss* Wal.), porijeklom od različitih matičnih jata, realizovan je u salmonidnom mrestilištu Klašnik - Banja Luka u trajanju od 134 dana (od 05.7.2012. do 16.11.2012.) i dio je istraživanja kojim su obuhvaćeni izbor matičnog jata, mrijest, analiza embrionalnog razvoja i karakteristika rasta dužičaste pastrmke do devet mjeseci starosti. U eksperimentu je korišćena dužičasta pastrmka starosti pet mjeseci porijeklom od pet različitih matičnih jata, a karakteristike rasta su praćene do uzrasta od devet mjeseci. Temperatura vode tokom realizacije eksperimenta prosječno je iznosila 10,93^{o}C, rastvoreni kiseonik u vodi 10,39 mg/l, zasićenje vode kiseonikom 94,8% i pH 7,57. Koeficijent kondicije (CF) analizirane mlađi dužičaste pastrmke uzrasta od devet mjeseci je u porastu, a stopa rasta (SGR) je u padu u svim eksperimentalnim grupama, dok koeficijent rasta za termičku jedinicu (TGC) ukazuje na različite tendencije. Dobijeni rezultati ukazuju na izraženu heterogenost karakteristika rasta mlađi dužičaste pastrmke porijeklom od pet različitih matičnih jata.

Ključne riječi: rast, dužičasta pastrmka, matično jato.
Keywords: growth, rainbow trout, broodstock

INTRODUCTION

Water potential of Republic of Srpska, Bosnia and Herzegovina is a great, especially if you take into account the fact that these are the waters of I and II class quality which is a fundamental prerequisite for successful fish farming. Territory of the Republic of Srska characterized predominantly commercial cultivation of rainbow trout (*Oncorhynchus mykiss*, Wal.), for favorable conditions, the quality and quantity of available water. In addition to the initial assumptions (the quality and quantity of water), it is important to take into account the choice of broodstock in order to obtain offspring with good production characteristics, disease resistance and utilization of nutrients. It is certainly important that the establishment of quality broodstock of rainbow trout adequate disease resistance and good growth characteristics with optimum utilization of food, in addition to the application of appropriate technological measures, one of the objectives whose fulfillment can improve production.

The characteristic of growth weight and body length young rainbow trout have been the subject of numerous studies (Uysal, 2002; Guzel and Arvas, 2011; Richardson, 2011; Kizak et al., 2011; Sevgili et al., 2012). Previous research in this area in our regions is symbolic, and their results are not widely applied in practice.

Understanding the importance of the broodstock in order to improve the production, we started investigation that included the tour fishpond of rainbow trout, the choice of broodstock which differed in origin and the conditions in which they are grown (temperature etc.), spawning selected broodstock, monitoring embryonic development and analysis of growth characteristics of rainbow trout to the 9 age of months under the same conditions for all experimental groups in the salmonid hatchery Klasnik - Banja Luka. Results embryonic development and characteristics of growth to 5 age of months indicate significant differences between rainbow trout from different fishponds (Savić et al., 2012, 2013).

The aim of this study was to analyze the characteristics of growth mass and the body length of rainbow trout (*Oncorhynchus mykiss* Wal.) age 5 to 9 months under the same environmental conditions and requirements dietary, originating from the 5 selected broodstock to determine difference characteristics of growth.

MATERIALS AND METHODS

The experiment was carried out for a period of 134 days (from 05.7.2012. to 16.11.2012.) in salmonid hatchery Klasnik - Banja Luka. Rainbow trout originating from five different broodstock is the populated in the 50 l tank with 100 fish in each experimental group.

Water temperature (^{o}C), content of dissolved oxygen (mg/l) and water oxygen saturation (%) were analyzed by digital oxy-meter Oxi 330i/SET 2b20-0011 WTW, and the pH value of a digital pH-meter pH 330i/SET WTW 2A20-1011 (Germany).

During the experimental period were followed characteristic growth of body mass (the scale Denver DL-501 payload 0.5 kg) and body length (ichtyo-meter). Useful volume of water in tanks, which were placed in the rotary pool and covered with a net to prevent fish jumping, was 50 l/tank with steady flow of water (roundabout) in all tanks.

Mass and body length were determined on a sample of 30 fish/exp group. On the basis of absolute indicators of mass and body length were calculated condition factor, specific growth rates and thermal-unit growth coefficient. Condition factor (CF) was calculated according to the formula: CF = (BW/L^3) x 100, where CF - condition factor; BW – body weight (g): L - length of fish (mm). A specific growth rate was calculated according to the formula: SGR = ((ln FBW – ln IBW) / D) * 100, where FBW - final body weight (g), IBW - initial body weight (g), ln - natural logarithm; D - days. Thermal-unit growth coefficient is calculated according to the formula: TGC = [FBW$^{1/3}$ – IBW$^{1/3}$] / Σ [TxD] x 100, where TGC- thermal-unit growth coefficient; FBW - final body weight (g), IBW - initial body weight (g), T - temperature (°C), D - days. Feed conversion ratio (FCR) was calculated according to the formula: FCR = F / G, where the F - food consumption, G - realized weight gain.

RESULTS AND DISCUSSION

The water temperature during the realization of the experiment averaged was it 10,93°C, content dissolved oxygen in water 10.39 mg / l, saturation water oxygen 94.8%, and pH 7.57. Table 1 show the average mass and total body length analyzed rainbow trout of different origin, standard deviation (SD) and coefficient of variation weight and body length, condition factor (CF), specific growth rates (SGR) and thermal-unit growth coefficient (TGC), rainbow trout aged between 5 and 9 months (05.7.2012. - 16.11.2012., 134 days).

Table 1. Growth performance rainbow trout from five different parent flock the same age and rearing under the same condition (age 5 to 9 months).

Age	Parameter	Experimental group (n = 30)				
		1	2	3	4	5
~ 5 months	Days post-hatch	161	154	154	153	149
	W±SD (g)	11,07±1,87	6,37±1,56	9,43±3,04	6,47±1,68	5,90±1,81
	CV W	16,94	24,57	32,19	25,92	30,63
	L±SD (cm)	9,77±6,00	8,07±6,00	9,01±8,73	8,24±7,16	7,96±7,00
	CV L	6,15	7,43	9,68	8,69	8,80
	CF	1,19	1,21	1,29	1,16	1,17
	SGR	2,44	1,64	3,08	1,69	1,84
	TGC	0,148	0,086	0,173	0,089	0,093
Days between 5 and 9 age months		134	134	134	134	134
~ 9 months	Days post-hatch	295	288	288	287	283
	W±SD (g)	57,13±9,62	39,57±5,97	42,60±18,64	49,20±9,56	46,23±9,44
	CV W	16,84	15,09	43,75	19,44	20,41
	L±SD (cm)	16,13±10,74	14,21±7,48	14,33±21,24	15,51±11,68	15,10±10,33
	CV L	6,66	5,26	14,82	7,53	6,84
	WG (g)	46,07	33,20	33,17	42,73	40,33
	LG (cm)	6,36	6,14	5,32	7,27	7,14
	FCR	0,81	1,15	1,00	0,76	0,85
	CF	1,36	1,38	1,45	1,32	1,34
	S G R	1,22	1,36	1,13	1,51	1,54
	T G C	0,109	0,105	0,093	0,121	0,120

W – body weight (g); CV W – coefficient variation of body weight; L– body lenght (cm); CV L – coefficient variation of body lenght; WG – weight gain (g); LG – length gain (cm); FCR – feed conversion ratio; FE – feed efficiency; CF – condition factor; SGR – specific growth ratio; TGC – thermal-unit growth coefficient.

There has been a decline in the coefficient of variation final weight in relation to the initial weight of the body, excluding the experimental group 3 in which the apparent pronounced variations. Similar trends are present in the case of variation of body length.

Condition factor at the start is lower and there are no significant differences between the analyzed groups, in contrast to the condition set forth in the final measurement where the more noticeable values of all analyzed groups, unlike SGR which is in decline in all analyzed groups in relation to the initial state, according to Uysal and Alpbaz (2002) that the SGR is not a constant value, in the early stages of development is higher but with increasing body size decreases.

The most significant decline SGR is present in groups 3 and was 1.13, although is to fifth month this age group recorded the highest growth rate of 3.08. Specific growth rates (SGR) analyzed fish is higher than quotation Richardson (2011) which states that the growth rate of rainbow trout aged 8 months in the control group (different spawning periods, September - December) 0.742 and 1.022, which is directly related to the actual higher body mass (73.1 g - 133.5 g) to 8 months of age, and the fact that with increasing size of fish growth rate declines, while Guzel and Arvas (2011) reported a similar growth rate with significantly greater body weight rainbow trout of the same age. On the other side Sevgili et al. (2012) and Kizaki et al. (2011) reported significantly higher SGR and body weight rainbow trout age of 5 months, which can be attributed primarily to the selection of broodstock, environmental conditions and the effects of feed (Savic et al., 2012, 2013).

In groups 1 and 3 is the highest growth SGR and TGC to 5 months of age, while those at the age of nine months have lower, and in experimental groups 2, 4 and 5 SGR is in decline, while TGC growing until the age of 9 months, which can be explained by compensation of growth, although all the experimental groups been in the same environmental conditions and on the same nutrition regime. In the groups 2, 4 and 5 the TGC have the growing trend while is in groups 1 and 3 in a significant decline. Based on the analysis of growth characteristics of rainbow trout originating from different broodstock, ntil the age of 9 months, is evident significant differences between the analyzed group, which indicates the heterogeneity of the analyzed broodstock, and the need for further research on the selection rainbow trout and improve sustainable cultivation.

CONCLUSION

The results showed significant heterogeneity of broodstock from selected fishpond. Future research should provide evidence of genetic variability of broodstock which together with these results, can be used as the basis for a serious approach to the selection of rainbow trout, which would certainly have a great impact on the improvement of production, the characteristics of growth rainbow trout and production results.

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DEVELOPMENT OF LIVE VACCINES FROM YERSINIA RUCKERI BY AROA AND AROC GENE MUTATIONS

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RAZVIJANJE VAKCINE PROTIV YERSINIA RUCKERI POMOĆU AROA I AROC GENSKE MUTACIJE

Apstrakt

Jersinioza koju izaziva *Yersinia ruckeri* je osnovni uzrok visokog mortaliteta i velikih ekonomskih gubitaka u slatkovodnoj i marinskoj akvakulturi. Da bi se bolest sprečila razvijena je živa atenuisana vakcina. U tu svrhu su geni *Y. ruckeri aroA* i *aroC* umnoženi i DNK fragmenti mutirani korišćenjem PCR metode kojom su klonirani i ubačeni u plazmid. Plazmid je prenet u *Y. ruckeri* da bi zamenio divlji tip gena mutiranim *aroA* i *aroC* genima, putem homologe rekombinacije. Virulenca mutiranog *Y. ruckeri* je određivana inficiranjem mlađi kalifornijske pastrmke mutiranim bakterijama i izračunavanjem mortaliteta i imunog odgovora riba. Efikasnost mutiranog *Y. ruckeri* je određivana vakcinacijom riba mutiranim bakterijama i izazivanjem obolenja kod iste ribe divljim tipom *Y. ruckeri* RB0708 6 meseci po vakcinaciji. Vakcinacija kalifornijske pastrmke sa *aroA* i *aroC* mutantima kao žive vakcine doprineo je značajnoj zaštiti protiv divljeg soja *Y. ruckeri*.

Ključne reči: sinteza aromatičnih amino kiselina, aroA, aroC, PCR, živa atenuisana vakcina

Abstract

Yersiniosis caused by *Yersinia ruckeri* is the main causes of high mortalities and severe economic losses in freshwater and marine aquaculture. To prevent this disease, a live attenuated vaccine was developed. For this purpose, *Y. ruckeri aroA* and *aroC* genes were amplified and DNA fragments mutated by overlap extension PCR was cloned into a suicide plasmid. This plasmid was transferred to *Y. ruckeri* for replacing wild type genes with mutated *aroA* and *aroC* genes via homologous recombination. Virulence of mutant *Y. ruckeri* was determined by infecting juvenile rainbow trout with mutant bacteria and calculating fish mortalities and immune responses in fish. Similarly, efficacy of mutant *Y. ruckeri* was determined by vaccinating fish with mutant bacteria and then challenging the same fish with wild type *Y. ruckeri* RB0708 6 months after vaccination. Vaccination of rainbow trout with the *aroA* and *aroC* mutant as a live vaccine conferred significant protection against the wild-type *Y. ruckeri.*

Keywords: Aromatic amino acid biosynthesis network (*aro*)*, aroA, aroC, overlap PCR, live attenuated vaccine*

FINDING OF THE NEMATODE LARVAE IN PREDATORY FISH SPECIES

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NALAZ LARVI NEMATODA KOD GRABLJIVIH VRSTA RIBA

Apstrakt

Neamatode iz roda *Eustrongylides* su paraziti ribojedih ptica. Njihov razvojni ciklus obuhvata dva prelazna domaćina, akvatične oligohete i planktofagne i bentofagne ribe npr. *Fundulus* ili *Gambosa*, *Neogobius*, ali i neke vrste iz porodice Cyprinidae koje kada pojedu inficiranu oligohetu postaju drugi prelazni domaćin. Grabljive vrste riba, kakav je som i smuđ, kada pojedu inficiranu ribu postaju paratenični domaćini dok njih ne pojedu ptice. Ovakvu ulogu mogu imati i vodozemci i gmizavci. Ustanovljeno je da ovi paraziti imaju uspešnu strategiju razvoja, s obzirom da jaje ostaje infektivno i do dve godine u spoljnoj sredini, a u prelaznom domaćinu može biti i preko godinu dana. Čovek nije tipičan domaćin ali se može zaraziti ukoliko jede sirovo ili nedovoljno termički obrađeno meso riba. U ovom radu prikazane su nematode koje se javljaju kod grabljivih slatkovodnih riba u Republici Srbiji. Istraživanja su sprovedena u periodu od 2011-2012 godine na kanalu Dunav-Tisza-Dunav, u gradskom području Novog Sada. Prikupljen je 21 uzorak smuđa (*Sander lucioperca*) težine od 250-500 g i 52 uzorka soma (*Siluris glanis*) težine 250-450 g. Urađen je postmortalni pregled abdominalne duplje, digestivnog trakta i drugih visceralnih organa. Utvrđeno je prisustvo nematoda kod 4 jedinke smuđa i 6 jedinki soma, što predstavlja prevalencu od 14.26%, odnosno 11.54%. Larve su bile prisutne u abdomenu, muskulaturi, lumenu želuca i želudačnom zidu gde su paraziti bili inkapsulirani. Broj parazita po ribi kretao se od nekoliko pa sve do 256. Sakupljene nematode fiksirane su u 70% etanol. Nakon fiksacije, svaka nematoda prosvetljivana je u mlečnoj kiselini radi morfološke observacije i identifikacije vrste. Relativni morfometrijski parametri i identifikacija parazita sprovedeni su prema ključevima Bauera (1987), Moraveca (1994) i Andersona (2000). Nematode su identifikovane kao *Eustrongylides* sp.- larveni oblici. Larve su bile crvenkaste boje. Dužina tela

larvi se kretala od 27 – 60.5 mm, a širina od 0.49 – 0.58 mm. Karakteriše ih prisustvo 12 papila na prednjem kraju, respoređenih u dva kruga po 6. Papile unutrašnjeg kruga su nešto izduženije Uzorci za patohistološki pregled uzeti su iz mišića i nodula koji su se nalazili u želudačnom zidu. Uzorci su bojeni standardnom metodom koristeći H&E. Paraziti konzumirani kod sveže i termički slabo obrađene ribe mogu predstavljati značajan rizik za ljude. Sveže meso ribe i tradicionalni riblji proizvodi pre nego što se nađu u prometu moraju biti pregledani na prisustvo nematoda. Adekvatna priprema ribljeg mesa jedna od najvažnijih mera opreza kao i podizanje javne svesti prilikom konzumacije ovih riba.

Ključne reči: Eustrongylides, nematode, predatorske ribe, larve
Keywords: Eustrongylides, nematodes, predatory fish, larvae

INTRODUCTION

Species from genus *Eustrongylides* have complex life cycles involving a definitive host and two intermediate hosts. Definitive hosts include aquatic birds mostly from order Ciconiiformes family Ardeidae, Anseriformes, Gaviiformes and Pelecaniformes (Spalding and Forrester 1993, Measures 1988). First intermediate hosts for *Eustrongylides sp*. are aquatic oligochaetes (Spalding et al. 1993). Second intermediate hosts are planktivorous and benthivorous fishes that could pass the infection on to fishes (paratenic hosts) and finally on to fish-eating birds (Moravec, 1994). Such exposure is usually common in larger fish species, like channel catfish - *Ictalurus punctatus* or pike-perch - *Sander lucioperca*, which, as predators, become infected with *Eustrongylides sp*. nematodes. In fish, these parasites are conspicuous as long, red, coiled individuals located in the body cavity or embedded in the muscle (Mitchum 1995, Overstreet 2003). They can produce grossly visible swelling or abdominal distention but mortalities from such infections are rarely reported and when they occur they usually involve secondary infections or suboptimal environmental conditions (Bursey 1982, Overstreet 2003).

In humans who have consumed raw or undercooked fish, *Eustrongylides sp.* have produced gastritis and intestinal perforation (Deardorff and Overstreet 1991; Cole 1999). Guerin et al. (1982) were the first to report a natural (accidental) human infection with *Eustrongylides sp.* The goal of this paper is to distinguish the presence of these types of nematodes in freshwater fish species in inland waters of Serbia and to indicate the need for adequate preparation of fish meat.

MATERIALS AND METHODS

Diagnostics and investigations were conducted in 2011-2012. Fifty two fish samples of European catfish *(Siluris glanis)* weighing 250-450 g and twenty one samples of zander (*Sander lucioperca*) were collected from eight locations on Danube-Tisza-Danube Canal. Fishes were parasitologically examined through cutting the body and the abdominal part, digestive tract and other ventral organs. Each fish was cut carefully from around the pectoral to the cloaca using scissors to observe the body cavity and to extract the viscera. All collected nematodes were were fixed in 70% ethanol for 24 h. After fixing, each nematode was cut at the anterior and posterior part of the body using a clean cover glass. Both ends of the body were cleared in lactic acid for morphological observation.

Relative parametars were measured and identification was performed using Bauer (1987), Moravec (1994) and Anderson (2000) keys. Samples for patohistological examination were taken from muscles and nodules in stomach walls. Samples were fixed in 10% water-buffered formalin and imbedded into paraffin blocks. 5μm thick slices were cut and stained with standard haematoxylin & eosin staining.

RESULTS AND DISCUSION

Presence of nematodes in the abdominal cavity (Figure 1), musculature (Figure 2), in the lumen of the stomach and encapsulated in stomach wall was revealed in 4 individuals of zander and 6 individuals of European catfish, what represented the prevalence of 14.26%, respectively 11.54%. The number of parasites per fish ranged from a few up to the 256. Parasites were determined as *Eustrongylides sp.* - larval form. This larva, was robust and pinkish red. Length of body was 27 – 60.5 mm, maximum width 0.49 – 0.58 mm. Buccal cavity 0.09 – 0.11 mm, oesophagus 0.907 – 1.35 mm. The larva retained a cuticle, and its twelve visible circle cephalic papillae were well defined. The number of papillae appeared to be six in each of two circles. The number of papillae was confirmed by apical observation with light microscopy. Inner circle papillae were spine-like apices and those of the outer circle were nipple-like apices. Patohistological observation of stomach and muscles are presented in Figure 3 and 4.

Figure 1. *Eusrongylides* sp. in the abdominal cavity of European catfish (*Siluris glanis*)

Figure 2. *Eusrongylides* sp. in the muscle of European catfish (*Siluris glanis*)

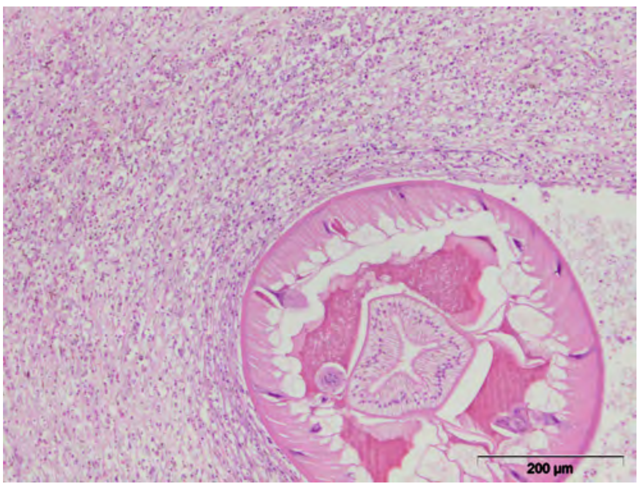

Figure 3. Cross-section of European catfish (*Siluris glanis*) stomach wall with *Eustrongylides* sp. larva (H&E).

Figure 4. Cross-section of European catfish (*Siluris glanis*) muscles with *Eustrongylides* sp. larva (H&E).

Fish parasites such as *Eustrongylides sp.* are highly important because they are capable to infect carnivorous organisms and humans who feed on them (Mohammad et al., 2011). Murrell (2002) suggested several control measures for preventing parasitic infections originating from freshwater, such as environmental control of surface water, hygienic aquaculture, and the control or elimination of the first intermediate hosts. FDA (2001) indicated that the effective methods for killing parasites are freezing, heating, adequate combination of salt content and storage time or hot smoking. On the other hand, brining and cold smoking may reduce the parasite hazard in fish, but they do not eliminate or minimize it to an acceptable level (Murrell, 2002). While health education is a key factor in combating zoonotic infections, experience in various countries has shown that for successful implementation of control measures, it is necessary, as Hughes (1992) points out, to have formal and informal cooperation between medical and veterinary interests at all levels of government, and with the community. According to Okumura et al. (1999) and Chieffi et al. (1992), the recommendation to avoid consumption of raw or poorly cooked fish is still the best preventive procedure.

CONCLUSIONS

Zoonotic nematodes can lead to human infection and special attention should be paid on them. Fresh fish meat and traditional fish product should be subjected to a visual examination for the purpose of detecting visible nematodes and other parasites before being placed in the trade. The consumption forms and the preparation of the fish food should be modified in a way that hazards to human health due to zoonotic parasites could be avoided. Health education is a key factor in combating zoonotic infections.

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SURVEY AND DIAGNOSTICS OF FISH DISEASES IN THE REPUBLIC OF SERBIA DURING THE PERIOD 2011-2012

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NADZOR I DIJAGNOSTIKA BOLESTI RIBA NA PODRUČJU REPUBLIKE SRBIJE U PERIODU OD 2011. DO 2012.GODINE

Apstrakt

Tokom dvogodišnjeg ispitivanja, preko 5000 uzoraka riba je ispitano na prisustvo bolesti. Tom prilikom je obuhvaćeno 56 šaranskih i 32 pastrmska ribnjaka. Na ribnjacima je vršen klinički pregled i odabir uzoraka za laboratoriju. Bakteriološka ispitivanja obavljena su zasejavanjem iz promenjenih unutrašnjih organa, kože i škrga na standardne i specifične hranljive podloge. Nakon 48^h vršen je pregled kolonija na oblik, boju, granulisanost, mukoznost, hrapavost i hemolitičnost. Determinacija izolovanih bakterija izvršena je na osnovu morfoloških i biohemijskih karakteristika. Za virusološka ispitivanja su korišćeni homogenati bubrega, slezine, jetre i škrga. Pulirani parenhimatozni organi i škrge su homogenizovani i centrifugirani na 2500 x g, 20 minuta. Za izolaciju, supernatanti su inokulirani na 24 sata stare kulture EPC i BF-2 ćelijskih linija. Inokulisane kulture su inkubirane na 15 - 20°C, tokom 7 dana i svakodnevno posmatrane na pojavu citopatogenog efekta. Identifikacija virusa je vršena PCR, ELISA testom i testom fluorescentnih antitela. Kao materijal za PCR je uziman homogenat organa i prva ili druga pasaža odgovarajuće ćelijske linije. DNK je ekstrahovana pomoću DNA mini kit prema uputstvu proizvođača (Qiagen, Nemačka). RNK je ekstrahovana pomoću RNA mini kita prema uputstvu proizvođača (Qiagen, Nemačka). PCR produkti su sekvencirani direktno, pomoću Big Dye Terminator v1.1 Cycle Sequencing Kit (Applied Biosystems, SAD) i ABI PRISM 3100-Avant Genetic Analyzer (Applied Biosystems). Dobijene sekvence su analizirane pomoću Sequencing Analysis Software 5.1 (Applied Biosystems). Tokom dvogodišnjeg ispitivanja utvrdili smo prisustvo zarazne nekroze gušterače (ZNG) i evropskog virusa somića (ECV). Utvrđena je pojava sindroma crvenih pega (RMS), jersinioze, renibakterioze, furunkuloze, septikemije izazvane pokretnim aeromonadama i sporadične pojave drugih oboljenja bakterijske i gljivične etiologije. Najčešće su utvrđene infestacije sledećim parazitima: *Trichodina domerguei, Chilodonella ciprini, Dactilogirus Girodactylus sp, Ichthyopthirius multifiliis, Diplo-*

stomum spathaceum, Caryophillaeus fimbriceps i Eustrongilus sp. U radu su opisane najvažnije bolesti riba dijagnostikovane su tokom 2011 i 2012 godine na ribnjacima u Srbiji.

Ključne reči: bolesti riba, dijagnostika
Keywords: fish diseases, diagnostics

INTRODUCTION

The intensive aquaculture is often characterized by high density of fish, poor water quality, 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 (Jeremic, 2003). The appearance and development of fish diseases is a consequence of the interaction of pathogen, host and environment. 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. Many diseases affecting farmed fish also represents a threat to natural fish populations (Thoesen, 1994). The relatively small number of pathogenic bacteria is responsible for major economic losses in cultured fish (Toranzo et al., 2005). 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). In this paper, the diseases diagnosed in fish farms in Serbia during the period 2011 - 2012 are described.

MATERIAL AND METODS

During two year period more than 5000 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. 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, H_2S 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 (Austin and Austin, 2007). The isolates were stored in broth with the addition of 15-20% glycerol at -80°C. For confirmatory identification immunofluorescence (FAT) and PCR were used.

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 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. For virus identification, ELISA, FAT, PCR, RT-PCR and sequencing were performed. 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, USA). RNA was extracted using RNA mini kit according to the manufacturer (QIAGEN, USA). PCR products (for SVC, IPN, ECV) were sequenced directly using Big Dye Terminator v1.1 Cycle Sequencing Kit (Applied Biosystems, 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

Each year national surveillance programmes are carried out for viral diseases i.e. infectious haemopoetic necrosis (IHN), viral haemorrhagic septicaemia (VHS), infectious pancreatic necrosis (IPN), spring viremia of carp (SVC), koi herpesvirus (KHV) and the bacterium *Renibacterium salmoninarum*. Testing for other infectious agents was also performed in various research projects, and on private basis, as part of the biosecurity program in some farms.

Infectious pancreatic necrosis (ZNG/IPN) - The disease was first noticed in Serbia in 1989 (Jeremic, 1989) and did not emerge until 2007, when the disease was confirmed at trout farm in Mačva district, in diseased rainbow trout, derived from imported fertilized eggs. The number of IPN affected localities in 2011 and 2012 was similar to previous years i.e. slightly over 10. All rainbow trout outbreaks were experienced during the juvenile stage. The disease is almost certainly under-diagnosed and therefore under-reported.

Viral Hemorrhagic Septicaemia (VHS) - All salmonid aquaculture facilities have been tested for VHS-virus every year as part of a national surveillance programme. The virus has not yet been detected. Serbia has therefore been considered a VHS-virus free zone.

Infectious Hematopoietic Necrosis (IHN) - All salmonid aquaculture facilities have been tested for IHN-virus every year as part of a national surveillance programme. The virus has not yet been detected. Serbia has therefore been considered a IHN-virus free zone.

European Catfish Virus (ECV) - The disease was first noticed in Serbia in 2008 and it is diagnosed every year since then in brown bullhead (*Ameiurus nebulosus*) and black bullhead (*Ameiurus melas*). Diseased fish showed erratic swimming. Slightly swollen abdomen and haemorrhagic spots on the external surface of the fish were present. Internally, liver ischemia, splenomegaly and frequently petechial haemorrhaging of mesenteric fat and internal organs. Based on the sequence analysis of the highly conserved major capsid protein, a positive diagnosis of epizootic hematopoietic necrosis caused by ECV was established.

Sleeping Disease (SD) - Sleeping disease is currently a matter of concern for salmonid fish farmers in most parts of the world. SD is characterized by a very particular "sleeping" behaviour with some of the fish in the affected group resting on their sides at the bottom of the tank. Histological observations of diseased fish show a chronological appearance of lesions in the pancreas, in the heart, and in the muscle at the last stage of the disease. Suspicious cases with characteristic signs of disease were recorded in two trout farms in 2012, but no virus was isolated, and no PCR positive samples were detected.

Spring Viremia of Carp (SVC) - Acute contagious viral disease caused by the *Rhabdovirus carpio*. Carp of all ages are affected and also other cyprinid fish species (Ahne et al. 2002). 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. No positive cases of SVC were detected during two year period in carp farms in Serbia.

Koi Herpes Virus (KHV) - All cyprinid aquaculture facilities have been tested for KHV-virus every year as part of a national surveillance programme. The virus has not yet been detected. Serbia has therefore been considered a KHV-virus free zone.

Furunculosis is a contagious disease caused by gram-negative bacteria Aeromonas *salmonicida subsp. salmonicida*. In 2011, the disease was diagnosed in fingerlings in five trout farms. In 2012 furunculosis with characteristic clinical symptoms was detected in six trout farms. *Aeromonas salmonicida subsp. salmonicida* was isolated from spleen and kidney of diseased fish on furunculosis agar.

Bacterial kidney disease (BKD) is characterised by a chronic progression with development of granuloma in various organs. In 2011, the disease was diagnosed in four trout farms. In 2012 BKD with characteristic clinical symptoms was detected in three trout farms. 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. Direct immunofluorescence test and PCR showed the presence of *R. salmoninarum*, a bacterium was isolated from the kidneys of diseased trout in KDM-2 agar.

Yersiniosis - In 2011, yersiniosis was detected in 8, and in 2012 in 11 fish farms. Several outbreaks have been associated with poor quality fish, high biomass, high water temperatures and low water availability.

Red mark syndrome (RMS) - In 2011, RMS was detected in 5, and in 2012 in 7 fish farms. **Other bacterial infections -** Outbreaks of septicemia caused by motile aeromonads were registered in 15 carp farms. All cases were associated with increased mortality. Detection of *Pseudomonas fluorescens* in fish farms is not unusual, and is often associated with poor water quality. In 2011, a higher number of such diagnoses were made compared to previous years and the situation will be monitored to establish whether this bacterium is increasing in virulence. A few cases of infection with *Flavobacterium psychrophilum* were identified in rainbow trout fry.

Parasitic diseases - The following parasites were recorded most frequently: *Trichodina domerguei, Chilodonella cyprini, Dactylogyrus sp., Gyrodactulus sp., Ichthyopthirius multifiliis, Diplostomum spathaceum, Caryophyllaeus fimbriceps and Eustrongilus sp.*

Fungal diseases - diseases caused by fungus, such as mycotic nephritis, swimbladder mycosis (various fungal spp.) and gill mycosis are registered sporadically. *Sapro-*

legnia sp. in eggs, gills and skin of carp and trout is a not uncommon finding and work continues towards prevention and treatment of these conditions.

Antibiotic resistance - routine testing of fish pathogenic bacteria isolated from fish in aquaculture during 2011-2012 identified new instances of reduced sensitivity to antibiotics licensed for use in Serbian aquaculture. Isolates of *Aeromonas hydrophila, Yersinia ruckeri* and *Pseudomonas spp.* with reduced sensitivity to antibiotics continue to be isolated from fish farms.

CONCLUSIONS

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.

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FISH-BORNE PARASITIC ZOONOSES WITH SPECIAL REFERENCE TO ANTHROPOGENIC IMPACT

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ZOONOTSKE PARAZITOZE RIBA SA POSEBNIM OSVRTOM NA ANTROPOGENI UTICAJ

Apstrakt

Parazitska oboljenja koja se prenose mesom ribe dugo vremena nisu dobijala zasluženu pažnju, pre svega jer su ove zoonoze bile vezane za zemlje tropskog i suptropskog pojasa, naročito azijske zemlje u kojima riba predstavlja veoma značajan izvor proteina za stanovništvo ruralnih sredina. Međutim, kao posledica globalnog zagrevanja dolazi do promena u ekosistemima voda i širenja geografskih granica u kojima se određeni paraziti pojavljuju. Širenju parazita doprinosi i fenomen globalizacije, razvijene saobraćajne mreže, komercijalni internacionalni hipermarketi, ali i demografske promene stanovništva od kojih najveći značaj imaju migracije. Na povećanje populacije pod rizikom utiču i promene kulturoloških navika i kulinarske prakse, naročito sve češća konzumacija sirove, marinirane, sušene ili na drugi način temperaturno nedovoljno obrađene ribe i proizvoda od ribe. Tokom poslednje decenija zabeležen je progresivan rast broja ljudi koji se hrane u restoranima, kioscima brze hrane ili kod uličnih prodavaca, koji često ne poštuju pravila o bezbednosti hrane. Ovakve navike posledica su brzog tempa života i sve većeg opterećenja današnjeg čoveka, ali i popularizacija raznih kuhinja, naročito azijske kuhinje, u svetu, ali i u našoj zemlji.

Prilagođavajući prirodu svojim potebama, čovek, kako na direktan, tako i na indirektan način, utiče na rasprostranjenost samih parazita. Izmena strujanja vode, koje nastaje pregrađivanjem reka branama prilikom izgradnje hidroelektrana i povišenje temperature vode, usled izgradnje termoelektrana, dovodi do supstitucije jednih vrsta fitoplanktona, zooplanktona i riba drugim vrstama. Promene flore i faune vodenog ekosistema ima direktan uticaj i na ptice koje se njima hrane, a koje su često pravi ili prelazni domaćini parazita. Ne retko, zbog narušavanja ekosistema i introdukcije novih vrsta riba javljaju se novi paraziti koji nisu karakteristični za neko područje. Zagađenje reka teškim metalima je jedan od značajnih antropogenih faktora koji utiču na prisustvo parazita, ali i na stepen akumulacije teških metala u mišićima riba. Naime, paraziti ne naseljavaju vodu zagađenu metil živom, ali istraživanja su pokazala da ukoliko su ribe već invadirane parazitima u trenutku zagađenja, akumuliraju živu u svom organizmu u manjim koncentracijama od one ribe koja nije inficirana.

U epidemiologiji zoonotskih parazitoza koje se prenose mesom riba najveći značaj imaju helminti, pre svega klase *Trematoda* vrste *Clonorchiosis sinensis* i *Opisthorchis spp. C. sinensis* ima izuzetan socialno-ekonomski značaj u određenim delovima Azije i predstavlja veliki zdravstveni problem. Procenjuje se da je oko 35 miliona ljudi u toku poslednje decenije inficirano ovim parazitom na globalnom nivou. *O. felineus* se nalazi u Kazakstanu, Rusiji, Nemačkoj, Italiji, Španiji, Grčkoj, Makedoniji i Albaniji, dok je *O.viverrini* karakteristična za područje Kambodže, Laosa, Vijetnama i Tajlanda. Prema podacima WHO iz 1995. godine preko 18 miliona svetskog stanovništva je obolelo od parazitoza izazvanim trematodama koje su prenete mesom riba, a njihova procena je da je pod rizikom od inficiranja više od pola milijarde stanovnika. Difilobotrijaza je parazitoza izazvana helmintima iz klase *Cestoda,* roda *Diphyllobothrium,* od kojih *Diphyllobothrium latum* predstavlja najčešći uzročnik infekcija ljudi. Ovi paraziti rasprostranjeni su u Evropi, Severnoj i Južnoj Americi i Aziji. U Evropi *D.latum* je rasprostranjen u oblastima oko alpskih jezera, a najveći broj slučajeva prijavljen je u Francuskoj, Švajcarskoj i Italiji. Od nematoda najveći značaj imaju kosmopolitski rasprostranjene *Anisakis simplex* i *Pseudoterranova decipiens*. Visok procenat parazitskih infekcija protiče subklinički ili asimptomatski, a manji broj se manifestuje kroz simptome poremećaja digestivnog trakta, dok se difilobotrijaza manifestuje hipovitaminozom B12 vitamina. Zbog velikog broja neprijavljenih slučajeva, ne uvek jasno izraženih kliničkih simptoma i manjka metoda za otkrivanje uzročnika, ove parazitoze se, uglavnom, ne dijagnostifikuju, naročito u delovima sveta za koji nisu karakteristične.

Ključne reči: helminti, ekosistem, klimatske promene, zagađenje vode
Keywords: helminths, ecosystem, climate changes, water pollution

INTRODUCTION

Compared with other well-studied parasitic diseases, fish-borne parasitic zoonoses have been public health orphans in the world of research funding, especially because these zoonoses have been limited for the most part to populations living in low- and middle-income countries (Chai et al., 2005). Zoonotic parasites are a significant food safety problem, particularly in Asia, because fish are a very important source of protein for people living in rural areas (Thien et al., 2009). In an attempt to change the nature, so they can adjust it for their needs, humans, directly or indirectly, affect parasites occurrence. As a result of global warming it is expected for some species to become locally extirpated and experience range contractions, while introductions of others, including potentially harmful pathogens, will occur in both freshwater and marine systems (Marcogliese, 2001.). Geographical barriers are slowly being breached by international travel developing into a major industry, by improved refrigerated food transport, by growing international markets, by increased tourism and by demographic changes such as migration (Chai et al., 2005; Dorny et al., 2009). Populations at risk are expanding also as a result of changes in cultural habits and culinary practices, such as the increase of eating raw, marinated, smoked, salted, pickled, airdried or undercooked fish meat and fish products. Eating sushi, sashimi, koi-pla, kinilaw and cevishe is becoming inceasingly fashionable in many countries (Macpherson, 2005). In the last decade it has been noted increasing in the number of people eating meals prepared in restaurants, canteens and fast food outlets as well as from street food vendors who do not always respect food safety (Dorny et al., 2009). All of these has led to a dramatic rise in the incidence of a large number of fish-borne zoonotic parasitic infections in previously uninfected ethnic groups (Macpherson, 2005). The role of some factors like ages, malnutrition, HIV infection and other underlying medical conditions should not be neglected in etiopathogenesis of fish-borne parasitic zoonoses (Dorny et al., 2009).

Fish parasitesTrematodiasis

Trematodiasis is the infection of humans caused by trematode parasites, known also as a liver flukes. The ones that cause the most common infection in humans are *Clonorchis sinensis*, *Opisthorchis viverrini* and *Opisthorchis felineus*. The life cycles of these trematode species are similar. Eggs are shed with the feces of the definitive host (man or other piscivorous animals). If the eggs reach water they develop into miracidia which are ingested by a snail, the first intermediate host, in which they develop and ultimately shed into the water as motile cercariae which penetrate into the muscle tissue of a fish, the second intermediate host. Human infection takes place through the consumption of under-processed fish containing the infective stage of the parasite (Santos and Howgate, 2011). *Clonorchis sinensis* (Chinese liver fluke) is widely distributed in East Asia and in this region present the most important species of fish-borne zoonotic parasite. *Opisthorchis viverrini* is highly prevalent in Southeast Asia including Thailand, Laos, Cambodia and Vietnam, and there are about 9 million people who are infected globally (Chai et al., 2005). *Opisthorchis felineus* is prevalent in Spain, Italy, Albania, Greece, France, Macedonia, Switzerland, Germany, Poland, Russia, Turkey, and in parts of the former Soviet Union (Chai et al., 2005, Macpherson, 2005). The major clinical problems in infected humans are cholangitis, choledocholithiasis, pancreatitis and cholangiocarcinoma and they are associated with the long chronic infections, but severe infections can cause jaundice, portal hypertension, ascites, gastrointestinal bleeding and formation of gallstones (Chai et al., 2005; Dorny et al., 2009).

Cestodiasis

Diphyllobothriasis is the most important fish-borne zoonosis caused by a cestode parasite. At least 13 of about 50 species of *Diphyllobothrium* have been reported from humans, but most important is *Diphyllobothrium latum*. The egg hatches in water, the motile embryo coracidium is ingested by a copepod, and develops to the procercoid. When an infected copepod is ingested by the second intermediate host (freshwater, anadromous or catadromous fish), the larva is released, enters the tissues of the host and develops to the plerocercoid which is infective for the final host (Chai et al., 2005). These parasites are found in Europe, North and South America and Asia. About 20 million people are infected worldwide (Dorny et al., 2009). In Europe, Scandinavian countries present 'hot spot', and Switzerland, Sweden, Finland and Estonia report more than 10 cases per year, while Lithuania, Poland, Hungary, Italy and France average 2–10 cases annually (Chai et al., 2005). Most of the cases in Switzerland, France and Italy occur in the Alpine Lakes region. Diphyllobothriasis in humans may often be asymptomatic, but in some cases it may cause diarrhea, abdominal pain and megaloblastic anaemia as a result of B12 deficency (Macpherson, 2005; Dorny et al., 2009).

Nematodiasis

Of all nematode parasites most commonly involved in human infections is *Anisakis simplex*, while *Pseudoterranova decipiens* is less frequent. Infections with the *Anisakis physeteris* and *Contracaecum spp*. are sporadic (Dorny et al., 2009). The life cycle of *Anisakidae* involve small crustaceans as the first intermediate host, fishes and cephalopods as the second host, and marine mammals as the definitive host. Humans are accidental hosts that become infected by eating larvae contained within the paratenic host (Lima dos Santos and Howgate, 2011). *A. simplex* and *Pseudoterranova spp.* are found in the northern, north east and north west Atlantic, northern Pacific, in the Mediterranean Sea, Norwegian Sea and Barents Sea. Anisakiasis is prevalent in north Asia and western Europe (Netherlands, Germany, France and Spain) (Chai et al., 2005). *A. simplex* causes an acute or chronic infection followed by abdominal pain, nausea, vomiting, and diarrhea and some patients develop syndromes exhibiting clinical manifestations of allergy (Lima dos Santos and Howgate, 2011).

Gnathostomiasis is primarily a disease of the skin caused by migration of the larvae of a *Gnatostoma* nematode and it is known to occur mainly in South East Asia, but has been reported as a cause of human infection in Peru, Ecuador and Mexico. It is often reported in tourists returning from endemic areas where they have eaten undercooked freshwater fish (Nawa et al., 2005; Dorny et al., 2009).

Anthropogenic impact

Climate change

Climate change can occur as a result of natural and anthropogenic causes. Temperature change, alterations in water levels, stratification, ice cover, acidification, ultraviolet radiation are some of the factors that affect parasits of aquatic organisms, their life cycles and distribution. Climate change has been predicted to be one of the major drivers of biodiversity change, especially in lakes (Marcogliese 2001). Global climate change with rising sea-levels and water temperatures that may result in changes in ocean circulation and a decrease in salinity may also cause measurable effects on fish parasite composition and biogeography. Climate warming affect interactions between host and parasits by increasing pathogen development rates, transmission and number of generation times per year, raising the overwinter survival rate of the pathogen and increasing the host susceptibility to thermal stressors. Also, increasing temperature alter the seasonality and biogeographical range of many species, including the hosts and the parasites (Palm, 2011).

Aquatic ecosystem pollution

Aquatic ecosystems present the most sensitive systems on earth (Palm, 2011).

Pollution can affect parasitism in fish in two different ways. It can increase parasitism if host defence mechanisms are negatively affected by increasing host susceptibility, or by simply increasing the population densities of suitable intermediate or final hosts. Some studies have focused on parasite-induced stress that was measured as an increased stress hormone concentration and it often present the first step in a series of physiological reactions such as a depressed immune response or other fundamental physiological reactions. Pollution can also decrease parasitism provided that infected hosts suffer more from environmental exposure than do uninfected hosts or pollution drives the necessary intermediate and final hosts to become extinct (Marcogliese, 2001). If the fish is living within a polluted environment where some pollutants also enter the fish, the concentration of these substances in the immediate surroundings of the parasite can also increase and accumulate in the parasite’s tissue, proving that fish parasites closely interact with the metabolisms of their host. Parasites are more susceptible to the particular pollutant, sush as haevy metals, than their host and some fish parasites can accumulate pollutants in a much higher concentration (to several thousand-fold higher levels) as their host organisms. That is one of the resons why parasites, especially helminths, can serve as biological indicators of water pollution (Palm, 2011; Marcogliese, 2001).

Industrialisation, technology and transport development

The creation of large freshwater reservoirs for hydroelectrical, irrigation, flood control or drinking water purposes present one of the most dramatic and widespread anthropogenic impacts on the natural environment (Morley, 2007). In Republic of Serbia the biggest hydroelectric power station and the biggest reservoir at the same time is Djerdap, Gate I, on the Danube River. Dams construction destabilizes the aquatic environment for a number of years which has serious implications for the aquatic area. There is a replacement of rheophilic fauna with limnophilic fauna. This changes affect not only phytoplancton and zooplancton but also a fish, especially because the dams make it difficult for migratory fish to enter the reservoir which are reflected to changes in their parasite fauna such as elimination, reduction or emergence of species which are not endemic for that area (Morley, 2007). There are anthropogenic impacts that may directly affect the physical parameters of the water and consequently parasites. For example, thermal power station Nikola Tesla (TENT) on the Sava River near Obrenovac, affect water temperature. As a result of cooling water from TENT A and B water temperature in Sava River is for 2 C° higher then water in Danube River. Because of higher temperatures there is no ice cover on Sava River. Missing of ice cover has a indirect influence on parasitic fish fauna. In these condition fish don’t reduce feeding and migratory birds may remain present during all seasons, creating the potential for parasite transmission throughout the year. Also, on the higher temperature duration of parasite life cycle is shorter (Marcogliese, 2001).

Demographic and tourism habits changes

As a result of globalisation, new tehnology and improved transport, in the last few decades it has been noted increasing number of immigrants from developing parts of the world to developed countries in search of opportunities and better life. This mobility implies also importing different cultures, health beliefs, food preferences that can present a risk factors not only for immigrants but for public health (Broglia and Kapel, 2011). An interesting variation on the potential risks associated with the immigration is the report of *O. felineus* infections in a family of Russian immigrants residing in Israel. One of the members of the family brought back from a visit to the family’s former home, in an endemic area of Siberia, an infected fishwhich was then consumed locally (Chai et al., 2005).

Some changes of the twentieth century, like tourism and international travel, have a great role in increasing number of not only fish-borne parasitic zoonoses, but of all parasitic diseases. In 2008, the World Tourism Organization reported that international tourist arrivals reached 924 million, and this number was expected to increase to 1.6 billion by the year 2020. Far and exotic destination that once was availble only for high class of society, nowadays present popular places for vacation. Travellers dining in street restaurants can be expected to have much higher risks of infections with various parasites, especially in Asia (Broglia and Kapel, 2011).

Changes in cultural habits, culinary practices and life style

Human behaviour and changes in live style may affect public health on many ways. Untill recent eating of raw fish was part of traditions, cultural and religious beliefs, but nowadays it presents part of everyday life, not only in Asia but all over the world. Eating sushi, sashimi (traditional Japanese dishes), koi-pla, kinilaw and cevishe is becoming increasingly fashionable in many countries as a result of globalisation and media, which play a significant role in popularisation of exotic foods (Broglia and Kapel, 2011; Macpherson, 2005). People more often consume raw or undercooked foods in the so called street-food restaurants or take-away, that do not always respect food safety standards (WHO, 2002). But it is not always a fast food products that present a risk factor for fish borne parasitic zoonoses. The increased awareness of healthy dietary regimes to prevent cardiovascular disease and cancer has raised fish consumption, including fish oil, omega-3 fatty acids, which has led to more aquaculture products from Asian markets being now globally traded and consumed (Broglia and Kapel, 2011).

Control Measures

A number of control measures are used to protect fish products from parasites (Orlandi et al., 2002). Evisceration of fish soon after catching prevents the larvae from migrating from the intestinal tract to the muscle tissue of the fish (Baltić et al., 2005). Moreover, if there are parasites in fish tissue they can be killed by using physical or chemical treatments. Salting and marinating are the chemical treatments most commonly used to inactivate viable helmints larvae, but their efficiency depends on concentration of salt and duration of treatment period. Vegetable products such as shogaol and gingerol extracted from Zingiber officinale are able to kill A. simplex, but only under specific conditions (EFSA, 2010). Freezing (-35C° for 15 h or -15C° for at least 96 h) and cooking (60C° for at least 1 min.-core temperature) remain the reference processes guaranteeing the destruction of larvae. Other physical treatments which can be used for killing helmints larvae are high hydrostatic pressure, irradiation (for some parasites) and hot smoking (EFSA, 2010; Orlandi et al., 2002). Low voltage current is patented in Spain in 2005, but this method is not currently available and requires further research (EFSA, 2010).

CONCLUSIONS

Some natural changes like global warming, but more important anthropogenic impacts such as pollution, dams construction or human behaviour affect parasites life cycles, distribution, and prevalence, making the multiple routes of transmission more complicate and difficult to understand. As a result of these impacts, fish borne parasitic zoonoses present global increasing, not only health but also economic problem.

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THE HEALTH STATUS AND EDIBILITY OF FISH FROM THREE HYPERTROPHIC IMPOUNDMENTS IN SOUTH AFRICA

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ZDRAVSTVENI STATUS I JESTIVOST RIBE IZ TRI HIPERTROFNE AKUMULACIJE U JUŽNOJ AFRICI

Apstrakt
Severozapadna oblast Južne Afrike je poznata po ekstenzivnim rudarskim i poljoprivrednim aktivnostima. Ove aktivnosti spiraju organska i neorganska jedinjenja iz zemljišta i za posledicu imaju pogoršanje kvaliteta vode u akumulacionim jezerima. Sledeće akumulacije iz severozapadne oblasti: Hartbeespoort Dam (HD), Klipvoor (KD) i Bospoort (BD) su poznate po visokim količinama nutrijenata i klasifikovana su kao hipertrofna jezera. Procena zdravlja riba i procena rizika po zdravlje ljudi čine važne komponente u uspostavljanju standarda kvaliteta vode i/ili dozvoljenih nivoa konzumiranja riba. Cilj ovog istraživanja bio je da se utvrdi: (1) da li su prisutni štetni efekti na jedinkama *Clarias gariepinus* i *Cyprinus carpio* iz jezera HD, KD i BD (2) da li su prisutni efekti na ljudsko zdravlje ukoliko se ovakva riba konzumira. Rezultati su poređeni sa kontrolnom lokacijom - jezerom Marico-Bosveld Dam (MD). Voda, sediment i ribe (n=20) su uzorkovani iz HD, KD i BD. Uzorci vode i sedimenta su analizirani na prisustvo neorganskih i organskih molekula. Riba je izmerena, urađena je disekcija i pregled, uzeti su uzorci krvi i određena je starost svakog primerka. Organi koji su korišćeni za histološku procenu stanja su bili: škrge, jetra, bubreg, testisi i jajnici Urađena je kvalitativna i polukvantitativna histološka procena. Mišićno tkivo svakog primerka je uzorkovano i izvršene su hemijske analize, koje su korišćene za procenu rizika po zdravlje ljudi. Makroskopskom analizom konstatovano je da je značajan broj riba iz jezera HD, KD i BD imao masnu degeneraciju i fokalnu promenu boje jetre, promene na koži i ozbiljnu infestaciju parazitima u visceralnoj šupljini. Vrednosti hematokrita su varirale od normalne do ispod i iznad normalnih vrednosti. Vrednosti leukokrita su bile u okvirima normalnih vrednosti, osim kod *C. gariepinus* iz jezera HD, koje su

pokazivale više vrednosti od normalnih. Vrednosti ukupnih proteina, factor kondicije i hepatosomatični indeks su bili u okviru normalnih vrednosti za obe vrste na svim ispitivanim lokacijama. Ribe iz jezera MD su bile starije od riba iz druga tri jezera. Histološke promene su primećene na jetri, bubregu i škrgama obe vrste, a jetra je pokazivala najviši stepen promena. pri klasifikaciji, bubreg i škrge su svrstane u grupu 1 (normalna struktura), dok je jetra svrstana u grupu 2 (struktura sa histološkim promenama). Visoki nivoi aluminijuma, silicijuma i hroma su detektovani u mišićnom tkivu. Nivo hroma je bio iznad preporučenih vrednosti. Rezultati hemijske analize mišićnog tkiva su pokazali da je u obe vrste bio detektovan *p,p,*-DDE i to na svim lokacijama, pa i na kontrolnoj. *C. gariepinus* u jezeru HD pokazivao više prosečne vrednosti *p,p*-DDD od propisanih 5µg/g/ jestivog dela ribe. Procena rizika po zdravlje ljudi je obuhvatala jestivi deo ribe (mišiće). Svi nivoi opasnih materija i rizik od izazivanja kancera su bili niski, sa izuzetkom hroma, ali ni količine ovog elementa su bile takve da se ne predviđa pojava zdravstvenih problema kod ljudi ukoliko se riba svakodnevno konzumira.

Ključne reči: histologija riba, procene rizika, zdravlje riba, eutrofikacija
Keywords: Fish histology, human health risk assessment, fish health, eutrophication

INTRODUCTION

The Hartbeespoort, (HD), Klipvoor (KD) and Bospospoort (BD) impoundments in the North West Province, South Africa are known to be polluted, being impacted from mining, industrial and agricultural activities. Excessive nutrient loads such as orthophosphates, resulted in these impoundments becoming hypertrophic and thus the quality of the water is a cause of concern. Pesticides are persistent, insoluble, are not broken down by light and maybe highly toxic to aquatic organisms and particularly those higher up the food chain such as fish, due to bioaccumulation. Fish kills have been reported in the HD, KD and BD indicating and highlighting the potential impacts of the elevated levels of eutrophication as a result of nutrient enrichment (Van Ginkel, 2007). This raised concerns, as both *C. gariepinus* and *C. carpio* from these impoundments are being used as a source of food by the local people and development of extensive commercial fisheries. The aim of this study was to determine (1) if *C. gariepinus* and *C. carpio* from the HD, KD and BD show adverse effects and (2) if consumed, pose a human health risk. The results were compared to the reference site, the Marico-Bosveld Dam (MD).

MATERIAL AND METHODS

Fish of each species *C. gariepinus* (n=20) and *C. carpio* (n=20) were sampled in the HD, KD, BD and the reference site MD using gill nets during 2009 and 2010. The fish were measured and weighed, blood collected for haematocrit, leukocrit and total protein determination. A necropsy was performed on each fish specimen to note any bodily abnormalities externally and internally. A scale from each *C.carpio* specimen and an otolith from each *C. gariepinus* specimen were removed for age determination and a piece of muscle for chemical analyses. The organs included for the histology-based fish health assessment were the gills, liver, kidney, testes and ovaries. The liver, spleen and gonads were weighed to determine the hepatosomatic (HSI), splenosomatic (SSI) and the gonadosomatic (GSI) indices respectively. Organs were processed for a qualitative and semi-quantitative (Bernet et al., 2004; van Dyk et al., 2009) histological assessment using standard techniques.

Physical water quality parameters were recorded. Water, sediment and fish muscle were analysed for inorganic and organic chemicals by accredited laboratories. The chemicals present in the fish muscle were used in the analyses to calculate if indigested fish muscle will pose a risk to human health (US EPA) (2012).

RESULTS

Macroscopically, a number of fish from HD, KD and BD impoundments exhibited liver with fatty change and focal discoloration as well as severe parasites within the visceral cavity. The haematocrit values varied from normal to below and above the normal range. Leukocrit values were within the normal range except for *C. gariepinus* from the HD, which was above the normal range. Total protein values were within the normal range for both species for all sites. Condition factor and hepatosomatic index (HSI) values were within the accepted range. For both species the HSI was the lowest in the reference site, MD.

Microscopically histological alterations were identified in liver, kidney and gills, while no alterations were identified in the gonads. When comparing the selected target organs the liver showed the highest frequency of alterations. The mean organ index values for all study sites fell within class 1 (normal structure) with the exception of the liver index from polluted sites which were in class 2 (structure with histological alterations). The frequency of alterations was more prevalent in *C. gariepinus* than in *C. carpio*. Fish from MD recorded a higher mean age when compared to fish specimen from polluted sites.

The pH values for the hypertrophic dams (HD, KD and BD) ranged from 9.5 to 10.6 while for the reference site (MD) was less than 9.5. Aluminium (HD: 174 mg/L; KD: 1924 mg/L; BD: 1000 mg/L; MD: 1540 mg/L) and Silikon (HD: 0; KD: 4110 mg/L; BD: 1842 mg/L; MD: 3645 mg/L) were the only metals with higher concentrations in the water from all the dams. Atrazine (1.2 µL) was found in the water and it was collected from the reference site, MD. None of the phenols were detected in the water sampled from the HD and MD. None of the inorganic chemical was above the guidelines. The hormones including estrone, estriol, 17-β estradiol and ethynylestradiol were below the detection limit of 10 ng/L. In the sediment Atrazine (10 µg/kg) and Technical nonylphenol (13.2 µg/kg) were found in HD and Ametryn (2.1 µg/kg) and Technical nonylphenol (22.4 µg/kg) were found in the reference site, MD.

In the fish muscle the levels of Al, Fe, Zn Cu, Cr, Ni, Sr and Mn found in the muscle tissue and should pose no threat to fish health or human safety when consumed. Silicon concentrations in muscle were high in all the sites in the muscle tissue of both species. Chromium was found to be higher than the expected guideline values in the muscle samples. Although the muscle samples were pooled the mean concentration op *p,p,*-DDE in tissue from both species was above the limit in fish from HD and the reference site MD. *C. gariepinus* had mean *p,p*-DDD levels higher than the set limit of 5 µg/g per edible portion.

In the Human Health Risk Assessments, the main focus was on the edible parts (muscle) of the fish. From the results of the hazard indices, none of the values of the pesticides are high enough to result in a significant health hazard. The inorganic chemicals (Al and Si) were high and show that it may be a health risk for the consumers, if inhaled. For the calculated cancer risk, the values are all below the 1-in-1 000 000 mark for the pesticides.

DISCUSSION

Evidently, on the basis of the macroscopic and histological results, the fish from polluted sites were more affected compared to fish from the reference site in terms of the parameters mentioned above. Microscopically histological alterations were identified in liver, kidney and gills (Van Dyk et al., 2009; Van Dyk et al., 2012) while no alterations were identified in the gonads (Van Dyk & Pieterse, 2008; Pieterse et al., 2010). Thus the gonads are proposed to be still in their functional state based on the parameters employed (Marchand et al., 2012; Wagenaar et al., 2012). When comparing the selected target organs the liver showed the highest frequency of alterations, which was expected as the liver is a detoxifying organ (Marchand et al., 2012, Van Dyk et al., 2012).

Chemical analysis findings showed that the detection of Atrazine levels in water samples from the MD was the main concern as this site is classified to be a near pristine site. It should be noted that there were agricultural activities next to the MD, which could explain the presence of these Atrazine in the MD. The high Al and Si concentrations in water and sediment are in line with other values reported from other South African waters (DWA, 1996). Silicon concentrations could be an issue, if inhaled. No health data is available for adverse effects, should Si be ingested.

From the results of the hazard indices, none of the values of the pesticides are high enough to result in significant health hazard. All the Hazard Quotients and risks of developing cancer are low with the exception of Cr which is an over prediction based on the VI versus III speciation, thus no predicted adverse health effects are anticipated based on consumption of fish on a daily basis.

CONCLUSIONS

Although macroscopic and microscopic alterations were identified in the selected fish species, it can be assumed that the organs are still in a functional state. The chemical analysis of *C. gariepinus* and *C. carpio* fillets indicated that the flesh of these species is adequate for human consumption, and that it complies with the minimum requirements set by relevant authorities.

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CONSUMERS' OPINION ABOUT EFFECTS OF REARING CONDITIONS AND STRESS ON FISH MEAT QUALITY

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MIŠLJENJE POTROŠAČA O UTICAJU USLOVA GAJENJA I STRESA NA KVALITET RIBLJEG MESA

Apstrakt
Dobrobit farmskih životinja predstavlja veoma aktuelnu temu u svetu, čemu je doprinelo inteziviranje proizvodnje i uočavanje povezanosti između uslova gajenja i pojave zdravstvenih, reproduktivnih i drugih problema. „Dobrobit" i "stres" su povezani i međusobno uslovljeni kod svih životinja, pa i riba. Iako su ribe veoma specifične već samim tim što žive u vodenoj sredini, dokazana je podudarnost sa kopnenim kičmenjacima po pitanju fiziologije stresa i njegovih posledica po zdravlje, produktivnost i kvalitet mesa. Najznačajniju ulogu u tome ima kvalitet životne sredine i hrane. Takođe, dokazano je da ribe mogu da osećaju bol, strah i patnju koje, pre svega uzrokuju postupci pri uzgoju, transportu i klanju.

U cilju dobijanja podataka o informisnosti javnosti u Srbiji o osnovnim činjenicama vezanim za uslove gajenja, stres i dobrobit riba anketirano je 235 punoletnih, slučajno izabranih lica. U radu je prikazan deo rezultata koji se odnose na povezanost uslova gajenja i kvalitet mesa riba. Statistička analiza odgovora izvršena je uz pomoć hi-kvadrat testa.

Približno 91% ispitanika znalo je da kvalitet mesa ribe zavisi od kvaliteta vode u kojoj riba živi, odnosno od kvaliteta hrane koju koristi (90%). Gotovo polovina je smatrala da zna i način na koji voda i hrana deluju na kvalitet mesa riba. O uticaju stresa, kao i načina na koji je riba usmrćena potpunu informaciju imao je najmanji deo anketiranih (22,98%, odnosno 25,96%), dok su nešto većem procentu anketiranih te činjenice bile poznate, ali ne i način delovanja. Veliki broj anketiranih izjasnio se da zna da ribe mogu da osećaju bol (72,77%), strah (70,21%) i patnju (44,68%), a ove emocije je 66,33% povezalo sa postupcima u toku uzgoja, transporta i klanja.

Na poznavanje efekta kvaliteta vode, hrane i stresa na kvalitet mesa ribe značajno je uticalo obrazovanja ispitanika ($p<0,001$) tj. veće znanje su pokazali ispitanici sa višim nivoom obrazovanja. Na poznavanje efekta kvaliteta hrane na sličan način je uticala i visina primanja (p=0,001), a na saznanje da na kvalitet mesa ribe utiče stres starosti ispitanika (p = 0,007), naročito onih između 30 i 49 godina. Shvatanje bola kod riba bilo je značajno povezano sa visinom primanja (p<0,037) i veličinom domaćinstva (p=0,038). Odgovori muškaraca i žena značajno su se razlikovali samo na pitanje da li znaju da riba može da oseća strah (p =0,04), prvenstveno zato što žene manje veruju u to. Preko 70% anketiranih iz gradova i 50% anketiranih iz seoskih naselja smatralo je da riba može da oseća bol. Procenat onih koji nisu čuli za to najveći je u selima (37,04%), pa u četiri najveća grada u Srbiji - Beogradu, Novom Sadu, Nišu i Kragujevcu (25,00%). Većina anketiranih iz svih tipova naselja smatralo je da su neki postupci u toku uzgoja, transporta i klanja uzrok bola, straha i patnje riba.

Na osnovu rezultata ovog istraživanja može se zaključiti da, uprkos znatnom broju ispitanika koji se izjasnio pozitivno u pogledu poznavanja efekata uslova života, ishrane i stresa na kvalitet ribljeg mesa, neophodno je bolje informisanje javnosti na ovu temu. Time bi se doprinelo poboljšanju kvaliteta ribljeg mesa, a svakako i dobrobiti farmski gajenih riba.

Ključme reči: stres, riba, kvalitet mesa, potrošači, Srbija

Keywords: stress, fish, meat quality, consumers, Serbia

INTRODUCTION

Welfare of fish has become an important issue in the world almost simultaneously with the development of intensive farming systems. Farmed fish are exposed to numerous stressors, they respond in the same way as other vertebrates, and can feel physical pain and unpleasant emotions (Ashley and Sneddon, 2008; Braithwaite and Boulcott, 2008; Lembo and Zupa, 2010; Wendelaar, 1997). These facts are generally reasons for all farmed animal welfare protection (Anon., 2009).

Food quality and safety for a long time represent a generally accepted concept of human and animal health protection. Violation of welfare principles may affect the safety of fish meat and products (Poli, 2009; Poli et al., 2005). Relation between animal welfare and food safety is mostly considered from an ethical aspects of food production, with requirements for humaneness use of procedures during fish harvesting (Cooke, 2001), as well as stunning and slaughter methods (EFSA, 2009), since these procedures contribute to manifestation of stress reaction and consequently unwanted processes in meat. Food safety can also be endangered by harmful substances, microorganisms and parasites that can get into live fish. Therefore, measures used to improve welfare and to reduce stress in fish, as well as provision of adequate quality of water and feed, stocking density, use of health protection measures, and careful handling with these animals have a positive effect on fish meat quality and safety (Relić et al., 2010).

In the last decade in Serbia great efforts has been directed to improvement of fish production and quality of fish meat (Marković et al., 2011, 2012). These efforts could be significantly supported if effects of rearing conditions and stress on the quality of fish meat are well known to the consumers. In this paper the results of preliminary research related to Serbian consumers' awareness about this topic is presented.

MATERIALS AND METHODS

This research was conducted in spring 2013 on a preliminary sample of 235 adult persons from the territory of the Republic of Serbia. The questionnaire consisted of four groups of questions, and in this paper questions from the first and the third group were analyzed. The first group consisted of questions related to gender and age, personal net monthly income, education, number of household's members, the presence of juveniles in the household, and character of the place of residence. In the second group were questions about frequency of fish consumption and about habits of Serbian consumers. The third group was represented by questions about terms related to rearing conditions and stress in fish i.e. understanding of effects of water (Question 1), feed (Question 2), stress (Question 3), and killing method (Question 4) on fish meat quality. In addition, consumers' opinion about existence of pain (Question 5), fear (Question 6), and suffer in fish (Question 7) and, finally, awareness that some handling procedures during rearing, transport and slaughter could cause pain, fear and suffer in fish (Question 8) were also analyzed.

Data analysis was performed by using Stat Soft 6.1., and Microsoft Excel 2007 softwares. Distributions of responses are presented graphically. For statistical analysis of the uniformity of answers through different categories and independence of the responses with respect to their demographic and other characteristics the chi-square test (x^2) was used.

RESULTS AND DISCUSSION

Analysis of answers to the first group of questions showed that in the sample of 235 persons, men (110) and women (125) were equally represented (x^2=0.96, P=0.328). Respondents were not equally distributed by age contingents (x^2=25.66, P<0.001). Most of the interviewed persons (71) were in the age group of 40 to 49 years. From contingents of age up to 40 a large number of people were surveyed (101) compared to contingents over 49 (63). Number of respondents with primary and secondary school (106) was not significantly different (x^2=2.25, P=0.134) from the number of better educated respondents (129). About the same number of people (x^2=1.23, P =0.267) participated in the survey with incomes below the (109) and above 40,000 RSD (126). In this survey, people from households with different number of members were represented in a different way (x^2= 36.13, P < 0.001). Similar to the situation in Serbia in respect to the average number of household members (Đorđević, 2008), respondents were mostly from households with three (67) and four (65) members. A significantly higher number of respondents (153) came from household without underage persons (x^2=21.45, P < 0.001), as well as from the four largest cities, 160, (x^2= 130.53, P < 0.001).

Further analysis showed the structure of the answers to three groups of two questions is the same (Fig. 1). Distribution of the respondents to the questions consents that they know that quality of fish meat is affected by quality of the water (Question 1) and fish feed (question 2), than by stress (Question 3) and method of killing (Question 4), as well as that fish can feel pain (Question 5) and fear (Question 6).

Approximately 91% of respondents knew that quality of fish meat depends on the **quality of water** as their environment and on the quality of their feed (90%). A slightly higher percentage of respondents knew how water quality affects the quality of fish meat (48.94%), in comparison to the percentage of persons aware of that fact, but did not know how it happens (42.13%). Similarly, slightly higher was incidence of respondents that knew the mode of action of **feed quality** on the quality of fish meat (47.66%) compared to those who knew about this influence, but did not know the mode of action (42.55%).

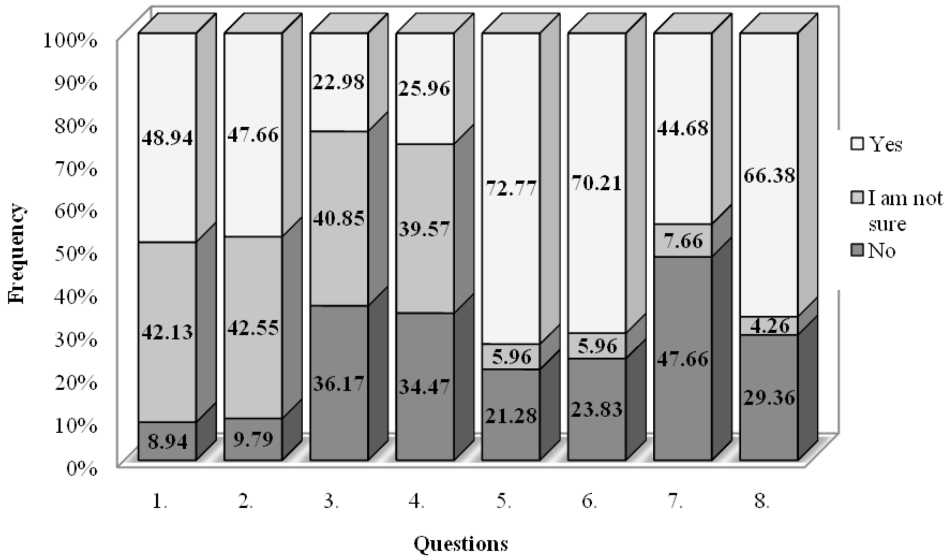

Figure1. Frequency of answers to the questions related to rearing conditions and stress in fish

Respondents were mostly aware that the quality of fish meat is associated with **stress** (40.85%) and the way the fish are killed (39.57%), but not the way of their actions. The smallest percent had complete information on the effect of stress (22.98%) and **killing method** on fish meat quality (25.96%). It is interesting that respondents knew that fish can feel **pain** (72.77%), **fear** (70.21%), and **suffer** (44.68%). Larger part of the respondents (66.33%) thought that some of the practices during rearing, transport and slaughter are the cause of these three emotions in fish.

The structure of the question on knowledge of the effect of water quality on the quality of fish meat depended on respondents level of education (x^2 x^2 = 22.59, P < 0.001). Half of the respondents (50.00%) that have completed primary or secondary school this fact was known, but without awareness about the mode of action. Persons with higher qualifications usually stated (61.24%) to know the relationship between the quality of fish and the quality of the water and how they are conditioned.

Knowledge of the respondents about impact of fish feed on the quality of fish meat depended on the level of education (x^2 x^2 =21.41, P <0.001) and their personal income (x^2 x^2 = 13.40, P=0.001). Similar to previous question about water quality effect, half of the respondents (50.00%) who completed primary or secondary school knew that food quality affects the quality of fish meat, but did not know in which way. More educated people mostly (59.69%) were aware of this relationship and their mutual dependence. Among respondents with lower incomes the highest percentage (47.71%) answered that they were aware the quality of fish meat is affected by the quality of fish feed, but did not know how. More than half of respondents (57.14%) with income over

40,000 RSD knew that the quality of fish meat depends on the fish diet, and they were familiar with the manner of that effect.

Awareness that stress affects the quality of fish meat very significantly depended on the age of the respondents (x^2=21.25, P=0.007), their education (x^2 =26.14, P<0.001), and monthly income (x^2=16.75, P<0.001). Among respondents younger than 29 the most numerous were those who did not know or were not sure that stress affects the quality of fish meat (63.64%), while in other age groups most of respondents (40.74 - 47.22%) knew this fact but did not know the mode of action. Only 6.82% of the youngest knew the fact of existence of stress and how it affects the quality of fish meat. Among respondents aware of the effect of stress 66.67% were between 30 and 49 years, and only 5.56% of younger.

52.83% of people with lower level of education did not know or were not sure about stress influence on fish meat, and 12.26% declared to know about stress and the mode of action. More educated respondents mostly were aware (45.74%) that the quality of meat depends on the stress, but did not know in which way. That was followed by persons who knew about stress effects and the manner of its action (31.78%). Knowledge of stress was positively correlated with the level of education.

Almost half of the respondents with income up to 40,000 RSD (48.62%) did not know that stress affects the quality of fish meat. Most of the respondents with incomes over 40,000 RSD (43.65%) were aware that the quality of fish meat is affected by stress, but did not know how, and 30.95% knew also the way of action. This means that the respondents with higher incomes were more informed about stress in fish.

Respondents which were classified into two groups according to income differed significantly by the knowledge that fish can feel pain. Although in both groups a higher percentage believed that fish can feel pain, among those who did not believe the answers were significantly associated with the level of income (x^2 =6.60, P < 0.037).

Independently of the household size, the largest part of respondents believed that fish can feel pain. However, the ratio of these values and the participation of those who have not heard that fish can feel pain suggest that knowledge about pain in fish was related to household size (x^2=16.30, P = 0.038).

Male and female responses were significantly different (x^2=6.34, P=0.04), only concerning the question of knowledge that fish can feel fear, especially because women believed in it less. One of the reasons could be small number of women who go for fishing, where they could have the opportunity to see manifestation of fear in fish.

Answers to the questions in the survey were independent of presence of children in the household.

Respondents from different types of settlements gave different answers on their awareness about fish fear (x^2=10.51, P = 0.033) and about influence of some practices during rearing, transport and slaughter on pain, fear and suffering in fish (x^2=12.93, P = 0.012). Over 70% of the citizens and 50% of respondents from rural areas believed that fish can feel pain. The percentage of those who have not heard about that was the greatest in rural areas (37.04%), and then in the four largest cities (25.00%). Opinion about fish feeling fear depended on the settlement of surveyed. However, respondents from all types of settlements, 59.68% in rural and 68.13% in Belgrade, Novi Sad, Niš and Kragujevac, believe that some of the practices during rearing, transport and slaughter cause pain, fear and suffering of fish.

Considering all data, this preliminary study is comparable with similar research carried out in the world (Honkanen and Olsen, 2009; Solgaard and Yang, 2011), at the first place in respect to the result that respondents' answers mainly depended on their knowledge about particular topic.

CONCLUSIONS

Results of this preliminary study showed that public in Serbia generally consider that life conditions, diet, stress, and procedures during rearing, transport and slaughter affect the quality of fish meat. With regard to the emotions of fish, people mostly recognize the feeling of pain and fear in fish.

Despite the considerable number of respondents that answered positively regarding the main factors affecting the quality of fish meat, better informing of public on this topic is necessary. This would also contribute to improving quality of fish meat, and certainly the welfare of farmed fish.

ACKNOWLEDGMENT

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EFFECT OF DIETARY VITAMIN C ON SURVIVAL AND GROWTH PERFORMANCE OF CASPIAN BROWN TROUT (*SALMO TRUTTA CASPIUS*) FINGERLINGS

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EFEKAT VITAMINA C IZ HRANE NA PREŽIVLJAVANJE I RAST MLAĐI KASPIJSKE POTOČNE PASTRMKE (*SALMO TRUTTA CASPIUS*)

Apstrakt

Ovo istraživanje je sprovedeno da bi se utvrdio uticaj vitamina C na rast, preživljavanje i hepatosomatski indeks (HSI) kaspijske potočne pastrmke (*Salmo trutta caspius*). Korišćeno je pet različitih vrsta hrane koje sadrže 0, 50, 100, 200 i 400 mg kg^{-1} vitamina C u obliku L-Askorbil-2-polifosfata. 600 jedinki pastrmske mlađi (9,6±0,6 g) nasumično je podeljeno u pet tretmana sa po tri ponavljanja, od kojih je svaki sadržavao 40 riba. Hranjenje je vršeno devet nedelja a pokazatelji rasta, uključujući prirast (WG), specifičnu stopu rasta (SGR), factor kondicije (CF), stopu efikasnosti proteina (PER), stopu konverzije hrane (FCR), i HSI izračunati sun a kraju eksperimenta. Rezultati su pokazali da je stopa preživljavanja riba u svim tretmanima bila100 %, a tokom eksperimenta kod mlađi kaspijske pastrmke nisu zabeleženi znaci nedostatka askorbinske kiseline. Značajne razlike između tretmana ($p<0.05$) utvrđene su u pogledu finalne težine (FW), prirasta (WG), specifične stope rasta (SGR), stope efikasnosti proteina (PER), stope konverzije hrane (FCR), faktora kondicije (CF) i hepatosomatskog indeksa (HIS). Rezultati su pokazali das u ribe koje su hranom dobijale 200 mg kg^{-1} askorbinske kiseline pokazale imale najbolje rezultate, iako nisu zapažene značajne razlike između tretmana sa 200 i 400 mg askorbinske kiseline. Dakle, za najbolji rast mlađi kaspijske potočne pastrmke optimalna količina vitamina C za svaki kilogram hrane je 200 mg askorbinske kiseline.

Keywords: Vitamin C, *Salmo trutta caspius,* preživljavanje, rast.
Keywords: Vitamin C, *Salmo trutta caspius,* survival, growth.

INTRODUCTION

Vitamin C (Ascorbic acid) is an essential vitamin which plays important roles in the physiological activities of animals like fish (Tolbert, 1979; Boyle, 2005). Due to the lack of L-Gulonolactone Oxidase enzyme, most of the teleosts cannot synthesize ascorbic acid and are depended to the diet supplementation (Fracalossi et al., 2001; Ai et al., 2004). Low levels of ascorbic acid will result in skeletal abnormality, impaired collagen formation, internal bleeding, reduced growth rate, and feeding appetite (Gouillou-Coustans et al., 1998; Oikawa et al., 2008). In cultured fish species diets with the lack of ascorbic acid may even leads to increased mortality rate (Ortuno et al., 2001; Lin and Shiau, 2005).

Adequate amount of ascorbic acid in fish diet, especially in life early stages, plays an important role in disease resistance followed by fish immunity and survival. However, ascorbic acid need is variable among different aquatic species and is accompanied by fish size, diet composition, and cultural system (Ortuno et al., 2001; Lim et al., 2002; Ai et al., 2004; Azad et al., 2007; Garcia et al., 2007). Therefore, determination of the vitamin requirements is necessary for normal growth and cultural performance.

The Caspian brown trout (*Salmo trutta caspius,* Kessler, 1877) is one of the salmonid species which is distributed in the south western coasts of the Caspian Sea and migrates to the freshwater rivers for spawning (Berg, 1949; Nikolskii, 1961). In spite of several studies on the nutritional requirement of the Caspian brown trout (Saber et al., 2005; Ramezani, 2009; Sotoudeh et al., 2011), little information is present about the vitamin needs as the most important factor in grows and immunity. Considering the vitamin C importance for reaching to suitable weight, the current research was done on the how different levels of ascorbic acid in fish diet will affect the survival, growth, hepatosomatic index of Caspian brown trout fingerlings.

Several studies have been done to preserve and restore the resources of the Caspian brown trout (Saber et al., 2005; Sayyad Burani et al., 2006; Zamani et al., 2007; Rahbar et al., 2009).

MATERIALS AND METHODS

Diets Preparation

Basal diet was prepared according to the Saber et al. (2005) suggestion for normal growth of Caspian brown trout to contain 49.60 % crude protein, 14.96 % lipid and 17.50 % ash. The other experimental foods were made by adding L ascorbyl-2-polyphosphate (LAPP) (25% ascorbic acid equivalent, Tiger, China) to obtain 0, 50, 100, 200 and 400 mg ascorbic acid equivalent kg^{-1} diet, respectively. The precise level of ascorbic acid in each experimental diet was analyzed by a reverse-phase high-performance liquid chromatogram (HPLC, KNAUER pump 1000, German).

Proximate Food Composition

The amount of dry matter was evaluated by drying an aliquot of each diet in a mechanical convection oven at 105 °C for 16 h to a constant weight. Ash content was determined based on AOAC (1998) method 938.08 by heating an aliquot of the samples in a muffle furnace at 550 °C for 3 h and weighing the remaining material. Protein content was also determined by converting the nitrogen content (N×6.25) based on the Kjeldahl method. Total lipid content was also extracted according to the Bligh and Dyer (1959) method using a chloroform-methanol (1:1 by vol.) mixture. All of the measurements were done according to the AOAC (1995) and the result expressed as g/100 g diet.

Ascorbic acid content was also analyzed according to Shiau and Hsu (1999) with some modifications. Approximately 3–5 g of grounded feed was treated with 25 ml chloroform and 100 ml distilled water, shacked for 25 min, settled for 25 min, and centrifuged for 5 min at 2739 ×g. One ml of the supernatant was buffered by 0.2 M acetic acid buffer (pH=4.8) and 0.2% DTT, kept in 37 °C bath for 2 h, and centrifuged for 6 min at 2739 ×g. After this, 20 μl of supernatants were sieved through a 0.22 μm pore size syringe filter and subjected to ascorbic acid analysis. The ascorbic acid content in the diets were determined by reverse-phase HPLC (KNAUER pump 1000, German) with an ODS column (4.6×25 mm, German). Mobile phase (flow rate 0.6 ml min^{-1}) was an aqueous solution of 0.05 M KH_2PO_4 (adjusted to pH 2.8 with phosphoric acid) and the effluent was monitored by a UV-detector (254 nm wave length).

Experimental Procedure

Six hundred Caspian brown trout fingerlings have been obtained from the Coldwater Fishes Research Center (CFRC), Tonekabon, Iran, and transferred to a local fish farm in Dohezar Road, Tonekabon. After fasting for initial adaptation to the experimental condition, the specimens were fed by artificial food (Behparvar Co., Karaj, Iran). Average weight of fish fingerlings at the beginning of the test was adjusted to 9.6±0.6 g and water was provided from a spring with flow of 6 L s^{-1} during the experiment.

This study was conductedin five raceway ponds (5×1×0.8 meter), and each had an input flow rate of 1 L s^{-1}. Each pond was divided to three equal parts by lace fabric with tiny holes and 40 fish fingerlings have been randomly distributed in these 15 testing areas. Mortality was not observed during periods of the adaptation and the experiment. The bioassay was in triplicate performed for nine weeks. The blocks were randomly divided into five treatments including 50, 100, 200 and 400 mg kg^{-1} ascorbic acid along with control treatment. Fish were hand-fed to an apparent satiation at 8, 12 and 16 o'clock in each treatment and the consumed amounts of food were recorded for each pond. It should be noted that food consumption by fingerlings fish was too fast and no food remained in water. The water temperature, dissolved oxygen, pH and electrical conductivity were measured every week. During the experiment period, the temperature ranged from 9.6 to 10.2, the pH ranged from 7.5 to 7.7, electrical conductivity ranged from 248.3 to 252.8 and the dissolved oxygen content was approximately 10.2 mg l^{-1}.

Growth parameters were determined during nine weeks. The fish were fasted 24 hours before harvest. The total body weight (0.01 g) and total length (0.1 cm) were determined after anesthesia by 250 ppm clove oil based on Soltani et al. (2001) recommendation.

Calculations and Statistical Analysis

The growth parameter including weight gain (WG), specific growth rate (SGR), condition factor (CF), protein efficiency rate (PER), feed conversion rate (FCR), and hepatosomatic index (HSI) were calculated for each treatment based on the formula suggested by Sotoudeh et al. (2011).

All statistical analysis was done using SPSS-16 software package. The means were subjected to the analysis of variances (ANOVA) after examination of normality by Colmogornov-Smirnov test. Where the differences were occurred, the Tukey's HSD test was used to determine the difference. Results are expressed as mean±SE and a P-values less than 0.05 were determined as significant.

RESULTS

Proximate composition of experimental diets

The results of the proximate composition for each experimental diet are presented in Table 1. No significant difference was found between the treatments in the amount of moisture, protein, lipid, and ash. Reduction in the amount of ascorbic acid in all experimental diet after food preparation could be related to the heating of diet during the process.

Table 1. Proximate composition of experimental diets (%)

	Moisture	**Protein**	**Lipid**	**Ash**	**Ascorbic acid**
Control	5.23±0.35	49.6±0.25	15.01±0.57	17.40±0.13	9.8
50 mg AA	5.57±0.32	49.7±0.24	14.80±0.48	17.33±0.09	43.8
100 mg AA	5.56±0.28	49.6±0.31	15.20±0.35	17.56±0.14	89.4
200 mg AA	5.62±0.30	49.5±0.23	14.88±0.43	17.43±0.18	188.5
400 mg AA	5.64±0.32	49.6±0.49	14.93±0.39	17.78±0.17	384.2

Similar letters at the same column show no significant difference between results for each treatment ($p<0.05$).
AA: Ascorbic Acid

Survival and growth

The survival rate of fish in all treatments was 100% and no symptoms of ascorbic acid deficiency in the Caspian brown trout fingerlings were observed during the 9-week experiment. The results showed that nine weeks of testing 200 mg AA kg^{-1} diet caused a significant increase in the growth compared with the control treatment and 50 mg AA kg^{-1} diet ($p<0.05$), however no significant difference was observed in final weight of fishes with 100, 200 and 400 mg treatment after nine weeks (Figure 1).

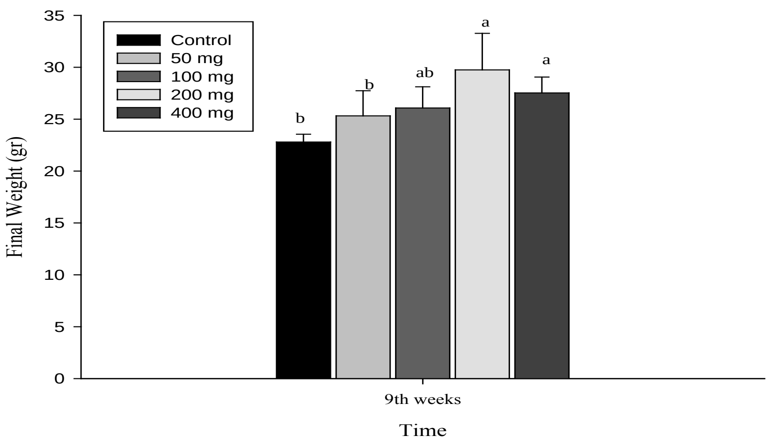

Figure 1. Effects of ascorbic acid on Final weight of Caspian brown trout (*Salmo trutta caspius*). Similar letters at the same column show no significant difference between results for each treatment ($p<0.05$).

Weight gain had also shown the same trend. The fingerlings fed by the control diet had the lowest weight gain, while the highest weight gain was found in the 200 mg AA treatment (20.10±0.98 g) after nine weeks. Moreover, the weight gain rate in 100 and 400 AA treatments were lower than 200 mg AA treatment ($p<0.05$), although no significant difference was recorded after the 9-week experiment (Table 2).

Specific Growth Rate (SGR) was also significantly different between treatments after nine weeks experiment ($p<0.05$). The 200 mg AA treatment had the maximum SGR with the rate of 0.77±0.02 % d^{-1}, while had no significant differences with 100 and 400 mg AA treatments. The control treatment had also the lowest rate of SGR by 0.59±0.03 % d^{-1}.

Specific Growth Rate in treatment of 200 mg ascorbic acid per 1 kg food was higher than in other treatments. However, there were no significant differences between treatments of 100 and 400 mg ascorbic acid per 1 kg food (Table 2).

Table 2. Weight gain and SGR of Caspian brown trout (*Salmo trutta caspius*) fed diets with graded levels of ascorbic acid for 9 weeks.

Target vitamin C supplementation (mg kg^{-1})	Analyzed ascorbic acid level (mg kg^{-1})	Growth response	
		Weight Gain (g)	SGR (% d^{-1})
Control	9.8	13.14±0.10^{c}	0.59±0.03^{b}
50 mg AA	43.8	15.66±3.74^{b}	0.64± 0.11^{b}
100 mg AA	89.4	16.42±1.27^{b}	0.68±0.03^{ab}
200 mg AA	188.5	20.10±0.98^{a}	0.77±0.02^{a}
400 mg AA	384.2	17.87±1.47^{b}	0.72±0.03^{a}

Similar letters at the same column show no significant difference between results for each treatment ($p<0.05$).
AA: Ascorbic Acid

The results showed a significant difference the amount of Protein Conversion Ration (PER) between the treatments ($p<0.05$). The lowest and highest PER was observed in control and 200 mg AA treatments with the rate of 1.32±0.12 and 2.02±0.09, respectively. However, no significant difference was found between 200 and 400 mg AA treatments. Besides, the 50 and 100 mg AA treatments showed no significant differences in the amount of PER with control treatment (Table 3). Fish fingerlings fed by control diet had the highest FCR with ration of 1/52±0/02 after the 9-week experiment, although no significant difference showed with 50 and 100 mg AA treatments. The lowest FCR was observed in the 200 and 400 mg AA treatment the ratio of 0.99±0.07 and 1.13±0.16, respectively (Table 3).

An increasing trend of HIS was attained by increase of AA in the feeding diet. The highest HSI of 1.55±0.05% was recorded in 200 mg AA treatment, although had no significant differences with 400 mg AA treatment. No significant difference was observed between 50, 100, and 200 mg AA treatments. There was no significant difference in the value of Condition Factor (CF) between treatments (Table 3).

Table 3. Growth response of Caspian brown trout (*Salmo trutta caspius*) fed diets with graded levels of ascorbic acid for 9 weeks

	PER	FCR	HSI	CF
Control	1.32±0.12[b]	1.52±0.02[a]	1.30±0.08[c]	1.00±0.04[a]
50 mg AA	1.57±0.37[b]	1.49±0.46[a]	1.40±0.12[b]	1.00±0.02[a]
100 mg AA	1.65±0.12[b]	1.23±0.16[a]	1.44±0.07[b]	1.02±0.05[a]
200 mg AA	2.02±0.09[a]	0.99±0.07[b]	1.55±0.05[a]	1.03±0.04[a]
400 mg AA	1.80±0.14[ab]	1.13±0.16[ab]	1.46±0.03[ab]	1.02±0.03[a]

Similar letters at the same column show no significant difference between results for each treatment ($p<0.05$).
AA: Ascorbic Acid

DISCUSSION

The Caspian brown trout is of the economically valuable species which the recruitment rate affects their population in the southern area of the Caspian Sea. According to an accepted theory, fish with higher growing rate will have higher survival rate by rapidly change in their role from hunt to hunter (Bergenius et al., 2002). It means that specimens with higher weight will have more opportunities for remigrating to the maternal rivers.

This study showed that vitamin C as an essential component of the diet increases the growing rate of Caspian brown trout fingerlings. The lower final weight of the fingerlings in control treatment indicates the direct effect of vitamin C on the growth of Caspian brown trout. Ascorbic acid is an essential coenzyme in Tyrosine amino acid oxidation and phenylalanine (Brander and Pugh, 1977) which can increase weight gain and protein efficiency rate. Accordingly, the higher levels of ascorbic acid lead to the faster growth rate by increasing protein content (Brander and Pugh, 1977; Faramarzi, 2012), although determination of amino acid profile in Caspian brown trout fingerlings muscles is necessary for common scenes. Ascorbic acid can also affect the synthesis of collagen as a structural protein which their synthetize can cause higher final weight gain (Smedsrød et al., 1993;Terova et al., 1998).

No significant difference in growth rate of Caspian brown trout fingerlings in 200 and 400 mg ascorbic acid treatments may be related to the supplement of ascorbic acid needs for biological activities and its saturation in storage tissues such as liver and muscle. However, continuous adding of ascorbic acid into fish feed can result in a lower amount of vitamin C needed to prevent symptoms of ascorbic acid deficiency in the Caspian Brown trout fingerlings, such as deformation of the gill operculum and spine, internal bleeding and reluctance to absorb food, and even high level of mortality (Lin and Shiau, 2005; Xie and Niu, 2006; Ibiyo et al., 2007; Tewary and Patra, 2008). No effects of ascorbic acid deficiency in control treatment could be also related to the previous vitamin C storage in the target tissues of the fingerlings (Ai et al., 2004; Li et al., 2007; Soltani et al., 2008). Therefore, longer period of vitamin C absence may cause deficiency signs of ascorbic acid in the Caspian brown trout fingerlings.

Higher rate of HSI in this study indicates the higher storage of glycogen assisting the Caspian brown trout fingerlings in energy saving, resistance to stressful situations and immunity against pathogens (Moon et al., 1989; Sampaio and Criscuolo, 2006). Confirming the previous studies, this study shows that ascorbic acid can impact on FCR (Ibiyo et al., 2007; Soltani et al., 2008; Adewolu and Aro, 2009). The best FCR in Caspian brown trout was found in 200 mg ascorbic acid treatment, although it had no significant difference with 400 mg ascorbic acid treatment. Therefore, 200 mg ascorbic acid can commercially be considered as the optimum amount for rearing of Caspian brown trout fingerlings by reduction of food costs and prevention of secondary contaminations.

The results of this study indicate that 200 mg ascorbic acid kg^{-1} diet of the Caspian brown trout fingerlings have significant effect on their SGR compared to the other treatments. In contrast, other salmonid members have been shown no significant differences of SGR with similar or higher levels of ascorbic acid (Dabrowski, 1991; Thompson et al., 1993). The dissimilarity in results could be due to differences in species specific characteristics, water temperature, fish density and other cultural conditions. The initial weight of the specimens, a critical factor affecting SGR, in this study was 9.6±0.6 g which was lower than initial fish weight in similar studies (Dabrowski, 2001; Ai et al., 2004; Handeland et al., 2008). Therefore, the higher SGR of Caspian brown trout fingerlings in this study can be due to their lower initial weight and the potential to use ????? as a supplementary factor in natural metabolisms and weight gain (Ibiyo et al., 2007; Soltani et al., 2008; Adewolu and Aro, 2009). The condition factor in ninth weeks of the experiment had no significant difference between the experimental diets supplemented with or without ascorbic acid. In support of previous studies, condition factor higher than 1 in the present research illustrated that increasing ascorbic acid in the diet will improve the growing condition of Caspian brown trout fingerlings (Cetinkaya and Sen, 2005; Ibiyo et al., 2007; Adewolu and Aro, 2009).

CONCLUSIONS

Results of the present study show that 200 mg ascorbic acid treatment have the best effect on growth rates of Caspian brown trout fingerlings compared with other experiment treatments. However, appropriate amount of ascorbic acid depends on the other factors such as age, weight, diet composition, physiological conditions, and the maturation stage (Dabrowski, 1986; Wang et al., 2002; Ai et al., 2004; Ibiyo et al., 2007). Further research particularly on the effect of ascorbic acid in non-specific immunity factors for longer periods will help to provide a more precise basis about the ascorbic acid necessity in Caspian brown trout diet.

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RELATIONSHIPS OF OTOLITH SIZE TO TOTAL LENGTH OF THE BURBOT (*LOTA LOTA)* FROM THE DANUBE RIVER

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ODNOS VELIČINE OTOLITA I TOTALNE DUŽINE KOD MANIĆA (LOTA LOTA) IZ DUNAVA

Apstrakt

Otoliti se rutinski koriste pri determinaciji vrsta, za određivanje starosti i rasta riba. Sve ove informacije su od velikog značaja za upravljanje i gazdovanje ribljim populacijama, kao i za istraživanja vezana za predator-plen odnose. Odnos između dimenzija otolita i dužine još uvek je nepoznat za većinu naših vrsta riba. Za ispitivanje ovog odnosa, odabran je manić (*L. lota*) koji je poznat kao dobra indikatorska vrsta degradacije sredine; kao vrsta koja je rani indikator uticaja klimatskih promena na hladnovodne vrste riba; kao vrste koja je u poslednje vreme (10-tak godina) postala predmet privrednog ribolova u Dunavu; kao vrsta koja predstavlja redovan i uobičajen plen u ishrani kormorana tokom njihove sezone prezimljavanja. Ispitivan je odnos dužine, širine i težine otolita i totalne dužine tela adultnih primeraka manića. Odnos sva tri morfološka parametra otolita i dužine bio je linearan, visoko korelisan ($r^2 > 0.700$) i statistički značajan (ANOVA, $P < 0.05$). Dobijeni rezultati ukazuju da je odnos širine otolita i totalne dužine tela najbolji prediktor procene dužine adultnih manića ($r^2 > 0.800$). Nije utvrđeno postojanje statistički značajnih razlika determinisanih odnosa za leve i desne otolite (t-test, $P < 0.05$) i određene su zajedničke jednačine regresione prave: $Y = 6.494X – 14.545$ za odnos dužina otolita – dužina tela, $Y = 13.964X – 11.762$ za odnos širina otolita – dužina tela i $Y = 17.006 + 0.559X$ za odnos težina otolita – dužina tela. Dobijene jednačine omogućavaju izračunjavanje totalne dužine adultnih manića na osnovu kompleta podataka o morfološkim karakteristikama otolita.

*Ključne reči***:** *manić, otolit, totalna dužina*
Keywords: burbot, otolith, total length

INTRODUCTION

Otoliths (ear stones) are calcareous structures in the form of aragonite in a protein matrix and commonly are used for determination of the taxon, age and size of fishes. Bony fishes have three pairs of otoliths (the sagittae, asteriscus and lapillus) placed in paired otic capsules on either side of the skull. In general, the sagittae are the largest and characterized with distinct growth rings, therefore being most frequently used by fisheries biologists for fish aging and growth studies. If a relation between otolith size and fish length exists it can be reliable tool in studies on length frequency distributions of fish stocks and commercial landings, as well as in predator-prey studies with aim to estimate original length and weight of consumed prey (Ross *et al.* 2005; Tarkan *et al.* 2007). The otolith size - fish length relationships and their significance may vary among species or among different stocks of the same species, as well as between different sizes of fish of the same species (Hunt, 1992).

The burbot (*Lota lota*), the only freshwater species among cods, is a Palearctic predator fish commonly found in rivers of the Danube basin throughout Serbia, where it inhabits both the lowland (cyprinid) and highland (salmonid) waters. Until recently, a burbot commercial fishery has developed on Danube and it became routinely fished during winter months. Moreover, it is considered as an excellent indicator species of the habitats degradation, as well as an early indicator of climate change on coldwater fish species (Edwards *et al.* 2011). Further, it is documented that burbot represents common food item of cormorants during their wintering season on the European waters (Keller, 1995). However, the status of burbot populations in Serbian waters is largely unknown and little biological information has been collected regarding them. The aim of the present study is to develop predictive relationships between morphological measures of sagittae and total length of the burbot (*Lota lota*) from the Danube, thus providing a useful tool for studies on fisheries and management issues related to this species. Additionally, these relationships may be used in studies on food habits of piscivores.

MATERIAL AND METHODS

A total of 76 burbot specimens were obtained from the commercial fisherman landings. Fish were caught in the Danube River at the Backa Palanka locality using hoop nets. The fish were measured for total length (TL) to the nearest 0.5 cm, weighed to the nearest 1.0 g and sex was determined. The sagittae were extracted, cleaned and stored dry in paper envelops. Three morphometric characters were considered: maximum length of the otolith (anterior-posterior axis), maximum width of the otolith (dorsal-ventral axis), and weight of the otolith. Otoliths length and width were measured using a digital caliper with resolution to 0.01 mm. Otoliths were weighed using a Sartorius digital balance with resolution to 0.0001 g. Only intact otoliths with no missing parts or fractures were utilised in data processing and statistical analysis. Therefore, otolith pairs of 69 fish were examined and the left and right otoliths were considered separately. The relationships between otolith measures and fish length (TL), with fish length considered as the dependent variable, were determined using a least-squares linear regression. The significance of the linear regression was tested using an analysis of variance (ANOVA). Differences between regression coefficients for the relationships of fish length and the morphological measures of left and right otoliths were tested using *t*-tests. All statistical analysis was performed using Statistica 6.0 package (StatSoft).

RESULTS AND DISCUSSION

Fish ranged from 23.5 to 63 cm including subadults and adults. The ranges for length, width and weight were 5.17-10.25 mm, 2.4-4.99 mm, 11.3-78.3 mg and 5.23-9.99 mm, 2.4-4.98 mm and 11.3-75.9 mg for left and right otolitihs, respectively (Fig. 1).

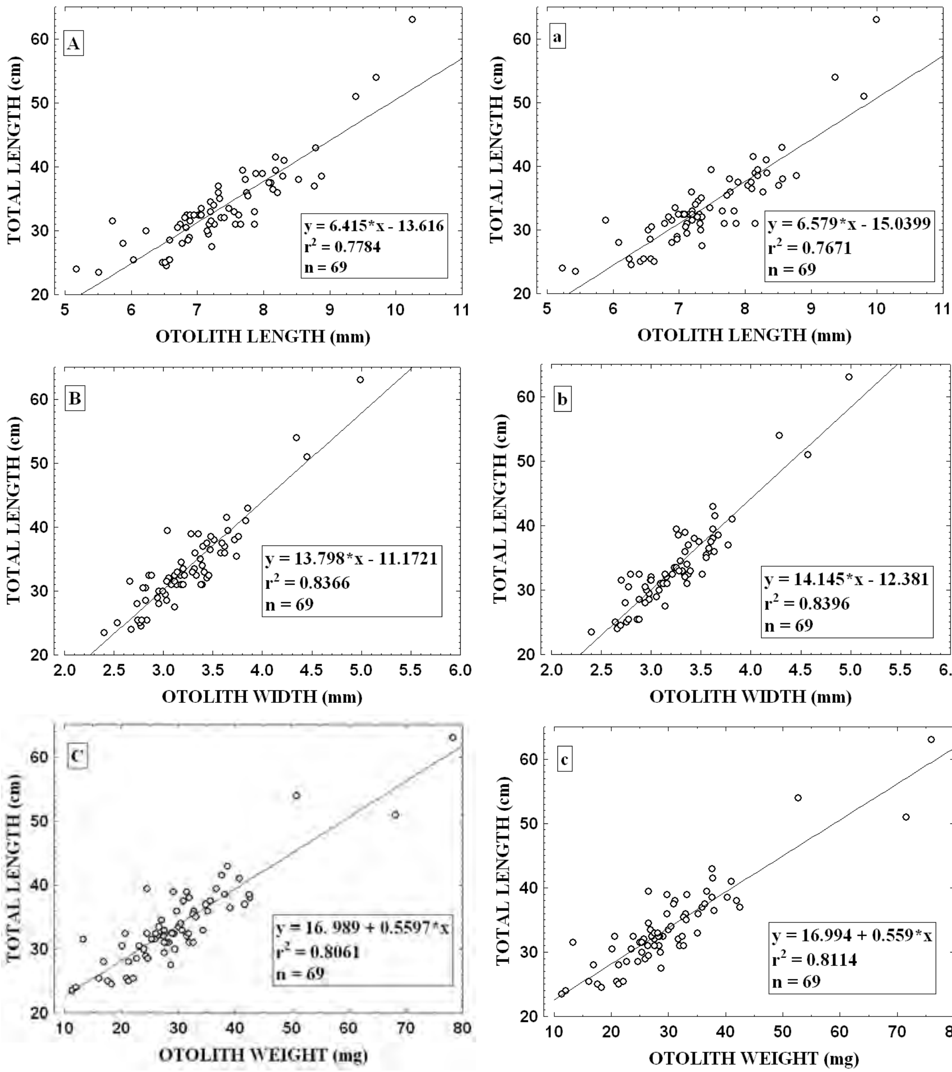

Figure 1. Linear relationships between otolith measures and total length of burbot (*L. lota*): A, B and C – left sided otoliths; a, b and c – right sided otoliths.

The relationships between all otolith morphometric measures and burbot size were linear and most of the variability were explained by obtained regression equations ($r^2 > 0.700$ in all cases; Fig. 1). All relationships were statistically significant (Tab.1), thus confirming that the linear regression model appeared to adequately describe the relationships between otolith dimensions and fish length.

Table 1. Regression analysis of different morphometric measurements in burbot sagittae and total fish length.

Otolith dimensions	Sum of squares	d.f.	F ratio	r^2	P
Length (left sided)	2348.065	1	235.339	0.778	0.00
Length (right sided)	2313.855	1	220.620	0.767	0.00
Width (left sided)	2523.778	1	343.147	0.837	0.00
Width (right sided)	2532.736	1	350.740	0.840	0.00
Weight (left sided)	2431.783	1	278.622	0.806	0.00
Weight (right sided)	2447.503	1	288.170	0.811	0.00

Regression coefficients of each otolith measure to fish length were not significantly different for left and right oriented otoliths (Tab. 2). Based on these results, and according to Zar (1984), a common linear regression equation was calculated for each of the otolith measure to fish length relationship. Thus, the common linear regression equations are $Y = 6.494X – 14.545$, $Y = 13.964X – 11.762$ and $Y = 17.006 + 0.559X$ for otolith length-fish length, otolith width-fish length and otolith weight-fish length relationship, respectively.

Table 2. Results of paired sample *t*-tests comparing regression coefficients of relationships of left and right sagittal otoliths measures versus fish total length.

	Otolith orientation			
Otolith measure	Left	Right	t	P
Length				
slope	6.415	6.579	0.269	>0.05
intercept	-13.616	-15.0399	0.385	>0.05
Width				
slope	13.798	14.145	0.327	>0.05
intercept	-11.172	-12.381	0.376	>0.05
Weight				
slope	0.5597	0.559	0.015	>0.05
intercept	16.989	16.994	0.052	>0.05

Depending on species and ontogenetic stadium, the linear curvlinear and multivariate models could describe relations between otolith size and fish length (Hunt, 1992; Tarkan *et al.* 2007; Zorica *et al.* 2010; Škeljo and Ferri, 2012). Significant linear relationships were found between otolith length, width and weight and total length of adult burbot. Each of the considered otolith morphometrics was strongly correlated with fish length, indicating that both otolith weight and linear dimensions could be used as indicators for burbot length estimation. Our results are consistent with findings on other species that all three otolith size variables are appropriate for predicting size of fishes (Hunt, 1992; Zorica *et al.* 2010; Škeljo and Ferri, 2012). According to Zar (1984) the relationships with the highest coefficient of determination are considered as the best predictor. Therefore, of the considered otolith dimensions width is the most and length the least suitable character for estimation length of burbot adults. Additionally, a strong relationship between otolith width and total length indicates that backcalculation of burbot growth rates based on otolith measurements is feasible, at least for the length ranges assessed here. The statistical testing on differences between left and right otolith are consistent with findings that in bilaterally symmetrical species the otolith pair do not vary markedly (Hunt, 1992; Loher *et al.* 2008). The lack of significant difference between otolith pairs allows the pooling both sided measurements, which is of special importance in estimating size of consumed burbots from measurements of otoliths recovered in stomachs or feces of piscivorous animals.

The common regression equations developed in the current study offer the opportunity of their use in future years to estimate burbot length from data sets of otolith measures. However, we recommend that the relationships be reviewed if data are collected from fish outside the range examined herein or from fish from different regions or different environment.

CONCLUSIONS

Results indicated that both weight and linear dimensions are linearly related to the total length of adult burbot, at least over the examined range of lengths.

Of the considered otolith dimensions, width is the most suitable character for estimation length of burbot adults.

Predicting size of burbot can be accomplished with fair reliability on the basis of developed regression equations.

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OPTIMIZATION MODEL OF FISH GUARD SERVICE IN ORDER TO PROTECT AQUATIC SYSTEMS

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MODEL OPTIMIACIJE RIBOČUVARSKE SLUŽBE U CILJU ZAŠTITE VODENIH SISTEMA

Apstrakt

Važan segment svakog upravljačkog sistema su ljudski resursi i pronalaženje modaliteta da se isti koriste racionalno i ekonomično. Cilj rada je da se na konkretnom primeru određivanja parametara koji utiču na optimalan broj ribočuvara po prvi put prikaže mogućnost primene skalarnog metoda ocenjivanja (SMO) u praktičnom upravljanju ribolovnim vodama. Kombinovanjem metoda SMO sa metodom analitičko hijerarhijskog procesa, moguće je upravljačke odluke na objektivan način valorizovati i učiniti metodološki primenljivim prilikom određivanja optimalnog broja ribočuvarske službe. Objektivnost definisanja kriterijuma i izbor alternativa u odnosu na postavljeni cilj zavise od dostupnih inicijalnih informacija i iskustva donosioca odluka, ali je ovaj nedostatak moguće otkloniti donošenjem alternativnih rešenja zasnovanim na principima višekriterijumske analize i matematičkog modelovanja.

U ovom radu SMO metod je prezentovan na primeru organizovanja ribočuvarske službe i daje mogućnost da se ciljni parametri odrede u kvalitativnom i kvantitativnom pogledu, kroz optimalizaciju broja potrebnih ribočuvara. Rezultati ovog rada ukazuju na potrebu inoviranja postojećih metoda pri donošenju upravljačkih odluka po pitanju or-

ganizovanja ribočuvarske službe. Iako na prvi pogled ovaj metod izgleda komplikovan, primenom odgovarajućeg softvera i korišćenjem tabelarnih kalkulatora, ovaj metod postaje izuzetno primenljiv i efikasan u donošenju pravilnih i realnih zaključaka.

Ključne reči: optimalizacija, upravljanje ribolovnim vodama, ribočuvarska služba, skalarni metod ocenjivanja

Keywords: optimalisation, management of fishery waters, fish guard service, scalar evaluation method

INTRODUCTION

A management organization of natural waters becomes complex and dynamic process. An important segment of any management system of resources is to find modalities to use them rationaly and economically. Users of natural waters have difficult task to, on relatively large space organize work of professional and guard services in order to meet contractual and legal obligations with less expenses. The aim of the study is to show, on defined example of determining the parametres which influence optimal number of fish guards, a possibility of applying multi-criteria mathematic modeling. By combining scalar evaluation method (SMO) with method of analytic hierarchy process AHP), management decisions becomes methodologically consistent and objective when making real conclusions.

MATERIAL AND METHODS

As a starting material for this study we used official reports of Ministry of Environment, Mining and Spatial Planning of Republic of Serbia (Table 1). Presented SMO combines inferential statistics methods and multi-criteria analysis (Srđević et al., 2000; Šarčević, 2011). To determine importance and priority in making management decisions we combined SMO with method of analytic hierarchy process (AHP). AHP of problem deciding presents necessary method when solving any complex problems in the field of multi-criteria decision.

Table 1. Annual report data from Ministry, sector of Supervision and Surveillance (2009)

USERS	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Number of issued licenses	2375	431	4600	3528	6322	7847	2484	10736	2090	349
Number of fish guards	17	5	14	5	25	19	10	56	23	6
Number of orders	930	633	1306	616	3300	807	479	3080	2400	790
Number of reports	335	2	18	32	48	16	60	106	44	1

USERS: 1-DOO ''Rivers Guard'' Jagodina; 2-Silver Leik Investment Beograd; 3-OOSR ''Dunavac'' Kostolac; 4- ZSR ''Timočka Krajina'' Zaječar; 5- JP ''Srbijašume'' Beograd; 6- ZOSR 'Južna Morava2' Niš; 7- Asocijacija ''Veternica'' i ''Vlasina; 8- OOSR ''Hristifor Perišić Kićo'', Kraljevo; 9- DOO ''Ekoribarstvo'' Valjevo; 10- OOSR ''Drina'' Ljubovija.

Mutual comparison of elements on each hierarchy level are based on forming the basic matrix, whereby the main diagonal entered values do not compare, and on positions symmetrical to the main diagonal are inverse values. In that way, we have got consistency of comparison and a result of comparison is made by vector of weighting values (Vtv) and determined criteria in relation to objectives. (Saaty, 1980; Jandrić and Srdevic, 2000; Suvočarev and Srdevic, 2007).

Results checking and consistency of pair comparison is calculated with consistency index CI, where λ_{max} is the maximum value of the matrix itself.

$$CI = \frac{(\lambda \max - n)}{(n-1)}$$

Consistency degree is determined by formula CR= CI/ RI, where RI presents random index statistically determined by the rows of the matrix. Using statistical correlation methods four parameters were analyzed, where the value of random index was 0,90. (Saaty, 1980). Forming of matrix criteria comparison is shown in Table 2.

Grade degree for the established criteria (parameters) and relational scalar value were determined based on statistical data analysis of relevant Ministry, using method presented in Šarčević (2011). The way we determine value of grade degree is shown in Table 3. For each grade degree by parametres (criteria), the vector of weighting values (Vtv) values were multiplied with relation scalar of belonging evaluation degree.

Table 2. Diagram of matrix criteria comparison

Criteria	**Parameter 1**	**Parameter 2**	**Parameter 3**
Parameter 1	**1,00**	a_1	A_2
Parameter 2	$1/a_1$	**1,00**	A_4
Parameter 3	$1/a_2$	$1/a_4$	**1,00**
Parameter 4	$1/a_3$	$1/a_5$	$1/a_6$

Table 3. Determining value of grade degree

Criteria	**Vector (Vtv)**	**Grade degree**					
		I	**II**	**III**	**IV**	**V**	**VI**
Parameter 1	**Vtv1**	***Vtv1* A1***	Vtv1* A2	Vtv1* A3	Vtv1* A4	Vtv1* A5	Vtv1* A6
Parameter 2	**Vtv2**	Vtv2* A1	Vtv2* A2	Vtv2* A3	Vtv2* A4	Vtv2* A5	Vtv2* A6
Parameter 3	**Vtv3**	Vtv3* A1	Vtv3* A2	Vtv3* A3	Vtv3* A4	Vtv3* A5	Vtv3* A6
Parameter 4	**Vtv4**	Vtv4* A1	Vtv4* A2	Vtv4* A3	Vtv4* A4	Vtv4* A5	Vtv4* A6
Relation	**scalar**	**A1**	**A2**	**A3**	**A4**	**A5**	**A6**

Using concrete data and comparing them to description of grade degree we performed adequate scalar assessment. Optimal number of fish guards was determined based on number of issued licenses, length of certain water flows, accessibility of terrain, number of issued fish guard orders and submitted reports.

RESULTS AND DISCUSSION

Results of correlation analysis, basic matrix and values of Vtv are shown in tables 4 and 5. During this research we established that there are significant correlations between fish guard's number and number of issued licenses (k = 0,828), as well as between fish guard’s number and number of issued orders (k = 0,796). We established insignificant correlation between fish guard’s number and number of submitted reports (k = 0,239).

Table 4. Correlation analysis of analyzed parameters

O.N.	Relation	Correlation coefficient	Correlation degree
1	Fish guard-licenses	**0,828**	significant correlation
2	Fish guard - order	**0,796**	significant correlation
3	Fish guard - report	**0,239**	insignificant correlation

Although at the first sight number of submitted reports can be more significant parameter for required number of fish guard’s analyses, orders of fish guards are statistically more significant for making decisions about required number of fish guards, that is also logical because orders have preventive role and give better long term results then repressive measures such as submitted reports.

Basic matrix and Vtv, show that consistency rating (CR) was lower than 0,10. According to Saaty-u (1980), tolerant value of CR is 0,10. Vtv for the number of issued licenses is 0,4656, for the length of water flow 0,2772, for the number of orders 0,1885 and for the accessibility of terrain 0,0687. In this research the above vectors which are clearly positioned the determination of the fish guards optimal number.

Table 5. Basic matrix of parameters comparison for defining the number of fish guards and values of the vector of weighting values (Vtv) for basic matrix criteria

Criteria	**Number of issued licenses**	**Number of orders**	**The length of water flow (km)**	**Accessibility of terrain**	**(Vtv)** CR= 0,06
Number of issued licenses	**1,00**	2,00	3,00	5,00	0,4656
Number of orders	1/2	**1,00**	1/2	3,00	0,1885
The length of water flow, km	1/3	2,00	**1,00**	5,00	0,2772
Accessibility of terrain	1/5	1/3	1/5	**1,00**	0,0687
Total					**1,0000**

In tables 6 and 7 we showed data description of scalar grade degree assessment and values of grade degree. Description of scalar grade degree assessment for these parameters are calculated according to existing statistics and specific conditions, and in that way we determined six degrees of the grade. This number is directly dependant on the relational scalar and it was determined based on minimum and maximum number of fish guards, as well as on regression analysis of the most important factor of this research, which is number of sold licenses in relation to the number of fish guards. Data we have got in table 6, show that parameter values ,vector of weighting value and relational scalar, as base for getting values of grade degree caused exponential form of grade degree, as it can be seen in table 7.

Table 6. Description of grade degree for established criteria

Parameter	Evaluation degree					
	1	2	3	4	5	6
Number of sold licenses	0 - 500	501-1500	1501-3750	3751-7500	7501-12500	>12500
Number of orders	0 - 1000	1001- 2000	2001- 3000	3001- 3500	3501- 4000	> 4000
The length of water flow, km	0 - 300	301 - 500	501 - 700	701 - 900	901 - 1200	> 1200
Accessibility of terrain	Flat terrain, little vegetation	Gently sloping terrain, little vegetation	Medium steep terrain, little vegetation	Steep, overgrown vegetation	Steep, densely overgrown vegetation	Partially hardly accessible terrain

Table 7. Values of grade degree

Parameter	Vector (Vtv)	Grade degree					
		I	II	III	IV	V	VI
Number of sold licenses	**0,4656**	0,93	2,33	9,31	18,62	27,93	32,59
Number of orders	**0,1885**	0,38	0,94	3,77	7,54	11,31	13,20
The length of water flow, km	**0,2772**	0,55	1,39	5,54	11,09	16,63	19,40
Accessibility of terrain	**0,0687**	0,14	0,34	1,37	2,75	4,12	4,81
Relation scalar		**2**	**5**	**20**	**40**	**60**	**70**

With grade degree formed values we accomplished final optimization of required number of fish guards (Šarčević, 2011), which is shown in tables 8 and 9.

Table 8. Practical example of determining the number of fish guards for 2 users

Users	Number of issued		The length of water flow, km	Accessibility of terrain	Fish guards (number)	
	licenses	orders			existing	optimal
I	18,62	7,54	11,09	2,75	25	≈40
II	27,93	7,54	19,40	1,37	56	≈56

USERS: I - PF ‘’Srbijašume’’ Belgrade; II- OOSR ‘’Hristifor Perišić Kićo’’, Kraljevo

Table 9. Review of existing and required number of fish guards

USERS	1	2	3	4	5	6	7	8	9	10	∑
Status	17	5	14	5	25	19	10	56	23	6	180
Required	**17**	**5**	**21**	**10**	**40**	**30**	**12**	**56**	**21**	**6**	**218**

USERS: 1- Doo ‘’ Rivers Guard’’ Jagodina; 2-Silver Leik Investment Beograd; 3-OOSR ‘’Dunavac’’ Kostolac; 4- ZSR ‘’Timočka Krajina’’ Zaječar; 5- JP ‘’Srbijašume’’ Beograd; 6- ZOSR ‘Južna Morava2’ Niš; 7- Asocijacija ‘’Veternica’’ i ‘’Vlasina; 8- OOSR ‘’Hristifor Perišić Kićo’’, Kraljevo; 9- DOO ‘’Ekoribarstvo’’ Valjevo; 10- OOSR ‘’Drina’’ Ljubovija

The data from table 8., clearly show that the user JP ‘’Srbijašume’’ in relation to OOSR ‘’Hristifor Perišić Kićo’’ issued 69,8% more fishing licenses, had the same number of orders, better accessibility of terrain , it had 73,93% longer length of water flow. Despite that, optimal number of required fish guards was 60 % lower.

In table 9 we showed data for existing and calculated number of guards for fishing waters for 10 users of those waters. Out of ten analyzed users, only four had required number of fish guards and one had more than required, which means that for the protection of aquatic ecosystems in the overall level, is necessary to engage another 38 (218-180=38) fish guard. Based on the research of all users we can conclude that the best results had four users, while the worst results were recorded with users number 5 and number 3.

Applied scalar evaluation method and organization of Fish Guard Service present, in an exact manner, simplicity of optimization for required number of fish guards and for the first time finds possibility of applying in sustainable management of natural waters. So far, it was applies in determining number of game wardens and assessment of generally useful functions of forests (Šarčević, 2011; Šarčević, 2012). A similar mathematic model of multi-parameter analysis in the field of environmental protection, and among other things land waters, indicate the work of Ridgley et al (1997). Practical significance of this method is elimination of two current assumptions, which are: the optimal number of fish guards depends only on number of issued fishing licenses, and the number of submitted reports can have significant influence on determining optimal number of fish guards.

CONCLUSIONS

Results of this study show that there is a need for upgrading of existing methods in making management decisions. Scalar evaluation method (SMO) presented on example of Fish Guard Service organization, gives us the ability to determine the target parameters in both quantitative and qualitative terms, through optimization of required number of fish guards. Although, at the first sight, this method may seem complicated, by applying appropriate software and by using tables, this method becomes very applicable and efficient in making management decisions.

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A CORRELATION BETWEEN ALKALINE PHOSPHATASE AND PHOSPHATE LEVELS WITH THE BIOMASS OF TROUT FARM EFFLUENTS

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KORELACIJA IZMEĐU NIVOA ALKALNE FOSFATAZE I FOSFATA U OTPADNOJ VODI RIBNJAKA SA MASOM PASTRMKI

Apstrakt

Otpadne vode iz pastrmskih ribnjaka se obično ispuštaju u nezagađene akvatične ekosisteme, s obzirom na to da su ribnjaci najčešće locirani na velikim visinama, gde nema drugog izvora antropogenog zagađenja. Cilj ove studije je da se istraži odnos između aktivnosti alkalne fosfataze (APH) izmerene u otpadnim vodama i mase riba u ribnjaku. Uzorci vode sa ribnjaka su prikupljani u proleće i leto i iz nje su određivani nivoi APH, fosfata (PO_4-P) i količine bakterija (CFU). Ustanovljena je pozitivna korelacija između mase riba i nivoa APH i PO_4-P u efluentu, dok sa druge strane, nije utvrđena korelacija između mase riba i CFU. Rezultati ove studije su pokazali da nivo APH može biti korišćen kao potencijalni indikator zagađenja vodenih ekosistema otpadnih voda iz pastrmskih ribnjaka.

Ključne reči: Pastrmski ribnjak, APH, bioindikacija, rečna ekologija

Keywords: trout farm, APH, bioindicator, rivers ecology

INTRODUCTION

Fish farming often results in a generation of effluents with high nutrient load with adverse effects on both the water quality and the aquatic ecosystem downstream. Fresh water Aquaculture activity of salmonids is frequently located by rivers at high altitudes. These aquatic ecosystems are frequently characterized by low pollution from agricultural or industrial activities and offer optimal temperature and water quality conditions for the farming of fish. A useful indicator of aquatic anthropogenic pollution is the bacterial load of aquatic ecosystems (Ackerman and Weisberg, 2003). At high altitudes and upstream of rivers, aquaculture may be the single anthropogenic source of aquatic pollution. Under intensive aquaculture conditions, the use of antibiotics is frequent and the high organic and bacterial load of aquaculture effluents may result in increased bacterial load downstream with antibiotic resistant strains (Gordon *et al.,* 2007). The effluents of land-based fish farms may vary in nutrient load according to the aquaculture management. For example, some farms may have effluent treatments of varying efficacy prior to discharge while others may vary in size and feeding regimes and thus use larger volumes of water and discharge more or less dilute waste (Rosenthal, 1994; Bergheim and Brinker, 2003).When fish farms are located upstream in river zones with no other human activity, the pollution generated by aquaculture can be easily assessed by monitoring environmental conditions upstream and downstream. Several chemical and biological indicators can be used to monitor the ecological status of a river (Camargo *et al.,* 2011). Frequently used chemical indicators include: pH, BOD, oxygen, nitrates, nitrites, phosphorus and ammonia levels (Tello *et al.,* 2010). Any possible alteration of these parameters between upstream and downstream river water of a fish farm can be attributed to the aquaculture activity. An additional index of fish farm effluents in river waters is the presence of extracellular enzymes released by the growing fish and the bacterial load of fish tanks. For example, Alkaline phosphatase (APH) activity in sediments downstream of fish farms may indicate higher bacterial load generated by the fish farm pollution (Baldock and Sleigh, 1988) but an additional source of APH is the excretion of farmed fish which can persist in fish farm effluents even after a septic tank treatment (Carr and Goulder, 1990). As a result, elevated levels of APH in rivers downstream of a fish farm is a combination of bacterial APH and excretion of farmed fish.

The aim of the present study was to investigate the hypothesis that the levels of free APH activity present in the outflow of fish ponds correlated with the biomass of the fish. This possible relationship would offer a tool to assess the aquaculture impact on streams and the potential capacity of fish farms to generate APH. This enzyme could under some circumstances contribute to elevated hydrolysis of organic phosphates and thus result in higher levels of inorganic PO_4 and accelerate eutrophication (Carr and Goulder, 1990).

MATERIALS AND METHODS

The study was carried out between May and June of 2010. Water samples were collected in sterilized glass bottles, at the outflows of fish tanks of a commercial rainbow trout fish farm in NW Greece (using a concrete raceways flow-through system of rearing).

The water samples were collected between 9:00 and 11:00 hours to measure phosphates (PO_4-P) according to APHA (2005). The determination of bacteria was carried out with the filtration technique and using agar plates, in triplicates. They were then incubated for 24h at 30°C. Alkaline phosphatase activity was assayed according to Cao et al. (2005). Triplicates of 5ml water samples were supplied with Tris-HCl buffer (pH 8.5, final concentration 13 mmol L^{-1}), Na_3N (final concentration 5mmol L^{-1}), and p-nitrophenyl phosphate (pNPP, final concentration 0.3 mmol L^{-1}), and the samples were incubated at 37°C for 12 h. The absorbance of p-nitrophenol was measured spectrophotometrically at 410 nm. APH activity was calculated as μmoles of p-nitrophenol released L^{-1} day^{-1}. The statistical significance of the regression between fish biomass, bacterial load, phosphorus and the Alkaline phosphatase activity was determined using ANOVA ($P<0.05$).

RESULTS

The biomass of fish in each tank ranged from 200 to 590 kg. The concentration of phosphorus in the effluents of trout farm tanks ranged between 0.13 to 0.43 mg L^{-1} (Fig. 1). There was a significant relationship between fish biomass and phosphorus load of the pond effluents ($R^2=0.22$, $F=5.36$, $P<0.05$). The levels of APH in the effluent of each tank ranged between 0.60 and 2.34 μmoles of p-nitrophenol L^{-1} day^{-1} (Fig. 2). There was a significant relationship between fish biomass and phosphorus load of the pond effluents ($R^2=0.30$, F=7,81, $P<0.05$).

The bacterial load (CFU 10^6 mL^{-1}) in the effluents of trout farm ponds with different biomass ranged between 0.08 to 1.40 (Fig. 3). There was no significant relationship between fish biomass and CFU in the effluents. Body mass correlated significantly with levels of APH and PO_4-P levels in the effluents. There was no significant relationship between fish biomass and CFU in the effluents.

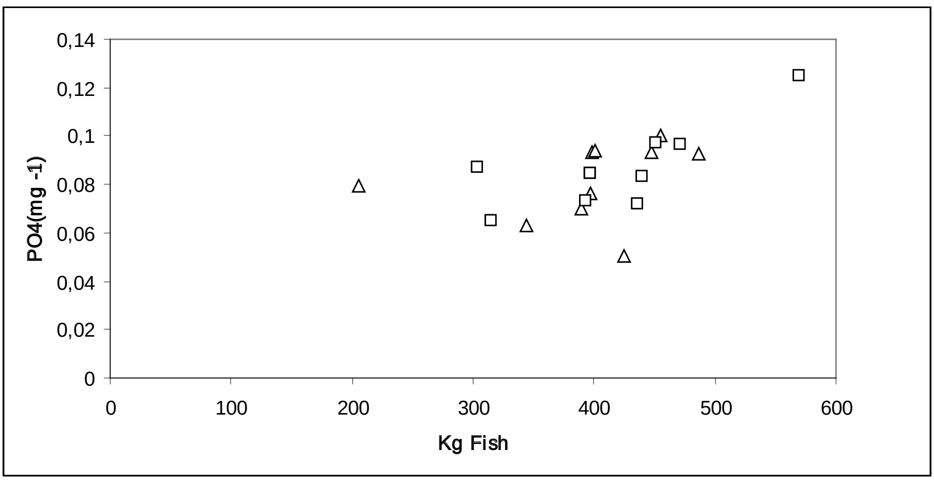

Figure 1. The concentration of phosphorus in the effluents of trout farm tanks with different biomass. Samples were collected in May (triangles) and June (squares) 2010.

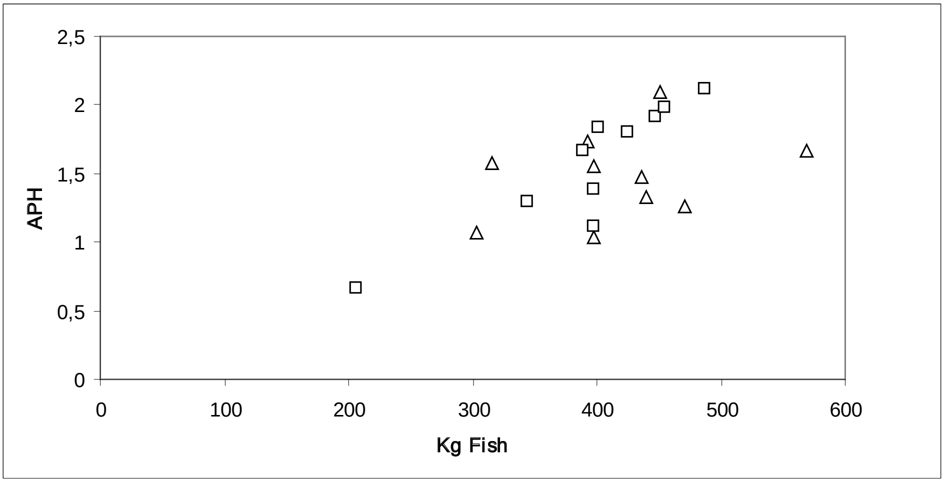

Figure 2. The activity of Alkaline Phosphatase (μmoles of p-nitrophenol L^{-1} day^{-1}) in the effluents of trout farm ponds with different biomass. During two different sampling sessions: May (triangles), June (squares).

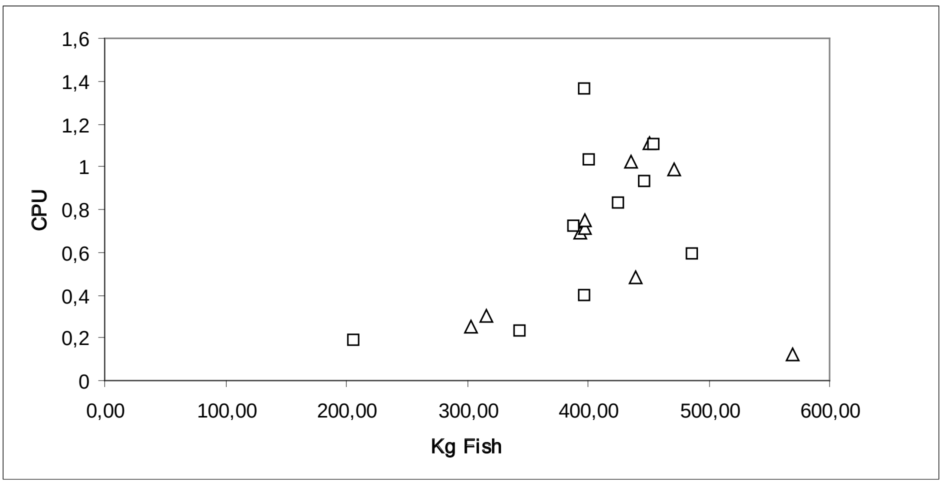

Figure 3. The bacterial load (CFU 10^6 mL^{-1}) in the effluents of trout farm ponds with different biomass. Samples were collected in May (triangles) and June (squares) 2010.

DISCUSSION

Both the activity of APH and the release of PO_4-P increased significantly with fish biomass. The activity of APH in the unfiltered effluents can be attributed to the combination of fish and bacteria present in the tanks whereas in the filtered water APH activity accurately represents the enzyme generated by the farmed fish (Carr and Goulder, 1990). The observed results support the relationship between fish tank biomass and APH activity in the effluents.

The PO_4-P released in fish tanks originates from fish feeds and excretion and can vary according to fish biomass and fish feed composition and feeding regimes (Satoh et al., 2003). There was no significant relationship between the bacterial load and fish biomass. The bacterial load of fish farm effluents stems from dissolved organic matter derived from uneaten feed and fish faeces as well as sediment flora (Bedwell and Goulder, 1997). Uneaten feed and faecal excretions of fish can vary in tanks according to the feeding rate regime, but sediment flora can vary according to tank conditions and cleaning procedures (Sindilariu, 2007).

According to standard aquaculture practices, during the growing season of trout farms, feed input in ponds varies according to fish biomass. The relationship observed in this study between the fish biomass and the bacterial and PO_4-P loads of fish pond effluents reflects a relationship between feeding rate and the potential environmental impact of land based fish farms (Boaventura et al., 1997). In several cases, even when river fish farm effluent are treated prior to discharge, the phosphates and microbiological parameters are significantly increased downstream from the fish farm (Ruiz-Zarzuela et al., 2009) with potential consequences for the aquatic ecosystem downstream of farm sites (Tello et al., 2010; Waring et al., 2012). The microbial load in the effluent may include antibiotic resistant strains with consequences for the public health (Naviner et al., 2011). The treatment of aquaculture water effluent, for example in settlement ponds for aquaculture water effluents, can reduce the organic load and microbial load (Snow et al., 2012) but regular pond management procedures such as fish harvesting and pond cleaning may result in sudden peaks of organic and microbial load of the fish farm effluents (Hinshaw and Fornshell, 2002).

The results of the present study indicate that the biomass of trout stocked in each tank correlated significantly with levels of APH and PO_4-P levels in the effluents but there was no correlation between fish biomass and the microbial load. The monitoring of APH levels could be used as a potential biomarker of fish farm pollution in the aquatic ecosystem.

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INTERNATIONAL COOPERATION PROJECTS IN THE RESEARCH OF THE ADRIATIC SEA

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PROJEKTI MEĐUNARODNE SARADNJE U ISTRAŽIVANJU JADRANSKOG MORA

Apstrakt
Prvi pisani dokumenti o naučnom istraživanju mora se pojavljuju u 16. vijeku i uglavnom se bave morskom dinamikom. Do 1950-ih godina, istraživanja su imala morfološke i sistematske karakteristike i bila su zasnovana na opisu i katalogizaciji vrsta. Moderna istraživanja sa širim implikacijama počinju u drugoj polovini 20. vijeka, nakon Drugog svjetskog rata, sa raznim ribolovnim ekspedicijama baziranim na istraživanju distribucije, biologije, ekologije vrsta, stanje pridnenih zajednica i mogućnosti za njihovu komercijalnu ali održivu eksploataciju. Prva ribarstveno-biološka ekspedicija u Jadranu je bila “HVAR” ekspedicija, organizovana 1948-1949 godine od strane Instituta za oceanografiju i ribarstvo u Splitu. Istraživanje je obuhvatilo većinu otvorenog Jadrana, a glavni cilj ekspedicije je bio da se stekne uvid u kvalitativni i kvantitativni sastav pridnenih zajednica na Jadranu, kao i procjena potencijala za komercijalno iskorištavanje takvih izvora. Od 2001. do 2007. istraživanja pridnenih zajednica su provedena u okviru projekta FAO ADRIAMED. Uz MEDITS program (Međunarodno straživanje pridnenih resursa Mediterana), Europska unija pokrenula je 1994 sveobuhvatno istraživanje pridnenih zajednica Mediterana i Jadrana na kontinentalnom šelfu i kontinentalnom slazu.

Ključne reči: Jadransko more, međunarodna saradnja, resursi ribarstva, odgovorno korišćenje
Keywords: Adriatic sea, international cooperation, fishery resources, responsible use of fisheries

INTRODUCTION

Evidence on use of natural resources from the Adriatic from prehistoric times clearly suggest the importance of this area for human population. Humans gathered their earliest experiences with the sea and related events upon their arrival on its shores following the great migrations. The first written records on scientific studies of the sea appear in the 16th century. Ichthyology (ancient Greek ichthys – fish, logos – science), a branch of zoology dedicated to the study of fish, their biology and ecology, developed during several periods. Human interest in fish begun the moment he became capable of research and understanding of terms and thoughts, as fish (like other animals) was a theme in human communication (images, symbols, and later the written text). Aristotle's "The History of Animals" represents the first written work in the study of fish.

The study of the ichthyofauna of the Adriatic has a long history. The first documents date back to the 16th century, from the time of the Republic of Dubrovnik. The 18th century is the period of Carl Linné's "Systema naturae" which brings the increased interest in categorisation and study of various species of the Adriatic. This interest continues into the 19th century with many scientists who worked in this field. The 20th century brought important works dealing with the cataloguing the Adriatic species. The most important check-list were compiled by Šoljan (1948, 1965, 1977), Jardas (1996) and Lipej and Dulčić (2010). Until 1950s, studies mostly dealt with morphological and systematic characteristics of the species, and were based on descriptions and check-lists. After the World War II modern, wide-encompassing studies begin with several scientific expeditions. These are based on studies of distribution, biology and ecology of the species, fish community conditions and the possibility for their commercial, but sustainable exploitation. FAO international scientific research projects in the Adriatic begin at the end of 1970s. Despite long tradition of research, the Adriatic ichthyofauna has still not been sufficiently researched, especially deep waters of the south Adriatic.

MATERIALS AND METHODS

The organised studies of the Adriatic for the advancement of fisheries begun in Trieste in the middle of the 19th century with the founding of the meteorological station and the City Natural History Museum. Several more station were founded afterwards. In 1891. a Zoological station of the Berlin Aquarium was established in Rovinj, which later became the station of the German–Italian Society. *Magyar Halyasati Biolocical Allomag* was established in 1904 in Rijeka. During the Kingdom of Yugoslavia, in 1930, the Biological–Oceanographic Institute is founded in Split (modern Institute of oceanography and fisheries), and in the Socialist Federal Republic of Yugoslavia, in 1961 Bureau od Marine Biology is founded in Kotor (modern Institute of Marine Biology).

Several scientific expeditions were organised at the beginning of the 20th century, "RUDOLF VIRCHOW", "ARGO", "ADRIA", "MONTEBELLO", "NAJADE" (Austria), "CICLOPE" (Italy), "THOR" (Denmark). Very important is the first expedition organised in Croatia, in the northern Adriatic in 1913 and 1914, "VILA VELEBITA". This expedition represents the most fruitful research in the Adriatic; a project which was spectacularly planned (for the period) and organised, with results that have (due to the multidisciplinary scientific approach) caused positive reactions of the scientific public. Unfortunately, this golden era came to an abrupt halt with the beginning of the Great War.

The first fishery–biological expedition conducted in the entire Adriatic was the "HVAR" expedition, organised by the Institute of oceanography and fisheries in Split in 1948/49. The expedition encompassed most of the open waters of the Adriatic, and the main goal was to provide a description of qualitative and quantitative composition of demersal benthic communities in the Adriatic, and the assessment of the possibility of commercial exploitation of those resources (Šoljan, 1977). Important data on hydrography and chemistry of the Adriatic were collected during the course of the expedition. This research was organised in the wake of the World War II, when the natural resources were preserved, without a significant influence from fishing activities. Fisheries were in their infancy in the Adriatic before the outbreak of WWII, and during the war, all commercial fishing activities were suspended. Such state of resources can be considered as intact, a "virgin state" of the populations, and as such can be used as a reference condition for the effect of fishing activities on the demersal resources of the Adriatic.

After the "HVAR" expedition, many other studies (temporally and spatially limited) were also organised (Crnković, 1959, 1965; Županović, 1961, Rijavec and Županović, 1965; Froglia, 1972; Jukić and Piccinetti, 1981; Županović and Jadrdas, 1989; Marano *et. al*., 1998; Vrgoč *et al.,* 2004; Regner and Joksimović, 2002).

Laboratory of marine biology in Fano (Italy) and the Institute of oceanography and fisheries in Split (Yugoslavia) started the "PIPETA" expedition in 1982, at that time the most detailed study of demersal communities of the Adriatic. Study area included the entire northern and central Adriatic, from the north to the Monte Gargano on the Italian coast, divided in 9 perpendicular transects. Sampling was done using the bottom trawl of the tartana type. After twenty years of research, the studies on the Italian (western) side of the Adriatic were continued until 2007 within the GRUND programme.

Figure 1. Scientific research expedition Deep Adriatic within the AdriaMed project, 2010

From 2001 to 2007, studies of the benthic communities in the eastern Adriatic were done within the framework of the FAO AdriaMed project (Scientific Cooperation to Support Responsible Fisheries in the Adriatic Sea) (Fig. 1). These research were organised annually, in the Autumn–Winter period with a standardised methodology in territorial waters of Slovenia, Croatia, Montenegro and Albania. Most stations were in the areas of the Jabuka Pit, northern Adriatic and the channel area of Croatian Littoral (Vrgoč, 2004).

The European Union started an all–encompassing study of benthic communities of the Mediterranean (including the Adriatic) in 1994 with the MEDITS project (Mediterranean trawl Survey). This project encompasses all trawling areas (continental shelf and slope). Samples are collected according to the standardised protocol, annually in the Spring–Summer period (Bartnard, 1995). The studies of the resources of small pelagic fish, anchovy and pilchard, using echosurvey and DEPM (Daily Egg Production Model) methods within the framework of AdriaMed and MEDIAS projects should also be mentioned.

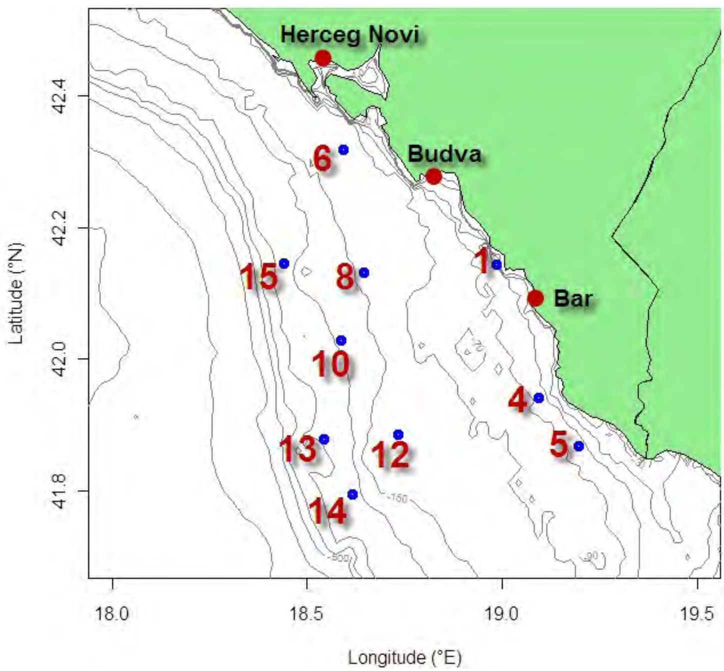

Figure 2. MEDITS stations in Montenegro

Recently, research using new methods have been conducted in the Adriatic, such as the research of the deepest areas of the South Adriatic Pit (1228 m; Deep Sea Survey), sole (*Solea solea*) community research (SOLEMON), research on Norway lobster in the Jabuka Pit using underwater TV (UWTV Survey) and genetic research of the exploited fish populations.

In addition to international research, national monitoring of commercial demersal, pelagic and artisanal fisheries are conducted in Croatia, Montenegro and Albania, while the EU member countries follow the Data Collection Framework (DCF).

All data collected in trawl fisheries are stored and processed in the ATrIS (AdriaMed Trawl Survey Information System) computer program (Gramolini, 2005), created within the AdriaMed project. Data on abundance, biomass and length frequencies are calculated using the swept area method, taking into account that the sampling was randomly stratified (Souplet, 1996), and the calculated values of abundance and biomass indexed are expressed as N/km^2 and kg/km^2, respectively.

RESULTS AND DISCUSSION

Results of the research are presented annually to the Resources Management Group of the GFCM (GFCM SubSAC Stock Assessment). Afterward, scientific recommendations are adopted by the Scientific Advisory Board (SAC) so the GFCM could send them to member countries to be implemented. The Mediterranean, including the Adriatic, is divided into sub–geographic areas (GSA), and all recommendations refer to specific GSAs. The Adriatic presents a special case, because its resources are shared among 5 countries, and is divided in two GSAa, 17 and 18. The data, especially those from AdriMed and MEDITS projects are then processed and published as annual reports, as has been previously mentioned.

An example of data and results obtained is presented using Montenegro as model. Research of demersal resources within the MEDITS project for 2012 have shown that an increase of the total biomass has occurred in the area compared to the results from 2011. In 2012, the total biomass of demersal resourceshas been estimated to 847.79 kg/km^2. A total of 123 species were registered during the survey. Abundance and biomass for the 25 most important commercial species are given in Table 1.

Table 1. The most abundant species in the survey area – MEDITS 2012.

VRSTA	MEDITS KOD	N/km^2
Spicara flexuosa	SPICFLE	14 872,52
Engraulis encrasicolus	ENGRENC	4075,35
Mullus barbatus	MULLBAR	3587,68
Spicara smaris	SPICSMA	3016,43
Merluccius merluccius	MERLMER	3010,36
Loligo vulgaris	LOLIVUL	1917,28
Trachurus trachurus	TRACTRA	1392,26
Illex coindetii	ILLECOI	1372,29
Parapenaeus longirostris	PAPELON	1349,25
Alloteuthis media	ALLOMED	1325,12
Lepidotrigla cavillone	LEPTCAV	1195,85
Sardina pilchardus	SARDPIL	1178,59
Scomber (Pneumatophorus) japonicus	SCOMPNE	917,74
Gadiculus argenteus	GADIARG	736,82
Serranus hepatus	SERAHEP	665,56
Sepia elegans	SEPIELE	522,27
Pagellus erythrinus	PAGEERY	454,71
Aspitrigla cuculus	ASPICUC	420,61
Macrorhamphosus scolopax	MACOSCO	403,58
Plesionika heterocarpus	PLESHET	401,36
Scyliorhinus canicula	SCYOCAN	394,37
Boops boops	BOOPBOO	341,95
Capros aper	CAPOAPE	310,57
Deltentosteus (Gobius) quadrimaculatus	GOBIQUA	290,67
Argentina sphyraena	ARGESPY	278,85

Analysis of the data presented in Tables 1 and 2 shows that the two picarel were most abnundant and had the highest biomass in demersal communities along the Montenegrin coast. In Montenegro, these two species are considered as discard, and usually returned to the sea, so their abundance is significant only from an ecological standpoint. Commercial species such as red mullet, hake, anchovy, common Pandora, black–bellied anglerfish, deep–water pink shrimp and bogue also show relatively high biomass indexes. The species mentioned are common in bottom trawl catches in Montenegro, and the increase in their biomass should have a positive financial effect with relevant subjects in Montenegrin fisheries sector. For comparison, the total estimated biomass increased from 616.08 kg/km^2 in 2011 to 847.79 kg/km^2 in 2012, which presents an increase of 37.6%. A parallel analysis of biomass indexes for commercial species in 2011 and 2012 is given in Fig. 3. The increase in biomass, sometimes as high as 400%, is obvious in most species.

Table 2. Species with the highest biomass in the survey area – MEDITS 2012.

VRSTA	MEDITS KOD	kg/km^2
Spicara flexuosa	SPICFLE	162,52
Mullus barbatus	MULLBAR	93,72
Engraulis encrasicholus	ENGRENC	60,10
Merluccius merluccius	MERLMER	59,19
Scyliorhinus canicula	SCYOCAN	51,63
Spicara smaris	SPICSMA	44,49
Illex coindetii	ILLECOI	35,48
Sardina pilchardus	SARDPIL	19,47
Pagellus erythrinus	PAGEERY	18,39
Todaropsis eblanae	TODIEBL	17,64
Helicolenus dactylopterus	HELIDAC	15,86
Aspitrigla cuculus	ASPICUC	15,85
Loligo vulgaris	LOLIVUL	14,63
Lophius piscatorius	LOPHPIS	14,50
Lepidotrigla cavillone	LEPTCAV	14,22
Trachurus trachurus	TRACTRA	13,53
Lophius budegassa	LOPHBUD	13,14
Scomber (Pneumatophorus) japonicus	SCOMPNE	13,03
Boops boops	BOOPBOO	10,79
Parapenaeus longirostris	PAPELON	9,73
Raja miraletus	RAJAMIR	8,56
Octopus vulgaris	OCTOVUL	8,31
Phycis blennoides	PHYIBLE	7,63
Trachurus mediterraneus	TRACMED	7,54
Zeus faber	ZEUSFAB	7,27

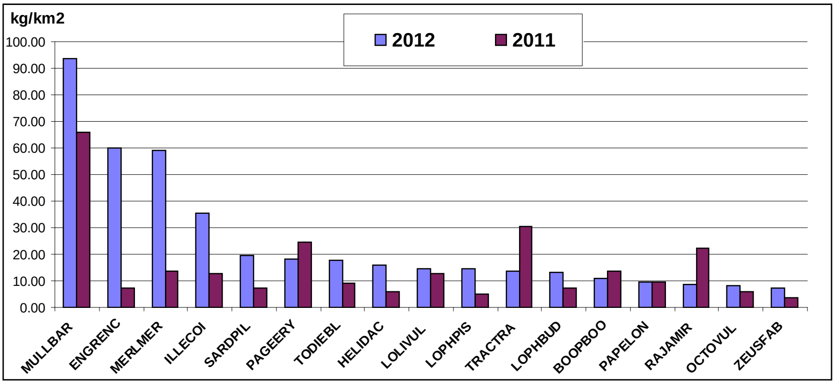

Figure 3. Parallel analysis of biomass indexes for commercial species in Montenegrin waters during the MEDITS survey in 2011 and 212

According to categories (fish, cephalopods, crustaceans), fish represent most of the biomass (87%), followed by cephalpods (12%) and crustraceans (1%). For comparison, the ratios were: fish 87%, cephalopods 8.4%, and crustaceans 3.6%.

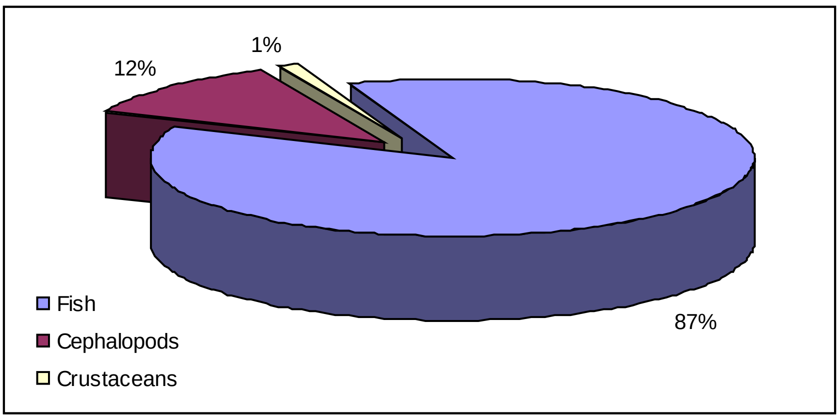

Figure 4. Percentage of biomass by category (fish, cephalopods, crustaceans) in Montenegrin waters, MEDITS 2012.

CONCLUSIONS

Adriatic and its living biological resources represent the shared resource of five Adriatic countries. This is the reason why international research and cooperation play an important role in the creation of fisheries activities, especially now when data indicate the disruption of balance between available resources and fishing effors, particularly on the west side. Biomass indexes and length frequency composition of commercial species can indicate certain (mostly negative) trends, which should lead to correct decisions on common conservation of resources. It is in that very segment that the importance of international cooperation can be seen in defining measures which will enable the renewal of resources (minimum mesh size increase, spatial and temporal fishing bans in certain areas). Naturally, it is the task of science to provide expert and scientific opinions on the status of the biological resources as well as the suggested measures for their long–term sustainable exploitation and conservation, whereas the politics shoud take the obligation and responsibility of making such measures effective in practice within the legal framework.

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TOWARDS THE IMPLEMENTATION OF EUROPEAN UNION'S NEW INTEGRATED MARITIME POLICY IN GREECE: BLUE GROWTH THROUGH MARINE AQUACULTURE FOR THE SUSTAINABLE DEVELOPMENT OF THE ISLANDS

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PREMA IMPLEMENTACIJI NOVOG INTEGRISANOG MARINSKOG ZAKONODAVSTVA EVROPSKE UNIJE U GRČKOJ: SPROVOĐENJE STRATEGIJE 'BLUE GROWTH' VEZANE ZA GAJENJE MORSKIH RIBA U CILJU ODRŽIVOG RAZVOJA OSTRVA

Apstrakt

Trenutna ekonomska situacija traži od Evropske Unije da brzo i efikasno nađe način da oporavi svoju privredu oslanjajući se na mudar, održiv i inkluzivni razvoj. Razvoj integrisanog pristupa primorskim pitanjima doprinosi jačanju Evropskog kapaciteta za povećanjem odrzivog korišćenja morske privrede. Ovaj pristup istovremeno garantuje bezbednost ljudi i zdravlje morskih ekosistema, s obzirom da je njihova zaštita važna za odrzivi razvoj i prosperitet. Nova strategija Grčkih ostrva – pod nazivom Integrisan Zakonodavstvo Ostrva - u potpunosti uključuje principe Integrisanog maritimnog zakonodavstva Evropske Unije, naročito njegovu 'Blue Growth' strategija koja se bavi razvojem kroz seriju marinskih aktivnosti i oslanja se na tri ideje: 'Kvalitetna' ostrva, 'Zelena' ostrva i ostrva gde svi imaju 'Jednake mogućnosti'.

Da bismo sproveli ovaj zakon moraju se razviti praktično primenljivi modeli, smernice i analitički okvir koji će pomoći donosiocima odluka i administrativnom osoblju da razlikuju postojeće aktivnosti, naročito na ostrvima. Oni ce takođe pomoći da se ograničeni finansijski resursi na pravi način usmere ka projektima ili regionima od kojih se očekuje najveća dobit. Metod opisan u ovom radu dozvoljava da izvršimo procenu nivoa održivosti u regionu ostrva i uticaj vodećih aktivnosti u ovoj oblasti, naročito kada je reč o gajenju morskih riba. Jedan deo ovog istraživanja koristi Delfi

Metod da bi naveo faktore koji utiču na gajenje morskih riba, i pokazatelje za merenje tih faktora.

Za potrebe razvijenog metoda (koji je baziran na zahtevu za zaštitu podataka programa za životnu sredinu UN-a) evaluacija aktivnosti zasnovana je na dva koraka: i) **učinkovitost po jedinici proizvodnje**, koja je vezana za dodatnu vrednost, radna mesta stvorena u toj oblasti, korisćenje vode, korisćenje energije, stvaranje otpada, i, ii) **raspon ispitivanih ljudskih aktivnosti u poređenju sa nosivim kapacitetom odredjene oblasti.** U ovom metodu održivi razvoj se tumači kao kontinuirani process koji istovremeno vodi do poboljšanja ekonomskih i društvenih uslova, kao i zaštite životne sredine koje su lokalna društva usvojila. Ocenjivanjem doprinosa svake ljudske aktivnosti, mozemo da predložimo odgovarajuće mere za određenu oblast i ispitamo mogućnost stvaranja novih farmi, ili širenje postojećih. Ovaj metod takođe može da se iskoristi da bi se utvrdile lokacije koje nisu odgovarajuće za projekte za razvoj akvakulture.

Ključne reči: održivi razvoj, ostrva, "blue growth", morska akvakultura
Keywords: sustainable development, islands, blue growth, marine aquaculture

INTRODUCTION

The present economic context calls for the European Union to find a fast and effective road to recovery based on smart, sustainable and inclusive growth. The development of an integrated approach to maritime affairs since 2007, consistent with other sectoral policies, contributes to the enhancement of Europe's capacity to maximise the sustainable use of the oceans, seas and coasts, while at the same time ensuring safety of people and the health of oceans and seas. Marine ecosystem goods and services and the protection of the marine environment are an important element for sustainable development and prosperity. This was reassured by the European Ministers with the "Limassol Declaration" (European Council, 2012). The new strategy for the Greek island regions - called Integrated Island Policy - is fully incorporating the principles of EU's Integrated Maritime Policy, especially its Blue Growth initiative, and relies on three (3) concepts: "Qualitative" Islands, "Green" Islands, and, Islands of "Equal Opportunities". Blue Growth is the contribution of the EU's Integrated Maritime Policy to achieving the goals of the Europe 2020 strategy for smart, sustainable and inclusive growth, and has identified five (5) specific areas with a particular potential for growth where targeted action could provide an additional stimulus: maritime, coastal and cruise tourism, blue energy, marine mineral resources, aquaculture**,** and, blue biotechnology.

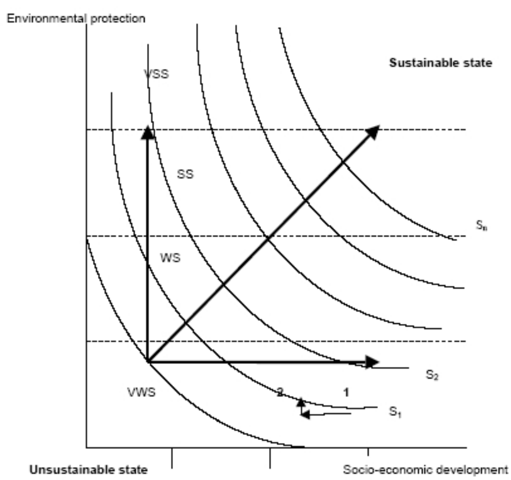
Resource constraints introduce a requirement for priority setting, which can be defined as the task of selecting a subset of issues, policies or projects towards which limited resources will be directed. The priority-setting task always involves trade-offs due to political, social, cultural, financial, legal & technological constraints. In multi-stakeholder and multi-objective settings the decision-making process is complex. The task is often made harder by incomplete or inaccurate datasets. Decision makers will aim to adopt a procedure that is analytically robust, auditable, transparent and understandable (Hajkowicz, 2002). This method, developed by the Laboratory for Local & Insular Development of the Department for Environment of Aegean University, allows the estimation of sustainability level in an island region and the footprint of the activities (driving forces) in this area. The method has been properly adapted to measure the contribution of marine fish farming to the sustainable development of island regions, letting us to proceed to faster implementation of "Blue Growth" initiative in Greece.

The sustainability analysis calls for the consensual setting of a "band of equilibrium" for a list of indicators making possible to evaluate the sustainability of the present situation in the target region and to determine what is desirable and what is unacceptable. The projection of these indicators also makes it possible to evaluate the region's sustainable development levels and thus its future sustainability (Spilanis et al., 2005). It is common practice to develop a single indicator of sustainable development, but this logic has not been adopted here. To have a better picture of the progress in each one of the three SD's dimensions separately, and to help policy makers to make clear suggestions, the overall number of factors (on economy, environment and society) is taken into consideration, without these factors appointed the same weight factor.

The purpose of the research is not to consider the prospect of sustainability of the industry (that is usually the research aim), but the development of a methodology and the proposal of an exclusive set of indicators to assess the contribution of marine aquaculture to sustainable development of the islands where they are installed, through the implementation of two different kinds of measurements, a) the performance and impact of marine fish farming, and, b) the factors that affect the performance and impact.

MATERIALS AND METHODS

For the purposes of the method developed (based on UNEP's DPSR), the evaluation of activities is based on two steps: i) **the performance per production unit**, that relates to the added value, the employment created at the area, water use, energy use, waste production, and ii) **the scale of the examined human activity compared to the carrying capacity of the host area**. In this method, the sustainable development (SD) considered a continuous process that leads simultaneously to the improvement of the economic, social and environmental goals adopted by each local society (this approach is shown in Figure 1, below).



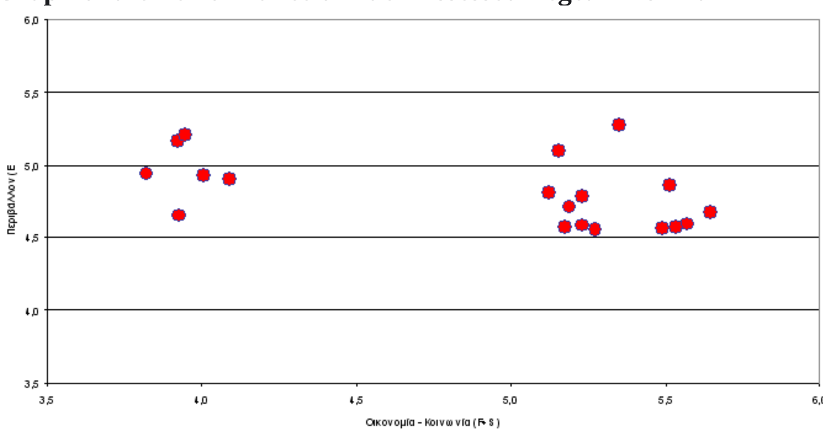
To measure the performance, and to locate and record the factors and parameters that affect performance (economic, social, environmental) of the marine fish farms that operate on islands, and the role of each one of these to their sustainability, **research has been made with structured questionnaires at the total number of fish farms of Aegean sea that use floating cages.** The questionnaire had a set of questions about the value of the proposed indicators and also on the factors that affect the performance and the sustainability of **each installation separately -** and not the company or group of companies in total - so, if the company had more fish farms in the study area (islands of Aegean sea) they had to complete one questionnaire for each one site. Totally, **31** questionnaires were sent by fax and e-mail to farms and finally **20** from them were answered. These 20 questionnaires relate to **80% of the fish farms** at Northern and Southern Aegean Sea that today are under operation, and they cover more than **90% of the production**.

Performance measured for all 3 dimensions of sustainable development: **Economic**, where the annual turnover per tonne of product (or per fry) is the critical index, **Social**, which takes into account the employment created (quantitative, number of employees, duration, wages) and qualitative characteristics (education level, gender, ethnicity), **Environmental**, where the per tonne (or per fry) resource consumption and waste generation, and the permanent change (deterioration) of the environment caused by infrastructure and facilities, are the parameters to be considered. The measurement uses in total **33 indicators** for the three (3) dimensions of sustainable development, spilt to **6** indicators for the **economic (F1 - F6),** to **9** indicators for the **social (S1 - S9)** and to **18** indicators for the **environmental (E1 - E18)** dimension of sustainability.

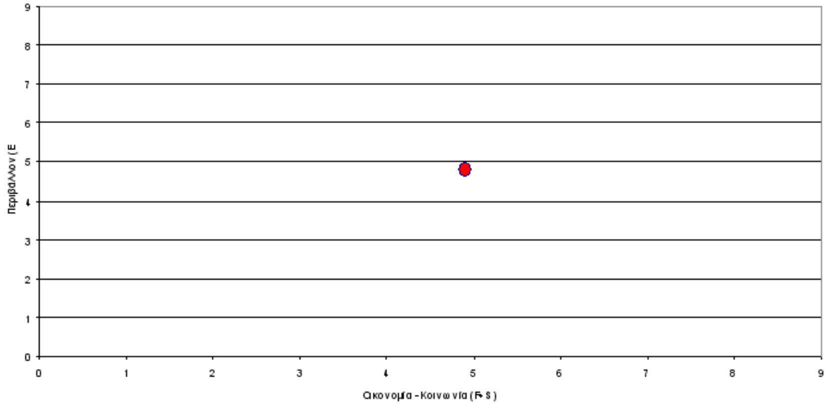
RESULTS

The quantitative and qualitative analysis of the answers gave us meaningful results, which are briefly presented here by these two (2) graphs, showing that we have two different group of farms, the one with relatively worst socio-economic status, but their total performance considered satisfactory. Having these data as a starting point we can now repeat the survey after a period of time and estimate if their development drives them to a more sustainable status.

Graph of the Performance of Each Assessed Aegean Fish Farm



Graph of the Total Performance of the Aegean Fish Farms



Next step was to locate the parameters that affect their performance, something that will give us the opportunity to actively involve and improve them. From our research we ended up with **14 parameters** and **77 indicators**. These parameters, with the number of their indicators in brackets, are: Quality Assurance (3), Product Differentiation (3), Environmental Protection (12), Fingerlings Production (2), Animal Welfare & Health (5), Biodiversity (4), Materials Supply (6), Site Selection (8), Employees (8), Public Image (7), Group of Companies (3), Standardization Level (9), Trading (3), Promotion (4).

We must notice that until today are not determined commonly accepted parameters that affect the performance of marine fish farming, and the indicators for their measurement. For that reason the parameters and their indicators, which occur from the research and analysis, it is possible to include a significant percentage of subjective opinion. To minimize that problem, the Delphi Method was used. To increase their possible acceptance, to classify their share separately at the three components of the sustainable development, and appoint their weight, a comprehensive matrix with the 14 parameters and the 77 indicators sent initially to **80 special scientists** working on research, production, and to administration, for examination, evaluation and completion. To the 2nd face of this exercise the panel of experts increased to **95 scientists**, and the parameters and their indicators finalised.

From original research determined that also **14 factors** should be measured when the Status (**State**) of an island region is estimated. These factors measured with different indicators, from which **9** indicators give the state of economy, **11** indicators the state of society, and **19** indicators the state of environment. These initial indicators were **supplemented** with more specialised indicators for use to productive aquatic ecosystems.

According to the applied theory, the **evaluation** of any human activity (actually, the **Pressure**) can be based on two criteria: 1) **The performance per unit of production**, which is linked to economic performance (value added), employment generated per unit, and the environmental burden (e.g. consumption of water, of energy, waste generation per unit, etc), and, 2) **The scale of the activity compared with the carrying capacity of the area**, for all the various human activities that take place in it.

The research proceeds to selection of proper indicators for the better measurement of the impact of marine fish farms, and to the **supplement** and **adjustment** of the existing ETNA’s methodology. The **2 additional** environmental indicators cover the case in which at the study area, the islands, **there is activities that are taking place in the marine environment**:

- **Π20 - Recording appearance of alien species, cultivation or farming of alien species.** Helps to estimate the pressure on marine ecosystems (e.g. from lessepsian immigrants), and the threat that consists human activities for the biodiversity, by type of ecosystem, and for the different ecosystems of the island.
- **Π21 - Quality of marine waters (marine environment).** Helps to estimate the quality of marine waters, which is particularly important in the case of marine fish farming, since it is the environment in which the production takes place, and is highly influenced by them, while at the same time the farms influence them back.

DISCUSSION

The specific characteristics of the islands (the "island phenomenon"), a combination of factors, affect always their economic development, and their sustainability level will be higher if their development is not dependent on a single activity: the economic risk then is lower and the stress on some of the natural resources is less important (Spilanis et al., 2005).

The new strategy for the Greek islands developed by the General Secretariat for Aegean & Island Policy (part of the Ministry of Shipping & Aegean) covers the whole island regions of the country and is called **Integrated Island Policy** because incorporates different sectoral policies targeting at the sustainable development of the islands through different activities, sushi as Marine Aquaculture, Tourism and Marine Tourism, Traditional Agriculture, etc. Its three (3) main pillars are:

1. **"Qualitative" Islands:** islands offering products & services of high quality. This can be done by developing action plans to utilise public and communal resources (e.g. public or municipal property etc.), incorporating the technical know-how and skilled work, as well as intangible assets for tourists with specialized interests.
2. **"Green" Islands:** islands that implement policies to reduce the use of the already limited water and energy resources. This includes better management of their natural resources (e.g. marine protected areas and forest biomass management), and the reuse of materials that are products of local recycling processes.
3. **Islands of "Equal Opportunities":** islands providing all the General Economic Interest Services. Offering "Equal Job Opportunities" to their inhabitants, "Equal Opportunities" to potential investors, and, will encourage and support innovative actions by providing proper motives (e.g. social services, IT technical know-how, scientific consultancy etc.).

An aquaculture development plan for an island region must have as an objective to provide a sound basis for development, while at the same time to conserve the unique environment of the islands for the present and future generations. The establishment of fish farms on islands is based on a comparative advantage: the farms, mainly in floating cages, use two recourses that are in a great abundance at the islands, (clean) sea and easy access from the (remote) beaches (Klaoudatos et al., 1996).

Therefore, even if today there are some difficulties, the investors will continue to show an interest in establishing fish farms at the island regions. There is, however, a question about **whether** and **how** this human activity can contribute to the islands sustainable development. Some aspects regarding the fast aquaculture development require further attention. Concerns relate to environment (Karakassis et al., 2005), health and animal welfare issues, and potential conflicts with fisheries and recreational activities.

Marine aquaculture (or Mariculture) is a new competitor for the same limited resources and this antagonism should be judged on the basis of the efficiency of resource utilize as well as the environmental compatibility. Mariculture has today an important role to play in rural development and in reversing decline in fishing communities (Burbridge et al., 2001). Common criteria should be used for evaluating all economic activities, and to include socio-economic and environmental costs & benefits is a good way to achieve it.

With this research a simple system is proposed that can monitor the ‘progress’ of each local society by calculating a number of indicators that can measure the state (S) and its change over time as pressure (P) comes from mariculture (DF). This approach reflects the fact that SD has a different content for different societies and comparisons can be misleading (Katranidis et al., 2003). Advantage of this approach is that compares similar states of sustainability for the same society and yields meaningful results (Chatziefstathiou et al., 2006).

CONCLUSIONS

The system of measurement is relatively simple, relying mainly on published or easily accessible data, and the selection and weighting of indicators made using the Delphi method (Caffey et al, 1998). The developed method could be used as tool capable in determining also the inappropriate sites for projects in areas that initially had been considered suitable for aquaculture development. Monitoring practices will ensure that the established activities will not lead in dew time to the deviation from the sustainability’s targets, and at the end of the policy period evaluation practices will determine whether the overall state of the SD of the island had been improved.

To diversify the activities we can proper assess the contribution of each activity and select the most desirables. In the case that this is aquaculture, we can propose the policy on which we must continue to examine the possibility of establishing new farms, or expanding the existing, with a way which will support the sustainable development of the islands through productive activities of the primary sector, while preserving the unique identity of each island (their ‘insularity’).

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USE OF ARTIFICIAL HABITATS AS RECRUITMENT SITES BY JUVENILE WILD FISH: EFFECTS OF FISH FARMS ON FISH DIET AND OTOLITH GROWTH

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VEŠTAČKA STANIŠTA KAO POTENCIJALNE NOVE NASEOBINE MLAĐI RIBA: EFEKAT RIBNJAKA NA ISHRANU I RAST OTOLITA

Apstrakt
Cilj ovog rada je identifikacija razlika u sastavu populacije i brojnosti morskih riba koje žive u blizini kaveznih sistema u poređenju sa kontrolnim lokacijama. Njihova identifikacija je pokušana analizom oblika otolita.

Ključne reči: oteoliti, stanište, marinski kavezni sistemi, morfometrija
Keywords: otolith, habitats, marine cages, morphometry

INTRODUCTION

Juvenile fish of more than 20 different species use off-shore floating sea cages as settlement habitats in the Mediterranean. These fish actively feed on the abundant zooplankton around the cages while, at the same time, they show a strong variation in the fatty acid profile of their tissues as an influence of the food pellets composition (Fernandez-Jover et al. 2009).

In the present work, surveys were conducted for identifying differences between the species composition and abundance of new settlers among farms and control rocky-shore environments and to test for variations in their diets. Some authors have pointed out that prey availability may influence otolith growth rates (Moksness et al. 1995, Morales-Nin et al. 1995), and recently, otolith morphology has been applied in order to differentiate reared and wild adult fish (Arechavala-Lopez et al. 2012). Consequently, otolith growth and morphology analysis were also applied in order to better understand which are the consequences for the ecology and growth performance of several fish species that use coastal farms as recruitment habitats in SW Mediterranean.

MATERIALS AND METHODS

The composition and abundance of juvenile fish around the cages was estimated at two farms situated off the coast of SE Spain (F1 and F2) and two control sites (C1 and C2) by means of transects of similar length (200 m^2). Fish were lately captured in order to confirm the species composition of the school, correct the visually-estimated size and to carry out diet and otolith analyses of the fish species *Atherina boyeri, Oblada melanura* and *Sarpa salpa*. In the laboratory, the left saggita of every fish was removed and a scaled photograph was taken for further image processing analysis. Otolith shape descriptors were measured from each otolith: area, perimeter, circularity, roundness, major and minor axes, aspect ratio and the standard length (SL)/Area ratio. Additionally, a closed-form Fourier analysis (Younker and Ehrlich, 1977) was applied to the otolith. This method decomposes the irregular shape of the contour into a series of orthogonal terms called the Elliptic Fourier Descriptors (EFDs) or harmonics. Additionally, otoliths of *A. boyeri* and *O. melanura* were further analyzed in order to identify differences in the rate of periodical growth zones deposition. An ANOVA test was used for univariate analysis and Linear Discriminant Analysis (LDA), based on previous Principal Components (PC) reductions of the variables (shape descriptors), was applied in order to detect if differences existed among farm-associated and control fish.

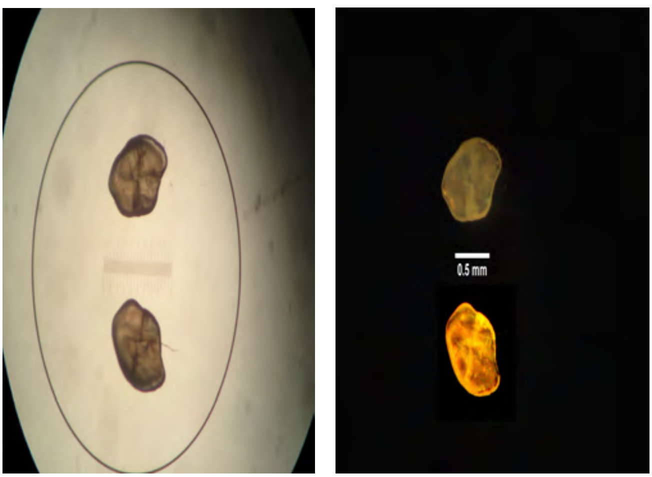

Figure 1. Example of image analysis for calculating the otolith shape descriptors.

RESULTS

No clear differences were found for the abundance and size distribution of fish among control and farm locations or diet composition, even diversity indexes were similar. However, a consistent pattern for all the studied species was a significant variation in shape descriptors principally identified through the aspect ratio index (Fig. 2), which was lower for farm-associated fish ($p<0.05$ for the three analyzed species). The otoliths shape modifications were effectively detected by the EFDs since the LDA correctly identified the farm or control origin for the 78.8%, 85.1% and 86.1% for *A. boyeri, O. melanura* and *S. salpa* individuals respectively. However, data on the rings deposition rate for *A. boyeri* and *O. melanura* was very variable and patters could not be concluded, as indicated by the lack of significant differences found for all the comparisons.

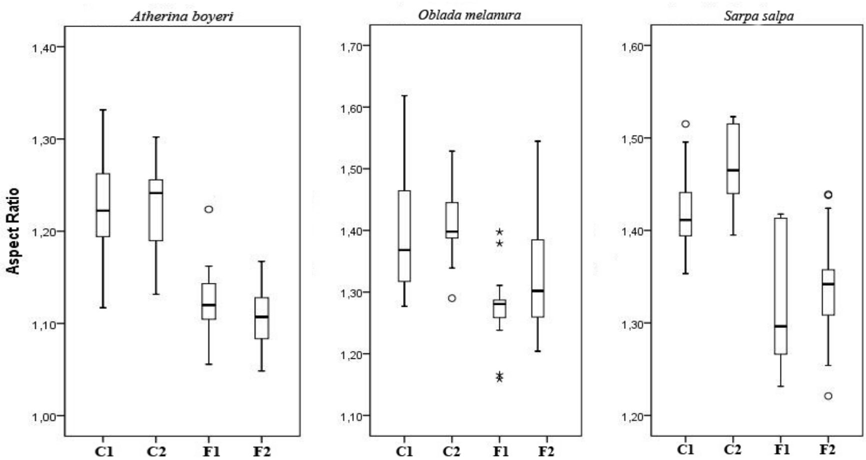

Figure 2. Results for the Aspect Ratio Index for control fish (C1 and C2) and farm-associated fish (F1 and F2).

DISCUSSION

Despite of failing to find differences in the diets of farm-associated fish, it has been nonetheless detected that there is an effect on the otolith morphology of these associated fish as shown by the modification in otoliths morphology, mainly reflected in the Aspect Ratio Index (a relationship between the major and minor axis of the otolith) since it reached higher values in control fish non-associated with fish farms. Differences were especially clear for *S. salpa* which also showed variations between control and farm-associated fish for the surface area of the otolith and its roundness.

Despite of this, it was not possible to detect differences in the periodical growth of the otolith rings. However, it is necessary to delve into this subject since farms act as both artificial reefs and enhanced fish attraction devices when aggregating a high number of fish of tens of different species, many of them of commercial interest. The use of artificial reefs has traditionally been applied to manage fish stocks for conservationist or resource management purposes, and presumably, cages can also have significant effects on local stocks if the synergistic effects of aquaculture enhance or damage these populations. Moreover, it is difficult to find similar artificial submerged structures with an equivalent size of a full production fish farm and with such an important capacity of fish aggregation.

CONCLUSION

In addition to the previously published effects on seasonal and spatial variations of associated species and the associated changes on fish physiology (Fernandez-Jover et al. 2009), it has been shown that there is also a change in the morphology of otoliths that can be effectively detected through the aspect ratio and the multivariate analysis of the EFDs. Despite of these results, it has not been demonstrated that the synergy of the observed changes entail negative effects on fish species that use floating cages as habitats for settlement.

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BILOGICAL CHARACTERISTICS OF ANCHOVY (*ENGRAULIS ENCRASICOLUS*) IN BOKAKOTORSKA BAY (MONTENEGRO)

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BIOLOŠKE KARAKTERISTIKE INĆUNA (*ENGRAULIS ENCRASICOLUS*) U BOKOKOTORSKOM ZALIVU (CRNA GORA)

Apstrakt

Inćun, *Engraulis encrasicolus*, 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 okaca (5-6 mm) u Bokokotorskom zalivu kao i malim mrežama plivaricama na ulazu u zaliv. Podaci predstavljeni u ovom radu rezultat su istraživanja sprovedenog u okviru Pilot studije AdriaMed projekta u periodu septembar 2007. – avgust 2011. 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 inćuna prikupljeni su u području Bokokotorskog zaliva (Sl. 1) 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, GSI, 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. Inćun je bio najzastupljeniji u ulovu u septembru i aprilu. Dužinski raspon iznosio je od 7.5 do 14.4 cm, sa srednjom vrednošću od 10.13 cm, dok je težinski raspon bio od 0.9 do 21 gram, sa srednjom vrednošću 6.4 g. Od ukupnog broja jedinki (2000) ženke čine 61%, mužjaci 33%, a 6% su jedinke kojima nije bilo moguće odrediti pol. Ženke su zastupljenije od mužjaka u svim dužinskim klasama, osim na 8 cm dužine gde je odnos polova 1:1. Najveći broj uzorkovanih jedinki bio je u drugom stadijumu zrelosti. Procenjeno je da je dužina pri kojoj 50% populacije inćuna dostigne polnu zrelost 9.1 cm, dok su $L_{25\%}$ i $L_{75\%}$ procenjeni na 8.6 i 9.7 cm. Koeficijent *b* dužinsko-težinskog odnosa kod mužjaka iznosio je 3.212, a kod ženki 3.437.

Ključne reči: inćun, biološke karakteristike, Bokokotorski zaliv
Keywords: anchovy, biological characteristics, Boka Kotorska Bay

INTRODUCTION

The FAO Project AdriaMed 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, so as to provide the basis for implementing an Ecosystem Approach to 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. to August 2011., with small interruption and on 12-monthly basis. The information on catch and effort of all the active fleet segments in the sampling ports were gathered by interviewing fishermen, also the biological samples of the main target species were taken to study their biological characteristics.The pilot study was developed on the basis of the existing monitoring practice and requirements in Mediterranean and European countries, in view of the membership of Montenegro in the FAO General fisheries Commission for the Mediterranean Sea (GFCM) and of the future entering of the Country in the European Union (EU).

MATERIAL AND METHODS

Since the industrial fishing at open sea is undeveloped in Montenegro, anchovy samples were collected from beach seine and small purse seine catches in the Boka Kotorska Bay (Fig. 1) on monthly base. This is one of the most productive areas of the Montenegrin coast and it seems to be a nursery ground for anchovy, sardine and other small pelagic fish species.

Figure 1. Map of the study area.

The following data were recorded for each specimen: total length (TL) with precision 0.1 cm, total body weight (TW) to nearest 0.01 g, sex, sexual maturity (according to MEDITS maturity scales - immature, maturing, mature and spent/resting, in stages 1, 2, 3, and 4, respectively), gonad weight (W_G) to nearest 0.01 g. This data collection scheme allowed for an estimation of size range and length frequency distribution of landings raised to the trimester of each sampling period, where possible. Sex ratio is given as a number of males, females or unsexed individuals over the total number expressed as percentage. Sex ratio by length is given as the number of males or females over a combined sum of the number of males and females, expressed as a proportion, for any given length category with a significant number of individuals (usually more than 10). Length at first maturity was calculated using the linear regression on a ratio of mature individuals (MEDITS subcategories 2b and above) over the total number of sexed individuals for a given length category, transformed using the

$$\ln\left(\frac{1}{P}-1\right)$$

expression (where P is the proportion of mature individuals over the total number of sexed individuals for any given length class). The regression gives the parameters α (intercept) and β (slope) of the maturity ogive. These parameters are then used to calculate lengths at which 25, 50 and 75% of the population reaches sexual maturity ($L_{25\%}$, $L_{50\%}$, and $L_{75\%}$, respectively). Maturity ogive is then estimated using the formula:

$$\frac{1}{1+e^{\alpha-\beta\cdot TL}}.$$

The gonadosomatic index is calculated according to the formula:

$$GSI=\frac{W_G}{TW}\cdot 100,$$

where W_G is gonad weight (g), and TW stands for total weight (g). The index is calculated for each mature individual (MEDITS sub-stage 2b and above) separately, and then averaged by sex and month. The relation between the length, TL and total weight (TW) of the specimens was determined according to the formula $TW = a\cdot L^b$.

RESULTS AND DISSCUSSION

A total of 2000 specimens of anchovy were sampled. The greatest abundance of anchovy in the landings was in September during the 2007/08, and in April during the 2009/10 and 2010/11 sampling periods. Length of the sampled individuals ranged from 5.0 to 14.4 cm TL (average of 10.13±1.33 cm TL), while the weight was in the 0.9–21.0 g range, with an average of 6.4±2.9 g. Minimum and maximum length of females was 6.5 and 14.4 cm TL, respectively, and averaged at 10.2±1.3 cm TL. Weight was in the 1.8–21.0 g range (average of 6.5±2.9 g). Males were, on average, slightly longer at 10.3±1.2 cm TL (7.5–13.1 cm TL) and somewhat heavier at 6.7±2.6 g (from 2.64–14.69 cm). Length frequency distributions were predominantly unimodal, with modes at 8–9 and 9–10 cm TL for the 1st and 2nd trimester of 2007/08, respectively. In 2009/10, the modes were at 10–11 cm TL (1st trimester), 7–9 cm TL and 9–11 cm TL (2nd trimester), and at 7–9 and 10–12 cm TL (3rd trimester). In 2010/11, modes were between 7 and 9 cm TL in 1st trimester, between 9 and 11 cm TL in 2nd trimester, and between 10 and 12 cm TL in 3rd.

There were 1217 females in the sample (61%), 667 males (33%), and 116 unsexed individuals (6%). Females strongly outnumbered the males in each of the sampling periods (Table 1).

Table 1. Sex ratio by sampling period

Sampling period	Females	Males	Unsexed
2007/08	58%	38%	3%
2009/10	62%	31%	7%
2010/11	62%	31%	7%

Generally, females were more numerous at each length class, except at 8 cm TL, where the male–female ratio was close to 1:1 (Fig. 2). Similar results are reported for the same area in period 2006-2007 (Djurovic, 2012), as well as in central Adriatic (Sinovčić, 2000; Sinovčić & Zorica, 2006).

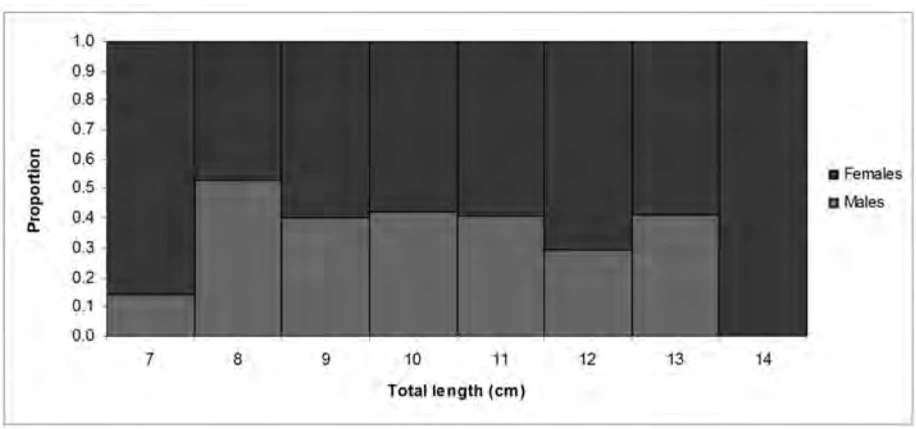

Figure 2. Sex ratio by length for the Montenegrin catch of *E. encrasicolus*

The majority of individuals were of gonad maturity stage 2 (66% of males and 61% of females), with a significant number in stage 1 (21% of males and 25% of females) which can be explained by the fact that younger individuals of anchovy inhabits coastal areas and bays and after the first spawning goes to deeper waters (Regner, 1973; Sinovčić, 1978; Sinovčić, 2000).

Length at first maturity was estimated at 9.1 cm TL for the total sample, or at 9.3 cm TL for females and 8.9 cm TL for males (Table 2., Fig. 3). Authors from Adriatic reported different results on length of first maturity for anchovy: 8.1 cm in north Adriatic (Rampa *et al*., 2005), 10.9 cm for females in central Adriatic (Muzinic, 1956), 9.7 cm and 7.1cm in central Adriatic (Sinovcic, 1978; Sinovcic and Zorica, 2006), and 10 cm in Albanian waters, south Adriatic (Kolitari, 2006).

Table 2. Length at first maturity and maturity ogive parameters by sex

Species *Engraulis encrasicolus*	Maturity ogive parameters		Length at maturity (cm) *(mm)		
	α	β	$L_{25\%}$	$L_{50\%}$	$L_{75\%}$
Total	18.8814	2.0684	8.6	9.1	9.7
Females	20.7300	2.2105	8.9	9.3	9.9
Males	15.6011	1.7377	8.3	8.9	9.6

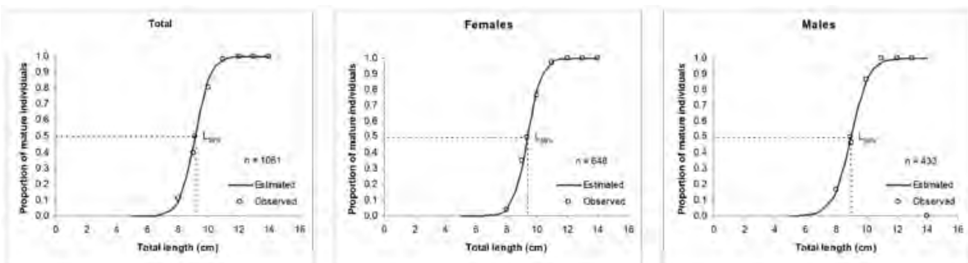

Figure 3. Maturity ogives for the total sample, females and males

The gonadosomatic index of both sexes peaked in May. For females range of GSI was from 0.3 in November to 4.74 in May, and for males from 0.18 in November to 4.97 in May. There were no individuals sampled in July–September period (Fig. 4). Those values are consistent with the results of other authors in Adriatic area; Djurovic (2012) reported 0.83-4.85 for females and 0.7-4.35 for males with the lowest values in winter months and highest in April-June period.

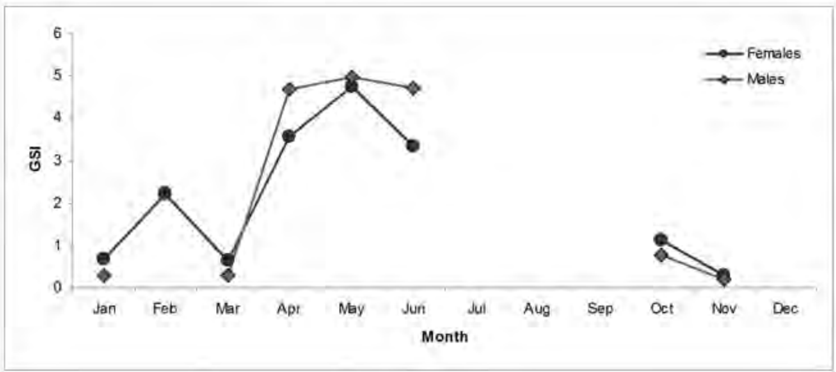

Figure 4. Gonadosomatic index of anchovy, by month

In length–weight relationship, the parameter *b* was consistently higher than 3 for males ($b_{♂}$ = 3.2118), females ($b_{♀}$ = 3.4365), and total sample (b_{TOT} = 3.3604) (Fig. 5). For the same area Djurovic (2012) reported b_{TOT} = 3.167 and b_{TOT} = 3.106, for period 2004-2005 and 2006-2007 respectively. Similar values are reported for Novigrad Sea, central Adriatic, b_{TOT} = 3.19 (Sinovcic, 1998) and b_{TOT} = 3.211 (Sinovčić and Zorica, 2006). Similar values of parameter *b* could be explained by similar conditions in these areas, especially in terms of eutrophication, since the amounts of nutrients are higher than in the open sea due to anthropogenic influence.

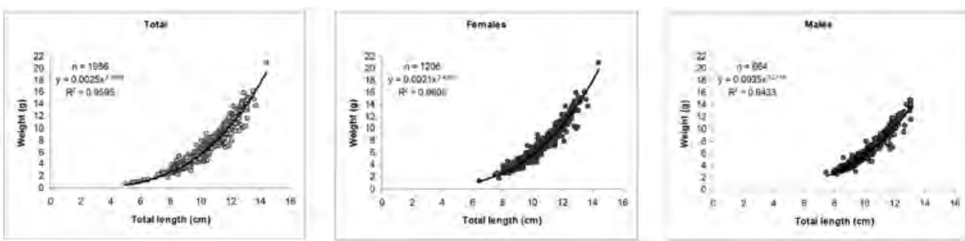

Figure 5. Length–weight ratio of anchovy, total sample, females and males

CONCLUSIONS

Fishery of anchovy and sardine with beach seines has several centuries of tradition in Boka Kotorska Bay. Since this type of fishery targets in certain ammount juvenile individuals of those species, therefore it is necessary to introduce measures for conservation of juvenile sardines and anchovies, in order to enable as many immature specimens as possible to reach sexual maturity.

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STATE OF BENTHIC (DEMERSAL) RESOURCES IN THE EASTERN COAST OF THE ADRIATIC SEA

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STANJE PRIDNENIH (DEMERZALNIH) RESURSA ISTOČNE OBALE JADRANSKOG MORA

Apstrakt

Pridneni (demerzalni) resursi predstavljaju jednu od najvažnijih komponenti morskog ribarstva u Jadranskom moru, kako u smislu količine ulova, tako i u njegovoj komercijalnoj vrijednosti. Trenutan ukupan ulov kočarskih organizama u Jadranu kreće se oko četrdesetak tisuća tona, od čega najveći dio ulovi talijanska kočarska flota (preko 85%). Prvim ribarstveno-biološkim istraživanjima u Jadranu smatra se ekspedicija „Hvar“ 1948/49. (Šoljan, 1997) koja je pokrila teritorijalna mora ex-Jugoslavije i Albanije, te najveći dio međunarodnih voda otvorenog Jadrana. Vrijednost ove ekspedicije leži u činjenici kako je ona provedena u vrijeme dok je kočarski ribolov bio u začetcima, ali i zaustavljen zbog ratnih aktivnosti 7-8 godina, te su resursi za vrijeme „Hvar“ ekspedicije bili u nultom stanju („virgin state“) i mogu služiti kao referentna točka za opisivanje kasnijih promjena koje su nastale kao posljedica intenzivne eksploatacije. U novije vrijeme intenziviraju se međunarodna istraživanja pridnenih resursa Jadrana. Istraživanja koja su tema ovog rada obavljena su u okviru međunarodne ekspedicije EU MEDITS u razdoblju od 2005. do 2010. godine u cijelom Jadranskom moru (osim najdubljih dijelova Južnojadranske kotline). Opisano je stanje pridnenih resursa kroz GIS prikaze indeksa biomase (ulov kg/km^2) u cijelom Jadranu i to za: ukupan ulov tijekom ekspedicije MEDITS (ribe, rakovi i glavonošci), te za MEDITS ciljane vrste (59 najzastupljenijih vrsta kočarskog ribolova), hrskavičnjače, kao i za gospodarski najvažnije vrste: oslić, trlja blatarica, arbun, kovač, mačka bjelica, škamp i kozica.

Ključne riječi: Jadransko more, pridneni resursi, ribolov, ekspedicija MEDITS
Keywords: Adriatic sea, natural resources, fishing, research expedition MEDITS

INTRODUCTION

Demersal resources represent one of the most important components of marine fisheries in the Adriatic Sea, both in terms of amount of catch, as well as its commercial value. The total catch of demersal organisms in the Adriatic Sea in recent years is approximately 40 000 tons per year, most of which (over 85%) is caught by Italian fleet. Large differences in the intensity of fishing and fishing effort in various parts of the Adriatic resulted in large differences in the state of demersal resources (Piccinetti *et al*., 2012). Investigations and assessment of demersal resources in the Adriatic Sea have a long tradition (Vrgoč et al., 2004), and the "Hvar" expedition in 1948/49 is considered the first systematic fishery-biological research in the Adriatic (Šoljan, 1997). "Hvar" expedition covered the territorial waters of the ex-Yugoslavia and Albania, and most of the international open waters of the Adriatic. The value of this expedition, apart from the quantity and quality of data collected, lies in the fact that it was conducted at a time when trawl fishing was in its infancy. World War II also stopped all trawling activities for 7 or 8 years, and the resources were therefore in the unexploited state ("virgin state") at the time of "Hvar" expedition. The results of the "Hvar" expedition can therefore be used as a reference point to describe the subsequent changes that have occurred as a result of intensive exploitation. After this expedition, numerous studies were organized in the Adriatic, but they were spatially and temporally limited. The most important studies of demersal resources along the eastern coast were conducted by Kirinčić and Lepetić (1954), Crnković (1959), Županović (1961), Županović and Jardas (1989), Vrgoč (2000), Jukic *et al*. (2001), Piccinetti *et al*. (2012) and others. In recent years, international research of demersal resources of the Adriatic was intensified, with the aim to describe qualitative and quantitative composition and the changes that occur as a result of exploitation. The most important research expeditions are EU MEDITS, FAO AdriaMed Trawl Survey, SoleMon project, UWTV survey of shrimps in the open sea and DeepSeaSurvey (Piccinetti *et al*., 2012).

MATERIAL AND METHODS

Studies presented in this paper were carried out within the framework of an international expedition EU MEDITS, in a period from 2005. to 2010. in the entire Adriatic (except the deepest areas of the South Adriatic Pit). Sampling was conducted using the scientific research net GOC 73 and according to the protocol of MEDITS expedition (Bernard *et al*., 2002). Research was performed yearly, in the spring-summer period at 260 stations; the duration of the hauls was 30 min at the stations with depths less than 200 meters and 60 min at stations with depths above 200 meters. Qualitative and quantitative analysis of catch and by-catch composition was done on board, while length and weight measurements and determination of sex and maturity stages of economically important species was done in the laboratory. All data were stored in a common database using the ATRIS computer program (Gramolini, 2005), which was used to calculate the biomass index (kg/km^2) of species. GIS distribution maps were made using ArcView GIS tools v. 3.2.

RESULTS AND DISCUSSION

A description of demersal resources is given as the GIS biomass index (kg/km^2) for the entire Adriatic for total catch during the MEDITS expedition (fish (including small pelagic), crustaceans and cephalopods) and for MEDITS target species (59 most abundant species in trawl fisheries), cartilaginous, and most important commercial species: hake, red mullet, common pandora, John Dory, small-spotted catshark, shrimp and deep water pink shrimp.

The biomass index for all species caught during MEDITS expedition in the study area (Fig. 1) shows that the highest population density is in the channel areas of the central Adriatic, and in the open waters of northern Adriatic (around the mouth of the River Po). This distribution pattern is primarily due to large catches of small pelagic fish, but also due to the fact that these are the areas of highest primary production (Buljan, 1964).

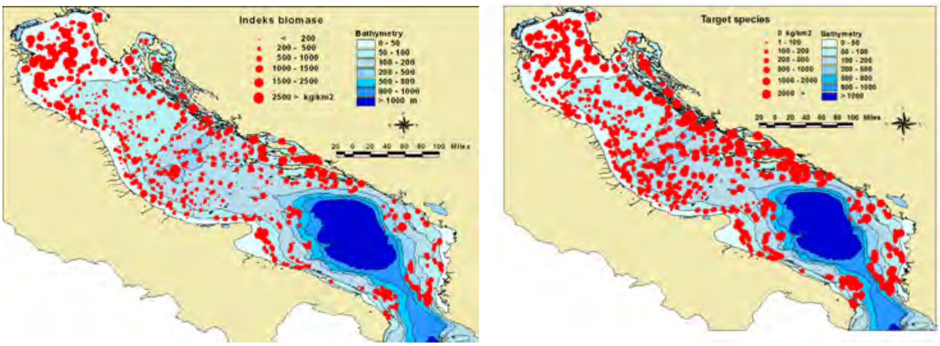

Figure 1. Biomass index for all species caught during the MEDITS expedition (left) and Biomass index of target species during MEDITS expeditions (right)

A much better view of the state of benthic communities is obtained if distribution of MEDITS target species is observed (Fig. 1, right). The highest density is found along the eastern Adriatic coast, mainly in the canal areas, and the lowest density in the open sea. Cartilaginous fishes are accepted as indicators of state of marine communities because they are extremely vulnerable due to their biological characteristics (slow growth, low reproductive potential) and they are the first species to be threatened by intensive fishing. Figure 2 shows that outside of territorial waters of the eastern Adriatic cartilaginous fishes are barely present in catches, which can be explained by intensive exploitation (Vrgoč, 2000; Jukic *et al.*, 2001).

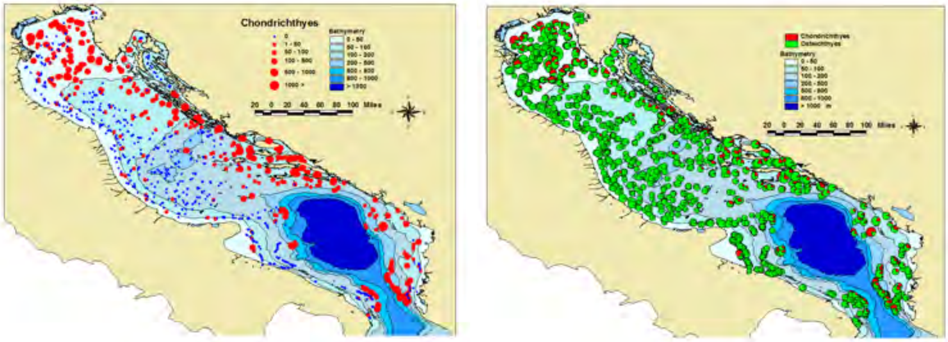

Figure 2. Biomass indexes of cartilaginous during MEDITS expeditions (left) and the ratio of biomass of cartilaginous and teleost during MEDITS expeditions (right)

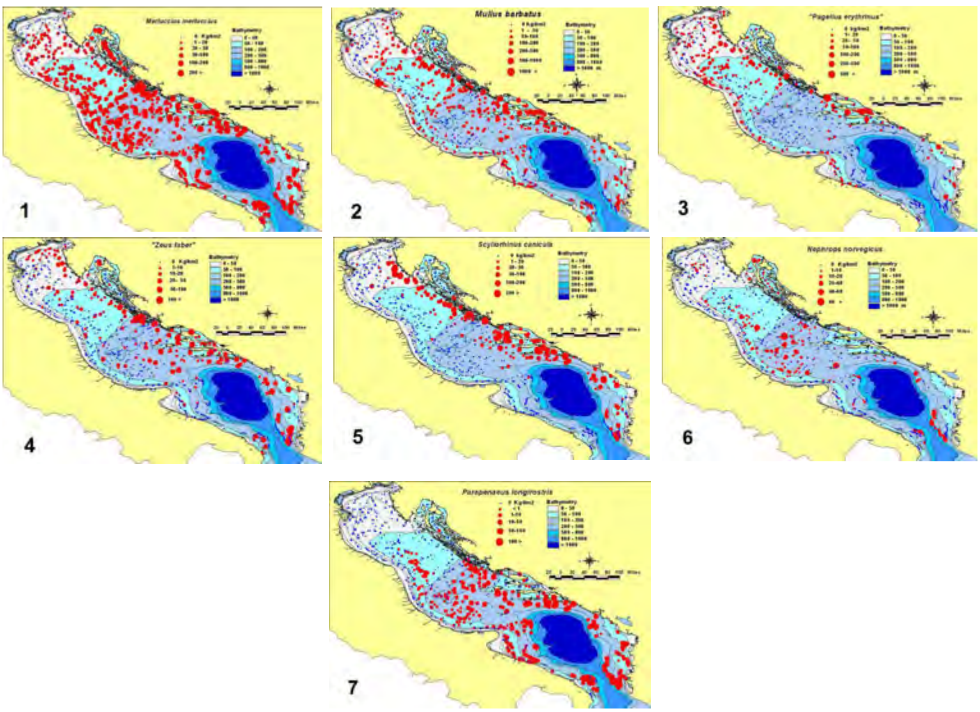

Figure 3. Biomass indexes of economically important species during the MEDITS expedition; 1 – *Merluccius merluccius*, 2 – *Mullus barbatus*, 3 – *Pagellus erythrinus*, 4 – *Zeus faber*, 5 – *Scyliorhinus canicula*, 6 – *Nephrops norvegicus*, 7 – *Parapenaeus longirostris*

Biomass index of economically important species (Fig. 3) shows that the population density is significantly higher along the eastern coast of Adriatic than in the open sea or along the western Adriatic coast. The highest population densities of hake can be found along the eastern coast of the Adriatic Sea and in Jabuka Pit, which is consistent with the fact that Jabuka Pit serves as a nursery and spawning area of this species (Županović and Jardas, 1989). Red mullet dominated in open waters along the eastern coast of the Adriatic, and in canal areas. This distribution is partly a result of periodic migrations of this species from the east to the west coast (where juveniles are located) of the Adriatic. Common pandora is a typical circalittoral species (Rijavec and Županović, 1965) which can be found on rocky bottom and at depths down to 100 m, which are common along the eastern coast of the Adriatic. The situation is similar with John Dory and small-spotted catshark, which are very scarce outside the territorial waters of countries on the eastern coast of the Adriatic. This is primarily due to intensive exploitation of those extremely vulnerable species along the western coast and in the open waters of the Adriatic, which is consistent with a significant drop in the biomass indexes recorded in the period between “Hvar” and MEDITS’s expeditions (Vrgoč, 2000; Jukic *et al.,* 2001). Distribution of shrimp in the Adriatic is related to the distribution of muddy types of sediment, and the densest settlements are located in the Jabika Pit, Velebit channel and part of southeastern Adriatic. Deep-water pink shrimp is a dominant species in deep south Adriatic and part of the central Adriatic, and population density is generally uniform throughout this area.

CONCLUSIONS

The recent state of benthic communities in the Adriatic Sea, described based on the data collected during the MEDITS expeditions shows uneven population densities between eastern and western Adriatic coast and in the open sea. Biomass indexes of the most important commercial species are often several times higher in territorial waters of eastern Adriatic countries. This situation can be explained by the uneven and over-intensive exploitation of demersal resources, primarily by the Italian fleet along the west coast and in the open sea, whose technical characteristics and fishing effort are greater than those along the eastern Adriatic coast. This should be taken into account in any future assessment of demersal fisheries resources and protection management measures in future regional management plans that are prepared within the GFCM.

ACKNOWLEDGMENTS

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DISTRIBUTION OF MUSKY OCTOPUS (*ELEDONE MOSCHATA* LAMARCK, 1798) (CEPHALOPODA: OCTOPODA) IN THE SOUTH-EASTERN ADRIATIC

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RASPROSTRANJENOST CRNOG MUZGAVCA (*ELEDONE MOSCHATA* LAMARCK, 1798) (CEPHALOPODA: OCTOPODA) U JUGOISTOČNOM JADRANU

Apstrakt

Procijenjene su sezonske varijacije u distribuciji i indeksima brojnosti i biomase crnog muzgavca na Crnogorskom primorju. Najveće vrijednosti zabilježene su u jesen (indeks brojnosti, 405.6 N/km^2) i zimu (indeks biomase, 26.0 kg/km^2) na dubinama manjim od 50 m, dok su na dubinama od 50 do 100 m za oba maksimumi bili u ljetnom periodu (126.0 N/km^2 odnosno 20.3 kg/km^2). Crni muzgavac nije nađen na dubinama većim od 100 m. Najveću gustoću naseljenosti pokazao je na području između Budve i Ulcinja na kojima prevladavaju dubine manje od 200 m. Postoje indikacije ograničenih sezonskih migracija, koje mogu biti uzrokovane povećanim dotokom slatke vode Bojanom u jesen i zimu.

Ključne reči: distribucija, muzgavac, Eledone moschata, Crnogorsko primorje

Keywords: distribution, musky octopus, Eledone moschata, Montenegrin coast

INTRODUCTION

Musky octopus, *Eledone moschata* (Lamarck, 1798), is an octopod cephalopod species distributed throughout the Mediterranean, including the Adriatic, southern coasts of Portugal, the Gibraltar, and the Gulf of Cádiz in the Atlantic Ocean (Roper *et al.*, 1984; Belcari & Sbrana, 1999). According to previous studies in south–eastern Adriatic, the musky octopus is found in shallow areas at depths down to 80 m, and only rarely at depths down to 100 m (Mandić *et al.*, 1982; Mandić, 1984; Pastorelli *et al.*, 1998; Vrgoč *et al.*, 2004). Musky octopus is one of the most important cephalopod species in bottom trawl fisheries in the south–eastern Adriatic (Belcari & Sbrana, 1999; Vrgoč *et al.*, 2004). *E. moschata* has been a subject of studies in different areas of the Mediterranean: Catalan Sea (Mangold–Wirz, 1963; Mangold, 1983), Gulf of Gabes (Ezzedine–Najai, 1997), Gulf of Cádiz (Silva *et al.*, 2004), Aegean Sea (Önsoy & Salman, 2004; Akyol *et al.*, 2007, Şen, 2007; Şen & Akyol, 2011) and the Adriatic (Krstulović Šifner, 2004; Krstulović Šifner & Vrgoč, 2009a, 2009b).

MATERIALS AND METHODS

The specimens of *E. moschata* were obtained from commercial bottom trawl catches. The hauls were trawled at 10 predetermined positions, following the MEDITS protocol (MEDITS, 2007). Sampling was done using the commercial trawl in the period between 30 minutes after sunrise and 30 minutes before sunset. The trawl was hauled for 30 minutes if the sampling depth was below 200 m, or 60 minutes at depths greater than 200 m. The codend mesh size was 20 mm, which is the legal minimum in Montenegro (Official Gazette of Montenegro, 56/09, 8/11). The sampling was performed seasonally, from April 2009. to April 2011.

Musky octopus catches were standardised according to the swept area method (Sparre & Venema, 1998) using the AtrIS computer program (AdriaMed, 2007), and the swept area was calculated according to the formula:

$$P = 0{,}001 \cdot a \cdot s$$

where P is the surface of the swept area [km^2], a is wing spread of the trawl [m], and s represents the distance travelled [km].

Obtained values of the swept area were then used to calculate biomass and abundance indices, which are used to express the catch according to area unit (kg/km^2 and N/km^2, respectively). The mean value of the catch can be expressed as:

$$\overline{X}_T = \frac{p_1\overline{X}_1 + p_2\overline{X}_2 + p_3\overline{X}_3 + \ldots + p_n\overline{X}_n}{p_1 + p_2 + p_3 + \ldots + p_n}$$

where $\overline{X}_T$ represents the mean value of the catch in the studied area (kg/km^2, N/km^2), $\overline{X}_{1,2,3,\ldots,n}$ is the mean value of the catch in a given depth stratum, and $p_{1,2,3,\ldots,n}$ is the surface area of a depth stratum (km^2).

RESULTS AND DISCUSSION

During the 2009–2011 study, the highest value of the abundance index (N/km^2) was found during the autumn season at depths down to 50 m (405.6 N/km^2), while the lowest value was found in spring (79 N/km^2) (Table 1, Fig. 1). Biomass indices (kg/km^2) at these depths ranged from 7.4 kg/km^2 in spring to 24.4 kg/km^2 in summer (Table 1, Fig. 2).

According to the results of MEDITS expedition in the north and central eastern Adriatic (1996.–2003.), done in the period between June and September (except in 2001, which started somewhat earlier, in May) (Petrić *et al.*, 2013)), the abundance index at depths up to 50 m ranged from 225 N/km^2 (1998) to 978 N/km^2 (2002), with a mean value of 623 N/km^2. The biomass index ranged from 20.36 kg/km^2 (1998) to 117.78 kg/km^2 (2002), with an average value of 65.85 kg/km^2 (Krstulović Šifner, 2004).

Table 1. Abundance (N/km^2) and biomass indices (kg/km^2) for *E. moschata* according to depth strata along Montenegrin coast, 2009–2011

Depth (m)	Index	Spring	Summer	Autumn	Winter
10–50	N/km^2	79	178.4	405.6	289.2
	kg/km^2	7.4	24.4	18.8	26.0
50–100	N/km^2	59.3	126.0	42.3	47.2
	kg/km^2	8.1	20.3	3.8	4.9

At depths between 50 and 100 m, abundance index ranged between 42.3 N/km^2 in autumn to 126 N/km^2 in summer (Table 1, Fig. 1). Biomass index ranged from 3.8 kg/km^2 in autumn to 20.3 kg/km^2 in summer (Table 1, Fig. 2). In central and northern Adriatic the biomass indices at 50–100 m depths were between 4.04 kg/km^2 (1998) and 90.07 kg/km^2 (2002), with a mean value of 24.09 kg/km^2, while the abundance index varied between 62 N/km^2 (1998) and 948 N/km^2 (2002), with an average of 295 N/km^2 (Krstulović Šifner, 2004).

During the present study, *E. moschata* was not found at depths greater than 100 m. In northern and central Adriatic, the mean biomass index at depths between 100 and 200 m was 1.73 kg/km^2, and the mean abundance index 20 N/km^2 (Krstulović Šifner, 2004).

According to Krstulović Šifner *et al.* (2005), musky octopus had greatest abundance in the north–eastern Adriatic, with mean abundance index of 240 N/km^2, and was found at depths down to 165 m. Tursi (1992) reports *E. moschata* at depths down to 400 m in the Ionian Sea, but notes that the abundance was greatest at depths under 50 m, and that the overall number of individuals in that study was very small. Belcari *et al.* (2002) note that *E. moschata* was very poorly represented or even nonexistent in the catches in the northern Ligurian Sea, north–eastern Corsica, Ionian Sea and in Moroccan waters, while catches in the north Adriatic (Slovenia) reached abundance index of 2396 N/km^2 and biomass index of 336.1 kg/km^2 (Belcari *et al.*, 2002).

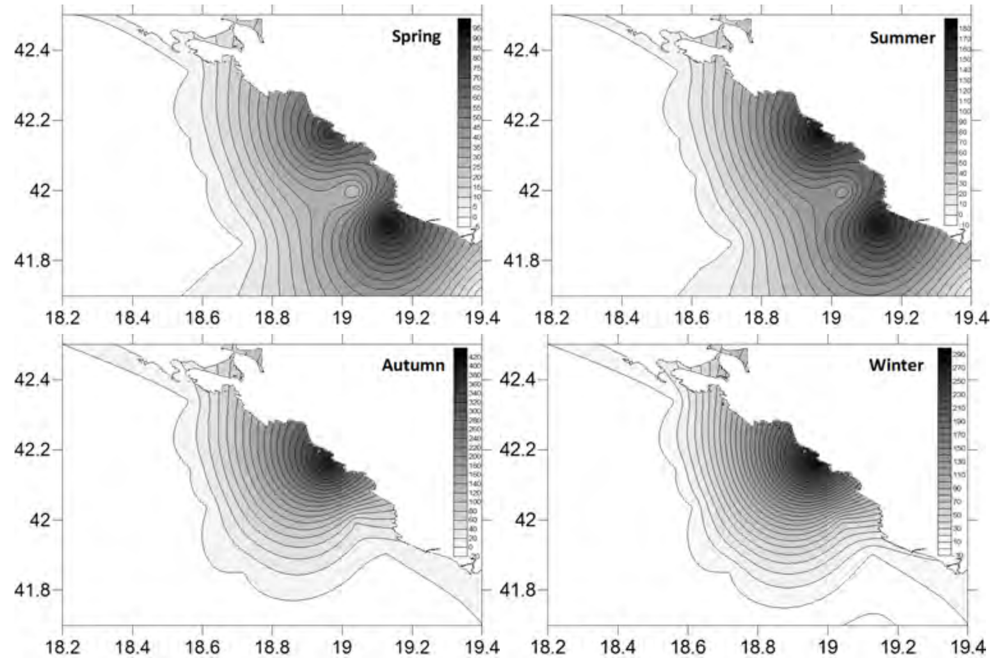

Figure 1. Distribution of *E. moschata* according to abundance index (N/km^2), by season along Montenegrin coast, 2009–2011

It should be noted that MEDITS results are not directly comparable to the results of this study because of different mesh sizes. MEDITS expeditions uses a 10 mm codend mesh size (IFREMER GOC 37 trawl type), while the legal minimum in Montenegro is twice that size (20 mm codend mesh size) (Official Gazette of Montenegro, 56/09; 8/11).

Along the Montenegrin coast, *E. moschata* had highest values in the area from Budva to the Bojana River estuary, which corresponds to the area with greatest shelf surface (depths under 200 m).

During spring and summer seasons, areas of increased values of abundance and biomass indices can be seen in front of Petrovac (Budva municipality, 41°12’20” N, 18°56’33” E) and the city of Ulcinj (41°55’12” N, 19°12’ E), while during autumn and winter increased values can be found only in front of Petrovac. This could indicate limited seasonal migrations, which could be explained by the increased inflow of freshwater via the Bojana river during autumn and winter. This could have a pronounced effect on these shallow areas around the city of Ulcinj (Mandić *et al*., 1982).

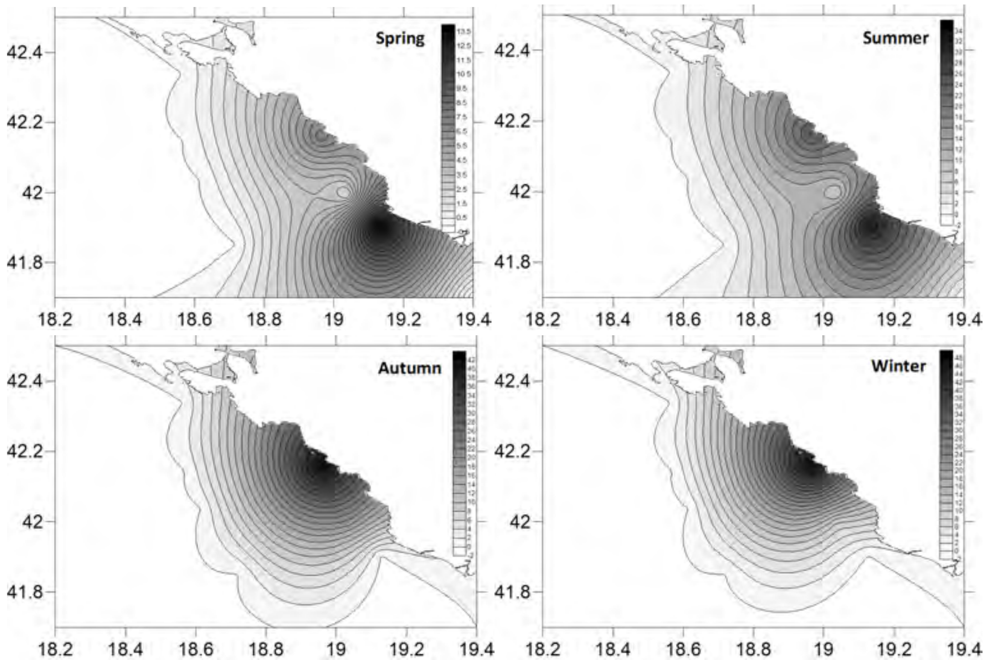

Figure 2. Distribution of *E. moschata* according to biomass index (kg/km^2), by season along Montenegrin coast, 2009–2011

CONCLUSIONS

The highest values of the abundance index for *E. moschata* were recorded in autumn and winter, and in summer and winter for biomass index, all at depths below 50 m, which is consistent with previous studies of this species, both in the Adriatic and in the Mediterranean. During this study in Montenegrin waters, musky octopus was not recorded at depths greater than 100 m. Generally, abundance and biomass indices of *E. moschata* were lower than in northern and central Adriatic. There are also indications of limited seasonal migrations. Further, more detailed studies are recommended for better understanding of distribution and biology of this economically important cephalopod species.

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EVALUATING THE HEALTH RISKS ASSOCIATED WITH CONSUMING OF CULTIVATED / WILD MUSSELS FROM BOKA KOTORSKA BAY

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PROCENA ZDRAVSTVENOG RIZIKA POVEZANOG SA KONZUMIRANJEM GAJENIH / DIVLJIH DAGNJI IZ BOKOKOTORSKOG ZALIVA

Apstrakt

Dagnje su veoma važne za ljudsku ishranu, jer predstavljaju jeftin izvor proteina, koji čine oko 60% suve mase mekog tkiva *Mytilus galloprovincialis*. Njihovim konzumiranjem omogućen je kako jeftin izvor proteina visoke biološke vrednosti, tako i minerala i vitamina. Sa aspekta hranljivosti, dagnja je važan izvor hrane bogat bitnim elementima (Ca i Fe), kao i određenim vitaminima (B1, B2 i B3). Međutim za dagnje je poznato da poseduju veliki kapacitet akumulacije zagađujućih materija pre svega esecijalnih i neesencijalnih elemenata čiji nedostatak, kao i višak može izazvati ozbiljne zdravstvene probleme kod čoveka.

Potrošnja ribe i morskih proizvoda u evropskim zemljama, Francuska (29,7 kg po glavi stanovnika godišnje), Nemačka (12,2), Grčka (22,7), Italija (23,1), Portugal (57,4), Norveška (50), Poljska (9,6), je i dalje niska. Kako ne postoje precizni podaci o prosečnoj potrošnji školjki po glavi stanovnika za većinu zemalja, pa ni za Crnu Goru, dozvoljeni nedeljni unos (*PTWI*) se koristi za izračunavanje koncentracije elemenata koje čovek može uneti u organizam bez posledica po svoje zdravlje.

Dagnja *M. galloprovincialis* široko je rasprostranjena u priobalnim vodama Bokokotorskog zaliva. Uzgaja se na preko 16 farmi stacioniranih unutar zaliva, ali se duž cele obale zaliva takođe mogu naći i divlje dagnje koje lokalno stanovništvo koristi za ličnu potrošnju. Kako je celokupan zaliv konstantno i sve više izložen negativnom antropogenom uticaju, nameće se potreba za redovnom kontrolom živih organizama iz mora koji se koriste u ishrani čoveka.

Cilj ovoga rada bio je da se odredi kvalitet dagnje *Mytilus galloprovincialis*, odnosno da se proceni eventualni zdravstveni rizik putem konzumiranja ove morske vrste (kultivisane/divlje) sa više lokacija Bokokotorskog zaliva. U svim uzorcima školjki određivane su koncentracije gvožđa, cinka, mangana i bakra.

Izmerene koncentracije cinka i bakra u kultivisanim/gajenim dagnjama Bokokotorskog zaliva ispod su vrednosti propisanih regulativom o hrani Crne Gore. Najveće koncentracije cinka i bakra izmerene su u uzorcima divljih dagnji sa lokacije Herceg Novi, dok je najveća koncentracija gvožđa izmerena u kultivisanim dagnjama sa lokacija Kukuljina i Krašići, a najveća koncentracija mangana u kultivisanim uzorcima sa lokacije Bijela.

Na osnovu minimalnih i maksimalnih izmerenih koncentracija gvožđa, cinka, mangana i bakra u uzorcima divljih/kultivisanih dagnji i podataka za maksimalno dozvoljeni unos ispitivanih elemenata (*PTWI*) izračunat je dozvoljeni interval nedeljnog konzumiranja dagnji u kilogramima bez posledica po ljudsko zdravlje za svaki pojedinačno ispitivani element. Nedeljna količina dagnji koju je neophodno konzumirati kako bi se dostigla propisana *PTWI* vrednost varira: za Fe 11.5 – 14.8 kg i 4.8 – 21.2 kg, za Mn 12.4 – 18.8 kg i 13.4 – 26.4 kg, za Cu 147.0 – 243.0 kg i 198.0 – 233.0 kg, i za Zn 7.1 – 21.7 kg i 14.2 – 17.6 kg za divlje i uzgajene dagnje, respektivno.

Na osnovi dobijenih podataka jasno je da se radi o velikim količinama dagnji koje treba konzumirati kako bi se dostigle granične vrednosti *PTWI*a. Ovo ukazuje da divlje/kultivisane dagnje iz Bokokotorskog zaliva, u odnosu na sadržaj Fe, Zn, Mn i Cu u njima, ne predstavljaju opasnost po zdravlje ljudi i bezbedne su za ljudsku upotrebu.

Ključne reči: dagnja, cink, gvožđe, bakar, mangan, PTWI, Bokokotorski zaliv
Keywords: mussel, zinc, iron, manganese, copper, PTWI, Boka Kotorska bay

INTRODUCTION

Aquaculture is the fastest-growing animal-food-producing sector of agriculture that includes the cultivation of aquatic organisms (FAO, 2010). Increasingly *Mytilus galloprovincialis* is used as a cultivated mussel species (Stankovic et al., 2012). This mussel is a native species of the Mediterranean Sea, the Black Sea and the Adriatic Sea (Gosling, 1992).

Montenegro is a Mediterranean country located in southeastern Europe on the Adriatic coast. In Boka Kotorska bay the harvesting and cultivation of marine organisms from the Adriatic Sea dates back centuries. Today, mussel *M. galloprovincialis* is cultivated on around 16 small farms located inside the Bay and each farm producing around 10 to 50 t of mussels per year (FAO, 2007). In addition to cultivated mussels, wild mussels are widespread throughout the Boka Kotorska bay, which are hand-collected for personal consumption.

Mussel *M. galloprovincialis* is a sedentary, filter feeding animal that through feeding, not only assimilates the food necessary for growth and development, but also accumulates contaminants present in the water. 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). Some trace elements, such as Fe, Zn, Cu and Mn, are essential for humans and need to be consumed in adequate amounts, but in excessive amounts they can be toxic (Nasreddine et al., 2010).

As the Boka Kotorska bay receives a heavy influx of sewage and industrial effluents, as well as domestic and agricultural wastes, (Jovic et al., 2011) the determination of the levels of trace metals in mussels is very important. Also in order to ensure the safe consumption of mussels, it is essential that measures are taken to reduce the risk to consumers. Hence, the aims of this study were to determine the levels of Fe, Zn, Mn and Cu in the soft tissue of wild and cultivated mussels *M. galloprovincialis* from Boka Kotorska bay in order to provide information on the amount of cultivated and wild mussels from the Bay that can be safely consumed.

MATERIALS AND METHODS

Fresh wild and cultivated mussels (*M. galloprovincialis*) were sampled from ten different locations in Boka Kotorska bay: Herceg Novi, Bijela, Ljuta, Perast, Sv. Stasija, Kotor, Opatovo, Tivat, Kukuljina and Krasici. At the each site, whether wild or cultivated mussels, more than 2 kg of mussels of similar length were collected, placed in plastic bags with sea water and transported to the laboratory. The 25-30 mussels from each station were pooled. Mussels were cleaned and rinsed with deionized water, dissected fresh and the soft tissue was rinsed with Milli Q water. Pooled samples were measured before and after freeze drying - lyophilization to determine the water content, pulverized and homogenized using a mill.

About 0.5 g of the mussel samples were digested with 7 ml of HNO_3 (65%), 2 ml of H_2O_2 (30%) in a microwave digestion system for 30 min and diluted to 25 ml with deionized water. A blank digest was performed in the same way. The concentrations of zinc, iron, manganese and copper in the samples of mussels were determined using a Flame Atomic Absorption Spectrometer (PerkinElmer, AAnalyst 200) with an air–acetylene flame.

The accuracy of the applied analytical procedure for the determination of trace elements in mussels was tested using SRM 2976 (Mussel homogenate; NIST) certified reference material.

RESULTS AND DISCUSSION

In the present study iron (Fe), zinc (Zn), manganese (Mn) and copper (Cu) were analyzed in wild and cultivated mussels from Boka Kotorska bay. The mean concentrations of the investigated elements in the soft tissues of *M. galloprovincialis* from ten sites are given in Table 1.

Table 1. Concentration of Fe, Zn, Mn and Cu (mean ± SD, mg kg^{-1} wet weight) in the soft tissues of mussels from Boka Kotorska bay

Location	Sample Nature	Water (%)	Fe	Zn	Mn	Cu
Herceg Novi	Wild	77.8	34.0±1.64	69.0±3.50	1,12±0.10	1.67±0.15
Bijela	Cultivated	78.2	22.2±1.67	34.5±1.50	2,01±0.17	1.05±0.05
Perast	Wild	86.6	26.5±1.58	22.6±0.13	1,43±0.05	1.61±0.07
Ljuta	Cultivated	81.0	18.5±1.12	29.2±1.04	1,44±0.21	1.24±0.04
Sv. Stasija	Wild	74.7	31.8±1.97	37.4±1.49	1,47±0.11	1.27±0.13
Kotor	Wild	76.8	27.8±1.58	27.4±2.87	1,49±0.13	1.16±0.06
Opatovo	Wild	80.3	29.6±1.39	28.0±0.89	1,24±0.18	0.99±0.08
Tivat	Wild	79.4	27.8±1.89	57.4±3.39	2,18±0.11	1.55±0.09
Kukuljina	Cultivated	81.7	82.4±2.21	27.9±0.50	1,02±0.03	1.19±0.07
Krasici	Cultivated	79.6	38.6±1.59	33.1±2.40	1,51±0.14	1.12±0.10

In comparison with the permissible limits set by the Montenegrin Food Regulation (Montenegrin Food Regulation, 2002) for Cu (30.0 mg kg^{-1} ww) and Zn (100 mg kg^{-1} ww), all concentrations (mg kg^{-1} ww) of these metals from all locations were lower than the limits. The highest iron concentrations were measured in the cultivated mussel samples from locations Kukuljina and Krasici (82.4 and 38.6 mg kg^{-1} ww, respectively). In the case of zinc and copper, the highest concentrations were measured in wild mussel samples from locations Herceg Novi and Tivat, and Herceg Novi, Tivat and Perast, respectively, Table 1. Locations Tivat (wild mussels) and Bijela (cultivated mussels) are extracted as the locations where measured the highest concentrations of manganese, 2.18 and 2.01 mg kg^{-1} ww, respectively.

Based on the minimum and maximum concentration (Table 1) and the data for the maximum allowable intake of analyzed elements, we calculate the allowed interval of weekly mussel consumption in kg, required to exceed the FAO/WHO provisional tolerable weekly intakes (*PTWI*), Table 2. The *PTWI* defines the amount of a substance that can be ingested every week throughout a person's life with no risk of negative health effects and was established by the Joint FAO/WHO Expert Committee on Food Additives (FAO/WHO, 2004).

Table 2. The amount of wild and cultivated *M. galloprovincialis* that would need to be consumed by a 70-kg adult (per week) to exceed the FAO/WHO limits

Sample Nature	Element	Concentration (mg kg^{-1} w.w.)	Amount of mussels per week required to exceed the limit (kg)
		Minimum and maximum for any one site	Interval based on the minimum and maximum
Wild	Fe	26,50 – 34,00	11,5 – 14,8
	Mn	1,43 – 2,18	12,4 – 18,8
	Cu	1,01 – 1,67	147,0 – 243,0
	Zn	22,60 – 69,00	7,1 – 21,7
Cultivated	Fe	18,50 – 82,40	4,8 – 21,2
	Mn	1,02 – 2,01	13,4 – 26,4
	Cu	1,05 – 1,24	198,0 – 233,0
	Zn	27,90 – 34,50	14,2 – 17,6

Based on the maximum and minimum Zn levels in the investigated mussels, the amount of mussels per week required to exceed the $PTWI_{Zn}$ value (7 mg/kg body weight/week) (FAO/WHO, 2007) varied from 7.1 to 21.7 kg for wild and from 14.2 to 17.6 kg for cultivated mussels. The amount of mussels consumed per week required to exceed the estimated $PTWI_{Fe}$ (5.6 mg/kg body weight/week) (FAO/WHO, 2007) varies between 11.5–14.8 kg and 4.8–21.2 kg for wild and cultivated mussels, respectively. The amount of consumed mussel per week required to exceed the $PTWI_{Cu}$ (3.5 mg/kg body weight/week) (FAO/WHO, 2007) and $PTWI_{Mn}$ (0,385 mg/kg body weight/week) (FAO/WHO, 2004) varies from 147 to 247 kg for wild and from 198 to 233 kg for cultivated mussels, and from 12.4 to 18.8 kg for wild and from 13.4 to 26.4 kg for cultivated mussels, respectively.

Calculations based on the element concentrations present in mussels collected from the Boka Kotorska bay suggest that a large amount of mussels would need to be consumed to exceed the prescribed *PTWI* values. It can be said that both wild and cultivated mussels from the Boka Kotorska bay are safe for human consumption regarding the Zn, Fe, Mn and Cu concentrations.

CONCLUSION

In terms of prescribed *PTWI* values for the tested elements (Fe, Zn, Mn and Cu), there is no limiting factor/element in the consumption of mussels from the Bay. Concentrations found for Fe, Zn, Mn and Cu indicate that the investigated mussels from Boka Kotorska bay pose no health risk to seafood consumers.

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EFFECT OF RAPESEED OIL IN FISH FEED ON MEAT QUALITY OF COMMON CARP

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UTICAJ ULJA ULJANE REPICE U RIBLJOJ HRANI NA KVALITET MESA ŠARANA

Apstrakt

Meso šarana, najzastupljenije ribe na ribnjacima u Republici Srbiji, predstavlja značajan nutritivni izvor n-3 visoko nezasićenih masnih kisleina (HUFA), pre svega eikozapentaenske (EPA) i dokozaheksaenske (DHA) kiseline, koje imaju važnu ulogu u bitnim fiziološkim procesima u organizmu i očuvanju zdravlja ljudi. Sadržaj masti i masnokiselinski sastav šarana su pod uticajem pola, godišnjeg doba, reproduktivnog statusa, uslova životne sredine, načina gajenja a posebno načina ishrane. Dobra tehnologija proizvodnje na ribnjaku je od nesumnjivog značaja za odgovarajuću strukturu planktonskih i bentosnih organizama, što igra veliku ulogu u dobijanju mesa šarana što boljeg hemijskog sastava. Riblje ulje je dugo predstavljalo glavni izvor masti u hrani za ribe, ali je zbog sve veće potrošnje, pa samim tim smanjene raspoloživosti i drastičnog povećanja cene istog došlo do povećanja upotrebe ulja biljnog porekla kao potpune ili delimične zamene za riblje ulje u hrani za ribe. Zamena ribljeg ulja sa biljnim uljima može imati i negativnih efekata na ribe iz uzgoja pošto je riblje ulje dobar izvor n-3 HUFA, što nije slučaj kada su u pitanju ulja biljnog porekla koja sadrže visok procenat 18C n-3 masnih kiselina, ali su siromašna ili potpuno lišena n-3 HUFA. Prema dosadašnjim istraživanima šaran ima veće potrebe za n-6, nego za n-3 masnim kisleinama za optimalan rast i razvoj. Uljana repica ima višestruku namenu: za ishranu ljudi, životinja i kao industrijska biljka. Odnos n-6/n-3 u ulju uljane repice je 2:1, što je pogodno sa aspekta zdravlja ljudi; bogato je oleinskom kisleinom i polinezasićenom masnim kiselinama (PUFA), pogotovo sa linolnom i linoleinskom kiselinom, ali ne poseduje n-3 HUFA. Često se upotrebljava u komercijalnim smešama za ishranu riba, bez negativnog uticaja na proizvodne parametre, ali može imati negativan uticaj na masnokiselinski sastav mesa šarana. Međutim, najveći broj istraživanja o zameni ribljeg ulja uljem uljane repice je izveden na salmonidnim vrtsama riba, pa su poželjna dalja ispitivanja o uticaju ovog ulja na kvalitet mesa i imunološki status šarana. Može se reći da meso šarana uglavnom poseduje povoljan masnokiselinski sastav i svrstati se u zdravu hranu za ljude. Gajenje šarana sa korišćenjem gotovih krmnih smeša bi trebalo da postane praksa na ribnjacima u Srbiji, kako bi se povećala proizvodnja po jedinici površine, dobio šaran što boljeg kvaliteta i postigla dugoročna ekonomska održivost. Takođe, potrebno je vršiti promociju šarana kao nacionalog zdravog proizvoda poželjnog u svakodnevnoj ishrani ljudi. Istraživanja o daljem unapređenju kvaliteta mesa šarana, kao i o načinu ishrane koji će zadovoljiti potrebe ove vrste, ali i ciljeve što ekonomičnije proizvodnje su neophodna u cilju dobijanja što jeftinijeg šarana, koji će zadovoljiti sve potrebe potrošača.

Ključne reči: šaran, ulje uljane repice, masti, masnokiselinski sastav
Keywords: common carp, rapeseed oil, lipids, fatty acid composition

INTRODUCTION

The prices of fishmeal and fish oil (FO) on the global market are increasing continuously rendering the sustainability of their use as fish feed components questionable (Naylor et al., 2000). Neither fishmeal nor fish oil is produced in Serbia, the import of these rises the production expenses, which poses a serious problem to and burdens fish farming; thus it is necessary to provide alternative feed components which will meet the needs of the reared fish, primarly common carp. The replacement of FO with vegetable oil (VO) can thus be rather challenging due to the fact that VOs are devoid of the n-3 HUFA which are abundant in FO including EPA and DHA. This review provides informations regarding the nutritional and feeding value of rapeseed oil, examines the literature on the implications of using rapeseed oil in common carp nutrition, and offers recommendations on future research needs.

COMMON CARP

Common carp is the most widespread fish species in Serbia. It is omnivore fish and very effectively uses food. Carp is tolerant to large variations of quality of ambient conditions, not susceptible to diseases and is tolerant to handling (Ćirković et al., 2010). Alike other fish species, common carp cannot synthesize the essential fatty acids of the n-6 and n-3 series. Hence, these fatty acids must be provided by the feed. Their original food sources are zooplankton, zoobenthos and detritus which naturally contain high levels of n-3 FA, including EPA and DHA (Ljubojević et al., 2012a). The main type of fish production in Serbia is the semi-intensivesystem for cyprinid production, carp being as the major species (Ćirković et al., 2011a). In addition to natural food, cereals are supplemented to meet energy requirement. Some fish farms increase production by introducing extruded completed feed for carp. The cost of inputs per unit of fish weight is higher than in extensive and semi-intensive farming with addition of cereals, especially because of the high cost of fish feed that contains a high level of protein with a balanced amino acid composition. High cost can be overcome by replacing animal origin feedstuffs with local available vegetable-derived ingredients, such as rapeseed oil (RO).

REQUIREMENTS OF COMMON CARP FOR ESSENTIAL FATTY ACIDS

Many cultured warm-water fish, including carp, require no meat or fish products in their diets (Ljubojević et al., 2012b). Carp require greater amounts of n-6 fatty acids than n-3 fatty acids for maximal growth (NRC, 1993). High levels of n-3 PUFA have been reported to depress the growth of carp (Du et al., 2008). The fatty acid requirement for carp was reported to range from 0.5 to 1% for both n-6 and n-3 PUFA (Takeuchi, 1997). The requirements for n-6 fatty acid can be met by the inclusion of linoleic acid, which is abundantly present in rapeseed oil. The relatively low requirement of those fish for n-3 fatty acids coupled with the low dietary lipid requirement (Du et al., 2008). Becausee of that facts, inclusion of fish meal and fish oil in carp culture is very low (Tacon et al., 2008) and therefore the substitution of fish meal and oil could be considerably easier than for carnivorous aquaculture.

FACTORS AFFECTING PRODUCTIVITY, LIPID CONTENT AND LIPID QUALITY OF COMMON CARP

Common carp meat is rich in protein and n-3 PUFA, including EPA and DHA (Steffens et al., 2005). Feeding extruded formula increased production per hectare of pond surface area almost doubled compared with feeding supplemental grains and almost thrice compared with feeding only natural food (Ljubojević et al., 2013b). Depending on age, rearing system, and food, fat content varies from 2.3 to 17% and protein content varies from 14 to 18% in carp (Ljubojević et al., 2013c). Supplementary feeding with grains leads to enlarged amounts of crude lipid in fish meat and it was doubled higher compared to supplementary feeding with extruded formula and three-fold higher compared to carp which ingested only natural food (Ljubojević et al., 2013b). In common carp, the n-3:n-6 ratio varies between 0.8 and 2.4 (Steffens et al., 2005). Other studies reported a smaller ratio, for example 0.92 (Ćirković et al., 2012); 0.54 (Ćirković et al., 2011b, Ljubojević et al., 2013a). Ćirković et al. (2011a) found that it is possible to influence the fatty acids composition of lipids through rearing conditions, particularly feed type. According to research conducted by Ćirković et al. (2012), cultured carp grown on natural food had a high content of both n-6 and n-3 fatty acids and Ćirković et al. (2011a) observed that PUFA/SFA ratio was the most favourable in carp fed complete feed mixtures, and the least in carp fed with maize and wheat.

RAPESEED OIL

Rapeseed is crushed to produce rapeseed oil for animal nutrition and industrial applications, and the resultant rapeseeds meal which is used as a protein sources in animal nutrition. Seeds contain 38-48% oil, 18-28% protein, eruic acid below 0,1%, and glucosinolates up to 10 μmol/g (Stanaćev et al., 2006). Rapeseed oil has a very low level of SFA (7%), relatively high content of MUFA (mostly OA, C18:1n-9), moderate amount of ALA (13%) and favorable ratio LA/ALA and n-3/n-6 1:2 and 1:2, respectively (Pickova et al., 2007) and is commonly used in feed for salmonids as a replacement for fish oil.

INFLUENCE OF RAPESEED OIL ON PRODUCTION PARAMETERS AND FLESH QUALITY

There were no adverse effects of diet on the growth performance, survival percent and whole body proximate composition of the cyprinid fish species in which RO did not affect the growth parameters of these animals over either short or long-term periods (Wiegand, 1993; Pozernick and Wiegand, 1997). The RO contains minor bioactive lipid compounds of which phytosterols might be the ones with most influence on fish metabolism. Although phytosterols are absorbed in small amounts, they may compete with cholesterol for cellular binding sites, resulting in lowered cholesterol levels (Ostlund, 2004). Common carp fingerlings fed diets supplemented with either 10% FO or RO did not show any significant differences in growth and feed conversion efficiency (Steffens et al., 1995).

CONCLUSION

Rapeseed oil can serve as a useful source of lipids for common carp. It is an excellent source of energy and essential fatty acids. The production performance of this vegetable oil fed carps is very often unchanged. The usage of rapeseed oil in common carp nutrition is still limited due to presence of antinutritional factors and disorders in fatty acid flesh composition. Using rapeseed oil as replacement fat sources can further improve productivity and nutritive value of carp. In general, common carp muscle has a favourable fatty acid composition and should be regarded as a healthy product in human nutrition.

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POSSIBLE REPLACEMENT OF FISH MEAL BY SOY CONCENTRATE IN FEED FOR TROUT

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MOGUĆNOST ZAMENE RIBLJEG BRAŠNA SOJINIM KONCENTRATOM U ISHRANI PASTRMKE

Apstrakt

U uslovima intenzivnog gajenja kalifornijske pastrmke (*Oncorhynchus mykiss)*, troškovi ishrane čine 50 do 70% ukupnih troškova u proizvodnji. Tokom višegodišnjih istraživanja, došlo se do zaključaka da proteinska hraniva biljnog porekla u određenoj meri mogu da zamene riblje brašno (FM) i smanje troškove proizvodnje. Najčešće korišćeni biljni proteini u ishrani riba su sojini proizvodi. Međutim, uspešnost zamene ribljeg brašna zavisi od vrste ribe koja se gaji, uzrasta riba kao i tipa korišćenog sojinog proizvoda. Kao jedan od boljih alternativnih izvora proteina navodi se sojin proteinski koncretat (SPC). SPC se dobija ekstrakcijom proteina iz sojinog brašna i sadrži oko 70% proteina. U akvakulturi predstavlja dobru zamenu za riblje brašno, jer sadržaj i svarljivost proteina u SPC su slični kao u FM. Neutralnog je ukusa i standardno je dobrog kvaliteta u odnosu na promenljiv kvalitet FM.

Istraživanje je sprovedeno u „Centru za ribarstvo i primenjenu hidrobiologiju" ODPF Radmilovac. Mlađ kalifornijske pastrmke, prosečne nasadne mase 33,62g, hranjena je smešama koncentrata (44/20 Extra, proizvođača Veterinarski zavod Subotica) sa različitim procentualnim učešćem FM:SPC, i to: 100:0, 75:25, 50:50, 25:75 i 0:100.

Na osnovu dobijenih rezultata, nakon 60 dana hranjenja riba smešama koncentrata sa različitim učešćem FM i SPC, mogu se konstatovati statistički vrlo značajne razlike u masi i dužini tela. Najbolju završnu masu, kao i BWG, SGR, TGC i FER uz najmanji FCR imale su ribe hranjene smešom gde je odnos FM:SPC bio 75:25. Nasuprot tome, ribe hranjene smešom koncentrata bez učešća FM, imale su statistički vrlo značajno niže vrednosti BWG, SGR, TGC i FER, pri čemu je FCR bio statistički vrlo značajno veći nego upotrebom drugih smeša.

Dobijeni rezultati ukazuju da je u ishrani mlađi kalifornijske pastrmke najopravdanije koristiti smešu u kojoj je odnos FM:SPC bio 75:25, kako zbog proizvodnih rezultata tako i zbog smanjenja cene koštanja samog proizvoda.

Ključne reči: Kalifornijska pastrmka, ishrana, riblje brašno, sojin proteinski koncentrat.

Keywords: Rainbow trout, nutrition, fish meal, soy protein concentrate

INTRODUCTION

As the aquaculture is in continuous development, expanding and intensifying (Bostock et al., 2009), it is essential to involve different components in fish feed (Tacon, 2005) and provide their maximal utilization. From the nutritional aspect, the best potential show components of animal origin, but are the most expensive, while components of plant origin have a lower nutritive potential, moderate prices and are more available at the market (Storebakken et al., 2000). In order to create better production results, due to high prices of certain components (Aas et al., 2009), it is essential to know requirements of different fish species. In this sense the nutrition and preparation of fish feed is highly important.

Rainbow trout (*Oncorhynchus mykiss)* as a carnivorous fish species requires high protein level in feed, mostly based on fish meal and owing to this feed accounts from 50 up to 70% of total production expenses (Tekinay at al., 2009). Due to the need to reduce production costs, replacement of fish meal by valuable plant proteins in fish feed has been a subject of many researches (Viola et al., 1982; Watanabe, 2002; Uran et al., 2008). A conclusion has been reached that protein rich feed of plant origin may be replaced by fish meal up to a certain degree (Refstie et al., 2005; Thiessen et al., 2005; Glencross et al., 2008; Marković et al., 2012).

The most frequently used plant proteins in fish feed are soy products. However the success in replacement of fish meal depends on the fish species, fish category and type of soy product used (Watanabe, 2002). Soy protein concentrate (SPC) is produced by the extraction of proteins from soy meal and contains around 70% of proteins, 20% of carbohydrates, 6% of ash and 1% oil. In aquaculture, SPC has many advantages as a alternative protein source in replacing proteins from fish meal (USSEC, 2008).The content of row proteins in SPC is similar to FM. The digestibility of proteins and the energy in soy protein concentrate are the same as in fish meal, and compared to soybean meal even higher (protein digestibility 96–97%). The level of amino acids in SPC is equal or higher than fish meal, with the exception of methionine and lysine. Soy protein concentrate has a considerably lower level of antinutritional factors than soybean meal, and indigestive or harmful carbohydrates (oligosaharides) are removed during SPC production process. It has a neutral taste due to oligosaharides removal and is of good quality standard compared to the variable quality of fish meal.

The main aim of research was to study the possibility of replacing fish meal by soy protein concentrate in diets for rainbow trout fry, i. e. the effects of diets containing different ratio of FM and SPC on production results and feed utilization.

MATERIAL AND METHODS

The research was carried out at the „Centre of Fishery and Applied Hydrobiology" ODPF Radmilovac. In 100 l aquariums equipped with water purification system and water flow of 2 l/min, ten individuals of rainbow trout (*Oncorhynchus mykiss)*, average weight of 33,62 g and14,46 cm in body length were placed. During 60 days fish were fed diets produced by Veterinary Institute JSC Subotica (44/20 Extra), with different share of fish meal and soy protein concentrate (FM:SPC), in ratio: 100:0, 75:25, 50:50, 25:75, 0:100. Fish were fed three times a day by manual distribution, according to the temperature and total ihtiomass in four replicates (aquariums) for each type of feed examined.

During the research, in 15 days intervals, the weight (Radwag THB – 600) and lengths of fish were measured using an ichtihyiometer. Following equations were used for calculations of growth parameters:

Body weight gain **(BWG)** = $W_t - W_o$;

Fulton's factor **(FF)** = $(W / L^3) \times 100$;

Specific growth rate **(SGR)** = $(100 \times (\ln W_t - \ln W_o) \times t^{-1})$;

Thermal-unit growth coefficient **(TGC)** = $(W_t^{(1/3)} - W_o^{(1/3)}) / (T \times t) \times 1000$;

Feed efficiency ratio **(FER)** = $(W_t - W_o) \times D^{-1}$;

Feed conversion ratio **(FCR)** = $D / (W_t - W_o)$,

where W_t is the average fish weight at the end of the experiment (g), W_o - the average fish weight at the beginning of the experiment (g), W - body weight (g), L - body length (cm), t - number of feeding days, T - water temperature (°C), D - the feed amonut (g).

The results were analyzed by one-way analyses of variance model with feed diet as the factor, and the significant difference of pairs of treatments were tested by Tukey HSD test.

RESULTS AND DISCUSSSION

The ANOVA shows that fish between treatments were not significantly different in weight ($F=0.443$; $p=0.776$), length ($F=1.679$; $p=0.207$) and Fulton's factor ($F=2.734$; $p=0.069$) at the beginning of experiment (Table 1). After 60 days of the experiment, fish were significantly different in weight ($F=42.975$; $p<0.001$) and length ($F=30.359$; $p<0.001$).

Table 1. Fish weight and length at beginning and end of experiment

Measurements	Fish diets (FM : SPC)	Weight		Length		Fulton's factor	
		$\overline{X}$	c_v	$\overline{X}$	c_v	$\overline{X}$	c_v
Beginning	100 : 0	33.61	0.57	14.40	0.56	1.12	2.15
	75 : 25	33.69	0.19	14.43	0.33	1.12	0.94
	50 : 50	33.58	0.30	14.58	0.78	1.07	1.46
	25 : 75	33.55	0.63	14.49	1.09	1.10	3.22
	0 : 100	33.60	0.47	14.46	0.68	1.11	2.25
Final	100 : 0	58.52^{ab}	4.65	17.17^{ab}	1.78	1.12	1.82
	75 : 25	64.55^{a}	1.25	17.78^{a}	1.49	1.15	3.36
	50 : 50	56.65^{ac}	5.63	16.96^{ac}	2.17	1.13	1.02
	25 : 75	51.86^{c}	8.55	16.37^{c}	2.55	1.15	4.39
	0 : 100	39.79^{e}	3.80	15.45^{e}	1.29	1.07	6.41

The same letters are denoted to treatments with no difference between

The fish fed by diets with 100% of SPC, had significantly ($p<0.001$) lower average weight and body length compared the fish fed by the remaining diets. By using feed with 75% FM and 25% SPC the highest average weight and length of fish has been achieved. Significantly ($p<0.001$) lower average weight has been gained after 60 days by using feed with 50% FM and 50% SPC, as well as with 25% FM and 75% SPC. By using fish feed with 100% of FM, significantly higher average weight has been gained ($p=0.032$) compared to the average weight of fish fed 25% FM and 75% SPC. Similar result was confirmed by Mambriniet al. (1999). A slightly lower effect on the average length ($p=0.018$) than on fish weight ($p<0.001$) had the diet with 50% FM, and 50% SPC.

The different ratio between FM and SPC provided significant difference between average values for all growth parameters and feed utilization (Zhao et al., 2010).The maximum average values for BWG, SGR, TGC and FER have been achieved in fish fed by 75% FM and 25% SPC, while the minimum with fish fed without FM in diet. Reverse was obtained for FCR (Table 2).

Table 2. Fish growth parameters and utilization of fish diets

Measurement	Fish diets (FM : SPC)	BWG		SGR		TGC		FER		FCR	
		$\bar{x}$	c_v	$\bar{x}$	c_v	$\bar{x}$	c_v	$\bar{x}$	c_v	$\bar{x}$	c_v
Final	100 : 0	24.91^{ab}	11.60	0.89^{ab}	9.17	1.17^{ab}	9.92	0.97^{ab}	5.42	1.03^{a}	5.20
	75 : 25	30.87^{a}	2.67	1.05^{a}	2.01	1.40^{a}	2.22	1.19^{a}	2.56	0.84^{a}	2.51
	50 : 50	23.07^{bc}	13.66	0.84^{bc}	10.73	1.10^{bc}	11.66	0.93^{b}	10.41	1.08^{a}	11.39
	25 : 75	18.31^{c}	24.38	0.70^{c}	19.88	0.90^{c}	21.31	0.74^{b}	27.15	1.46^{a}	**36.72**
	0 : 100	6.18^{e}	25.03	0.27^{e}	23.03	0.33^{e}	23.46	0.28^{c}	25.18	3.75^{e}	26.72
	Rez. ANOVE	F=41.562; $p<0.001$		F=45.390; $p<0.001$		F=44.109; $p<0.001$		F=40.001; $p<0.001$		F=22.222; $p<0.001$	

The same letters are denoted to treatment with no difference between

The average values of the growth parameters in fish fed by diets with 100% of SPC were significantly different from average values of all growth parameters of fish fed by diets with lower FM content. Also fish fed with 75% FM and 25% SPC differed significantly in all growth parameters, except for FCR, from fish fed 25% FM and 75% SPC. Similar research by Drew et al. (2007) showed that by replacing FM up to 29% with canola protein concentrate normal growth was observed in salmon fry.

Average BWG, SGR and TGC were significantly ($0.01<p<0.05$) different in fish fed 100% FM compared the fish fed 25% FM and 75% SPC. Significantly different ($0.01<p<0.05$) were average values of BWG, SGR, TGC and FER in fish fed FM:SPC in ratio 75:25 and 50:50.

CONCLUSION

Obtained results in this study show that there are no significant differences in the final fish weight, growth parameters and feed utilization if FM is replaced by SPC up to 50%. By replacement of FM with over 75% SPC, rainbow trout fry stops feeding after one month thus provide lower values of growth parameters.

By observing the production characteristics, the best growth (30.87 g) and feed utilization rate (1.19) with the lowest conversion coefficient (0.84), has been obtained when fish were fed with diet where the ratio between fish meal and soy protein concentrate was 75:25.

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COMPARISON OF THE NUTRITIVE VALUE OF WHEAT AND TRITICALE IN FISH DIET

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UPOREĐIVANJE NUTRITIVNIH VREDNOSTI PŠENICE I TRITIKALE ZA ISHRANU RIBA

Apstrakt

Žitarice predstavljaju osnovni izvor energije za ishranu ljudi i domaćih životinja zbog visokog sadržaja skroba i drugih ugljenih hidrata. Dominantu ulogu među njima imaju kukuruz, pirinač i pšenica. Demografska eksplozija uslovljava da se za ovu namenu, uključujući ishranu riba, sve više uključuju druge vrste žitarica među kojima je i tritikale.

Žitarice se prema nutritivnoj vrednosti i upotrebljivosti za ishranu domaćih životinja mogu rangirati sledećim redosledom: pšenica - tritikale - kukuruz - ječam – raž.

Tritikale (x *Triticosecale*) je hibrid pšenice (*Triticum* spp.) i raži (*Secale cereale* sa nizom poboljšanih kvantitativnih karakteristika u odnosu na roditelje. Tolerantnost na bolesti i nepovoljne agroekološke uslove omogućuju gajenje u područjima manje pogodnim za gajenje komercijalno značajnijih žitarica. Zrno tritikale prema ukupnom sadržaju proteina (do 20% suve mase) i pojedinih esencijalnih aminokiselina (posebno lizina) prevazilazi druge žitarice. Sem toga, karakteriše ga povećana iskoristljivost fosfora uslovljena višim sadržajem enzima fitaze. Zrno tritikale ima neznatno manju energetsku vrednost u poređenju sa pšenicom. Povećan sadržaj celuloze smanjuje njegovu svarljivost. Ekstrudiranjem i drugim termičkim postupcima moguće je izvršiti želatinizacija sirovog skroba i povećati stepen svarljivosti.

Veći broj istraživanja sprovedenim na šaranu (*Cyprinus carpio*), ukazuju na mogućnost delimične zamene pšenice i kukuruza, kao i ribljeg brašna, tritikaleom u smešama za ishranu šaranskih vrsta.

Ključne reči: akvakultura, pšenica, tritikale, ishrana riba
Keywords: aquaculture, wheat, triticale, fish nutrition

INTRODUCTION

A large part of the human population has improper nutrition, implying inadequate intake of high-quality foodstuffs, primarily those of animal origin. Fish is known for its high quality attributed to high levels of proteins, vitamins (A and D in particular), minerals, polyunsaturated fatty acids and other nutrients (Vladau et al., 2008). Due to the above, intensification of fish production is underway on a global scale. In contrast to open water catches, aquaculture involving fish farming as its major activity shows a continued growth tendency.

Cereals in feeds for fish (notably cyprinids) are used as major sources of energy due to their high content of starch and other carbohydrates. In 2010, maize, rice and wheat accounted for more than 90% of world cereal production (Anonymous, 2013). The three cereal crops provide subsistence and serve as the major source of feed for livestock. Therefore, minor cereals including barley, rye, millet and other crops such as triticale are increasingly used in livestock and fish feed formulations.

WHEAT AND TRITICALE – BASIC CHARACTERISTICS

Wheat (*Triticum* sp.) is the world's most common crop (grown on over 240 x 10^6 ha, with a production of 651 x 10^6 t in 2010). Wheat originates from south-western Asia. There are about 30 wheat species, with common wheat (*T.aestivum* 6n = 42) and durum wheat (*T.durum* 4n = 28) as the predominant ones. The high nutritional value of wheat grain (average content of carbohydrates 71%, proteins 13%, fats 2.5%, high amounts of minerals and vitamins, particularly B-complex vitamins) and relatively high resistance to changing agroenvironmental conditions are reasons for the dominant role of wheat in human diet and its importance in livestock feeds.

Triticale (x *Triticosecale*) is a crop species developed in the second half of the 19th century from a cross between wheat (*Triticum* spp.) and rye (*Secale cereale*). Combining the genomes of the two crops resulted in genotypes incorporating the high yield potential and quality of wheat and the tolerance of rye to unfavourable environmental conditions (primarily low temperatures and pathogens) (Janković et al., 2011). Early triticale cultivars contained high levels of antinutritional factors such as trypsin inhibitors. Breeding work has led to the creation of hybrids (mostly 2n = 42) exhibiting improvement in qualitative and quantitative traits in terms of decreased amounts of harmful substances, an increased protein level (up to 20% dry weight basis) and a favourable amino acid profile (Table 1.). Apart from its intended use as a livestock feed and, to a lesser extent, as a foodstuff in human diet, triticale grain has been increasingly used as a feedstock for biofuel production. The total world triticale production in 2010 was 13.2 x 10^6t (Anonymous, 2013).

Table 1. Average composition of some cereal feed grains (Anonymous, 2011)

	Triticale	Wheat	Barley	Sorghum
Available energy (MJ/kg as fed)	12.9	13.1	12.9	14.6
Crude protein (% as fed)	12.9	10.4	11.3	9.7
Lysine (% as fed)	0.44	0.31	0.38	0.20
Methionine (% as fed)	0.20	0.15	0.16	0.12
Threonine (% as fed)	0.42	0.29	0.37	0.27
Starch (% dry matter)	63	66	58	74
Non-digestible fibres (% dry matter)	15.9	15.9	21.3	12.7

WHEAT AND TRITICALE IN FEEDS FOR CYPRINIDS

According to their nutritive value and usefulness in livestock nutrition, cereal crops can be ranked as follows: wheat – triticale – maize – barley – rye (Przybyl and Mazurkiewicz, 2004). The nutritive value of cereal crops is assessed using a number of parameters, notably available energy, protein content and digestibility.

The energy value of triticale grain is slightly lower compared to wheat. Grain protein content in cereal crops shows high variation, depending on species, cultivar, cultural operations and agroenvironmental conditions. Cereal proteins generally have a low content of essential amino acids compared to some other crops (e.g. soybean). The average levels of proteins and some essential amino acids (lysine, in particular) are higher in triticale grain than in some other cereals (Table 1.). Moreover, triticale grain has high phosphorus use efficiency due to the high phytase content (Janković et al., 2011).

Digestibility of cereal grains is affected by total carbohydrates, primarily starch and fibre. In semi-intensive carp farming, cereals account for 35-45% of feeds on average, with their crude starch and fibre content being 60-70% and 2-5%, respectively. Crude starch digestibility in carp is about 70%, whereas fibre is generally non-digestible. Thermal processing (primarily extrusion) enables starch gelatinisation, increases starch digestibility (Ćirković et al., 2002; Jovanović et al., 2005) and improves protein use efficiency. Compared to other cereal crops, wheat meal shows the highest apparent digestibility in carp diet (Degani et al., 1997). A high fibre content (4% on average) reduces the digestibility of triticale grain (Janković et al., 2011).

Justification for the use of triticale in feeds for cyprinids has been confirmed in many studies. Vacha et al. (2007) evaluated the effect of different cereal crops on flesh yield and quality of common carp over a period of 8 months. Common carp were in their third year of life, and their average initial weight was 1.13 kg. The fish were cultured in four ponds using, respectively, natural food only (control group), maize, wheat and triticale. A decrease in yield and feed conversion ratio was observed in carp fed triticale in their diets compared to carp receiving wheat and maize (with no significant differences observed). The average percentage of n-3 PUFA (polunsaturated fatty acids) in carp muscles was 2.5% ± 0.36 for maize, 3.1% ± 0.39% for triticale and 3.38% ± 0.44 for wheat, suggesting a high nutritive value of triticale.

Fish meal is the primary source of protein in most fish feeds (Stanković et al., 2011). The stagnation of fish catches from open waters and economic reasons demand the use of other sources of protein. The use of triticale as a partial fish meal replacement has been justified. In a 50-day experiment, Mazurkiewicz (2009) analysed the body weight of carp diets containing different amounts of fish meal, legume-rapeseed mixture, triticale meal and rye bran (Table 2.).

Table 2. Experimental diets for market carp (Mazurkiewicz 2009)

	Diet			
Ingredient (%)	I	II	III	IV
Fish meal	12.2	7.7	5.1	2.5
Legume-rapesed mixture	-	17.0	27.0	37.0
Triticale meal	25.2	31.9	36.9	41.3
Rye bran	45.0	24.0	12.0	-
Monocalcium phosphate	-	0.8	1.4	1.6
Standard components in all diets: Yeast (8%), Erythrocyte meal (5%), Rapeseed oil (1%), Soy bean lecithin (0.5%), Premix[1] (1.5%), Vitazol AD_3EC^2 (%), Chalk (1.3%), Choline chloride (0.2%)				

[1] Polfamix W, BASF Ltd, Kutno Poland; BIOWET Drwalew, Poland

Table 3. Growth of carp body weight during rearing (Mazurkiewicz 2009)

Days of test	Weight (g)			
	I	II	III	IV
Start	970 ± 1.1	969.3 ± 5.8	972.3 ± 7.2	965.7 ± 9.3
10	1125.3 ± 4.0	1124.7 ± 15.9	1146.0 ± 18.7	1115.0 ± 26.2
30	1307.0 ± 19.2	1350.7 ± 32.1	1386.3 ± 29.4	1364.0 ± 27.1
50	1522.0 ± 29.6	1568.3 ± 42.6	1650.7 ± 40.3	1592.7 ± 50.1

Table 3. shows data on growth of carp body weight. The tested diets for market carp did not have a significant impact on the quantitative and sensory characteristics of the individual carp analysed. The results suggest the possibilities of substituting fish meal protein with plant protein.

CONCLUSIONS

Cereals serve as a major source of energy in both human and livestock nutrition due to their high content of starch and other carbohydrates. According to their nutritive value and usefulness in livestock nutrition, these plants can be ranked as follows: wheat – triticale – maize – barley – rye.

Triticale is a hybrid of wheat and rye bred for improved quantitative characteristics of its parents. The total content of proteins and some essential amino acids is higher than that of other cereal crops. Its tolerance to diseases and unfavourable agroenvironmental conditions ensures adaptability to regions less favourable for the cultivation of commercially important cereals. Research on carp suggests the potential to use triticale as a partial substitute for wheat, maize and fish meal in feeds for cyprinids.

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APPLICATION OF A NEW CROP-QUINOA FOR FISH FEED

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PRIMENA NOVE KULTURE - KVINOJE U ISHRANI RIBA

Apstrakt

Kvinoja (*Chenopodium quinoa*, Willd.) je pseudocerealija koja se tradicionalno gaji na malim plantažama u ruralnim oblastima Južne Amerike u regionu Anda. Kvinoja je ratarska kultura, gajena za domaću upotrebu siromašnog stanovništva. Zbog sposobnosti prilagođavanja različitim agro-ekološkim uslovima, kvinoja može da se gaji u različitim regionima. Agrotehnika zasnovana na principima organske poljoprivrede i primenjena je u Danskoj, Italiji i Makedoniji. Zahvaljujući visokoj nutritivnoj vrednosti seme se koristi u ljudskoj ishrani, usled čega je poslednjih godina porastao interes za gajenje i preradu kvinoje, kao funkcionalne hrane. Poznato je da i lišće biljke poseduje značajne nutritivne vrednosti, pa se koristi kao zamena za spanać. Biljka može da poraste 1-3m visine, a plodovi su u obliku okruglog, malog semena, koje je obavijeno perigoniumom različite boje (bledo žute, do svetlo crvene). Perigonium se mehanički lako odvaja, kada je seme suvo. U perikarpu semena nalaze se saponini, nosioci karakterističnog gorkog ukusa semena kvinoje, zbog čega je potrebno iste odstraniti, pre upotrebe u ljudskoj ishrani.

U ovom radu predmet istraživanja je bila danska sorta KVL 37, gajena u okolini Beograda. Ispitivan je nutritivi sastav sirovog i oljušćenog semena kvinoje, kao novog useva i mogućnost njegove primene u ishrani riba. Poznato je da viskoka nutritivna vrednost semena kvinoje potiče od sadržaja proteina, različitih minerala i vitamina, i to E vitamin i vitamini B grupe. Prosečan sadržaj proteina varira od 8%-22%, a glavne proteinske frakcije čine albumini i globulini (44-47% ukupnih proteina). U ovom radu sadržaj proteina je varirao od 15,5% do 16,8%, u zavisnosti stepena čistoće semena. Seme poseduje odličano izbalansiran sastav amino kiselina, a izdvajaju se lizin, treonin i metionin, amino kiseline koje su uglavnom deficitarne u biljnim sirovinama. Glavnu komponentu semena kvinoje čine ugljeni hidrati, čiji sadržaj varira od 67% do 74%. Skrob čini oko 52-63%, dok su ostali ugljeni hidrati, kao i sirova vlakna malo zastupljeni. Kvinoja sadrži 2% do 10% lipida, a dokazano je i prisustvo esencijalnih masnih kiselina, kao sto su linolenska, oleinska i palmitinska. Značajan je sadržaj minerala tj. kalcijuma, gvožđa i cinka, ali se njihov sadržaj kvantitativno smanjuje u daljim postupcima ljuštenja, pranja i poliranja semena. U humanoj ishrani saponini i fitinska kiselina predstavljaju glavne nedostatke kvinoje. Ljuštenjem i daljim prečišćavanjem seme kvinoje je našlo primenu u ishrani ljudi kao varivo, hrana za doručak, za kolače za proizvodnju brašna, kao i za ishranu životinja u formi mekinja ili pogača. U našem radu, kod oljuštenog zrna, dokazano je značajno povećanje sadržaja ulja, dok je sadržaj sirovih vlakana i ukupnog pepela smanjen za oko tri-puta. Sadržaj skroba je u očekivanim graničnim vrednostima. U pogledu sadržaja minerala nije bilo većih promena. Imajući u vidu veličinu zrna, laku pripremu, nutrtitivni potencijal, kao i novu kulturu u našoj regiji, pokazano je da seme kvinoje može da nađe primenu i kao hrana za ribe.

Ključne reči: kvinoja seme, kvalitet proteina, osobine ishrane
Keywords: quinoa seeds, protein quality, nutrition properties

INTRODUCTION

Quinoa (*Chenopodium quinoa* Willd.) is a seed crop traditionally cultivated in the Andean region for several thousand years. Quinoa is considered as a multipurpose agricultural crop. Genetic variability of quinoa is huge, with cultivars being adapted to growth from cold, highland climates to subtropical conditions, which makes it possible to select, adapt and breed cultivars for a wide range of environmental conditions (Bertero et al., 2004). In addition, the quinoa plants show tolerance to frost, salinity and drought, and have the ability to grow on marginal soils. Quinoa was successfully trialed in typical agro-climatic conditions of South Eastern Europe region. Quinoa has been tested in diverse climatic regions of USA, Canada, India, England, Denmark, Greece, Italy and other European countries (Bhargava et al., 2007; Jacobsen et al., 2003; Pulvento et al., 2010). The planting season varies from August in the Andean highlands, extending through December and in some areas from January to March. Quinoa is harvested at physiological maturity, which may be reached within 70 to 90 days after flowering. Depending on the variety, plants take between 5 and 8 months to mature. Quinoa plant grows 1-3 m high. The fruit of quinoa is in the form of the seeds which are small, round and flattened, measure about 1.5 mm in diameter and about 350 seeds weigh 1 g and can germinate very fast. They are covered by perigonium, which is of the same color as the plant: white, yellow, gray, light brown, pink, black or red. It is easily removed when it is dried (Demin et al., 2012).

The quinoa seeds, also known as Inca-rice, were traditionally used as a basic component in the diet. The seeds may be utilized for human food, in flour products and in animal feedstock because of its high nutritive value (Repo-Carrasco et al., 2003). Saponins and phytic acid are the main disadvantageous factors in quinoa. Quinoa contains saponins in the amount from 0.1 to 5%. The pericarp of the quinoa seed contains saponins which may impart a bitter taste. Their separation is easily accomplished by rinsing the seed in cold alkaline water or by mechanical abrasion (Jancurova et al., 2009).

The aim of this work was to examine the nutritional potential of the quinoa seeds, as a new crop in the fish feed application. Protein quality, starch properties and other nutrients of the quinoa seeds have been studied.

MATERIALS AND METHODS

Materials. Quinoa (*Chenopodium quinoa* Will.) seeds were produced in the vicinity of Belgrade, in a field of the agroindustrial complex "Stara Pazova", harvest season was 2010. Quinoa (*Chenopodium quinoa* Willd.) variety used for investigation was Puno (KVL 37), provided by the University of Copenhagen. The variety has recently been registered as a new quinoa variety in Europe, bred from Chilean and Peruvian landraces and selected for earliness and adaptation to European conditions. The raw and flaky quinoa seeds were manually dehulled to remove the pericarp. Manual dehulling was done by using abrasive action in the pestle and mortar, and the hulls were separated carefully by sieving to avoid inclusion of other seed portions (Chauhan et al. 1992).

Chemical analyses. The seeds were milled in Cemotek Sample Mill Foss, Sweden and the flour was further examined. Standard AOAC methods (AOAC, 1997) numbers 925.10, 923.03 and 920.87 were used to determine moisture, ashes and protein (Kjeltec 2300 system) contents, respectively. The nitrogen to proteins conversion factor of 6.25 was used for the calculation of crude protein content. Automatic extraction method AOAC number 920.39 (FOSS-TEKATOR SOXTEC AVANTI) was used for oil content. Fibertek 2010 System was used to describe crude fiber content, using 962.09 AOAC describe method. According to Grosso et al., 2000 the total starch content was calculated.

RESULTS AND DISCUSSION

The quinoa seeds produced in the field experiment were evaluated for nutritive value in order to investigate her potential as a new fish feed. The main chemical and nutritive parameters of row and dehulled quinoa seeds that were estimated are presented in Table 1.

Table 1. Chemical and mineral composition of the Quinoa seeds (Grams per 100g of the fresh weight samples)

Composition (%)	Row Quinoa seeds	Dehulled Quinoa seeds
Moisture	10.70	10.56
Protein	**16.68**	**15.61**
Oil	2.42	4.06
Crude fiber	9.89	2.98
Ash	7.67	2.99
Starch	52.64	63.80
P (g/kg)	0.38	0.22
Ca (g/kg)	0.44	0.35
NaCl	0.46	0.37
Na	0.18	0.14

High nutritional value of quinoa seeds is mainly due to the high protein content and additionally the wide range of minerals and vitamins. The protein content of quinoa seeds varies from 8% to 22% and is higher in comparison with wheat, rice or other cereals, i.e. 8-14%. Here protein varied from15,5% to 16,8% depending of the the degree of purification. Quinoa also has a good balance of the amino acids. The seed proteins are rich in amino acids like lysine, threonine and methionine (Fleming and Galwey, 1995, Stikic et al., 2012).

The main component in quinoa seeds consists of carbohydrates and varies from 67% to 74% of the dry matter. Starch is the main component in both seeds makes about 52-63%. The other carbohydrates were found in small amounts (Jancurova et al., 2009). Purification method which was undertaken significantly reduced the crude fiber and ash content for about tri-fold times. On the contrary, the oil content increased for 60%. Quinoa contains from 2% to 10% oil. The overall fatty acid composition of the whole quinoa seeds was similar to the other cereal grains with essential linoleic, oleic and palmitic acids as the major acids present. Quinoa seeds contain calcium, magnesium, iron and zinc, as the common cereals. Finally, reducing the mineral content is expected, as we previously reported (Stikic et al., 2012).

CONCLUSION

Quinoa (*Chenopodium quinoa* Will.) seeds were obtained from the fields in the vicinity of Belgrade. Chemical composition revealed the potential of the row and dehulled seeds as a valuable source of protein, crude fiber and calcium. The results showed the possibility of development of a nutritionally valuable crop, as a rich source of high quality protein and oil in the application for fish feed.

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POTENTIAL OF SECONDARY RAW MATERIAL – SOYBEAN OKARA FOR USE AS FISH FEED

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MOGUĆNOST PRIMENE OKARE KAO SEKUNDARNE SIROVINE U PROIZVODNJI HRANIVA ZA RIBE

Apstrakt

Poslednjih godina istraživanju soje poklanja se velika pažnja zbog povoljnih zdravstvenih efekata. Mnoge studije su utvrdile u zrnu soje prisustvo komponenti poput proteina, dijetalnih vlakana, masnih kiselina, izoflavona i drugih fitohemikalija, koje povoljno deluju na organizam. Sirova okara, poznata i kao "sojina pulpa", je nusprodukt u priozvodnji sojinog mleka. To je belo-žućkasta materija koja se sastoji od nerastvorljivih delova semena soje (uglavnom semenjače) koji ostaju na filteru pri filtriranju usitnjenog kuvanog zrna soje pri proizvodnji sojinog mleka (Jimenez-Escrig *et al*., 2008).

Cilj ovog rada je bio da se proceni uticaj metoda polupogonskog postrojenja koje koristi hidrotermičku obradu sojinog zrna u proizvodnji sojinog mleka (HTC obrada; visoka temperatura i povišen pritisak/kratko vreme) na sadržaj i aktivnost hranljivih komponenti okare, pripremane od šest različitih genotipova soje, kao i da se proceni mogućnost korišćenja okare u pripremi hrane za ribe. Može se pretpostaviti da će primenjeni HTC postupak, koji je sličan procesu ekstrudiranja, koji se najčešće koriste u proizvodnji hrane za ribe (visoka temperatura/kratako vreme), dati okaru povoljnih svojstava, pogodna za ishranu riba. Pored toga, obzirom da je optimalna pH vrednost vode za uzgoj riba od 7.0 do 8.0 (Zhanga *et al*., 2011), različita od izoelektrične tačke (Ip) glavnih proteina okare može se pretpostaviti da se proteini okare neće taložiti u mulju, već će ostati da plutaju u vodi.

Glavne komponente okare dobijene primenjenim HTC postupkom su ugljeni hidrati (51.25-59.25%) i proteini (31.81-40.36%). Mateos-Aparicio *et al*. (2010b) ističu veoma povoljnu antioksidativnu aktivnost okare, koju uglavnom pripisuju polisharidima semenjače sojinog zrna, pre svega pektinima, iako ističu da se ne može isključiti ni doprinos belančevina. Ovakva istraživanja ukazuju na mogućnost delovanja okare kao komponete u ishrani riba u smislu odbrane od antioksidativnog stresa. Visok sadržaj proteina, čini okaru potencijalno dobrim izvorom biljnih proteina, niske cene, za ishranu ljudi i životinja. Visok sadržaj i vrlo dobre funkcionalne karakteristike proteina okare (Mateos-Aparicio *et al*., 2010a), ukazuju na to da mogu biti pogodni kao dopuna hrane za ribu u smislu vezivnog medijuma za druge aktivne i hranljive komponente u proizvodu, pri čemu doprinose nutritivnoj vrednosti hraniva. Štaviše, odnos esencijalnih amino kiselina u ukupnom aminokiselinskom sastavu proteina okare je sličan kao u sojinom mleku i tofuu (Vang i Cavins, 1989). Glavni proteini okare su bazni, 7S globulin (Bg7S; 24.61-28.37%) i glicinin (11S globulin; 28.49-33.11%). Poznato je da je Bg7S glikoprotein bogat cisteinom (Omi *et al*., 1996) što povećava nutritivnu vrednost proizvoda. Proteini soje nisu nutritivno idealni proteini, obzirom da ispoljavaju neželjeni efekat na metabolizam nakon konzumiranja sirove sojine sačme, što se pripisuje prisustvu tripsin inhibitora (TI) i lektina. Tripsin inhibitorska aktivnost ispitivanih uzoraka je veoma mala (4.82-7.99%) što ukazuje da okara ne bi ispoljavala antinutritivni efekat na organizam, tim pre što se veruje da postizanje zadovoljavajućeg nivoa TIA dovoljno smanjuje aktivnost lektina, obzirom da su inhibitori termički stabilniji nego lektini (Friedman i Brendon, 2001).

Ključne reči: okara, hrana za ribe, HTC obrada, nutritivna vrednost
Keywords: okara, fish feed, HTC processing, nutritional values

INTRODUCTION

In recent years soybean has gained much attention because of its health-promoting properties. Many studies have reported on the beneficial components of soybean such as protein, dietary fiber, fatty acids, isoflavones and other phytochemicals. Soy foods and feeds are becoming more popular as low cost substitutes of traditional products and an ideal nutritional supplement for protein rich products. Raw okara, also called "soy pulp" is a byproduct of the soymilk industry. It is a white-yellowish material consisting of the insoluble parts of the soybean seeds which remains in the filter sack when pureed soybeans are filtered for the production of soymilk (Jiménez-Escrig *et al*., 2008). The main components of okara are protein 25.40-40.36% (O'Toole, 1999; Stanojevic *et al*., 2012) and dietary fiber, 14.5-55.4 g/100 g on dry basis (O'Toole, 1999). High protein content of about 30% makes okara a potential source of low cost plant protein for production of food and feed. Moreover, the ratio of essential amino acid to total amino acids in okara is similar as in soymilk and tofu (Wang and Cavins, 1989). The basic 7S globulin (Bg7S) and 11S protein are two main proteins in okara (Stanojevic *et al*., 2012). It is known that Bg7S is a cysteine-rich glycoprotein (Omi et al., 1996) which increases the nutritional value of the product.

Soy protein is not an ideal protein because adverse nutritional effects following consumption of raw soybean meal have been attributed to the presence of trypsin inhibitors (TI) and lectins and to their poor digestibility. Since the TI are cysteine-rich proteins (Wolf, 1977) heat treatment should aim to preserve their content and to reduce their activity to make them nutritionally valuable. Therefore, we assessed the influence of a pilot plant method that uses high-pressure hydrothermal processing for soymilk production (HTC processing, high temperature and high pressure/short time) on content and activity of nutritional components in okara prepared from six different soybean genotypes and also, the possibility of using okara as a fish feed.

MATERIALS AND METHODS

Materials. For okara preparation six commercial soybean genotypes grown in field conditions were used: ZPS-015, Krajina, Novosađanka, Balkan, Nena and Lana. The genotype Lana lacked the Kunitz type of trypsin inhibitor. **Okara processing.** Okara was made on the pilot plant scale using the production method which includes hydrothermal cooking (HTC; Wang *et al.,* 2003) for soymilk preparation, modified by Stanojevic *et al.,* (2011). **Applied analysis.** Dissociating electrophoresis (SDS-PAGE) for all samples were performed according to the Fling and Gregerson procedure (1986), detailed by Stanojevic *et al*. (2011, 2012). Trypsin inhibitor activity (TIA) of okara was estimated according to the method of Liu and Markakis (1989), and was expressed as residual activity (TIA in percents) relative to defatted soybean flour or in trypsin units inhibited (TUI) per milligram of the dry sample. Total nitrogen content in samples was determined by the micro-Kjeldahl method (AAOC, 2000) and total protein content was calculated (Nx6.25). Total fat content in samples was determined by extraction with diethyl ether in a Soxhlet system (AOAC, 2006). Ash content was calculated by AOAC method (1984). Moisture and volatiles were determined by standard AACC procedure (2000). Carbohydrate content was calculated by subtracting moisture, protein, fat, ash and cellulose values from 100%.

RESULTS AND DISCUSSION

All investigated samples of okara were characterized by high content of total protein (31.81-40.36%; Table 1) what indicated significant nutritional value of these products. The high protein content in okara and very good functional characteristics of okara proteins (Mateos-Aparicio *et al*., 2010a) indicate that okara may be suitable for fish feed supplement in terms of connective medium for active and nutrient components incorporation in the product. In addition to protein, okara contained mainly carbohydrates (51.25-59.25%), Table 1. Mateos-Aparicio *et al*. (2010b) reported that, potential antioxidant activity of okara cell-wall polysaccharides could be attributed to pectins, although the contribution of residual proteins cannot be ruled out. Such research suggests the possibility of okara activity as a component in the fish feed in terms of oxidative stress defense.

Table 1. Proximate composition of okara flour[1] (%)

Genotype	Moisture	Solids	Protein	Ash	Fat	Carbohydrate[2]
Nena	6.50[c]	93.50	35.07[d]	0.86[c]	0.25[d]	57.32
Kraijna	6.71[a]	93.29	35.27[c]	1.02[b]	0.50[c]	56.50
Novosadjanka	6.62[b]	93.38	40.36[a]	0.90[bc]	0.52[c]	51.60
Balkan	6.53[c]	93.47	35.41[c]	5.69[a]	1.03[b]	51.25
ZPS - 015	6.54[c]	93.46	37.32[b]	1.13[b]	0.91[b]	54.10
Lana	6.63[b]	93.37	31.81[e]	1.06[b]	1.25[a]	59.25

[1] Means in the same column with different superscript roman letters are significantly different ($p<0.05$), all analyzes were carried out applied to the defatted okara flour; [2] Carbohydrate by difference

One of the most advanced technological operation in fish feed production is the extrusion process. It increases digestibility of diet components thus enhancing nutritional value of fish feed (Sørensen *et al.*, 2005). Based on this, it can be assumed that the high temperature and high pressure in short time that were applied in our HTC procedure gave okara with favorable properties for use in fish feed. In addition, the optimal pH value of water for fish cultivation is from 7.0 to 8.0 (Zhanga *et al.*, 2011), which is different from the isoelectric point (Ip) of okara major proteins, Bg7S (24.61-28.37%) and 11S globulins (28.49-33.11%), Stanojevic *et al.*, (2012), Figure 1, B. It can be assumed that okara proteins wouldn’t settle down in the mud, but would tend to float in the water. Moreover, our previous results (Stanojevic *et al.*, 2012) showed that okara extractable soluble protein (ESP) content was 27.83−32.53%, which led to very high okara protein extractability (79.29−90.45%). High values for ESP content and protein extractability may be prerequisites for good nutritional and functional properties of okara protein.

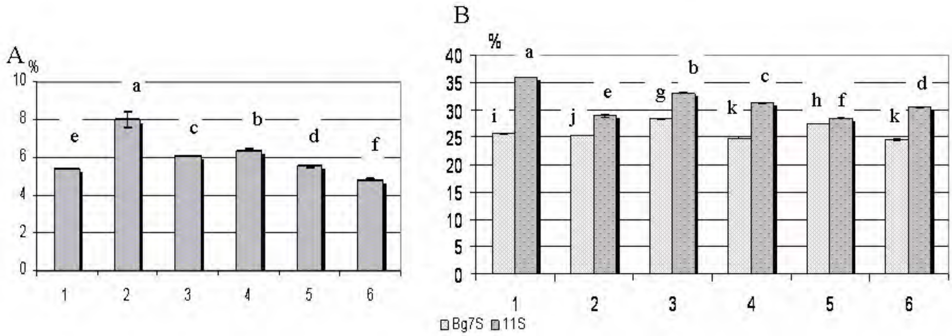

Figure 1. A. Trypsin inhibior activity of okara from investigated genotypes; **B.** Content of the major storage proteins in okara prepared from investigated genotypes; Bars with different letters are significantly different (p<0.05); 1 - Nena, 2 - Krajina, 3 - Novosadjanka, 4 - Balkan, 5 - ZPS-15, 6 - Lana.

Our results for trypsin inhibitor activity (TIA; Figure 1, A) in okara that were much lower (4.82-7.99% or 4.61-14.93 TUI/mg) than some literature data might be the consequence of the applied HTC processing. For example, Vishwanathan *et al.* (2011) reported significantly higher levels of TIA in okara produced using membrane technology (33.75 TUI/mg). Such low TIA in okara suggested that okara obtained by hydrothermal cooking could be applied for human consumption and for the fish feed, too, since it featured less than 20% of residual TIA. Friedman and Brandon (2001) reported that the TIA below 20% isn’t antinutritional. Since the inhibitors are more heat-stable than the lectins, it is believed that by achieving a satisfactory level of TIA sufficiently decreases the activity of lectins (Friedman and Brandon, 2001).

CONCLUSIONS

The investigated soybean genotypes all produced by HTC process okara with high contents of protein and carbohydrates, as well as with very balanced contents and activity of bioactive components, such as trypsin inhibitors and lectins. Less than 20% of residual TIA indicated that okara was heated adequately to inactivate antinutritional factors. Higher content of sulfur-containing amino acids and lysine in okara protein than in β-conglycinin or glycinin favors its use for supplementation of different food and feed products because plant proteins are deficient in these amino acids. Moreover, the high quantity of plant protein in okara (>35%) and very high protein extractability (>80%) prove this byproduct to be very interesting for potential application in food or feed fortification as a functional ingredient. In addition, it favors okara use as ingredient for extruded aquafeed production.

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CURRENT STATUS AND PROSPECTS IN BURBOT, *LOTA LOTA* L., AQUACULTURE

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TRENUTNO STANJE I PERSPEKTIVE RAZVOJA AKVAKULTURE MANIĆA *LOTA LOTA* L.

Apstrakt

Manić, *Lota lota* L., je jedini slatkovodni predstavnik familije Gadide koji živi u Holarktiku. Ova vrsta je nedavno postala važan kandidat u procesu diverzifikacije slatkovodne akvakulture zbog potrebe za niskom temperaturom vode, brzog rasta i velike komercijalne vrednosti.

Effikasnost produkcije riba u akvakulturi u RAS-u je zavisna od mnogo parametara i svaki korak u proizvodnji se karakteriše određenim teškoćama. Najvažnije je obezbediti optimalne uslove za gajenje (npr. fototermalni režim, količina i kvalitet hrane, kvalitet vode) koje mogu do dovedu do najvećeg prirasta. Međutim, još uvek se ne zna dovoljno o idelanim uslovima za gajenje manića. Shodno tome, cilj ovog rada je da razmotri trenutni status akvakulture manića i da se ukaže na moguće načine razvoja intenzivnog sistema gajenja ove vrste.

Poznato je da je najbolja temperatura za gajenje larvi manića 12 ^{o}C. Na ovoj temperature tri dana nakon izvaljivanja kod larvi (DPH – day post hatch) počinje punjenje mehura, a 5 dana nakon izvaljivanja, larve kod kojih ne dođe do punjenja mehura ne moga da preplivaju rastojanje od 10 cm do površine vode.

Kao i većina larvi riba i larve manića moraju da se hrane živom hranom na početku svoje egzogene ishrane. Utvrđeno je da nema potrebe larvama manića davati zooplankton jer se uspešno mogu hraniti sa naupliusima artemije od samog početka. Na 12 ^{o}C naupliuse treba davati od 9 do 10 dana nakon izvaljivanja.

Prelazak larvi sa žive hrane na veštačku hranu je jedna od najproblematičnijih, ali i najvažnijih faza u gajenju riba i direktno je vezana za povećan mortalitet jedinki. Jedna od glavnih teškoća u gajenju larvi manića je njihov prelazak na kompletna hraniva. Istraživanja pokazuju da je potrebno gajiti larve manića do oko 50 dana na temperaturi od 17 ^{o}C dok ne dostignu preko 25 mm dužine i 0.20g težine, što se smatra za "početnom tačkom" za prelazak na kompletna hraniva.

U gajenju juvenila i adulta manića nema većih problema s obzirom da imaju brz prirast, dobro iskorišćenje hrane i 100% preživljavanje kod riba koje se hrane kompletnim hranivima. Podaci jasno pokazuju da je najefikacije hraniti juvenile manića na 17 °C sa učešćem hrane od oko 2% u odnosu na ihtiomase dnevno. Uticaj osvetljenja na juvenile nije utvrđen. Takođe je utvrđeno da je najveća efikasnost u unosu hrane za adulte manića temperatura od 11 °C.

S obzirom da je manić postao jedan od značajnijih kandidata u proizvodnju riba u akvakulturi, postoji još uvek potreba za unapređivanjem postojećih metoda gajenja kao i razvijanje novih. Najvažnije je da se nastavi sa istraživanjem biologije i ponašanja manića koja mogu pomoći u osmišljavanju novih eksperimenata i rešavanju postojećih problema. Osim toga, razvoj tehnologije komercijalne proizvodnje riba zahteva utvrđivanje brojnih faktora, kao što su sastav hrane, temperatura, optimalno osvetljenje, koji imaju uticaja na efikasnost gajenja u intenzivnim uslovima, a još uvek su nedovoljno objašnjeni i razumljivi kod ove vrste.

Ključne reči: Lota lota, gajenje larvi, zalučivanje, gajenje riba
Keywords: Lota lota, larviculture, weaning, fish farming

INTRODUCTION

In recent years, increased attempts in the diversification of freshwater aquaculture have been observed. This has led to thorough research on the possibilities of intensive production (in recirculating aquaculture systems [RAS]) of few important candidates among the commercially valuable fish species. It regards, among others, perch, *Perca fluviatilis* L., pikeperch, *Sander lucioperca* (L.) and burbot, *Lota lota* L. (Teletchea *et al.* 2008, Fontaine *et al.* 2009). In the case of the latter, the lowest progress has been observed to date.

Burbot, *Lota lota* L., is the only freshwater representative of the Gadidae family, found almost in the entire Holarctic (e.g. Teletchea*et al.* 2006). Recently, burbot became an important candidate for diversification of freshwater aquaculture due to low temperature requirements, fast growth rate and high commercial value Edsall*et al.* 1993; Trabelsi*et al.* 2011; Wocher*et al.* 2011).

The effectiveness of aquaculture production of finfish in RAS is dependent on many variables and different obstacles characterize each of the production steps. Shortly after hatching, the most important thing is to provide suitable conditions for swim bladder inflation and the right amount and kind of food securing high growth and survival rates (Dąbrowski 1984; Czesny *et al.* 2005). Usually, the next step is to wean the larvae onto commercial (dry) diet in the most efficient way (Jensen *et al.* 2011; Palińska-Żarska *et al.* 2013). Further on, it is necessary to secure optimal culturing conditions (e.g., photothermal regime, amount and quality of feed, water condition) that will provide the highest growth (Trabelsi *et al.* 2011). However, there is not enough data on the most suitable culturing conditions for burbot production. On the other hand, the published data are sometimes ambiguous or incomplete. Therefore, the aim of this paper is to review the current status of burbot aquaculture and to indicate the possible ways of development considering intensive production of this species.

INITIAL REARING OF LARVAE

The best temperature for burbot larvae rearing is 12 °C (Wolnicki *et al*. 2002). It is very important, from the burbot production point of view, that the larvae do not have major problems with inflation of their swim bladders, like some larvae of Perciformes (Rieger *et al*. 1998; Czesny *et al*. 2011). It was observed that (at 12 °C) larvae begin to inflate their swim bladder on the 3 day post hatch (DPH), while on 5 DPH none of the larvae without an inflated bladder are able to swim up the distance (10 cm) to the water surface (Palińska-Żarska *et al*. 2012).

Another important matter during the initial rearing of fish larvae is the question what kind of food should be given and when is the best moment to feed the larvae? Like most of the fish larvae, burbot larvae also need to be fed with live food from the beginning of the exogenous feeding. Since burbot larvae are one of the smallest among freshwater species (at the moment of hatching they have around 4 mm), trials to feed them with zooplankton organisms were performed (Harzevili *et al*. 2003; Lahnsteiner *et al.* 2012). However, it was found that there is no need to feed them with zooplankton since they could be successfully fed with *Artemia* nauplii from the very beginning (at 12 °C the nauplii should be given from 9-10 DPH) (Żarski *et al.* 2009; Palińska-Żarska *et al.*2012).

WEANING

The transition of larvae from live food to artificial diet is one of the most problematic, yet most important stages in fish culture and is strictly associated with increased mortality (Jensen *et al.* 2011; Wocher *et al.* 2011). However, from an economic point of view, it is very important to wean larvae at the earliest possible stage of culturing since the cost of feeding with live food is very high (Baskerville-Bridges & Kling 2000). However, considering larviculture of burbot, the biggest bottleneck is weaning the fish onto compound diet. The obtained results indicate that it is necessary to ensure intensive rearing of burbot larvae for about 50 days (at 17 °C) until they reach over 25 mm and over 0.20 g, what may be considered as a “starting point” for the weaning procedure. The described results clearly indicate that the appropriate length and weight of larvae, not the age, are the parameters that are considered the for effective transition to artificial feed (Palińska-Żarska *et al.* 2013).

JUVENILES REARING

There are not a lot of papers dealing with rearing burbot juveniles, since there are no big problems at this stage. High growth rate, good feed utilization and 100% survival rate of fish fed with dry compound diet are recorded. The latest findings concern the optimal food ratio for juvenile burbot or the light intensity and temperatures during rearing of juveniles (Wolnicki*et al.* 2001; Trejchel*et al.* 2012).

The obtained data clearly demonstrate that the most effective feeding level for burbot juveniles reared at 17 °C is about 2% of fish biomass per day (Trejchel *et al*. 2012). Finally, the data demonstrate that there is no effect of light conditions (1800 lx or 4 lx) on the rearing of burbot juveniles (Trejchel *et al*. 2012). These data supplement already published data, where light conditions for burbot juveniles remained unclear (e.g. Jensen et al. 2011; Trabelsi et al. 2011). However, for culturing purposes of juveniles burbot, constant light conditions may be recommended.

LIVING IN RAS

There are almost no data about adult burbot growth in RAS. One of the main reasons is that there are not many difficulties related to this, therefore not many experiments are conducted on adult burbots. There are some data dealing with the optimal temperature, both for feeding burbot (Pääkkönen&Marjomäki 2000) and for its reproduction (Żarski *et al*. 2010). It was confirmed that the highest efficacy of food intake for the adult burbot is around 11 °C (Pääkkönen&Marjomäki 2000). It was described that the synchronization of the spawning could be reached only under controlled thermal regimes, when spawners are kept at 6°C before spawning while after spawning the temperature is decreased to 1°C. This restrictively controlled thermal regime during reproduction of burbot in RAS provided the most synchronous spawning of females (Żarski *et al*. 2010).

FUTURE PROSPECTS IN BURBOT AQUACULTURE

Since burbot become a very promising candidates for aquaculture production there is still a need to improve the existing methods of rearing, as well as to develop new ones. The most important is to carry on with the observation of burbot biology and behavior, which helps to design new experiments and solve existing problems. Besides, the development of comprehensive commercial fish production technology requires determining numerous factors that may have an impact on the efficacy of rearing burbot in intensive culture conditions. Feed composition (exclusively for burbot) and the optimal light intensity still seem to be poorly explained and understood in this species. Furthermore, the main bottleneck in burbot aquaculture is still weaning. That is why the biggest challenge is the economic optimization of costs connected with such long feeding of larvae with live food. Data attained till this day are a very good basis for further research on the optimization of all factors (e.g., temperature, type of feed, other methods of feed change such as co-feeding) affecting burbot production effectiveness.

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THE EFFECTS OF DIFFERENT STRIPPING TERMS ON SPERM QUALITY PARAMETERS OF RAINBOW TROUT *(ONCORHYNCHUS MYKISS)*

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UTICAJ PERIODA ISTISKIVANJA MLEČA NA PARAMETRE KVALITETA SPERME KALIFORNIJSKE PASTRMKE (*ONCORHYNCHUS MYKISS*)

Apstrakt
U istraživanju su praćeni parametri kvaliteta sperme mužjaka kalifornijske pastrmke (Oncorhynchus mykiss) kojima je istiskivan mleč u januaru, februaru i martu. Rezultati nisu pokazali značajnu razliku u vrednostima koncentracije i gustine spermatozoida, pH, zapremine i spermatokrita mleča, kao i trajanje pokretljivosti spermatozoida ispitivanih muških matica ($P>0.05$). Statistički značajna razlika utvrđena je za vrednosti pokretljivosti spermatozoida u različitim periodima ($P<0.05$). Utvrđeno je opadanje svih parametara kvaliteta od januara do marta.

Ključne reči: parametri kvaliteta sperme, kalifornijska pastrmka, Turska
Keywords: Sperm quality parameters, rainbow trout, Turkey

INTRODUCTION

The use of high quality gametes is of great importance for ensuring the production of viable larvae, but gamete quality is difficult to assess in a quantitative and meaningful manner (Kjorsvik et al., 1990). It is very important to control and examine performances of broodstocks in hatchery stations of fish farms for their quality and productivity of gametes will enter into aquaculture systems. The determining quality of sperm is necessary to define its quality. Motility, duration of motility and density of active spermatozoa, pH, spermatocrit and seminal plasma contents of sperm has been used to determine quality of sperm (Billard and Cosson, 1986; Linhart et al., 1991). It determined that reproduction performance has been affected on level of stocking, quality, amount and rate of feeding, quality of physicochemical parameters of water, method of spawning and stripping, age and size of salmonid broodstocks (Büyükhatipoğlu and Holtz, 1984).

The objectives of this research were (1) to assess of effects by different term stripping on sperm quality parameters, (2) to determine reproduction performance of male broodstocks in a trout farm.

MATERIALS AND METHODS

Sperm samples were obtained during 2010 spawning seasons from broodstock supplied at Fishery Department in Erzincan University, Kemaliye Hacı Ali Akın Vocational High School in Turkey. The mean weight and total length of the fish was 3156.64±434.32 kg and 61.00±2.45 cm, respectively. For this research, 15 mature males took out and stocked into 500-L fiberglass tank at reproduction term in this farm. Tank was supplied with a constant flow of well water. Fish for experiment adapted in this tank for 2 weeks. Feeding stopped 2 days before stripping. Water temperature (^oC), dissolved oxygen (DO) (mg^{-L}) and pH were measured in ponds daily (APHA, 1985). Stripping was performed by massage from the front to back of the fish abdomen. Freshly stripped milt was stored on ice in 30-ml plastic containers until used. A total of 10 usable milt samples were collected from 10 males in January (A) (14.01.2010), February (B) (17.02.2010) and March (C) (14.03.2010).

Sperm concentration was measured through a spectrophotometric method after dilution (2µl sperm:1998µl NaCl, 0.7%) by 605 nm (Ciereszko and Dabrowski, 1993). Sperm was sampled into 50-ml calibrated glass tubes and the volume was expressed as ml. Sperm pH was measured using standard pH-electrodes. Spermatocrit was determined using milt collected into microhaematocrit tubes without heparin (75 mm length, 1.1–1.2 mm inner diameter) and centrifuged at 10000 rpm for 5 minutes. Motility was evaluated at 400X magnification. The sperm motility duration was taken as the time at which 50% of the activated spermatozoa ceased movement. This was obtained using a stop-watch. This procedure was repeated tree times for each of males (Viveiros et al., 2003).

In statistics, ANOVA (with Duncan) was used to determine the significance of the observed all data by SPSS 15 software. Statistical difference was indicated when the p value was less than 0.05.

RESULTS

Sperm quality parameters in Rainbow trout in different stripping terms (January-A, February-B and March-C) have been showed in Table 2 in this study. It determined density, motility, duration of motile in spermatozoa and pH, spermatocrit and volume in sperm for each fish species. In the study, water temperature, dissolved oxygen (DO) and pH values were measured daily. Throughout the research period, water temperature was 10±0.1^oC, pH 7.3±0.2 and DO recorded as 9.5±0.31 mg/L. During the experimental period, water temperature, pH and DO values did not show much variations and this difference was identified statistically ($p>0.05$) as insignificant. All our results showed that were up and down value in volume of sperm, slightly decrease in pH, duration of motility, spermatocrit and density of spermatozoa but distinctly decrease in motility of spermatozoa at post stripping in this study.

Motility of spermatozoa was significantly ($p<0.05$) lower in C than A and B but was not significant ($p>0.05$) in all other parameters for stripping terms (Table 2-3, Fig.1).

Table 1. ANOVA test for sperm quality parameters between different stripping terms

		Sum of Squares	Mean Square	F	Sig.
Sperm Volume (ml)	Between Groups	1931.258	965.629	1.838	0.177
	Within Groups	15232.617	525.263		
pH	Between Groups	0.247	0.124	1.305	0.287
	Within Groups	2.745	0.095		
Motility (%)	Between Groups	21.169	10.584	14.735	0.000*
	Within Groups	20.831	0.718		
Duration (sec)	Between Groups	201.241	100.621	1.226	0.308
	Within Groups	2381.023	82.104		
Spermatocrit	Between Groups	148.728	74.364	1.445	0.252
	Within Groups	1492.072	51.451		
Density of Spermatozoa (x 10^9/ml)	Between Groups	7.510	3.755	0.276	0.761
	Within Groups	394.482	13.603		

* Significant difference as statistically, $p<0.05$

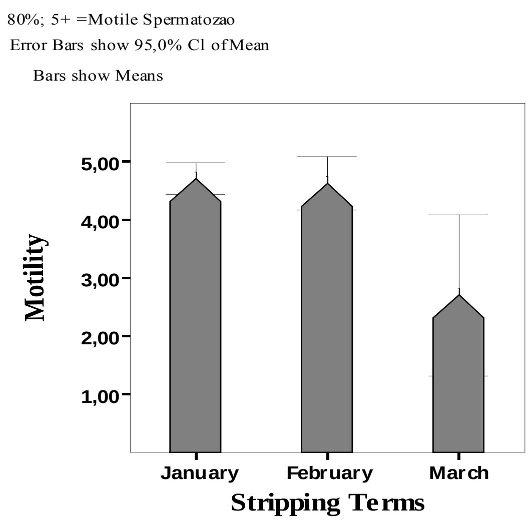

Figure 1. The values of motility of spermatozoa between different stripping terms

Table 2. Descriptive Statistics of sperm quality parameters between different stripping terms

		N	Mean±Std.Dev.	95% Confidence Interval for Mean		Min.	Max.
				Lower Bound	Upper Bound		
Sperm Volume (ml)	**A**	10	29.79±20.57[a]	17.91	41.66	9.00	79.00
	B	10	46.64±21.61[a]	32.12	61.16	11.00	70.00
	C	10	31.43±29.04[a]	4.57	58.29	5.00	87.00
pH	**A**	10	7.36±0.21[a]	7.23	7.48	7.00	7.75
	B	10	7.45±0.15[a]	7.35	7.56	7.00	7.50
	C	10	7.21±0.57[a]	6.69	7.74	6.50	8.00
Motility	**A**	10	4.71±0.47[b]	4.44	4.98	4.00	5.00
	B	10	4.64±0.67[b]	4.18	5.09	3.00	5.00
	C	10	2.71±1.50[a]	1.33	4.10	1.00	5.00
Spermatozoa Duration (second)	**A**	10	27.08±5.19[a]	24.08	30.07	20.32	42.02
	B	10	25.27±4.11[a]	22.51	28.03	20.24	33.05
	C	10	20.53±17.62[a]	4.23	36.82	5.20	58.58
Spermatocrit (%)	**A**	10	25.02±9.09[a]	19.77	30.27	8.70	37.50
	B	10	20.88±4.03[a]	18.18	23.59	13.70	27.01
	C	10	26.02±6.53[a]	19.98	32.05	18.52	35.29
Spermatozoa Density (x 10^9/ml)	**A**	10	9.86±3.18[a]	8.03	11.70	3.78	13.16
	B	10	10.44±2.07[a]	9.05	11.83	6.49	13.50
	C	10	9.12±6.06[a]	3.51	14.72	1.08	18.00
Fish Weight (g)		15	3156.64±434.32			2591	3852
Fish Length (cm)		15	61.00±2.45			56.00	65.00

DISCUSSION

Density of spermatozoa, motility and duration of motility are the most commonly used parameters to evaluate sperm quality. From comparison between species, sperm production can be expressed in 10^9 sperm per g body weight: it was 7 for rainbow trout, 4 for carp, 2.7 for guppy, 0.6 for pike and 0.1 for *Leporinus*. Measurements of beat frequency on three species show that the duration of motility is very short in trout (20-25s) and lasts slightly longer than 1 min in carp and halibut. The beat frequency of the majority of sperm declines progressively within 20-25s (trout) and 80-90s (carp) (Billard et al., 1995).

In this study, the determined density of spermatozoa was 9.86(A), 10.44(B) and 9.12(C) x10^9 per/ml and duration of motility was 27.08(A), 25.27(B) and 20.53(C) second, respectively. Density of spermatozoa obtained in this study are close to the results reported by Billard et al. (1995), for rainbow trout. . Other authors reported for rainbow trout a density of spermatozoa 11.8 (Ciereszko and Dabrowski, 1993), 8.9 (Geffen and Evans, 2000); for Atlantic salmon, *Salmo salar*, 3.5 (Aas et al., 1991), 12 (Truscott and Idler, 1969). Several studies have mentioned that the differences in sperm production could be related to many factors including the age and weight of the male, ecology and spawning behavior of broodstock and sampling period and method (Piironen and Hyvarinen, 1983; Suquet et al., 1994, Suquet et al., 1998). Volume and pH of sperm were also investigated as 29.79(A), 46.64(B) and 31.43(C) ml and 7.36(A), 7.45(B) and 7.21(C), respectively. The data of volume of sperm in this study was highest in term B and included to the normal standards for the sperm quality parameters which were reported by authors (Munkittrick and Moccia, 1987; Geffen and Evans, 2000).

Variation in sperm quality across the spawning season has been previously reported for a number of freshwater and marine fishes (Billard 1986; Munkittrick and Moccia 1987; Beirao et al., 2011). Results have shown that within and across species, seasonal changes in sperm quality can differ. Increases in sperm density throughout the spawning season have been found in Atlantic salmon (Piironen, 1985), and Atlantic cod (Butts et al., 2010). In contrast, studies on rainbow trout (Büyükhatipoğlu and Holt, 1984), snow trout, *Schizothorax richardsonii* (Agarwal and Raghuvanshi 2009), brown trout, *Salmo trutta* (Hajirezaee et al., 2010) and Atlantic salmon, *Salmo salar* (Aas et al., 1991) found that sperm density decreased throughout the season. As stated by Billard (1986), in salmonids, gametogensis is a discontinuous process where sperm is released from the sperm ducts over several months, aging throughout the spawning period.

According to our results, the values of semen volume and pH, motility, duration and density of spermatozoa declined by increasing of stripping frequency as previously reported for Atlantic salmon (Aas et al., 1991) and rainbow trout (Sanchez-Rodriguez et al., 1978).

As a conclusion, good suggestions on obtaining sperm quality parameters from broodstocks have been offered. It is hoped that this research would contribute to more production of this important species of fish in our country.

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COMBINED METHODS FOR ARTIFICIAL REPRODUCTION OF PIKEPERCH, *SANDER LUCIOPERCA*

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KOMBINOVANI METODI VEŠTAČKE REPRODUKCIJE SMUĐA *(SANDER LUCIOPERCA)*

Apstrakt

Smuđ, *Sander lucioperca*, je vrsta koja ima dobru perspektivu u procesu diverzifikacije evropske kopnene akvakulture. Iako su različite tehnike njegovog razmnožavanja (prirodni mrest, veštačka i polu-veštačka propagacija) vrlo dobro opisane i dalje postoji širok prostor za razvoj i usavršavanje mnogih detalja. Do sada je objavljeno nekoliko publikacija o indukciji završnog sazrevanja gameta ove vrste korišćenjem različitih hormonalnih supbstanci (Ronyai., 2007; Kristan et al 2012, Zakes et al., 2012; Zarski et al., 2012). Najčešće upotrebljavani hormoni su ekstrakt šaranske hipofize - CPE, (Carp Pituitary Extract) i humani horioni gonadotropin (hCG, Human chorionic gonadotropin). Prirodni mrest, bilo u jezerima ili kavezima (Demska-Zakes and Zakes, 2002, Schlumberger i Proteau, 1996; Ruuhijarvi and Hyvarinen, 1996, Steffens et al., 1996), izgleda najjednostavniji, ali u isto vreme, manje pouzdan metod, kako je uspeh mresta visoko zavisan ne samo od stadijuma zrelosti matica, već i od nekontrolisanih spoljašnjih ekoloških faktora (npr. temperatura i kvalitet vode). Nasuprot tome, veštačka, ili poluveštačka propagacija bi mogli biti u prednosti za mnogo pouzdaniju i sinhronizovaniju produkciju larvi. Ove tehnike mogu biti izvedene bilo mrestom u tanku ili ručnim istiskanjem (Kucharczyk et al., 2007). U slučaju reprodukcije u veštačkom okruženju , vreme latencije (LT -, izračunat kao interval od prve hormonalne injekcije do ovulacije)

je vrlo nesinhronizovano u većini studija. Zarski et al. (2011) su razvili novu klasifikaciju preovulatornih stadijuma sazrevanja ovocita za smuđa sa svrhom bolje sinhronizacije ovulacije. Iako postoji poboljšanje sinhronizacije, period od prve do poslednje ovulacije varira 10-25 časova. Nepredvidljivo vreme ovulacije uzrok je ozbiljnom problemu pri ručnom istiskanju ikre kod smuđa, što ponekad dovodi do spontane ovulacije i gubitka ikre u tanku (Zarski et al., 2011). U ovoj studiji korišćena je kombinovana metoda prezentovana od strane Rónyai (2007). Izbor ovakvog metoda je u cilju sprečavanja spontane ovulacije ženki u tanku, ali i kako bi, se dobile živi gameti za eksperimentalne i produkcione svrhe. U cilju realizacije eksperimenta, 4. aprila 2013. 16 parova matica gajenih u jezeru prebačeno je u RAS Instituta za ribarstvo, akvakulturu i irigaciju (HAKI, Sarvaš, Mađarska). Matice su aklimatizovane sa temperature vode u jezeru (7.5^{0}C) na optimalnu temperaturu za reprodukciju (16^{0}C) postepenim zagrevanjem (2^{0}C/dan). 8. aprila merene se mase matice i uzet uzorak ovocita ženki uz pomoć katetera (unutrašnji prečnik 1.2mm) korišćenjem metode po Kucharczyk et al. (2007). Uzorak ovocita razbistren je u Serinom rastvoru (etanola, formaldehid i glacijalna kiselina u odnosu 6:3:1,) i pod mikroskopom je određen stadijum sazrevanja, kao što je objašnjeno od strane Zarski et al. (2011). Nakon toga, svi parovi matica smešteni su u zasebna odeljenja za mrest oformljena deljenjem 4 tanka ‘’raceway” tipa, zapremine 4m^3 plastičnom mrežom na 4 dela. Temperatura vode je održavana na 16.2±0.3^{0}C tokom svih procedura mresta. Zasićenje kiseonikom je mereno svaka 3 časa i održavano iznad 80%. Tokom svih procedura ribe su anestezirane u rastvoru ulja karanfilića. 10. aprila data je prva inekcija ženkama: osam ženki 3mg/kg CPE, a drugih osam 200 IU/kg hCG. 11. aprila, druga hormonalna inekcija data je svim ženkama (3mg/kg CPE). U isto vreme su tretirani mužjaci sa 2mg/kg CPE. U trenutku obe injekcije uzet je uzorak ovocita ženki i određen je stadijum sazrevanja. Nakon druge inekcije, gnezda su ubačena u svako odeljenje i observacija mresne aktivnosti je vršena svaka 2 časa dok iz 4 ženke nije istisnuta ikra (navedeni broj istiskivanih ženki bio je potreban za dalja istraživanja). 12 matica se mrestilo i ostala gnezda su proveravana svaka 2 časa radi utvrđivanja perioda latencije.

Srednji period latencije ženki, istiskivanih i mreščenih, bio je 57 ± 9 časova. Periodi latencije ženki u različitim stadijumima sazrevanja ovocita iznosio je 66±6, 55±6, 47±0 i 44±7 časova za II, III, IV i V stadijum, respektivno. Period latencije ženki indukovanih različitim sredstvima bio je 51±7 za CPE+CPE i 62±7, za hCG+CPE. Prosečni pseudogonadosomatski indeks (PGSI) istiskivanih ženki iznosio je 9.7±4.6%. Stepen fertilizacije istiskivanih i mreščenih riba određen u stadijumu neurulacije bio je iznad 80% u svim grupama. Stadijum sazrevanja ovocita na dan uzorkovanja i vreme latencije svih ženki dati su u tabeli 1.

Radi sinhronizacije ovulacije, ženke u nižim stadijumima maturacije su tretirane prvom injekcijom hCG praćenom sa CPE, što izaziva najkraće i najsinhronizovanije vreme latencije, (Rónyai,2007). Uprkos tome, LT se razlikovao između grupa, a što potvrđuje da je stadijum sazrevanja najbitniji faktor za predviđanje ovulacije. Dobijeni srednji PGSI je u saglasnosti sa ranije izvedenim istraživanjima (Zakes and Demska Zakes, 2005, Ronyai, 2007), ali trebalo bi napomenuti da je od četiri istiskivane ženke, jedna počela mrest u tanku, dok su ostale nastavile mrest nakon vraćanja u mresne komore. Obzirom da je istiskivan potreban broj ženki i da nije bilo značajnog gubitka jaja, može se zaključiti da je kombinovan metod veštačke reprodukcije bio uspešan za ovu svrhu. Ručno istiskanje ženki omogućava izvođenje efektnih odgajivačkih programa uz manipulaciju genoma i korišćenje krioprezervirane sperme (Bokor et al., 2007; Bokor et al., 2008).

Ključne reči: veštačka reprodukcija, smuđ, hormoni, period latencije
Keywords: artificial reproduction, pikeperch, hormones, latency time

INTRODUCTION

Pikeperch, *Sander lucioperca* L., is a possible candidate for diversification of European freshwater aquaculture. Although its different breeding techniques (natural spawning, artificial and semi-artificial propagation) are generally well described, still there is a room for the development of many details and to their refinements. Several papers have been published on the induction of final gametes maturation of this species using different hormonal substances (Rónyai, 2007; Kristan et al., 2012; Zakes et al., 2012; Zarski et al., 2012, Steffens et al., 1996). Most commonly used hormones are Carp Pituitary Extract (CPE) and human Chorionic Gonadotropin (hCG). Natural spawning either in ponds or cages (Demska-Zakes and Zakes, 2002; Schlumberger and Proteau, 1996; Ruuhijiirvi and Hyviirinen, 1996), seems to be the most simple, but - at the same time - less reliable method as the spawning successes highly depend not only from the maturity stage of the breeders, but also from the uncontrollable external ecological factors (i.e. water temperature and water quality). In contrast, artificial, or semi artificial propagation could give advantages for more reliable and synchronized larval production. These techniques could be performed either by tank spawning or hand stripping (Kucharczyk et al., 2007).

In case of reproduction in artificial environment the latency time (LT, calculated as the time interval from first hormonal injection to ovulation), was much unsynchronized in most of the studies. Zarski et al. (2011) developed a new classification of pre-ovulatory oocyte maturation stages in pikeperch with a purpose of better synchronization of ovulation. Although there was improvement of synchronization, range from first to last ovulation varied between 10-25 hours. The unpredictable time of ovulation cause a serious problem with hand-stripping of this species. This phenomenon could lead to spontaneous ovulation and loosing eggs in the tank (Zarski et al., 2011).

In our study we applied combined method formerly documented by Rónyai (2007). Reason for choosing this method was from one side to prevent spontaneous ovulation of females in tank, and - from the other side - to make attempts to obtain stripped viable gametes either for experimental or production purposes.

MATERIALS AND METHODS

For purpose of this study, on April 4, 16 pairs of pond reared breeders were transferred to the recirculating aquaculture facility of the Research Institute for Fisheries, Aquaculture and Irrigation (HAKI, Szarvas, Hungary). Breeders were acclimatized from the actual pond water temperature (7.5^{0}C) to the optimal one for reproduction (16^{0}C) by gradual (2^{0}C/day) warming up. On April 8 weight of spawners was measured and a sample of oocytes was taken with a catheter (inside diameter of 1.2 mm) from each female as described by Kucharczyk et al. (2007). Sample of oocytes was clarified in a Sera's solution (ethanol, formalin and glacial acetic acid in the ratio of 6:3:1, respectively), placed under the microscope and final oocyte maturation (FOM) stage was determined, as described by Zarski et al. (2011). Furthermore all pairs were stocked in individual spawning "compartments", which were formed by dividing 4x4 m^3 raceway type tanks in four parts with mesh walls.

On April 10 the first injection was given to females. Eight females were treated with 3mg/kg of CPE and the other eight with 200 IU/kg of hCG. On April 11, second hormonal injection was given to all females (3mg/kg of CPE) and at this time all males were injected with 2mg/kg of CPE. At the time of both injections, a sample of oocytes was taken from each female and the FOM stage was determined (Table 1). After the second injection, nests were introduced into each compartment and observation of spawning activity was performed every two hours until four females could be stripped. (This number of stripped females was needed for further research). All the remaining 12 pairs were spawned and remained nests were checked every two hours for the LT determination.

Water temperature was maintained at 16.2±0.3^{0}C during the whole spawning procedure. Oxygen saturation was monitored every 3 hours and maintained above 80%. During all procedures fish were anaesthetized in clove oil solution.

RESULTS AND DISCUSION

Mean LT either to stripping or spawning was 57±9 hours. The LT values for females with different FOM were 66±6, 55±6, 47±0 and 44±7 hours for II, III, IV and V stage, respectively. For females injected with different hormonal substances mean LT was 51±7 and 62±7 hours for CPE + CPE and hCG + CPE respectively. For the stripped fish the mean pseudo-gonadosomatic index (PGSI) was 9.7±4.6% (Table 2). Fertilization ratios both for stripped and spawned fish were determined at the neurulation stage and were above 80 % in each batches. The stages of FOM on the sampling dates and latency time of each female is given in Table 1.

Table 1. FOM stages and LT for females

Female	April 8	April 10 (first injection)	April 11 (second injection)	Latency time (hours)
1	II/III	III	IV/V	51
2	II	III/IV	III	55
3	II	III	III	62
4	I	II	III	66
5*	II	III	IV/V	52
6	IV	IV	V	47
7	II	II	III	64
8	I	II	III	68
9*	I	II	III	75
10	III	III	IV/V	47
11*	II/III	III	IV	57
12	II	III	IV	52
13	IV/V	V	VI	39
14*	IV	V	V/VI	49
15	II	III	IV	60
16	II	II	II	51

*- fish which were stripped

Table 2. Body weight, eggs weight and PGSI for stripped females

Female weight [g]	Weight of eggs [g]	PGSI [%]
2320	100	4.3
2460*	183	7.4
1780	225	12.6
1900	272	14.3

*-started spawning on the nest

For the purpose of ovulation synchronization, females in lower FOM stages were injected with primary injection of hCG followed with CPE, as it is described by Rónyai (2007) to induces shortest and more synchronized LT. Even though, LT was different between these groups. This confirms that FOM stage is the most significant factor for prediction of ovulation. Obtained mean PGSI is in agreement with former studies (Zakes and Szczepkowski, 2004; Zakes and Demska Zakes, 2005; Rónyai, 2007), but we should notice that from the four stripped females, one started to spawn on the nest prior to stripping and other three spawned again after returning to the spawning chamber. Muller-Belecke and Zienert (2008) reported very different mean commercial fecundities between the groups of preseason spawning with use of similar method of artificial propagation. Even though mentioned studies were conducted in the different conditions, reported PGSI makes a question about stripping efficacy of pikeperch females and leaves the open door for further investigation.

CONCLUSIONS

With concern to strippe needed number of females and to avoid significant lost of eggs, it could be concluded that combined method of artificial reproduction of pikeperch was successful for this purpose. However, hand stripping enables effective breeding programs with genome manipulations and egg fertilization with cryopreserved sperm (Bokor et al., 2007; Bokor et al., 2008).

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EFFECTS OF WATER CURRENT ON SOME GROWTH FACTORS AND WATER QUALITY IN A CLOSED TROUT CULTURE SYSTEM

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EFEKTI PROTOKA VODE NA NEKE FAKTORE RASTA PASTRMKE I KVALITET VODE U ZATVORENIM SISTEMIMA GAJENJA

Apstrakt
Efekti nivoa protoka vode na uzgoj pastrmke ispitani su u toku 35 dana eksperimenta. U tankove su naseljene ribe mase 5,5 g i dužine 6,7 cm. Četiri različite brzine vode (0; 3,5; 7; 10,5 cm/s) ispitane su kroz tri ponavljanja. Ovi različiti protoci obezbeđeni su ponovnim korišćenjem izlazne vode iz svake uzgojne jedinice. Kod oglednih riba ocenjena je dužina, težina, dnevni prirast (DGR), specifična stopa rasta (SGR), factor kondicije (CF) i stopa preživljavanja (SR). Takođe, istovremeno su ispitane promene NO_2, NO_3, NH_3, NH_4^+, ukupne tvrdoće i pH vode u svakom tretmanu. Analiza varijanse podataka o kvalitetu vode pokazala je veoma značajne razlike između svih tretmana u toku prve nedelje ($p<0,01$). Međutim, ovi rezultati nisu zabeleženi u toku nastavka eksperimenta. Krajnji rezultati pokazali su veoma značajne razlike ($p<0,01$) u svim tretmanima u pogledu faktora koji su ispitivani kod mlađi. Na osnovu rezultata Dankanovog testa, najbolja stopa preživljavanja (97%), dnevni prirast (1), SGR (6%) i prosečna težina (24 g) postignuti su pri brzini protoka od 10,5 cm/s.

*Ključne reči***:** *brzina protoka, zatvoren sistem, kalifornijska pastrmka, Iran*
Keywords: water current, closed system, rainbow trout, Iran

INTRODUCTION

Rainbow trout production has been expanded in Iran through last ten years so that its amount grew from 9000 mt in 2000 to 62630 mt in 2008. Aquaculture increment could be one of the main reasons for water pollution in the world. So water quality protection in fish culture is important and this leads industry to use modern systems such as Recirculation Aquaculture System in order to get maximum production without water polluting. The possibility of outlet water refining is the main advantage of these systems and pH, temperature and bacterial disease controlling are their other advantages (Willoughby, 1999). However they are very expensive. But what happens if some functions of RAS (Recirculating Aquaculture System) were been eliminated and water velocity through the culture unit is increased? Larger fishes can stand against more water velocity (Sedgwick, 1990). Therefore an experiment was conducted to assess the effects of water velocity, physical filtration and aeration in a closed rainbow trout culture system. This experiment was done through Randomize Complete Blocks design with 4 treatments and 3 repeats. Different water velocities (0, 3.5, 7, 10.5 cm/s) along with twelve plastic aquariums (200×40×15 cm) formed treatments and plots respectively.

MATERIALS AND METHODS

The experiment was carried out during 35 days in Khojir Natural Resources Station of Tehran-Iran. Each plot was contained 60 liters water with a Renault air pump for aerating. Fry were provided from a private farm in Semnan province and transferred to the station in oxygenated plastic bags. 48 hours before transferring, fry feeding had been stopped. After adaptation 12 fry were introduced to each aquarium. New feeding started 48 hours after fry introduction to the new environment. Initial fry weight and length were 5.5±0.32gr and 6.7±2.41cm respectively. Water recirculation has been done by external electrical pumps through the each plot which was connected to 30μ mesh size filter bags in order to physicaly filtrate the water. These filter bags were cleaned daily by fresh water and manually. Evaporated water in the each plot was replaced by isotherm fresh water. The water was gathered from the bottom of each plot by pump and recirculate to aquarium after filtering. Fry stocking density in this experiment was 200 fish/m^2, which is more than two fold of average density at Iranian farms. 48 hours after introducing fishes to plots, feeding operation was started with commercial food. Feeding was conducted 5 times a day among 7 am and19 pm. Food was provided from Biomar Company in France and was combined from 54% crude protein, 18% crude fat, 0.5% fiber, 10% ash and 1.4% phosphor. Feeding amount had been considered to 4% (Jeffrey, 1999) of fry weight so that all foods were used by fry. Water temperature and pH were measured daily during the experiment. At the same time, fry weight, length, specific growth rate (SGR), daily growth rate (DGR), survival rate (SR), and condition factor (CF) were weekly measured as follows (EIFAC, 1980):

DGR: [(final weight (gr) - initial weight (g)) ÷ experiment days]×100
SGR: [(Ln final weight- Ln initial weight) ÷ experiment days]×100
CF: (weight (gr) ÷ $lenght^3$)×100
SR: (live fry÷ total plot fry)×100

Fry length and weight were measured by an ordinary ruler (1mm accuracy) and a Sartorious digital scale, Ek-120A model (0.01gr accuracy), respectively. pH of the water was also determined by waterproof pH-meter pen model YTH 10 that was made in the United States of America.

Data analysisiswas done by SPSS software, version 14 and Duncan's averages comparing test was used to determine the best fishes indexes averages among different treatments.

RESULTS

Water temperature average was 18±1.19°C during experimental period. Average oxygen demand was 7, 7.5, 8.5, and 10.5 in 0, 3.5, 7 and 10 cm/s treatments respectively.

Table 1. Results of ANOVA and Duncan's test through the first week

Factors	F_s	Treatment			
		1T	2T	3T	4T
(g) Weight	**16.823	7.8731^{c}±0.30	0.38±8.3867^{bc}	9.0191^{b}±0.23	9.8938^{a}±0.42
(cm) Length	**11.173	0.17± 7.4961^{c}	0.48±8.0231^{bc}	8.3046^{ab}±0.18	8.8276^{a}±0.18
SGR%	**16.892	0.55±5.1172^{c}	0.65±6.0173^{bc}	7.0590^{b}±0.53	8.3791^{a}±0.62
DGR%	**16.814	0.04±0.3389^{c}	0.05± 0.4123^{bc}	0.5026^{b}±0.04	0.6276^{a}±0.06
CF%	**6.608	0.10±1.8666^{a}	0.21±1.6381^{b}	1.5751^{b}±0.04	1.4381^{b}±0.02
SR%	3.27^{ns}	100.000^{a}±0	100.000^{a}±0	100.000^{a}±0	100.000^{a}±0

** Very significant differences in $p<0.01$; ^{ns} not significant differences

Table 1 shows very significant differences about studied fry factors among all treatments ($p<0.01$) except survival rate. Based on Duncan's test results, it seems the fourth and the first treatments have been caused maximum (a rank) and minimum (c rank) averages in week 1, respectively. The changes of fry weight, length, and specific growth rate are summarized in figs. 1-4.

Table 2. Results of ANOVA and Duncan's test through the second week

Studied indicators	F_s	Treatment			
		1T	2T	3T	4T
(g) Weight	9.162**	10.7598^{c}±0.67	12.5396^{bc}±0.32	13.4600^{ab}±0.70	14.7584^{a}±0.98
(cm) Length	1.531^{ns}	9.0186^{a}±0.75	9.3664^{a}±0.46	9.5305^{a}±0.28	9.8107^{a}±0.17
SGR%	3.884*	4.4502^{b}±0.55	5.7005^{a}±0.86	5.7027^{a}±0.22	5.7131^{a}±0.33
DGR%	5.600*	0.4095^{b}±0.07	0.5932^{a}±0.13	0.6341^{a}±0.05	0.6949^{a}±0.07
CF%	0.115^{ns}	1.4943^{a}±0.29	1.5242^{a}±0.06	1.5558^{a}±0.06	1.5616^{a}±0.05
SR%	8.296**	83.300^{b}±8.3	97.200^{a}±4.84	100.000^{a}±0	100.000^{a}±0

Table 3. Results of ANOVA and Duncan’s test in the third week Studied indicators

	F_s	Treatment			
		1T	2T	3T	4T
(g)Weight	$^{**}6.783$	$14.3287^{c}\pm1.71$	$17.7351^{bc}\pm2.80$	$19.8966^{ab}\pm1.87$	$22.2434^{a}\pm2.39$
(cm) Length	8.033^{**}	$10.0897^{c}\pm0.40$	$10.5445^{bc}\pm0.21$	$10.9010^{ab}\pm0.20$	$11.0927^{a}\pm0.20$
SGR%	1.852^{ns}	$4.0416^{a}\pm0.82$	$4.8838^{a}\pm0.83$	$5.4926^{a}\pm0.59$	$5.5548^{a}\pm1.20$
DGR%	$^{*}5.057$	$0.5097^{b}\pm0.14$	$0.7421^{ab}\pm0.21$	$0.9194^{a}\pm0.16$	$1.0692^{a}\pm0.20$
CF%	3.286^{ns}	$1.3903^{b}\pm0.009$	$1.5054^{ab}\pm0.14$	$1.5329^{ab}\pm0.06$	$1.6252^{a}\pm0.08$
SR%	5.735^{*}	$74.967^{b}\pm8.35$	$86.067^{ab}\pm9.58$	$94.400^{a}\pm4.84$	$97.200^{a}\pm4.84$

** Very significant differences in $p<0.01$; * significant differences in $p<0.05$; ^{ns} not significant differences

Table 4. Results of ANOVA and Duncan’s test indexes in the fourth week

Studied indicators	F_s	Treatment			
		1T	2T	3T	4T
(g) Weight	$^{**}6.850$	$16.7387^{c}\pm2.78$	$21.8137^{bc}\pm4.91$	$25.4217^{ab}\pm3.27$	$29.9999^{a}\pm4.36$
(cm) Length	6.636^{**}	$10.7669^{b}\pm0.33$	$11.3195^{b}\pm0.48$	$11.3698^{b}\pm 0.32$	$12.2400^{a}\pm0.46$
SGR%	6.922^{**}	$2.1568^{c}\pm0.67$	$2.8973^{bc}\pm0.51$	$3.4641^{ab}\pm0.49$	$4.2246^{a}\pm0.60$
DGR%	6.808^{**}	$0.3442^{c}\pm0.15$	$0.5826^{bc}\pm0.19$	$0.7892^{ab}\pm0.20$	$1.1080^{a}\pm0.28$
CF%	9.790^{**}	$1.3323^{c}\pm0.10$	$1.4724^{bc}\pm0.13$	$1.6270^{ab}\pm0.08$	$1.7460^{a}\pm0.05$
SR%	6.016^{**}	$61.100^{c}\pm12.73$	$74.967^{bc}\pm8.35$	$83.300^{ab}\pm8.3$	$94.433^{a}\pm9.64$

** Very significant differences in $p<0.01$

Table 5. Results of ANOVA and Duncan’s test in the fifth week

Studied indicators	F_s	Treatment			
		1T	2T	3T	4T
(g)Weight	$^{**}7.604$	$18.7571^{c}\pm3.66$	$25.8052^{bc}\pm5.75$	$32.2331^{ab}\pm5.75$	$40.2558^{a}\pm7.26$
(cm) Length	2.617^{ns}	$12.1281^{b}\pm0.43$	$12.2315^{ab}\pm0.48$	$12.4044^{ab}\pm0.54$	$13.0214^{a}\pm0.38$
SGR%	12.696^{**}	$1.5753^{b}\pm0.42$	$2.3826^{ab}\pm0.52$	$3.4561^{a}\pm0.69$	$4.1440^{a}\pm0.52$
DGR%	8.594^{**}	$0.2883^{c}\pm0.12$	$0.5701^{bc}\pm0.22$	$0.9730^{ab}\pm0.35$	$1.4651^{a}\pm0.41$
CF%	13.811^{**}	$1.0449^{c}\pm0.14$	$1.3964^{b}\pm0.17$	$1.6773^{ab}\pm0.11$	$1.8103^{a}\pm0.18$
SR%	15.568^{**}	$41.633^{b}\pm8.35$	$58.300^{b}\pm8.3$	$77.773^{a}\pm12.72$	$91.633^{a}\pm8.35$

** Very significant differences in $p<0.01$; ^{ns}not significant differences

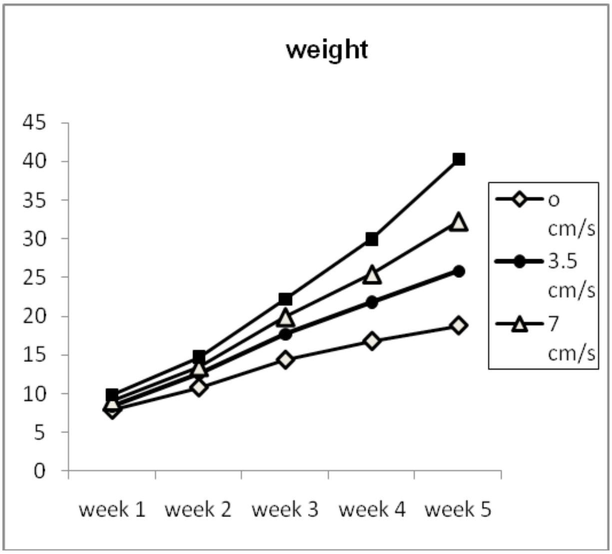

Figure 1. Changes of fry weight in different treatments during the experiment’s period

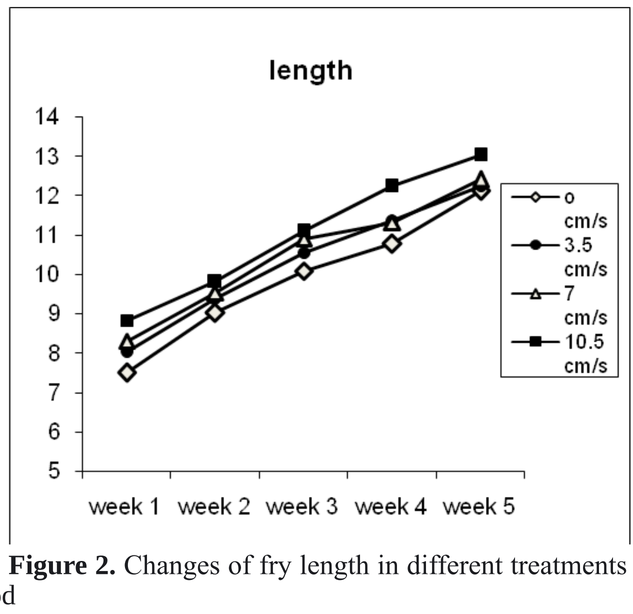

Figure 2. Changes of fry length in different treatments during the experiment’s period

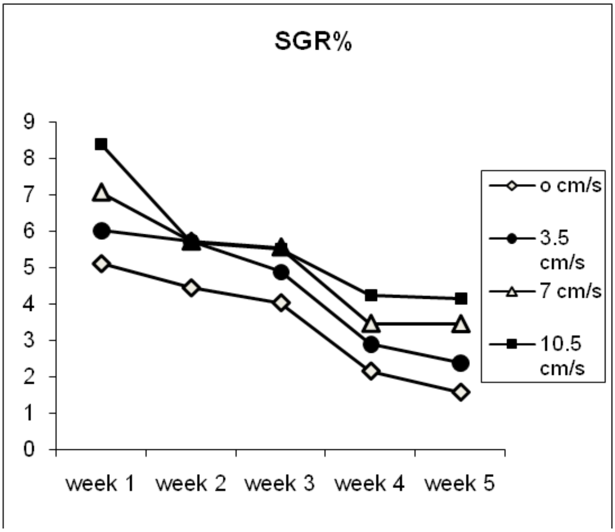

Figure 3. Changes of fry SGR% in different treatments during the experiment’s period

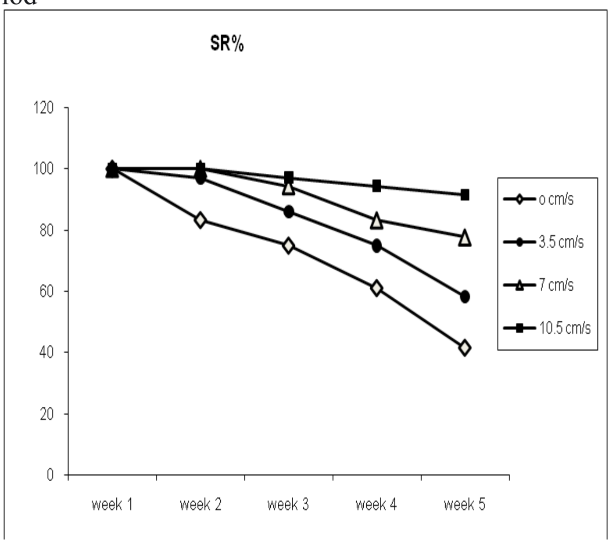

Figure 4. Changes of fry SR% in different treatments during the experiment’s periodTable6.Results of ANOVA and Duncan’s test indexes during 35 days of the experiment

Table 6.

Studied indicators	F_s	Treatment			
		1T	2T	3T	4T
(g) Weight	**7.617	13.6915^{c}±1.82	17.2561^{bc}±2.89	20.0061^{ab}±2.38	23.4302^{a}±3.08
(cm) Length	4.815*	9.9239^{b}±0.41	10.3250^{ab}±0.39	10.4920^{ab}±0.29	10.9985^{a}±0.28
SGR%	7.059**	3.4682^{c}±0.56	4.3767^{b}±0.66	5.0494^{a}±0.49	5.5881^{a}±0.64
DGR%	7.605**	0.3781^{c}±0.10	0.5800^{bc}±0.16	0.7637^{ab}±0.16	0.9929^{a}±0.20
CF%	11.336*	1.4257^{b}±0.03	1.5073^{ab}±0.04	1.6124^{a}±0.02	1.6174^{a}±0.06
SR%	10.615**	72.200^{c}±6.73	83.3066^{bc}±6.007	91.0866^{ab}±5.09	96.6533^{a}±4.42

** Very significant differences in $p<0.01$; * significant differences in $p<0.05$

DISCUSSION

Concerning high fry density in this survey water velocity had been increased till dissolved O_2 and CO_2 increases and decreases during water fall respectively. Summerfelt et al. (2000) showed that water oxygenation along with CO_2 elimination are necessary factors in water reused aquaculture systems. Our results implied that water velocity increment by outlet water reuse can also moderate these two factors. Clark (2003) suggested that oxygen injection could increase fish raceway capacity, although our results showed this matter could be done by water velocity increment through reuse filtered outlet water. Water velocity increment increases mixing level of air and water. This could result in better balance of dissolved O_2 and CO_2 in the water. CO_2 concentration could be bearable till 24 mg/lit by rainbow trout in culture unit (Good et al., 2010), but we have never record such CO_2 concentration during the experiment. At the same time, CO_2 reduction in the faster treatments was more evident. Based on the Martins et al. (2009), insoluble and dissolved matter concentration could be inhibitor factor in recirculation aquaculture systems so their high amount could been resulted in fry mortality. Results of this experiment showed that stocking density level could be different based on water velocity in fry bearable limitation. In our project, water velocity increment is provided by outlet water reuse after physical filtration and aeration only. Our results justify Colt (2005) findings who has introduced water speed as an effective factor on reducing water pollution. It was seen that water pollution accursed gradually and it is reusable after aerating and Total Solid Sediment (TSS) elimination. This matter implies previous findings (Summerfelt et al., 2004; Summerfelt et al., 2006; Stewart et al., 2006). In this experiment stocking density was more effective on fish growth and survival rate rather than water quality and this matter have reported already (North et al., 2006; Person et al., 2008). They showed that desire water quality covers stocking density problems. In spite of stocking density increment, our results showed that water speed increment could reduce the stocking rate problems.

At the same time, previous findings imply that fish density does not lead to considerable effect on fish growth and survival (Lefrancois et al., 2001; North et al., 2006). Roque d’orbcastel et al. (2009) found that only water recirculation could supply fish survival without necessity to water exchange. These results were justified by our findings in current water treatments, although this result was not observed in control treatment (0 cm/s).

In this experiment, weekly biometry showed some differences which could not be seen during all 35 days period. The reason for this matter can be seen by comparing tables 1-5 to table 6 . Duncan’s test results justify that in the first week the fourth treatment (10 cm/s) took the best rank (a) in assessed fry factors, excluding Condition Factor (CF), which its reason was not distinctive and needs more studies. At the same time, such result was not recorded in other weeks (table 2-5). However, the weakest results were observed in static water treatment (control).

Concerning the information in tables 1-5, the fourth treatment (10 cm/s) provided better results than the other treatments. It seems water velocity in our experiment was more important than water resource and its quality, so water speed increment could effect on water quality and adjust it to some extent.

This survey was done in laboratory condition, so its examination in farm condition could be a good point for future studies. It is recommended that other more water speeds are been examined in order to determine limitation velocity for trout culture under laboratory condition.

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DETERMINATION OF VHS VIRUS TITER IN EXPERIMENTALLY INFECTED RAINBOW TROUT, *ONCORHYNCHUS MYKISS*

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ODREĐIVANJE TITRA VIRUSA VHS U EKSPERIMENTALNO INFICIRANIM PASTRMKAMA, *ONCORHYNCHUS MYKISS*

Apstrakt
U radu se opisuje infekcija različitih tkiva kalifornijske pastrmke virusnom hemoragične septikemije i poređenje titra VHS virusa u različitim tkivima inficirane pastrmke u cilju određivanja najboljeg organa za izolaciju virusa i identifikaciju faze bolesti. Rezultati pokazuju da je mozak dobar organ za izolaciju VHS virusa u fazi bolesti kao i tokom inkubacije i da je značajan koliko i bubrezi i srce za detekciju virusa. Srce i bubrezi su organi koji su imali najviši titar virusa, jetra, škrge, pilorus i koža najmanji, dok su mozak i slezina između te dve grupe.

Ključne reči: VHS virus, virus titar, kalifornijska pastrmka, *Oncorhynchus mykiss*
Keywords: VHS virus, virus titer, rainbow trout, Oncoryhinchus mykiss

INTRODUCTION

Viral haemorrhagic septicaemia (VHS) is a serious viral disease affecting a range of fish species (Wolf, 1988). The organs affected during an infection with both VHS and IHN viruses are usually kidney and spleen, though most organs and tissues are affected in later stages of the disease. Hematopoetic tissue in kidney and white pulp of the spleen are the most frequently tissues affected, but there are cases in which the virus was isolated from the brain only (Yasutak & Amend, 1972; Kinkelin et al., 1979; Wolf, 1988). Leukocytes and endothelial cells are anticipated to be important sites for virus replication (Yamamoto et al., 1989; Yamamoto & Clermont, 1990). It has been demonstrated that VHS virus effectively infects by waterborne challenge. Both gills and intestine are suggested as a primary site of infection (Neukirch, 1986; Yamamoto et al.,1989). Yamamoto et al.(1992) showed that epithelial cells from skin and gills are capable of supporting early VHS virus and IHN virus replication. Kidney and spleen have the highest titers in the acute or chronic phase. Brain should also be sampled in fish in the convalescent stage (Noga, 1996). Kidney and spleen are the organs recommended for isolation of VHS virus from epizootic specimens. Brain is suggested if survivors are to be assayed (Wolf, 1988). A confirmed diagnosis of VHS can be made only by isolating and serologically identifying the causative virus in an appropriate cell culture system (Jørgensen 1974). Most of the studies referred that immunohistochemical techniques reveal fewer positive samples than virus cultivation. Cultivation is more sensitive than immuno-histochemistry for detection of VHS virus (Øystein et.al 1994). **The purpose of the present investigation was to compare VHS virus titer in different tissues in experimentally infected rainbow trout in order to find the best organ for virus isolation and identification in disease phase of viral haemorrhagic septicemia in rainbow trout.**

MATERIALS AND METHODS

140 apparently healthy fish average weight 75 g were collected from a local rainbow trout farm. The farm is approved VHS, IHN and IPN free. During the study the fish were fed once daily with commercial feed. The fish were divided in to two equal groups (I ,II) in 120 liter tanks supplied with dechlorinated tap water. The water temperature was 10±2 ^{o}C. Tanks were aerated to maintain sufficient O_2 concentration during infection.

A VHS virus isolate (DK – 3592 B) with two passage in BF–2 cells, serologically similar to the reference strain F_1 and with proven high pathogenicity in rainbow trout was used.

Isolation of VHS virus in cultures of a number of fish cell lines is well documented by Olesen and Jørgensen (1992), Lorenzen et al. (1999). The fish cell line BF-2 and RTG-2 are recommended. Alternatively, EPC or FHM cells may be used, but are in general less susceptible than BF-2 and RTG-2**.**

The access infection was through a room with in and out path. Group II, containing 70 fish, was infected by bathing in the concentration of 10^{-3} TCID50 VHSV ml^{-1} of water**.** 0.5 ml of virus suspension was mixed with 49.5 ml of Eagle's medium without fetal calf serum and added to a tank with no water renewal for two hours at 12°C to achieve desired virus concentration per ml in the tank. Fish from group I, considered as control, were exposed to an equal volume (50 ml) of virus free medium and handled as the infected fish. Before the water was discharged from the facility, it received a heat treatment in a pasteurization unit at 120°C during 2 minutes. Shoes and laboratory coats were changed before entering to the fish laboratory. Disposable gloves were worn during the work. Dead fish were removed daily from each tank. The number of dead fish and clinical signs of VHS were recorded every day. One week after the mortality initiation, at the days 12,13 and 14 post infection, dead fish were collected. They were opened aseptically and 0.1g of each organ including brain, gills, heart, kidneys, liver, pyloric caeca, spleen and skin from five fish were measured and placed in 8 eppendorf tubes (8 pool), diluted to a ratio of 1:10 in a dilution medium. Samples were homogenized with the tissue mixer, treated with Gentamicin and stored in refrigerator 4°C over night. Half of samples were used for virus titration and remaining was stored in -20°C for virological examinations. During the sampling a piece of aluminum foil, new gloves and a new sterile pair of scissors have been used for every new sample to avoid contamination.

Samples were diluted in a dilution plate from 10^{-1} - 10^{-4} and subsequently added to the 24-hour old monolayer of BF- 2 cell line. The inoculated tissue culture plates were incubates at 15°C and monitored for viral cytopathic effect (CPE). When CPE was completed, virus titers were calculated in different organ (Tab 1). Tubes were placed in cold tap water and when thawed. 100µl from each tissue was collected in a eppendorf tube and inoculated on BF-2 cell in 24-well plate as described previously (Mortensen et al. 1999). The 8 fold dilution from undiluted to 10^{-7} were incubated at 15°C and inspected regularly with microscope for the occurrence of cytopathic effect (CPE). When CPE was evident, supernatants were tested by *ELISA to confirm the presence of VHS virus.*

The ELISA test used to detect VHS virus was performed according to general principles of direct ELISA described by (Olesen & Jørgensen 1991). Virus is trapped by rabbit anti-VHSV antiserum which is coated on to the wells of an ELISA plate. The virus is identified by a monoclonal antibody against the VHSV N- protein (Mab IP5B11) coupled to the biotine / streptavidine - horseradish peroxidase system. Culture media from cells cultures showing evidence of CPE were analyzed with direct ELISA.

Statistical analyses

Significant difference between the data obtain from tissues were analyzed using Duncan mean comparing test. The data were significant at $p \leq 0.05$.

RESULTS

No mortality or presence of virus was observed in the control fish group. 6 days following the challenge experiment mortality was reported. The number of dead fish at beginning was high but a decreasing in mortality was noticeable after five days. Few mortality cases were observed during day 11 to 21 with no death reported after that. The Elisa test confirmed that VHS virus was the cause of death in experimental fish. The clinical signs in dead fish were dark color, pettechial haemorrhages at thebase of the fin, peduncle, skeletal muscles, adipose tissues, anal region, swim bladder, eyes and brain and also hyperemia of kidneys and paleness in liver and gills. The present study revealed that there was a significant difference between the virus tropism in various organs ($p \leq 5\%$). Heart and kidney showed the highest amount of virus, liver, gills, pyloric caeca and skin showed the lowest quantities while brain and spleen were in between (Table 1 and fig.1).

DISCUSSION

The titration results for each organ are summarized in Table 1. In the end stage of disease phase (12,13 and 14 d p.i.), virus was detectable in all samples but heart and kidney showed the highest viral titres. The present observation shows that brain samples are valuable for VHS virus isolation in disease phase and carrier stage. However, brain samples are almost equal to kidney and heart for virus detection (fig1). Previously brain was suggested as an organ for virus detection in convalescent phase of VHS disease by Wolf (1988) and Noga (1996). Our finding are in contrast with Øystein et al. (1994) who reported that virus is not detected in the brain section at any stage of the disease. The interaction of rhabdovirus with a cell depends on the presence of a receptor(s) molecule(s) on the cell and of a binding molecule (G) on the virion (Col, 1995).

The rate of virus titer about two weeks post infection was in the order kidney= heart > brain> spleen > liver > pyloric caeca > skin > gill (Table 1 and fig.1). The general opinion is that the gills are the prime portal of entry when virus is transmitted in water (Wolf 1988), although one report has focused on the epidermis and epidermal tissues as a possible site for entry and early virus replication (Yamamoto et al. 1992). It seems that virus elimination in skin and gills is faster than than in other organs but this still needs better characterization of virus target cell in VHS virus infections and virus elimination during the disease and reconvalescense phase of viral haemorrhagic septicaemia.

Table 1. *Oncorhynchus mykiss*. $TCID_{50}$/ml in different organs about 2 weeks after challenge by bathing in concentration 10^{-3} TCID50 VHSV ml^{-1} of water

Organs	Range of virus titers	Mean ± SD
Heart	2.8-4.4	3.5 ± 0.86
Kidney	2.9-4.6	3.47±.0.66
Brain	2.6-4.1	3.24±0.60
Spleen	2.4-3.9	3.17±1.07
Liver	1.4-3.1	2.54±0.67
Pyloric caeca	<1.3-2.9	2.33±0.73
Gill	1.3-2.8	2.27±1.01
Skin	<1.3-2.6	2.30±0.7

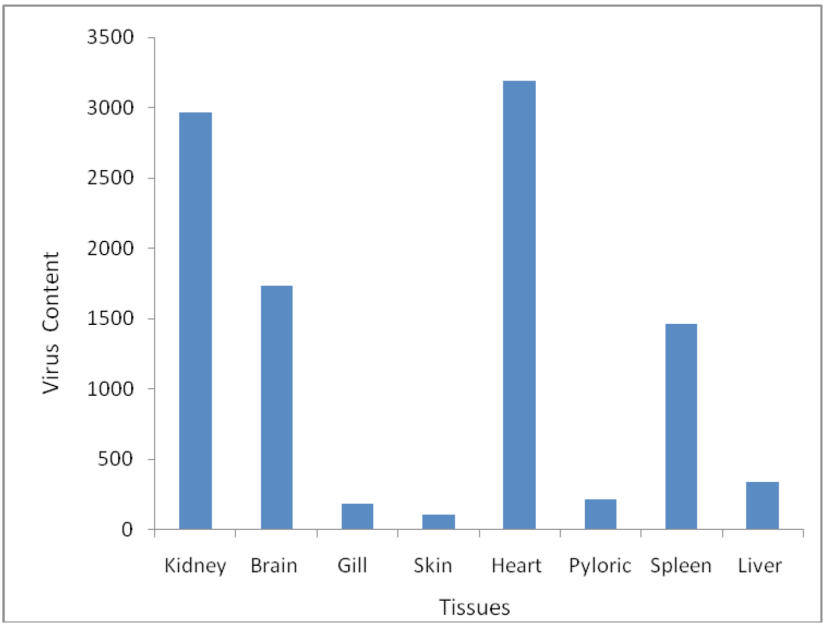

Figure 1. *Oncorhynchus mykiss*, Comparison of VHSV titer in different organs about 2 weeks after challenge by bathing in concentration 10^{-3} TCID50 VHSV ml^{-1} of water.

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THE INFLUENCE OF DIFFERENT THERMAL CONDITIONS ON THE PROXIMATE COMPOSITION OF RAINBOW TROUT FLESH FROM TWO FISH FARMS IN NW GREECE

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UTICAJ RAZLIČITIH TERMALNIH USLOVA NA HEMIJSKI SASTAV MESA KALIFORNIJSKE PASTRMKE SA DVE FARME U SEVEROZAPADNOJ GRČKOJ

Apstrakt

Kalifornijske pastrmke su gajene u različitim termalnim režimima. Jedna farma (A) se nalazila pored reke u planinskom regionu Epira, a druga (B) se nalazila u nizijskom delu, na obalskom delu Preveza. Na farmi A su preovlađivale niže temperature (10-11 ^{o}C prema 16-16.6 na farmi B). Analiza hemijskog sastava ukazuje da su različiti temperaturni režimi doveli do značajnih razlika u kvalitetu fileta I cele aksijalne muskulature. Ruba sa farme B je imala veću stopu rasta I sadržaj lipida u filetima. Ovo istraživanje je ko finansirano od strane Evropske Unije (Evropski socijalni fond, European Social Fund – ESF) i Grčkih nacionalnih fondova kroz Operativni program «Obrazovanja i celoživotno učenje - Education and Lifelong Learning «Nacionalnog Strateškog referentnog okvira (NSRF) – Program finansiranja istraživanja ARCHIMEDES III – Investiranje u društvo znanja kroz Evropski socijalni fond.

Ključne reči: kalifornijska pastrmka, akvakultura, mišić riba, temperatura

Abstract

Rainbow trout from two fish farms were reared under different thermal regimes, Farm Awas located by a river in a mountain region of Epirus and farm B was located in a lowland location at the coast of Preveza. Lower temperature prevailed in farm A (10-11 °C vs. 16-16,6 in farm B). Proximate composition analysis indicated that the different thermal conditions resulted in significant differences in the filleting yield and the proximate composition of rainbow trout axial musculature. The fish from the farm B exhibited higher growth rate and fillet lipid contents. This research has been co-financed by the European Union (European Social Fund – ESF) and Greek national funds through the Operational Program “Education and Lifelong Learning” of the National Strategic Reference Framework (NSRF) - Research Funding Program: ARCHIMEDES III. Investing in knowledge society through the European Social Fund.

Keywords: rainbow trout, aquaculture, fish muscle, temperature

THE PRESENCE OF ZINC IN MUSCLE TISSUE OF PRUSSIAN CARP AND BREAM IN THE GRUŽA AND BOVAN RESERVOIRS

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PRISUSTVO CINKA U MIŠIĆNOM TKIVU BABUŠKE I DEVERIKE U AKUMULACIJAMA GRUŽA I BOVAN

Apstrakt

Zagađenje slatkovodnih ekosistema širokim spektrom zagađivača postalo je veoma važno pitanje u poslednjih nekoliko decenija, ne samo zbog uticaj na vodosnabdevanje, već i zbog štete koju nanosi vodenim organizmima. Smatra se da su metali najvažniji oblik zagađenja voda zbog toksičnosti, perzistentnosti i akumulacije u vodenim organizmima. Vodeni organizmi su u širokoj upotrebi biološkog monitoringa promena nivoa antropogenih zagađujućih materija. Poznavanje koncentracije metala u ribama je važno kako za pravilno planiranje upravljanja vodama tako i u pogledu ljudske ishrane. Zn je esencijalni metal i kao takav igra važnu ulogu u biološkim sistemima, ali takođe prekomernim unosom može proizvesti toksičan efekat.

Akumulacije Gruža i Bovan su formirane osamdesetih godina za vodosnabdevanje, ali su danas i značajne ribolovne vode. Antropogeni uticaj na ove akumulacije je vrlo izražen. Konstantno je direktno ispuštanje neprečišćenih otpadnih voda, okolno zemljište se intenzivno obrađuje uz primenu invazivnih agrotehničkih mera (prekomerna upotreba pesticida, herbicida i fungicida), ali i širenje vikend naselja koja uglavnom nemaju regulisano skladištenje i ispuštanje otpadnih voda.

Babuška (*Carassius gibelio*) i deverika (*Abramis brama*) su prikupljene iz akumulacija Gruža i Bovan, a uzorci mišićnog tkiva su analizirani na prisustvo cinka (Zn) kako bismo utvrdili da li postoji razlika u akumulaciji ovog metala u mišićnom tkivu između ove dve vrste, kao i između iste vrste u dve različite akumulacije. Koncentracija Zn u mišićnom tkivu babuške je bila znatno veća nego u mićnom tkivu deverike u obe akumulacije, ali nije postojala bitna razlika u koncentracijama između dve akumulacije. Koncentracija cinka u mišićima obe vrste u obe akumulacije je na prihvatljivom nivou za ljudsku ishranu.

Ključne reči: babuška, deverika, akumulacija cinka, akumulacije Gruža i Bovan
Keywords: bream, Prussian carp, Zn concentrations, reservoirs Gruža i Bovan

INTRODUCTION

Water contamination with metals is a very important problem in the contemporary world, and their presence in the aquatic environment is a serious issue that threatens not only the aquatic ecosystems but also human health (Jianguo et al., 2007). Metals from natural and anthropogenic sources are continually being released into aquatic ecosystems, and they are a serious threat because of their toxicity, long persistence and capacity for bioaccumulation (Papagiannis et al., 2003). Mainly pollution sources leads to fish contamination with metals originated from industrial and domestic wastewaters (Tuzen, 2009). Zn is essential metal, since it play an important role in biological systems, but also can produce toxic effects when its intake is elevated excessively. Aquatic organisms are widely used to biologically monitor variation in environmental levels of anthropogenic pollutants (Farkas et al., 2003). Knowledge of metal concentrations in fish is important both with respect to nature management and human consumption of fish (Ebrahimpour and Mushrifah, 2010). Muscles are often a major tissue of interest for routine environmental monitoring, considering their implications for human consumption and potential health risk.

The aim of the present study was to assess the concentration status of Zn in two fish species (Prussian carp and bream) in two reservoirs (Gruža and Bovan) in order to determine whether there is a difference in the metal accumulation between species and between reservoirs. Moreover, the metal loading in fish muscle was determined to assess the risk to humans from the consumption of fish.

MATERIALS AND METHODS

The field work was conducted during the July of 2011 (Gruža Reservoir) and the May of 2012 (Bovan Reservoir). Two fish species: Prussian carp (*Carassius gibelio*) and bream (*Abramis brama*) were collected with nets of different lengths, widths and mesh diameters. The species were selected as those that are regular in fisherman catch in the study reservoirs. Specimens were sacrificed with a quick blow to the head, measured for their total weight (g) and standard and total body length (cm), and subsequently dissected. Mean values (±SD) for total length and weight of fishes in Gruža Reservoir were: 43 ± 16.57 cm and 1,120 ± 560.50 g for Prussian carp and 38 ± 9.09 cm and 608 ± 365.10 g for bream, respectively. Mean values (±SD) for total length and weight of fishes in Bovan Reservoir were: 35.33 ± 11.11cm and 883.66 ± 534.42 g for Prussian carp and 26.5 ± 2.17 cm and 585.33 ± 213.71 g for bream, respectively. Samples were removed from the right dorsal muscle, washed with distilled water and transferred to the laboratory.

In the laboratory, fish samples (~1.5 g) were digested in an Advanced Microwave Digestion System (ETHOS 1, Milestone, Italy) using a mixture of 65% nitric acid and 30% hydrogen peroxide (Merck, Darmstadt, Germany, 10:2 v/v) at 220ºC for 20 min. After cooling to room temperature and without filtration, the solution was diluted to a fixed volume (volumetric flask, 25 ml) with deionized water. Concentration of Zn was measured in fish muscle in triplicate using a Thermo Scientific iCAP 6500 Duo ICP instrument (Thermo Fisher Scientific, Cambridge, United Kingdom). The potential presence of trace elements in chemicals used in sample preparation was resolved by using a number of blank samples. Multi-elemental plasma standard solution, Multi-Element Plasma Standard Solution 4, Specpure®, 1000 µg/ml certified by Alfa Aesar GmbH & Co KG, Germany, was used to prepare calibration solutions for ICP-OES. The detection limit for Zn was 0.109 mg kg^{-1} mg kg^{-1}.

All concentrations were expressed as mg kg^{-1} wet weight (ww).

RESULTS AND DISCUSSION

Zn, as an essential mineral for both animals and humans, showed a protective effect against the Cd and Pb toxicity (Malik et al., 2010). On the other hand, in high concentrations can be toxic for aquatic organisms as well as for the human population.

Average concentration (mg kg^{-1} ww) of Zn found in the muscle tissue of Prussian carp and bream in two Reservoirs is given as Figure 1. As can be seen, Zn concentrations were generally lower in bream (4.49 and 4.56 mg kg^{-1}) than in Prussian carp (14.49 and 12.83 mg kg^{-1}). The investigated species are of the same family (Cyprinidae). Prussian carp (e.g. *Carassius gibelio*) is zooplankton feeder (Ebrahimpour et al., 2011), while bream (e.g. *Abramis brama*) is benthophagus. Because of breams diet mainly consists of invertebrates -chironomid larvae and other benthic organisms, the metal levels detected in bream muscle tissue reflect the pollution level of the sediment and its biota, rather than the prevailing pollution state of the water (Rajkowska and Protasowicki, 2013). Considering that the main food of carp is free-floating zooplankton, metal concentrations in the muscle tissue of carp reflects the degree of water pollution. In such conditions, according to previous studies (Farkas et al., 2002), the metal uptake from food is predominant.

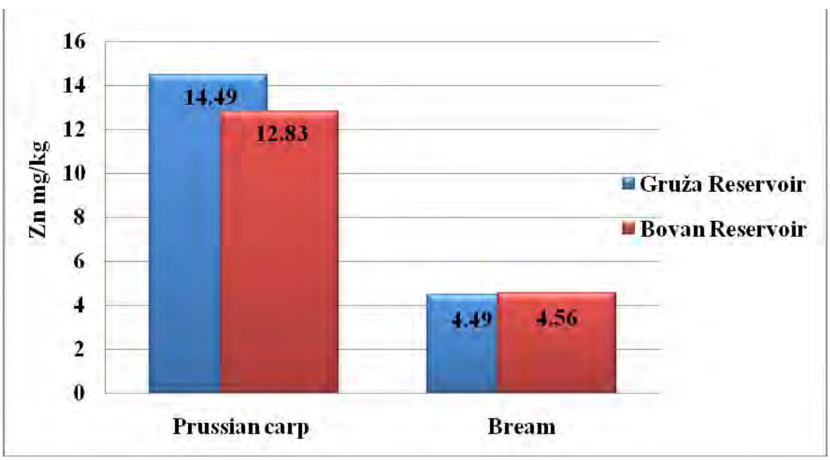

Figure 1. Comparative Zn concentrations in two fish species in two reservoirs

According to Ranković and Simić 2006, Gruža Reservoir is eutrophic reservoir as well as Bovan Reservoir (Ostojić, 2006). As seen in Figure 1, there were no differences in metal accumulation between two reservoirs which can be associated with same trophic level of reservoirs.

Concentrations of Zn were below national permitted concentrations (for canned fish meat, 100.0 mg kg^{-1} ww) in all analyzed muscle samples of two analyzed fish species, which indicates that the meat of studied species should be safe for utilization in human diet.

CONCLUSIONS

There was significant difference on Zn concentrations between species but no significant difference established between reservoirs. Also, meat of this two fish species from both reservoirs is safe for human consumption.

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CONCENTRATIONS OF 16 ELEMENTS IN TISSUES (LIVER, MUSCLE, SCALES) OF PRUSSIAN CARP (*CARASSIUS GIBELIO*, BLOCH, 1782) IN MEDJUVRŠJE RESERVOIR, SEASONAL ASPECT

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KONCENTRACIJA 16 ELEMENATA U TKIVIMA (JETRA, MIŠIĆ, ŠKRGE) BABUŠKE (*CARASSIUS GIBELIO*, BLOCH, 1782) AKUMULACIJE MEĐUVRŠJE, SEZONSKI ASPEKT

Apstrakt

Akumulacija Međuvršje nalazi se u izlaznom delu Ovčarsko-kablarske klisure. Dužina akumulacije iznosi 9.3km, a površina 1.5km^2. Najveća širina iznosi 272m, dok je maksimalna dubina do 12m zabeležena neposredno ispod brane. Dno akumulacije je najvećim delom muljevito, i u manjoj meri peskovito. Aktivnost HE Međuvršje dovodi do oscilovanja u litoralnoj zoni od 20 do 30 cm. Stalno taloženje rečnog nanosa dovodi do stvaranje malih zaravni koje usporavaju tok i menjaju izgled rečnog korita. Kako je akumulacija oivičena branama HE Ovčar i HE Međuvršje, od kojih ni jedna ne poseduje objekte za prelaz riba (tzv. riblje staze), uzvodne i nizvodne migracije i kontakti ribljih populacija su praktično onemogućeni što akumulaciju čini jedinstvenim zatvorenim sistemom.

Prema podacima koji su prikupljeni tokom terenskih istraživanja 2012. godine, akumulaciju Međuvršje naseljavaju 19 vrsta riba iz ukupno 6 familija. Akumulacija Međuvršje ima sastav ribljeg naselja koji ukazuje da je to ribolovna voda sa osobinama i elementima gornjeg toka šaranskih riba ili region rečne mrene (epipotamon) i srednjeg toka šaranskih riba ili region deverike (metapotamon). Riblju zajednicu akumulacije Međuvršje karakteriše dominacija uklije *(A.alburnus),* gavčice *(Rodeus sericeus)* i ba-

buške *(Carasius gibelio)*, uz zadovoljavajuću brojnost bodorke *(Rutilus rutilus),* skobalja *(Ch.nasus),* grgeča *(Perca fluviatilis)* i klena *(S.cephalus)*. Nepovoljnoj strukturi ihtiofaune doprinose „novi članovi" – alohtone vrste, babuška, cverglan (*Ameiurus melas)*, čebačok (*Pseudorasbora parva)* i sivi tolstolobik *(Hypophthalmichthys nobilis*) (Simović 2001).

Babuška (*Carassius gibelio* bloch 1782) živi u stajaćim i sporotekućim nizijskim vodama. Naseljava deo Azije i srednju Evropu, sa izuzetkom Italije, Švajcarske i južne Francuske. Nalažena je i u Sibiru sve do reke Lene. U naše predele introdukovana je iz jugoistočne Azije. Ima je u rekama Dunavskog sliva, a najbrojnija je u plavljenim zonama Dunava, Save, Tise, Begeja, Tamiša, u kanalskoj mreži Dunav-Tisa-Dunav, starim koritima Velike Morave i šljunkarama pored Dunavskih pritoka.

Ribe su često izložene visokom stepenu zagađenja u vodi, što može dovesti do čitavog niza različitih promena, od biohemijskih na nivou ćelija, do promena na nivou celih populacija (Bernet et al. 1999). S obzirom da se ribe nalaze na vrhu lanaca ishrane u vodenoj sredini, često u organizmu akumuliraju velike količine pojedinih teških metala (Yilmaz et al. 2007). Takođe se smatraju i jednim od najosetljivijih akvatičnih organizama na prisustvo toksičnih materija u vodi (Alibabić i sar. 2007).

Do sada je većina istraživanja pretežno bila usmerena na akumulaciju teških metala u mišićnom tkivu riba, pošto je to osnovni deo ribe koji se koristi u ishrani (Storelli et al. 2006; Keskin et al. 2007). Kao rezultat mehanizama absorpcije, regulacije, skladištenja i ekskrecije, tkiva se međusobno razlikuju po stepenu akumulacije, kao i po svojoj ulozi u ovim procesima (Storelli et al. 2006). Mišićno tkivo ne predstavlja uvek dobar indikator celokupne kontaminacije organizma, pa je stoga važno u analizu uključiti i druge organe, kao što su jetra i škrge (Has-Schön et al. 2006).

Tokom ihtioloških istraživanja na akumulaciji Međuvršje 2012. godine ispitivao se nivo akumulacije 16 metala u tkivima (mišić, jetra, škrge) babuske. Škrge su bile centar akumulacije Ba, Mn, Sr, Zn i B, dok je jetra centar akumulacije gvožđa i bakra. Mišići, su generalno imali niži nivo akumulacije u odnosu na druga dva tkiva. U mišićima su zabeležene koncentaracije žive iznad maksimalno dozvoljenih evropskom i nacionalnom legislativom.

Ključne reči: babuška, teški metali, koncentracija, akumulacija Međuvršje
Keywords: Prussian carp, heavy metals, concentrations, Međuvršje reservoir

INTRODUCTION

The heavy metals presence in aquatic ecosystems, in recent times, is paid considerable attention due to their toxicity and effects on living organisms (Dural et al. 2006). Contamination of aquatic ecosystems (e.g. lakes, rivers, streams) with heavy metals and trace elements has been receiving increased worldwide attention (Mansour and Sidky, 2002). Metallic elements are environmentally ubiquitous, readily dissolved in and transported by water and readily taken up by aquatic organisms. These elements enter in aquatic environment by atmospheric deposition, by erosion of the geological matrix, or through anthropogenic sources, such as industrial effluents and mining wastes, agriculture (Alam et al., 2002, Kumar Singh et al., 2007). They are a serious threat because of toxicity, bioaccumulation effects, long persistence, and biomagnifications in the food chain (Erdogrul and Ates, 2006).

Fish are at the top of the food chain in aquatic ecosystems, which is the reason why some heavy metals accumulate in their body (Yilmaz et al., 2007, Mansour and Sidky, 2002). Fish is also considered as one of the most sensitive groups of organisms in the presence of toxic substances in the water (Alibabić et al., 2007). Fish meat is an essential component of the human diet, there is therefore a great interest in the scientific community for the presence of heavy metals in water and their accumulation in fish tissues of (Dural et al., 2006; Storelli et al., 2006; Alibabić et al., 2007 ; Erdogrul and Erbilir, 2007; Keskin et al., 2007). Most of the research, so far, has been mainly focused on the muscle tissue of fish (Storelli et al., 2006; Keskin et al., 2007). But muscles are not always the best indicators of overall contamination in the body, and therefore it is important to include other organs such as liver and gills in the analysis (Has-Schön et al., 2006). Monitoring of heavy metal contamination of commercially important fish species is of great importance (Erdogrul and Erbilir, 2007; Yilmaz et al., 2007).

Prussian carp is a non-indigenous, invasive fish species, introduced in Europe (European part of USSR) in 1948 (Maletin et al., 1981). In Serbian open water prussian carp appeared at the begining of the 1960s (Plancic, 1967), and in Medjuvrsje reservoir it was registered for the first time in 1984 (Simović, 2001). After that, prussian carp has been mass present in all fish fauna inventories (Marković, 2003, 2007; Skorić and Đikanović, 2012), and it is the most common species in the catch of recreational fishermen on the reservoir.

Medjuvrsje reservoir is located in the output section of Ovčarsko-Kablarska gorge. It is one of the oldest reservoirs in Serbia, formed in 1953 by damming the Zapadna Morava River. Water quality of Medjuvrsje reservoir is greatly influenced by many pollutants of industrial, municipal and rural wastewater.

MATERIAL AND METHODS

Collection of fish samples was performed during 2012. with seasonally dinamic (April, June, November) using a set of standing gill-nets with buds (mesh) diameter of 30 - 50 mm. The total weight (g) and total body length (cm) of each fish specimen were measured. Samples of muscle, liver and gills were removed on field and frozen prior to analysis. In laboratory, samples were dried by Freeze Dryers Rotational-Vacuum-Concentrator, GAMMA 1-16 LSC, Germany, and sample portions between 0.2 and 0.5 g dry weight were subsequently processed in a microwave digester (speedwave MWS-3þ; Bergof ProductsþInstruments GmbH, Eningem, Germany), using 6 ml of 65% HNO_3 and 4 ml of 30% H_2O_2 (Merck suprapure) at a food temperature program (100–170 1C). Potential presence of analyzed elements was resolved by using a number of blank samples. Following a cooling to a room temperature, the digested samples were diluted with distilled water to a total volume of 25 ml. The analysis was performed by inductively-coupled plasma optical spectrometry (ICP-OES). It included the assessment of concentrations of the following 16 elements: As, B, Ba, Cd, Co, Cr, Cu, Fe, Hg, Li, Mn, Mo, Ni, Pb, Sr and Zn. The quality of the analytical process was controlled by the analysis of BCR-185R reference material of bovine liver, as well as IAEA-336 Lichen reference material. All elemental concentrations were expressed as mg g^{-1} dry weight (dw).

Statistical analysis included comparisons of elemental concentrations between three tissues of the prussian carp individulas (Discriminant Analysis - Multivariate Exploratory Techniques), as well as among different elemental concentration in tissues per season (ANOVA - Tukey test).

RESULTS AND DISCUSSION

During this study 30 individuals of prussian carp have been analysed. Mean body weight of individuals was 184.51 ± 92.43 g (mean ± standard deviation, range 66 – 446 g), total body length 22.07 ± 3.57 cm (range 16 – 30 cm). The determined concentrations of heavy metals and trace elements in three tissues analyzed are shown in Table 1. The analysis showed that the Cd in all samples was below the detection limit.

Table 1. Heavy metal and trace elements concentrations in muscle of selected fish specie s (mean values±SD). Concentrations are expressed as µg/g of dry weight.

		APRIL	JUNE	NOVEMBER
As µg/g	liver	0.976±0.121	0.803±0.536	1.885±1.846
	gills	0.375±0.224	0.357±0.264	1.748±1.13
	muscle	0.693±0.379	0.618±0.317	2.103±1.166
B µg/g	liver	19.746±4.039	33.667±15.950	8.193±7.894
	gills	29.281±8.917	18.099±2.503	10.766±9.209
	muscle	20.815±7.737	13.687±0.745	7.788±8.018
Ba µg/g	liver	4.490±2.622	7.470±2.493	6.255±3.372
	gills	31.662±12.288	22.169±4.389	31.652±8.135
	muscle	3.258±2.908	0.533±0.196	0.713±0.665
Co µg/g	liver	0.180±0.028	0.189±0.128	0.052±0.062
	gills	0.210±0.079	0.167±0.022	0.169±0.076
	muscle	0.055±0.035	0.050±0.029	0.020±0.025
Cr µg/g	liver	0.284±0.175	0.628±0.465	0.487±0.302
	gills	0.660±0.281	0.657±0.297	1.161±0.335
	muscle	0.068±0.066	0.118±0.056	0.105±0.091
Cu µg/g	liver	80.783±81.257	130.744±94.97	20.484±18.737
	gills	15.213±31.116	3.943±0.662	5.807±0.618
	muscle	3.651±1.013	2.674±1.002	8.593±18.833
Fe µg/g	liver	255.133±31.666	399.693±280.945	236.519±118.476
	gills	250.6769±97.911	285.192±106.438	496.082±182.12
	muscle	29.866±12.55	24.890±15.754	60.399±111.182
Hg µg/g	liver	3.832±1.297	8.619±3.906	2.300±1.545
	gills	5.611±2.23	4.506±0.663	2.993±1.636
	muscle	4.487±0.986	4.112±0.24	2.946±1.641
Li µg/g	liver	2.822±0.990	6.368±3.125	1.529±1.349
	gills	4.151±1.558	3.613±0.978	3.785±0.618
	muscle	2.742±0.422	2.684±0.219	1.609±1.48

Mn µg/g	liver	2.629±1.520	3.763±1.299	2.783±4.245
	gills	25.344±8.870	23.863±4.006	32.895±6.447
	muscle	1.444±0.431	1.160±0.395	1.262±0.574
Mo µg/g	liver	3.705±0.674	7.406±3.626	3.261±1.454
	gills	4.644±1.963	3.540±0.598	2.946±0.541
	muscle	3.468±0.597	2.864±0.285	2.952±0.384
Ni µg/g	liver	0.038±0.0363	0	0.161±0.225
	gills	0.132±0.172	0.033±0.060	0.954±0.840
	muscle	0.201±0.450	0	0.035±0.110
Pb µg/g	liver	0.649±0.226	1.044±0.728	3.432±2.247
	gills	1.115±0.992	1.078±1.104	11.588±6.771
	muscle	0.535±0.346	0.406±0.284	0.453±0.498
Sr µg/g	liver	0.415±0.69	1.95±0.914	1.118±1.018
	gills	110.56353±22.85251	98.058±24.712	103.753±18.637
	muscle	5.794±10.101	2.443±1.251	3.076±2.033
Zn µg/g	liver	111.46±31.383	111.46±31.383	52.363±13.06
	gills	225.06±73.758	225.06±73.758	278.821±72.334
	muscle	55.536±25.412	55.536±25.412	56.883±18.882

In analyzed fish tissues, As was distributed like liver>muscle>gills, while Cu was staggered muscle>liver>gills. By tissues, Hg was distributed uniformly.

The extent of accumulation significantly differed among the three studied fish tissues (Discriminant Analysis) (Table 1, Figure 1). In this study, gills were the center of accumulation of Ba, Mn, Sr, Zn and B. Liver was the center of accumulation of Fe, Cu while the muscle had the high levels of Fe and Zn. The muscle generally had lower elemental levels when compared with those detected in other tissues. Other elements do not show a clear pattern of accumulation already occurring seasonal variations of concentrations in different tissues. These results are similar with concentration of the same studied elements in research of relation between great cormorants and their prey (prussian carp) (Skoric et al., 2012).

To compare observed heavy metal levels with the maximum acceptable concentrations (MAC) in fish meat provided by both EU and national regulation, all concentrations were recalculated to wet weight. According to the European Commission Regulation (1881/2006/EC), the MAC for Pb, Cd and Hg in fish meat for human consumption are, respectively, 0.3, 0.05 and 0.5 µg/g wet weight. National regulation of Republic of Serbia prescribed 0.3, 0.05, 0.5 and 2 µg/g wet weight as MAC for Pb, Cd, Hg and As in fresh fish meat, respectively (Anonimus, 2011). National MAC for Zn, Cu and Fe are 100.0, 30.0 and 30.0 µg/g wet weight, respectively (for canned fish meat). Hg concentrations in the prussian carp muscle were above MAC for fish meat provided by EU and national regulation of the Republic of Serbia, while the levels of other heavy metals were below the permissible levels.

Remarkably higher concentration of Cu in liver with regard to other analyzed tissues, were registered also in some other researchers (Rashed 2001; Storelli et al. 2006; Wu et al. 2006; Farag et al. 2007; Yilmaz et al. 2007; Uysal et al. 2009).

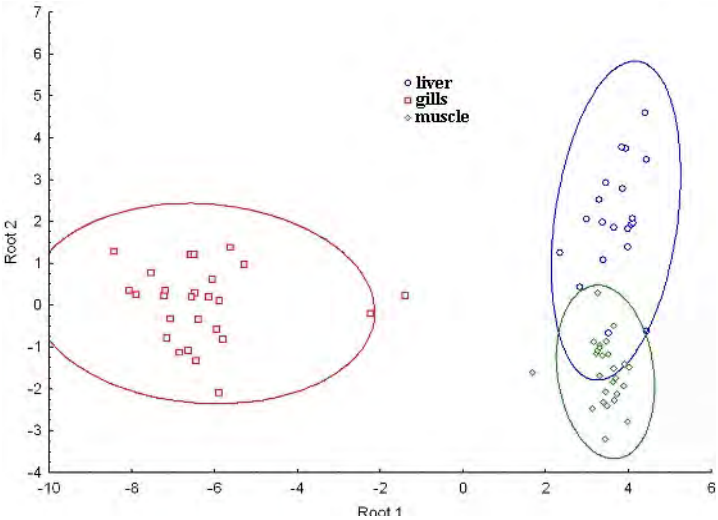

Figure 1. Distribution of elemental concentration in prussian carp tissues (liver, gills, muscle).

Comparison of the extent of accumulation among analyzed fish tissues showed that the tissue concentrations significantly differed ($p<0.05$; Table 1) for five of the assessed elements (B, Cu, Fe, Sr, Zn), depending of the sampling season. Concentrations of Zn, Fe, Sr were higher in gills with maximum values in November. The highest concentrations of these heavy metals in liver were in June. In muscle concentrations were similar while Fe was the highest in November.

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CONTENT AND DISTRIBUTION OF HEAVY METALS IN ORGANS AND TISSUES OF FRESHWATER FISH IN THE ZAPADNA MORAVA RIVER

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SADRŽAJ I DISTRIBUCIJA TEŠKIH METALA U ORGANIMA I TKIVIMA SLATKOVODNIH RIBA ZAPADNE MORAVE

Apstrakt

Uzimajući u obzir da se u vodenoj sredini ribe nalaze na vrhu lanca ishrane, česta je pojava da u svom organizmu akumuliraju određene količine teških metala zbog čega se smatraju jednim od najosetljivijih akvatičnih organizama na prisustvo toksičnih materija. Pošto meso ribe predstavlja jednu od značajnih komponenti ljudske ishrane, prekomerna akumulacija teških metala u muskulaturi riba čini ovu namirnicu zdravstveno nebezbednom za ishranu ljudi.

U radu su analizirane koncentracije teških metala kadmijuma (Cd), olova (Pb), žive (Hg) i cinka (Zn) u mišićnom tkivu i jetri bentosnih i omnivormih vrsta riba, uzorkovanih u ekosistemu Zapadna Morava. Cilj rada bio je dobijanje potpunijeg uvida u stepen akumulativnosti ili opterećenosti teškim metalima, kako vode kao ambijenta u kojoj ribe žive, tako i ribljeg mesa kao namirnice.

Na osnovu ispitivanja prisustva teških metala u srebrnom karašu (*Carassius gibelio*) i deverici (*Abramis brama*) utvrđeno je prisustvo sva četiri analizirana metala, ali u koncentracijama ispod maksimalno dozvoljenih količina (MDK). Na osnovu utvrđenih rezultata može se konstatovati da ekosistem Zapadne Morave pokazuje trend poboljšanja kvaliteta u pogledu opterećenosti antropogenim zagađenjem i da je meso ispitivanih vrsta riba zdravstveno bezbedno i higijenski ispravno za ishranu ljudi. Takođe, relativno niski sadržaji teških metala u jetri riba jasno ukazuju da je u ovom ekosistemu došlo do znatnog sniženja stepena zagađenosti vode i sedimenta u odnosu na ranije periode ispitivanja.

Ključne reči: teški metali, reka Zapadna Morava, deverika, srebrni karaš
Keywords: heavy metals, Zapadna Morava River, bream, silver carp

INTRODUCTION

Fish meat is highly appreciated food in human diet. Besides nutritional value in terms of consumer, its hygienic and health safety is also significant. It is well known that the fish population from land waters (open and closed) is important as natural biological resource of animal proteins needed in human diet. Although man usually use fish meat supplies from the sea and aquaculture, it is not insignificant amount of fish which we got by commercial and recreational fishing in freshwaters.

Most of freshwater ecosystems are, due to urbanization, industrialization and other forms of human activity, permanently exposed to the influence of various forms of water pollution. Because of that, biota representatives in it, and especially fish populations, suffers serious consequences, but also a man as their consumer. Fish come to contact with these pollutants through skin or gills and by feeding (plankton, benthic fauna, detritus, plant material), while predatory fish species intake metals into the body through other fish with which they are fed. This way, metals enter directly through bloodstream into the liver, kidneys and muscle tissue, where they are, more or less, accumulated (Has-Schön et al. 2006).

According to available literature data, fish population of The Zapadna Morava River ecosystem are systematically exposed to influence of numerous polluters, especially toxicants of anthropogenic origin, generally known as POPs - *Persistent organic pollutants* (Veljović and Spasojević, l992; Lazić et al., 2003; Marković and Lenhardt. 2007).

The main goal of this researche is to determine accumulation level of heavy metals (lead, cadmium, mercury and zinc) in muscle tissue and liver of Prussian carp (*Carassius gibelio*) and bream (*Abramis brama*). Thus we would be able to estimate possibility of using different fish tissue and organs as heavy metals pollution indicator, as well as assessment of health safety of their meat in human diet. At the same time information about concentration of metals stored in the fish liver would indicate the degree of water and sediment pollution in the ecosystem of The Zapadna Morava River.

MATERIAL AND METHODS

Heavy metal contamination of muscle tissue and the liver of fish species were analyzed, for Prussian carp as allochthonous species and bream as autochthonous species in ecosystem of The Zapadna Morava River, profile site Stančići 9 km location downstream from Čačak.

In choosing this fish species it was taken care of its trophic level and their representativenes in aquatic ecosystem. Both examined species are benthic and omnivorous, as such they accumulate the highest concentration of examined heavy metals. As for the choice of heavy metals whose content was analyzed in fish muscles and liver, we chose cadmium and lead as highly toxic, zinc because of its competitive bond with cadmium and highly toxic mercury whose toxic effect on fish manifests at concentrations of 0,003 mg/l in water.

The fish were caught by using nets with different mesh size, so that there would be specimen representatives of different age and size in the sample. In total we caught 19 specimens, 11 individuals of Prussian carp and 8 individuals of bream. After dissection we took samples of muscle tissue (left dorsal muscle) and liver, by which the collective composite samples of muscle tissue and liver were prepared for each fish species separately. Analyses were conducted according to standard methodology for quantitative analysis for heavy metals, by which we used necessary instrumental techniques. By atomic absorption spectrometry method (flame technique - FAAS) we determined contents of Cd, Pb and Zn, while the content of Hg in tissues and organs was determined by HGAAS method (absorption spectrometry method - hydride technique). Determined concentrations of heavy metals in edible parts of the fish were compared to allowed values of maximum acceptable concentrations (MDK) provided by national regulation (Anonymous 2011Gazette Republic of Serbia 25/2010, 28/2011).

RESULTS AND DISCUSSION

Results of analyzed parameters in fish muscle and liver are shown in Table 1.

Table 1. Content of heavy metals in fish samples wet weight, mg/kg

Fish species	Organ	Cd	Pb	Hg	Zn
Prussian **carp** n = 11	muscle tissue	0,004	0,324	0,135	17,392
	liver	0,084	0,372	0,152	18,190
Bream n = 8	muscle tissue	0,005	0,193	0,098	19,286
	liver	0,012	0,231	0,112	32,152

Based on this table results, we can conclude that the concentration of heavy metals in examined fish muscle mass was lower in relation to their content in the liver. The lowest values of metal in muscle tissue were established for cadmium and they varied from the range of 0,004 mg/kg in Prussian carp to 0,005 mg/kg in bream. These are below the prescribed MDK.

Reference concentrations are determined in terms of the content of analyzed metals in fish muscles. Average value of lead was 0,324 mg/kg in Prussian carp, while it was 0,193 mg/kg in bream. Maximum acceptable lead concentration, according to regulations is 0,3mg/kg. Mercury concentration varied in range of 0,098 mg/kg in bream to 0,135 mg/kg in Prussian carp. Upper limit of MDK for this parameter in edible parts of the fish is 0,5 mg/kg. Content of zinc was also acceptable and it varied from 17,392 mg/kg in Prussian carp muscle mass to 19,286 mg/kg in bream. By the book of regulations, the allowed concentration for this metal was not determined, but according to the same legislation, maximum content of Zn in fish products and tin containers is up to 100 mg/kg.

Comparing the average values of content for examined metals in muscle tissue of Prussian carp and bream, to the data quoted by other researchers (Veljović etal., l992;

Lazić et al., 2003; Marković and Lenhardt 2007), it can be concluded that accumulation of metals in fish from The Zapadna Morava River is decreased in comparison to previously determined values.

The part of the research related to metal content in liver of analyzed fish species clearly show that aquatic ecosystem of The Zapadna Morava River is not loaded with pollutions from industrial and communal waste, although this ecosystem is their main recipient. The reason for this is lower level of industrialization and closing of many factories.

Determined concentrations of heavy metals in Prussian carp and bream liver (Table 1) varied within normal physiological concentrations for all four chemical elements and were below the level of tolerance for human consumption (Biro et al., 1991). This means that the water flow of The Zapadna Morava River on examined profile,can be considered relatively pollution free in terms of loading with heavy metals (Cd, Pb, Hg and Zn). According to Oloj et al., (2005) because of biological degradability, and ability to accumulate along the food chain, heavy metals are considered to be one of the main pollutants of internal fish organs and entire aquatic system.

The results of this research point to the necessity of continuous sampling and analyzing this water flow in order to prevent accidental situations and maintaining health safety of fish meat in this water flow. To be able to make the final assessment of the hygienic quality of fish meat for human consumption and the ecological status of this ecosystem, it is necessary to conduct complex analyses, which would consider: determination of heavy metals content in water, sediment and commercially important fish species from ichthyofauna of the Zapadna Morava River. The necessity of this type of research suggest some other authors as well (Đukić et al., 1998., Teodorović et al.1999., Farkas et al., 2002).

CONCLUSIONS

Considering that the fish are usually at the top of the food chain, they often accumulate huge amounts of certain heavy metals, so they are considered to be one of the most sensitive aquatic organisms to presence of toxic substances. Since the fish meat presents one of the essential components of human nutrition, excessive accumulation of heavy metals in fish muscles make this food unsafe for human consumption.

The content of heavy metals (Cd, Pb, Hg and Zn) in muscle tissue of benthic and omnivorous fish species of Prussian carp and bream from The Zapadna Morava River, was within MDK limits and in accordance with established legal acts on the contents of metals and other toxic substances that can be found in food.

Based on conducted researche, we can conclude that the ecosystem of The Zapadna Morava River shows the trend of improvement in the quality of the load in terms of anthropogenic pollution, for the registered amounts of heavy metals stored in the liver of silver carp and bream varied in the range of normal physiological concentrations.

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METAL POLLUTION INDEX AS A TOOL FOR ASSESSING WATER QUALITY OF BOKA KOTORSKA BAY

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INDEKS ZAGAĐENJA METALIMA KAO SREDSTVO ZA PROCENU KVALITETA VODE ZALIVA BOKA KOTORSKA

Apstrakt

Zaliv Boke Kotorske nalazi se u jugoistočnom delu Jadranskog mora i jedan je od najlepših zaliva sveta. Uzak i dugačak zaliv koji se u kopno urezuje u dužini od 20 km ima oblik strele i sastoji se od četiri manja zaliva: hercegnovski, tivatski, risanski i kotorski zaliv. Svaki od ovih zaliva ima specifične hidrografske i reljefne karakteristike i, u odnosu na otvoreni deo crnogorskog primorja, ove vodene površine pokazuju veliku različitost, a samim tim poseduju i specifičan morski život. Kao i ostala područja Mediterana i Jadranskog mora, i Bokokotorski zaliv je pod velikim uticajem antropogenog faktora i aktivnosti koje se odvijaju na njegovoj obali. Poslednjih decenija evidentna je sve veća urbanizacija i industrijalizacija, koja je dovela do obimnog naseljavanja crnogorskog primorja, naročito Bokokotorskog zaliva, što je uslovilo zagađenje životne okoline zaliva, naročito morske vode za koju je karakteristično slabo strujanje i cirkulacija sa otvorenim morem. Otpad iz različitih industrija, brodogradilišta, hotela i bolnica stacioniranih unutar samog zaliva, ispuštaju se u more i predstavljaju konstantan izvor zagađenja vodene sredine. Ova regija je poznata kao područje sa najvećom prosečnom količinom padavina u Evropi, pa udeo zagađujućih meterija koje spiranjem zemljišta (vodena erozija, rastvaranje stena i abiogene podloge), usled velikih količina atmosferskih padavina, dospevaju u more nije zanemarljiv. Na ovaj način u morsku sredinu se unose različite vrste zagađujućih materija, među kojima su najopasniji teški metali, kao što su živa, olovo, kadmijum, kobalt, nikl, itd. Teški metali predstavljaju ozbiljne zagađujuće materije morske sredine, pre svega zbog svoje toksičnosti, teške biorazgradivosti i dugog zadržavanja u biogeohemijskom ciklusu.

Neki vodeni organizmi, među kojima i dagnja *Mytilus galloprovincialis*, imaju sposobnost da apsorbuju razne zagađujuće materije iz vode i predstavljaju veoma pogodan indikator za analizu zagađenja sredine koju nastanjuju, odnosno, u slučaju dagnji, mogu se koristiti kao sredstvo za procenu kvaliteta vode.

Cilj ovoga rada bio je određivanje sadržaja teških metala (Pb, Ni, Co, Cd i Hg) u dagnjama *M. galloprovincialis* sa određenih lokacija Bokokotorskog zaliva u tri sezone 2008. godine. Na osnovu dobijenih vrednosti koncentracija teških metala određivan je indeks zagađenja metalima (*MPI*) kao sredstvo za procenu kvaliteta vode zaliva Boka Kotorska.

Indeks zagađenja metalima predstavlja ukupni kvalitet posmatrane životne sredine u odnosu na koncentraciju teških metala i njegova upotreba se pokazala kao veoma korisno sredstvo u proceni nivoa zagađenja. Ovaj indeks pruža mogućnost da se na jednostavan način uporedi stepen zagađenja različitih lokacija i mesta uzorkovanja.

Najveće koncentracije ispitanih teških metala u većini ispitanih uzoraka izmerene su u zimskom periodu 2008. godine. Ovaj podatak se može objasniti smanjenjem mase jestivog dela dagnje tokom zimeodnosno činjenicom da se zbog smanjene količine hrane u zimskom periodu masa jestivog dela školjke smanjuje, dok sadržaj već akumuliranih teških metala ostaje isti, pa se stoga koncentracija teških metala u odnosu na masu jestivog dela školjke povećava.

Na osnovu vrednosti *MPI*a možemo reći da se lokacije iz Tivatskog zaliva, pre svega lokacija Tivat, izdvajaju kao lokacije sa najvećim indeksom zagađenja metalima u odnosu na druge ispitivane lokacije. Ovaj podatak ne iznenađuje, jer se radi o zalivu sa najvećim brojem antropogenih izvora zagađenja, pogotovu lokacija Tivat (aerodrom, vojna luka, brodogradilište, poljoprivredna aktivnost). Hercegnovski zaliv je sledeći po zagađenju morske vode, dok je najmanje zagađenje dobijeno za lokaciju Sv. Stasija iz Kotorskog zaliva.

Na osnovu dobijenih podataka, a na osnovu njihove analize može se zaključiti da indeks zagađenja metalima predstavlja korisno i moćno sredstvo za procenu kvaliteta vode zaliva Boka Kotorska.

Ključne reči: dagnja, olovo, nikl, kobalt, kadmijum, živa, MPI, Bokokotorski zaliv
Keywords: mussel, lead, nickel, cobalt, cadmium, mercury, MPI, Boka Kotorska bay

INTRODUCTION

Living organisms have been used as water pollution bioindicators (Lafabrie et al., 2007; Kouba et al., 2010; Yap et al., 2011, Stanković and Jović, 2012). Many microelements are found in trace amounts in sea water, but often at elevated levels in aquatic organisms, making them even more attractive for bioindication (Morillo et al., 2005). Currently, there is a great interest in the use of living organisms as pollution bioindicators in aquatic ecosystems in order to evaluate the quality of a marine environment.

Human and industrial activities in the coastal area of the southeastern Adriatic have increased and resulted in the different types of pollutants. As the Montenegrin coastal area, especially the Boka Kotorska bay, receives a heavy influx of sewage and industrial effluents as well as domestic and agricultural wastes (Jović et al., 2011), it is a great entry of a diverse array of polluting agents including trace metals, which are potentially toxic. Due to its specific structure, semi-enclosed bay system and low flow of water through the Bay, the anthropogenic impact is more pronounced and this pollution leaves direct effects on this unique ecosystem and organisms.

Mussel *Mytilus galloprovincialis*, native species of the Mediterranean Sea, of the Black Sea and of the Adriatic Sea, is a sedentary, filter-feeding animal that through feeding, not only assimilates the food necessary for growth and development, but also accumulates contaminants present in the water (Jović et al., 2011). The Metal Pollution Index which represents the overall quality of environmental compartments with respect to metals and its use has proved to be a very useful tool in evaluating pollution level (Usero et al., 2005; Wang,.2007; Sharma et al., 2008).

The aims of this study were to determine the concentrations of Pb, Hg, Ni, Co and Cd in the soft tissue of mussels *M. galloprovincialis* from Boka Kotorska bay and to calculate the Metal Pollution Index (*MPI*) in order to compare the pollution status of different places and to establish locations in Boka Kotorska bay with the highest or lowest level of pollution.

MATERIALS AND METHODS

Fresh mussels (*M. galloprovincialis*) were sampled from five different locations within the Boka Kotorska bay: Herceg Novi (H1), Sv. Stasija (K1), Krasici (T1), Tivat (T2) and Opatovo (T3). From each location more than 2 kg of mussels of similar length were collected, placed in plastic bags together with the sea water and transported to the laboratory. The 25–30 mussels from each station were pooled. Mussels were cleaned and rinsed with deionized water, dissected fresh and the soft tissue was rinsed with Milli Q water. Pooled samples were pulverized and homogenized using a mill.

About 0.5 g of the mussel samples were digested with 7 ml of HNO_3 (65%), 2 ml of H_2O_2 (30%) in a microwave digestion system for 30 min and diluted to 25 ml with deionized water. A blank digest was performed in the same way.

Analyses of lead (Pb), cobalt (Co), nickel (Ni) and cadmium (Cd) were performed using Graphite Furnace AAS (Perkin-Elmer, 4100ZL, with Zeeman background correction). Cold vapor technique was used for analyses of mercury (Hg) (PerkinElmer, AAnalyst 200). The accuracy of the applied analytical procedure for the determination of trace elements in mussels was tested using SRM 2976 (Mussel homogenate; NIST) certified reference material.

RESULTS AND DISCUSSION

The concentrations (range and mean) of Pb, Hg, Ni, Co and Cd found in *M..galloprovincialis* sampled from different locations in the Boka Kotorska bay during different seasons of 2008 are given in Table 1.

Table 1. Range and mean metal concentrations (mg kg^{-1} dry wt) in *Mytilus galloprovincialis* in winter, spring and fall 2008

	Pb	Hg	Ni	Co	Cd
winter	(2.0–5.5) *3.6*	(0.61–1.2) *0.86*	(4.11–8.65) *5.74*	(8.02–12.0) *9.63*	(2.76–3.67) *3.31*
spring	(1.5–5.0) *3.1*	(0.12–0.85) *0.34*	(1.50–4.11) *2.51*	(3.05–4.81) *3.51*	(1.05–1.52) *1.26*
fall	(2.5–9.0) *5.1*	(0.20–0.57) *0.30*	(1.52–3.40) *1.99*	(3.05–6.55) *4.57*	(1.50–1.95) *1.65*

The overall metal content of mussels from the investigated locations was compared using the Metal Pollution Index (*MPI*). MPI is obtained by the following equation:

$MPI = (C_1 \times C_2 \times \ldots \times C_n)^{1/n}$,

where C_n is the concentration of the metal n in mussels and n is the total number of metals (Usero et al., 2005).

Values of the *MPI* for the investigated mussel samples are shown in Table 2.

Table 2. Metal Pollution Index (*MPI*) of each site in different seasons

	Metal Pollution Index		
	winter	**spring**	**fall**
T1	2.91	1.56	1.52
T2	4.03	1.84	2.40
T3	3.89	1.50	1.98
K1	2.85	1.26	1.41
H1	3.77	1.35	1.68

Nickel, lead, cobalt, cadmium and mercury, the most commonly analyzed toxic trace elements in aquatic environments, are quite sensitive to anthropogenic influences (e.g. urbanization, industrialization, agriculture). *MPI* index is easily evaluated in order to compare the pollution status of different locations and for assessing water quality.

The highest values of *MPI* were obtained for the winter period. Explanation for this could be found in the reduction of dry weight of the edible part of *M. galloprovincialis* with respect to shell weight during the winter. Due to decreased food assimilation in winter the edible body mass decreases and the metal content remains the same, but concentration expressed in relation to edible body mass therefore increases (Klaric et al., 2004).

Comparing the *MPI* values by locations, it can be seen that the highest concentrations and *MPI* values were recorded for the site T2 in Tivat bay for the all three seasons. It comes as no surprise that the highest concentrations of toxic elements were found in mussels from Tivat bay, because the anthropogenic activities (airport, military harbor, shipyards, oil tankers, agricultural, *etc*.) are the highest on this location. According to the obtained metal concentrations as well as of the *MPI values,* location Herceg Novi in Herceg Novi bay is the next most polluted part of the Boka Kotorska bay. This bay also has pollution problems, primarily with urban, touristic and industrial effluents. In the case of Herceg Novi bay, which is located near to the entrance in Boka Kotorska bay, a major impact on the reducing of contamination with toxic metals is because of the mixing of the water from the Bay with the water from the open sea. Location Sv. Stasija in Kotor bay showed the lowest contamination by the investigated metals.

CONCLUSION

Investigated organism, mussel *Mytilus galloprovinciails,* indicated that the highest polluted area is Tivat bay, while the lowest polluted area is Kotor bay. The site Tivat within the Tivat bay stands out with the highest *MPI* values for all three studied seasons.

Based on all, Metal Pollution Index (*MPI*) has proved to be a very useful and powerful tool for assessing water quality of Boka Kotorska bay.

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ORIGIN AND GENETIC STRUCTURE OF SEVERAL HUNGARIAN WILD AND DOMESTICATED BROWN TROUT POPULATIONS BASED ON PCR-RFLP AND MICROSATELLITE MARKERS

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POREKLO I GENETIČKA STRUKTURA NEKOLIKO MAĐARSKIH DIVLJIH I DOMESTIFIKOVANIH POPULACIJA POTOČNE PASTRMKE NA OSNOVU PCR-RFLP I MIKROSATELITSKIH MARKERA

Apstrakt

U Evropi je, na osnovu studija mitohondrijalne DNK, identifikovano pet evolutivnih linija potočne pastrmke *(Salmo trutta m. fario* L. 1758): Atlanska, Dunavska, Mediteranska, Jadranska i Mramorna. Mađarske linije bi teorijski trebalo da pripadaju Dunavskoj liniji na osnovu hidrogeografije zemlje, ipak, ovo nije potvrđeno genetičkim studijama. Korišćeni su molekularni markeri da bi se ispitalo genetička pozadina populacije potočne pastrmke u Mađarskoj. Istraživanja su uključila jedini matični nasad potočne pastrmke u Mađarskoj, kao i po jednu populaciju u planinskim vencima Bükk, Aggtelek, Börzsöny i Visegrádi, po jedna populacija u svakom. Genetička analiza je do sada sprovedena na 533 individue, isečak peraja je uzorkovan sa svake ribe i PCR-RFLP (kontrolni region mitohondrijalne DNK, laktat dehidrogenaza i somatolaktin geni), kao i analiza mikrosatelitnih markeri (BFRO002, OMM1064, Ssa408uos, SsoSL417, SsoSL438) su bili korišćeni da bi se razlikovale Dunavska i Atlanska linija potočne pastrmke. Na osnovu genetičke analize mitohondrijalne DNK divljih populacija, udeo Dunavskog haplotipa je nizak (< 10 %), sa izuzetkom potoka Apátkúti u planinama Visegrádi, gde je nađena relativno visok udeo Dunavskog haplotipa (34%). 401 analizirani primerak matičnog jata farme je skoro u potpunosti Atlanski haplotip, što ukazuje na efekat osnivača. Iako su kasnije prikupljeni primerci iz obližnjeg potoka i pridodati matičnom jatu, njihov broj je bio ograničen jer su uglavnom svi bili mužjaci. Budući da je jedino matično jato u Mađarskoj, ribe sa ove farme se koriste za poribljavanje od strane ribolovaca, što može dovesti do značajnog uticaja na prirodne populacije. Na osnovu analize nuklearnih markera sve populacije su veoma heterogene. Veliki udeo (60-80%) Atlanskih alela primećen za ove markere na svim lokacijama gde je obavljano uzorkovanje ukazuje na efekat intenzivnog poribljavanja mađarskih salmonidnih regiona. Analize mikrosatelitskih markera su ukazale na visoku heterozigotnost i Hardy-Weinberg-ovu ravnotežu svih populacija.

Ključne reči: potočna pastrmka, PCR-RFLP, mikrosateliti, populaciona genetika, Mađarska

Keywords: Brown trout, PCR-RFLP, microsatellite, population genetics, Hungary

INTRODUCTION

Brown trout (*Salmo trutta m. fario*) is a salmonid species native to the freshwater streams of Eurasia. Analyses of variation in the mitochondrial DNA revealed the existence of five major evolutionary lineages in the native range of the species: Atlantic, Danubian, Mediterranean, Adriatic and *marmoratus* (Bernatchez, 2001). The species is cultured primarily for stock enhancement of natural waters and those managed by anglers. As most hatchery-maintained broodstocks originate from the Atlantic lineage (Jug et al, 2005), several European populations have been affected by hybridization and introgression with stocked trout of this lineage (Snoj et al, 2002, Marić et al 2010).

Hungary is a landlocked country with all its streams and rivers belonging to the Black Sea drainage through the river Danube, therefore, it is expected that local brown trout would also belong to the Danubian lineage. Moreover, due to the relative scarcity of typical salmonid waters in the country, currently there is only one hatchery that has a constant brown trout broodstock. Unless fish are imported from other countries, all brown trout stocked in the country theoretically originates from this broodstock.

The objective of this work was to elucidate the origin and genetic composition of the only Hungarian brown trout broodstock and that of natural populations in some streams using mitochondrial and nuclear (PCR-RLFP and microsatellite) markers.

MATERIALS AND METHODS

Brown trout broodstock was maintained at the fish farm of Hoitsy & Rieger Kft in Lillafüred, Hungary. Fish were sampled between June and November of 2011. In total, 401 fish of the broodstock were samples. Samples from wild populations were collected at the Bán stream in the Bükk mountains (25 fish), the Jósva stream in the Aggtelek mountains (33 fish, both on April 26th, 2012), the Kemence stream in the Börzsöny mountains (24 fish on October 25th, 2012) and finally the Apátkúti stream in the Visegrád mountains (50 fish on February 13th, 2013). On all locations, fish were anesthetized in a 0.04% solution of 2-phenoxyethanol, then laid on a wet towel. Fin clips of approximately 1 cm^2 were collected from each fish and stored in a 1,5-mL microcentrifuge tube in 96% ethanol. A photo was taken of each fish. Collected samples were shipped to the laboratory of the Department of Aquaculture of Szent István University, Gödöllő, Hungary where DNA isolation and a part of the genotyping work was conducted. A part of the genotyping work of broodstock samples was performed at the Biotechnical Faculty of the University of Ljubljana in Domžale, Slovenia. Results received at the two laboratories were compared and verified using test samples. Samples collected from natural streams were processed entirely at the Department of Aquaculture of Szent István University.

Whole DNA isolate was made from the tissues according to Kovacs et al. (2001).The quality and concentration of DNA were assessed by a photometer (IMPLEN). The DNA concentration were adjusted to 50 ng/µL before PCR amplification.

Primers were synthesized to the flanking regions of five microsatellites (BFRO002, Sušnik et al, 1997, OMM1064, Rexroad et. al., 2002, Ssa408uos: Cairney et al, 2000, SsoSL417, Slettan et al, 1995, SsoSL438, Slettan et al, 1996), two genomic PCR-RFLP (lactate dehydrogenase: LDH-C1; McMeel et al, 2001 and somatolactin: SL Ford, 1998), and one mitochondrial PCR-RFLP (CRmtDNA; Bernatchez and Danzmann, 1993).

During the microsatellite analyses in Slovenia, fluorescently labelled primers were used, while tailed primers (Shimizu et al, 2002) were employed in the investigations in Hungary. In this latter method, a 17-bp tail (5'-ATTACCGCGGCTGCTGG-3') was attached to the 5' end of one of the forward primers. A third FAM, NED, PET or VIC dye labelled primer corresponding to the sequence of the tail was used in the PCR reactions.

Amplification of microsatellite alleles were performed according to Bogataj (2010) with tailed primer pair (250 nM) and an additional tail specific labeled (50 nM) primer.

For separation of microsatellite alleles, labeled PCR products of individuals were separated on the ABI 3130 sequencer with 38cm long capillaries, POP7 polymer, GeneScan™-500 LIZ®. size standard, The length of PCR products was determined by Genotyper software (Applied Biosystems). Allele sizes read during the fragment analyses were reduced by the length of the tail (17 bp). Results received with the two methods were compared and verified using test samples.

The PCR-RFLP analyses of CRmtDNA and LDH-C1were performed according to Sušnik et.al (2008), while PCR-RFLP Somatolactin were made according to Marić at al. (2010).

The digested PCR products were separated on 3% agarose gels, containing 0.5 µg/ml ethidium bromide. Band patterns were photographed under UV light.

Statistical analyses of allele frequencies, genetic diversity, Hardy-Weinberg equilibrium per locus were calculated using the free software GenoDive version 20b22 for Mac OS X (Patrick G Meirmans, the Netherlands).

RESULTS AND DISCUSSION

To date altogether 533 brown trout individuals were genotyped. Antlantic and Danubian alleles were distinguished according to Bogataj (2010). The proportion of the mitochondrial haplotype characteristic of the Danubian lineage was low (< 10 %), with the exception of the Apátkúti stream with a relatively high percentage of Danubian haplotype (34 %). The 401 analysed individuals of the broodstock almost uniformly displayed the Atlantic haplotype. Analyses of nuclear markers showed a varying proportion of alleles characteristic of the Atlantic or Danubian lineages although those representing Atlantic origin dominated in every population (Table 1).

Table 1. Proportions of alleles characteristic of the Danubian or Atlantic lineages of brown trout in the analyzed populations at 3 tested PCR-RFLP loci (mtDNA, SL and LDH-C1)

Locus	mtDNA		SL		LDH	
Population	Danubian	Atlantic	Danubian	Atlantic	Danubian	Atlantic
Broodstock	0,002	0,998	0,221	0,779	0,370	0,630
Bán	0,080	0,840	0,300	0,580	0,300	0,660
Jósva	0,091	0,909	0,167	0,803	0,106	0,894
Kemence	0,083	0,917	0,417	0,583	0,292	0,708
Apátkút	0,340	0,660	0,180	0,820	0,190	0,810

Results indicate that the original broodstock that was introduced to the farm following its construction in 1933 was of the Atlantic lineage female. The low proportion of Danubian genes observed originated probably from male individuals collected from local streams. In addition, stocking of natural waters with fish of non-native origin was conducted in the past, as revealed by the results.

Overall 99 alleles were found across the five microsatellite loci and the effective number of alleles varied greatly at all locations. Microsatellite OMM 1064 was found to be the most polymorphic whereas, BFRO002 displayed the lowest PIC values. On population level, the populations are effectively in Hardy-Weinberg equilibrium for both PCR-RFLP and microsatellite markers.

CONCLUSIONS

Overall we found a remarkably high proportion of allochthonous Atlantic alleles in the Hungarian brown trout populations as a clear indicator of the import and stocking of non-native populations. According to these findings, further stocking of brown trout in Hungary should be conducted in more controlled conditions.

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ICHTYOFAUNA OF KRIVAJA RIVER CATCHMENT AREA

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IHTIOFAUNA SLIVNOG PODRUČJA REKE KRIVAJE

Apstrakt

Terenski dio ihtioloških istraživanja rijeke Krivaje i njenih pritoka obavljen je u periodu oktobar-novembar 2010. godine na području opština Olovo, Vareš i Zavidovići za potrebe izrade Ribarske osnove za područje Zeničko-dobojskog kantona. Izlov ribe je obavljen elektroagregatima marke “Honda”: „FEG 15.000“ snage 15 kW i „OHV 5.5“ snage 3,0 kW. Elektro-ribolov je izvršen na 18.870 m vodotokova, odnosno na 591.140 m^2 ukupne vodene površine. Prikupljeni uzorci ribe obrađeni su na terenu i vraćeni u njihovo prirodno stanište, dok je manji broj reprezentativnih primjeraka fiksiran u 4%-tnom formaldehidu i dopremljen u laboratorije Centra za akvakulturu i ribarstvo Poljoprivredno-prehrambenog fakulteta Sarajevo na dalju analizu. Sistematska determinacija riba je vršena po Vukoviću i Ivanoviću (1971) i Sofradžiji (2009). Najveću brojnost u mješovitoj populaciji riba u rijeci Krivaji imale su jedinke iz porodice *Cyprinidae* sa procentualnim učešćem od 93,92%. Ostale evidentirane vrste iz porodica *Thymalidae, Cottidae, Salmonidae* i *Cobitidae* bile su zastupljene od 0,33-2,64%. Najveću brojnost iz pritoka rijeke Krivaje imale su vrste iz porodice *Cyprinidae* sa procentualnim učešćem od 54,93%. Nižu brojnost u istraživanim pritokama rijeke Krivaje imale su vrste iz porodice *Salmonidae* 31,54% i *Thymallidae* sa 9,34%. Najnižu brojnost imale su vrste iz porodice *Cottidae* sa 4,53%. Na osnovu dobivenih podataka o kvantitativno-kvalitativnom sastavu ihtiofaune sliva rijeke Krivaje, generalno se može zaključiti da ovo istraživano područje ima zadovoljavajuće ekološke uslove za život mnogih vrsta riba.

Ključne riječi: Krivaja, populacija, brojnost, ihtiouzorak, sliv

Keywords: Krivaja, populations, abundance, fish sample, catchment

INTRODUCTION

Freshwater fish fauna of Bosnia and Herzegovina, by the riches of interesting species, is a unique phenomenon in our continent. Ichthyofauna of Bosnia and Herzegovina can be divided into two parts, each very different in composition: ichthyofauna of the Adriatic catchment area, which is characterized by a number of endemic forms, and ichthyofauna of the Black Sea catchment area, where there is no endemic form but the species that have a wider distribution in Europe. First research of ichthyofauna in Bosnia and Herzegovina was carried out in mid and late nineteenth century, when Heckel, Kner and Steindachner described a number of interesting and hitherto unknown to science forms from the waters of Bosnia and Herzegovina (according to: Vuković and Ivanović, 1971). The second period of research of the ichthyofauna in the former Yugoslavia, and thus also in Bosnia and Herzegovina, occurs in the first half of the twentieth century. In this period, the most significant are works of Karaman (1924, 1928 and 1938), who described a number of species and subspecies of the family *Salmonidae*, *Cyprinidae*, *Cobitidae* and *Gasterosteidae*, especially from the Vardar river catchment, Prespa and Ohrid lakes and some flowing rivers in Yugoslav areas of the Adriatic catchment. Significant contribution to the understanding species composition of the Bosnia and Herzegovina was also given by other researchers: Čurčić (1938), Taler (1945 and 1953), Zaplata and Taler (1932). In their works, they mostly dealt with distribution of certain freshwater fish.

Modern ichthyofauna research in the second half of the twentieth century have been sustained, complex and particularly organized. Intensive study of ichthyofauna (with various aspects) in Bosnia and Herzegovina began in the 50s of XX century. Investigations of fish during this period to the present day include also knowledge of the composition and structure of fish populations and the different impacts on their living conditions.

Waters in catchment area of the Krivaja River belong to the Black Sea catchment and they are inhabited by fish species that have the widest range area in Europe. Although in this catchment area has no endemic form, it does not diminish the importance of these ichthyofauna researches.

Krivaja is a river in Central Bosnia, which does not have its source crest, but is created by connecting two mountain rivers: Bioštica and Stupčanica that drain the surface water and groundwater, and the two rivers joining in Olovo city. Krivaja River flows in north-west through the canyon-gorge valley. After a long course of 72 kilometers, Krivaja River flows into the Bosna River in urban zone of Zavidovići as its right tributary. Krivaja River catchment area drains surface water from the central part of Eastern Bosnia. It is located on the border between geographic regions, Central and Northern Bosnia. Southeastern parts of this catchment area drain surface water from Central Bosnia and lower parts of this catchment area from the southern parts of Northern Bosnia.

With an area of 1,350.8 km^2, Krivaja River catchment ranks among the medium-sized catchment areas of Bosnia and Herzegovina. This catchment has a length of 94.5 km and an average width is 14.7 km. The maximum width of 30 km catchment refers to the area of the source crests of Bioštica and Stupčanica, and minimum of 5.4 km to its lower part. Catchment area is quite symmetrical, which indicates symmetry coefficient of 1.4.

Ichthyologic research in the Krivaja River catchment area made in the Fish base study for the Bosna river catchment area (Kosorić *et al.* 1983) and Fish base study for the area of Zenica-Doboj Canton (Muhamedagić *et al.* 2011). The obtained data about state of ichthyo-populations are taken directly from these scientific research projects and presented and argued in this paper.

MATERIAL AND METHODS

The field work part of ichthyologic research of Krivaja River and its tributaries was carried out October and November 2010 in Olovo, Vareš and Zavidovići municipalities for the purpose of Fish base study for the area of Zenica-Doboj Canton. Assessment of the structure of fish populations by longitudinal sequence of catchment area of the Krivaja river and its tributaries treated at 10 locations; 6 locations in the municipality Olovo (Boganovići, Čuništa, Maoča, Bioštica, Stupčanica and Duboštica), 2 locations in the municipality of Vareš (Očevlja and Tribija) and 2 locations in the municipality of Zavidovići (Krivaja and Skroze).

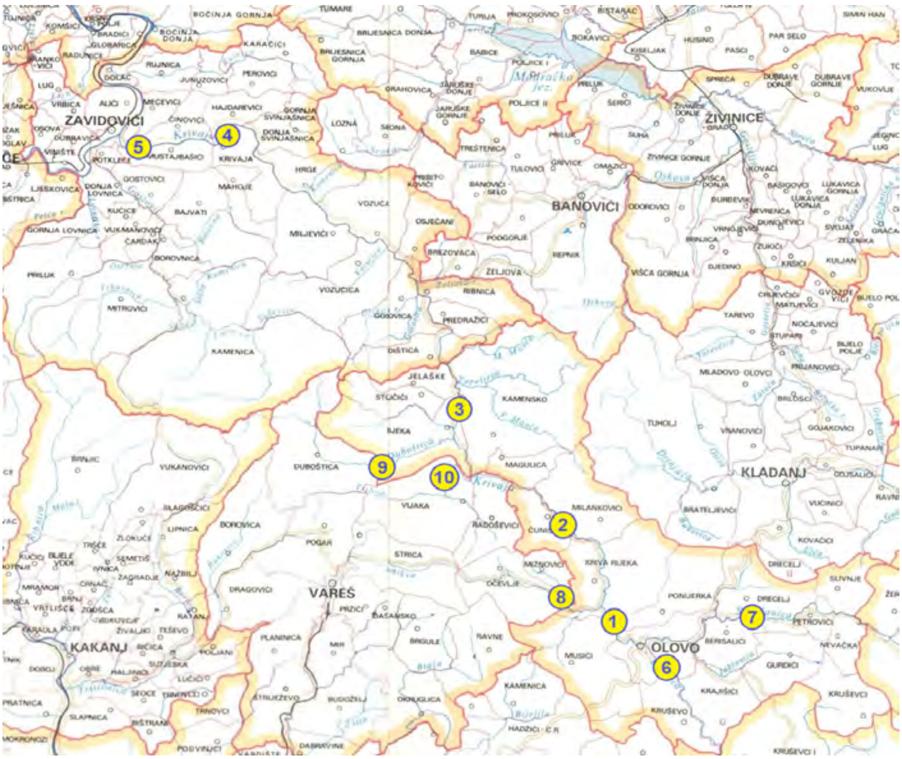

Figure 1. Investigated locations (1. Boganovići, 2. Čuništa, 3. Maoča, 4. Krivaja, 5. Skroze, 6. Bioštica, 7. Stupčanica, 8. Očevlja, 9. Duboštica i 10. Tribija)

Catching of fish is done by "Honda" brand aggregates: "FEG 15,000", with power of 15 kW and "OHV 5.5", with power of 3.0 kW. Electro-fishing is carried out on 18,870 m of watercourses, or on 591,140 m^2 of total water surface.

Collected fish samples were processed on the field and returned to their natural habitat, while a smaller number of representative specimens fixed in 4% formaldehyde and brought to the laboratory of Center for Aquaculture and Fisheries at Faculty of Agricultural and Food Sciences in Sarajevo for further analyses.

Systematic determination of fish is done by Vuković and Ivanović (1971) and Sofradžija (2009). Statistical analysis was performed according to Petz (1985).

RESULTS AND DISCUSSION

Results of the ichthyologic research of watercourses in Krivaja River catchment area (in municipalities Olovo, Vareš and Zavidovići) are presented in the following tables and charts.

Krivaja River

Results of qualitative and quantitative composition of ichthyo-populations in the Krivaja River from area of municipalities Olovo and Zavidovići are presented in Table 1.


Figures 2 and 3. Krivaja River: headwaters and its mouth to Bosna River


Figure 4 and 5. Part of ichthyofauna of Krivaja River and Danube salmon (*Hucho hucho* Linnaeus, 1758)


Figure 6 and 7: Part of ichthyofauna of Krivaja River: Common nase (*Chondrostoma nasus* Linnaeus, 1758) and Danube barbel (*Barbus balcanicus* Kotlík, Tsigenopoulos, Ráb & Berrebi, 2002)

Table 1. Qualitative and quantitative composition of ichthyo-populations in the Krivaja River

Family	Fish species	Locations					Total	
		Boganovići	Čuništa	Maoča	Krivaja	Skroze		
		n	n	n	n	n	n	%
Salmonidae	Danube salmon - *Hucho hucho* (Linnaeus, 1758)	2	3	1	1	2	9	0.6
	Brown trout - *Salmo trutta* Linnaeus, 1758	–	4	–	–	–	4	0.27
Thymallidae	Grayling - *Thymallus thymallus* (Linnaeus, 1758)	20	14	5	–	–	39	2.64
Cyprinidae	Chub - *Squalius cephalus* (Linnaeus, 1758)	16	16	15	102	123	272	18.4
	Common nase - *Chondrostoma nasus* (Linnaeus, 1758)	30	50	7	12	54	153	10.4
	Danube barbel - *Barbus balcanicus* Kotlík, Tsigenopoulos, Ráb & Berrebi, 2002	15	14	16	22	18	85	5.75
	Schneider - *Alburnoides bipunctatus* (Bloch, 1782)	122	116	140	202	142	722	48.9
	Gudgeon - *Gobio gobio* (Linnaeus, 1758)	–	–	–	6	–	6	0.4
	Danubian longbarbel gudgeon - *Romanogobio uranoscopus* (Agassiz, 1828)	8	7	–	–	–	15	1.01
	Belica - *Leucaspius delineatus* (Heckel, 1843)	–	–	–	37	62	99	6.7
	Bitterling - *Rhodeus sericeus* (Pallas, 1776)	–	–	–	22	–	22	1.49
	Eurasian minnow - *Phoxinus phoxinus* (Linnaeus, 1758)	13	–	–	–	–	13	0.88
Cottidae	Bullhead - *Cottus gobio* Linnaeus, 1758	19	13	–	–	–	32	2.16
Cobitidae	Balkan loach - *Sabanejewia balcanica* (Karaman, 1922)	–	–	1	2	2	5	0.33
Total catch (in number of individuals)		**245**	**237**	**185**	**406**	**403**	**1,476**	**100.00**

Ichthyo-populations of the Krivaja River are presented with fourteen species from five families: *Salmonidae*, *Thymallidae*, *Cyprinidae*, *Cottidae* and *Cobitidae*. Cyprinids are the largest fish group in this river. In total, it was registered nine species: Chub, Common nase, Danube barbel, Schneider, Gudgeon, Danubian longbarbel gudgeon, Belica, Bitterling and Eurasian minnow. *Salmonidae* family was presented with two species: Danube salmon and Brown trout. Other families are represented by one species. Family *Thymallidae* was presented by Grayling, *Cottidae* by Bullhead and *Cobitidae* by Balkan loach.

By representative sampling at five locations in Krivaja River (Boganovići, Čuništa, Maoča, Krivaja and Skroze), 1,476 individuals of various fish types were catch in total. Ichthyology analysis of the number of individuals of certain fish species was provided data on the relative abundance of fish populations in the Krivaja River. Results of the quantitative structure of fish families in the studied river are shown in Chart 1.

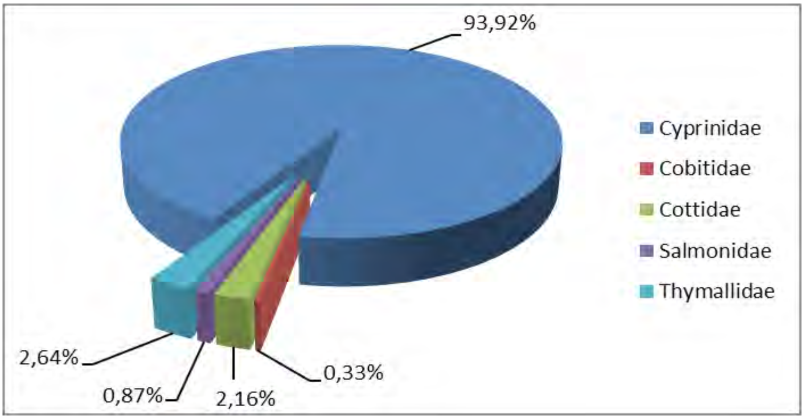

Chart 1. Percentage of families in the total fish catch in Krivaja River

In total fish population of Krivaja River representatives of cyprinids have registered in the highest number of caught individuals, and constitute 93.92% of the total number, while families *Thymallidae* makes 2.64%, *Cottidae* 2.16% and *Salmonidae* 0.87%. *Cobitidae* family makes the lowest share of ichthyo-populations with only 0.33%.

Among cyprinids, and generally in the overall fish population, population of Schneider is presented by the largest number of individuals (722), and makes 48.90% of Krivaja River fish population. By significant number of individuals, it was also registered population of Chub with 271 individuals, which is 18.40% in relative terms. Population of Common nase stands with high abundance. It was caught 153 individuals, so in the total fish population Common nase accounts for 10.40%. Danube salmon has been registered in the whole course of the Krivaja River and in total it was caught nine specimens of this species (0.6%). The lowest number was recorded for population of Brown trout (4 individuals or 0.27%), Balkan loach (5 or 0.33%) and Gudgeon (6 individuals or 0.40%).

Tributaries of Krivaja River

In order to determine the current state of fish populations in tributaries of Krivaja River, fish samples were collected from five tributaries: Bioštica, Stupčanica, Očevlja, Duboštica and Tribija. Rivers Bioštica and Stupčanica are source crests that merge in Olovo city and so create Krivaja River, while Očevlja, Duboštica and Tribija are left tributaries of Krivaja River. The results of the quantitative and qualitative composition of fish populations in these tributaries are shown in Table 2.


Figures 8 and 9. Location 6 - Bioštica and location 7 - Stupčanica

Table 2. Qualitative and quantitative composition of ichthyo-populations in tributaries of Krivaja River

Family	Fish species	Tributaries of Krivaja River					Total	
		Bioštica	Stupčanica	Očevlja	Duboštica	Tribija		
		n	n	n	n	n	n	%
Salmonidae	Danube salmon - *Hucho hucho* (Linnaeus, 1758)	2	–	2	–	–	4	1.13
	Brown trout - *Salmo trutta* Linnaeus, 1758	43	35	28	–	–	106	30.02
Thymallidae	Grayling - *Thymallus thymallus* (Linnaeus, 1758)	12	15	6	–	–	33	9.34
Cyprinidae	Chub - *Squalius cephalus* (Linnaeus, 1758)	–	5	–	–	8	13	3.68
	Barbel - *Barbus barbus* (Linnaeus, 1758)	12	–	–	–	–	12	3.39
	Danube barbel - *Barbus balcanicus* Kotlík, Tsigenopoulos, Ráb & Berrebi, 2002	36	7	7	3	4	57	16.14
	Schneider - *Alburnoides bipunctatus* (Bloch, 1782)	–	–	14	–	39	53	15.01
	Eurasian minnow - *Phoxinus phoxinus* (Linnaeus, 1758)	10	16	–	33	–	59	16.71
Cottidae	Bullhead - *Cottus gobio* Linnaeus, 1758	4	–	12	–	–	16	4.53
Total catch (in number of individuals)		**119**	**78**	**69**	**36**	**51**	**353**	**100.00**

After conducted ichthyological research, it is concluded that fish populations in tributaries of Krivaja River is consist of nine fish species from four families: *Salmonidae, Thymallidae, Cyprinidae* and *Cottidae*. Family *Cyprinidae* is the largest with five representatives: Chub, Barbel, Danube barbel, Schneider and Eurasian minnow. Danube salmon and Brown trout were recorded from *Salmonidae* family, grayling from *Thymallidae* and Bullhead from family *Cottidae*. Sampling from five tributaries of Krivaja River: Bioštica, Stupčanica, Očevlja, Duboštica and Tribija, has resulted in caught of 353 individuals of different fish species in total. Relative abundance of fish populations in the studied tributaries is shown in Figure 2.

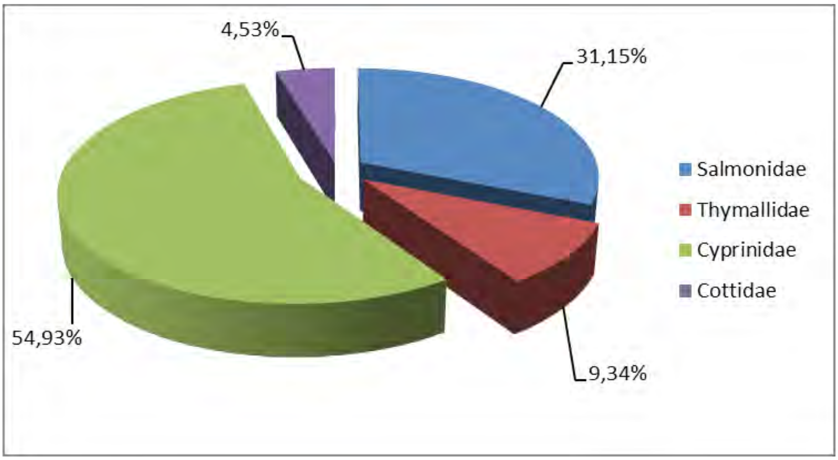

Chart 2. Percentage of families in the total fish catch in tributaries of Krivaja River

Cyprinids in the tributaries of the Krivaja River, with 194 individuals caught, constitute 54.93% of the total number. A significant number have fish of the *Salmonidae* family, with 110 specimens, which made 31.15% of the total number. Family *Thymallidae* with 33 grayling makes 9.34% of the total number. The smallest number has fish species from *Cottidae* family, with 16 individuals (4.53%).

Among salmonids, and generally in the overall fish settlement of these tributaries, the population of Brown trout is the largest with 106 specimens, which in relative terms is 30.02% of the total number. The second largest is population of Eurasian minnow with 59 registered individuals in the sample or with 16.71% share in the total fish number. They are followed by Danube barbel population with 57 individuals, which makes 16.14% and Schneider populations with 53 individuals caught or 15.01% of the total number.

Other species are recorded by much smaller number of individuals, including their participation numerically less. The lowest number was recorded in populations of Danube salmon, only four specimens, which makes 1.13% of the total number of fish in these tributaries.

Based on the obtained results it can be concluded that in the Krivaja river and its tributaries: Bioštica, Stupčanica, Očevlja, Duboštica and Tribija primarily represented cyprinid fish species: Chub, Common nase, Danube barbel, Schneider, Eurasian minnow, etc.

The data obtained on the qualitative and quantitative composition ichthyo-population of Krivaja River catchment are coincide with earlier data known from the literature on the composition and structure of the ichthyofauna of the Bosna River catchment area, such as: Mučibabić *et al*. (1967 and 1971), Kosorić (1976), Kosorić *et al*. (1980), Kosorić and Mikavica (1981), Proha (1997), Sofradžija *et al*. (2003), Korjenić (2003), etc.

Also, the data fit the literature data, which relate to the composition and structure of the ichthyofauna of Neretva River catchment area and Drina River catchment area, such as: Kosorić and Vuković (1966), Kosorić (1974 and 1978), Vuković *et al*. (1987), Škrijelj (1995), Mikavica, Sofradžija and Dizdarević (1991), Mašović (2000), Sofradžija *et al*. (2003), etc.

On the basis of the presented data on quantitative and qualitative composition of ichthyofauna Krivaja River catchment area and comparisons with earlier published data of this and other catchment areas in Bosnia and Herzegovina, in general it can be concluded that this studied catchment area has sufficient environmental living conditions for many fish species.

CONCLUSIONS

Based on the results of qualitative and quantitative analyses of fish population's structure in investigated Krivaja River catchment area, it is possible to present the following basic conclusions:

In the analyzed samples provided by study of Krivaja River catchment area it was observed the presence of 15 fish species that are classified into five families of freshwater ichthyofauna: *Salmonidae*, *Thymallidae*, *Cyprinidae*, *Cottidae* and *Cobitidae*.

During field research in Krivaja River, 1,476 individuals were caught in total. The largest number in a mixed fish population had individuals from *Cyprinidae* family with share of 93.92%. Other recorded species from families *Thymallidae*, *Cottidae*, *Salmonidae* (Danube salmon is registered in the whole course of Krivaja River) and *Cobitidae* were represented from 0.33 to 2.64%.

In tributaries of Krivaja River, 194 individuals in total were caught during our research. The highest abundance was for species of *Cyprinidae* family with proportional share of 54.93%. Lower abundance in the studied tributaries of Krivaja River had species of families *Salmonidae* (31.54%) and *Thymallidae* (9.34%). Lowest abundance had species from family *Cottidae* with share of 4.53%.

Comparing the results from 1983 with results from the 2004 it can be concluded that the Krivaja river and its tributaries predominantly inhabited by cyprinids species, but their numbers declined (in 1983 cyprinid species were represented with 68% and in 2004 with 59.33%), while the number of salmonid species grows (in 1983 they were represented with 10% and in 2004 with 33.47%). The number of species from families *Cobitidae* and *Thymallidae* is also decreases, compared to the data from 1983 (in 1983 *Thymallidae* family was represented with 7% and in 2004 with 1.44%, while *Cobitidae* family in 1983 was represented by 6% and in 2004 with 5.74%).

Based on the results of the quantitative and qualitative composition of ichthyofauna of Krivaja River catchment area, it generally can be concluded that the studied area has favorable environmental conditions for the life of many fish species.

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FISH BIODIVERSITY IN THE RIVER KRUŠNICA

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BIODIVERZITET RIBA U RECI KRUŠNICI

Apstrakt

Ribe su rasprostranjene u vodama širom svijeta i većina ih se upotrebljava u ljudskoj ishrani. Osim toga, ribe se sve intenzivnije danas izučavaju sa različitih aspekata biologije i drugih primjenjenih nauka, ali i rekreativnog sporta i turizma. Zbog sve intenzivnijeg antropološkog uticaja na životnu sredinu, monitoring ribljih populacija je neizostavan iz više praktičnih razloga: razumjevanja mehanizama zagađenja, prikupljanja podataka o prirastu ribljih vrsta koje se koriste u uzgoju i akvakulturi, procjenu stanja vodenih biotopa i dr. Sposbnosti osjetljivih ribljih vrsta za bioakumulaciju zagađenja, koje nastaje kao posljedica ljudskih aktivnosti, danas je veoma primjenljivo u zaštiti i unaprijeđenju životne sredine onih biotopa koji su se našli na udaru raznih hemijskih i drugih polutanata. Polazne osnove za bilo kakvo fiziološko, genetičko, ekološko ili bilo koje drugo stanovište proučavanja populacija određenih vrsta jeste određivanje biodiverziteta nekog užeg ili šireg područja vodenog staništa. Ihtiofauna Bosne i Hercegovine je relativno dobro proučena, o čemu svjedoči veliki broj objavljenih radova, što se ne može reći za sliv rijeke Une, pogotovo njene manje pritoke, u kojim inventarizacija ihtiofaune ni do danas nije u potpunosti urađena. Uzevši u obzir činjenicu da je ihtiološko ispitivanje ribljih populacija u rijeci Krušnici, desnoj pritoci rijeke Une, važno za ovaj predio Bosne i Hercegovine gledajući iz različitih uglova, provedna su istraživanja tokom perioda jesen-zima 2001. godine i proljeće-ljeto 2002. godine. Praćena su kvantitativna i kvalitativna obilježja izlovljenih ihtiopopulacija u ovom vodenom biotopu sa ciljem registrovanja prisutnih i najbrojnijih vrsta riba. Dio izlovljenog materijala se direktno analizirao na terenu, kratko odmah nakon izlova, a veći dio je fiksiran i prenesen u laboratorij Biotehničkog fakulteta, Univerziteta u Bihaću, gdje su nastavljena morfološko-anatomska istraživanja. Ispitivanja osnovnih pokazatelja kvalitativnog i kvantitativnog sastava ihtiofaune rijeke Krušnice provedena su na tri lokaliteta, izvorištu rijeke, na srednjem toku i u području ušća. Pri tome praćene su fizičko-hemijske karakteristike vode, temperatura, pH, tvrdoća vode, prisustvo otopljenih soli, količina kisika, količina ugljične kiseline, osnovne osobine režima vode, mikroklimatske karakteristike područja, prikupljeni su uzorci fitobentosa i zoobentosa koji su također važni za određeni ekosistem, jer od sastava planktonskih vrsta i bentosa ovisi i sastav ribljih vrsta. Izlovljeno je ukupno 108 jedinki, osam različitih vrsta riba koje su klasifikovane prema standardizovanim ključevima za određivanje slatkovodnih vrsta riba u pet familija: *Salmonidae, Thymallidae, Cyprinidae, Esocidae* i *Cottidae.* U radu su primijenjene standardne statističke metode obrade podataka. Kod svih ulovljenih vrsta riba, njihova relativna brojnost, morfološke, ekološke i druge karakteristike, unatoč evidentnim inavazivnim uticajima ponajviše nezakonitog ribolova, predstavljaju zadovoljavajuće rezultate do kojih se došlo ovim radom.

Ključne riječi: riba, populacija, rijeka
Keywords: fish, population, river

INTRODUCTION

Ichthyofauna of water systems in Una-Sana basin is considered to be vaguely explored, making them interesting for research. The Krušnica River is a right tributary of the Una river and rises near the village Gudavac, municipality of Bosanska Krupa, below the mountain massif of Grmeč. Krušnica is 6,8 km long, with an average width of 20 m, and average depth 5–7 m. Freshwater ichthyofauna of former Yugoslavia includes relatively large number of species–135 (Vuković i Ivanović, 1971), and Bosnia and Herzegovina 108 species (Vuković, 1977). The research of stream bed of the Una river (Fishing Report for Fishing area "UNA I" 1984) identified a large number of animal species, more precisely 68 species. The research of zoobenthos in the Krušnica river published in Fishing Report for Fishing area "UNA I" in 1984, provided data on 138 individual animals of different systematic categories. The newest comprehensive research on biological community of phytobenthos (Sofradžija et al., 2002) included quantitative analysis on content of phytobentos in the Una river and provided data on the presence of 36 species. The references on the Krušnica River provide data on presence of 4 families: *Salmonidae, Thymallidae, Cyprinidae* i *Cottidae*. *Salmonidae family* is presented by 2 species: *Salmo trutta morfa fario* and *Hucho hucho*. *Thymallus thymallus* is a representative of *Thymallidae* family. Three species of *Cyprinidae* family are registered: *Leuciscus cephalus*, *Barbus barbus* and *Rutilus pigus virgo. Esox lucius* is the only representative of *Esocidae* family.

MATERIALS AND METHODOLOGY

The field research was conducted in October–November 2001, and by the end of May 2002. Qualitative and quantitative structure of ichthyofauna in longitudinal profile of this river was determined at three locations–source-location of Vranjska, middle course-Zalug and river mouth-Pazadžik. Catching was conducted by means of a fixed net. A small number of caught fish was field tested, while the rest was fixated and transferred to the laboratory of Biotechnical faculty of University of Bihać for further examination. Determination of caught fish was conducted according to the relevant reference books on freshwater fish, Vuković i Ivanović (1971). The total body length of fish was measured with ichthyometer, and laboratory scale was used to determine the weight. Age determination of caught fish was conducted by counting natural growth rings (annuli) on the scales taken from the area below the dorsal fin. Gender determination was conducted after dissection by examination of gonads. The obtained data served to calculate number and mass portion of populations of certain fish species, providing relative (percentual) figures of number and mass, for each location, as well as for the entire river course. Water quality parameters were determined according to standard methods for the examination of waters and wastewaters that are in compliance with the Law on water resources. The results were statistically elaborated according to Petz (1985). Ichthyofaunal heterogeneity of the examined ecosystem was evaluated according to Kerovec (1988) based on two parameters: diversity index (d) using the equation d=S/N (S=number of species, N=number of individuals in specific area) and coefficient of similarity (QS) using the form QS=(2cx100)/(a+b) (c=number of similar fish species in certain ecosystems, a=number of species in ecosystem *a*, b=number of species in ecosystem b).

RESULTS AND DISCUSSION

Many authors (Zelikoff, 2000; Farkas et al., 2003; Lukin et al., 2003; Licata et al. 2003.) consider monitoring of water biotope to be important, since it provides data on the state of an ecosystem. The source of the Krušnica river corresponds to bonitation class I, except for the oxygen saturation level which amounts to 83,2% and an increased amount of ammonium (0,12 mg/L in the first and 0,11 mg/L in the second season) at the river mouth, which can be considered as relatively low amounts, however indicating the presence of anthropogenic influence. According to all other physical and chemical characteristics, waters of all location correspond to bonitation class I. The temperature of water at the examined area is balanced at all locations and corresponds to the climate of the area. According to Northcote (1995) optimal water temperature for sustenance of *Salmonidae* species, including grayling is 8-10ºC. *Salmonidae* species are able to tolerate changes of temperature in maximum decrease to 5ºC in winter period to an increase up to 18ºC in summer periods. This facts confirms the statement that the examined waters are salmonid (Sofradžija et al., 2002), which was proved by this research. Physical and chemical characteristics of the Krušnica river are in great measure similar to those of the Test river (United Kingdom) which is considered to be one of the most suitable salmonid rivers in the world (Ajanović, 1999). Being an indicator of conditions in water ecosystem (Mol, 1982), zoobenthos was examined in 24 samples, where 14 taxons with 786 individuals were determined. Ichthyological research conducted at three locations resulted in number of 108 individuals of different fish species. A number of 30 fish was caught in the season of autumn-winter, and 78 individual fish of different species in season of spring-summer. Two species of family *Salmonidae* were found in the Krušnica: brown trout-*Salmo trutta morfa fario* (Linnaeus, 1758), caught in both seasons, and rainbow trout-*Oncorhynchus mykiss* (Walbaum, 1792) caught only in autumn-winter season 2001. Only one species of *Esocidae* family was found: pike-*Esox lucius* (Linnaeus, 1758) caught only in spring–summer season 2002. The representative of *Thymallidae* family was grayling-*Thymallus thymallus* (Linnaeus, 1758) and was caught in both seasons. According to the number of recorded species, most frequent is family *Cyprinidae* with three species–chub-*Leuciscus cephalus* (Linnaeus, 1758), common minnow-*Phoxinus phoxinus* (Linnaeus, 1758), and the Danube roach-*Rutilus* *pigus virgo* (Heckel, 1852). The Danube roach was caught in both seasons, while chub was present only in autumn–winter season of 2001, and common minnow in season spring-summer of 2002. The representative of *Cottidae* family is bullhead *Cottus gobio* (Linnaeus, 1758), present in both seasons with the largest percentage in total number of samples (Figure 1).

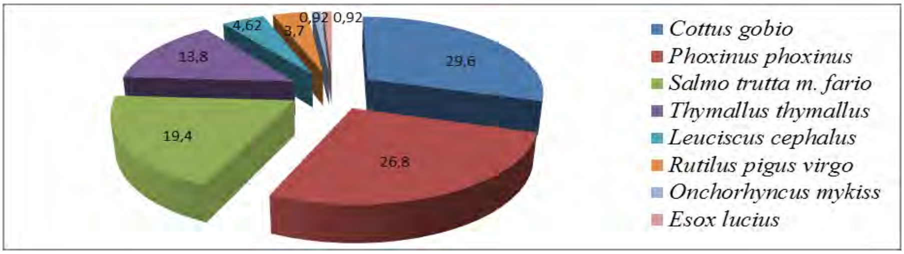

Figure 1. Relative number of certain fish species in the total sample caught from the Krušnica river within two seasons (percentages)

The highest value of diversity index was calculated at the middle course of the Krušnica d=0,31, and the lowest at the source 0,06, while at the river mouth the value was 0,23. Lower values of diversity index in a biotope indicate an decrease in optimal values of ecological conditions, which is in compliance with conclusions made in relevant resource books (Kerovec, 1988.). According to the data it is possible to conclude that ecological conditions at the source are farthest from the optimal values, while the middle course has the lowest rate of decrease in optimal values. By comparing the values of diversity factor, an influence of ecological factors on fish population can be considered as balanced. Regarding the presence of species at the locations and in research seasons, it can be stated that the grayling individuals were identified in both seasons and at all three locations of the Krušnica River. Other species are diversely distributed at different locations and in different research seasons. If we consider the coefficient of similarity, the highest level of ichthyofaunal similarity exists between locations Pazadžik and Vranjska–QS=60,0%. Ichthyofaunal similarity between locations of Vranjska and Zalug is lower than 50%, while between locations Zalug and Pazadžik QS=54,4%. The data indicates that the ecological factors at the locations are inconsiderably different, thus being rather stable.

CONCLUSIONS

According to the analysis of data obtained during the field research of fish biodiversity, the following can be stated: The most numerous family is *Cyprinidae*, represented by three species: chub-*Leuciscus cephalus*, Linnaeus 1758, Common minnow-*Phoxinus phoxinus*, Linnaeus 1758, The Danube roach-*Rutilus pigus virgo,* Heckel 1852; there were two representatives of *Salmonidae* family: Brown trout-*Salmo trutta morfa fario*, Linnaeus 1758, and rainbow trout-*Oncorhynchus mykiss*, Walbaum 1792; *Esocidae*, *Thymallidae*, and *Cottidae* families each have one representative: pike-*Esox lucius*, Linnaeus 1758, grayling-*Thymallus thymallus*, Linnaeus 1758, and bullhead-*Cottus gobio*, Linnaeus 1758.The most numerous species is bullhead–*Cottus gobio* with 29,6% of total number of samples; the least numerous species are pike–*Esox lucius* and rainbow trout-*Oncorhynchus mykiss*; brown trout-*Salmo trutta morfa fario* has the largest ichthyomass in the Krušnica with 36,3% of the total ichthyomass; and the lowest ichthyomass is that of pike–*Esox lucius*; diversity index values are rather low, while the coefficient of similarity is over 50% in two of three instances.

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FINDING OF STERLET (*ACIPENSER RUTHENUS*) IN THE SAVA RIVER NEAR ZAGREB

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NALAZ KEČIGE (*ACIPENSER RUTHENUS*) U RIJECI SAVI KOD ZAGREBA

Apstrakt
U ovom radu se potvrđuje prisustvo dva odrasla primerka kečige, nađenih 20 km jugoistočno od Zagreba u reci Savi. Familija Acipenseridae spada među najstarije zrakoperke. Populacija kečige u reci Savi je doživela značajan pad u toku XX veka, a u delovima reke Dunav koji prolaze kroz Austriju i Nemačku, ova vrsta je skoro izumrla i njen opstanak zavisi samo od kontinuiranog poribljavanja. Tokom marta 2011. godine dva odrasla primerka kečige veličine 431 i 570 mm su ulovljeni u reci Savi. Analizirano je šesnaest morfometrijskih i tri merističke karakteristike. Starost kečiga je procenjivana korišćenjem delova pektoralnog peraja, dok je za analizu crevnog sadržaja korišćena prva trećina ukupne dužine creva. Procena crevnog sadržaja je urađena koristeći frekvenciju pojavljivanja (F%) i brojnost (N%) različitih komponenti hrane. Iz crevnog sadržaja je evidentno da su glavna hrana vrste *Acipenser ruthenus*, Chironomidae, kao i različite larve insekata, pre svih Trichoptera i Odonata.

Ključne riječi: kečiga, Acipenser ruthenus, Sava, Hrvatska
Keywords: sterlet, Acipenser ruthenus, Sava, Croatia

INTRODUCTION

The sturgeons (family Acipenseridae) are among the most ancient ray-finned fishes. This extremely endangered group of animals should be the object of biological conservation programmes (Birstein, 1993) which, to be efective, require preliminary study of the specific status of sturgeons in the diferent regions of their distribution area. Sterlet (*Acipenser ruthenus* L.) is a fluvial fish which inhabits rivers and their tributaries (Vostradovsky, 1974). Occurs in large rivers, usually in the current and in deep water. In the first place, sterlet is potamodromous (Lucas and Baras, 2001) and moves to flooded areas for feeding (Kottelat and Freyhof, 2007). Like other sturgeons, it aggregates in bottom holes in winter and exhibit little activity. In spring, when ice breaks, it rises from the bottom holes and moves upstream for spawning. Spawns in habitats with strong-current on gravel, rarely on gravel-sand bottom or in flooded sites. Juveniles stay in riverine habitats during their first summer. This species is classified as endangered.

Populations of sterlet in the Sava River have experienced serious decline during the 20th century (Ristić, 1969), and sterlet has been almost extirpated from the German and Austrian section of the Danube River, where its presence depends on continuous stocking efforts (Reinartz, 2002). It has a limited distribution in the basin of the Middle and the Lower Danube (Guti and Gaebele, 2009), and there are ongoing stocking activities with the majority of fish released by Hungary, and to a small extent by Slovakia and Bulgaria (Holčik et al., 2006). In Serbia there is a captive broodstock of *A. ruthenus* at the Danube river, upon which controlled reproduction has been successfully performed since 1988. Specimens of this stock have been imported to Danube river. The objective of the present study is to confirm the occurrence *A. ruthenus* in a Sava river.

MATERIALS AND METHODS

During the March 2011, two adult specimens of *A. ruthenus*, ranging from 431 to 570 mm total length (TL), were captured by local fishermen using hook in a water body about 20 km southeast of Zagreb City. Specimens were examined and identified according to Kottelat and Freyhof (2007). All morphometric and meristic characters were taken following Copp and Kováč (2003) and measurements recorded to the lowest millimetre using a digital calliper, as shown in table 1. All morphometric measurements related to the head region as a proportion relative to head length, and those not related to the head region as proportions of the standard length.

Sterlet age determination was performed using pectoral fin spine sections according to Stevenson and Secor (1999). Briefly, we cut the spines with a laboratory saw and then polished it with emery paper, until sections were transparent enough for microscopic observation. Age was estimated by counting annuli in the fin ray sections. For the analysis of stomach contents the anterior third of the gut was used, with the food organisms still recognizable, weighed and fixed in 4% formaldehyde. When possible, the different food items in the gut were identified to the family or genus level. The identification and the counting were made using a binocular microscope.

Assessment of the diet was based on the frequency of occurrence (F%) and numerical frequency (N%) of the different diet components, using the following formulas:

$$F\% = \frac{fi}{\sum_f} x100$$

fi = number of stomachs containing each prey items and Σ_f = total number of stomachs with food;

$$N\% = \frac{ni}{\sum_n} x100$$

ni = total number of one food item, Σ_n = total number of food items consumed by the fish (Hyslop, 1980).

RESULTS

Morphometric and meristic traits are given in table 1. *A. ruhtenus* exhibited the following set of characterists: narrow and pointed snout with four long and fringed barbels. Back and flanks are beige. Five rows of scutes, first dorsal scute not fused with head. Ventrals and laterals are very light-colored, nearly white. Inferior lip clearly slit.

Table 1. Morphometric measurements and meristic counts and age of *A. ruhtenus* from Sava river

Morphometric and meristic traits	Specimen 1	Specimen 2
Total length (mm)	431,00	570,00
Standard length (mm)	348,00	453,00
Fork length (mm)	108,82	109,49
Preanal length (mm)	74,70	73,28
Predorsal length (mm)	75,88	77,483
Prepectoral length (mm)	27,05	24,94
Pectoral fin length (mm)	19,70	17,21
Head length (mm)	25,88	26,04
Preorbital length (mm)	50,00	50,84
Preoral length (mm)	62,50	61,016
Prebarbel length (mm)	39,77	40,67
Mouth width (mm)	15,90	16,94
Distance between eyes (mm)	28,40	31,35
Maximum head width (mm)	51,13	43,22
Head width at barbel base (mm)	25,00	23,72
Head width at mouth level (mm)	35,22	34,74
No. of dorsal scutes	11	12
No. of lateral scutes	61	60
No. of ventrolateral scutes	15	13
Age (years)	+5	+8

From the stomach content it is evident that Chironomidae and various insect larvae are primarily food of *Acipenser ruthenus,* of which are important Trichoptera and Odonata. Since the specimens were caught on earthworms (*Lumbricus terrestris*), the same one were found in the stomachs of the analyzed samples (Table 2).

Table 2. The frequency of occurrence (F%), numerical frequency (N%), pergentage of weight of stomach content (W%) of *Acipenser ruthenus* from Sava river

Taxa	F%	N%	W%
Chironomidae	100,00	51,09	1,93
Lumbricus terrestris	50,00	1,09	1,84
Other Insecta	100,00	33,70	5,42
Trichoptera larvae	50,00	10,87	8,85
Odonata anisoptera	50,00	2,17	9,74
Bivalvia	50,00	1,09	0,01
detritus	-	-	72,20

DISCUSSION

The fish from family Acipenseridae are economically extremely valuable and threatened by overfishing. Unreasonable fishing in the Danube River, based on the catch of the younger age categories that are not sexually mature, presents a serious risk for from this family. The major factor driving unsustainable legal and illegal sturgeon fisheries in this region is the fact that beluga caviar is one of the most prized fish products worldwide (Nikčević et al., 2003). Changes in natural habitats caused by human activity, such as pollution, sand digging and regulation of water flows adversely affected the natural sturgeon population. It was noted that the construction of dams and reservoirs affects the propagation of sturgeon because of the population increase in the number of elderly individuals that do not reproduce (Mrakovčić et al., 2006). Also, dams block access to many sturgeon-spawning grounds and, therefore, are considered to be one of the main reasons for the decline of their stocks (Lenhardt et al., 2004).

A. ruthenus is consider endangered by IUCN Red List (Gesner et al., 2010) in Croatia, and listed as vulnerable by the Red book of Freshwater Fish of Croatia (Markovčić et al., 2006).

Nowadays in Croatia a relatively good conditions exist for a start of common action among state representatives, scientists, holders of fishing rights, fishermen and private sturgeon hatcheries to solve the problem of sturgeon extinction risk.

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AGE STRUCTURE OF POPULATION OF ENDEMIC SPECIES *CHONDROSTOMA PHOXINUS* HECKEL, 1843 (TELEOSTEI: OSTARYOPHYSI, CYPRINIDAE) FROM BUSKO LAKE

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UZRASNA STRUKTURA POPULACIJE ENDEMIČNE VRSTE *CHONDROSTOMA PHOXINUS* HECKEL, 1843 (TELEOSTEI: OSTARYOPHYSI, CYPRINIDAE) IZ BUŠKOG JEZERA

Apstrakt

Raspolažući aktualiziranim podacima o kvantitativnom i kvalitativnom sastavu različitih ribljih populacija u Buškom jezeru, kao i činjenicom opasnosti od dominacije alohtonih ribljih vrsta nad autohtonim, posebna pažnja je i u pogledu analize dobne i spolne strukture ciljano usmjerena prema endemskim ribljim vrstama jezerskog ekosistema među kojima je svakako posebno mjesto imala vrsta *Chondrostoma phoxinus*.

Vrsta *Chondrostoma phoxinus* Heckel, 1843 (poznatija kao podbila) je opisana na osnovu primjeraka iz Sinja (Hrvatska), a nešto kasnije je pronađena i na teritoriji Bosne i Hercegovine, preciznije rečeno u Buškom jezeru (vještačkoj akumulaciji formiranoj 1974. godine na južnom dijelu Livanjskog polja), koja je prethodno postojala kao prirodni hidrografski, močvarni ekosistem.

Materijal, vrste *Chondrostoma phoxinus* Heckel, 1843, je sakupljan periodično od aprila 2003. do septembra 2004. godine te tokom 2007. i 2008. godine standardnim ihtiološkim metodama. Izlov je rađen na prethodno označenim i pristupačnim lokalitetima gdje je postavljeno ukupno 200 m mreže različitih promjera okaca. Detaljnija obrada ihtiomaterijala, sastojala se u utvrđivanju uzrasnih kategorija pregledom krljušti, skidanih uvijek s desne strane trbuha odmah ispod osnove leđnog peraja. Nakon toga, brojanje skleritnih prstenova rađeno je pomoću binokularnog mikroskopa *Binokulares LED Schulmikroskop BA 658*. Za statističku obradu podataka korišten je softverski program „SPSS for Windows 15,0". Činjenice jesu da u piramidi brojnosti pojedinih uzrasnih klasa, najmlađe uzrasne klase, pod normalnim uslovima su rasprostranjenije, dok kod

starijih, prirodna smrtnost ili, pak, smrtnost prouzrokovana spoljašnjim uticajima smanjuje brojnost jedinki (Georgiev, 1986).

Pregledom svih jedinki podbile (252), izlovljene u periodu istraživanja, samo 5 je izlovljeno u uzrasnoj klasi 1^+, pri čemu su to 4 mužjaka i jedna ženka. U uzrasnoj klasi $2^{+,}$ izlovljeno je ukupno 17 jedinki – 12 mužjaka i 4 ženke. U uzrasnoj klasi 3^+, brojnost izlovljenih jedinki je veoma visoka i iznosi 44, od čega su 32 mužjaka i 12 ženki. Struktura spolova u brojčanom omjeru se naglo mijenja u višim dobnim klasama, te je tako u uzrasnoj klasi 4+, od ukupno 79 jedinki, 60 jedinki ženskog spola, a samo 19 jedinki muškog, itd. Detaljnija statistička analiza, uz korištenje χ^2, pokazuje postojanje statistički značajne razlike između posmatranih jedinki podbile određenog spola i utvrđene starosne dobi. Za ovu analizu uzete su samo jedinke podbile kod kojih je određena i spolna i uzrasna kategorija, što se odnosi na 205 jedinki te vrste. Također su logički grupirane uzrasne klase prema reproduktivnom periodu na nivou mlađih jedinki, reproduktivno zrelih i starijih jedinki s izraženim periodom stagniranja. Statistička analiza u ovom slučaju, pokazuje granicu značajnosti ili signifikantnosti kod istraživanih jedinki podbile na nivou 0,004, ili 99,6 %.

Iz toga proizilazi da je najveći broj jedinki muškog spola izlovljen u starosnoj kategoriji 3^+, što predstavlja 36,8% ukupnog broja utvrđenih jedinki muškog spola (87) u analiziranom materijalu. S druge strane, najmanji broj jedinki muškog spola zabilježen je u uzrasnoj klasi 7^+, odnosno samo jedna, ili 1,1%. Razmatrajući zastupljenost jedinki podbile ženskog spola u određenim uzrasnim kategorijama, iznenađuje činjenica o izrazitoj brojnosti ženki starosne dobi 4^+. Registriranih 60 jedinki, u ukupnom broju jedinki ženskog spola, ima udio od visokih 50,8%. Procentni odnos ženki u uzrasnoj klasi 4^+, prema ukupnom broju od 79 izlovljenih jedinki u toj uzrasnoj kategoriji, prilično je visok i iznosi 75,9%. I, konačno, taj broj jedinki ženskog spola u uzrasnoj klasi 4^+, u poređenju s ukupno izlovljenim brojem jedinki podbile (252), izražen procentualno, iznosi 23,8%. Ovaj podatak, koji se odnosi na dominaciju ženki u ukupnoj populaciji, a posebno u reproduktivno izražajnijem periodu (klasa 4^+), daje pozitivne rezultate u trendu rasta i dinamike opstanka populacije. Slijedeći dalje rezultate analize odnosa spolova u preostalim uzrasnim kategorijama, zapaža se izrazitija brojnost jedinki ženskog spola. Tako uzrasna kategorija 5^+ (30 ženki) u ukupnom broju registriranih ženki u izlovu čini udio od 25,4%, a u uzrasnoj kategoriji 6^+, (11 ženki) predstavlja 9,3%.

Slična razmatranja mogu se iskazati i za jedinke muškog spola u uzrasnim klasama 2^+, 3^+, 4^+, 5^+, 6^+. U uzrasnoj klasi 1^+ prisutne su 4 jedinke muškog spola, u klasi 2^+ prisutno je 12 jedinki, a 32 jedinke muškog spola prisutne su u uzrasnoj klasi 3^+, iz čega proizilazi zaključak o nešto većoj brojnosti mužjaka u odnosu na ženke u tim uzrasnim klasama. S druge strane, nešto manje (19) jedinki muškog spola u uzrasnoj klasi 4^+, 13 u uzrasnoj klasi 5^+, te 6 mužjaka podbile u uzrasnoj klasi 6^+, znači konstataciju znatnije brojnosti i zastupljenosti jedinki ženskog spola u reproduktivno zrelijoj dobi i ponovni dokaz pozitivnog trenda rasta populacije te vrste. Veća brojnost jedinki podbile ženskog spola, može se usko povezati i s mrijesnim periodom (kasno proljeće), kad je i brojčano najbogatiji izlov tokom cijelog perioda istraživanja.

S obzirom da je distribucija jedinki određenih uzrasnih kategorija vrlo usko povezana sa sezonskim pojavnostima, brojnost jedinki podbile u pojedinim izlovnim sezonama tokom dvije godine istraživanja varira – 79 jedinki dobi 4^+, 44 jedinke dobi 3^+, 43 jedinke u uzrasnoj kategoriji 5^+ itd. Pri tome je posebno uočljiva brojnost jedinki izlovljenih u sezoni proljeće – ljeto, 2007. godine, gdje je opisano ukupno 70 jedinki, od kojih je najveća zastupljenost uzrasne klase 4^+ (31 jedinka), što je vezano uz aktivnost očekivanog mrijesnog perioda. Veliki broj jedinki izlovljen je i u sezoni jesen – zima, 2007. godine, tačnije, 65 jedinki skoro podjednako zastupljenih u svim uzrasnim klasama, dok je iduća proljetna sezona, 2008. godine, obilježena nešto manjim brojem registriranih jedinki (45), također s većom brojnošću jedinki starosne dobi 4^+.

U ovako profiliranim radovima koji obrađuju autohtonu, a posebno endemičnu ihtiofaunu naše Zemlje, krajnji cilj u sveopćem monitoringu biološke raznolikosti jeste utvrditi stepen narušenosti ekoloških niša usljed degradacijskog učinka abiotičkih i biotičkih faktora koji su izražajniji u novonastalom akumulacijskom bazenu Buško jezero. Direktna je povezanost i posljedična reakcija narušenog staništa i ugroženosti autohtonih ribljih populacija čijim smanjenjem direktno mijenjamo sliku biodiverziteta tog područja.

Ključne riječi: Chondrostoma phoxinus, podbila, dobna struktura, Bosna i Hercegovina

Keywords: Chondrostoma phoxinus, Buško lake, age structure, *Bosnia and Herzegovina*

INTRODUCTION

Buško Lake, former natural, hydrographical, floodplain system, the largest of its kind in the fields of Bosnia and Herzegovina, became an artificial accumulation in 1974. It forms the southern tip of the vast Livanjsko Field and belongs to the system of the River Cetina and the central part of the Adriatic catchment basin. It sits at an altitude of 700 m and exhibits pronounced oscillations in water levels, surface area and volume (the total surface of Buško Lake is 28 km^2 at minimum water level, whereas at maximum water level it is 56.7 km^2).

The diverse ecosystem of the water basin became the original habitat of the endemic species *Chondrostoma phoxinus* Heckel, 1843 described for the first time by Heckel (1843) near Sinj in Dalmatia and somewhat later discovered on the territory of Bosnia and Herzegovina, i.e. in Buško Lake (Livanjsko Field).

All earlier statements on species of the genus *Chondrostoma* Agassiz, 1835 speak in favour of their inhabiting very specific ecological niches and occupying an important place both in the ecology and in the biosystematics and morphology of fishes. Some 18 species have been described to date, all of them in the area of Europe (except Great Britain, Scandinavia, Finland and the Northern Sea basin), the Black and the Caspian Sea drainage basin, the Orontes, the Tigris and the Euphrates.

More detailed descriptions of the *Chondrostoma phoxinus* species, the minnow-nase, in terms of biosystematics have been provided by various other authors as well, referencing Heckel and Kner’s (1858) data and giving comparative overviews of results achieved by using different scientific methods (cytological, biochemical, physiological, genetic, comparative-anatomical, embryological, paleontological, and other). By studying the literature references, one comes to realise that a number of authors have been interested in the meristics and morphometrics of the species in question, i.e. its biosystematics in general, as well as the status of the minnow-nase in the area it inhabits, its feeding habits, age categories, sex ratios and other. However, despite of the data from the extensive research papers on the *Chondrostoma phoxinus* species in the waters of Bosnia and Herzegovina, there is still a lack of a more comprehensive monographic research, as well as the most recent validation of the existing results.

The earliest findings and results of the *Chondrostoma phoxinus* species research are provided by Ćurčić (1916), Karaman (1923, 1928), Kišpatić (1938) Taler (1954), whereas somewhat more relevant data come from authors like Vuković (1966, 1977), Aganović (1969), Vuković and Ivanović (1971), Veledar and Kosorić (1972, 1974), Seratlić - Savić (1976), Aganović et al. (1974), Aganović and Kapetanović (1978), Vuković et al. (1978), Kosorić (1985), Elvira (1987), Habeković, et al. (1987), Mikavica and Kosorić (1987), Mikavica and Zovko (1989) and others.

The present-day research in this field is certainly aimed at gaining a more comprehensive knowledge of this endemic species, about which not enough is known, including its endangered status and confirmation of its distribution on the territory of Bosnia and Herzegovina. Kerovec et al. (1998) published a work on the occurrence of the *Chondrostoma phoxinus* species in Croatia. Žujo (2005) in her master’s thesis reveals the results on the biosystematic position and population status of the *Chondrostoma phoxinus* species in Buško Lake (Livanjsko Field). Valuable accounts from our area are provided by Mrakovčić et al. (2006) as part of the Red Book of Freshwater Fish of Croatia, in which they especially point out the endangered status of the endemic species *Chondrostoma phoxinus.* Kottelat and Freyhof (2007) specify the basic details on the morphology, distribution, habitat and biology of the species noting also its conservation status. Ćaleta et al. (2009) published a work on the threatened fishes of the world with an accent on the *Chondrostoma phoxinus* species. With all of the relevant research conducted so far as the starting point of her work, Žujo Zekić (2009-2010) introduces her own research conducted as part of her doctoral dissertation in which she took a particular interest in the endemic species *Chondrostoma phoxinus, Telestes (Leuciscus) turskyi* and *Aulopyge huegelli* while establishing the biological diversity of the fish population in Buško Lake.

MATERIALS AND METHODS

The material, species *Chondrostoma phoxinus* Heckel, 1843, was collected periodically from April 2003 to September 2004 and during 2008 and 2009 using standard ichthyological methods. During the first visits to the site, the specimens were collected from four locations on Buško Lake: Prisoje, Pećina, Marinovac and Široka Draga where the ichthyosamples consisted of 71 specimens of the species *Chondrostoma phoxinus* Heckel, 1843 (Figure 1). The field research continued during spring-summer season in 2007 and autumn-winter season in 2007, as well as the same seasons in 2008. The representative samples of ichthyofauna for the relevant period were collected on ten marked locations: Korito Ričine, Vrbovi Panji, Marinovac, Mukišnica, Brana Kazaginac, Široka Draga, Matkovača, Kanal, Bilo Polje and Golinjevo (Figure 1). During collection of fish two types of nets were used: single bottom gillnets (so called *popunice*) with different mesh sizes ranging from 10 mm, 16 mm, 28 mm, 32 mm to 50 mm and so called “*Baracuda”* nets with the mesh size of 70 mm, 80 mm, 90 mm, 110 mm and 120 mm. An attempt was made to avoid the selectivity of ichthyomaterial samples during fishing by using different available fishing tools. The caught specimens were then fixed in 4% formaldehyde solution and transported to the laboratory for further analysis. The biosystematic identification of the caught fish was performed according to Vuković and Ivanović (1971), Vuković (1977), and Kottelat and Freyhof (2007). The detailed analysis of the ichthyomaterial consisted of determining the age categories. To analyse the age structure fish scales were collected always from the right side of the belly right underneath the dorsal fin which were then cleaned with 10% KOH. This was followed by counting the sclerite rings (annuli) using the binocular microscope *Binokulares LED Schulmikroskop BA 658.* Software programme *SPSS for Windows 15.0* was used to perform the descriptive statistics in this paper.

Figure 1. The chart of fishing locations at Buško Lake (prepared by Drešković, N.)

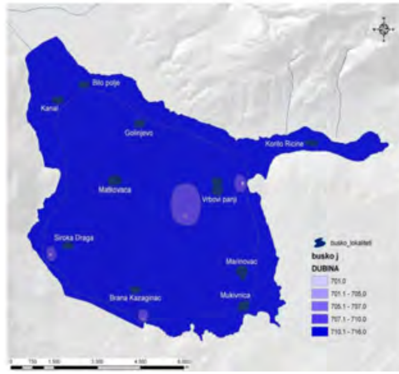

Figure 2. *Chondrostoma phoxinus* Heckel, 1843from Buško Lake (photo: Žujo Zekić D.)

RESULTS AND DISCUSSION

In the processes of change of natural ecosystems and living conditions that govern such a water habitat, whether caused by the systematic fish stocking or damming of watercourses (construction of reservoirs), it is essential to know the quantitative and the qualitative composition of the biotic communities (fish populations) and their age and sex structure. The established age structure of the analysed ichthyopopulation is a dynamic character that indicates most clearly the growth trend and dynamics of specimens, which affects directly the status of the entire population. The age structure analysis of the random sample from specific collection locations included the total of 206 specimens. The age was established only for those specimens that had pronounced sclerite rings (annuli). In the analysis dictated in such a manner, different movement patterns and directions were observed, tracking the oscillations in the seasonal growth rates and sex attributes.

In the first original ichthyosample (subsample) from 2003-2004, collected on four locations of Buško Lake, the age class 3^{+} was determined in the majority of the 71 analysed specimens of minnow-nase. Only three specimens belonged to the age class 4^{+}. One of the most likely reasons is the selectivity of sampling using the 28 mm mesh size net. Furthermore, the length of the caught fish was considerably uniform which confirms the stated reason.

The study of the fish growth patterns, including minnow-nase growth patterns in Buško Lake waters, provides answers concerning the sexual maturity, reproductive ability and the dynamics of the researched population including also its rational fishing, which is of both theoretical and practical importance to the monitoring of the ichthyofauna.

The research period was continued during 2007, 2008 and 2009 when more precise results were determined concerning the age class of the *Chondrostoma phoxinus* specimens fished out in specific seasonal intervals during the research period (Table 1).

Table 1 – The share of *Chondrostoma phoxinus* Heckel, 1843 specimens across specific age categories during researched seasons on Buško Lake

SEASON			AGE CLASSES							TOTAL
			1^+	2^+	3^+	4^+	5^+	6^+	7^+	
Spring-summer	2007	No. of specimens	0	5	14	31	13	6	1	**70**
		% in season	0,0%	7,1%	20,0%	44,3%	18,6%	8,6%	1,4%	**100,0%**
Fall-winter	2007	No. of specimens	2	8	10	16	19	10	0	**65**
		% in season	3,1%	12,3%	15,4%	24,6%	29,2%	15,4%	0,0%	**100,0%**
Spring-summer	2008	No. of specimens	3	4	13	19	6	0	0	**45**
		% in season	6,7%	8,9%	28,9%	42,2%	13,3%	0,0%	0,0%	**100,0%**
Fall-winter	2008	No. of specimens	0	0	7	13	5	1	0	**26**
		% in season	0,0%	0,0%	26,9%	50,0%	19,2%	3,8%	0,0%	**100,0%**
TOTAL		**Total number of specimens**	**5**	**17**	**44**	**79**	**43**	**17**	**1**	**206**
		% in all seasons	2,4%	8,3%	21,4%	38,3%	20,9%	8,3%	0,5%	100,0%

The numbers show that of the total of 252 collected minnow-nase specimens the age class was not determined for 46 of them, for the previously mentioned reasons, and therefore the age structure analysis for that population included the total of 206 specimens of the species *Chondrostoma phoxinus*.

Given the fact that the distribution of specimens from specific age categories is closely related to seasonal processes, Table 1 lists the numbers of minnow-nase specimens of specific age in specific fishing seasons during two years of research. The presented numbers of the minnow-nase specimens of different age varies across the researched seasons – 79 specimens of the age 4^+, 44 specimens of the age 3^+, 43 specimens of the age 5^+ and so on.

The numbers of the specimens collected during season spring-summer, 2007 are especially notable, where the total of 70 specimens have been described, most of them of the age class 4^+ (31 specimen), which is directly related to the activities of the expected spawning period. The numbers of specimens fished out during season fall-winter, 2007 is also large, *i.e.* the total of 65 specimens divided almost equally across all age classes, whereas the next spring season of 2008 had somewhat smaller number of registered specimens (45), also with larger numbers in the 4^+ age of specimens.

When observing the percentage share of the minnow-nase specimens of certain age across specific seasons, we can notice a high share of the 4^+ age specimens, 44.3% or 31 specimens in the spring season of 2007. The percentage share of the specimens of that same age is also high during spring season of 2008 or 42.2%. The percentage of the 4^+ age specimens is also high during fall-winter, 2008 season (50.0%), but only because of the small number of collected minnow-nase specimens in other age classes during the said season.

The table overview (Table 2), which shows seven registered age classes of minnow-nase specimens collected from Buško Lake, presents the correlation between the established age of the specimens and their sex attributes. It can be noted that the four-year old minnow-nase specimens are the most numerous compared to the total population composition of this fish species in the relevant aquatic complex, as many as 79 specimens or 31.35%.

The 3^+ age class has 44 minnow-nase specimens, with males prevailing, whereas the 5^+ age class registers 43, predominantly female, specimens. Expressed in percentages, the aforementioned values total 17.46% and 17.06%, respectively. Considerably lower number of minnow-nase specimens was registered in the second and the sixth age class, whereas the number of specimens in the age classes 1^+ and 7^+ is insignificant. While examining the presence of female minnow-nase specimens in specific age categories, it was surprising to find a large number of females in the age class 4^+. 60 registered specimens, of the total number of female samples, comprise a value of 50.8%. The percentage share of females of the age class 4^+ in the total number of 79 collected specimens in that age category is rather high and amounts to 75.9%. Finally, the number of female specimens in the age class 4^+, compared to the total number of collected minnow-nase specimens (252), amounts to 23.8% in percentages. This piece of data, which reflects the dominance of females in the total population, especially during a reproductively significant period, gives positive results with regard to the growth pattern and the population's survival dynamics. When examining further results on the ratio of sexes in other age categories, we can observe that the female specimens are more numerous. The age category 5^+ (30 females) comprises 25.4% of the total number of registered females in the collected sample, and the age category 6^+ (11 females) comprises 9.3%.

Similar reflections can be given for male species in age classes 2^+, 3^+, 4^+, 5^+, 6^+. The age class 1^+ has 4 male specimens, the age class 2^+, 12 male specimens and the age class 3^+, 32 male specimens. A conclusion can be made that in the said age classes males are somewhat more present than females. On the other hand, a slightly lower number (19) of male specimens in the age class 4^+, 13 males in the age class 5^+, and 6 minnow-nase males in the age class 6^+, leads to a conclusion that females are more numerous and more represented in the reproductively more mature age, which is another proof of positive growth trend of the population of this species. Higher numbers of female minnow-nase specimens can be closely related to the spawning period as well (late spring), when the largest number of samples was collected compared to the entire research period. By examining all minnow-nase specimens (252) collected during the research period, only 5 specimens belong to age class 1^+, with 4 males and one female. The total of 17 specimens – 12 males and 4 females – was collected in the age class 2^+. Very large number of specimens, 44 in total, was collected in the age class 3^+ of which 32 are males and 12 are females. The structure of sexes in terms of ratio changes abruptly in higher age classes and therefore in the age class 4+ of the total number of 79 specimens, there are 60 females and only 19 males, etc. A more detailed statistical analysis by using χ^2 test can demonstrate the existence of a statistically significant difference between observed minnow-nase specimens of a particular sex and established age. Only the minnow-nase specimens specified in terms of their sex and age category have been included in this analysis, the total of 205 specimens. The age classes have been also logically grouped according to their reproductive age, i.e. younger specimens, reproductively mature specimens and older specimens with a pronounced stagnation period. The statistical analysis in this case demonstrates the significance level for the tested minnow-nase specimens of 0.004 or 99.6 %.

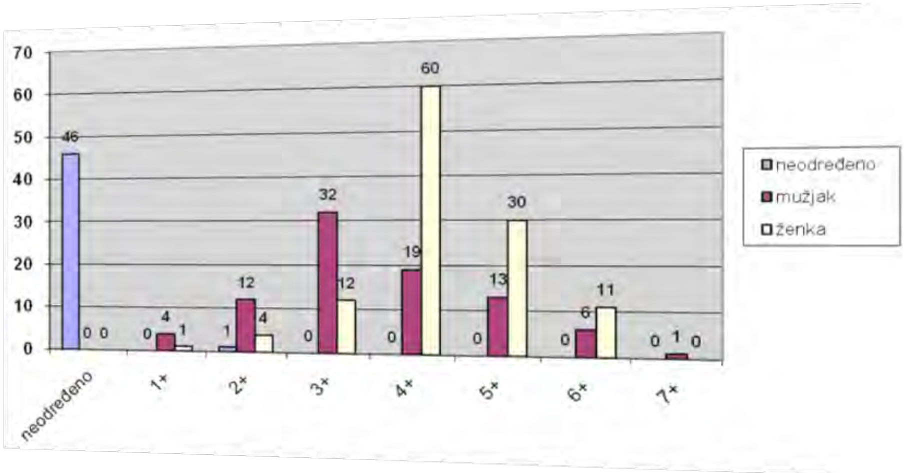

Diagram 1. Correlation of age and sex structure of *Chondrostoma phoxinus* specimens

DISCUSSION

During the research of the *Chondrostoma phoxinus* population in Buško Lake we did not come across a sufficient number of new data and valid results against which the results actualised in this paper could be compared. Therefore, the final conclusions and the discussion are mostly based on some general data on the status of the minnow-nase population in Livanjsko Field watercourses.

Veledar and Kosorić (1972, 1974) present results according to which the analysed minnow-nase specimens from Livanjsko Field waters achieve the greatest body length growth rate during the second and third year of life, which was also concluded in reference to the highest weight growth rate. Furthermore, a hypothesis was stated that certain age classes were unevenly distributed across different sections of Buško Lake, which needs to be confirmed by the results of some future research planned for this reservoir.

Our research presents the seasonal dynamics of occurrence of minnow-nase specimens of specific age classes in Buško Lake reservoir. We have observed that reproductively mature minnow-nase specimens in the age classes 4^+ and 5^+ were more present in the spring – summer season during the two years of research, as was expected since it relates to the spawning period.

Numerous examples from the research of the age and sex structure of mixed fish populations in Buško Lake are provided by M u č i b a b i ć et al. (1973), as part of their detailed limnological research of Buško Blato reservoir. They determined the age structure for the analysed specimens of *Chondrostoma phoxinus* population in Buško Lake and found that 31.16% of minnow-nase specimens belonged to the age class 6^+, which, according to the authors, was inexplicable. The share of the age class 3^+ specimens was negligibly low, lower than 10%, which moreover indicates that our results present a more positive image of the actual status with significant numbers of minnow-nase specimens in the age classes 4^+ (79), 3^+ (44) and 5^+ (43 specimens).

The results of similar analyses have been found in the paper by Cvijović and Kosorić (1985) who concluded that the weight growth was more pronounced in younger age classes and that the highest growth rate was achieved in the specimens in the fourth and fifth year of life.

By analysing the age structure of Buško Lake minnow-nase, Mikavica and Kosorić (1987) established 7 age classes from 3^+ to 9^+, with most specimens belonging to the age class 5^+ (50 specimens, 33,33%). As part of their paper, they recorded 70 males and 80 females from the total number of analysed specimens (150) and they concluded that females were more numerous in older age classes. Because there were no occurrences of younger age classes, the authors predicted the stagnation of the minnow-nase population in future in terms of its reproduction, which cannot be validated at present by the updated data.

CONCLUSION

Research tasks which involve monitoring of the population of *Chondrostama phoxinus* species, as an endemic species of the Adriatic catchment area, present an invaluable scientific contribution to the ichthyological communities of both Bosnia and Herzegovina and Europe. The waters of Bosnia and Herzegovina are inhabited by a large number of species from the Cyprinidae Bonaparte, 1840 family many of which are endemic. The species of the genus *Chondrostoma* Agassiz, 1832 are particularly interesting.

By examining the referenced literature and systematically comparing the current data on the status of fish communities prior to, during and following the formation of the aforementioned Buško Lake reservoir, very obvious and concise conclusions can be made as to the partial degradation of the ecosystem and the endangered status of the endemic ichthyopopulations caused by the distinct interspecies competition encouraged in this case by human activities (uncontrolled fish-stocking – exploitation of the autochthonous fish communities).

At present, the most numerous specimens, with respect to the composition of the entire population of Buško Lake *Chondrostoma phoxinus* species, are the minnow-nases in their fourth year of life, totalling 79 specimens or 31.35%.

The percent share of females in the age class 4^+, compared to the total number of minnow-nase specimens is quite high and amounts to 75.9%. This piece of data, which reflects the dominance of females in the total population, especially in their more reproductive period, provides positive results in terms of the population's growth trend and survival dynamics.

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ALGAE AS WATER QUALITY BIONIDICATORS OF THE RIVER DJETINJA

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ALGE KAO BIOINDIKATORI KVALITETA VODE REKE ĐETINJE

Apstrakt
U radu su prikazani rezultati algološke i saprobiološke analize reke Đetinje u periodu jun - septembar 2008. godine. Na odabranim lokalitetima uzvodno od Užica i u samom Užicu, kao i u Volijačkom potoku, identifikovano je 142 taksona iz 4 razdela algi. Voda reke Đetinje pre uliva Volujačkog potoka pripada II kategoriji, dok se neposredno po njegovom ulivu pogoršava kvalitet (III kategorija). Na lokalitetu „Plaža" u samom Užicu vrednosti koncentracije hlorofila *a* su niske, kao i ukupna brojnost individua/ćelija fitoplanktona po jedinici zapremine i ne ukazuju na značajniju primarnu produkciju fitoplanktona.

Ključne reči: fitoplankton, fitobentos, kvalitet vode, Đetinja
Keywords: phytoplankton, phytobenthos, water quality, river Djetinja

INTRODUCTION

In order to assess the quality of surface water it is usually necessary to take complex physico-chemical and biological examination to obtain the most complete review of the situation and events in the aquatic ecosystem. In most aquatic ecosystems algae are ecologically very important group of organisms as primary producers of organic matter and oxygen. It is possible to notice the first signs of environment degradation and their causes because algae react rapidly to the wide range of pollutants. The possibility of continuous monitoring through space and time and predicting changes in the ecosystem before the occurance of serious damages are criteria which gives advantage to algae as bioindicators over other organism indicators (McCormick & Cairns 1994).

The Djetinja River originates in the southwest of Mountain Tara, under the peak Runjeva Slavica at 1438m altitude. It is 75,4 km long and its catchment area is 1208 km^2. On its way to the city of Užice it partly runs through 8 km long and over 300m deep gorge (partly canyon) valley (Marković 1980). It runs through the city of Užice, and near the town of Požega it connects with the Moravica River and creates the Zapadna Morava River on that way. There are 3 artificial lakes and 2 hydropower plants on the river. The Djetinja River has one large tributary (Skrapež) and several smaller. The relief of the Djetinja River's catchment makes very steep slopes of the mountain massifs, valley is narrow and expends only at the mouth of the Skrapež River and at the part where it meets the Moravica River. The Djetinja River has a character of fast-flowing mountain river and flow rate at the site where it meets the Moravica River is 11,8 m^3/s (Gavrilović, Dukić 2002).

The Djetinja River (at the site Gorobilja) is included in the monitoring program of surface water quality of RHMZ of Serbia (RHMS of Serbia 2008). Available data on the water quality from 1972 based on biological analysis indicate that the water of the Djetinja River was at the transition from α-mezosaprobic to polisaprobic water (Todić 1972). However, algae of the Djetinja River were not systematically studied. Available literature data indicate that there was detailed analysis of diatoms community only at the site Djetinja-Gorobilje (Tomašević 2000).

MATERIAL AND METHODS

The water samples for the qualitative analysis of phytoplankton were collected by plankton net (Ø 22 μm). Phytoplankton samples for quantitative analysis and determination the chlorophyll-*a* concentration were collected using a Rutner's bottle (1 l) from 1 m depth on the site Djetinja „Plaža". Phytobenthos samples were collected by scraping the stone surface or by taking a sample from the bottom surface of the Djetinja River with a pipette and from stone walls on the site „Plaža" as well as by collecting sludge and sand. Also, samples of macrophytes and macroscopic covers were collected from sludge and stones. All samples were immediately fixed with formalin solution to the final concentration of 4%. Qualitative analysis of phytoplankton and phytobenthos were performed with a Zeiss AxioImager.M1 light microscope with software AxioVision 4.8. Quantitative analysis of phytoplankton was done by using Utermöhl`s method (1958) with a Leica inverted microscope and is expressed as number of individuals/l and cells/l. Chlorophyll-*a* concentration was determined with spectrophotometric method according to ISO 10260:1992 (E).

Saprobiological analysis was performed using the Pantle-Buck (1955) and Zelinka, Marvan, Kubiček's (1959) method that is based on algae as indicators of the saprobity degree. Diatom pollution index (DAIpo) (Watanabe et al. 1986) is based on benthic diatoms as indicator and is used as an ecological indicator of organic pollution in aquatic ecosystems.

RESULTS AND DISCUSSION

In the examination of the algological samples, the total number of 142 taxa was identified from four divisions: Cyanobacteria, Chlorophyta, Euglenophyta and Bacillariophyta. From the total number of taxa, there are 81 present in the phytoplankton, and 131 in the phytobenthos. In both communities, the highest species diversity was observed within Bacillariophyta.

Quantitative analysis of phytoplankton

The total number of individuals in the phytoplankton at the site Djetinja „Plaža" ranged from 18.240 individual/l in July to 144.320 individual/l in June. Also, the maximum number of cell/l was observed in June (1684.960 cell/l), and the minimum number in July (54.560 cell/l) (Fig. 1). Changes in the number of individuals/cells/l in phytoplankton do not indicate a significant level of primary production. In June, a significant difference was observed between the number of individuals and number of cells per unit volume which is caused by the greater presence of colonial and trichal algae. In the phytoplankton of the Djetinja River, during the first 3 months of examination, in all samples expressed as number of individual/l, diatom *Fragilaria vaucheriae* dominated. In September diatom *Stephanodiscus hantzchii* became dominant, while *F. vaucheriae* changed to subdominant.

Phytoplankton quantitative analysis expressed as number of cells per unit of volume indicates a different situation. In the samples significantly less individuals of trichal blue-green algae were present, but when it was recalculated to the number of cells these individuals took absolute dominance. During the whole studied period absolute dominance of trichal blue-green algae *Phormidium tergestinum* was recorded in phytoplankton. This algae was probably found in the phytoplankton at the site „Plaža" due to erosion of the Djetinja River's benthos, downstream from the mouth of Volujac Stream.

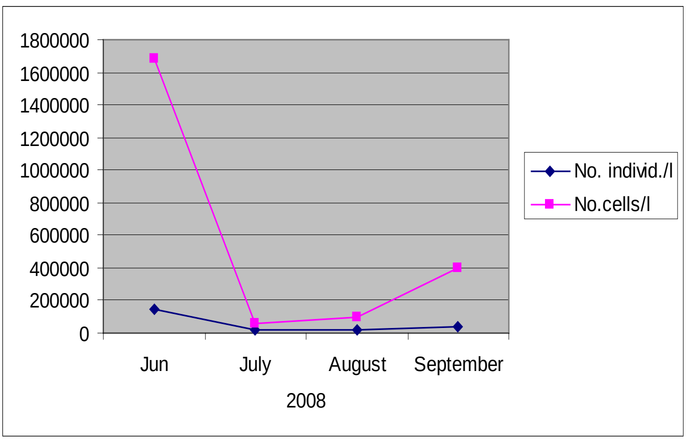

Figure 1. Abundance of phytoplankton community at the site Djetinja „Plaža"

Analysis of chlorophyll-a concentration

The highest chlorophyll-*a* concentration was recorded in July (6,6 µg/l), at 1 m depth, and the lowest in August (3 µg/l) (Fig. 2). The values of the chlorophyll-*a* concentration are low and do not indicate a significant phytoplankton production at the site Djetinja „Plaža" in the studied period.

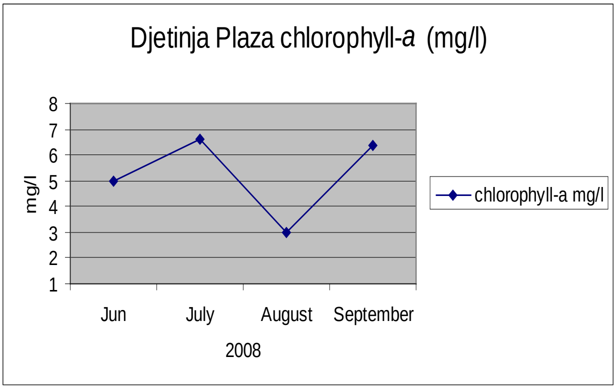

Figure 2. Chlorophyll-*a* concentration at the site Djetinja „Plaža“ in the studied period

Water quality analysis of the Djetinja River based on diatom pollution index (DAIpo)

The values of DAIpo index at the first site Djetinja above Volujac Stream indicate that the water was β-mesosaprobic (category II) exept in September (α-mesosaprobic, category III). During the studied period there was a slight decrease in the value of DAIpo index at this site as well as decrease of water quality (Fig. 3). The second site Djetinja under Volujac Stream is only ten meters downstream from the first site. The values of DAIpo index indicate that the water was α-mesosaprobic (category III). At the third site Djetinja "Plaža“ DAIpo index also indicate that the water is α-mesosaprobic (category III).

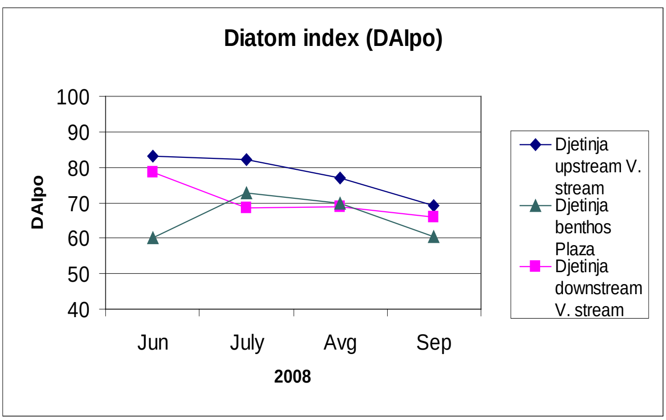

Figure 3. Diatom pollution index (DAIpo) of the Djetinja River at the studied sites

The data from DAIpo index clearly show deterioration of water quality and organic load of the Djetinja River during the studied period, immediately after the influx of Volujac Stream. Also, general decrease in the water quality of the Djetinja River is observed in the study.

Saprobiological analysis of the Djetinja River

Saprobity index according to Pantle-Buck (S) at all studied sites during the whole study was in the β-mezosaprobic zone (shows that the water of the Djetinja River is in category II, fig 4.). However, it is clear that the water of Djetinja River above Volujac Stream has slightly lower saprobity (better quality then downstream part of the river).

The resulting saprobic valence of saprobity index in June at the studied sites based on 43 indicator taxa are in β-mesosaprobic zone. The Djetinja River’s site above Volujac Stream has the best quality. Immediately after the influx of Volujac Stream it has the lowest quality with a high α-mesosaprobic value (Fig. 5).

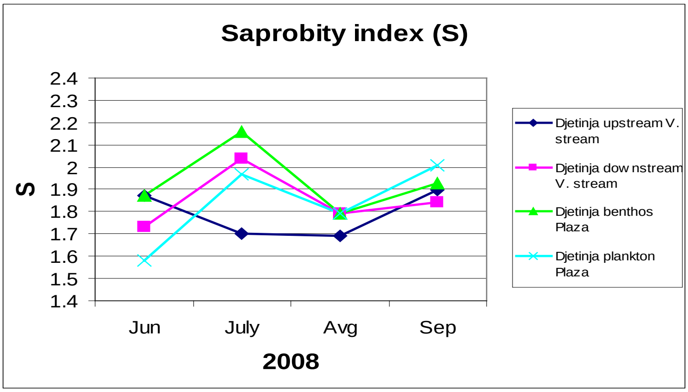

Figure 4. Saprobity index (S) of the Djetinja River at studied sites

Forty indicator taxa were determined at the studied sites in July 2008. The water above Volujac Stream has the best quality (β-mesosaprobic zone, category II of water) with the highest ratio of oligosaprobic value. The Djetinja River’s water has significantly lower quality just after the influx of Volujac Stream with an almost equal ratios of α-mesosaprobic and β-mesosaprobic values. Benthos analysis at the site „Plaža“ (Fig. 5) shows low water quality (α-mesosaprobic). This confirms the results of quality and quantitative analysis from the site Djetinja “Plaža“. These results show that erosion from the benthos of Djetinja (downstream from the influx of Volujac Stream) leads to precipitation of large amount of sludge and creation of favorable conditions (water temperature and nutrients) for the development of blue-green algae and diatom community. Eventually, these organisms reach the surface where they decompose and form a scum on the water sufrace.

Forty-seven indicator taxa were determined at the studied sites in August 2008. The resulting saprobic valence at all studied sites were in β-mesosaprobic zone. Figure 5 shows that the lowest quality was recorded at the site placed immediately after the influx of Volujac Stream.

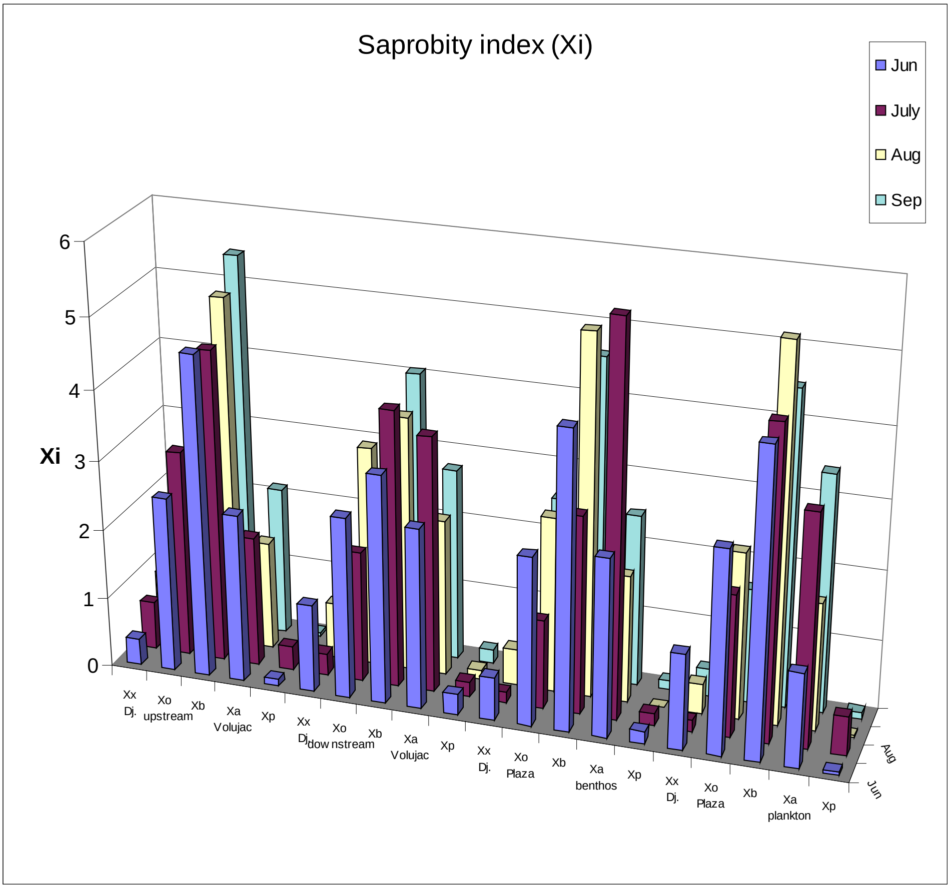

Figure 5. Saprobity index (Xi) of the Djetinja River.

Forty indicator taxa were determined at the studied sites in September 2008. The resulting saprobic valence at all studied sites were in β-mesosaprobic zone. Figure 5 shows that the lowest quality was recorded at the site placed just after the influx of Volujac Stream in the Djetinja River.

CONCLUSION

According to the obtained algological and saprobiological water analysis of the Djetinja River and Volujac Stream in the period from June to September 2008 at the studied sites, it can be concluded that the water of the Djetinja River is in II category just before the influx of Volujac Stream. Hydrological conditions (widened and deepened bottom and a significant deceleration of flow) enable widespread development of macrophytes and epiphytic community, which further contribues to the polution load of the river. The water quality of the Djetinja River immediately after the influx of the Volujac Stream has a deteriorating status and is in the transition from category II to III according to S and Xi, category III according to DAIpo. The Volujac Stream brings a large amount of suspended particles (sludge) to the Djetinja River which leads to the blurring of the water and the creation of thick layers of sludge at the river bottom. Blue-green cover is made by blue-green algae *Phormidium tergestinum* and green algae *Cladophora glomerata* and it is found in the suspended material of the Djetinja River, which completely covers the bottom and enables the development of epiphytic community. The chlorophyll-*a* concentration level indicates a low primary production of phytoplankton at the site „Plaža" in the studied period. The total number of phytoplankton individuals/cells per unit volume at the site „Plaža" does not indicate a significant level of production. The occurrence of dominant benthic forms in the upper parts of the river, indicates a significant influence of upper flow on water quality at the site „Plaža". Macroscopic floating formations at the site „Plaža", consist of the communities of blue-green algae, diatoms and suspended sludge particles which are generated by lifting from the bottom as a consequence of algae over- reproduction.

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WATER QUALITY ASSESSMENT IN THE RAŠKA RIVER BASED ON ZOOBENTHOS AND ZOOPLANKTON ORGANISMS AS BIOIDICTORS

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KVALITET VODE U RECI RAŠKOJ NA OSNOVU ORGANIZAMA ZOOBENTOSA I ZOOPLANKTONA KAO BIOINDIKATORA

Apstrakt

U cilju ispitivnja kvaliteta vode reke Raške, organizmi zoobentosa i zooplanktona korišćeni su kao bioindikatori. Istraživanje je sprovedeno od aprila 2011. godine do maja 2012. godine, u vremenskim intervalima od dva meseca. Odabrano je ukupno pet lokaliteta na dužinini toka od 2.5 km. Između drugog i trećeg lokaliteta lociran je pastrmski ribnjak, čiji je uticaj na zajednice organizama praćen. Na lokalitetima koji su obuhvaćeni istraživanjima konstatovano je 57 taksona makrozoobentosa (34 vrste, 21 roda, 1 familija i 1 klasa) i 75 taksona zooplanktona (58 vrsta, 15 rodova, 1 red i 1 klasa). Srednje vrednosti indeksa saprobnosti ukazivale su na manje razlike u dobijenim vrednostima korišćenjem zoobentosa i zooplanktona. Na osnovu saprobioloških analiza, kada su kao bioindikatori korišćeni organizmi bentosa, kvalitet vode u reci Raškoj je druge klase kvaliteta, ili na prelazu između prve i druge klase, uglavnom na lokalitetima iznad ribnjaka. Organizmi zooplanktona su pokazali da voda celom dužinom toka pripada prvoj klasi kvaliteta. Najmanje vrednosti indeksa saprobnosti zabeležene su na prvom, referentnom lokalitetu 1.453±0.098 (zooplankton), a najviše na četvrtom lokalitetu 1.88±0.021 (zoobentos). Na lokalitetu ispod ribnjka, gde je zabeležen pad u koncentraciji kiseonika, dominiraju organizmi bentosa koji tolerišu veći stepen organskog zagađenja (Chironomidae, Oligochaeta, Simuliidae). Iako su koncentracije ukupnog fosfora i ortofosfata rasle od trećeg ka petom lokalitetu, njihove koncentracije nisu premašivale dozvoljene vrednosti za salmonidne vode. Međutim, srednje vrednosti koncentracije nejonizovanog amonijaka ($NH4^{+}$) su iznad referentnih vrednosti na trećem i četvrtom lokalitetu.

Razlog nepodudaranja vrednosti indekasa saprobnosti za dve istraživane grupe, može biti usled nestabilnih zooplanktonskih zajednica zbog izraženog efekta drifta, kao i usled činjenice da će zbog procesa sedimentacije dospelih organskih materija, organizmi dna biti izloženiji njihovom uticaju. Zajednice bentosa, kao znatno stabilnije i u vremenu i u prostoru, pogodnije su za procenu kvaliteta tekućih voda.

Ključne reči: *zoobentos, zooplankton, kvalitet vode, bioindicators*
Keywords: zoobenthos, zooplankton, water quality, bioindicators

INTRODUCTION

Freshwater pollution represents one of the major global problem of the modern age. Various human activities, particularly those related to agriculture, have a great impact on the amount of organic matter found in freshwaters. In Serbia, some first and second order streams are under the influence of trout farms that are built close to their sources. Organic matter in the form of degraded fish feed and feces are drained into the environment usually without previous treatment and have a big impact on water quality of the recipient (Marković and Poleksić, 2011). Chemical parameters, as indicators of water quality, do not provide the state of biological communities and therefore cannot properly indicate the status of freshwater ecosystems (Camargo *et al.,* 2011). Over the last decades, biological methods have been recommended as a useful tool for the assessment of water quality (Hynes, 1970). Together with chemical parameters, they are used in monitoring freshwaters.

In addition to various types of biotic indices, saprobiological analysis of water ecosystems is one of the main methods for the assessment of water quality using aquatic organisms. Each water quality class has a corresponding saprobic state based on the the amont of certain organic material susceptible to degradation (Moog, 2002). Different groups of animal and plant species such as algae, macrophytes, zooplankton, macrozoobenthos, meiobenthos and fish can be used in biological monitoring.

In first and second order streams biological monitoring, is usually based on zoobenthos as the most stable and the most diverse of all aquatic communities. In many lotic systems they form communities with over a hundred species from many phyla such as Arthopoda, Mollusca, Annelida, Nematoda i Platyhelminthes (Turbellaria) (Hose *et al.*, 2004). As a group also used in biological monitoring, zooplankton offer several adventages: they have worldwide distribution and their communities are sensitive to changes in environmental factors as well as the amount of organic matter in aquatic habitats, therefore can indicate water quality (Arimoro & Oganah, 2010). However, zooplankton is not often used in monitoring especially in smaller rivers, due to intense drift effect on its communities. Though, many studies show that they may be present in such water bodies, particularly within the phylum Rotatoria. According to Kobayashi *et al.* (1998) flow velocity is the main factor affecting the diversity of zooplankton in lotic systems.

The aim of this study was to determine the effects of a trout farm on the water quality downstream from the recipient, using macrozoobenthos and zooplankton as bioindicators. Additionally, we compared the values of saprobic index and chemical parameters in order to determine the level of correspondence between the results.

MATERIAL AND METHODS

Study area

The Raška River is a left tributary of the Ibar River, located in the southwestern part of Serbia. Its length is 39 km, width 10-25 m, depth 0.3-1 m and the catchment area is 1040 km^2 (Gavrilović i Dukić, 2002). The source of the Raška River is a specific geomorphological phenomenon. Karst Koštam field is a shortened source of the river Raška and is located 950 m a.s.l. (Marković, 1988). Across the bottom of the Koštam field are sinks of the Točilovska, Delimeđska and Likovska River. Waters of Koštam field are drained and flow through underground channels to emerge from the cave in the form of a strong spring, in the vicinity of the monastery Sopoćani. This water is used for electricity production and partly for trout farm supply in the area of Ras, in the village Pazarište.

This research was conducted during 2011 (April, June, August, October, December) and 2012 (March and May). Sampling of macrozoobenthos and zooplankton was carried out at five localities of a channel formed by redirecting the Raška River from it's source (2.5 km in length). (Figure 1).

The first locality (RŠ1) is situated near the river source at 557 m a.s.l. RŠ2 locality is 400 m away from the RŠ1, at 548 m a.s.l. RŠ3 locality is placed 10 m below the outlet of the channel owned by the trout farm "Vekoslav Vukićević". Communities of aquatic macrophytes are dominant at this site. Two hundred meters below the third locality is RŠ4 locality, on 535 m a.s.l. RŠ5 is situated just before the confluence with the main stream of the Raška River, 1.7 km from the forth locality, at 540 m a.s.l. Pebble, gravel and stones are the dominant substrate type at all localities.

Figure 1. Map with marked localities on the chanell of Raška River

Methods

Macrozoobenthos samples were collected with Surber net, with a catchment area of 300 cm^2 and mesh size of 250 µm. Two samples were taken per locality. The collected material was placed in plastic bottles and preserved with 96% alcohol. The samples were examinad using ZEISS Discovery V8 stereomicroscope in the laboratory of Institute of Zoology, Faculty of Biology. Macroinvertebrates were identified to genus or species level according to following taxonomic keys: Rozkošny1980, Waringer and Graf 1997, Lechthaler and Stockinger 2005, Nilsson 1996, Edington and Hildrew

1981. Zooplankton samples were taken by collecting 30 l of water at each locality using a plastic bucket, and filtering through sieve with 23 µm mesh size. Lentic areas with dense vegetation were chosen. Samples were preserved using 4% formalin and examined under optical microscope Leica Galen III with maximal magnification of 160x. Zooplankton was identified to species or genus level using standard keys for identification (Flosner 1972, Koste 1978). Quantitative samples were analyzed using subsampling technique, where the number of identified species was recalculated to the volume of 1 liter

Saprobity index (S) was calculated according to Pantle-Buck (Pantle and Buck, 1955) method:

$$S = \frac{\sum h \cdot s}{\sum h}$$

h – absolute abundance of individual taxa, s – individual taxa saprobic value. Saprobity values were used from the list of indicator species given by Wegl (Wegl, 1983) for zooplankton and Moog (Moog, 2002) for macrozoobenthos.

Physical and chemical parameters

Basic physical and chemical parameters were measured directly in the field and at the laboratory of the Institute for Chemistry, Technology and Metallurgy, Belgrade, Serbia. Measurements of temperature, pH, dissolved oxygen and conductivity were done using water field kit PCE-PHD meter (Germany). Analyses of total phosphorus, orthophosphates (OP) and ionized ammonia (NH_4^+) were preformed according to APHA protocols (APHA, 1998).

Data analysis

In order to estimate the differences in the saprobity index between localities, t-test was applied using Sigma Stat program (version 2).

RESULTS AND DISCUSSION

A total number of 57 taxa of zoobenthos were found at all studied localities. Of the 57 taxa, 34 were determined to the species level and 21 taxa to the genus level. Oligochaeta and Chironomidae were determined to the class i.e. family level. Aquatic insects were the most diverse group of macroinvertebtares in the Raška River, especially Diptera, with 17 identified taxa (29.82%), followed by Trichoptera (13 taxa, 22.81%.), Mollusca (5 taxa, 8.77%), Coleoptera (3 taxa, 5.26%) and Platyhelminthes (1 taxon, 1.75%). The same number of taxa were recorded for Ephemeroptera and Plecoptera (7 taxa, 12.28%) as well as for Hirudinea and *Gammarus* (2 taxa, 3.51%). The diversity of organisms was high at most localities and in all seasons (*Ancylus fluviatilis, Valvata cristata, Pisidium* sp., *Gammarus balcanicus, Gammarus fossarum, Baetis* sp., *Ephemerella ignita, Ephemera vulgata, Rhyacophila fasciata, Rhyacophila tristis, Adicella* sp.), but their abundance varied. Taxa sensitive to changes in the aquatic environment (reduced oxygen concentration, high concentration of organic matter) like *Dinocras cephalotes, Rhitrogena* sp., *Sericostoma personatum* were found at localities downstream from the trout farm, but were not abundant.

During the research period, a total of 75 zooplankton taxa were found in the Raška River. Rotifers were the most diverse group (65 taxa, 86.67%). Representatives of the zooplankton community also belong to Cladocera (7 taxa, 9.33%), Copepoda (2 taxa, 2.67%) and Ostracoda (1 taxon, 1.33%,) The highest number of identified species are among following genera: *Lecane*, *Brachionus*, *Cephalodella* i *Lepadella*. Species common for all studied sites are: *Collurella adriatica*, *Encentrum putorius*, *Euchlanis dilatata*, *Lepadella patella*, *Trichocerca porcellus*, *Mytilina mucronata*, *Alona affinis* and *Alona guttata*.

The composition of zoobenthos communities clearly reflects values of saprobity index. Mean values of the saprobity index along localities ranged between 1.631±0.02 to 1.88±0.021 (Figure 2). The highest values were recorded at localities below the trout farm (RŠ3 and RŠ4), particularly in August (1.91 and 1.94). The saprobity index at RŠ5 was lower and similar to the reference locality RŠ2 (Fig.2). Significant differences in the value of the saprobity index were recorded between fourth and all other localities ($p<0.05$), i.e. its values clearly indicate a changes in the water quality. The results of the saprobity index show that the water quality along the investigated localities is between the first and second class, i.e. from oligo to beta mesosaprobic.

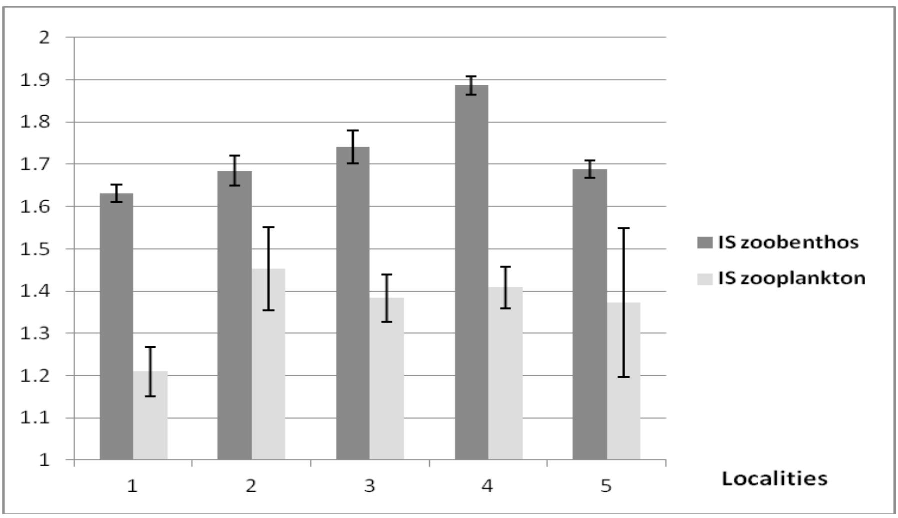

Figure 2. Saprobity index values (mean±standard error) for zoobenthos and zooplankton communities

Index of saprobity obtained from the analysis of zooplankton communities indicate a rather different trend. The results of t-test show that the differences of the saprobic index values are almost absent and the only small difference is between first and second locality ($p=0.049$). The highest mean value is recorded at the second locality (1.453±0.098), whereas the lowest values, as in the case of zoobenthos, are observed at the first locality. When using zooplankton organisms, the saprobity index value indicates the first class of water quality along the course, i.e oligosaprobic.

Mean values of basic chemical parameters such as temperature, pH and conductivity, were rather constant at all localities (Figure 3). The exception was a remarkable decrease in the mean concentration of dissolved oxygen at the third locality. This was expected since RŠ3 is the first locality below the fish farm that regularly discharges water used for trout farming. Concentration of orthophosphates and total phosphorus were below the recommended upper limit (TPECD, 2006) (Figure 4). Downstream from the recipient a slight increase in the concentration of these two parameters was observed. Ammonia concentration varied considerable during the research period (Figure 4). During August and October at RŠ1 and RŠ2 localities values of this parameter were below the limit of detection (<0.01 mg/l), whereas in other months its concentration ranged from 0.01 to 0.05 mg/l. Rapid increase in the concentration of ammonia was noted at the localities below the trout farm. At the localities RŠ3 and RŠ4, the mean values of this parameter were higher than recommended for salmonid waters (Fig. 4)(TPECD, 2006).

By comparing the results obtained from chemical and biological analysis, we can conclude that the values of chemical parameters correlate strongly with the saprobic index of macrozoobenthos. This holds especially for ammonia and oxygen concentration (Figure 3 and 4). Sudden changes in these parameters clearly indicate the influence of the trout farm on the water quality as well as on zoobenthos communities. Oligocaheta, Chironomidae, *Gammarus* and *Simulium* as one of the most tolerant taxa to organic pollution were dominant at RŠ3 locality.

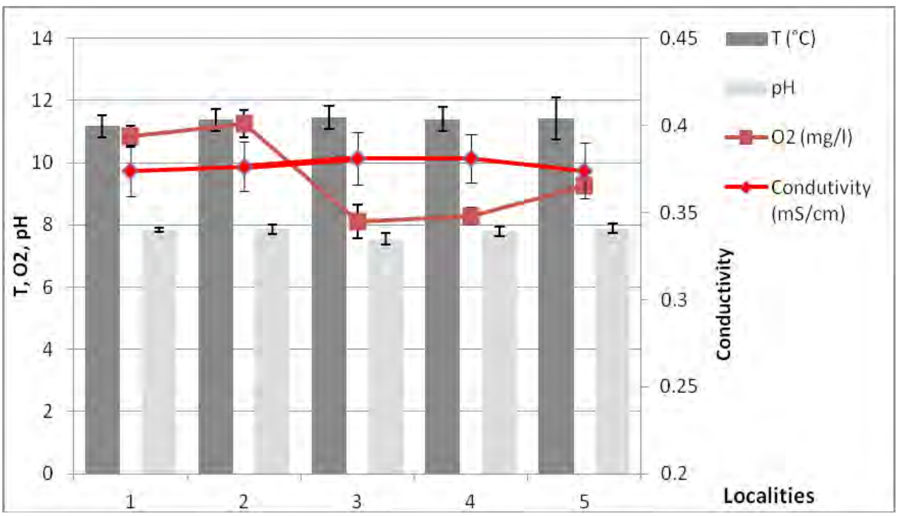

Figure 3 Mean values (±standard error) of pH, oxygen, temperature and conductivity at studied localities

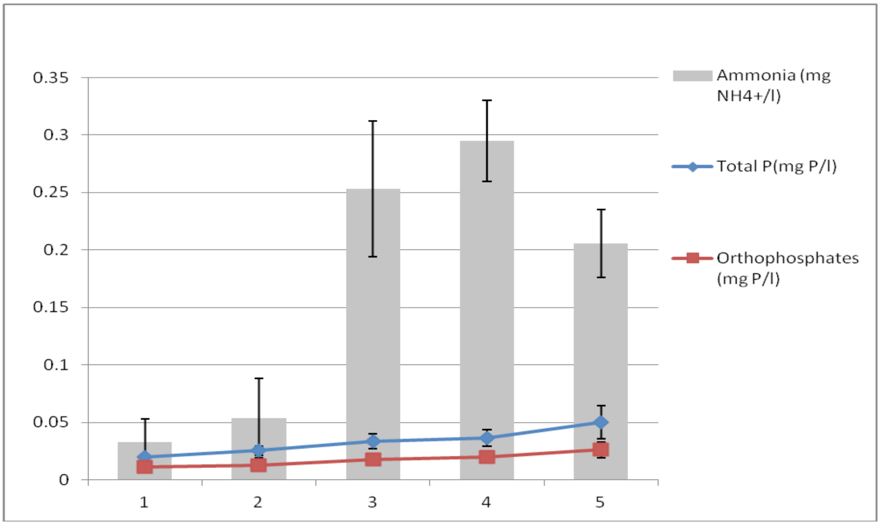

Figure 4. Mean values (±standard error) of total phosphorus, orthophosphates and ammonia at studied localities

Majority of sampled zooplankton organisms belong to the I and II water quality class (Wegl, 1983). The exception is genus *Brachionus*, usually present in waters of second and third class of quality. At the localities downstream from the trout farm, only one species, *Brachionus calyciflorus*, was recorded, while other species of this genus were found at the localities upstream from the trout farm (*B. angularis*, *B. diversicornis*, *B. falcatus*, *B. rubens*). Although 75 taxa of zooplankton were found at the investigated localities, Rotifers were the absolute dominating group. One of the reasons for their prevalance in lotic systems, could be that most of the species found are those adapted for living in intersticial sediments of running waters, especially those with gravel and peble sediments (Schumid-Araya, 1998). A lot of authors reported high abundance and diveristy of Rotifers in streams and rivers of various orders and can constitute from 35 to 85% of the meiofaunal community (Palmer, 1990a,b, 1992; Schmid-Araya, 1997, 1998)

CONCLUSIONS

Taking into account biological and chemical parameters, water quality of the Raška River was between the first and the second class of the quality.

Results of the saprobiological analysis of zooplankton showed better water quality (oligosaprobic) while macrozoobenthos communities showed that water was betamezosaprobic at all studied localities. This difference was probably due to drift effect in running waters affecting zooplankton communities. Additionally, changes in their populations are more subtile and might need a longer period of investigation to provide comparable results.

Chemical parameters showed correspodence with the macrozoobenthos index of saprobity. The slight increase in total phosphorus and orthophosphates concentrations from the third to the fifth locality, highest ammonia concentration at the third and forth locality and decline of oxygen concentration, especially at the third locality were followed by a adequate decrease in the saprobity index.

ACKNOWLEDGEMENTS

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EFFECTS OF DIFFERENT ORGANIC MANURES ON BODY SIZE AND PRODUCTION OF *DAPHNIA MAGNA*

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EFEKTI RAZLIČITIH VRSTA ORGANSKOG ĐUBRIVA NA VELIČINU TELA I PRODUKCIJU *DAPHNIA MAGNA*

Apstrakt

U akvakulturi se već decenijama masovno proizvodi zooplankton kao živa hrana za gajenje mlađi riba, rakova i mekušaca. Za slatkovodne ribe se osim naupliusa artemije i rotatorija može koristiti i veliki broj drugih organizama, naročito iz grupe Cladocera (*Daphnia magna, D. carinata* i dr.). Korišćenje organiskog đubriva (stajnjaka) za proizvodnju žive hrane, može biti ekološki (uklanjanje nutrijenata) i ekonomski isplativo. Stajnjak je bogat izvor azota, fosfora i ugljenika kao i organskih materija i kao takav se može koristiti kao alternativni izvor nutrijenata za gajenje žive hrane za potrebe akvakulture.

Cilj ovog istraživanja je bio ispitivanje efekata 4 različite vrste stajnjaka (goveđi, ovčiji, koziji i riblji) na produkciju i veličinu tela D. magna. Eksperiment je realizovan u laboratorijskim uslovima, u 1L čašama, u dozi od 1g/L stajnjaka. Pored praćenja hemijskih parametara (kiseonik, elektroprovodljivost, pH, ukupan fosfor, nejonizovani amonijak), određivana je i ukupna brojnost dafnija kao i njihova veličina iz celih uzoraka. Tri veličinske klase dafnija (mala, srednja i velika) su ispitivane da bi se dobio uvid u populacionu strukturu i reproduktivni potencijal dafnija za čije su gajenje korišćeni različiti stajnjaci. Značajno veća brojnost dafnija ($P<0.05$) dobijena je u tretmanu sa goveđim stajnjakom, u odnosu na tretmane sa ovčijim i kozijim stajnjakom. Između ostalih nije bilo značajnih razlika. Kada se razmatraju veličinske klase, statistički značajno najveću brojnost su imale male *D. magna* u svim tretmanima u odnosu na velike. Na osnovu dobijenih rezultata, može se reći da je tretman sa goveđim stajnjakom pokazao najbolje rezultate i kada je u pitanju ukupna brojnost, ali i brojnost po veličinskim klasama (prosečna brojnost malih, srednjih i velikih dafnija je bila najveća). Osim toga ovaj tretman se izdvajao od ostalih i po svim hemijskim parametrima. U njemu je izmerena najveća prosečna koncentracija kiseonika, elektroprovodljivosti i pH, a najmanja koncentracija ukupnog fosfora i nejonizovanog amonijaka. U zaključku se može reći da je goveđi stajnjak obezbedio najbolje uslove za rast i reprodukciju *D. magna*.

Ključne reči: Daphnia magna, stajnjak, živa hrana, slatkovodne ribe
Key words: Daphnia magna, manure, live food, freshwater fish

INTRODUCTION

Mass production of zooplankton as live food feeding of fish larvae, fry and adults, crustaceans and mollusks has been used in the aquaculture industry for decades (Hertrampf and Piedad-Pascual, 2000; Southgate, 2003).

For freshwater fish larvae except Artemia salina nauplii and rotifers a wider range of zooplankton can be used as live food especially cladocerans. *Daphnia magna* has been extensively used as live food for a range freshwater fish species due to their easy maintenance and adaptability to survive in low water quality conditions (Haisy and Porter, 1977; Dinges, 1982). Using animal manures for live food production can be ecologically (removing nutrients) and economically feasible. Animal manures are a rich source of nitrogen, phosphorus and carbon as well as organic mater and have been used as an alternative to other sources of nutrients for live food production (Wohlfarth and Schroeder, 1979).

The aim of the present investigation was to study the effect of four different organic manures (cow, sheep, goat and fish) on the production and body size of *Daphnia magna*. The three size classes of investigated organisms will give insight into the population structure and reproduction capability of daphnia fed different diets. The organic manure that provides the best conditions for growth of *D. magna* in terms of abundance and body size can be used either as live food for fish larvae and fry in hatcheries or as exogenously added zooplankton for juveniles or adult freshwater fish especially in ornamental fish production.

MATERIAL AND METHODS

The experiment was conducted by using a laboratory clone of *Daphnia magna* obtained from RECETOX (Masaryk University) initially grown on a combination *of Scenedesmus quadricauda* and bakers yeast. *D. magna* was reared in 1L beakers with 4 different organic manures (cow, sheep, goat and fish) during 30 days. Before the beginning of the experiment manures were left to dissolve in dechlorinated water for 12 days at a dosage of 1g/L, in glass beakers. The beakers were kept in a water bath at 24 °C and were thoroughly mixed every day. Every type of manure was applied in triplicates. On the 13th day each beaker was inoculated with 5 medium size adult *D. magna* individuals. The light regime was 12 hr day and 12 hr night.

Water quality parameters such as temperature, dissolved oxygen (DO), pH and conductivity (COND) were measured every other day using WTW MULTI 340i/SET (WTW, Germany). Water samples for laboratory analysis of total ammonia nitrogen and total phosphorus (TP) were taken once a week from all three replicates per treatment in equal amounts (50 ml) as an integral sample (150 ml). Total ammonia nitrogen and total phosphorus were measured following APHA (1998). Un-ionized ammonia (UIA) was calculated from total ammonia nitrogen taking into account pH and temperature (Alabaster and Lloyd 1980).

At the end of the experiment, water from every beaker was filtered through a 25 μm sieve and fixed with 4% formalin. All individuals of *Daphnia magna* from samples were counted and body length measured from the tip of the head to the base of the tail spine in Sedgwick Rafter chamber using a microscope ocular micrometer. According to the measurements animals were grouped into three size classes: small (700 - 1400 μm), medium (1400 - 2000 μm) and large (2000 - 3200 μm).

Statistical analysis

All data are presented as mean ± SE. Two-way ANOVA was used to test differences ($P<0.05$) in *Daphnia magna* total densities and densities between size classes and type of manure. One-way ANOVA was used to test differences ($P<0.05$) in water chemistry parameters depending on type of manure used for rearing. In the cases where water chemistry parameters showed linear change over time paired t-test was used to test for statistical differences ($P<0.05$). All statistical tests were performed with Sigma Plot version 9 software (Systat Software, Inc.).

RESULTS

Water temperature was constant during the experiment in all treatments, 22°C. One way ANOVA showed significant differences between all measured chemical parameters except for un-ionized ammonia. Mean values of all parameters are show in Table 1. In all treatments, excluding beakers with goat manure, dissolved oxygen had an increasing trend from the beginning to the end of the experiment (Figure 1.a). A striking increase of this parameter was evident after day 18 in fish and goat manure and ranged from 3.54 and 4.24 mg/L up to 9.12 and 9.35 mg/L, respectively (Table 1.). On average, the highest level of dissolved oxygen was obtained in the treatment with cow manure (Table 1.).

Table 1. Average values (mean ± standard error) of chemical parameters, number of *Daphnia magna* individuals in three size classes and their total number in all treatments

Types of manure	DO	COND	pH	TP	UIA	Small size	Medium size	Large size	Total number
Cow	6.7 ± 0.2^{a}	659 ± 4^{a}	8.78 ± 0.02^{a}	0.27 ± 0.05^{a}	0.20±0.06	155±71	88±26	41±19	95 ± 28^{a}
Sheep	6.0 ± 0.2^{a}	818 ± 10^{a}	8.70 ± 0.02^{b}	0.33±0.09	0.20±0.05	14±5	62±8	7±3	28 ± 9^{a}
Fish	4.5 ± 0.4^{a}	778 ± 22^{a}	$8.35\pm0.08^{a,b}$	0.59 ± 0.10^{a}	0.34±0.09	93±34	42±17	10±2	48±16
Goat	5.4 ± 0.5^{a}	830 ± 20^{a}	8.57 ± 0.04^{a}	0.61 ± 0.08^{a}	0.24±0.10	22±15	14±10	11±4	16 ± 6^{a}

* data in the same column sharing the letter ($^{a,\,b,c}$) are statistically different ($p<0.05$).

Conductivity showed an increasing trend during the investigation period in all four treatments (Figure 1b). Overall, significantly lower ($P<0.05$) average values of COND were noted in cow manure. When paired t-test was applied conductivity of fish manure was significantly lower ($P<0.05$) than goat manure. pH values increased during the experiment in all beakers apart from those with cow manure where a slight decrease of this parameter was noted (Figure 1c). On average, treatment with cow manure had significantly higher ($P<0.05$) values of pH than treatments with fish and goat manures. In paired t-test pH of fish manure was significantly higher than goat manure. A notable decrease of total phosphorus was evident in all treatments until day 15, while thereafter increase in fish and goat treatments was observed (Figure 1d). Significantly higher average values (P<0.05) of TP were evidenced in fish and goat treatments compared to the cow manure treatment. Un-ionized ammonia was the only parameter that showed no significant differences between treatments. A decreasing trend of this parameter was observed in all treatments with a slight increase in the second half of the study (Figure 1e).

Two-way ANOVA showed that there was significant difference in total number of *D. magna* between treatments (P<0.05). However, treatments and size classes of daphnia were statistically independent. The total number of daphnia in treatment with cow manure was significantly higher (P<0.05) than in sheep and goat manure. However, no significant difference was found between other treatments. Among the three size classes of daphnia, significantly higher numbers were obtained in the small class compared to the large size class in all treatments.

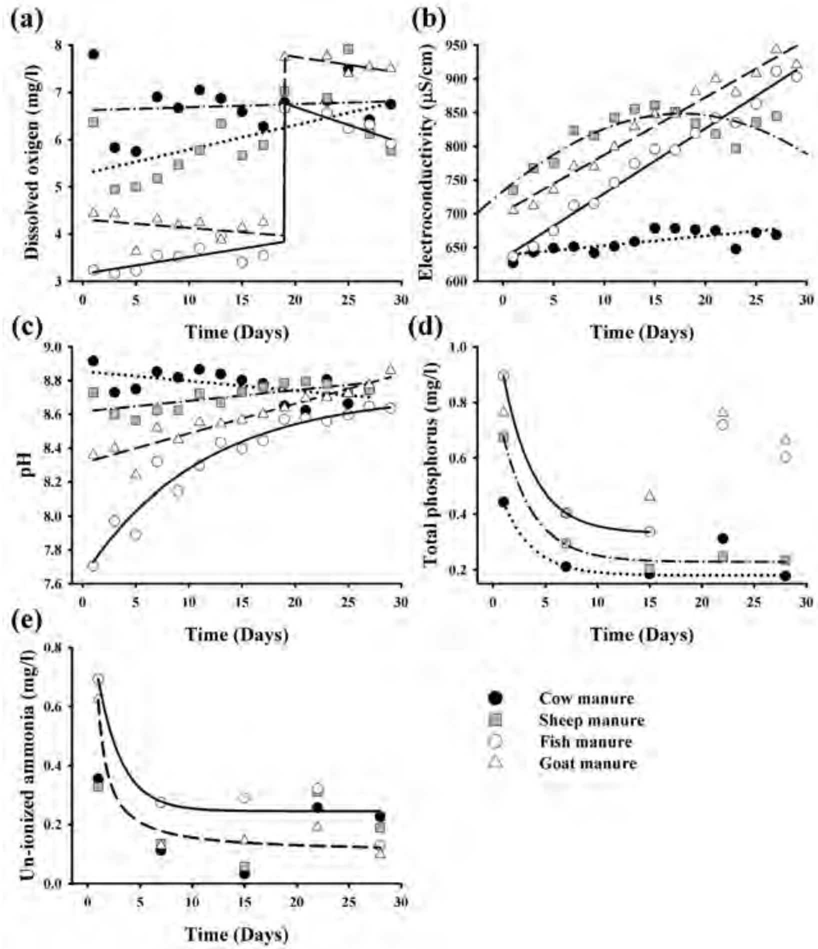

Figure 1 a–e. Measured chemical parameters (dissolved oxygen, electroconductivity, pH, total phosphorus and un-ionized ammonia) in four treatments during the 30 day experiment

Between other size classes no significant differences was observed. Overall, total number of *D. magna* in all size classes were on average higher in treatment with cow manure that in other three treatments.

DISCUSSION

Dissolved oxygen in all four treatments had a general decreasing trend from the beginning towards the end of the experiment. This was expected due to higher consumption rates on bacteria and ciliates present in manures by increased number of daphnias over time. Similar results were obtained by other authors (Jana and Chakrabarti, 1993, Srivastava et al. 2006). Srivastava et al. (2006) investigated effect of 4 different dosages of a mixture of cattle manure and poultry droppings on the production of *Ceriodaphnia carinata*, where irrespective of manure dosage a subsequent increase of DO towards the end of the experiment was observed.

Daphnia magna is often found in wastewater treatment ponds loaded with organic matter (Kibria et. al. 1999, Cripp and Kumar, 2003). This species is known for its high utilization of nitrogen ammonia and total phosphorus. Therefore *D. magna* can significantly improve the water quality due to their high filtering capabilities while grazing on pathogenic and fecal bacteria as *E. coli* (Kima et al., 2003, Shiny et al., 2005, Jung et al., 2009,). The results of our study are in line with above mentioned investigations. *D. magna* reduced the amount of total phosphorus in all types of manures regardless of their initial levels. This holds for un-ionized ammonia as well, a clear decrease in all treatments was evident throughout the present study. While being a more toxic form of ammonia for most aquatic animals, zooplankton, especially cladoceran can sustain high concentrations of UIA.

Considering the effect of treatments on the production and body size of daphnia, it seams that cow manure treatment provided the ideal conditions for growth and reproduction of *D. magna*. The overall highest number of individuals was obtained in cow manure. Additionally, on average, all size classes of daphnia were more abundant in this treatment than in the remaining ones.

In conclusion, cow manure was distinctively different from the rest of the manure treatments in all chemical parameters showing highest dissolved oxygen, conductivity and pH and the lowest total phosphorus and un-ionized ammonia. These findings select cow manure as the best growth media for culturing *Daphnia magna*.

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PRODUCTION OF MACROZOOBENTHOS IN THE RAČA RIVER UPSTREAM AND DOWN STREAM FROM TROUT FARM

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PRODUKCIJA MAKROZOOBENTOSA U RECI RAČI UZVODNO I NIZVODNO OD PASTRMSKOG RIBNJAKA

Apstrakt

Biomasa (produkcija) makrozoobentosa je odabrana kao osnovni pokazatelj za praćenje promena kvantitativnog sastava naselja dna na lokalitetima uzvodno i nizvodno od pastrmskog ribnjaka u reci Rači.

Istraživanje sekundarne produkcije makrozoobentosa reke Rače sa sedam lokaliteta, obavljeno je u periodu 2011. (april, jun, septembar, oktobar, decembar) i 2012. godine (februar i maj). Dominantne grupe u biomasi makrozoobentosa su Hirudinea (Annelida), Mollusca, Gammaridae (Crustacea) i Trichoptera (Insecta).

Vrednosti biomase zoobentosa kretale su se u svim mesecima istraživanja u intervalu od 3,2001 g/m^2, na lokalitetu RČ2 (u februaru) ,do 216,7120 g/m^2, na lokalitetu RČ3 (u februaru).

Biomasa faune dna najveća je u svim mesecima istraživanja na lokalitetu RČ3, koji je lociran nizvodno od pastrmskog ribnjaka. Na ovom lokalitetu biomasa makroinvertebrata se kretala od 87,8643 g/m^2 (u aprilu 2011. godine) do 216,7120 g/m^2 (u februaru 2012. godine).

Ključne reči: makrozoobentos, biomasa, sekundarna produkcija, pastrmski ribnjak
Keywords: macrozoobenthos, biomass, secondary production, trout farm

INTRODUCTION

Freshwater ecosystems - rivers primarly - are one of the most important resource for mankind. By using this resource (for water supply, energy production, food source etc), man directly and indirectly makes an influence on the change of structure and function of aquatic ecosystems. Building a trout farms on rivers is definitely one of these activities.

Trout farming is increasing in Serbia. Trout farms cover approximately 14 ha in our country (Marković and Poleksić, 2011). Spring water (or water from streams and rivers) used for trout farm supply is often disposed into the environment after use without being treated by sedimentation or purification (Marković and Poleksić, 2011).

The negative influence of trout farms on such streams is mainly caused by the release of fish food remains and fecal matter (Liao, 1970), leading to deterioration of the water quality and changes in structure of the stream bottom.

Trout farms also influence the structure of macroinvertebrate communities. This is reflected through the level of secondary production of bottom fauna.

Zoobenthos biomass usually increases due to the greater amount of fish food remains, serving as food supply. On the other hand, wastewater from trout farms causes decline of diversity, replacement of more sensitive species by less sensitive ones, and changes of trophic structure due to increased abundance of collectors and reduced abundance of scrapers and shredders (Živić *et al.*, 2009).

Biomass is one of the basic parameters for quantification of the level of secondary production (Mason et al., 1985; Živić *et al*., 2000, 2008). This makes it very important for understanding the functioning of fresheater ecosystems, to establish a quantitative relationships between the number and activity of zoobenthic organisms (Zivić *et al.*, 2002) since they represent an essential link in the food webs of aquatic biocenoses (Cummins, 1973).

In this paper, macrozoobenthos biomass was used to monitor changes of quantitative composition of bottom fauna in the Rača River. Localities were seleceted upstream and downsteram from the Rača River trout farm.

MATERIAL AND METHODS

Description of the Study Area

The Rača River emerges on the slopes of Mt. Tara (Western Serbia, Figure 1). Rača River has several sources, best known is alkaline spring Lađevac at an elevation of 498 m a.s.l. (Marković, 1998). The basin of the Rača River is of a highland nature, building a deep canyon. It flows into the Drina River, a second order tributary of the Danube, near the town of Bajina Bašta.

The study was conducted at seven localities on the Rača River (Figure 1) Locality RČ1 is situated at 353 m a.s.l., at the point where the canyon passes through dense forest complex. The river bottom consists mostly of larger and smaller stones, rarely rocks. Locality RČ2 – 10 m upstream from the intake of water for the fish farm, with coarse bottom substrate, mostly pebble and stones. Locality RČ3 – 10 m downstream from the trout farm effluent. It predominantly features gravel substrate type and aquatic macrophytes. Locality RČ4 and RČ4’ are situated at about 340 m and 320 m a.s.l., downstream from the effluent, passing through a dense forest complex, with sharp and steep banks. The dominant substrate type is stone. Locality RČ5 – 1.5 km downstream from the trout farm, at 306 m a.s.l., with pebble and stones as substrate type. Locality RČ6 – 4 km downstream from the farm, at 245 m a.s.l. with coarse bottom substrate. Water from this locality is directed to another trout farm, situated 5m downstream.

The "Ihthos" trout farm is situated in the upper part of the Rača River (within the Rača Monastery), between second and third locality of the study (Figure 1). Fish farm consists of water intake and an open type main input channel. Its capacity is small, with two fish raceways for pre-consumer grade trout and two pools for consumer-grade trout nurturing.

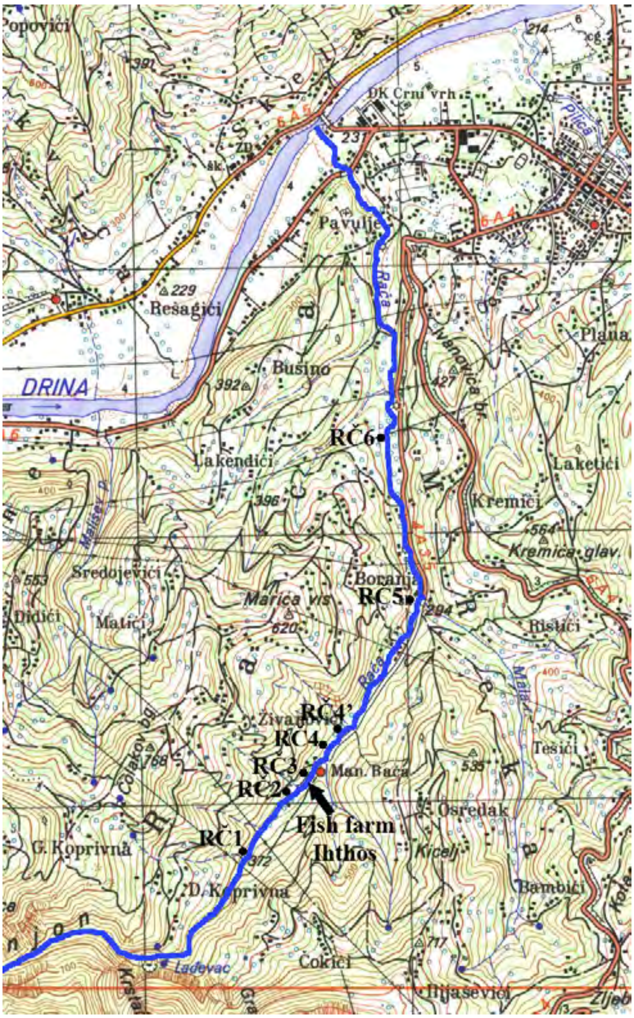

Figure 1. Studied localities in the Rača River and position of the "Ihthos" fish farm

Sampling

Macrozoobenthos samples were collected at seven localities on the Rača River, during 2011 and 2012 (April 4^{th} 2011, June 20^{th} 2011, 28^{th} September 2011, 16^{th} October

2011, 10 th December 2011, 26 th February 2012, 6 th May 2012). Samples were collected with a Surber net with the catchment area of 300 cm^2 and mesh size of 250 μm. The collected material was placed in plastic bottles and fixed with 96% alcohol. Three samples were taken with Surber net from every locality each month of the research. A sample is defined as material obtained from the river by a single sweep of the Surber net.

The fresh weight of macroinvertebrates was measured with a KERN ABS 80-4:ABS/ABJ analytical balance with a percision of 0.0001 gr, in order to estimate the total biomass of macrozoobenthos at localities above and below the trout farm.

Chemical parameters

The dissolved oxygen concentration, water temeperature, pH and conductivity were measured using PCE-PHD device (Germany). Measurements were performed each month, at each locality. Also, water was taken for chemical analysis of total phosphorus, orthophosphates and ammonia.

RESULTS AND DISCUSSION

Freshwater ecosystems of the temperate zone are characterized by relatively constant biomass of macroinvertebrates, in which aquatic insects are dominant, together with molluscs, annelids and crustaceans (Cummins, 1973). It is possible to notice certain seasonal variation in zoobenthic biomass (Hynes, 1970), resulting from different adaptations of the life cycle of aquatic insects (egg laying-emergence) to environmental factors, primarily water temperature.

Macrozoobenthos biomass varied annualy in the Rača River, in the interval from 3.2001 g/m^2 at locality RČ2 (February) to 216.7120 g/m^2 at locality RČ3 (February) The greatest mean value of biomass was recorded in June (108.1430 g/m^2), while the second part of the year was characterized by decline of secondary production from 55.2918 g/m^2 in September and 61.5764 g/m^2 in October (Figure 2).

The reason of this production can be explained by the increasing number of major groups of macrozoobenthos, but also by the fact that emergence of larger forms of Ephemeroptera and Diptera has not occurred. Also, increased abundance of Hirudinea was recorded. There was a decrease in abundance and biomass of benthic organisms in August, due to emergence of insect groups. Caused by abiotic factors of the environment (especially decrease in temperature), the development cycle of bottom organisms enters a phase of slow biochemical processes, resulting in reduction of seconadary production (September and October, Figure 2). The lowest production of macrozoobenthos was registered during the winter (56.3485 g/m^2 in February) and early spring (56.9956 g/m^2 in April). Unexpectedly high level of bottom fauna biomass was recorded in December (103.6084 g/m^2). Result of such growth is related to RČ3, RČ4 i RČ4’ localities. Great abundance of trichopteran larvae were registered at locality RČ4 and RČ4’, like *Hydropsyche, Rhyacophila* and *Sericostoma* genera, which overall contribute to the increase in biomass at these localities. The increase in biomass at RČ3 locality (208.2071 g/m^2) was connected with a great abudance of taxa less sensitive to organic pollution, overall Hirudinea and *Gammarus* genus.

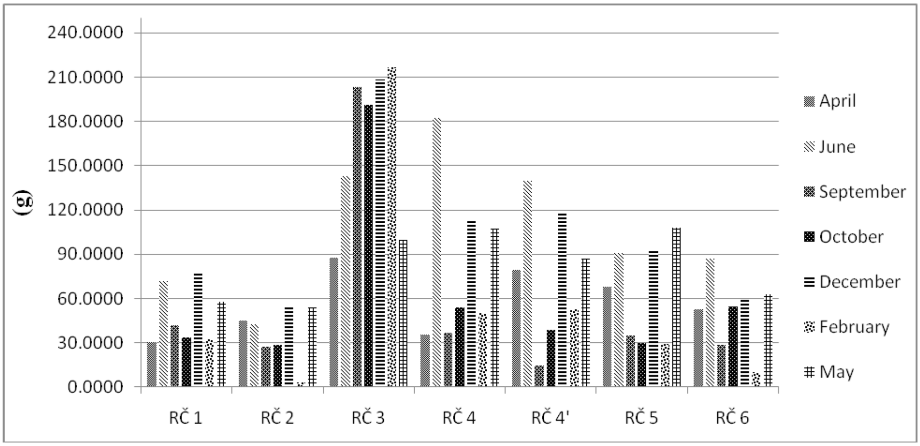

Figure 2. Macrozoobenthos biomass (g/m^2) at seven localities in the Rača River

According to the results of bottom fauna biomass, rapid growth trend can be seen at locality RČ3, right below the trout farm, relative to localities RČ1 and RČ3 situated above the farm. Macrozoobenthos biomass was in the interval from 87.8643 g/m^2 (in April 4th 2011) to 216.7120 g/m^2 (26th February 2012) at RČ3 locality. Comparing the average macrozoobenthos biomass between localities upstream from the trout farm (RČ1 - 49.3482 g/m^2; RČ2 - 36.5903 g/m^2) and downstream from the farm (RČ3 - 164.3381 g/m^2) it is evident they are significantly lower.

The maximum values of zoobenthos biomass at RČ3 locality can be explained by the high inflow of fish food remains in the recipient used by bottom organisms as food source. Due to deterioration of water quality (also because of the wastewater influx from the trout farm) only tolerant macroinvertebrate groups were found, which are larger and therefore contributed to the biomass of the community. Dominant groups collected at this locality were Hirudinea (Annelida), Mollusca (*Lymnea* sp.), Gammaridae (Crustacea) and Trichoptera (Insecta).

Figure 3 and 4 represents mean values (mean ± standard error) of chemical paremeters measured at localities. It is evident that temperature increases from the first to the sixth locality, while the concentration of oxygen decreases from the first to the third locality, in order to increase again from the fourth locality (Figure 3). However, these changes in oxygen concentration were not drastic, as its concentrations remained high (10.3-10.0 mg/l). The consequence – high oxygen concentration during the entire study period - was probably due to good river aeration and field configuration (steep banks). Conductivity and pH were more constant throughout the study period (Figure 3). Their higher values indicate that substrate type of the Rača River is primarily limestone. Concentration of total phosphorus and orthophosphates stayed below the recommended upper limit (Figure 4), although their higher concentrations were expected at the localities below the trout farm. Mean value of orthophoshates in the Rača River reached up to 0.017 mg/l and the recommended level of 0.02 mg/l (Dulić, 2010) are characteristic for salmonid waters. The exception was the concetration of orthophosphates at the RČ 4' locality with the highest concentarion of 0.0317 mg/l. The maximal permissible concentration of total phosphorus is 0.2 mg/l for salmonid waters (TPECD, 2006) and its highest value was observed at the RČ4' locality (0.072 mg/l) in February. Ammonia concentrations varied considerably during the research period (Figure 4), especially at the locality below the trout farm (RČ3) where the highest mean concentartion was observed (0.18 mg/l). Taking into account the parameters that were measured at all studied localities, we can conclude that only high concentration of ammonia at the third locality made an imact on zoobenthos biomass, in terms of the presence of taxa less sensitive to such changes. Those taxa, especially Mollusca and Hirudinea, have the lagrest single biomass.

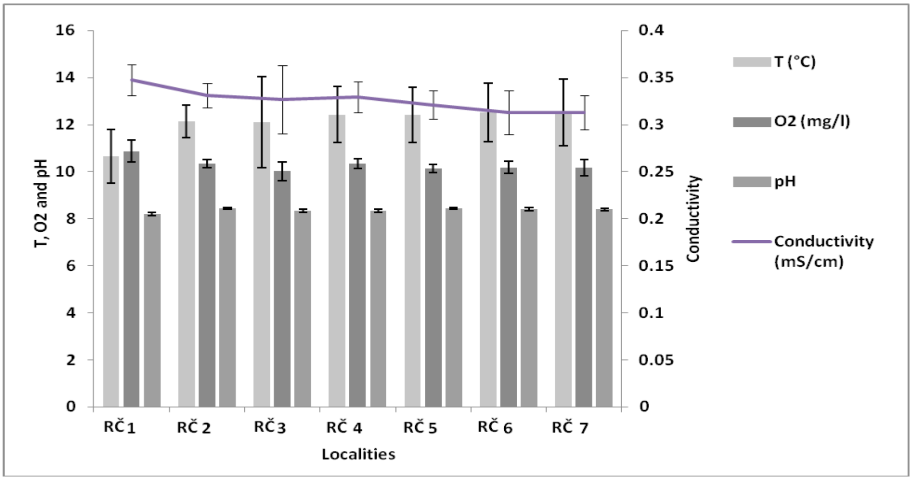

Figure 3. Mean values (with standard error) of temperature, dissolved oxygen, pH and conductivity

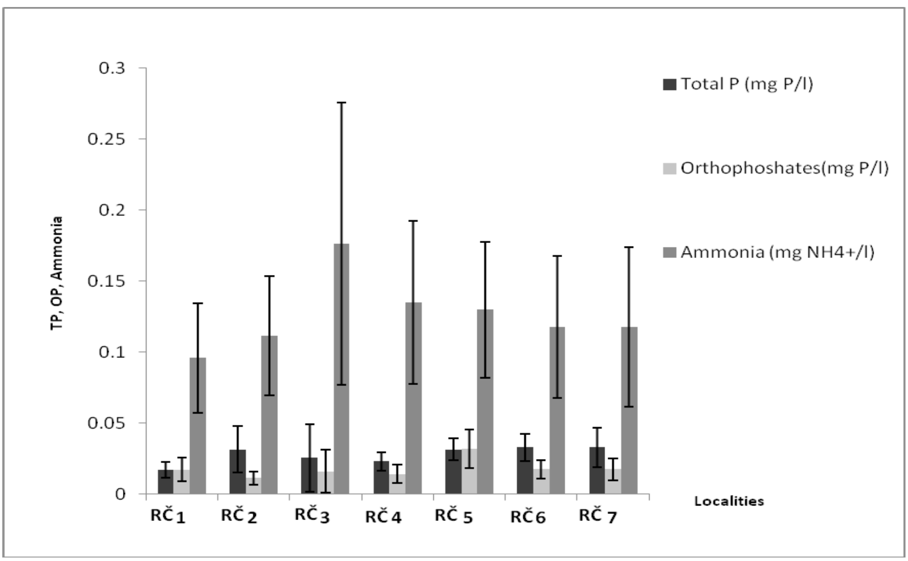

Figure 4. Mean values (with standard error) of total phosphorus, orthophosphates and ammonia

CONCLUSIONS

The research of macrozoobenthos secondary production was conducted during 2011 and 2012 in the Rača River (in a length of 4 km), upstream and downstream from the trout farm.

The greatest mean value of biomass was recorded in June (108.1430 g/m^2). The second part of the year was characterized by decline of secondary production, except in November, with the lowest biomass of organisms during the winter (56.3485 g/m^2 in February) and early spring (56.9956 g/m^2 in April).

Macrozoobenthos biomass varied annualy in the Rača River, in the interval from 3.2001 g/m^2 at locality RČ2 (February) to 216.7120 g/m^2 at locality RČ3 (February).

Locality RČ3, situated downstream from the trout farm, was characterized by the greatest bottom fauna biomass. At this locality macrozoobenthos biomass was in the interval from 87.8643 g/m^2 (in April 4th 2011) to 216.7120 g/m^2 (26th February 2012).

Mean values of chemical parameters remained below the recommended upper limit for salmonid waters. Concentration of total phosphorus and orthophosphates were higher than recommended level at RČ4' locality.

Ammonia concentrations varied considerably during the research period. The highest mean concentartion was observed at the locality below the trout farm (0.18 mg/l). High concentration of ammonia at the third locality made an imact on zoobenthos biomass, in terms of the presence of taxa less sensitive to such changes, who also have the largest single biomass.

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ASSESSMENT OF MARINE WATER QUALITY: EFFECT OF AQUACULTURE AND DOMESTIC SEWAGE DISCHARGE

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PROCENA KVALITETA MORSKE VODE: EFEKTI AKVAKULTURE I OTPADNIH VODA IZ DOMAĆINSTVA

Apstrakt

Cilj ovog istraživanja je bio procena uticaja morskih ribnjaka i otpadnih voda iz domaćinstva na kvalitet vode na obali Rize lociranoj na jugoistoku Crnog Mora. U tu svrhu je voda uzorkovana mesečno sa 5 tačaka oko kaveza sa ribama, 2 tačke sa mesta izliva otpadnih voda i jedne referentne tačke bez efekta kaveza i otpadnih voda, od juna 2007 do aprila 2009. Merenja su izvođena standardnim metodama. Podaci su prikazani kao minimum i maksimum sledećim redom: obalske tačke, kavezi sa ribama i referentna tačka. Dobijeni su sledeći rezultati: za temperaturu vode 7.83-27.51, 7.79-27.41, 7.72-27.00 °C; za rastvoreni kiseonik 7.60-11.00, 7.00-11.2, 7.80-11.60 mg/L; za pH 8.04-8.43, 7.93-8.38, 7.62-8.33; za salinitet 61-18.60, 16.77-19.03, 16.78-18.97 ppt; za silikate 0.29-14.64, 0.25-16.43, 0.25-11.70µM; za ortofosfate 0.10-1.71, 0.09-1.50, 0.10-1.95 µM; za ukupni fosfor 0.24-3.73, 0.12-3.30, 0.10-3.52 µM; za ukupne suspendovane materije 1.70-26.40, 1.00-20.40, 1.00-11,80mg/L; za hlorofil-a 0.46-3.26, ND-3.25, 0.10-4.99 µg/L, gore navedenim redom. Na osnovu dobijenih rezultata nisu konstatovani značajni efekti kaveznih sistema na kvalitet vode. Na osnovu vrednosti pomenutih parametara u ovom istraživanju manje je negativnih efekata na kvalitet vode koji potiču od delatnosti uzgoja riba u odnosu na otpadne vode iz domaćinstva.

Ključne reči: marinska akvakultura, uticaj na životnu sredinu, Jugoistočno Crno more, Rize

Abstract

The aim of this study was to determine influence of marine fish farm and sewage on water quality in Rize coast located at southeastern Black Sea. For this purpose, water samples were taken monthly from 5 points around fish cages, 2 points effected sewage areas, a reference point without effects of fish farm and sewage,from June 2007 to April 2009. Measurements were performed according to standard methods. Data was given as min-max for coastal, fish cage and reference points, respectively. Results were for water temperature7.83-27.51, 7.79-27.41, 7.72-27.00 °C; for dissolved oxygen7.60-11.00, 7.00-11.2, 7.80-11.60 mg/L; for pH 8.04-8.43, 7.93-8.38, 7.62-8.33; for salinity16.61-18.60, 16.77-19.03, 16.78-18.97 ppt; for silicate 0.29-14.64, 0.25-16.43, 0.25-11.70µM; for orthophosphate 0.10-1.71, 0.09-1.50, 0.10-1.95 µM; for total phosphorus 0.24-3.73, 0.12-3.30, 0.10-3.52 µM; for total suspended solids 1.70-26.40, 1.00-20.40, 1.00-11-,80mg/L; for chlorophyll-a 0.46-3.26, ND-3.25, 0.10-4.99 µg/L, respectively. According to the results, no significant effects of fish farm on water quality were observed in field study. Based on levels of these parameters observed in the study, it was defined that the fish farming was less effect to water quality than domestic sewage discharge.

Keywords: marine aquaculture, environmental impact, Southeastern Black Sea, Rize Coast

ASSESSMENT OF NORWAY LOBSTER - *NEPHROPS NORVEGICUS* (LINNAEUS, 1758) POPULATIONS IN THE ADRIATIC SEA USING ALTERNATIVE METHODS

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PROCJENA POPULACIJE ŠKAMPA - *NEPHROPS NORVEGICUS* (LINNAEUS, 1758) U JADRANSKOM MORU ALTERNATIVNIM METODAMA

Apstrakt

Škamp (*Nephrops norvegicus*) spada u gospodarski najvažnije vrste rakova na području Mediterana i severnoistočnog Atlantika kojeg se godišnje izlovi preko 60000 t. Rasprostranjen je u istočnom Atlantiku i diljem Mediterana od 20 do 800 m dubine. Obitava na muljevitim sedimentima u kojem iskopava karakteristične tunele. Najveća gustoća populacije u Jadranskom moru zabeležena je na području Jabučke kotline, Velebitskom kanalu, Kvarneru i Kvarneriću. U južnom delu Jadrana gustoća populacije je osetno manja. Škamp se u Jadranskom moru intenzivno izlovljava uglavnom povlačnom pridnenom mrežom koćom od strane mnogobrojne ribarske flote svih jadranskih zemalja. Usled toga došlo je do negatvinih promena ukupne biomase, te demografske strukture populacije škampa. Da bi se uspostavilo odgovorno i održivo ribarstvo, te zaštitila populacija škampa provode se brojna istraživanja i monitorinzi, te procene stanja populacije. Ova istraživanja uglavnom se temelje na ribarstveno biološkim metodama kao što su praćenje totalnog ulova, kretanje CPUE ili analitčkih metoda (VPA, LCA and yield-per-recruit analysis). Glavni nedostatak ovih metoda je što se temelje na pretpostavci reprezentativnog uzorkovanja populacija što kod škampa, zbog njegovih bioloških karakteristika, nije slučaj. Obzirom da škamp obitava u tunelima, koje iskopava u sedimentu, on može biti uzorkovan samo kada se nalazi izvan njih, a izlazak varira ovisno o sezoni, dobu dana, veličini, spolu te stadiju zrelosti.

Da bi se postiglo reprezentativno uzorkovanje u svrhu dobivanja tačne procene stanja populacije u poslednje vreme sve više se koristi alternativna metoda istraživanja upotrebom povlačne podvodne kamere (UWTV). Ova metoda se zasniva na promatranju morfoloških karakteristika morskog dna da bi se identifikovale vrste koje obitavaju u tunelima na osnovu karkterističnog izgleda otvora tunela. Da bi se tačno idenetifikovala vrsta koja obitava u tunelima treba promatrati oblik, dijametar, razdaljinu, orijentaciju, grupiranje otvora, prisustvo vrste ili tragova te drugih značajnih parametra. Dobivene vrednosti se zajedno sa rezultatima ribarstveno bioloških istraživanja analitičkim metodama preračunavaju se u indeks biomase po površini. Metodologija istraživanja provodi se upotrebom specijalnih podvodnih kamera montiranih na sanje koje se istraživačkim brodom povlače po morskom dnu određenim vremenom i brzinom. Video snimka, zajedno s ostalim zabeleženim oceanografskim podacima, se putem optičkog kabla u realnom vremenu prenosi u kontrolnu jedinicu na brodu. Snimljeni materijal se analizira prema međunarodnom protokolu (ICES) da bi se omogućila ispravna usporedba podataka. UWTV metoda zajedno s drugim ribarstveno biološkim metodama značajno doprinosi sistematskom praćenju stanja i procjene populacije škampa, te uvođenju odgovornog i održivog iskorištavanja bioloških obnovljivih resursa zasnovanog na znanstvenim saznanjima.

Ključne reči: UWTV, Jadransko more, procena populacija, ribarstvena biologija
Keywords: UWTV, Adriatic sea, population estimation, fishery biology

INTRODUCTION

Norway lobster (*Nephrops norvegicus*) is one of the most important commercial crustacean species throughout Mediterranean and the NE Atlantic with the annual catch of more than 60000 t according to the FAO fisheries statistics (FAO, 2012) This species is distributed in the eastern Atlantic, from Morocco to Norway and Iceland, and in the Mediterranean from 20 to 800 m of depth (Fisher et al., 1987). Norway lobster mostly inhabits muddy sediments in which it digs its characteristic burrows. It is widely distributed in the Adriatic Sea at depths from about 30 m in the northern Adriatic Sea to 400 m in the southern part of the Adriatic (Karlovac, 1953; Marano et al., 1998; Piccinetti et al., 2012). The most important fishing grounds, with high population density, are in the Jabuka Pit region and in the Velebit Channel, Kvarner and Kvarnerić region along the Croatian coast (Karlovac, 1953; Crnković, 1965). In the southern Adriatic, along the western (Italian) and eastern (Albanian and Montenegrin) coasts, the settlements are not so dense (Karlovac, 1953; Marano et al., 1998) (Fig 1).

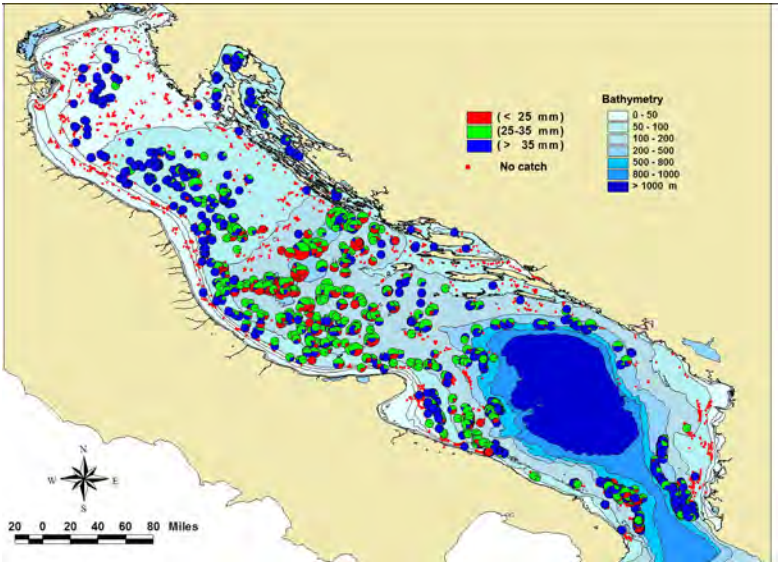

Figure 1. Distribution of Norway lobster (*Nephrops norvegicus*) in the Adriatic Sea (source: Isajlović)

Population of Norway lobster, together with other commercially important species, in the Adriatic is mostly exploited by bottom trawlers of all Adriatic countries in which larger Italian fleet achieved the majority of total catch. Due to that, Adriatic Sea trawling grounds have been classified as fully exploited to overexploited (Sardà, 1998) with respect to Norway lobster which shows decreasing trends both in the total catch and also in the demographic structure since 1993 (Piccinetti et al., 2012).

Since Norway lobster represents one of the most commercially important demersal species in Mediterranean, therefore also in the Adriatic Sea, monitoring of the fisheries pressure and assessment of the population is key factor in applying fisheries management based on responsible and sustainable exploitation. Assessment of Norway lobster population in Mediterranean usually relies almost uniquely on fishery-dependent techniques based on the use of catch and CPUE trends or on analytical methods such as VPA, LCA and yield-per-recruit analysis (Morello et al., 2007). These methods are highly dependent on selectivity of fishing gear which is used as a sampling tool (Fiorentini et al., 1998.) and the main downfalls are that they rely on assumptions such as equal capture availability and stock redistribution following capture. These assumptions do not hold for this species because of its specific biological characteristics. Norway lobster can be caught only when it emerges from its burrow and emergence may vary with time of day, season, animal size, sex, and reproductive status (Morello et al., 2009). Therefore sampling natural population in a certain area or time can result that sample is not qualitatively or quantitatively representative and in this case data obtained by fishery or scientific trawl surveys may represent only part of the population. For these reasons, fishery-independent methods are of particular importance for this species and the most practical of these uses under water video system “UWTV” for burrow counts as an index of stock abundance. If appropriately integrated with trawl hauls, burrow counts can be converted into biomass estimates.

UWTV METHODOLOGY

The first experimental UWTV surveys were carried out by Scottish scientist in the early 1990s and up to day it has become the standard method of assessment for NE Atlantic stocks (ICES, 2007). UWTV has received detailed attention in a series of ICES workshops aimed at standardizing methodologies and quantifying the uncertainties associated with the method (Campbell et al., 2009). This techniques is based on examination of a surface features to identify the occupant of a given burrow observed by UWTV and in most cases it could be determined to species level. Recognition of burrows and identification of their occupants is based on observing the main morphological features such as shape of burrow openings, presence of tracks and trails, presence of characteristic clusters, diameter of burrow openings, distance between openings of a given burrow, orientation of the burrow, presence of the occupant and any other additional helpful information. Norway lobster dig burrows in a muddy sediment which are consisted of a tunnel with a front entrance opening within a crater-like depression and smaller rear aperture opening on flat surface (Fig. 2). Often Y-shaped burrows are found with 3 openings as well as complex burrows with more openings, which are generally associated with the presence of juveniles, known to attach their burrows to the ones of adults.

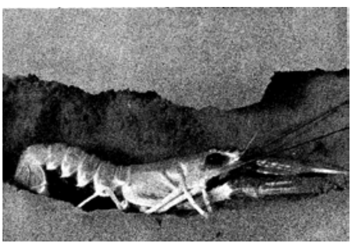

Figure 2. Morphological characteristics of Norway lobster (*Nephrops norvegicus*) burrows system (from CEFAS training material provided by Jim Atkinson)

The surveys are conducted using under water TV camera which is mounted on a sledge and towed by research vessel on the sea bed at a speed of 1 knot. The position of the sledge at each minute, depth, current, turbidity and other oceanographic data are monitored by data-logger systems which are synchronized with the camera deck unit (Fig 3.). The UWTV tows should be carried out during day time at least for 20 minutes at each UWTV station (Martinelli et al., 2013).

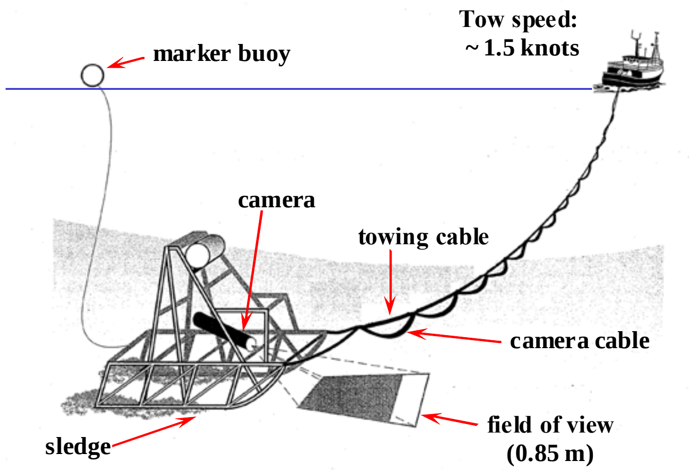

Figure 3. Description of UWTV system and investigation methodology

Analysis of video footage and Norway lobster burrow identification and quantification should be carried out following ICES protocols (ICES, 2008) by several independent trained readers with a minimum of 8 ‘good’ (easy to read) minutes per station as a threshold fixed to accept the validity of each station.

The first experimental UWTV investigations of Norway lobster settlements in the Adriatic Sea were held in the western part of Jabuka pit during the end of 1990s (Froglia et al., 1997). Following their achievement from 2009 systematic UWTV investigations are conducted in wider area of central Adriatic in order to carry out an evaluation of the Norway lobster stock under the framework of the FAO – ADRIAMED project.

CONCLUSION

Following the negative changes both in population stock biomass and demographic structure of Norway lobster population in the Adriatic Sea special consideration should be given to the exploitation of this species. Since it is one of the most important commercial species exploited by large fisheries fleet of all Adriatic countries, primary goal of fisheries scientist is to provide recommendations for sustainable exploitation of Norway lobster based on scientific conclusions. Use of the UWTV methodology together with trawl surveys in the future would allow systematic assessment of the Norway lobster stocks and contribute to identification and monitoring of year-to-year variation in biomass and size composition.

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DISTRIBUTION, ABUNDANCE AND POPULATION STRUCTURE OF THE COMMON CUTTLEFISH, *SEPIA OFFICINALIS* LINNAEUS, 1758, IN THE ADRIATIC SEA

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RASPROSTRANJENOST, BROJNOST I SASTAV POPULACIJE OBIČNE SIPE *SEPIA OFFICINALIS*, LINNAEUS, 1758, U JADRANSKOM MORU

Apstrakt

Sipa, *Sepia officinalis*, je glavonožac rasprostranjen u istočnom Atlantiku, od Shetlandskog otočja i južne Norveške do sjeverozapadne Afrike, te u Mediteranu. Obitava na svim dubinama do 200 m, ali najviše je zastupljena na dubinama do 100 m. Vrsta je velikog gospodarskog značaja, poglavito za zemlje u području njezine rasprostranjenosti. Izlovljava se većim brojem ribolovnih alata, u gospodarskom ribolovu najviše pridnenom povlačnom mrežom koćom. Ukupni svjetski ulov ove vrste u 2011. godini iznosio je 26 701 t, a u ukupnim ulovima najviše je zastupljen Tunis u Mediteranu. U radu su korišteni podaci istraživanja provedenih u Jadranu u sklopu programa EU MEDITS u razdoblju od 1996. do 2008. godine. Uzorkovanja su provedena na svim koćarskim područjima Jadrana (GSA 17 i GSA18) korištenjem eksperimentalne pridnene povlačne mreže GOC 73, posebno konstruirane za ovaj tip istraživanja. Srednje vrijednosti indeksa biomase i indeksa brojnosti sipe (N/km^2 i kg/km^2), koji su dobiveni korištenjem "swept area" metode, izračunati su po godinama, po dubinskim stratumima kao i za cijelo razdoblje istraživanja. Srednja vrijednost indeksa biomase i brojnosti ove vrste u Jadranu iznosila je 0,90 kg/km^2 i 11,42 N/km^2. Analiza raspodjele po dubinama pokazala je da vrsta preferira plitka područja te su srednje vrijednosti oba indeksa bile najviše u dubinskom stratumu 10 do 50 m. Ukupno je analizirano 1069 primjeraka sipe, od toga 500 mužjaka, 352 ženke i 217 jedinki kojima pol nije bilo moguće odrediti. Omjer mužjaka i ženki bio je 1,42, odnosno u uzorcima su dominirali mužjaci što je najvjerojatnije posljedica masovnog pomora ženki nakon razmnožavanja. Raspon dužina plašta lovljenih primjeraka kretao se od 30 do 215 mm, a njihova srednja dužina iznosila je 81,23 mm (st.dev.=33,04). Prosječne godišnje vrijednosti indeksa biomase i brojnosti pokazuju značajne međugodišnje oscilacije što je uobičajeno za kratkoživuće vrste, poput sipe, koje izrazito ovise o uvjetima okoliša. Međutim, kod sipe se uočava i slabi negativni trend indeksa biomase u razdoblju 1996.-2008. godina a takve promjene u populaciji mogu biti posljedica prevelikog ribolovnog napora kojemu je vrsta izložena, posebice uzme li se u obzir i sinergijski učinak većeg broja ribolovnih alata kojima se ova vrsta izlovljava. Stoga je neophodno provoditi monitoring uzimajući u obzir što veći broj relevantnih parametara kao i sve dionike u ribolovu ove vrste radi predlaganja i provođenja mjera kojima će se osigurati dugoročno održivo korištenje ovog važnog ribolovnog resursa u Jadranskom moru.

Ključne reči: obična sipa, Sepia officinalis, distribucija, populacija, Jadransko more

Keywords: common cuttlefish, Sepia officinalis, distribution, population, Adriatic Sea

INTRODUCTION

The common cuttlefish, *Sepia officinalis*, is a cephalopod species distributed in the Eastern Atlantic, from the Shetland Islands and southern Norway to northwestern Africa and in the Mediterranean Sea. It can be found from subtidal waters to 200 m, but it is most abundant in upper 100 m, with larger animals at greater depths (Nesis, 1987; Jereb and Roper, 2005).

This species has a significant commercial value and it is one of the most important species for cephalopod fisheries in many countries. It is caught with several selective fishing gears in artisanal fishery, and in the industrial fishery it is primarily fished with trawls (Jereb and Roper, 2005). Total world catches in 2011 were 26 701 t with highest catches recorded for Tunisia in the Mediterranean Sea (FAO, http://www.fao.org/fishery).

Although *S. officinalis* is one of the most studied cephalopod species, there are scarce published papers dealing with the biology of this speciesor the impact of fishery on its population in the Adriatic Sea (ManfrinPiccinetti and Giovanardi, 1984; Fabi, 2001; Vrgoč et al., 2004). The paper deals with the series of data from the scientific biological-fishery survey EU MEDITS between 1996 and 2008, comprising data from the whole Adriatic Sea with an attempt to describe the population structure and distribution patterns of this species in the Adriatic Sea and to analyze recent trends of mean annual biomass indices in this period.

MATERIAL AND METHODS

Biological samples used for this study were collected in the whole Adriatic Sea (GSA17 and GSA18), in the period from 1996. to 2006., in the scope of the MEDITS programme. Surveys were conducted each year in spring-summer period, except for the year 1999 so this year was excluded from the analysis. Sampling stations were chosen based on the depth-stratified sampling scheme, taking into account the surface of each depth stratum: 10-50 m, 50-100 m, 100-200 m, 200-500 m and stratum over 500 m. An experimental synthetic bottom trawl net GOC 73, with a large vertical opening and 20 mm stretched mesh size at cod-end was used (Bertrand et al., 2002). Catches of all species at each sampling station were standardized, using swept area method, as number of individuals and weight per square kilometer (N/km^2, kg/km^2) (Sparre and Venema, 1998).The average annual biomass and density indices for *S. officinalis*were presented and recent biomass trends tested for significance with Pearson's correlation coefficient. All data were stored in a common database using the ATrIS information system (Gramolini et al., 2005) and GIS distribution maps were made using ArcView GIS tools v. 3.2.

RESULTS AND DISCUSSION

The common cuttlefish is distributed along the shelf of the Adriatic Sea both in the Northern and Central (GSA17) and in the Southern part (GSA18) (Fig. 1). The depth range of the distribution of *S. officinalis* in the Adriatic Sea is between 16 and 173 m.

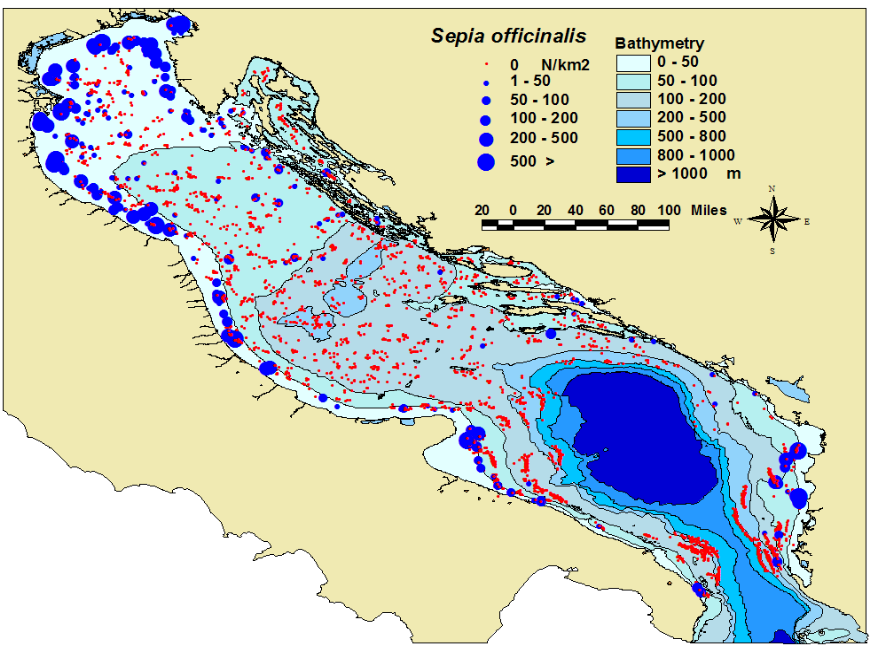

Figure 1. Distribution of the abundance of *Sepia officinalis* in the Adriatic Sea

Comparison of data obtained for the GSA17 and GSA18 prove that this species is more abundant in the Northern and Central Adriatic (1.25 kg/km^2 and 11.21 ind/km^2) than in the South Adriatic Sea (0.44 kg/km^2 and 7.09 ind/km^2). Abundance indices are higher in the northern and north-western part of the investigated area at least in the spring-summer period when surveys were done, and similar was observed for the species in previous studies (Krstulović Šifner et al., 2005, 2011).

Length frequency distribution of the species was obtained measuring 1069 individuals of *S. officinalis*, including 352 females and 500 males and 217 individuals of undetermined sex. The sex ratio was 1.24 in favor of males, which is probably a result of the postspawn mass mortality among adult females (Jereb and Roper, 2005). The average mantle length of all caught individuals was 81.23 mm (st.dev.=33.04). Mantle length in females ranged between 35 and 195 mm with average 93.14 mm (st.dev.=32.78), while in males ranged between 30 and 215 mm, with mean ML 80.06 mm (st.dev.=31,12) (Fig. 2). The highest mantle lengths in the sample were lower than the maximum values recorded in other areas: 490 mm in temperate waters and 300 mm in the subtropics (Jereb and Roper, 2005).

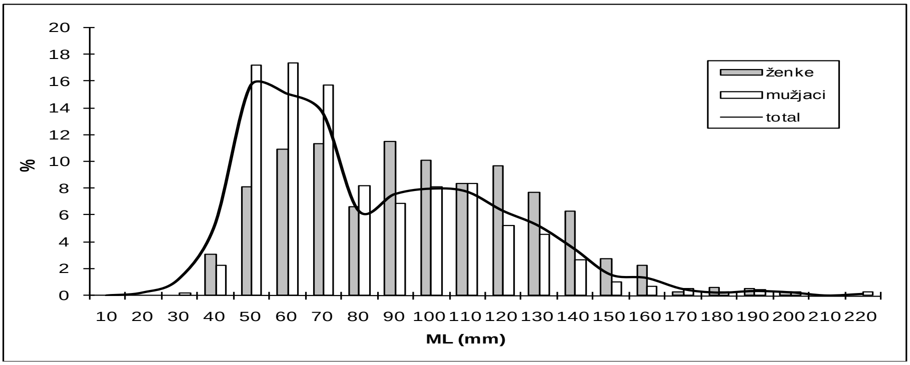

Figure 2. Length-frequency distribution of *S. officinalis* in the Adriatic Sea

The highest values of abundance and biomass indices were recorded in the shallowest stratum (2.98 kg/km^2 and 37.31 ind/km^2) (Figs. 3 and 4). Catches were much lower in stratum 50-100 m (0.24 kg/km^2 and 3.33 ind/km^2) and in stratum 100-200 m the common cuttlefish was caught only sporadically with low values of both indices (0.04 kg/km^2 and 0.74 ind/km^2).

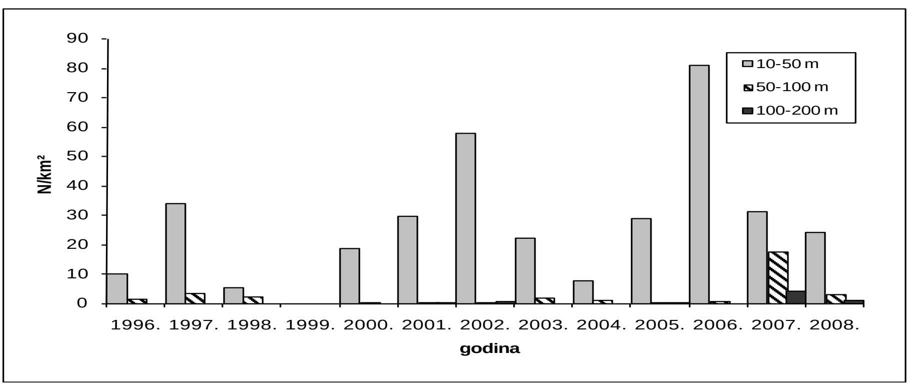

Figure 3. Mean annual abundance indices (ind/km^2) of *S. officinalis* in three depth strata

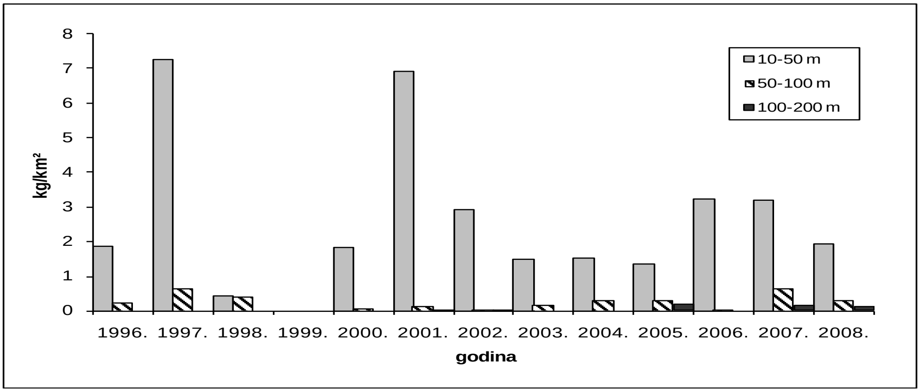

Figure 4. Mean annual biomass indices (kg/km^2) of *S. officinalis* in three depth strata

Seasonal migrations have been described for this species, with adults migrating in shallow waters in spring–summer period, while in colder season they migrate in deeper waters (Grubišić, 1982), so in autumn-winter season distribution of this species probably shows different pattern, being more concentrated at medium shelf grounds. The average biomass and density indices calculated for the whole period were 0.90 kg/km^2 and 11.42 ind/km^2, respectively. The highest value of biomass index was recorded in 1997. (2.25 kg/km^2) and the highest abundance index in 2006. (22.89 ind/km^2), in latter year survey being performed much later which explains the larger number of smaller individuals and high mean abundance index. Regarding mean biomass indices in the investigated period for this species a negative trend was observed, with no statistical significance ($r=-0.194$, $p=0.545$). *S. officinalis* is a short living organism, very much dependent on environmental conditions and also a species exposed to a very high fishing effort with synergistic impact of several fishing tools used for its exploitation in commercial and artisanal fishery of the Adriatic Sea. It is additionally vulnerable as it prefers shallow waters where it is more exposed to the human fishing activities and furthermore its fecundity is much lower than in some other commercially important cephalopods (e.g. squids). These changes in population abundance and structure should be carefully monitored using all available data on biology and fishery of this species.

CONCLUSIONS

S. officinalis is a neritic, benthic species distributed in shallow waters along the coasts of the Adriatic Sea, with higher abundances in the Northern and Central (GSA17) than in the Southern Adriatic Sea (GSA18). This species is very important in the fishery of the Adriatic Sea but it is exposed to the high fishing effort with synergistic effect of several fishing gears. The results indicate some negative changes in the period between 1996 and 2008, so the population of *S. officinalis* should be carefully monitored and fishing activities properly regulated to ensure the long-term sustainable exploitation of this species in the Adriatic Sea.

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THE INFLUENCE OF DIFFERENT ENVIRONMENTAL CONDITIONS ON FLESH QUALITY INDICES OF FARMED SEA BASS, IN NW GREECE

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DELOVANJE RAZLIČITIH USLOVA SREDINE NA KVALITET MESA FARMSKI GAJENOG BRANCINA U SEVEROZAPADNOJ GRČKOJ

Apstrakt

Ispitivan je hemijski sastav i kvalitet lipida farmski gajenog brancina iz područja Severozapadne Grčke. Uočene su razlike u hemijskom sastavu fileta brancina farmski gajenog u različitim uslovima sredine. Ribe gajene u lagunama imale su viši sadržaj vlage i niži sadržaj masti u poređenju sa ribama gajenim u morskim plutajućim kavezima. Rezultati su pokazali da postoje variranja u kvalitetu mesa gajene ribe koji se mogu pripisati razlikama u sistemima gajenja i uslovima akvakulture. Istraživanje je bilo kofinansirano od strane Evropske Unije (Evropski Socijalni Fond, European Social Fund – ESF) i Grčkih nacionalnih fondova kroz Operativni program “Obrazovanje i celoživotno učenje” (“Education and Lifelong Learning”) nacionalnog strateškog referentnog okvira, National Strategic Reference Framework (NSRF) – programa za finansiranje istraživanja ARCHIMEDES III Investiranje u društvo znanja kroz Evropski Socijalni Fond.

Ključne reči: *akvakultura, kalifornijska pastrmika, brancin, kvalitet mesa, riblji lipidi*

Abstract

The chemical composition and lipid quality of farmed sea bass from NW Greece was investigated. A difference in the proximate composition and filleting yield of farmed sea bass reared in different environmental conditions was observed in the present work. Fish reared in aquaculture lagoons exhibited higher moisture and low lipid compared to the fish farmed in marine floating cages. The results of the present work indicate that farmed fish exhibit variability in the flesh quality which can be attributed to differences

in the rearing systems, aquaculture conditions with consequences for the nutritional benefits of consuming farmed fish. This research has been co-financed by the European Union (European Social Fund – ESF) and Greek national funds through the Operational Program “Education and Lifelong Learning” of the National Strategic Reference Framework (NSRF) - Research Funding Program: ARCHIMEDES III. Investing in knowledge society through the European Social Fund.

Keywords: aquaculture, rainbow trout, sea bass, flesh quality, fish lipid

TEHNOLOGY OF MUSSEL (*MYTILUS GALLOPROVINCIALIS*) AND OYSTER (*OSTREA EDULIS*) FARMING IN BOKA KOTORSKA BAY

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TEHNOLOGIJA UZGOJA DAGNJI (*MYTILUS GALLOPROVINCIALIS*) I KAMENICA (*OSTREA EDULIS*) U BOKOKOTORSKOM ZALIVU

Apstrakt

Razvoj marikulture u Crnoj Gori je jedno od strateški važnih pitanja u programima proizvodnje hrane za domaće tržište i za izvoz. Program podrazumijeva proizvodnju, odnosno uzgoj nekoliko vrsta školjaka i riba, dok je u planu i uzgoj rakova. Marikultura je sve značajnija privredna grana u svijetu jer nadoknađuje smanjene potencijale hrane iz prirodnih izvora.

I pored naglašenog značaja marikulture, izuzetnih bioloških i ekoloških karakteristika priobalnog mora Crne Gore, naučnog-stručnog znanja, moramo konstatotovati da je uzgoj morskih organizama na navedenom području tek u inicijalnom začetku, čak i kada se radi o uzgoju školjaka, čija je sadašnja proizvodnja oko 200 t. godišnje, što ostvaruje 16 uzgajivača u Bokokotorskom zalivu- uglavnom u Kotorskom i Tivatskom dijelu. Dakle, cijela marikultura u Crnoj Gori koncentrisana je na područje Bokokotorskog zaliva, dok je otvoreno more, u tom smislu, ostalo potpuno neiskorišćeno.

U davna vremena način uzgoja školjaka bio je vrlo jednostavan. Grane hrasta, trešnje, masline i ostalog raspoloživog materijala su se sakupljale i bacale u more. Na tako bačene grane hvatala se mlađ školjaka (kamenica i dagnji), a nakon tri godine i nekoliko faza uzgoja proces je bio kompletan, školjke su se vadile iz mora, te otpremale na tržište.

Cilj ovog rada je da damo svojevrsni pregled, počevši od prvih istraživanja mogućnosti uzgoja dagnji i kamenica u Bokokotorskom zalivu do današnjeg stanja i pravaca u kojima bi marikultura trebala da se razvoja u Crnoj Gori.

Ključne riječi: tehnologija uzgoja školjki, razvoj marikulture, Bokokotorski zaliv
Keywords: technology of bivalve farming, development of mariculture, Boka Kotorska Bay

INTRODUCTION

First investigation on the possibility of mussel (*Mytilus galloprovincialis*) and oyster (*Ostrea edulis*) farming in Boka Kotorska Bay goes back to the early seventies of the last century. Farming process implied three stages of cultivation for oysters and two phases of mussels farming. Bundles of branch, so-called “fašine”, were placed in the sea, and young oysters were caught on them after six months. Then oyster fries were extracted from the sea for second phase. Second phase implied processing and beam forming braids with diluted branches. The third phase involved the removal of branches, cementing and interference in the final braids.

Mussel farming was somewhat easier compared to oyster farming, and it was implied of two stages – collecting young on old ropes, so-called “kadena” that were placed horizontally below the surface. Collecting the fries was followed by a second phase - the removal of young mussels from ‘kadena” and involvement in braids, which have been positioned on the floating park in the space of 35-40 cm. Breeding parks and piers were a steel structure, and these experimental parks were placed in positions with a very shallow depth (5-10 m).

Total time of oysters growing lasted from 28-30 months, while mussel reaching market size in 2-3 years, depending on the size of the individual (Stjepčević, 1974).

Today, the farming technology of mussels and oysters in the Boka Kotorska Bay uses floating buoys and ropes, which proved that it meets all the necessary conditions for safe production (Mandić, 2008).

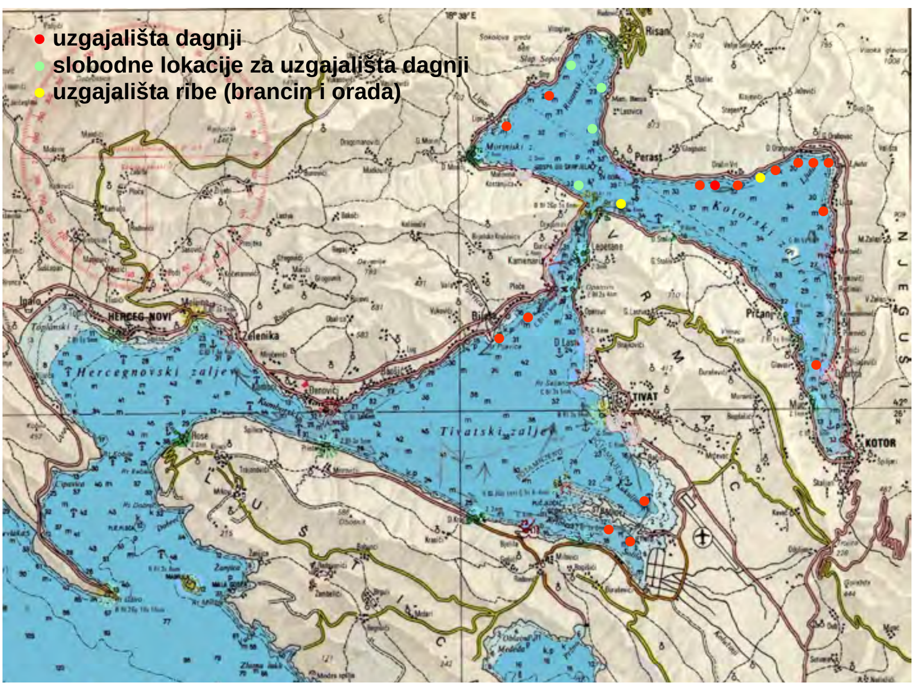

Figure 1. Boka Kotorska Bay and locations that are used for shellfish (red dots), fish (yellow dots), as well as sites that are designed to be favorable for aquaculture program (green dots).

In Boka Kotorska Bay (Fig 1) currently there are 16 mussel farms, one oyster farm and two fish (sea bass and sea bream) farms.

The total annual production of mussels in Montenegro is about 200 tons, while the amount of oysters is still too early to say since the first commercial farm these shells started working in 2009, and the first consumer oysters were on the market during the 2012. The entire farming of both species takes place on floating parks, and therefore that technology will be described in this paper.

MATERIAL AND METHODS

Technical solution for floating parks

To build a floating parks need the following items:

- Plastic buoys - polyethylene buoys with the size of 80 -160 liters (smaller or bigger are also appropriate, depending on the ecological conditions on the locality)
- Polypropylene rope 0, 6, 12 and 16 mm;
  - Braids or so-called “pergolar”, or mesh in the form of sleeves made of synthetic material, 2 m long, 20 cm in diameter, with a mesh size of 2-3 cm (for the first phase of treatment) and 3-5 cm (for the second phase of treatment);
  - anchorage of reinforced concrete weighing 500 to 1000 kg.

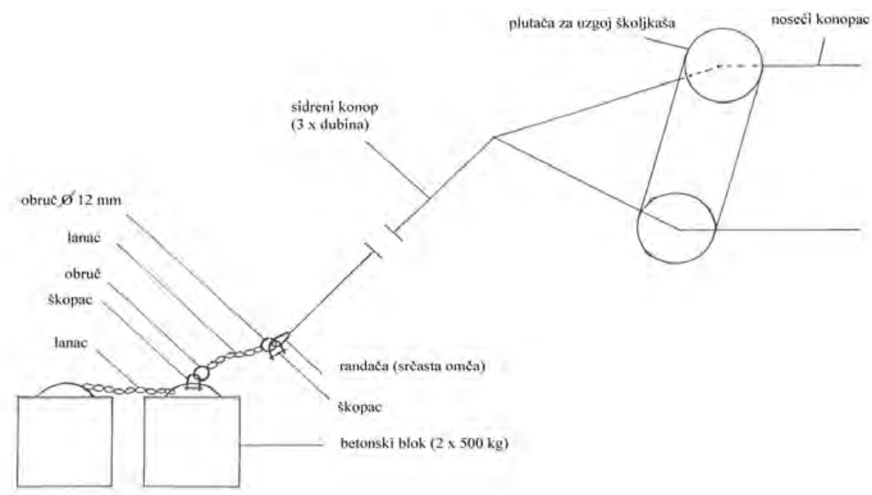

Figure 2: Schematic presentation of anchor line (Teskeredžić *et al.,* 2004)

Figure 3. Installation of the line of the park.

Technology of oyster farming

Oyster is very sensitive and delicate seashell, and these characteristics for its cultivation require large power consumption, which causes, together with the meat quality, its high price (Dujmušić, 1992). For technological processes of control breeding of oysters a floating line for mussels is required (eg 10 buoys at a distance of 10 m - total length 100 m, 3 m security fence to side and end) with anchoring equipment, collectors to accept younger oysters as well as ropes and / or boxes for the cultivation of oysters.

Depending on the wishes of producers, farm on an area of 1 ha can be partly used for the cultivation of mussels, and partly for the cultivation of oysters.

Cementing of oyster is the traditional method in the Adriatic Sea. Comparative studies on quality of consume oysters reared on braids, in plastic boxes or ropes have shown that the best ones are cemented, but their percentage of mortality is greatest. Specifically, oysters are unprotected and exposed to predators and the substantial amount of fouling, but the growth, the overall weight and condition index are the best using this technology of farming.

The most common period for oyster cementing is late August or early September, in order to have oysters ready for the market next year.

DISCUSSION

From the period of the first experimental studies on the possibilities of mussel and oyster farming in the Boka Kotorska Bay until today, studies of water quality and potential localities for cultivation showed that the bay area is very favorable for the cultivation, not only of these species, but for other edible shellfish (Mandić *et al*., 2001). The fact that this relatively small bay in the early seventies of the last century was participated with 6.6% of all mussels production in the former Yugoslavia, shows that little has been done on the development of mariculture, especially if mention that the open part of the Montenegrin coast remained completely unused in this regard .

One of the biggest obstacles to increase production are predators (*Sparus aurata, Diplodus puntazzo, Diplodus sargus*) that make huge damage on the farms by feeding on shells. One of the priorities in the development of this sector in Montenegro is to protect farms from predators, increase production and to introduce new native species in the process of growing.

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MAGNETIC RESONANCE IMAGING - A NEW APPROACH IN INVESTIGATIONS OF ENDANGERED MARINE BIVALVES

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MAGNETSKA REZONANCIJA - NOVI PRISTUP U ISTRAŽIVANJU UGROŽENIH MORSKIH ŠKOLJKAŠA

Apstrakt
Magnetska rezonancija (MR) sve se više koristi u istraživanjima na području biologije. Upotreba i implementiranje ove metode u svrhu nedestruktivnog proučavanja anatomije i fiziologije ugroženih vrsta morskih školjkaša daje veliki značaj ovakvim istraživačkim tehnikama. Nove moderne tehnologije omogućavaju bolje razumijevanje građe i oblika mekih struktura (npr. gonade, želudac, probavna žlijezda, mišići, škrge), te prepoznavanje anatomskih odlika i međusobni odnos organa kod živih školjkaša. Zbog svoje biološke važnosti, ugrožene vrste školjkaša predmet su mnogih istraživanja. U okviru zaštite vrsta, potrebno je izvršiti preliminarnu procjenu tehnoloških mogućnosti MR. Cilj ovog istraživanja je napraviti prikaz mogućnosti i ograničenja primjene MR u procjeni anatomskih struktura i razvoju gonada kod prstaca, *Lithophaga lithophaga* L., zaštićenog školjkaša iz Jadranskog mora. Ovaj rad predstavlja primjenu moderne tehnologije u svrhu istraživanja komparativne anatomije te je, uz dosadašnja saznanja, predstavljen originalan način kako bi se istražile morfološke osobitosti ugroženih vrsta.

Ovo izvješće obuhvaća preliminarne MR snimke prstaca. Na dobivenim fotografijama moguće je razlučiti i izračunati indekse mase mišića, funkciju probavne žlijezde, gonada i aduktornih mišića te škrga u različitom razdoblju razvoja školjkaša. Primjenom ove nedestruktivne metode u svrhu mjerenja rasta gonada i spolnog sazrijevanja prstaca moguće je procijeniti individualni razvoj, kao i usporediti pojedine žive jedinke.

Ključne riječi: MRI, in vivo anatomija, fiziologija, ugrožene vrste, prstac

Abstract

Magnetic Resonance Imaging (MRI) is increasingly used in biology and the appearance of such imaging and structural findings devoted to endangered marine bivalves shows the recent keen interest in these research techniques. New imaging techniques are ideal for understanding the normal form of soft structures (e.g. gonad, stomach, digestive gland, muscle, gills), and identifying anatomical positions and landmarks of structures in vivo.

Because of its biological importance, endangered bivalve mollusks are the subject of much research in physiology. In terms of protection of species, it seemed necessary to perform a preliminary technological evaluation of the MRI possibilities.

Aim of this research was to make a preliminary exploration of the possibilities and limits of MRI for assessing anatomical structures and gonad development of the date mussel, *Lithophaga lithophaga* L., an endangered bivalve from Adriatic sea. This paper presents a new imaging technique used in comparative morphology, with examples of recent applications, and will present original research demonstrating a use of this technique to investigate morphological structures in endangered species. This report therefore presents the preliminary results on MR imaging of the date mussel. From the obtained images, variations in the volumes of the flesh, digestive gland, gonad and adductor muscle, and the surface of the gills could be measured during growth.

Gonad measurement by MRI presents a non-destructive method and therefore makes it possible to assess live individual development as well as to compare live individuals.

Keywords: MRI, In vivo anatomy, Physiology, Endangered species, Date mussel

LEUKOCYTES OF COMMON BREAM (*ABRAMIS BRAMA*, LINNEAUS, 1758) FROM THE ARTIFICIAL MODRAC LAKE (BOSNIA & HERZEGOVINA) IN VARIOUS SEASONS

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ANALIZA LEUKOCITA DEVERIKE (*ABRAMIS BRAMA*, LINNEAUS, 1758) IZ HIDROAKUMULACIJE MODRAC (BOSNA I HERCEGOVINA) TOKOM SEZONE

Apstrakt

Analiza hematološko-biohemijskih parametara krvi riba pruža mogućnost uvida utjecaja okolinskih faktora na fiziološko stanje riba. Također, je i odličan pokazatelj kondicije i zdravstvenog stanja ovih organizama. Karakteristika ektotermnih kičmenjaka je širok raspon variranja hematoloških parametara i velike individualne varijacije, zbog izraženog uticaja okolinskih faktora. Hematološki status ribe je dobar indikator odgovora organizma ribe na navedene uticaje. Istraživanja raznih autora ukazuju na jako velike sezonske varijacije u vrijednostima pojedinih hematoloških parametara. Sezona utiče kako na broj leukocita, tako i na vrijednosti pojedinih vrsta leukocita u okviru diferencijalne krvne slike, koji su analizirani tokom sprovedenog istraživanja. Broj leukocita je manji kod zdravih riba. U dostupnoj literaturi nema podataka o pokazateljima zdravstvenog stanja deverike iz hidroakumulacije Modrac. Za potrebe istraživanja prikupljeno je ukupno 200 jedinki deverike (lat. *Abramis brama*, Linnaeus, 1758) iz akumulacije Modrac, odnosno po 50 jedinki u četiri sezone. Tokom istraživanja na izlovljenom uzorku određen je ukupan broj leukocita, kao i vrijednost diferencijalne krvne slike. Krv je uzeta punkcijom srca, te je za dalju analizu korištena nativna krv bez dodavanja antikoagulansa. Ukupan broj leukocita određen je u Bürcker-Türckovoj komorici na uvećanju 40x. Za određivanje diferencijalne krvne slike pripremljen je krvni razmaz, obojen metodom po Pappenheimu. Najniža prosječna vrijednost broja leukocita u krvi *Abramis brama* iz hidroakumulacije Modrac utvrđena je tokom ljeta u iznosu od 1,607 x 10^9/L, dok je najviša vrijednost zabilježena u proljeće 3,613 x 10^9/L. Limfociti su leukociti koji pokazuju najveću relativnu zastupljenost u krvi *Abramis brama* iz akumulacije Modrac, slijede nesegmentirani neutrofili, heterofili, monociti, segmentirani neutrofili, te eozinofilni granulociti. Najmanju relativnu zastupljenost od svih leukocita, očekivano, pokazuju bazofilni granulociti, te nisu utvrđeni kod jedinki izlovljenih u periodu ljeta i jeseni. Korelativnom analizom je utvrđeno da monociti i nesegmentirani neutrofili pokazuju najveću signifikantnu pozitivnu korelaciju ($r = 0.4723$; $p < 0,01$). Dok limfociti negativno koreliraju sa nesegmentiranim neutrofilima ($r = - 0,8318$), heterofilima ($r = - 0,5365$) i monocitima ($r = - 0,6230$), pri čemu su ove povezanosti statistički visoko signifikantne ($p < 0,01$). Za testiranje razlika između utvrđenih vrijednosti analiziranih parametara u različitim sezonama istraživanja primjenjen je t-test. Primjenom navedenog testa utvrđeno je da su proljeće i zima sezone koje se statistički značajno razlikuju u najvećem broju analiziranih varijabli (broju leukocita, procentu segmentiranih neutrofila, nesegmentiranih neutrofila, eozinofila, heterofila, te procentu agranulocita). Proljeće i jesen su sezone između kojih su, primjenom t-testa, utvrđene signifikantne razlike u najmanjem broju analiziranih parametara (broju leukocita, procentu segmentiranih neutrofila i bazofilnih granulocita). Utvrđen je manji broj leukocita u krvi *Abramis brama* iz jezera Modrac u odnosu na broj leukocita koji su dobili različiti autori, pri analizi navedenog parametra kod istih ili sličih vrsta riba.

Ključne riječi: leukociti, Abramis brama, hidroakumulacija Modrac
Keywords: white blood cells, Abramis brama, artificial Modrac Lake

INTRODUCTION

A characteristic feature of all ectothermic vertebrates is a wide physiological range of blood composition and a large individual variation, resulting among others from the fact that they are under a great effect of environment. Their homeostatic system and mechanisms of its control are much less specialised compared to mammals, and thus maintaining some parameters within a narrow range of the so called physiological standard is very difficult (Homątowska et al., 2002). The physiological status of fish body is directly related to inner and outer factors, biotic and abiotic influences which acting on the organism (Stosik et al., 2002). Fish blood cells (RBC, WBC counts) are good indicators of systemic response to external stimulus and any changes are therefore reflected in their morphology and distribution in the blood (Srivastava and Choudhary, 2010). Count of leukocyte depends on the age of the fish, sex, sexual activity, nutritional status, season, temperature changes of water and health of fish (Bogut et al., 2006). It is known that leukocyte cells are normally lower in healthy fishes and could be used as a significant indicator for infectious diseases (Jamalzadeh et al., 2009). Research of many authors suggest a large variation in the values of some haematological parameters during different seasons. The artificial Modrac Lake is built in year 1964, and it is the largest water management facility of this kind in Bosnia and Herzegovina (Arnautalić, 2006). In the recent literature there is no data of the health status indicators of common bream from the accumulation Modrac.

MATERIALS AND METHODS

For research purposes, we collected a total of 200 samples of common bream (*Abramis brama*, Linnaeus, 1758) from the accumulation Modrac: 50 individuals in each of four seasons. Samples of blood were taken by puncture of the heart, using a sterile needle wide diameter 1.1 mm, with a place that was previously disinfected. For further analysis we used native blood without adding anticoagulant. The total count of leukocytes was determined in 20 µl of blood and added to a test tube with 4 ml Natt Herrick's solution. Content is homogenized and poured into Bürcker-Türck chamber for leukocytes counting. Leukocytes were counted at magnification of 40x. Differential blood picture is a relative representation of certain types of white blood cells. To determine parameters of differential blood picture prepared blood smear, stained according to the Pappenheim. For statistical analysis, we used the value of the relevant software programs: Statistica 8, SPSS and Microsoft Excel. Seasonal difference between the arithmetic mean of the analyzed variables we tested by t-test. Correlation for all analyzed parameters according to the seasons is determined.

RESULTS AND DISCUSSION

Table 1. presents the mean count of leukocytes and the mean relative abundance of granulocytes in the blood smear of common bream identified during the different seasons. The lowest average value of the number of leukocytes in the blood of *Abramis brama* from the reservoir Modrac is determined during the summer, while the highest value was recorded in the spring. Basophils shows lowest relative number of all leukocytes. Basophils were not observed in the blood of individuals who have been caught during the summer and fall.

Table 1. The total count of leukocytes and the relative distribution of granulocytes in the blood of common bream

Variable	Season	Number of fish	Mean	SD	Min	Max	Coefficient of variation	Courtosis	Skewness
Leukocyte number (10^9/L)	Winter	50	2,330	0,997	0,600	4,350	42,769	-0,785	0,286
	Spring	50	3,613	1,341	1,450	7,700	37,119	1,087	0,896
	Summer	50	1,607	0,542	0,650	3,050	33,699	-0,339	0,451
	Autumn	50	1,804	0,960	0,600	4,000	52,239	-0,599	0,724
Segmented neutrophils (%)	Winter	50	4,060	4,177	0,000	20,000	102,876	3,932	1,752
	Spring	50	1,340	2,125	0,000	12,000	158,566	12,125	2,937
	Summer	50	0,780	1,075	0,000	4,000	137,765	2,070	1,591
	Autumn	50	2,300	2,597	0,000	12,000	112,917	3,811	1,796
Nonsegmented neutrophils (%)	Winter	50	3,260	4,222	0,000	17,000	129,523	2,553	1,763
	Spring	50	15,700	10,708	0,000	40,000	68,204	-0,599	0,618
	Summer	50	12,000	10,753	0,000	42,000	89,611	0,338	1,046
	Autumn	50	19,760	11,644	0,000	47,000	58,925	-0,209	0,431
Eosinophils (%)	Winter	50	0,140	0,351	0,000	1,000	250,364	2,684	2,14
	Spring	50	0,480	0,886	0,000	4,000	184,620	4,951	2,171
	Summer	50	0,380	2,547	0,000	18,000	670,165	49,673	7,038
	Autumn	50	0,320	0,621	0,000	2,000	193,978	2,086	1,799
Basophils (%)	Winter	50	0,020	0,141	0,000	1,000	707,107	50	7,071
	Spring	50	0,160	0,510	0,000	2,000	318,438	8,929	3,165
	Summer	50	0,000	0,000	0,000	0,000	0,000		
	Autumn	50	0,000	0,000	0,000	0,000	0,000		
Heterophils (%)	Winter	50	2,620	3,392	0,000	16,000	129,466	5,589	2,227
	Spring	50	9,320	6,678	0,000	29,000	71,647	0,803	1,064
	Summer	50	17,560	9,719	0,000	40,000	55,346	-0,219	0,548
	Autumn	50	7,140	5,421	0,000	21,000	75,926	0,168	0,841

Besides granular leukocytes in blood smears stained according to Pappenheim, we determined the relative number of agranular leukocytes: monocytes and lymphocytes (Table 2). In contrast to basophils, lymphocytes showed the highest prevalence levels in blood of *Abramis brama*. In some instances values of lymphocytes in common bream amounted to 100%.

Table 2. Relative representation monocytes and lymphocytes in the blood *Abramis brama*

Variable	Season	Number fish	Mean	SD	Min	Max	Coefficient of variation	Courtosis	Skewness
Monocytes (%)	Winter	50	1,440	1,248	0,000	4,000	86,668	-1,386	0,208
	Spring	50	7,640	7,136	0,000	28,000	93,409	0,725	1,306
	Summer	50	3,900	3,786	0,000	18,000	97,087	3,422	1,685
	Autumn	50	7,640	4,952	1,000	21,000	64,815	-0,261	0,662
Lymphocytes (%)	Winter	50	88,460	8,885	55,000	98,000	10,044	4,169	-1,794
	Spring	50	65,280	16,054	32,000	93,000	25,592	-0,836	-0,312
	Summer	50	65,380	16,638	18,000	100,000	25,448	0,192	-0,376
	Autumn	50	62,840	14,947	32,000	88,000	23,785	-0,881	-0,228

To test the difference between the determined values of the research parameters analyzed in different seasons we applied t-test. Those results are shown in Table 3. The biggest difference is found between the spring and winter seasons, contrary to difference between spring and autumn, which was smallest.

Table 3. T-test values between different seasons

	Seasons	Leu	Segm. neutrophils	Nonsegm. neutrophils	Eosinophils	Basophils	Heterophils	Monocytes	Lymphocytes
t-test Sig. (2-tailed)	Spring / Summer	0.000	0.099	0.088	0.794	0.029	0.000	0.001	0.976
	Summer / Autumn	0.209	0.000	0.001	0.320	-	0.000	0.000	0.424
	Summer / Winter	0.000	0.000	0.000	0.511	0.320	0.000	0.000	0.000
	Spring / Autumn	0.000	0.046	0.073	0.298	0.029	0.076	1.000	0.433
	Spring / Winter	0.000	0.000	0.000	0.013	0.064	0.000	0.000	0.000
	Autumn / Winter	0.008	0.013	0.000	0.077	0.320	0.000	0.000	0.000

Correlation analysis of between different type of leukocytes in total sample is presented in Table 4. At the same shows the values of the coefficient correlation (r) and statistical significance of the correlation (p). Monocytes and nonsegmented neutrophils showed the highest positive correlation.

The number and relative distribution of white blood cells is an important parameter that indicates the health of the fish. The average value of the number of leukocytes of *Squalius cephalus* from the river Krupica is higher than that of common bream and

Squalius cephalus from the River Željeznica (Mitrašinović, Suljević, 2009). Also, Stosik et al., 2002 found higher values of leukocytes in common bream (*Abramis brama*) from Lake Dąbie and Szczecin in relation to the common bream from reservoir Modrac. These authors found that *Abramis brama* from Lake Dąbie and Szczecin has the highest value of lymphocytes, in the analysis of differential blood picture, which is consistent to the results of our research.

Table 4. The coefficients correlation (r) between different types of leukocytes in the total sample

Variable	Leu	Segm. neutrophils	Nonsegm. neutrophils	Eosinophils	Basophils	Heterophiles	Monocytes	Lymphocytes
Leukocytes	1.0000	.0120	-.0196	-.0217	.0660	-.1442*	.1416*	.0383
Segmented neutrophils		1.0000	-.0238	-.0690	.0554	-.1916**	-.1203	-.0190
Nonsegmented neutrophils			1.0000	-.0242	.0822	.0939	**.4723****	**-.8318****
Eosinophils				1.0000	.0271	.0305	.0525	-.0828
Basophils					1.0000	.0228	-.0561	-.0736
Heterophiles						1.0000	.0615	**-.5365****
Monocytes							1.0000	**-.6230****
Lymphocytes								1.0000

* p < 0,05; ** p < 0,01

The negative correlation is established between lymphocytes and nonsegmented neutrophils, heterophiles and monocytes (p <0.01).

CONCLUSIONS

White blood cells in the blood of common bream from the accumulation Modrac is showing the lowest average values during the summer, while the highest values were recorded in spring;

Basophils showed lowest relative number of all cells determined in this study and were not noticed in animals collected during the summer and fall;

Lymphocytes are the most common white blood cells in the blood of common bream;

Spring and winter are the seasons that show statistically significant differences in the values of the majority of the analyzed parameters;

Variables that are positively correlated to nonsegmented neutrophils were monocytes, while lymphocytes were significantly negatively correlated to nonsegmented neutrophils, heterophils and monocytes.

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REPRESENTATIVES OF TAPEWORMS (CESTODA) OF FISHES IN BELGRADE SECTION OF THE DANUBE RIVER

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PREDSTAVNICI PANTLJIČARA (CESTODA) RIBA BEOGRADSKOG SEKTORA DUNAVA

Apstrakt
U radu su izneti rezultati dvogodišnjih istraživanja unutrašnjih parazita različitih vrsta riba Dunava u beogradskom sektoru, i u tom cilju je urađena identifikacija i prikazan je spisak crevnih parazita iz grupe pantljičare (Cestoda). Tokom istraživanja pregledene su ukupno 802 jedinke 22 vrste riba. Pregledom crevnog trakta riba, utvrđeno je da je parazitima zaraženo 140 jedinki 12 vrsta riba, odnosno 17.46%. U inficiranim jedinkama riba nađena su ukupno 13 crevna parazita koji pripadaju grupi Cestoda (pantljičare). Predstavljen je spisak vrsta faune crevnih panljičara, po vrsti ribe, međusobni odnos parazitskih vrsta, kao i njihova dinamika prisustva u jedinkama riba. Prikazane su vrednosti intenziteta (broj parazita po ribi) i ekstenziteta infekcije (% zaraženosti, broj zaraženih riba određenim parazitom).
Pantljičare su pljosnati, dorzo-ventralno spljošteni crvi, bez crevnog trakta. Telo im je podeljeno na članke (proglotise). Prednji kraj tela je skoleks i na njemu su smešteni organi za pričvršćivanje (pijavke, kukice). Iza njega je suženje (vratni region), a zatim sledi niz proglotisa. Razviće ovih parazita odvija se kroz smenu generacija (prelazni domaćin je oligoheta).
Tokom istraživanja, izdvojile su se određene parazitske vrste pantljičara ("*hit paraziti*"), koje inficiraju veliki broj vrsta riba. To su vrste *Proteocephalus torulosus* (7 vrsta riba), *Caryophyllaeides fennica* i *Caryophyllaeus laticeps* (6 vrsta riba). Vrsta ribe koja je inficirana sa najvećim brojem parazita je *Abramis brama* (12). U njenom crevu je identifikovana vrsta parazitske pantljičare sa najvišim intenzitetom infekcije - *Caryophyllaeus laticeps* (1-165).
Istraživana ihtiozajednica beogradskog sektora Dunava još uvek ima sastav koja se može okarakterisati autohtonom, uz napomenu da u toj fauni, nakon izgradnje HEPS

„Đerdap“, nema više anadromnih jeseterskih vrsta (Acipenseridae). U zajednici se zapaža izrazita dominacija mirnih riba, pre svega krupatice (*Bllica bjoerkna*), bodorke (*Rutilus rutilus*) i deverike (*Abramis brama*). Većina konstantovanih vrsta riba mogu se smatrati predstavnicima zajednice gornjeg potamona, sa pojedinim elementima – vrstama riba zajednice donjeg ritrona.

Sprovedena studija je pokazala da je inficiranost riba Dunava u beogradskom regionu značajna, s obzirom na brojnost i raznovrsnost identifikovanih crevnih parazita grupe pantljičara. Podaci o konstatovanoj fauni crevnih pantljičara upotpunjuju i potvrđuju dosadašnja sporadična saznanja o njihovom rasprostranjenju u otvorenim vodama Srbije. Do sada je, u 75 godišnjim izučavanjima parazitofaune riba površinskih voda Srbije, nađeno ukupno 170 parazita (ekto- i endo-). Od toga, grupi pantljičara (Cestoda) pripada 19 taksona.

Ključne reči: *slatkovodne ribe, crevni paraziti, Cestoda, infekcija, beogradsko područje Dunava*

Keywords: freshwater fish, intestine parszite, Cestoda, infection, Belgrade section of the Danube River

INTRODUCTION

The catchments area of the Danube River in the Belgrade region is a part of the middle sector of the Danube River Basin – the largest sector of the river’s watercourse from Bratislava to the Iron Gate dams (Serbia/Romania) (Babic-Mladenovic et al., 2010). With its main tributaries, the Danube represents the most significant Serbian water resource. Ichthyofauna of the Serbian section of the Danube River consists of 68 fish species from 50 ordos and 16 families (Simonovic and Nikolic, 1996). In the meantime, in this river section, have been found three more allochthonous, invasive gobid species (family Goobidae), introduced fish species *Syngnathus abaster* (family Syngnathidae) and species *Perccotus glenii* (family Odontobutidae). Composition of fish fauna in the Belgrade section of the Danube River still have autochtonous character. It can be seen the dominance of still water fishes, like white bream (*Blicca bjoerkna*), roach (*Rutilus rutilus*) and bream (*Abramis brama*). The construction of a hydro-energetic system „Djerdap“ caused changes in ichthyocenosis and exstinction of anadromous sturgeon fish species (Acipenseridae) in this river section (Lenhardt et al., 2006).

Parasitic species of freshwater fishes represent a large group of organisms comprising either adults or larval stages. During last 75 years, systematic parasitofauna investigations have been carried out and a total of 170 parasites of fish species in Serbian open waters (Danube River Basin) have been reported (Djikanovic et al., 2011).

Cestoda (tapeworms) are long, extremely dorso-ventraly flattened hermaphroditic parasites, without intestines. These typeworms as adults are found in the intestine of vertebrates. Larvae are not segmented with scolex, usually encysted; adults are segmented, with flattened body with scolex (attachment organ). These parasites generally have larval forms encysted in an intermediate host (Oligochaeta).

In this work, the aim is to present results of freshwater fish intestine parasites (Cestoda) investigation in the Belgrade section of the Danube River. Checklist of identified endoparasites per fish are presented, relation of identified parasitic species, as well as their presence in specimens of different fish species.

MATERIAL AND METHODS

Ichthyoparasitological samples were collected during period November 2007 to November 2009 along the course of the Danube River through the Belgrade Region, by nets of different size of mesh (32 – 50 mm), from two sampling sites, Zemun (1.173 rkm) and Visnjica (1.162 rkm). Fish were transported to the laboratory, where the analysis of their intestines for endoparasites was conducted. The intestines were examined under an Olympus binocular microscope and Olympus stereomicroscope. Parasitic species found were fixed first with hot 4% formaldehyde and after two weeks put into 70 % alcohol for determination and collection. Identification was carried out to the species level, except in case of juvenile individuals. Parasites were identified using identification keys (Bykhovskaya-Pavlovskaya et al., 1962; Kakacheva – Avramova, 1983; Bauer, 1987) to the lowest taxonomic level (species, ordo). The level of parasitic infestation was studied by analyzing of number of parasites per fish specimen. It has been analyzed prevalence (number of fish infected by certain parasite, % of infestation,) and intensity (number of parasites per fish) of infection.

RESULTS AND DISCUSSION

During the ichthyoparazitological study 22 freshwater fish species from families Cyprinidae (14), Esocidae (1), Percidae (4), Centratchidae (1), Siluridae (1) and Gadidae (1) have been collected and examined. 802 fish specimens have been examined and 17.46% were infected. In intestine of 12 fish species 13 species/taxa of Cestoda (*Caryophyllaeides fennica* (Schneider, 1902), *Khawia sinensis* (Hsü, 1935), *Caryophyllaeus brachycollis* (Janiszewska, 1951), *Caryophyllaeus laticeps* (Pallas, 1781), *Caryophyllaeus fimbriceps* (Annenkova-Khlopina, 1919), *Caryophyllaeus* sp. juvenile (Gmelin, 1790), *Triaenophorus nodulosus* (Pallas, 1781), *Triaenophorus* sp. juvenile (Rudolphi, 1793), *Proteocephalus torulosus* (Batsch, 1786), *Proteocephalus sp.* juvenile (Weinland, 1858), *Ligula intestinalis* (Linnaeus, 1758), *Diphyllobothrium* sp. (Cobbold, 1858) and Cestoda-cysts) have been found. During the investigation, few parasitic species have been dissociated ("*heat parasites*"), infested a large number of fish species. These parasitic species are: *Proteocephalus torulosus* (7 fish specimens), *Caryophyllaeides fennica* and *Caryophyllaeus laticeps* (6 fish specimens). Fish species with the greatest number of intestine parasitic species was *Abramis brama* (12). In its intestine parasite with the highest intensity of infection - *Caryophyllaeus laticeps* (1-165) have been identified. Fish species that had been infected by a great number of cestodes were *Abramis sapa* (8), as well as *Abramis ballerus* and *Vimba vimba* (5), while others were infected with two or three tapeworms. In intestine of *Rutilus rutilus* and *Sander lucioperca* only one parasitic species was found. Number of infected fish species, prevalence and intensity of infection per each cestode are presented in Table 1.

Table 1. Number of infected fish species, prevalence and intensity of infection per each identified cestode

Parasitic species/taxa / Fish species	***Abramis brama***			***Abramis ballerus***			***Abramis sapa***		
	Extensity of infection		Inten. of infec.	Extensity of infection		Inten. of infec.	Extensity of infection		Inten. of infec.
	Number of infected	Prev (%)		Number of infected	Prev (%)		Number of infected	Prev (%)	
Caryophyllaeides fennica (Schneider, 1902)	16	2.00	1-9	6	0.75	1-19	12	1.50	1-11
Khawia sinensis (Hsü, 1935)									
Caryophyllaeus brachycollis (Janiszewska, 1951)	5	0.62	1-9						
Caryophyllaeus laticeps (Pallas, 1781)	17	2.12	1-165	7	0.87	1-10	12	1.50	1-9
Caryophyllaeus fimbriceps (Annenkova-Khlopina, 1919)	7	0.87	1-13						
Caryophyllaeus sp. juvenile (Gmelin, 1790)	15	1.87	1-17				1	0.12	0-4
Triaenophorus nodulosus (Pallas, 1781)	1	0.12	0-3				1	0.12	0-27
Triaenophorus sp. juvenile (Rudolphi, 1793)	4	0.50	1-2						
Proteocephalus torulosus (Batsch, 1786)	2	0.25	2-18	3	0.37	1-2	5	0.62	1-2
Proteocephalus sp. juvenile (Weinland, 1858)	4	0.50	1-8	2	0.25	1-15	1	0.12	0-2
Ligula intestinalis (Linnaeus, 1758)	3	0.37	1-10				1	0.12	0-1
Diphyllobothrium sp. (Cobbold, 1858)	3	0.37	1-9						
Cestoda-ciste	6	0.75	1-5	2	0.25	0-1	3	0.37	0-6
Parasitic species/taxa / Fish species	***Bllica bjoerkna***			***Aspius aspius***			***Barbus barbus***		
	Extensity of infection		Inten. of infec.	Extensity of infection		Inten. of infec.	Extensity of infection		Inten. of infec.
	Number of infected	Prev (%)		Number of infected	Prev (%)		Number of infected	Prev (%)	
Caryophyllaeides fennica (Schneider, 1902)	3	0.37	1-4						
Khawia sinensis (Hsü, 1935)									
Caryophyllaeus brachycollis (Janiszewska, 1951)							4	0.50	3-9
Caryophyllaeus laticeps (Pallas, 1781)	1	0.12	0-4	1	0.12	0-9			
Caryophyllaeus fimbriceps (Annenkova-Khlopina, 1919)									
Caryophyllaeus sp. juvenile (Gmelin, 1790)	2	0.25	1-2				2	0.25	9-11

Triaenophorus nodulosus (Pallas, 1781)									
Triaenophorus sp. juvenile (Rudolphi, 1793)									
Proteocephalus torulosus (Batsch, 1786)									
Proteocephalus sp. juvenile (Weinland, 1858)									
Ligula intestinalis (Linnaeus, 1758)									
Diphyllobothrium sp. (Cobbold, 1858)									
Cestoda-ciste				1	0.12	0-4			
Parasitic species/taxa / Fish species	***Chondrostoma nasus***			***Leuciscus idus***			***Gymnocephalus schraetzer***		
	Extensity of infection		Inten. of infec.	Extensity of infection		Inten. of infec.	Extensity of infection		Inten. of infec.
	Number of infected	Prev (%)		Number of infected	Prev (%)		Number of infected	Prev (%)	
Caryophyllaeides fennica (Schneider, 1902)				1	0.12	0-2			
Khawia sinensis (Hsü, 1935)									
Caryophyllaeus brachycollis (Janiszewska, 1951)				8	1.00	1-6			
Caryophyllaeus laticeps (Pallas, 1781)									
Caryophyllaeus fimbriceps (Annenkova-Khlopina, 1919)									
Caryophyllaeus sp. juvenile (Gmelin, 1790)									
Triaenophorus nodulosus (Pallas, 1781)									
Triaenophorus sp. juvenile (Rudolphi, 1793)									
Proteocephalus torulosus (Batsch, 1786)	1	0.12	0-9	2	0.25	2-12	2	0.25	1-1
Proteocephalus sp. juvenile (Weinland, 1858)							1	0.12	0-1
Ligula intestinalis (Linnaeus, 1758)									
Diphyllobothrium sp. (Cobbold, 1858)									
Cestoda-ciste	3	0.37	0-11				1	0.12	0-1
Parasitic species/taxa / Fish species	***Sander lucioperca***			***Rutilus rutilus***			***Vimba vimba***		
	Extensity of infection		Inten. of infec.	Extensity of infection		Inten. of infec.	Extensity of infection		Inten. of infec.
	Number of infected	Prev (%)		Number of infected	Prev (%)		Number of infected	Prev (%)	
Caryophyllaeides fennica (Schneider, 1902)							5	0.62	1-2

Khawia sinensis (Hsü, 1935)				1	0.12	0-2			
Caryophyllaeus brachycollis (Janiszewska, 1951)									
Caryophyllaeus laticeps (Pallas, 1781)							3	0.37	1-18
Caryophyllaeus fimbriceps (Annenkova-Khlopina, 1919)									
Caryophyllaeus sp. juvenile (Gmelin, 1790)							1	0.12	0-2
Triaenophorus nodulosus (Pallas, 1781)									
Triaenophorus sp. juvenile (Rudolphi, 1793)									
Proteocephalus torulosus (Batsch, 1786)							1	0.12	0-1
Proteocephalus sp. juvenile (Weinland, 1858)	1	0.12	0-12						
Ligula intestinalis (Linnaeus, 1758)									
Diphyllobothrium sp. (Cobbold, 1858)									
Cestoda-ciste							2	0.25	2-7

In previous ichthyoparasitological studies in Serbian open waters, representatives of tapeworms were identified. Babic (1935) determined tapeworms from the Danube and Sava River fishes. Seasonal dynamic appearance of Cestoda *Ligula intestinalis* metacercariae in schneider (*Alburnoides bipunctatus* Bloch, 1782), in the Borsko Lake (eastern Serbia) were examined by Petrovic et al. (1975). Andric (1984) provided detail data on fish parasites from the Obedska Bara swamp and reported five cestodes from 7 fish species. Cakic and Hristic (1987) reported *Triaenophorus nodulosus* of white grasscarp, bream and carp in Pancevacki Rit channels. Kiskaroly and Tafro (1988) examined fishes in the Danube River and DTD channel and recorded *Amphilina foliacea, Caryphyllaeides fennica, Caryophyllaeus laticeps, C. branchicollis, Proteocaphalus torulosus, Silurotaenia siluri* and *Ligula intestinlis.* Fish parasite studies in highland rivers and lakes of Sjenicko-Pesterska plateau (Uvac River Basin) (Cakic, 1992) determined nine Cestoda species. . Examination of sterlet specimens in Serbian part of the Danube River identified one representative from Cestoda (*Amphilina foliacea*) (Cakic et al., 2008). Composition of identified tapeworm community was similar to results of our study.

CONCLUSION

Results obtained in this ichtyoparasitological study will contribute considerably to complete data on fish helminths composition and their distribution in Serbian open waters, which is of benefit for scientific (academic) public and economic fisheries and its development. In addition the data on intestine fish parasites are important for evaluation of conditions and general effect of the level of parasitism on the community structure.

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HEMATOLOGICAL EVALUATION OF RAINBOW TROUT (*ONCORHYNCHUS MYKISS)* FINGERLINGS FROM DIFFERENT HATCHERIES

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HEMATOLOŠKA PROCENA MLAĐI KALIFORNIJSKE PASTRMKE (*ONCORHYNCHUS MYKISS)* IZ RAZLIČITIH MRESTILIŠTA

Apstrakt

Za praćenje zdravlja i kondicije riba u prirodnim staništima kao i riba u akvakulturi veliku važnost imaju istraživanja koja se odnose na krv i tjelesne tečnosti. Parametri eritrocitne i leukocitne loze predstavljaju veoma značajne pokazatelje stanja organizma riba i njihove vrijednosti su specifične za svaku vrstu. Osnovne karakteristike normalne krvne slike zdravih jedinki svake vrste moraju biti dostupne, prije njihovog korištenja u procjeni zdravstvenog stanja riba. Svaka vrsta ima krakterističan broj i veličinu eritrocita, koncentraciju hemoglobina, i hematološke indekse. Pored toga, postoje fiziološke varijacije svih parametara koje su pod uticajem pola, reprodukcije ili se pojavljuju tokom različitih faza životnog ciklusa vrste. Dnevne i sezonske oscilacije su takđe veoma dobro izražene. Sve vrijednosti hematoloških parametara kao i njihove varijacije su specifične za vrstu i/ili rasu i varijetet. Iz tog razloga smo u ovom radu ispitali dužičastu pastrmku porijeklom iz pet različitih matičnih jata. Mrijest je obavljen na pet izabranih ribogojilišta, nakon čega je oplođena ikra dopremljena u mrestilište Klašnik gdje su obezbijeđeni isti uslovi sredine tokom embrionalnog razvoja i gajenja za svih pet grupa jedinki. Ukupno je analizirano 50 jedinki koje su podijeljene u 5 grupa. Jedinke su bile raspoređene u bazene, sa protočnom vodom. Hranjene su proporcionalno masi tijela odgovarajućom količinom hrane istog proizvođača. Iz svake grupe za analizu je uzeto po 10 jedinki. Hematološki parametri koji su praćeni u radu predstavljeni su parametrima eritrocitne loze: brojem eritrocita, koncentracijom hemoglobina, hematokritom, srednjom vrijednost zapremine eritrocita (MCV),srednjom vrijednosti količine hemoglobina u eritrocitu (MCH)i srednjom vrijednosti hemoglobina u litri eritrocita (MCHC). Vrijednosti broja eritrocita značajno su niže u drugoj grupi nego kod jedinki iz grupa IV (p = 0,024) i V (p = 0,020). Vrijednosti hematokrita kod riba prve grupe značajno su više nego kod grupa II (p = 0,001), IV (p = 0,002) i V (p = 0,008). Takođe, vrijednosti kod III grupe riba su signifikantno više nego kod grupa II (p < 0,001), IV (p < 0,001) i V (p = 0,001). Najniže vrijednosti koncentracije hemoglobina konstatovane su kod jedinki iz četvrte grupe i značajno su bile manje u poređenju s ostalim grupama (I p = 0,016, II 0,004, III p = 0,004, IV p = 0,013). Vrijednosti prosječne zapremine eritrocita značajno su bile manje kod riba prve grupe nego kod jedinki iz grupa II (p = 0,007), IV (p = 0,002) i V (p = 0,005). Takođe, signifikantno više vrijednosti konstatovane su u grupi tri u poređenju sa vrijednostima grupa II (p = 0,001), IV (p < 0,001) i V (p < 0,001). Najmanje vrijednosti MCH utvrđene su kod grupe IV i značajno su bile manje u poređenju sa grupama I (p = 0,014), II (p = 0,001), III (0,003). Vrijednosti MCHC značajno su bile niže kod jedinki iz prve grupe u poređenju sa vrjednostima iz grupa II (p = 0,001) i V (p = 0,035). Vrijednosti ovog parametra su bile značajno više kod druge grupe u odnosu na grupe III (p = 0,001) i IV (p = 0,007). Takođe, značajno viša vrijednost zabilježena je kod grupe V u odnosu na grupu III (p = 0,028). Uočene razlike mogu se jedino objasniti različitim porijeklom od različitih matičnih jata.

Ključne riječi: *kalifornijska pastrmka, matična jata, mrijest, hematologija*
Keywords: rainbow trout, breeding stock, hatching, hematology

INTRODUCTION

Fish are in close contact with the environment, so they are very sensitive to physical and chemical environmental changes, which can lead to changes in blood components (Wilson and Taylor, 1993; Adhikari et al., 2004; Maheswaran et al., 2008). In the same time fish hematological parameters represent indicators of the state of the environment (Dekić et al., 2009). Research of blood and body fluids are of a great importance for monitoring the fish health and the conditions in natural habitat and in aquaculture (Ivanc et al., 2005). Changes of environmental conditions lead to specific or unspecific changes in hematological state of certain fish species, and these changes along with seasonal changes affect the blood physiology and biochemistry. Erythrocyte and leukocyte parameters represent significant indicators of fish health, and its values for certain species contribute to the knowledge of the limits of its variations in different phases of life cycle and determination of normal values typical for that species (Ivanc and Miljanović, 2003). According to Rehulka and Adamec (2004), erythrocytes reflect the state of an organism during long period of time, and in the same time are significant and reliable indicators of different stress sources.

The goal of this research was to determine hematological status of *Oncorhynchus mykiss* individuals originating from different breeding stock and different fish farms, but grown under the same conditions in order to access their quality in the production aspect.

MATERIAL AND METHODS

Individuals of rainbow trout used in this research originate from different breeding stock and different fish farms. Spawn was done in five chosen fish farms, after which the fertilized roe was transported to Klašnik hatchery where the same environmental conditions for embryonic development and cultivation were provided for all five groups of individuals. Individuals were distributed in pools, with circulating water flow, and proportional to the mass with the same amount of food of the same supplier. From every group 10 individuals were analyzed. Monitored hematological parameters were: number of erythrocytes (RBC), hemoglobin concentration, packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC).

Hematological analysis. Blood for hematological analysis was collected by heart puncture using sharp and wide sterile needle (1.0 to 1.2 mm), by applying the rules of the sterile work. Native blood, without an anticoagulant, is used for further analyses. Erythrocyte count was performed in hemocytometer using diluent by Kekić and Ivanc (1982). Hemoglobin concentration (Hb) was determined by hemoglobin cyanide method using Drabkin reagent (Blaxhall and Daisly, 1973). Hematocrit (Hct) was determined by microhematocrit centrifuge. Hematological indices were calculated using values of hematocrit, erythrocyte number and hemoglobin concentration.

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

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

Statistical analysis. Statistical analyses were done using statistical programs Microsoft Excel 2007 and SPSS 11.5. Comparison was done using ANOVA and LSD test.

RESULTS AND DISCUSSION

Results of conducted analyses of rainbow trout blood by different groups are present in table 1.

Table 1. Mean values of erythrocyte parameters *Oncorhynchus mykiss* from different parent flocks – group I to V

Group		RBC x 10^{12}/l	Hb (g/l)	Hct (l/l)	MCV (fl)	MCH (pg)	MCHC (g/l eryt.)
I	Mean	1.012	82.222^{4}	$0.591^{2,4,5}$	$587.842^{2,4,5}$	81.854^{4}	$141.987^{2,5}$
	Standard deviation	0.078	8.152	0.092	105.472	11.780	25.245
	Coefficient of variation - %	7.750	9.914	15.535	17.942	14.391	17.780
II	Mean	$0.978^{4,5}$	85.185^{4}	$0.449^{1,3}$	$463.511^{1,3}$	88.366^{4}	$193.049^{1,3,4}$
	Standard deviation	0.100	10.620	0.068	89.603	17.371	33.009
	Coefficient of variation - %	10.257	12.467	15.122	19.331	19.658	17.099

III	Mean	1.000	84.815^{4}	0.622^{2,4,5}	627.039^{2,4,5}	85.572^{4}	140.395^{2,5}
	Standard deviation	0.085	10.966	0.092	109.836	14.396	35.544
	Coefficient of variation - %	8.524	12.930	14.725	17.517	16.823	25.317
IV	Mean	1.063^{2}	68.889^{1,2,3,5}	0.468^{1,3}	445.003^{1,3}	65.381^{1,2,3}	151.079^{2}
	Standard deviation	0.087	8.765	0.093	102.189	10.925	26.904
	Coefficient of variation - %	8.202	12.723	19.817	22.964	16.710	17.808
V	Mean	1.066^{2}	82.593^{4}	0.484^{1,3}	456.079^{1,3}	77.476	174.227^{1,3}
	Standard deviation	0.046	18.064	0.084	85.233	16.249	42.493
	Coefficient of variation - %	4.337	21.872	17.395	18.688	20.974	24.389

Numbers in superscript denotes groups with significantly different mean values of the given parameter ($p \leq 0.05$)

Comparison of the results of five examined groups of rainbow trout showed the presence of significant differences in most of the parameters. Values od erythrocyte count were significantly lower in group II comparing with groups IV ($p = 0.024$) and V ($p = 0.020$). PCV values were significantly higher in first group comparing with groups II ($p = 0.001$), IV ($p = 0.002$) and V ($p = 0.008$). Group three had also significantly higher values of PCV comparing with groups II ($p <0.001$), IV ($p < 0.001$) and V ($p = 0.001$).

The lowest values of hemoglobin concentration were determined in individuals from fourth group and were significantly lower comparing to the other groups (I $p = 0.016$, II $p = 0.004$, III $p = 0.004$, IV $p = 0.013$).

Values of mean corpuscular volume were significantly lower in individuals from first group comparing with those from groups II ($p = 0.007$), IV ($p = 0.002$) and V ($p=0.005$). Also, significantly lower values were established in group three comparing with values determined in groups II ($p = 0.001$), IV ($p < 0.001$) and V ($p < 0.001$).

The lowest values of MCH were determined in group four and were significantly lower comparing with groups I ($p = 0.014$), II ($p = 0.001$), III ($p = 0.003$). MCHC values were significantly lower in individuals from the first group comparing with those from groups II ($p = 0.001$) and V ($p = 0.035$). Values of second group were significantly higher comparing with values from groups III ($p = 0.001$) and IV ($p = 0.007$). Also, significantly higher values were established in group V comparing with group III ($p = 0.028$).

Looking at the results of monitored parameters it is evident that individuals of rainbow trout from group IV had lower values of hemoglobin concentration, PCV, MCV and MCH.

Given that individuals were under the same conditions and treatment, observed differences can be correlated with fish origin from different breeding stocks. Svobodova et al. (2008) showed similar differences, but among spawners of eight carp breeds, for hemoglobin level (Hb), haematocrit value (PCV), mean corpuscular volume (MCV) and mean corpuscular haemoglobin (MCH).

CONCLUSIONS

All experimental gropus of rainbow trout had been provided with the same conditions for embrionic development and cultivation. Given that individuals were under the same conditions and treatment, observed differences can be correlated with different origine from breeding stock.

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PATHOLOGICAL CHANGES WHICH *THELOHANELLUS NIKOLSKII* CAUSE ON THE FINS, SCALES AND SKIN OF COMMON CARP

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PATOLOŠKE PROMENE NA PERAJIMA, KRLJUŠTIMA I KOŽI ŠARANA IZAZVANE *THELOHANELLUS NIKOLSKII*

Apstrakt

Telohaneloza je obolenje koje se javlja kod svih kategorija šarana. Uzročnik je protozoa *Thelohanellus nikolskii*. Manifestuje se u dva oblika. Prvi se javlja kao telohaneloza na perajima kod jednogodišnjih mladunaca šarana, a drugi kao telohaneloza krljušti i kože kod dvogodišnjih, trogodišnjih i višegodišnjih kategorija ribnjačkog šarana (*Cyprinus carpio*). Istraživanjima koja su trajala u periodu od 2008-2012. godine obuhvaćeno je 22 ribnjaka, od čega se 18 nalaze u Srbiji a 4 u BIH, Republika Srpska. Praćenjem epizootiološke rasprostranjenosti utvrđeno je da su oba oblika oboljenja bila prisutna na svim ispitivanim ribnjacima. Kod telohaneloze krljušti prevalenca se kretala od 2-75% a intenzitet infekcije 2-206 cisti po jedinki, dok su iste vrednosti kod telohaneloze na perajima iznosile 3-30%, odnosno 2-84 ciste. Promene na krljuštima bile su prisutne tokom aprila i maja, a na perajima tokom jula i avgusta. Tokom epizootiološkog praćenja i istraživanja promena na krljuštima zapaženo je da na njima dolazi do formiranja cisti okruglastog oblika koje su dostizale veličinu i do 3 mm. Promene na krljuštima zabeležene su kod dvogodišnjih mladunaca koji su najčešće bili inficirani, ali i kod trogodišnjih i četvorogodišnjih konzumnih i matičnih kategorija šarana, dok kod jednogodišnje mlađi nisu ustanovljene ni u jednom slučaju. Ciste su bile prisutne kako kod mnogoljuskavih šarana, koji su potpuno prekriveni krljuštima, tako i kod maloljuskavih gde su se promene nalazile na dorzalnom redu krljušti. Takođe je uočena i pojava cisti na koži, a ne i na krljušti kod četvorogodišnjih matičnih riba. Tokom čitavog

vegetacionog perioda na perajima su primećivane malformacije i nedostaci dela peraja kod mladunaca. Jedinke kojima su otpala repna peraja postajale su plen ribojedih ptica. Najveći broj cisti nalazio se na repnom peraju, zatim na leđnom, grudnom, trbušnom i analnom. Merenjem i određivanjem morfoloških karakteristika spora izolovanih iz plazmodijuma sa krljušti dvogodišnjih mladunaca i plazmodijuma sa peraja jednogodišnje mlađi dobijene su vrednosti dužine i širine spora, kao i dužine i širine polarnih kapsula. Prilikom determinacije patohistoloških karakteristika utvrđena je struktura plazmodijuma, gde se u centralnim delovima nalaze sporame, dok se na periferiji nalaze razvojni stadijumi i pansporoblasti, te se na osnovu dominacije pojedinih ćelija unutar njega može utvrditi da li se radi o mladim ili o zrelim cistama. Plazmodijum se razvija unutar kalcifikovane kolagene kapsule, koja predstavlja tanak sloj kalcifikovanog kolagena oko kojeg se nalaze izdužene ćelije – skleroblasti, koji su zaduženi za formiranje ciste. Molekularnim tehnikama i BLAST analizom potvrđeno je da je etiološki uzročnik *Thelohanellus nikolskii* isti za oboljenje jednogodišnjih mladunaca na perajima i višegodišnjih šaranskih riba na krljuštima i koži. Značajan momenat širenja telohaneloze predstavlja promet mladunaca između ribnjaka gde se ne vodi računa o postojanju ove bolesti. Drugi bitan faktor je hidrografska povezanost ribnjaka, gde se ispuštanjem i upuštanjem vode omogućava prenošenje uzročnika bolesti. S obzirom na malu udaljenost ribnjaka ribojede ptice takođe predstavljaju bitan vektor širenja bolesti. Pošto ne postoji ni jedno adekvatno terapeutsko sredstvo, kontrola telohaneloze i dalje se bazira na pridržavanju osnovnih sanitarno–profilaktičkih mera, kao što su isušivanje objekata, izmrzavanje, mehanička obrada tla i dezinfekcija krečom.

Ključne reči: Thelohanellus nikolskii, patološke promene, krljušt, peraje, Cyprinus carpio

Keywords: Thelohanellus nikolskii, pathological changes, scales, fin, Cyprinus carpio

INTRODUCTION

The myxosporidian *Thelohanellus nikolskii* was described for the first time by Achmerov (1955) from common carp living in river Amur. In Europe the parasite was first detected in Hungary more than 30 years ago (Jeney, 1979). In Serbia *Thelohanellus nikolskii* was present and reported since the beginning of the eighties (Ćirković et al., 1983). Wolf and Markiw (1984) were brought a turning point in developmental cycle of myxosporidia. They determined that an intermediate host is necessary for infection on *Myxobolus cerebralis* life cycle. The pathological changes and ultrastructure caused by *Thelohanellus nikolskii* present in the form of cysts on the fins of one year old carp fingerlings were studied by Molnar (1982), Desser et al. (1983) and Ćirković et al. (1997). Moshu and Molnar (1997) described for the first time *Thelohanellus* infection of the scales in the 2-4 years old European wild carp (*Cyprinus carpio carpio*). Ćirković et al. (2009) and Novakov (2013) have detected and described thelohanellosis on the scales and skin in common carp (Cyprinus carpio) from Serbia. The aim of this paper is to describe pathological changes which *Thelohanellus nikolskii* causes on the fins, scales and skin of pond cultured common carp.

MATERIALS AND METHODS

The investigation was carried out from 2008 to 2012 on 18 fish farms in Serbia (Bač, Svilojevo, Kolut, Sombor, Srpski Miletić, Ruski Krstur, Susek, Despotovo, Bečej, Žabalj, Lukino Selo, Mošorin, Sečanj, Banatski Dvor, Novi Itebej, Jazovo, Novi Kneževac, Kanjiža) and 4 fish farms in Bosnia and Hercegovina (Prijedor, Prnjavor, Bardača, Brod). Fish material included 1- to 4 yr-old pond-cultured common carp (*Cyprinus carpio*). Sampling at the fish farms was conducted between April and October with the intervals of 7-14 days. During each visit of pond, clinical examinations were performed on a hundred of fish, and for laboratory examination complete parasitological dissection was performed on 21 specimens of common carp. Light microscopy examinations of fresh smears from different organs were conducted during dissection by compression between 2 slides to look for myxosporean parasites. The location of cysts was recorded. Measuring of fresh spores and capsules length and width was performed by using the software program Cell B. Pathohistological examination was conducted by standard techniques: following fixation with Bouin's solution, the tissue samples were processed, sliced to 5-µm-thick sections, mounted and stained in haematoxylin and eosin. Photographs were taken in a Olympus BX51 microscope.

RESULTS AND DISCUSION

During the five-year investigation, round cysts reaching 3 mm in diameter (Fig. 1) were found on scales of 2 to 4 year-old pond-cultured common carp. The cysts were located at the outer margin of scales. The cysts sizes were related to different stages of infection. They were significantly smaller at the beginning of infection than in the final stage, when reaching a maximum size. Plasmodia on scales were present from the beginning of April until the end of May, while characteristic changes were not observed during the rest of the year. In 2008, the cysts were observed on scales in Susek, Žabalj, Jazovo and Bač fish farms, while in the period of 2009-2012 scale changes were present in all investigated farms. The prevalence of infection ranged from 2 to 75%, while its intensity was 2 - 206 cysts per fish.

Figure 1. Cysts present on scales of two year old common carp fingerling

Changes on scales were found in two-year-old fingerlings that were the most often infected, but also in three-year and four-year old common carp individuals. Cysts were not observed in one-year-old fry. The cysts were mainly present in carps, which were completely covered with scales, but also in mirror carps, where plasmodia were located in the dorsal row of scales. Also sporadic, cysts were detected on the skin of four-year-old spawn carp (Fig. 2)

Figure 2. Four year old spawn carp with cyst located on skin

Thelohanellosis on the fins was also present in all investigated fish farms. Cysts were noticed in the 60 days old carp fingerlings. The prevalence of infection ranged from 3 to 30 %, while its intensity was 2 -84 cysts per individual. Cysts were present in all fins (Fig. 3).

Figure 3. Cyst present on the common carp fins.

Plasmodia develop on scale's surface inside a calcified collagenic capsule (Fig. 4). Cartilaginous parts around the plasmodia break into pieces and calcified islets appear inside the connective tissue around the cyst (Fig. 4). The wall of the cysts around the plasmodium contains a thin layer of calcified collagen, as Moshu and Molnar (1997) called them elongated cyst-forming scleroblast cells. The central parts of plasmodia are filled with spores, while in the periphery vegetative developmental stages and pansporoblasts are present. Based on the dominance of individual cells within the plasmodium, it can be determined whether it is young or mature cysts.

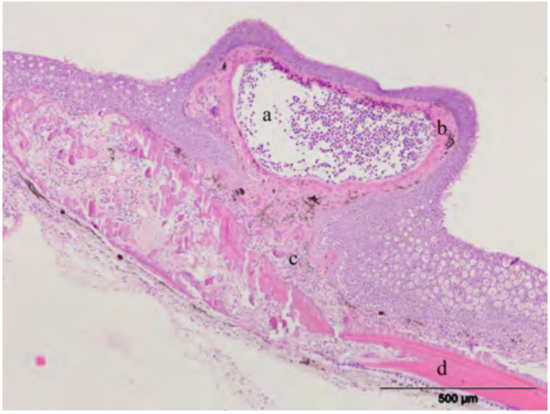

Figure 4. *Cyprinus carpio*. Cross section of scale with present plasmodium (a), cartilage of scale (b), calcified islets (c) and collagenic capsule (d). H&E

For measurements and morphological characteristics determination fresh spores were taken from plasmodia of scales and fins. Isolated spores were oval, with a large polar capsule in which turns of polar filaments can be detected. Measurement results of fresh spores and capsules length and width are shown in Table 1.

Table 1. Measurement values of spores isolated from scales and cysts.

Localization of spores	Spore lenght (µm)	Spore width (µm)	Capsule lenght (µm)	Capsule width (µm)
Scales	17,7 (13.5-20.3)	10.9 (10-12.1)	7.1 (6.1-8.7)	6.2 (5.8-6.6)
Fins	17.2 (13.4-20.6)	10.8 (9.2-13.3)	6.5 (5.8-7.6)	5.9 (5.2-6.8)

The shape and size of spores from the scales plasmodia correspond to *Thelohanellus nikolskii* spores from fins. Pathohistological structure of the cysts from scales of common carp that is presented here is almost identical with those described in paper by Moshu and Molnar (1997). Although *Thelohanellus nikolskii* cause changes on scales and fins, clinical manifestation, the age of fish and the period of occurrence are different. In one year old fingerlings location of plasmodia are fin rays, while in two years old and older carps cysts are located on the scales and sporadically on the skin. According to Moshu and Molnar (1997) in older fish –due to advanced calcification of fin rays-the cartilage is less suitable for plasmodium formation than the scales. Since infection was presented in all investigated fish farms, to its prevalence contributed the fact that the infection of the fins was also present in the investigated ponds (Novakov, 2013). Spreading of thelohanellosis was also made possibly by movement of fry between fishponds where this disease was not taken into account. The second important factor is hydrographical connection between fishponds, where water circulation enables transmission of infective agent. Taking into account small distance between fishponds, fish eating birds are also an important factor in disease spreading. Also, the prevalence and intensity of infection were higher in facilities where stocking densities were higher, and therefore immunity and conditions of fish were weaker.

CONCLUSIONS

Thelohanellosis manifests on fins in one-year-old carp fingerlings during July and August, on scales and sporadically on skin in two-year-old, three-year-old and older categories of pond-cultured common carp during April and May. During investigation, it has been concluded that infection was present in all investigated fishponds. Histopathological changes of plasmodia and spores from scales and fins are very similar. Losses from thelohanellosis on fins can be significant when disease results with drops of fins, while in the case of thelohanellosis on scales direct losses were not determined. Control of thelohanellosis is still based on compliance with basic sanitary-prophylactic measures such as drying of objects, freezing, mechanical cleaning and disinfection with lime.

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BACTERİAL DİSEASE SCREENİNG OF CULTURED HORSE MACKEREL (*TRACHURUS MEDİTERRANEUS*) İN SEA- CAGES İN SOUTHERN BLACK SEA REGİON OF TURKEY

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PRIKAZ BAKTERIJSKIH BOLESTI MEDITERANSKOG ŠNJURA (*TRACHURUS MEDİTERRANEUS*) GAJENOG U MORSKIM KAVEZIMA U JUŽNOM CRNOMORSKOM REGIONU TURSKE

Apstrakt

Cilj ovog rada je da prikaže bakterijske bolesti šnjura gajenog u morskom ribnjaku u Južnoj crnomorskoj regiji Turske. Bakterijska flora riba ispitivana je jednom mesečno a uzorci su prikupljani od šnjura gajenog u morskim kavezima od novembra 2011 do oktobra 2013. U toku istraživanja nije utvrđeno prisustvo parazita, dok je iz uzoraka iz gajenih riba izolovano nekoliko vrsta Gram-negativnih vrsta bakterija, uključujući *Aeromonas hydrophila*, *Chryseobacterium indologenes*, *Vibrio vulnificus*, *Bulkholderia cepacia*, *Photobacterium damselae damselae* i *Vibrio alginolyticus*. Pedeset procenata ili više bakterija bile su otporne na streptomicin, sulfametoksazol, gentamicin, cefalotin i ampicilin. Kao najefikasniji antibiotici pokazali su se florfenikol i hloramfenikol. Najčešće utvrđeni geni rezistencije bili su beta-laktamski (blaTEM-OT3-4) i tetraciklinski (tetB).

Ključne riječi: šnjur, bakterijske bolesti, geni rezistencije na antibiotike, morski kavez

Abstract

This study aimed to screen bacterial disease in cultured horse mackerel in the marine fish farm of the Southern Black Sea Region of Turkey. Fish bacteria were surveyed monthly and collected in November 2011 to October 2013 from cultured horse mackerel in the sea-cages. During the study, no parasite was found while several Gram negative bacterial species including *Aeromonas hydrophila, Chryseobacterium indologenes, Vibrio vulnificus, Bulkholderia cepacia, Photobacterium damselae damselae* and *Vibrio alginolyticus* were isolated from cultured fish. Fifty percent or more of the bacteria were resistant to streptomycin, sulfamethoxazole, gentamycin, cephalothin, and ampicillin. The most effective antibiotics were florefenicol and chloramphenicol. The most prevalent resistance genes were found to be beta-lactam (blaTEM-OT3-4) and Tetracycline (tetB).

Keywords: Horse mackerel, bacterial disease, antibiotic resistance gene, sea-cage

DISTRIBUTION OF SULFONAMIDE RESISTANCE GENES IN BACTERIA ISOLATED FROM RAINBOW TROUT IN TURKEY

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DISTRIBUCIJA GENA OTPORNOSTI NA SULFONAMIDE KOD BAKTERIJA IZOLOVANIH IZ KALIFORNIJSKE PASTRMKE U TURSKOJ

Apstrakt
Cilj ovog istraživanja bio je da se ispita otpornost bakterija na sulfonamide i učestalost pojave gena otpornosti na sulfonamide kod bakterija izolovanih iz kalifornijske pastrmke (*Oncorhynchus mykiss*) sa farmi u Rizi i Trabzonu, Turska. Izolovano je 13 bakterijskih vrsta i sve bakterije su pokazale otpornost na sulfametoksazol. Među genima otpornosti na sulfonamide najčešće je utvrđen *sul1* (24%), zatim *sul2* (20%) i *sul3* (12%). Geni višestruke antimikrobne otpornosti otkrivene su kod 3 od 33 (9%) bakterija. Dva *Aeromonas hydrophila* soja sadržavala su oba gena, *sul1* i *sul2*. Ovim istraživanjem utvrđeno je da su bakterije otporne na sulfonamide i njihovi geni rezistencije prisutni u različitim vrstama. Ovo istraživanje takođe ukazuje da je vodena sredina rezervoar *sul* gena, u kojoj ovi geni mogu biti prenošeni ne samo na bakterije koje žive u vodi, nego i na one koje su povezane sa bolestima ljudi.

Ključne reči: otpornost bakterija; patogeni riba, sulfonamidi, farme kalifornijske pastrmke

Abstract
The aim of this study was to investigate the antimicrobial resistance to sulfonamide and the prevalence of sulfonamide resistant genes of bacteria isolated from rainbow trout (*Oncorhynchusmykiss*) farms in Rize and Trabzon, Turkey. 13 bacterial species were isolated and all of the bacteria exhibited resistance to sulfamethoxazole. Among sulfonamide resistance genes, *sul1*(24%) was found in the highest frequency followed by *sul2* (20%) and,*sul3* (12%). Multiple antimicrobial resistance genes were detected in 3 of 33 (9%) bacteria. Two *Aeromonashydrophila* strains contained both *sul1* and *sul2*

genes.This study revealed that sulfonamide resistant bacteria and their resistance genes were distributed in different species. This research also suggests that the aquaticenvironment is a reservoir of *sul* genes, in which the genes may be transferred among not only aquatic bacteria but also human related bacteria.

Keywords: Antimicrobial resistance; Fish pathogen; Sulfonamide; Rainbow trout farms

HELLENIC MARICULTURE SAFETY AND HEALTH: INITIAL REPORT ON WORK RELATED INJURIES AND ILL HEALTH

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ZDRAVLJE I BEZBEDNOST ZAPOSLENIH U SEKTORU GAJENJA MORSKIH RIBA U GRČKOJ: INICIJALNI IZVESTAJ O POVREDAMA NA RADU I ZDRAVSTVENOM STANJU

Apstrakt

U ovom radu predstavljene su analize povreda na radu u industriji gajenja morskih riba. Ove analize služe kao osnova za dalje unapređenje zdravlja i bezbednosnih uslova rada. Ovaj inicijalni pristup navodi, izmedju ostalog, indikatore, kao što je učestalost nezgoda tokom sezone i kako one utiču na proizvodnju. LEONARDO DA VINCI program Evropske Unije je finansijski podržao ovaj projekat (Izvršilac za INTRANEMMA projekat, Ugovor br. 2010-1-GR1-LEO 05-03986). Akvakultura u istočnom delu Mediterana se jako brzo razvila u proteklih 20 godina, što je dovelo do povećanog broja ribnjaka, kao i do povećane proizvodnje koja je dostigla 100000 t (u 2001. godini). Takođe, očekuje se trostruki porast proizvonje u bliskoj budućnosti. Ova stopa rasta povećava broj povreda na radu u industriji gajenja. Grčka je najveći proizvođač morskih riba u Mediteranu gde ukupan broj radnika dostize skoro 0.2% ukupne radne snage u Grčkoj, od kojih je 20% zaposljeno u marinskom sektoru, naročito na farmama riba sa kaveznim sistemima. U ovoj studiji razmatran je ukupan broj radnika u oblasti akvakulture u toku jedne godine, iako je, između ostalog, broj radnika u marinskom sektoru sezonski promenljiv. Izveštaji o povredama na radu u ovom sektoru su već dati u godišnjim izveštajima koje je objavila Grčko Društvo za Ispitivanje Rada (SEPE). Analiza trenutne studije zasnovana je na podacima iz 2009-2011 datim u ESAW 2001 (Eurostat, Evropska statistika za nezgode na poslu). Do 2009. godine, bilo je teško dobiti rezultate analiza o nezgodama na radu u akvakulturi i ribarstvu, zato što su Grčke vlasti davale izveštaj o tim podacima zajedno sa podacima o preradi i pakovanju poljoprivrednih i ribljih proizvoda. Iako su nezgode kao i bolesti na radu najvažniji za opisivanje i ustanovljavanje uslova za zdravlje i bezbednost na radu, ne možemo dobiti pouzdane rezulate za ovaj period. Jedini način na koji možemo da stvorimo sliku o ovom sektoru za ovaj izveštaj je da se oslonimo na međunarodne publikacije. U ove nezgode i bolesti spadaju mišićno skeletni poremećaji, efekti UV radijacije, itd. Dalje analize radi dobijanja podataka o nezgodama na radu su već isplanirane. One ce uključiti više faktora koji utiču na uslove pod kojima se nesreće dešavaju. Na primer, slučajevi povreda na radu vezani za klizanje i spoticanje (39%), rukovanje (5%) i objekte koji su pali (16%) nisu sasvim precizni zbog nedostatka izvestaja. Ipak, podaci koje imamo ukazuju da su ove povrede učestalije u ovom sektoru nego u drugim sektorima u Grčkoj (na primer 18%, 23% and 10%, za svaku od ovih nesreca u 2011. godini). Zbog ovoga možemo zaključiti da diskrepance u izveštajima o nesrećama na radu u sektoru akvakulture mogu da dovedu do pogrešnih tumačenja uslova za zdravlje i bezbednost.

Ključne reči: akvakultura, opasnost, bezbednost, zdravlje, Grčka
Keywords: aquaculture, hazards, safety, health, Greece

INTRODUCTION

Aquaculture activities in the Eastern Mediterranean have been rapidly expanded during the last 20 years leading to an increase of both the number of fish farms and their production reaching 100,000 t in 2011, whereas a three-fold increase is expected in the future years. These facts also increase the numbers of work related incidences within the workers of the farming industries.

MATERIALS AND METHODS

The analysis of the present study based on the data reported (SEPE annual report 2011) during 2009-2011 through the ESAW 2001 (Eurostat, European Statistics on Accidents at Work). In contrast, prior to 2009, analyses on work accidents data in aquaculture and fisheries are rather difficult to take place, because Hellenic (Greek) authorities reported the corresponding data together with the processing and packaging of agricultural and fishery products.

RESULTS

Injuries

During 2009-11 within the Aquaculture sector occurred:

- 3 fatalities representing less than 1.5% of the total numbers of reported fatalities to SEPE, which they account to 25% of the Agriculture, Forestry and Fisheries sector (SEPE annual report 2011)
- no contractor worker or third party employee was injured
- the percentage of the serious injuries and/or no injuries per year was fluctuated without any trend (this percentage was also estimated to be higher than the percentage of the total number of injuries reported per each year in all sectors)
- and a decreasing trend was shown for the reported work related fatal injuries which was also depicted by an increasing trend for the total number of all injuries in the farming sector

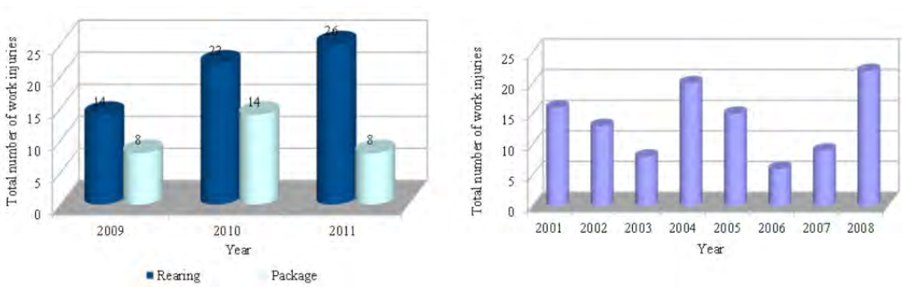

Figure 1. Annual work related injuries in Aquaculture during 2009-2011 (ESAW 2001) and 2001-2008 (National Statistics)

Fatalities

During 2009-2011 3 fatal injuries occurred (Fig. 2) accounting for 10 per 100,000 workers. This number is stable throughout the three-year period.

Serious injuries

Positive trends were shown for the total number of accidents within the Aquaculture sector and for the rate of the total number of accidents per 100,000 workers during 2009-2011. These seemed to be comparable to the number of work related injuries in the Agriculture, Forestry, Fisheries and Aquaculture sector during 2001-2008.

Over 3 days absence

The decrease in the percentage of the number of injuries at work that leaded to over three day of absence in 2011 was compared with those in 2010, but it is rather optimistic to consider that this is attributed to the improvement in Safety and Health standards and policies implemented by the sector companies. More data is needed in order to better analyse the influencing factors.

Seasonal and monthly distribution

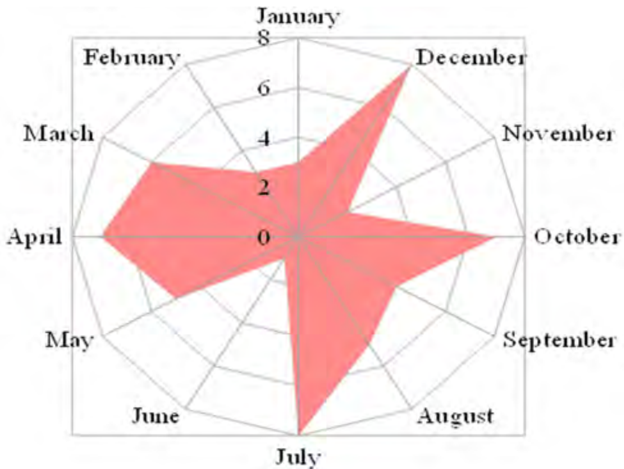

Figure 2. Cumulative monthly distribution of the total number of accidents in mariculture during 2009-2011.

Accidents follow a monthly and seasonal pattern (Fig. 6) that seemed to coincide with the sector's production cycle (e.g. stocking, raising, fishing, etc)

DISCUSSION

Work related accident analyses in Mariculture Industry are presented herein, serving as a reference base for further improvement of health and safety. This initial approach proposes, among others, indicators, such as seasonal distribution of accidents that they also correlated with the production line.

Greece is the largest Mariculture fish producer in the Mediterranean.

The total number of workers in the Greek farming industries reaches almost the 0.2% of the total workforce in Greece, of which 20% is employed within the Mariculture sector and in particular on cage farms.

In the present study the total annual number of Aquaculture employees was taken into account due to the fact that, amongst other factors, the number of aquaculture workers is seasonally fluctuated.

Work related hazards within the sector have been already reported through the annual reports of the Hellenic Labour Inspection Body (SEPE).

CONCLUSIONS - INDUSTRY PROFILE

Hellenic (Greek) Aquaculture sector shows lower rate (1 in 400 workers) in work related accidents when compared to other European Countries (Eurostat).

Throughout Europe, the Agriculture, Forestry, and Fisheries are the most Hazardous sectors showing high rates for work related injuries (Eurostat). Hellenic Aquaculture as a whole represents less than 2 per thousand of the total work force, 3 per thousand of all serious injuries and 16 per thousand of fatal injuries during 2009-2011.

Future analyses on work related accident data has been already planned to account for incorporating more factors influencing the conditions under which accidents occurred. In this context, work injuries related with factors such as slips and trips (39%), handling (5%) and fallen objects (16%) includes high uncertainty due to the absence of reports, even though there are higher when compared with the remaining sectors in Greece (i.e. 18%, 23% and 10%, respectively for 2011).

Thus, discrepancies in the reports of the work related accidents in the Aquaculture sector might lead to false interpretations about the health and safety conditions.

ACKNOWLEDGEMENTS

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DRESSING PERCENTAGE OF 3-YEAR OLD CARP FROM CAGE PRODUCTION SYSTEM

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RANDMAN TROGODIŠNJEG ŠARANA IZ KAVEZNOG SISTEMA UZGOJA

Apstrakt

Uzgoj šarana (*Cyprinus carpio L.)* u kaveznom sistemu predstavlja posebnu vrstu intenzivne proizvodnje. Odlikuje se malim početnim ulaganjima u izgradnju kaveznog ribnjaka, ne zahteva značajno angažovanje radne snage i obezbeđuje veliku proizvodnju po jedinici zapremine.

Izbor lokacije je od velikog značaja jer utiče na ekonomsku održivost, proizvodnju i mortalitet šarana. U odnosu na druge uzgojne sisteme, utvrđeno je da u kaveznom sistemu morbiditet i mortalitet mogu značajno varirati i u slučaju odstupanja vrednosti parametara kvaliteta vode od optimalnih, gubici mogu nastupiti brzo i biti dramatični.

Nedostatak kaveznog sistema gajenja ogleda se u olakšanoj transmisiji bolesti i zagađenju vodenog ekosistema u kome je kavezni sistem postavljen, što se može sprečiti uvođenjem zaštitnih sistema. Uz poštovanje načela dobre proizvođačke prakse, održavanjem dobrog kvaliteta vode i optimalno izbalansiranu hranu primerenu starosnoj kategoriji uzgajanog šarana, očekivan mortalitet je 1-5%.

Zahtevi savremenog tržišta su sve više usmereni ka obrađenoj ribi, posebno filetima. Uklanjanjem kože i odstranjivanjem unutrašnjih organa i intramuskularnih kostiju, fileti šarana i ostalih ciprinida koje se gaje u polikulturi sa šaranom, postaju visokovredni obroci, koji se lako i brzo spremaju. Randman riba je ekonomski parametar čija je vrednost značajna pri svim tehnološkim operacijama vezanim za preradu ribe. Iskazuje se kao odnos primarno obrađenog trupa u odnosu na masu trupa žive ribe.

Odnos jestivog i nejestivog dela trupa ribe može značajno da varira u zavisnosti od vrste, mase i veličine ribe i sezone ulova). Od njega direktno zavisi ekonomičnost proizvodnje i neopravdano je zanemaren kao parametar u istraživanjima.

Razlika u randmanu između različitih linija i njihovih hibrida javlja se zbog različitog oblika, dužine glave, debljine i širine tela. Randman riba značajno je povoljniji od randmana kod ostalih vrsta životinja. Delovi koji se odbacuju mogu sadržati sve ili samo neke delove glave, krljušti, kože, creva, gonada i peraja.

Cilj istraživanja bio je određivanje randmana kod trogodišnjeg šarana poreklom iz kaveznog sistema uzgoja. Uzorkovanje je obavljeno iz dva kavezna sistema na Tikveškom jezeru (Makedonija) (41°20′51″N 21°57′58″E) i iz jednog na Bilećkom jezeru (Bosna i Hercegovina) (42°49′31″N 18°26′17″E). Ishrana riba vršena je kompletnim krmnim smešama različitih proizvođača. Iz svakog kaveznog sistema uzorkovano je po 8 jedinki muškog i 8 jedinki ženskog pola (ukupno po 24 jedinke svakog pola).

Morfometrijske osobine riba utvrđene su sa ciljem da se ispita uticaj pola na randman trogodišnjeg šarana gajenog u kaveznom sistemu. Nakon što su izlovljeni, šarani su držani na ledu. Krljušt je skinuta ručno nazubljenim nožem. Glava je odsečena cirkularnim rezom ispred pojasa pektoralnog peraja tako da je peraje ostalo na trupu. Peraja su odsečena na početku perajnih žbica. Egzenteracija organa zajedno sa gonadama obavljena je ručno. Dobijeni su obrađen trup, koji podrazumeva trup ribe bez krljušti, peraja, unutrašnjih organa i glave. Posle odstranjivanja krljušti, unutrašnji oragni, gonade, glava, peraja i obrađen trup su izmereni. Rezultati su obrađeni t-testom kako bi se utvrdio uticaj pola na randman trogodišnjeg šarana gajenog u kaveznom sistemu proizvodnje.

U odnosu na pol (ženke naspram mužjaka šarana) pažnja je usmerena na parametre koji su važni sa aspekta gajenja i što povoljnijeg randmana.

U našem istraživanju randman je određen kao odnos mase cele ribe i mase trupa bez glave, krljušti, peraja i unutrašnjih organa. Rezultati dobijeni u našem istraživanju pokazuju veću težinu ženskih u odnosu na muške jedinke (FW), ali nemaju statističku značajnost.

Ukupna dužina (TL), standardna dužina (SL) i dužina trupa (CL) ženki u odnosu na mužjake imaju statistički značajnu razliku ($p < 0,01$), dok razlike u telesnoj masi ženki i mužjaka nisu statistički značajne. Rezultati dobijeni merenjem težine trupa (CW) i težine unutrašnjih organa (VW) ženki u odnosu na iste parametre kod mužjaka imaju statistički značajnu razliku ($p < 0,01$). Vrednosti dobijene merenjem težine glave (HW), težine gonada (GW) mužjaka, kao i vrednosti gonadosomatskog indeksa (GSI) u odnosu na vrednosti istih parametara kod ženki imaju statistički značaj ($p < 0,01$). Razlike u dužini glave (HL), visini tela (BH), masi peraja (FW), masi jedinki (FW) i obrađenih fileta (FilletW) mužjaka i ženki, nemaju statistički značaj. Vrednosti dobijene izračunavanjem relativne težine fileta i randmana uzorkovanih trogodišnjih jedinki šarana muškog i ženskog pola nemaju statistički značajnu razliku.

Ključne reči: Šaran, kavezni sistem, pol, randman
Keywords: carp, female, male, dressing percentage

INTRODUCTION

Carp (*Cyprinus carpio* L.) is domesticated in every continent except Antarctica, owing to its ability in adapting to different values of water quality parameters, including tolerance to very low dissolved oxygen concentrations. Considering the benefits that are reflected by the extensive reproductive abilities, breeding and prime selection potentials, and high resistance to various diseases, it is not surprising that carp represents the dominant species in all counties with prerequisites for extensive, semi-intensive and intensive freshwater fish production. The carp growth in different production systems depends on many factors, such as varietal, density, welfare and fish feed with proper nutrients along with geographic location.

Cage production system for carp is a special type of intensive production. It is characterized by lower initial investments in cage construction and minimal labor engagement, ensuring high production yield per volume capacity of water contained in the constructed units. Modern cages are constructed from metal and synthetic polymers (Mihailovic et al., 2007). Cage has to be robust enough to endure associated hazards such as winds and waves, uncomplicated servicing and maintenance. Location choice is critical as it affects the production and mortality of the produce (Carp) directly impacting the profitability of the production unit.

Below listed are three-selection criterion considered for selection of sites for cage culture (Bogut et al., 2007).

1. Physical and chemical parameters including temperature, dissolved oxygen concentration, water flow, pollution and algal blooms.
2. Factors relating to the choice of location taking into account weather conditions, shelter, substrate, water currents and the degree of blur. Additionally the shape and size of the cage and the depth to which they are installed.
3. Profitability of the farm, and includes legal aspects, accessibility, facilities, security, economic and social conditions.

Superior production practices, quality of water and optimally balanced diet appropriate for the cultured carp category, expected mortality rate is 1-5%. Compared to other breeding systems, it was found that the cage system morbidity and mortality could vary significantly in the case of deviation in the values of water quality parameters from the optimum, the losses can occur quickly and be dramatic. (Orajić et al., 2007)

Shortcoming of cage production system is reflected through allowing of disease transmission and aquatic ecosystems contamination where the cage system is set up, this can be prevented by protective systems introduction.

Modern market demands are increasingly directed towards processed fish, especially fillets (Hough, 1993; Vallod, 1995). By removing the skin, internal organs and intramuscular bones, fillets of carp and other cyprinids grown in polyculture along with the carp, become high value meals that are easily and quickly prepared (Lin et al., 1989).

Dressing percentage of fish is an economic parameter with significant value attached to it, in all technological operations related to fish processing. It is expressed as the ratio of the primary processed carcass (dressed) relative to carcass weight of live fish.

The ratio of edible and non-edible part of the fish carcass can vary significantly depending on the species, weight, size of the fish and season catch (Baltic and Teodorović, 1997). Since the dressing percentage directly influences the economy (profitability) of production, it is unjustly neglected as the parameter in scientific research/studies (Ljubojević et al, 2012).

The difference in yield of different lines and their hybrids occur due to different body shape, head length, width and fat of the body. Dressing percentage of fish is significantly higher comparatively than the yield of other animal species (Ćirkovic et al., 2002). Parts that are rejected may include all or some parts of the head, scales, skin, intestine, gonads and fins.

According to Dunhamu et al. (1983) fish carcass primary handling involves removal of the head, scales and internal organs, while Lovell (1981) reference states that primary treatment involves the removal of skin, dorsal and pectoral fins, head and abdominal organs cavity, with the tail present. Naumovski (1991) and Tumbas (1976) define fish carcass primary handling as a process which include removal of the tail fin.

In this study, yield was determined as the ratio of the fish weight and the carcass without the head, scales, fins and internal organs.

MATERIALS AND METHODS

The specimens of 3-year old carp from cage production system were sampled in the Tikveš lake in Macedonia (41°20′51″N 21°57′58″E) (two groups) and from the Bilećko Lake in Bosnia and Herzegovina (42°49′31″N 18°26′17″E) (one group). Fish were fed with a completed feed mixture from different manufacturers. From every cage system, 8 male and 8 female samples are taken (24 individuals of each sex). For the statistical analysis of the results, software package Microsoft Office Excel 2010 and Data Analysis ToolPack, were used. Statistical t-test was used for mean values determination. Morphometric characteristics determination, (described by Baltić and Teodorović, 1997) of 3-year old carp specimens from cage production system is conducted in order to evaluate the dressing percentage in relation to sex. After being harvested, the carp were kept on ice. The scales are removed with a serrated knife. The head is cut off with a circular incision in front of the pectoral fins (Gela and Linhart, 2000). The internal organs along with the gonads were removed manually and a processed carcass (without scales, fins, internal organs and head) is obtained. After dissection, the internal organs, gonads, head, fins and carcass were measured. The obtained results were used to determine the relative percentage ratio in relation to the initially measured fish body weight.

RESULTS

The biometric parameters of 3-year old male and female carp specimen are presented in the Table 1: (TL-total length, SL-standard length, CL-body length, HL-head length and BH-body height), and weight parameters (FW-fish weight, CW-carcass weight, FW- fillet weight, HW- head weight, FW-fin weight, GW-gonad weight, VW- viscera weight). After dissection acquired data were used to calculate the percentage of fillets, head, fins, viscera and waste per fish weight, as well as important indices such as dressing percentage (DP), gonadsomatic index (GSI) and relative fillet weight. The obtained results were used to determine the relative percentage ratio of each parameter in relation to the initially measured fish body weight. For the statistical analysis of the results, software package Microsoft Office Excel 2010 and Data Analysis ToolPack, were used. Statistical t-test was used for mean values determination.

Table 1. The values of biometric parameters in carp (*Cyprinus carpio*) in relation to sex (females, males) Values in the table are the mean values ± SD (n = 24), values in the same row with different letters in superscript differ significantly at p < 0,01.

Parameter	Unit	Female n=24 mean±S.D.	Male n=24 Mean±S.D.
		Biometric parameters	
Total length (TL)	mm	457,5±13,72[a]	438±8,18[b]
Standard length (SL)	mm	393,88±16,76[a]	369,87 ±21,83[b]
Carcass length (CL)	mm	322,5±13,72[a]	308±8,18[b]
Head length (HL)	mm	87,92±2,61	87,7±2,8
Body height (BH)	mm	126±4,8	125,54±6,51
		Weight parameters	
Fish weight (FW)	g	1550,23±55,59	1495,47±109,09
Carcass weight (CW)	g	1010,12±19,57[a]	947±5±72,68[b]
Fillet weight (filletw)	g	750,42±33,81	727,5±51,29
Head weight (HW)	g	235,96±30,98[b]	263,89±27,35[a]
Fins weight (FW)	g	40,05±4,59	38,88±5,4
Viscera weight (VW)	g	267,18±57,13[a]	222,14±24,64[b]
Gonad weight (GW)	g	56,57±3,21[b]	59,61±2,89[a]
		Calculated parameters	
GSI		3,66±0,26[b]	4,01±0,4[a]
Dressing percentage	%	65,57±2,25	63,57±5,49
Fillet weight relative	%	48,41±1,29	48,6±2,02

The results obtained in our study showed slightly higher body height (BH) and head length (HL) values in the females compared to the males. Weight parameters obtained (fish weight, fin weight and fillet weight) had no statistically significant difference ($p > 0.05$) Total length (TL), standard length (SL), and carcass length (CL) of females compared to males had a statistically significant difference ($p < 0.01$). In female carp, significantly higher carcass weight (CW) and viscera weight (VW) were found ($p < 0.01$). Significant differences ($p < 0.01$) were found in head weight (HW) and gonad weight (GW) as well in gonadosomatic index value (GSI) in the favor of male carps.

DISCUSSION

Carp (*Cyprinus carpio* L.) belongs to the species that have expressed a high degree of sexual dimorphism. The results of research conducted by Bay et al. (2006) showed almost the same growth rate of male and female 3-year carp from four different sub-groups examined. The faster growth of female carp was confirmed by research of Cherfasa et al. (1996). Analysis of the relation between sexual maturity and growth rate of the Chinese and European carp conducted by Hulata et al. (1995) showed that the beginning of sexual maturity has a negative impact on the physical growth of many species of fish. The production of sex hormones during sexual maturation, especially their accumulation, inhibits growth as a result of the reaction between sex hormones and growth hormones. Due to the different time of reaching sexual maturity amongst different sexes, the results of this study are consistent with the conclusion that males attribute of reaching sexual maturity earlier than females, utilize more nutrients for development of the gonads, while sexually immature female specimen(s) during the maturation period go through intensive physical growth.

CONCLUSION

The results of this study demonstrate greater weighing females compared to males (FW), but of no statistical significance.

The results gonad weight (GW) of males and values of the gonad somatic index (GSI) of males had a statistically significant difference compared to the values of the same parameters in females ($p < 0.01$). Total length (TL), standard length (SL) and body length (CL) of females compared to males had a statistically significant difference ($p < 0.01$), while the difference in weight of females and males were statistically of little significance. Results obtained by measurements of carcass weight (CW) and the weight of the internal organs (VW) females compared to males with the same parameters demonstrated statistically significant difference ($p < 0.01$). The difference in values obtained by measuring the weight of the head (HW), gonad weight (GW) of males, and gonad somatic index GSI values compared to the values of the same parameters in females demonstrated variance of statistical significance ($p < 0.01$).

Analytical Differences in the head length (HL), body height (BH), body weight of individuals (FW) and fin weight (FW) of males and females demonstrated variance of no statistical significance.

Values obtained by calculating the relative weights and fillet yield of three-year sampled carp male and female demonstrated no variance of statistical difference. Production of 3-year old carp in cage production system is economically unprofitable compared to the production of a 2-year old carp, due to utilization of more nutrients and energy received in feed during sexual maturation for development of the gonads.

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LACTIC ACID BACTERIA: EFFECT ON THE QUALITY AND SAFETY OF FISHERY PRODUCTS

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MLEČNO KISLEINSKE BAKTERIJE – UTICAJ NA KVALITET I BEZBEDNOST PROIZVODA OD RIBE

Apstrakt
Prisustvo mlečno kiselinskih bakterija (MKB) u mesu ribe dugo vremena bilo je zanemarivano s obzirom na njihov nizak broj u mesu ribe, što je uslovljeno specifičnostima ribljeg mesa. Međutim, promena navika u ishrani ljudi u poslednjoj dekadi prošlog veka, kao i sve veći zahtevi potrošača po pitanju kvaliteta hrane koju konzumiraju doveli su do toga da se industrija hrane sve više razvija u pravcu proširenja asortimana proizvoda. To se naročito odnosi na povećanu proizvodnju "ready-to-eat" hrane, kao što su blago konzervisani proizvodi od ribe (BKPR). Za ove proizvode, karakteristično je da se u procesu proizvodnje dodaju određene količine sastojaka, kao što su šećer i so, što dovodi do promena u svojstvenim karakteristikama mesa ribe (npr. snižava se a_w vrednost mesa). Ovakve promene u mesu ribedovode do inhibicije rasta mikroflore koja je odgovorna za nastanak kvara mesa ribe i proizvoda od ribe, istovremeno stimulišući rast mikroflore, kao što su mlečno kiselinske bakterije. Takođe, i najčešći načini pakovanja ovih proizvoda, a to su vakuumiranje i pakovanje u modifikovanoj atmosferi, favorizuju rast mlečno kiselinskih bakterija, a inhibiraju rast mikroflore karakteristične za meso ribe. Samim tim, poslednjih godina, raste i interesovanje za ispitivanje značaja prisustva mlečno kiselinskih bakterija i njihovog efekta na kvalitet i bezbednost ovih proizvoda od ribe, pakovane na različite načine. Proces u kojem se u hranu dodaju veće količine odabranih mikroorganizamau cilju inhibicije nepoželjnih mikroorganizama naziva se biokonzervacija. MKB su upravo mikroorganizmi od izbora za ovu tehnologiju, s obzirom na širok spektar i veliki broj inhibitornih komponenti koje nastaju kao posledica njihovog metabolizma. Sa aspekta mikrobiološke bezbednosti blago konzervisanih proizvoda od ribe, najveći rizik predstavlja prisustvo Listeria monocytogenes. Za pojedine sojeve mlečno kiselinskih bakterija utvrđeno je da veoma uspešno inhibišu L. monocytogenes u ovim

proizvodima. Međutim, za iste je utvrđeno i da nemaju nikakvog uticaja na odlaganje pojave prvih znakova kvara, tj da nemaju uticaja na održivost ovih proizvoda. Takođe, primena mlečno kisleinskih bakterija u blago konzervisanim proizvodima od ribe ograničena je iz razloga što BKPR nisu fermentisani proizvodi, te bi primena MKB-a kao protektiva mogla da dovede do promene organoleptičkih svojstava i nutritive vrednosti krajnjeg proizvoda koji bi, na taj način, bio neprihvatljiv za potrošača. Podaci iz literature upućuju na to da je jednostavnije kontrolisati rast patogenih mikroorgaizma primenom pojedinih sojeva mlečno kiselinskih bakterija, tj. osigurati bezbednost proizvoda, nego poboljšati kvalitet BKPR. Stoga su brojna istraživanja usmerena kako bi se pronašli oni sojevi koji bi imali zaštitno delovanje u smislu mikrobiološke bezbednosti, zatim pozitivan uticaj na kvalitet, odnosno održivost, a da pri tome ne bi menjali organoleptičke osobine i nutritivna svojstva, od čega bi nesumnjive koristi imali kako potrošači, tako i proizvođači. Poslednjih godina sprovode se istraživanja u cilju primene mlečno kisleinskih bakterija kao probiotika za živu ribu, koja su dala pozitivne i ohrabrujuće rezultate. Nauka o primeni probiotika u akvakulturi je na samom početku i za cilj ima proizvodnju hrane za ribe koja ima pozitivan efekat na zdravlje ribe.

Ključne reči: kvalitet, bezbednost, blago konzervisani proizvodi od ribe
Ključne reči: quality, safety, light preserved fish products

Lactic acid bacteria

Lactic acid bacteria are widespread in nature and can be found in many foods (dairy products, meat, fruit, vegetables, etc.), as well as in the intestinal tract of humans and animals. People have used the lactic acid bacteria for thousands of years in natural fermentation of milk, meat, vegetables and fruit. Acidification process as a result of the creation of lactic acid is one of the most desirable effects of lactic acid bacteria growth, with the inhibition of other microorganisms, including pathogenic microorganisms to humans. The presence of lactic acid bacteria in fish meat has been neglected for a long time due to their low number caused by the specific traits of the fish meat. However, changes in eating habits in the last decade of the last century, as well as the existing and growing consumer demand, have led to developing of the food industry in order to expand the range of products, particularly relating to increased production of ready-to-eat foods, such as light preserved fish products (LPFP).

For the production process of this type of product it is characteristic to add certain amounts of ingredients, such as sugar and salt, which leads to changes in the intrinsic properties of fish (e.g., lower a_w value of meat). These changes in the fish meat lead to inhibition of growth of microflora responsible for the occurrence of the spoilage of fish and fish products, while at the same time stimulate the growth of microflora, such as lactic acid bacteria (Leroi, 2010). Therefore, with the increase in production of fish products, especially light preserved products such as cold smoked fish, there is growing interest in testing for the presence of lactic acid bacteria and for their influence on the quality and safety of these fish products.

Lactic acid bacteria in fish meat and meat products

Microflora of live fish is a reflection of the microflora of the environment from which the fish is caught. Muscles of live fish are sterile. The bacteria are found on the skin, gills and alimentary tract (Baross and Liston, 1970), whose number and type depend on numerous factors such as the type of fish, water temperature and water salinity, the amount of soluble oxygen, the degree of pollution, diet, stress, etc. A typical microflora of the skin and gills of fish is generally represented with aerobes (Simidu et al., 1969). Research shows that the microflora isolated from the gastro intestinal tract of fish mostly (50 to 90%) consists of gram-negative bacteria (*Vibrio, Enterobacteriaceae, Acinetobactrer*) (Huber et al. (2004). Though atypical, it is generally accepted that lactic acid bacteria are a normal microflora of the intestinal tract of fish (Yang, 2007).

Fish meat has such properties which stimulate the growth of microorganisms. Despite the low content of carbohydrates (0.2-1.5%), fish meat is abundant in non-protein nitrogen compounds of low molecular weight, which are rapidly metabolized by bacteria. The high *post-mortem* pH of meat (around 6), and low content of carbohydrates favour the growth of gram-negative psychotropic bacteria present in the meat of fish, which, in the first few days, can reach a large number compared to the LAB (Leroi, 2010). Careless handling of raw material during primary processing and improper and unhygienic production can cause the contamination of fish with microorganisms that can be found in fish meat products (Gudbjornsdottir et al., 2010).

Slightly preserved fish products are characterized by the fact that these products are not or only slightly heat treated, with the salt content of less than 6% in the aqueous phase, with a_w value below 0.96, and with the pH greater than 5. Different types of marinated fish, fish in brine, cold smoked fish, etc. are classified into this group of products. In the production of these products the fresh fish is most commonly used. Technological process of production of LPFP features a few extra steps, which increase the possibility of cross contamination, and the characteristics of the production process are such that, on one side, they have impact on the reduction of the number of microorganisms, but not enough to completely eliminate them (Leroi et al., 1998 ; Gonzales-Rodriguez et al., 2002). These products are perishable; they are kept in a cool regime and they are usually packed in vacuum or in a mixture of gases, in order to prolong viability. Initial microflora depends on the hygiene in the production facility, but most often dominated by gram-negative bacteria that are typical for fresh fish. Adding of NaCl at a concentration of 5,5 - 6.5% in the aqueous phaselowers a_w value to 0.96. These values of the water activity in the product have an inhibitory effect on gram-negative bacteria, but the growth of microorganisms, such as LAB, is undisturbed, and the research results show that at the end of the storage periodLAB become the dominant microflora in these fish products (Truelstrup Hansen et al., 1995). Also, smoking as a stage in the production of cold smoked fish affects the final number of LAB in these products, because with the amount of smoke of 10 mg/kg the number LAB count increases. (Leroi and Joffraud, 2000). Packaging of fish and fish products in a vacuum or gas mixturesleads to major changes in the growth, composition and number of certain microorganisms that lead to spoilage. In the process of packaging of food in a vacuum microaerophilic environment is created, and carbon dioxide accumulates partly, thus inhibiting the growth of aerobic gram-negative bacteria and providing a better shelf life of meat (Soccol and Oetterer, 2003). In such circumstances, microflora is developing and lactic acid bacteria dominate during the entire period of storage of vacuum-packed products, up to the occurence of the product spoilage (Leroi et al., 1998; Truelstrup Hansen and Huss, 1998). However, the role of LAB, in the development of the spoilage of LPFP, where they constitute the dominant flora, is not fully understood.

The predominant microflora of cold smoked fish products packed in a mixture of gases is the one that is resistant to CO_2 (Siverstvik et al., 2002). Basically, the gram-negative bacteria are much more susceptible to the action of CO_2, and are also the most inhibited microorganisms in products packed in a mixture of gases (Jay et al., 2005). Gram-positive bacteria, such as lactic acid bacteria are not susceptible/sensitive to the action of carbon dioxide, and in fish products packed in a mixture of gases they become the dominant bacteria, in addition to being the species that has less potential to cause spoilage (McMillin, 2008). In the results of our tests, it was established that the dominant microflora of cold smoked trout packed in a mixture of gases formed during storage were LAB (approximately 7 log CFU / g after six weeks of storage), while in the samples of cold smoked vacuum packed trout somewhat lower lactobacilli count was recorded. Statistically significantly higher count of lactobacilli in cold smoked trout fillets packed in a mixture of gases can be explained by the fact that carbon dioxide exerted inhibiting effect on certain groups of microorganisms, especially on *enterobacteriaceae*, which contributed to the undisturbed growth of lactobacilli and to their higher count compared to vacuum packaged trout fillets (Kilibarda Nataša, 2010).

Bio-preservation and antagonism

The interaction between different bacterial species is a well-known phenomenon. Competition for substrate and antagonism are thought to be important in the selection of microflora in a particular ecological niche (Gram, 1993). Several kinds of interactions may occur in the food at the same time, leading to the final characteristics of the finished product. Lactic acid bacteria are mentioned as one of those responsible for the growth inhibition of pathogenic microorganisms. Acidification as a result of the creation of lactic acid is one of the best known mechanisms which lead to the inhibition of the undesirable microorganisms occurring as the result of LAB activity. It is known that lactic acid in its undissociated form can induce changes in the electrochemical proton gradient in the membranes of sensitive bacterial cells (Bower and Hiatela, 2010).

In the literature are also cited other mechanisms, such as the creation of inhibitory molecules, competition for substrate which could lead to the inhibition of pathogens in food. The process during which selected micro-organisms in large numbers are added into foods, in order to inhibit undesirable microorganisms, is called biopreservation (Leroi, 2010). LABs are microorganisms of choice for this technology, given the wide range and large number of inhibitory components that occur as a result of their metabolism (organic acids, hydrogen peroxide, diacetyl and bacteriocins). However, their application in fish products has not yet been practiced, partly because LPFP are not fermented products, and the application of LAB as protectionist could lead to changes in the organoleptic properties and nutritive value. Therefore, numerous studies have focused in recent years to identify those strains that could have a protective effect and in the same time do not modify the organoleptic and nutritional properties.

Safety and LAB

The greatest microbiological risk when it comes to LPFP is the *Clostridium botulinum* type E and *Listeriamonocytogenes*. The presence of *C. botulinum* in these products can be adequately controlled by the combination of parameters of production and storage (3.5% of salt in aqueous phase and storage temperature below 5^0C) (Heinitz and Johnson, 1998). However, *L. monocytogenes* can grow at low temperatures (0 C, at a pH of 4.5 and low a_w value). Though it can be found in the raw material, the presence of L. monocytogenes in the final product often is a result of cross-contamination during the production process, and, therefore, it is almost impossible in the production of LPFP to consistently have the final product without the presence *L. monocytogenes (Rorvick et al., 1995)*. The characteristics of the technological production process are not sufficient to eliminate the presence of *L. monocytogenes* in the final product (Ribeiro Neunlist et al., 2005). For this reason, the use of protective microorganisms would have very important role in preventing *L. monocytogenes* in the final products of this type. In the literature, the LAB of genus *Carnobacterium* are often mentioned, which do not have the ability to create acid (aciduric bacteria non) and thus cause spoilage, and create bacteriocins which lead to inhibition of pathogenic microorganisms, primarily *L.monocytogenes*.

Sustainability/shelf life and LAB

Another important approach is that the use of protective cultures increases shelf life, or delays signs of spoilage. In the research, it was shown that bacteria of the genus *Carnobacterium* which have been shown to have an inhibitory action on the *L.monocytogenes* have an inhibitory effect on other microflora, and therefore do not delay the appearance of signs of spoilage in LPFP (Brillet et al., 2005). These data suggest that it is easier to control the growth of pathogenic cultures using the protective microorganismsthan to improve the quality of LPFP. The reason should be sought in the fact that the occurrence of spoilage of food is a result of a complex ecosystem, which consists of a variety of bacteria whose number and types vary from product to product. Although the interest in the application of bioprotectives aimed to improve the quality and safety of LPFPhas been present in the past twenty years, it is of great importance to perform further research in determination of those strains of LAB which would not change the organoleptic properties and nutritional value of the product, and would have positive effect on the quality and safety.

LAB – as probiotics

Probiotics are live microorganisms, mainly LAB, which when entered into the body in large quantities have a positive effect on human health. The use of probiotics is usually associated with dairy products. LPFP, although they contain a significant number of LAB, have never been considered as probiotics for human use, since they are not consumed in large amounts, as is the case with dairy products. In recent years research was carried out focused on the application of LAB as probiotics for live fish, which yielded positive results. Study on the application of probiotics in aquaculture is at the very beginning, and its aim is to produce fish feed that has a positive effect on the health of fish (Azad and Ai-Marzouk, 2008).

CONCLUSION

The modern consumer is looking for high-quality food which has maintained the sensory characteristics and the nutritional value of the raw materials from which it is produced, which has retained a natural appearance, taste and aroma, and is also safe for his/her health. LPFP packed in vacuum or mixture of gases largely meet these requirements, which is why their production is increasing from year to year. In these products, the environmental conditions are such as to favour the growth of LAB, which explains the great interest of the scientific community in the last decade, in order to investigate the influence of these microorganisms on the quality and safety of LPFP. It is necessary to examine in future research if LAB in products derives from fresh fish or their presence is a result of contamination during the manufacturing process, in order to better understand the way of contamination and therefore to control the contamination. The role of LAB in fish products is complex and depends on the type of fish, the characteristics of the production process, storage conditions, and on the bacterial species and strains and interaction between different bacterial species. The most important effect of their presence in LPFP is the impact on their safety, given the inhibitory effects on pathogenic microorganisms. Also, many studies are conducted to determine the effect of LAB on product quality in terms of extending its sustainability/shelf life, and therebythe presence and activity of LAB does not influence the organoleptic properties of the product. Similarly, research on the use of LAB as probiotics for live fish gave encouraging results, which will enable faster development of the market for LAB originating from fish.

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A NON-INVASIVE METHOD FOR TRACING ORGANIC FISH

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NEINVAZIVNA METODA ZA UTVRĐIVANJE ORGANSKI GAJENE RIBE

Apstrakt

U poslednjih nekoliko godina, prisutne su drugačije tendencije u akvakulturi, koje pre svega imaju za cilj plasiranje novih proizvoda, od kojih je jedan i riba gajena u organskoj akvakulturi. Razlikovanje ribe gajene u organskoj akvakulturi od one gajene na konvencionalni način je teško, ali se razlika može napraviti preko njenog izgleda. U ovom eksperimentu brancin je hranjen konvencionalnom i organskom hranom i u toku gajenja, pravljene su fotografije primeraka. Nakon kalibracije boje, određene su merne tačke na svakoj fotografiji, a nakon toga su geometrijskim i morfometrijskim metodama dobijene RGB matrice. Tako dobijena matrica (195x135,225) je prvobitno analizirana korišćenjem 50-50 MANOVA metode, a nakon toga su urađeni diskriminantna analiza i na kraju dendrogram. Svi uzorci su klasifikovani korišćenjem tri diskriminantna modela. Tako je dendrogram sa ukupno 9 različitih klasa pokazao da se ribe koje su gajene u organskoj akvakulturi slične ribama uzorkovanim iz prirodnih populacija. Rezultati su pokazali i da dve grupe riba, hranjenih različitim komercijalnim hranama u ovom eksperimentu, jedne u organskoj, a druge u konvencionalnoj akvakulturi mogu biti prepoznate po boji njihovog tela. Šta više, što duže vremena ribe provedu u jednom od ova dva načina gajenja, to se boja njihovog tela više razlikuje. Deo tela koji pokazuje najveće promene u boji jeste glava, koja je značajno svetlije boje u grupi riba gajenih u organskoj akvakulturi. Tako je dokazano da analiza boje tela može biti iskorišćena za razlikovanje riba koje su gajene u drugačijim uslovima korišćenjem različitih protokola i načina gajenja. Međutim, ovaj zaključak se može primeniti samo na ove, konkretne podatke, ne može biti generalizovan i ne može se primeniti na sve ribe gajene u organskoj akvakulturi.

Ključne reči: Dicentrarchus labrax, brancin, akvakultura, organska proizvodnja, morfologija
Keywords: Dicentrarchus labrax, sea bass, aquaculture, organic farming, morphology

INTRODUCTION

In the last 20 years, fish aquaculture companies have tried to find ways of diversification at every level of the production pipeline, from the start - by introducing alternative species or developing niche and "minor" productions (Bianchini and Palmegiano, 1994) up to the consumer end, e.g. offering fillets, fish patties, ready-to-cook preparations, etc. (Bianchini et al., 2010). One form of product diversification could also be implemented during the raising phase by employing methods of organic farming (Cataudella et al., 2001).

Organic fish farming is still a small fraction of the aquaculture total output (less than 1%), but nevertheless it is worth more than 50 million euros annually (IFOAM, 2010).

Organic certification and labeling (IFOAM, 2010) goes together; in fact, without long and expensive analyses, it would otherwise be impossible to discriminate a fish grown organically from another raised with conventional practices. On the other hand, appearance is used throughout all production stages, for sorting by size (Costa *et al*., 2013a) or for defects (Bianchini *et al*., 1994), but also as a primary mean for judging the quality of individual units of product, and might be useful in tracing the fish origins.

This study aims to test whether the color of European sea bass (*Dicentrarchus labrax* L.) reared using an organic protocol is different from that resulting from a conventional approach.

MATERIALS AND METHODS

The experiment was carried out in an experimental facility (Costa *et al*., 1999) on the Lake of Sabaudia (LT, Italy), a coastal lagoon 100 km SE of Rome.

Besides different densities, sea bass in the organic protocol were fed with a special diet (EcoLife Pearl 864, 4.5 mm), while fish in the conventional protocol were supplied commercial feed (Ytelse M 664, 4.5 mm) of the same manufacturer (BioMar sas, Nersac, France). Basic ingredients, proximate composition and main energy characteristics are reported in Table1.

Table 1. Ingredients, proximate composition and energy characteristics of organic (EcoLife Pearl 864) and conventional (Ytelse M 664) sea bass feeds (from manufacturer's notice).

		organic	**conv.**			**organic**	**conv.**
proteins	%	46.0	44.0	total energy	MJ	20.0	22.5
lipids	%	15.0	20.0	digestible energy	MJ	17.0	19.7
carbohydrates	%	17.0	22.0	energy from proteins	%	58.0	51.0
ash	%	11.6	6.5	energy from fats	%	32.0	41.0
water	%	10.4	7.5	proteins/energy	g/MJ	24.3	21.7
EcoLife: fish meal, organic peas, organic soya cake, fish oil, minerals, vitamins							
Ytelse: corn gluten, peas, soya cake, fish meal, rapeseed cake, fish oil, rapeseed oil, peanut cake, minerals, vitamins							

The experiment started in March 2011, when a sample of unweaned fish was measured and photographed, and the differential feeding began (organic (O) *vs*. conventional (C)). In order to measure the fish color pattern, the sea bass were checked 4 times (from T_1 to T_4), and a total of 195 images of individual fish were validated. The number of images for each rearing treatment and time is reported in Table 2, together with the mean standard length (SL, cm). The increase in weight of the sea bass fed organic or conventional protocols were very similar, as well as their respective condition factors.

Table 2. Sampling dates, number of valid images, mean standard length (SL) and mean condition factor (CF) of sea bass reared under organic and conventional protocol.

	date	**n. organic (O)** **mean SL±StD (cm)**	**n. conventional (C)** **mean SL±StD (cm)**
T_0	25/03/2011	19 21.6±1.8	17 21.1±1.1
T_1	06/05/2011	14 22.6±1.3	13 22.9±1.3
T_2	17/06/2011	26 22.7±1.3	24 22.5±0.9
T_3	27/07/2011	21 23.9±1.2	16 24.1±1.4
T_4	14/09/2011	22 24.6±1.2	23 24.6±1.5

Color calibration and validation was carried out using a 32 color-patch ColorChecker (the GretagMacbeth ColorChecker 24 color-patches together with other 8 patches which better overcompensate the RGB color space; Costa *et al*., 2013b) as reference standard. Matlab was used to perform the image calibration based on TPS-3D calibration (Menesatti *et al*., 2012). For each patch of the ColorChecker the spectral reflectance values from 400 to 700 nm was extracted using a portable integrated-sphere D50/2 spectrocolorimeter. Spectral reflectance values were converted in sRGB (Furusawa *et al*., 2010) using Matlab OptProp.

After color calibration, a total number of 16 landmarks were digitized (Fig. 1) on each fish image, in order to allow the comparison of the entire body fish area. The first 12 landmarks were used to contour the region of interest (ROI) to be compared among samples. Following the landmarks configuration, the image RGB matrices were warped through a geometric morphometry procedure (Costa *et al*., 2009; 2013a; Menesatti *et al*., 2010). This way, each pixel inside each ROI could be compared with the one in the same position of the other samples. For each individual, the 3 RGB values of the 45,075 pixels composing the ROI were decomposed in a single row (135,225 values).

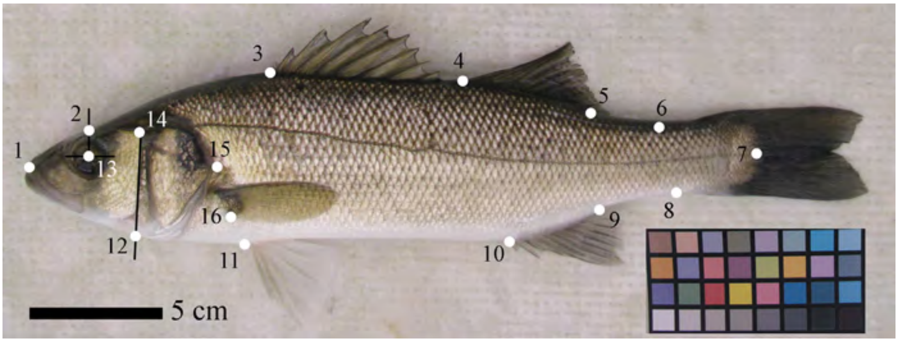

Figure 1. Landmarks (white dots) on sea bass, and the 32 color-patch ColourChecker.

The whole matrix (195 x 135,225 elements) representing the RGB color values inside the ROI of each fish was first analyzed with a 50-50 MANOVA procedure (Langsrud, 2002). A partial least square regression (PLS) was applied on the whole matrix in order to observe the relationship between color and size (SL) or condition index (CI = 100 * weight * length^{-3}). A partial least square discriminant analysis (PLSDA) was used to build a model discriminating between: *i*. T_0 *vs*. T_4 (O *vs*. C) (3 classes), *ii*. T_4 O *vs*. T_4 C (2 classes), *iii*. all the combinations between rearing treatments (O *vs*. C) and sampling times (from T_0 to T_4) (9 classes, considering T_0 O and T_0 C as the same class).

A dataset partitioning (75% to the model building, 25% for the testing procedure) was carried out by: 1) a partitioning algorithm that takes into account the variability in both X- and Y-spaces called sample set partitioning based on joint x-y distances (SPXY; Harrop Galvão *et al*., 2005) for PLS approach; 2) an extraction function based on distances and on the Kennard-Stone algorithm for PLSDA approach (Kennard & Stone, 1969).

The degree of estimation accuracy in quantitative prediction (PLS) must be inferred by the direct comparison between the measured and the estimated response variable, by calculating different parameters of the prediction efficiency: coefficient of correlation (r) between measured and predicted values, RMSE (root mean square error); SEP (standard error of prediction). The PLSDA analysis provides the percentage of correct classification of each class. This analysis expresses also the statistical estimates indicating the modeling efficiency arising from sensitivity and specificity parameters (Costa *et al*., 2008). RPD is the ratio between the standard deviation of the measured data and the RMSE, and was calculated on both the training and the validation set. The load of each pixel (X-block), in first latent vector (LV), was extracted (Costa *et al*., 2009) in order to determine the pixels contribution to the PLSDA classifications.

For each ROI the mean RGB values for each rearing treatments (O *vs*. C) and sampling times (from T_0 to T_4) (9 classes, considering T_0 O and T_0 C as the same class) were calculated. A dendrogram (single linkage) based on the mean Euclidean distances, between each rearing treatment (O *vs*. C) and sampling times (from T_0 to T_4) (9 classes, considering T_0 O and T_0 C as the same class), based on the 3 RGB values decomposed in a single row (135,225 values), was finally built.

RESULTS

The results of the 50-50 MANOVA are reported in Table 3. It is possible to observe that condition index, sampling time and their interaction all appear significant, using 32, 16 and 33 PCs, respectively.

Table 3. Results of the whole MANOVA. df: degrees of freedom. exVarSS: (Sum of SS for each response) / (Sum of total SS for each response). nPC: number of principal components used. nBuf: number of PCs used as buffer components. exVarPC: variance explained by nPC components. exVarBuf: variance explained by (nPC+nBuf) components. p-values: 50-50 MANOVA p-values.

source	df	exVarSS	nPC	nBuf	exVarPC	exVarBuf	p-value
rearing treatment (RT)	1	0.05526	32	75	0.504	0.810	>0.00001
sampling time (ST)	4	0.22829	16	83	0.501	0.818	>0.00001
RT*ST	4	0.05918	33	74	0.501	0.803	>0.00001
error	185	0.65967					

The summary of the PLS regression is reported in Table 4. Following the classification proposed by Viscarra-Rossel *et al*. (2007), the RPD_{RMSE} values of the testing procedure of both SL and condition index range from 1.0< RPD <1.4, indicating a poor prediction model, and, even if the RPD_{RMSE} values of the testing procedure are high, the models show a scarce relationship between color and SL or condition index. These results were confirmed also by the low r values of the testing procedure (0.69 and 0.76, respectively).

Table 4. Results of partial least squares (PLS) regression to relate color and SL or condition index. r= correlation coefficient; SEP= standard error of prediction; RMSE= root mean squares error; RPD_{RMSE}= ratio of percentage deviation for both model and testing sets.

	model		**testing**	
	SL	**condition index**	**SL**	**condition index**
n. latent vectors	8	4	8	4
r	0.9993	0.9617	0.6862	0.7569
SEP	0.059	0.059	1.591	0.199
RMSE	0.059	0.059	1.601	0.225
RPD_{RMSE}	27.175	3.651	1.296	1.304

The principal results of the PLSDA models are presented in Table 5 for: *i.* T_0 and T_4 (O *vs*. C) (T_0 *vs*. T_4), *ii.* T_4 O and T_4 C (T_4), *iii.* all the combinations between rearing treatments (O *vs*. C) and sampling times (from T_0 to T_4) (Total). The 3 models correctly classify all samples within their own classes, both in model and in testing datasets (only one individual T_3 O in the Total testing dataset was misclassified as T_4 C). Scores of the Total testing samples on first 2 LVs are reported in Figure 2; note how the organic fishes (filled symbols) were more similar to the T_0 (i.e., wild; crosses) samples than to the conventional ones (empty symbols).

Table 5. Results of partial least squares discriminant analysis (PLSDA) for: T_0 vs. T_4 (O *vs.* C) (T_0 *vs.* T_4), T_4 O *vs.* T_4 C (T_4) and all the combinations between rearing treatments (O vs. C) and sampling times (from T_0 to T_4) (Total).

	T_0 *vs.* T_4	T_4	total
N	81	45	195
n. of latent vectors	3	3	7
n. of classes	3	2	9
Sensitivity	1	1	1
Specificity	1	1	0.98
% probability of random assignment of an individual	33.3	50.0	11.1
% correct classification in the model building (75%)	100	100	100
% correct classification in the testing procedure (25%)	100	100	97.8

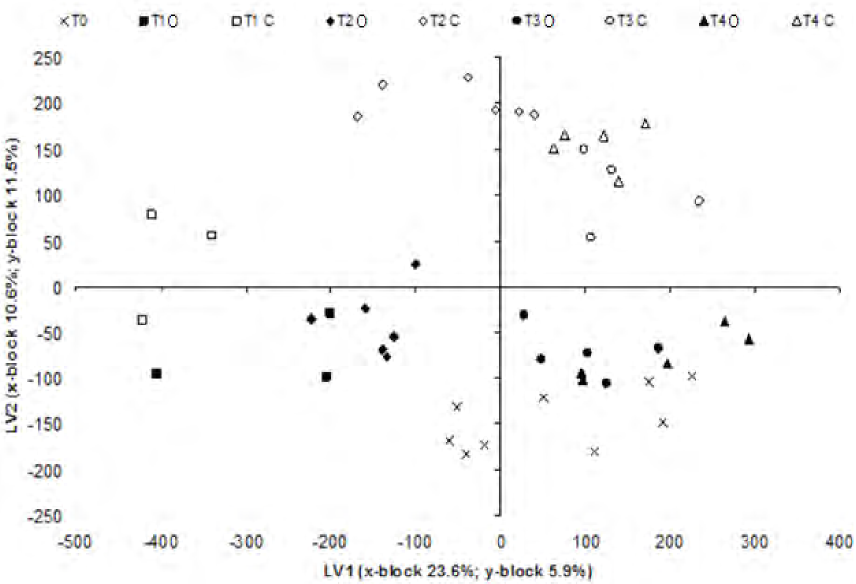

Figure 2. PLSDA Total testing dataset: scores of the samples belonging to each group on the first 2 latent vectors.

In Fig. 3, a dendrogram (single linkage) of the mean Euclidean distances, between each rearing treatment (O *vs.* C) and sampling time (from T_0 to T_4) (9 classes, considering T_0 O and T_0 C as the same class), based on the 3 RGB values decomposed in a single row (135,225 values) together with the relative mean RGB values within each ROI, is reported. It is possible to observe that the organic samples at T_2, T_3 and T_4 cluster together with T_0 (wild), while both samples at T_1 were much more distant; note also how the head region is darker in the reconstructed representations of conventional fish (especially at T_2, T_3 and T_4).

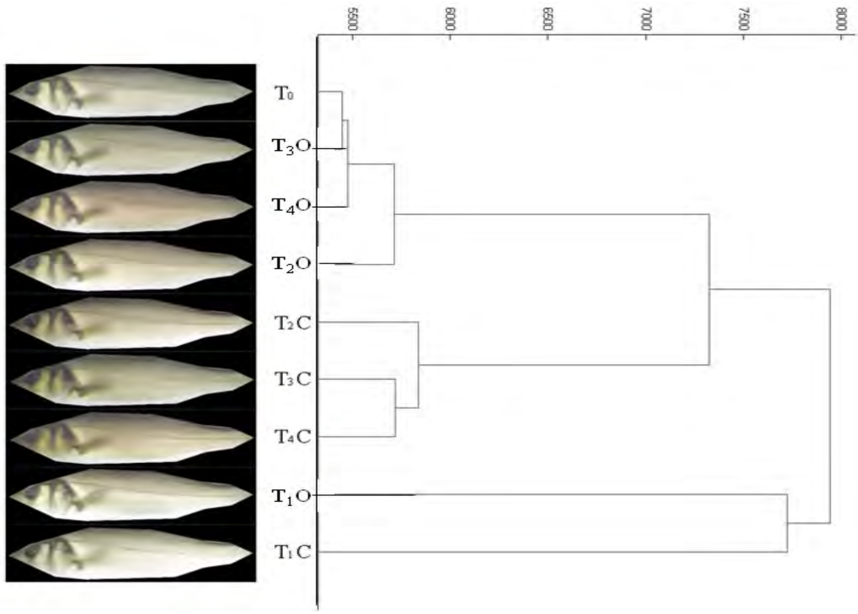

Figure 3. Dendrogram (single linkage) of the mean Euclidean distances between each rearing treatment (O *vs*. C) and sampling time (from T_0 to T_4), based on the RGB values decomposed in a single row, together with relative mean RGB values within each ROI.

DISCUSSION AND CONCLUSIONS

The preceding outcomes show that the two batches of fishes raised in different environmental conditions and fed different artificial diets, in the specific case following the organic vs. the conventional protocols, can be set apart also through their morphological characteristics, using their color appearance: moreover, the longer the time spent under the different regimens, the stronger the evidence of this outcome.

The body part that displays the greatest difference between the two groups is the head, which is of lighter color in fishes raised organically. Besides that, wild fishes are lighter than those arising from conventional aquaculture, at least in the present situation; nevertheless, data are not sufficient to generalize the similarity of appearance of natural and organic individuals.

It should be noted that the first samples under treatment (T_1), while being already distinct between themselves, are even more different from the following ones (T_2 to T_4), probably because the young fishes were still "weaning" (adapting to the artificial feeds) and thus presenting sub-optimal body conditions.

The present results are preliminary and apply only to the current data - which are limited in number, time and space - and therefore cannot be generalized to whole organic vs. conventional aquaculture. They may suggest a morphological difference between organic and conventional fishes, but in fact they prove only that the two specific and peculiar farming conditions under study generated measurable differences. Those consequences are promising, but further experiments are required, longer in time, spread on many farms in different locations, with more batches of various origins and/or hatcheries, employing greater sample numerosity, and maybe other species. In fact, a similar approach may allow: to verify the coherence and stability of the measurements; to identify whether any specific parameter (e.g., origin, breeding density, feed, water quality, season, etc.) has dominant influence in determining the body color; to transfer the results obtained on the sea bass to other aquaculture species; to extend the methodology to the control of other quality "certifications" (e.g., besides the "organic aquaculture" label, the "protected geographical indication" IGP, the "controlled denomination of origin" DOP, the "sustainable agriculture" PAS, and so on).

In conclusion, a non-invasive colorimetric analysis, carried out with geometric morphology studies, can be used to discriminate sea basses arising from different, complex, raising protocols, and in this specific case to certify the fish as organically grown.

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THE SURVEY OF SOME BACTERIAL CONTAMINATION IN FROZEN SEMI-COOKED READY TO EAT SEA FOOD PRODUCTS IN ALBORZ PROVINCE- IRAN

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ISPITIVANJE NEKIH BAKTERIJSKIH KONTAMINACIJA SMRZNUTIH GOTOVIH POLUKUVANIH MORSKIH PREHRAMBENIH PROIZVODA U PROVINCIJI ALBORZ U IRANU

Apstrakt

Socijalne i ekonomske promene i način života u Iranu uzrokovali su i promene u ishrani. Tako su potrošači sve više skloni konzumiranju svih vrsta gotove hrane (engl. ready–to-eat, RTE), kao što su morski proizvodi. Kontaminacija ovih hraniva psihrofilnim i patogenim bakterijama je opasna i smatra se da ozbiljno ugrožava zdravlje ljudi. Šesdeset devet smrznutih polukuvanih RTE morskih prehrambenih proizvoda trinaest različitih proizvođača (po tri uzorka od svakog tipa proizvoda) kupljeni su između januara i marta 2012. iz supermarket i lanca hipermarketa u provinciji Alborz, a zatim prebačeni do laboratorije za higijenu hrane Islamskog Univerziteta Azad u Karaju. Izvršena je identifikacija i izolacija *L. monocytogenes,* kao i utvrđivanje broja *Staphylococcus aureus* i psihrofilnih aerobnih bakterija. Srednja vrednost ± SD (Log_{10} CFU/g), minimalni i maksimalni broj aerobnih psihrofilnih bakterija i *Staphylococcus aureus* u svim smrznutim polukuvanim RTE morskim proizvodima iznosili su, redom: 3,79±1,66; 3,59±0,23; 0/0,6/11 i 0/0,6/86 (Log_{10} CFU/g). Takođe, 26,1% uzoraka bili su kontaminirani sa *L. monocytogenes*. Izgleda da su RTE morski prehrambeni proizvodi bili izloženi patogenim bakterijama i uzročnicima kvara hrane. Oni predstavljaju pretnju za ljudsko zdravlje. Prema tome, čišćenje, sanitacija opreme i osoblja, odgovarajuća temperatura za vreme i posle prerade i transporta, kao i odgovarajuće skladištenje može da smanji kontaminaciju i brojnost mikroorganizama koji su uzrok kvara namirnica.

Ključne reči: zamrznuti kuvani gotovi morski prehrambeni proizvodi, *Staphylococcous aureus, Listeria monocytogenes,* aerobne psihrofilne bakterije, Iran

Keywords: Frozen cooked ready to eat sea food products, *Staphylococcous aureus, Listeria monocytogenes, Aerobic psychrophilic bacteria,* Iran

INTRODUCTION

The increasing growth of world population has changed the production pattern and technology in food industry. RTE food products have been considered more by customers, because they are prepared and consumed very quickly. RTE Sea foods have an important role in human health due to high protein levels, unsaturated fatty acids, easy digestion and general health benefits. However, improper manipulation of sea foods can cause cross-contamination during and after processing. Also, abuse time-temperature during processing, machine transportation and markets freezers may be an important factor for growth of bacterial spoilage and pathogens such as *psychrophilic bacteria,* and *Staphylococcus aureus* and *Listeria monocytogenes*. *L. monocytogenes* is a pathogenic bacteriawith widespread presence in nature, affecting a wide range of animals and humans (9, 13). In the vast majority of human cases, listeriosis is the result of consumption of contaminated food, with a high fatality rate. *S. aureu*s is a Gram-positive microorganism and salt-tolerant. It is estimated that 50% of the human population are long-term carriers of *S. aureus*, which can survive as part of the normal skin flora, and nasal passages (5). So, the handling of sea products during the manufacturing process involves a risk of contamination and causing food borne human intoxication (4). In research of Vitas et al. psychrophilic growth occurs 7 to 10 days of incubation with maximum bacterial number of 10^6cfu/g. Improper usage of time-temperature during production and storage at refrigeration or freezing is the most important factor in the growth of psychrophilic bacteria (10). So, we surveyed the contamination of frozen semi-cooked RTE sea food products in Iran with *L.monocytogenes, S.aureus and psychrophilic bacteria*.

MATERIALS AND METHODS

Sample collection: 69 frozen semi-cooked sea food samples including fried shrimp, shrimp burger, fish finger, baked trout fish, fish batter, fish strudel, roast skate fish, fish sausage, pasta fish, ball fish, fried fish fillets, Pot roast vegetables fish, fish burger, minced potatoes fish and smoked salmon, from thirteen different factories in Alborz province were purchased randomly between January-March 2012. All samples were transported to the laboratory of food hygiene,beside cool box and kept in -18°C.

Bacterial analysis: The modified Canadian version of the U.S. FDA Listeria isolation protocol was used. Briefly, 25 g/225 ml was homogenized in LEB containing KSCN and nalidixic acid (30°C/24-48h) and loopful from LEB was then streaked onto Listeria selective agar containing nalidixic acid and PALCAM LSA with supplement. Suspected colonies were confirmed by Gram staining, motility test (hanging drop), catalase and ß-hemolysis tests and sugar fermentation tests for rhamnose, xylose and mannitol (8). For enumeration of *psychrophilic aerobic bacteria* were used surface method with 10 fold serial dilution in King agar media (1-4°c/$_{1\text{-}7\ days}$). For isolation of *S. aureus* including enrichment of a 1-g sample in 10ml cooked meat medium (37°c/$_{24h}$) streaking a loopful of the enrichment culture on Baird–Parker agar (37°c/$_{24h}$) with using surface plate technique and subsequent confirmatory coagulase test of jet-black colonies (8).

For statistical analysis: The SPSS 16 for Windows software was used, and $p<0.001$ was considered statistically significant**.**

RESULTS

Mean±SD (Log_{10}cfu/g) of *Aerobic psychrophilic bacteria* and *S. aureus* in sixty-nine frozen semi-cooked RTE sea food samples were 3.79±1.66 and 3.59±0.23 respectively. 75% of counted *psychrophilic bacteria* and 70% of counted *Staphylococcus aureus* were >2/9 $10^3\times$ and > $10^3\times$8/1Log_{10}*CFU/g*. Min and max of them were (0/0.6/11) and (0/0.6/86) Log_{10}*CFU/g*, respectively. Also, in nine samples *L. monocytogenes identified* (fig. 1,2,3)

DISCUSSION AND CONCLUSIONS

In Iran, consumption of RTE foods is increasing due to the economic, social and cultural changes. RTE foods include convenient, ready, instant and fast foods. Health quality of these kinds of food due to the way of production, processing, transportation; storage and improper use of time-temperature have been constantly facing the risk of contamination with pathogenic and spoiling microorganisms. *L. monocytogenes* be able to survive and even multiply in numerous food and may be lead to outbreaks of listeriosis. So, the pathogen has become of much concern to the food processing industry, public health professionals and regulators. According to Basti et al. (2006) some types of salt smoked fish may be considered as risk of *L. monocytogenes* and *S. aureus* infection and intoxication for Iranian consumers (1). Also in different researches, it has reported *L. monocytogenes* contamination in RTE salmon and RTE seafood(3.31.28)%, respectively (2,5,7). In our research, it was 27%. It seems that raw materials, environment, the factory design and sanitation procedures during and post by cross-contamination are to be source of *L. monocytogenes* contamination for RTE semi-cooked sea food products(7,10).

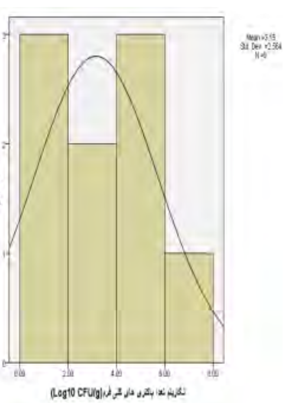

Figure 1. *Aerobic psychrophilic bacteria* contamination in sea food samples (Log_{10}cfu/g)

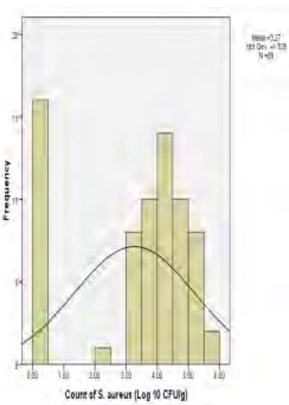

Figure 2. *Staphylococcus aureus* contamination in sea food samples (Log_{10}cfu/g)

Figure 3. The *Listeria monocytogenes* contamination in sea food samples

High *S. aureus* counts (10^4-10^5g-1) occurred in cold-smoked fish proceesing. According to FDA, (2004) up to 10^4 *S. aureus* cells.g-1 can be tolerated in RTE seafoods (3). In this survey 70% of counted *S. aureus* were >10^3×8/1Log_{10}*CFU/g*. The high presence of it, as a result of improper handling and hygiene might lead to the contamination of food and this eventually affects the health of the consumers (4,8). Abuse usage of time-temperature during of production and storage in refrigerated and freezer cooked samples were the most important factor in the growth of spoilage bacteria (9). In Iran, consumption of these products may pose risk of food borne infections and intoxication. So Standards of sanitation, handling and time/temperature of holding them by application of the correct principles of GMP and GHP with HACCP and ISO 22000 will be most effective in reduction of these food-borne pathogens and spoilage.

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THE MARKETING CHANNELS OF FISH CAUGHT IN LARGE SERBIAN RIVERS

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MARKETINŠKI KANALI RIBE ULOVLJENE NA OTVORENIM VODAMA U SRBIJI

Apstrakt
Ribolov na otvorenim vodama u Srbiji obuhvata privredni, rekreativni i sanacioni ribolov. Zakonski je definisano da se prometom mogu baviti proizvođači, privredna društva i preduzetnici registrovani za promet ribe, kao i privredna društva i preduzetnici koji obavljaju privredni ribolov. Istraživanje je bazirano na prikupljanju podataka o prometu, anketiranjem prodajnih objekata (ribarnice i restorani u Beogradu) i anketiranjem ribara koji ribare na Dunavu. Cilj rada je da istraži marketinške kanale prodaje ribe ulovljene na otvorenim vodama u Srbiji, obrađivanjem podataka prikupljenih anketiranjem različitih nivoa prodaje. Aktivnosti vezane za ribolov su u nadležnosti 4 ministarstva što stvara ambijent koji je za ribare netransparentan. Organizovan otkup ribe ne postoji, najveći deo ulova ide tokovima sivog tržišta. Većina prometa ribe ulovljene na Dunavu prolazi kroz kratke marketinške kanale, zbog čega ribari ostvaruju malu zaradu.

Ključne reči: *privredni ribolov, plasman ribe, otkup, izvoz i uvoz ribe*
Keywords: commercial fishing, marketing of fish, exports and imports of fish

INTRODUCTION

Fishing in Serbia includes: commercial, recreational and cropping fishing (to prevent the development or reproduction of non-native species). The Law on the Protection and

Sustainable Use of Fish Resources (''Off. Gazette of RS", 36/2009), which regulates fishing activities, does not proceed to regulate trade of the catch, except for the section that defines who can participate in fish trade. Trade may be conducted by producers, companies and entrepreneurs registered for fish trade, as well as companies and entrepreneurs engaged in commercial fishing. There is no official purchase of the fish catch, so most of the catch is traded through the grey market. There are only individuals who have managed to resolve the secure sale of their catch, as well as fish processing and marketing of products.

The current organization of the entire fisheries sector is divided between four different Ministries in Serbia. The Ministry of Natural Resources, Mining and Spatial Planning are responsible for planning and organization of commercial and recreational fishing to comply with sustainable use, as well as supervision and inspection of fish stock usage. The Ministry of Energy, Development and the Environment covers the domain of natural resources protection. The Ministry of Agriculture, Forestry and Water Management regulate aquaculture, and define who may engage in aquaculture and trade, as well as fish traffic. This Ministry also covers the veterinary control of fish and fish products. The Ministry of Finance and Economy deals with the responsibilities that follow from the following definition: A fisherman must be an entrepreneur.

MATERIALS AND METHODS

The fish trade was analyzed by combining data collected from relevant institutions: the Ministry of Agriculture, Forestry and Water Management, the Belgrade Chamber of Commerce, Chamber of Commerce of Serbia, and the Statistical Office of the Republic of Serbia and surveys of 35 fish markets (26 free market fish shops, 1 reseller, 7 fish departments within supermarkets, 1 commercial fisherman's shop), 9 restaurants in Belgrade and 122 commercial fishermen on the Danube. Two types of questionnaires were created: one on the sale of fish, and another for the survey of commercial fishermen. The surveys were conducted between March 2010 and September 2011. Analyses were done in SPSS and they are partly descriptive.

RESULTS

Fishmongers

The demand for freshwater fish in Serbia usually is associated with the Christian Orthodox fasting periods. In 70% of the fish markets included in this study, fish sales were highest in December and January, which coincides with the period of fasting. When asked whether there are required standards for fish to be marketable, the majority of fish retailers (65.7%) stated that they have required standards, which usually include fish health. For 14.3% of the retailers there were no specific standards required, and 20% of the fish retailers did not respond the question.

Most fish markets have a mixed assortment of freshwater (domestic and imported) and marine fish. Most (63.1%) of the freshwater fish come from fish farms, while 23.7% have redeemed Danube fish from fishermen. Some interviewees (13.2%) did not answer what is origin of fish they sell.

Best-selling domestic freshwater fish species are common carp and trout, wells, bighead carp, with pike-perch, Prussian carp, sterlet, and grass carp that follow. According to the responses of employees in the fish markets, firstly price (45.7%), followed by species (40%), and size of fish (5.7%), determine costumers' choice of fish purchase. The price affects the fish consumption. For example, the average retail price of carp during the survey was 253 RSD/kg, but the price in the fasting period was 160 RSD/kg. Average price of fish vary at different levels of sales, as shown in Figure 1 (vertical chain of sell). Differences in prices of fish through a vertical chain vary, starting from 3.6%, 25%, 30%, to as much as 6 times larger prices at the end of chain in the restaurants.

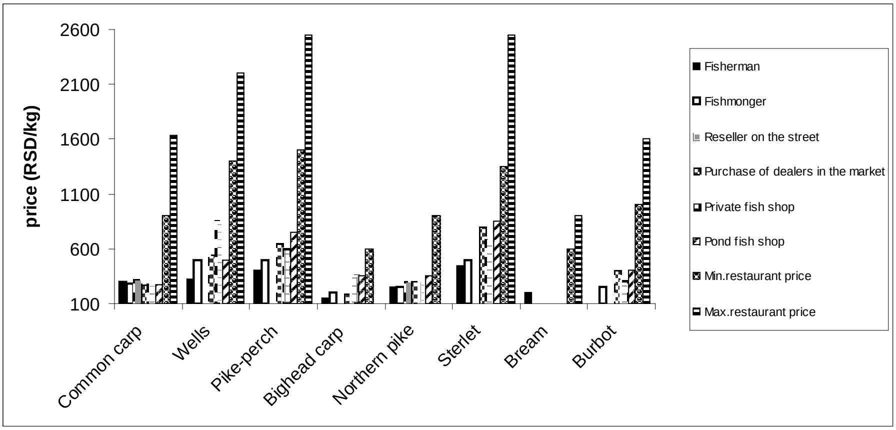

Figure 1. Average price of fish at different levels of sales (vertical chain).

Prices of fish are slightly different among markets in Belgrade. According to the fish sellers and owners, the difference in price and the offer of fish are most related to the economic structure of the population in certain parts of the city.

The amount of imported fish is increasing. Striped catfish (*Pangasius sp.*) is on third place among the most wanted species in fish markets, just after the carp and the trout. With low cost and simple way of preparing, it has become the most competitive fish on the market.

Restaurants

When it comes to restaurants, fish sales are highest during the summer months. In restaurants, the fish that are most in demand are wells and pike-perch, and then comes trout. Certain restaurants were previously exclusively oriented towards offering freshwater fish, but now their menus offer meats and marine fish as well. Of the 9 restaurants surveyed, 6 (66.6%) served freshwater fish purchased from Danube fishermen. Restaurants usually buy fish from a number of fishermen.

Marketing channels of fish caught on the Danube

Marketing of their catch is one of the most important problems for fishermen. Most fishermen (75.4%) sell their catch through personal networks, having their own regular customers (Figure 2) and their fish do not pass to the official fish market. Most of the catch is sold through the grey market, as the result of a lack of organized purchasing. Rent of the market place for the sale of fish, according to the opinion of fishermen is expensive. For example, in Zemun market monthly renting in 2010 was 30 000 RSD (price includes a gig, electricity, water).

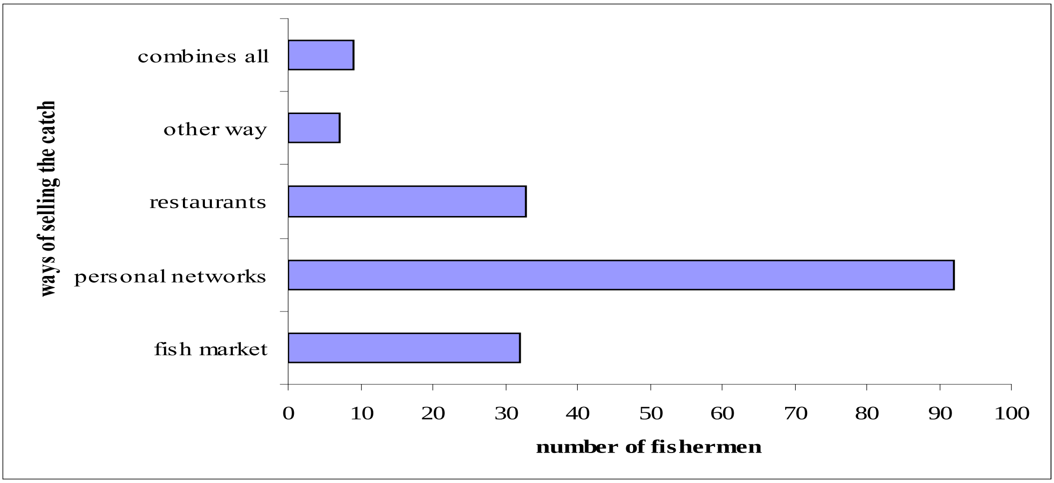

Figure 2. Ways that commercial fishermen sell their catch.

If the answers on the method of catch are crossed with the amount of catch in one year, significant correlations can be obtained ($r = -0.266$, $p = 0.027$). The fishermen who sell their catch through their own channels had a lower catch. Fishermen who had to sell their catch independently needed to set aside additional time for organizing their sales.

DISCUSSION

In Serbia, the fish market is unregulated and disorganized. There is a problem in the division of responsibilities between the four different Ministries. The sale of freshwater fish is usually conducted by pond shops and retailers who purchase fish from fishermen. Serbia increasingly imports fish and fish products, and the low prices of imported fish compete with the marketing of domestic fish. In 2003, the price of fish from Argentina was 1.2 $/kg (http://www.fao.org). Most fish sold in Serbia are imported from Vietnam, Argentina and Norway. In 2008 the most imported fish was the Argentine hake (*Merluccius hubbsi* Marini, 1933), and in 2011 the Striped catfish (*Pangasius hypophthalmus,* Sauvage, 1878) (Miščević *pers.comm*. 2011). By comparing data, regarding the realized trade of fish in Serbia and the funds engaged in the process, we find that Serbia imports fish far more than it exports (Figure 3).

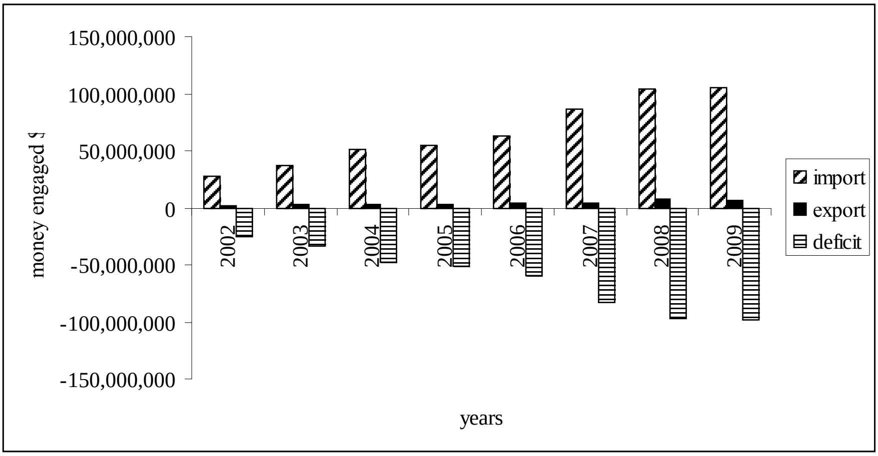

Figure 3. Trade in fish and fishery products according to the Ministry of Agriculture, Forestry and Water Management.

It is interesting that a kilogram of imported fish in 2002 cost 1.23$ and domestic fish was exported at 1.37$/kg, while in the 2009, 1 kg of imported fish cost 2.67$ and 1 kg of exported fish cost 5.12$. Comparing the prices of imported and exported fish, it is not surprising that more and more fish are imported and the price of imported fish is more accessible to customers.

For comparison, data were taken from the FAO (2013) on the turnover of fish in Bulgaria and Romania, since we share the same fishery resources. In Romania, supply comes from commercial marine and freshwater fishing, and saltwater and freshwater aquaculture. Fish distribution is carried out through various channels. Most of the fish pass through at least two separate ''operators'' before being sold to the consumer. This usually involves a producer or fisherman who sells directly to future sales or through an intermediary. The fish market is very low in Romania, as well as in Serbia, resulting in side-channel selling of fish. Many of these short marketing chains in the sale of fish, due to the high cost of transport and distribution, increases the price of fish. Local market in Bulgaria has a limited demand, and a ban on exports of fish products from Bulgaria to the European Union (starting in 2000) contributed to the reduction of production and catch. Most of the exports now go to Eastern and Central Europe (http://www.fao.org).

Fishing in Serbia is in the process of transition from a centrally planned to a market economy. Change to the dominance of the private sector, instead of the state, led to the problem in the fisheries sector. The shift from a centralized state structure to a private market system has occurred within the context of low incomes, limited investment possibilities, rudimentary market institutions and lack of government subsidies for the development of the fishing industry.

In Serbia, in the 2011 there were only two registered facilities to export fish to the EU and 7 facilities for fish exports to Russia (Miščević *pers. comm.* 2011).

CONCLUSION

1. Most of the fish caught in the Danube pass through short-marketing channels, causing fishermen to make a small profit. 2. Fishing is under the regulation of 4 ministries, which complicates the organization of the market and creates an environment that is not transparent to fishermen. 3. If one wants to engage in fishing, a fisherman must be registered. This creates (according to fishermen) significant costs and push fish marketing through gray market channels. 4. Due to low demand, improving the situation of fishermen can be achieved primarily through changes in the structure of supply and reducing the cost of business and creating opportunities for the use of more complex marketing channels. 5. To improve the position in the marketing chain, fishermen will (in the future) likely seek joint ventures, and their bargaining power with customers will thus be stronger than working individually.

ACKNOWLEDGMENT

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EFFECT OF STRESS ON PRESENCE OF DEVELOPING NEPHRONS OF COMMON CARP REARED IN SEMIINTENSIVE SYSTEM

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EFEKAT STRESA NA REGENERACIJU NEFRONA ŠARANA GAJENOG U POLUINTENZIVNOM SISTEMU

Apstrakt
Regenerišući nefroni (RN) u bubregu se često koriste kao indikator zdravstvenog stanja riba. U ovom radu je evaluiran efekat stresa koji šarani doživljavaju na početku sezone gajenja na bubrege koristeći broj RN. Ribe su hranjene različitim tipovima dodate hrane i gajene tokom dve sezone u vodi različitog kvaliteta, ali nijedan od ovih faktora nije uticao na pojavu RN. Međutim, ukoliko se prati vremenski tok sezone gajenja, broj RN se smanjuje. Njihov broj je najviši u maju i junu, dok na kraju sezone gajenja uopšte nisu bili prisutni u bubregu riba.

Ključne reči: histologija, regenerišući nefroni, šaran, indikatori
Keywords: histology, developing nephrons, common carp, indicators

INTRODUCTION

Handling and transport of fish is a risk for their health. At the start of the growing season, in semiintensive system, fish is either transported to fish farm or from winter pond to rearing pond. These activities are source of stress to fish (Iversen et al., 1998; Acerete et al., 2004). Effects of these activities and stress intensity could be monitored using different methods: biochemistry, gene expression, or histology. While biochemical parameters and gene expression are methods of choice in short-term monitoring, histology is important for long-term monitoring of fish health. Frequently used histological changes in kidney, after stress period, include presence of necrosis, eosinophilic granule cells, rodlet cells, neoplasia, and increased number of melanomacrophage centers. Aim of this manuscript is to evaluate presence of developing nephrons (DN) in common carp

(*Cyprinus carpio* L., 1758) during growing season, after experiencing stress at the start of the growing season.

MATERIALS AND METHODS

Detailed experimental design is already published elsewhere (Rašković et al., 2011; Rašković et al., 2013). Briefly, at the start of the growing season fish is transported from fish farm Neuzina to the Center for Fishery and Applied Hydrobiology in a plastic bags, filled with oxygen. Distance from fish farm to CEFAH is 95 km, while the trip lasted 90 minutes. After a one-month period of adaptation to new ponds, fish were placed in three earthen ponds, each measuring (L x W) 36 x 25 m. Different water supply was applied each of the two study years: in first year (Y1), water supply was from the nearby stream, while in second year (Y2) water from a tube well, 125 m deep, was used. In each of the three ponds, fish were fed with feed supplements: raw cereals (CF; 1:1:1 ratio mixture of wheat, corn and barley grains), pelleted (PF) and extruded (EF) compound feed. Water quality parameters were measured regularly and their values are reported in Rašković et al. (2013). From each of the ponds, three fish were sampled each month and five at the end of experiment, a total of 20 fish per pond. Specimens were sacrificed with a quick blow to the head, and kidney samples were 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 5mm. Sections were stained with hematoxylin and eosin (H/E) (Humason, 1979). Microphotographs were taken with a Leica DM LS microscope with the Leica DC 300 camera. Scoring system proposed by Bernet et al. (1999) was applied for the assessment of appearance of DN. Minimum values of appearance were 0, while maximum were 6. Comparisons of number of regenerative nephrons between ponds were performed by non-parametric Kruskal-Wallis test. The difference between years was compared using Mann-Whitney U test.

RESULTS

DN are easily distinguished from normal nephrons in fish kidney (**Figure 1a** and **1b**). The main characteristic of developing tubules and glomeruli were intensive basophilic staining and smaller dimensions compared to normal nephrons. Beside basophylia, developing cells have large and euchromatic nuclei (**Figure 1b**).

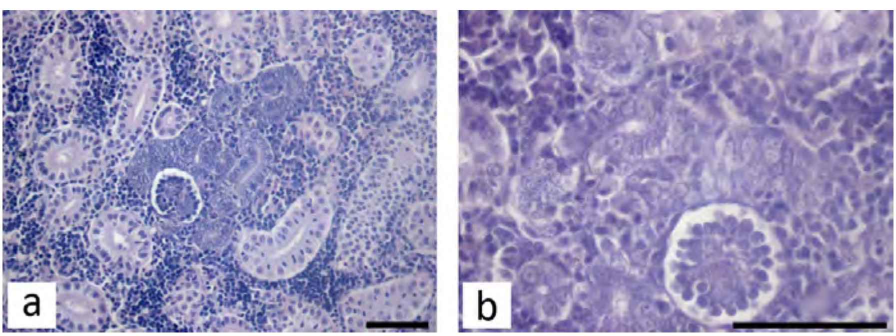
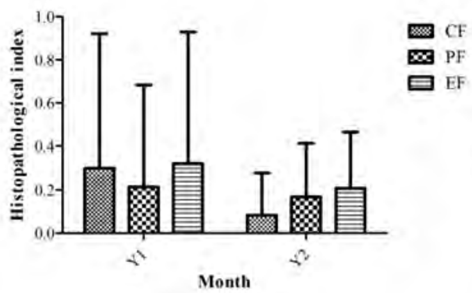


Figure 1. Developing tubules and glomerulus; a) H/E x400; b) H/E x1000; bar = 50 µm

Number of DN was higher during the first year of experiment, but that difference was not significant (p=0.210; **Figure 2**). Their number was not different between groups, neither in first year of experiment (p=0.871), nor in the second year of experiment (p=0.422).



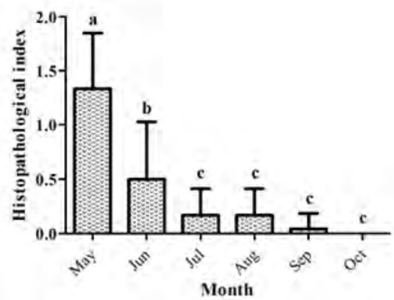


Figure 2 (left). DN number during Y1 and Y2 in different feeding groups; **Figure 3 (right)**. Frequency of developing nephrons during both growing seasons; different letters denounce significant difference between months (P<0.05) (p<0.05)

The presence of DN was higher during first two months of the growing season (p<0.05; **Figure 3**), with values reported in May higher than those in June (P<0.05). This trend was characteristic for both years in all three feeding groups. During last month of experiment presence of DN was not observed.

DISCUSSION

In this study the presence of DN was confirmed at the start of experiment. Fish kidney has an ability of a new nephron development throughout life, in contrast to mammals, although they are more frequently present in young fish (Reimschuessel, 2001). DN are good markers of chronic toxicity, because time for their full development is estimated to 9 weeks (Salice et al., 2001). The presence of DN is reported in fish recovery from application of gentamicin (Watanabe et al., 2009), and in polluted freshwater ecosystems (Camargo and Martinez, 2007; Lukin et al., 2011). Increased number of DN found during first two months of the growing season in both study years point at probable effect of stress during fish transport. Lower number of DN in subsequent months confirms acceptable water quality for common carp culture. We also confirmed that different water supply and different feed applied do not influence kidney regeneration in fish, in contrast to other histopathological changes observed in this study (Rašković et al., 2013).

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SEASONAL VARIATIONS AND DIVERSITY OF CLADOCERA (CRUSTACEA) IN NATURAL, DOMESTIC WASTEWATER TREATMENT LAGOONS

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SEZONSKA VARIJABILNOST I DIVERZITET CLADOCERA (CRUSTACEA) U LAGUNAMA ZA PRIRODNO PREČIŠĆAVANJE OTPADNIH VODA IZ DOMAĆINSTAVA

Apstrakt

Cladocera predstavljaju najznačajniju komponentu zajednice zooplanktona u većini stajaćih slatkovodnih ekosistema. Kao filtratori, hrane se algama, bakterijama, protozoama i organskim česticama, koje obiluju u netretiranim otpadnim vodama iz domaćinstava. Krupne Cladocera se proteklih decenija uveliko primenjuju u biomanipulaciji kao biološko sredstvo za prečišćavanje organski opterećenih otpadnih voda i smanjenje primarne produkcije u vodenim ekosistemima.

Istraživanje predstavljeno u ovom radu realizovano je na istraživačkom poligonu "Mali Dunav" Centra za ribarstvo i primenjenu hidrobiologiju, Poljoprivrednog fakulteta Univerziteta u Beogradu. U tri lagune, namenski napravljene na potoku Šugavac za prirodno prečišćavanje kanalizacionih voda poreklom iz uzvodno lociranog naselja, obavljeno je ispitivanje sastava i brojnosti zajednice zooplanktona. Pored bioloških ispitivanja, praćeni su hemijski parametri kvaliteta vode u trajanju od godinu dana, posmatranih kroz 4 perioda od po tri meseca. Istraživanje je sprovedeno sa ciljem ispitivanja dinamike populacije Cladocera u organski visoko zagađenim, plitkim, stajaćim vodenim ekosistemima i mogućnosti opstanka vrste *Daphnia obtusa* u datim uslovima sredine.

Prema parametrima kvaliteta vode, lagune su se značajno razlikovale samo u pogledu prosečnih vrednosti pH i rastvorenog kiseonika. Diverzitet Cladocera tokom jednogodišnjeg perioda istraživanja bio je relativno visok obzirom na smanjen kvalitet vode, sa ukupno 9 identifikovanih vrsta. Kruskal-Wallis test pokazao je da između laguna ne postoji statistički značajna razlika u ukupnoj brojnosti vrsta tokom perioda ispitivanja. Međutim, ukupna brojnost i diverzitet značajno su se razlikovali u svakoj laguni u zavisnosti od perioda posmatranja, što ukazuje na izražena sezonalna variranja u pogledu kvalitativnog i kvantitativnog sastava zajednice. Ova sezonska varijabilnost ogleda se u maloj brojnosti i potpunoj dominaciji sitne vrste *Chydorus sphaericus* tokom prvog perioda posmatranja (od kraja februara do sredine maja 2012.), praćenoj povećanjem brojnosti i diverziteta vrsta u drugom periodu (od sredine maja do sredine avgusta 2012) i dominaciji krupne vrste *Daphnia obtusa* u trećem periodu (od sredine avgusta do kraja novembra 2012.), čija je brojnost ponovo opala nakon dostizanja letnjeg maksimuma, uz ponovno pojavljivanje *C. sphaericus* u četvrtom periodu (od decembra 2012. do kraja februara 2013. godine). Vrsta koja je na svim tačkama na kojima je obavljeno ispitivanje dostigla najveću brojnost bila je *Daphnia obtusa*. Njeno prisustvo tipično je za vodene ekosisteme u kojima odsustvuju ribe kao predatori (Vadstein, 1993). Kruskal-Wallis test nije pokazao statistički značajne razlike u brojnosti ove vrste između laguna tokom jednogodišnjeg perioda istraživanja, dok su u svim lagunama utvrđene statistički značajne razlike u brojnosti posmatrane u različitim periodima. Rezultati pokazuju da je *D. obtusa* bila dominantna vrsta zooplanktona u lagunama (dostižući prosečnu maksimalnu brojnost od 1083 - 1444 ind/l tokom perioda ispitivanja), veoma tolerantna na niske vrednosti rastvorenog kiseonika (u proseku 0.32 – 6.90 mg/l) i visoko organsko opterećenje (BOD 26.65 – 33.09 mg/l).

Ključne reči: Cladocera, prirodno prečišćavanje otpadnih voda, Daphnia obtusa, organsko zagađenje

Keywords: Cladocera, natural wastewater treatment, Daphnia obtusa, organic pollution

INTRODUCTION

Currently worldwide, among the biggest ecological concerns are eutrophication and organic pollution coming from agriculture and urbanization. In recent decades, various biomanipulation methods for wastewater treatments have been developed, including constructed wetlands and wastewater stabilization ponds. Untreated domestic wastewaters contain different kinds of chemicals, high concentrations of phosphates and nitrates, organic matter, bacteria, protozoans and metazoans. Zooplankton, particularly Cladocerans, is capable of reaching high densities feeding on organic wastes and bacteria (Roche 1998, Nandini et al. 2004). Due to their high filtering rate, large daphnids such as *Daphnia magna* are commonly used for water purification in biomanipulation studies (Cauchie et al. 2002, Nandini et al. 2013).

The structure of zooplankton communities has been the subject of many studies, but only few research have been performed in polluted environments (Utz et al., 2008). This type of studies have an important role in obtaining knowledge on species diversity, variability and dynamics in stressed aquatic ecosystems. The aim of presented research was to investigate the dynamics of zooplankton communities, with emphasis on cladoceran species developing within shallow natural wastewater treatment lagoons.

MATERIAL AND METHODS

This research was conducted in three earthen lagoons at the experimental site „Little Danube" of the CEFAH, Faculty of Agriculture, University of Belgrade. The lagoons cover a total area of 0,4 ha, with average depth ranging from 0.4 to 0.5 m. Ponds are fed by surface water from the stream Sugavac and domestic wastewater coming from upstream households through an inlet pipe. The wastewater runs through lagoon one (L1), two (L2) and three (L3), respectively. In two lagoons (L2 and L3), a gravel layer 60 cm wide is built laying 1.5 m from the inlet, so that the inflow water from the previous lagoon runs through this filtering barrier while entering the pond. In each lagoon, emergent vegetation dominated by *Typha spp.* is planted and is present from the end of April until the beginning of December. Field sampling was conducted biweekly, from February 2012 till February 2013, at 4 sampling points: two points were located in L1 and L2 each, while two were located in L3: at the inlet and the outlet point. Environmental variables (temperature, pH and dissolved oxygen DO) were measured using a water field kit WTW MULTI 340/i (WTW, Germany) while total phosphorus (TP), total ammonia nitrogen (NH_4^+) and biological oxygen demand (BOD_5) were analyzed according to APHA (1998). Zooplankton samples for quantitative analysis were taken by collecting 30 l of water at each point using a plastic bucket, and filtering through sieve with 23 µm mesh size. Qualitative samples were taken by pulling plankton net (mesh size 75 µm) through surface layer in the centre of each lagoon. Samples were preserved using 4% formalin and examined under optical microscope Leica Galen III with maximal magnification of 160x. Zooplankton was identified to the level of species according to standard key for identification (Šramek-Hrušek et al., 1962). Quantitative samples were analyzed using subsampling technique, where the number of identified species was recalculated to the volume of 1 liter. Statistical analysis of obtained results was carried out using software STATISTICA v.6.0. Water quality parameters were logarithmically transformed ($\log_{10}$ x for BOD and NH_4^+, $\log_{10}$ (x+0.5) for temperature, $\log_{10}$ (x+1) for TP, $\log_{10}$ (x+1.5) for DO) except for pH (coefficient of variation < 30%) and evaluated using ANOVA and Tukey test. Biological parameters were evaluated using Kruskal-Wallis and Mann-Whitney U tests. In order to analyze the data easier, the investigation year was divided into four periods each lasting three months: period 1 – end of February till mid May of 2012, period 2 – mid May till mid August of 2012, period 3 – mid August till end of November of 2012 and period 4 – from December of 2012 till end of February of 2013.

RESULTS AND DISCUSSION

Analysis of variance of the environmental variables showed statistically significant difference between lagoons for pH ($p = 0.001$) and DO ($p = 0.023$), while other chemical parameters examined were not significantly different. Tukey test showed statistically significant difference between sampling points 1 and 3 ($p = 0.019$) 1 and 4 ($p = 0.001$) for pH, as well as 1 i 4 ($p = 0.018$) for DO. The results are presented in Table 1.

Table 1. Basic statistical data and results of ANOVA and Kruskal-Wallis tests

Parameter	Sampling point	Mean* ± SE	Min	Max	Cv (%)	*P* value**
Total count (ind/l)	1	226.32^{a} ± 83.22	0.00	1546.91	180.13	0.999
	2	231.63^{a} ± 88.40	0.00	1628.20	186.96	
	3	443.87^{a} ± 146.61	0.00	2914.00	161.81	
	4	348.96^{a} ± 104.80	0.00	1422.60	147.13	
Daphnia obtusa (ind/l)	1	346.00^{a} ± 136.48	0.49	1307.78	136.64	0.829
	2	366.54^{a} ± 122.41	0.52	1195.70	110.76	
	3	452.04^{a} ± 143.38	0.97	1444.00	114.36	
	4	478.87^{a} ± 146.47	0.18	1083.00	101.44	
pH	1	7.74^{aA}± 0.02	7.53	7.88	1.18	**0.001**
	2	7.78^{ab}± 0.02	7.55	7.90	0.97	
	3	7.82 ^{b}± 0.03	7.57	8.20	1.59	
	4	7.85 ^{B}± 0.02	7.62	8.10	1.36	
T (°C)	1	11.13^{a} ± 1.34	2.70	23.70	58.87	0.960
	2	11.74^{a} ± 1.45	3.50	25.70	60.57	
	3	12.23^{a} ± 1.50	3.40	26.60	60.05	
	4	12.46^{a} ± 1.55	3.40	27.90	61.12	
DO (mg/l)	1	2.11^{a} ± 0.23	0.32	5.73	52.59	**0.023**
	2	2.30^{ab} ± 0.26	0.47	6.90	54.61	
	3	2.53^{ab} ± 0.27	0.74	6.80	51.60	
	4	3.06 ^{b}± 0.23	1.43	6.30	36.99	
NH_4^+ (mg/l)	1	6.28^{a} ± 0.49	2.33	9.8	38.35	0.785
	2	6.03^{a} ± 0.43	2.09	9.9	34.78	
	3	5.67^{a} ± 0.40	2.46	9.8	34.15	
	4	5.62 ± 0.41	2.3	9.7	36.10	
TP (mg/l)	1	3.61^{a} ± 0.30	0.65	7.20	41.07	0.894
	2	3.35^{a} ± 0.26	0.60	5.50	37.40	
	3	3.24^{a} ± 0.27	0.64	5.40	41.18	
	4	3.29^{a} ± 0.25	0.42	5.00	37.94	
BOD_5 (mg/l)	1	33.09^{a} ± 3.18	16.22	59.81	41.92	0.462
	2	30.56^{a} ± 2.98	15.37	55.30	42.55	
	3	29.34^{a} ± 3.01	14.38	56.00	44.78	
	4	26.65^{a} ± 2.81	13.90	54.40	45.91	

*significant difference between values marked by different letters: a, b ($P < 0.05$), and A, B ($P < 0.01$); There is no significant difference between values marked by the same letter ($P > 0.05$), **bolded values are significant

Species diversity of Cladocera was relatively high throughout the examination period in all three lagoons taking into account the poor water quality. In total 9 species were identified in the samples (*Daphnia obtusa, Moina micrura, Scapholeberis aurita, Chydorus schaericus, Simocephalus vetulus, Alona quadrangula, Bosmina longirostris, Ceriodaphnia cornuta* and *Pleuroxus striatus)*. Kruskal-Wallis test showed no significant difference for total count of cladoceran species determined between the lagoons ($p = 0.9998$). However, significant statistical difference was found in each lagoon for total count of cladocerans between the periods (sampling point 1 $p = 0.0057$, 2 $p = 0.002$, 3 $p = 0.0030$, 4 $p = 0.0114$). Similar results were obtained for presence of *Daphnia obtusa*: no significant difference was found between the lagoons ($p = 0.8288$), while statistically significant differences were shown between the periods in each lagoon (sampling point 1 $p = 0.0065$; 2 $p = 0.0039$; 3 $p = 0.0039$; 4 $p = 0.0065$). Significance of differences in average total count as well as *Daphnia obtusa* count between the periods were tested by U test and the results are presented in Table 2.

Table 2. Effect of the period on the average total count and *D. obtusa* count in the lagoons

Period	*P*-level*							
	Total count (ind/l)				***Daphnia obtusa*** (ind/l)			
	1	2	3	4	1	2	3	4
1 and 2	0.260	0.228	**0.050**	0.319	0.317	0.317	0.317	-
1 and 3	**0.004**	**0.004**	**0.003**	**0.004**	**0.002**	**0.002**	**0.002**	**0.002**
1 and 4	**0.025**	**0.010**	**0.003**	**0.016**	**0.007**	**0.022**	**0.007**	**0.007**
2 and 3	0.055	**0.016**	0.261	0.261	**0.003**	**0.003**	**0.003**	**0.002**
2 and 4	0.873	0.873	0.873	0.873	**0.049**	0.074	**0.021**	**0.007**
3 and 4	**0.006**	**0.004**	**0.004**	**0.006**	**0.006**	**0.004**	**0.004**	**0.006**

*bolded values are significant

The seasonal variations were characterized by absolute domination and low abundance of small cladoceran *Chydorus schaericus* in the period 1, followed by diversity and production increase in the period 2, leading to maximum production values and domination of large *Daphnia obtusa* in the period 3. After reaching summer peak of production, gradual decrease of abundance occurs, followed by reappearance of *Chydorus schaericus* in the period 4.

CONCLUSIONS

Taking into account the poor water quality in all three natural wastewater treatment lagoons, species diversity of Cladocera was relatively high throughout the examination period. Strong seasonal variations for both abundance and species diversity were found between the three-month periods which were evaluated. Maximum diversity was reached during the period 2 (from the mid May till the mid August of 2012), with production peak in the period 3 (from the mid August till the end of November of 2012). *Daphnia obtusa* was shown to be well adapted to environmental conditions in these shallow wastewater lagoons, tolerant to low DO of 0.32 – 6.90 mg/l, reaching maximum production of 1444 – 1083 ind/l throughout the investigation period. Due to the potential for growing in domestic wastewater, *D. obtusa* may be adequate for use in biomanipulation studies.

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TOTAL COLIFORM AND FECAL COLIFORM COMMUNITY DYNAMICS IN NEWLY BUILT WASTWATER TREATMENT LAGOONS

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DINAMIKA ZAJEDNICA UKUPNIH KOLIFORMNIH I FEKALNIH KOLIFORMNIH BAKTERIJA U NOVOIZGRAĐENIM LAGUNAMA ZA PREČIŠĆAVANJE VODE

Apstrakt

Konstruisani vetlandi se koriste za prečišćavanje otpadnih voda zbog svoje efikasnosti, isplativosti izrade sistema i pozitivnog uticaja na životnu sredinu. Fekalne koliformne bakterije predstavljaju indikatore zagađenja voda jer ukazuju na potencijalno prisustvo patogenih mikrooganizama kao i drugih zagađujućih materija, a dospevaju u vodene ekosisteme direktno iz kanalizacionih sistema, ali i difuznih humanih i životinjskih otpadnih materija. Zbog toga je ovo istraživanje usmereno na praćenje dinamike preživljavanja ukupnih koliformnih i fekalnih bakterija u otvorenom površinskom konstruisanom vetlandu sa emerznom vegetacijom. Preživljavanje mikroorganizama, kao i njihova distribucija u vetlandima, zavisi od tipa vetlanda, ali i drugih ekoloških pojava koje utiču na njihov razvoj, gubitke i uništenje. U pogledu prečišćavanja otpadnih voda, vetlandi imaju učinak kao biofilteri, jer se u njima kombinovanjem fizičkih, hemijskih i bioloških procesa vrši uklanjanje kontaminanata. Fekalne koliformne bakterije se vezuju za suspendovane čestice, koje se zadržavaju na biljkama iz vetlanda i po njihovoj žetvi se zajedno sa biljkama uklanjaju iz sistema. U ovoj studiji je ispitivana efikasnost novoizgrađenog vetlanda, koji se nalazi na istraživačkom poligonu "Mali Dunav" u Centru za ribarstvo i primenjenu hidrobiologiju (CEFAH) Poljoprivrednog fakulteta, Univerziteta u Beogradu, fakultetskog dobra "Radmilovac". U tu svrhu je konstruisan vetland koji se sastoji iz jedne ulazne lagune, namenjene za predtretman vode i još dva bazena, u kojima se prirodno tretira površinska otpadna voda i otpadna voda iz domaćinstava. U ovom radu ispitivane su vrednosti ukupnih i fekalnih koliformnih bakterija, petodnevna biohemijska potrošnja kiseonika (BPK_5), i ukupne suspendovane materije. Istraživanje je započeto u martu 2012.godine i završilo se u februaru 2013.godine. Za praćenje indikatorskih organizama, kao što su ukupne i fekalne koliformne bakterije korišćene su standardne mikrobiološke metode uzorkovanja i tretiranja uzoraka u laboratoriji. Tokom jednogodišnjeg perioda praćenja parametara na mesečnoj bazi, vrednosti BPK_5 su varirale od 15 mg/L do 52 mg/l, a vrednosti TSS od 2.0 mg/L do 89.6 mg/L. Na godišnjem nivou prosečne vrednosti ovih parametara nisu prelazile propisani limit. Mesečno opterećenje sistema sa ukupnim koliformnim bakterijama se kretalo od 5.0×10^2 CFU/ml do 5.19×10^5 CFU/ml, a opterećenje fekalnim koliformnim bakterijama je variralo od 5.0×10^1 CFU/ml do 1.4×10^3 CFU/ml. Prosečna godišnja vrednost opterećenja sistema ukupnim koliformnim bakterijama je iznosila 9.93×10^3 CFU/ml, a fekalnim koliformnim 4.05×10^3 CFU/ml. Na kraju perioda ispitivanja prosečna vrednost ukupnih koliformnih bakterija u efluentu je umanjena za 50%, a prosečna vrednost fekalnih koliformnih bakterija za isti period je redukovana za 97%. Smanjenje brojnosti fekalnih koliformnih bakterija ukazuje da je primena konstruisanih vetlanda opravdana za korišćenje u svrhe obuhvaćene ovim istraživanjem. Radi dodatnog poboljšanja efikasnosti rada vetlanda potrebno je primeniti neke od sledećih mera: povećati raznovrsnost emerzne vegetacije, formirati dodatne ćelije kojima će se poboljšati hidraulički proticaj u vetlandu, da bi se dostigle još niže vrednosti fekalnih koliformnih bakterija u efluentu.

Ključne reči: *konstruisani vetlandi, ukupne koliformne, fekalne kolifomne,* BPK_5, *TSS*

Key words: constructed wetlands, total coliforms, fecal coliforms, BPK_5, *TSS*

INTRODUCTION

Low food supply and water scarcity worldwide has brought great attention to water quality and its safe usage in various human activities in last decades. In this sense constructed wetlands have been widely used and recognized as environmentally friendly, very efficient and cost effective means for wastewater treatment. In Serbia, high percentage of rural and suburban households use septic tanks for disposal of domestic wastewater. Septic tanks are considered to be highly effective and environmentally safe, when properly designed and well maintained. Unfortunately, there is a lot of evidence for majority of septic tanks in suburban areas to be either improperly made or malfunctioned - leading to their leakage and consequently to polluting of surrounding soil, waterbodies and ground water.

An extensive literature exists on the design and use of treatment wetlands for different kinds of wastewater and water quality applications (e.g., Reed et al. 1995; Kadlec and Knight 1996, Vymazal. J 1998; Tchobanoglous et al. 2003, Crites et al. 2006; Kadlec and Wallace 2009). Diverse factors such as temperature, ultraviolet radiation, unfavorable chemical conditions, sedimentation that occurs in constructed wetlands makes it a hostile environment for pathogenic organisms (E.Smith et al., 2005). The survival, faith and distribution of microorganisms in wetlands depend on the type of constructed wetland as well as associated phenomena influencing their death, losses and growth.

Constructed wetlands in this sense act like biofilters combining physical, chemical and biological processes (Kadlec and Knight, 1996, Werker et al., 2002) in which fecal coliforms are being removed from water by attaching to suspended solids that are further trapped by wetland vegetation.

In regard to this widespread problem, the aim of this investigation was to study the efficiency of a newly built constructed wetland located at the experimental site „Little Danube" of the CEFAH, Faculty of Agriculture School Estate "Radmilovac", aiming to reduce pollution of a little stream that exists in this small suburban area of Belgrade. The investigation conducted in this research is dealing with dynamics of total coliform and fecal bacteria in free-water surface constructed wetland with emergent vegetation.

MATERIAL AND METHODS

A free-water surface constructed wetland consisted of one pretreatment lagoon and two cells, treating surface runoff and domestic wastewater, were evaluated in this study for total and fecal coliform bacteria presence, BOD_5 values and total suspended solids (TSS). Two of the three cells were planted to cattail (*Typha spp.*).

Monitoring of total and fecal coliforms started in March 2012 and was completed in February 2013, in all wetland cells (i.e.: L_1 – pretreatment lagoon, L_2 – first treatment lagoon, L_{3in} – inlet of second treatment lagoon, L_{3out} – outlet of second treatment lagoon). Samples were collected on a monthly basis at the influent ends of each system's cell and after the filters (60 cm in width), positioned at 1.5 m distance from the inlet pipe, in sterile sample plastic bags, and transported to the microbiological laboratory of Faculty of Agriculture on ice to minimize population changes in the tested water. Samples were prepared by transferring 20 ml of water sample in 180 ml flasks with peptone solution, in sterile conditions and homogenized in rotation mixer set at 250 rpm for 20 minutes. The samples were afterwards diluted serially and analyzed for total and fecal coliforms by pour plate technique. Appropriate sample volumes, in triplicate were poured onto the plates with Brilliance E.coli/coliform Selective Agar (Oxoid CM1046) and incubated for 24h at 37°C to enumerate total coliforms and for 24 h at 44°C for enumeration of fecal coliforms and expressed in log_{10}CFU/ml.

The standard 5-day BOD (BOD_5) test was used to assess reduction of biochemical oxygen demand. Samples were stored at less than 5°C for 24 hours, and after standardized procedure of solution buffering were incubated at 20°C in BOD incubator.

TSS were determined gravimetrically following filtration through glass-fiber membrane filters (APHA, 1992).

RESULTS AND DISCUSSION

The average concentrations of total coliforms entering the constructed wetland in pretreatment lagoon, L_1, were 9.98×10^3 CFU/ml during the examined year. Outlet concentrations of total coliforms for the investigated period were 4.40×10^4 CFU/ml.

Bacterial indicators of fecal contamination showed average influent concentrations of 4.05×10^3 CFU/ml for the examined period, and 1.13×10^1 CFU/ml average concentrations at the outlet end of wetland. This indicates that the reduction of *E.coli* at the outlet end of wetland was 97% at the end of conducted observations (Keith R.Hench et al., 2003).

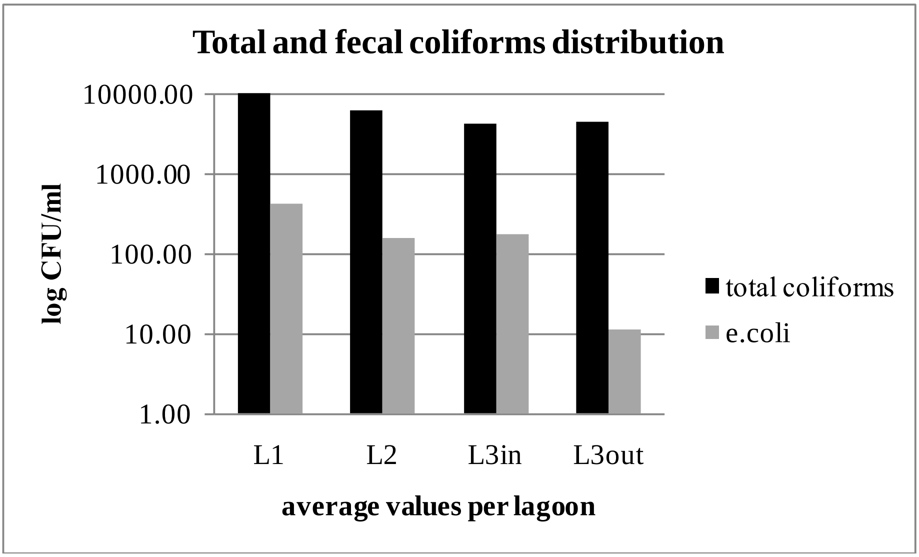

Figure 1. – Distribution of total and fecal coliforms

Influent BOD_5 levels varied insignificantly between system's lagoons, ranging from 15 mg/l to 52 mg/l through the examined year. The highest mean concentrations were in March, in year 2012, and slightly exceeded recommended values by EPA (30 mg/l) in March, April and June of the same year. All the other values in investigated period were below the threshold (Table 1).

Table 1. Average levels of BOD_5 and TSS

Lagoons	BOD5 mg/L	TSS mg/L
L1	31,8	30,1
L2	28,5	18,1
L3in	27,3	18,9
L3out	25,4	26,1

Wetlands are known to be effective in reducing suspended solids. The observed average values for total suspended solids (TSS) have never exceeded permitted limits of 30 mg/L at the outlet end of CW (Fig. 2).

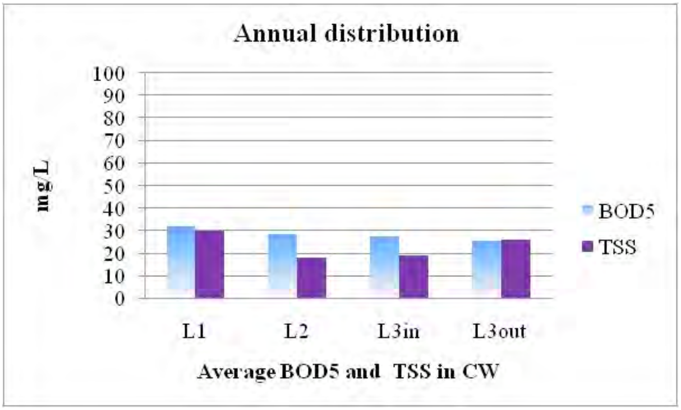

Figure 2. Annual averages of BOD_5 and TSS in wetland cells

Results demonstrated that reduction of fecal coliforms was high regardless the season of the year, and were reduced comparing to wetland loading.

CONCLUSIONS

Constructed wetlands are undoubtedly very effective and low cost systems for domestic wastewater treatment in suburban areas where standard septic tanks fail to produce environmentally safe surroundings. This study has justified the application of constructed wetlands for wastewater treatment, since the reduction of fecal coliforms was significant over the investigated period, but also has proven that further monitoring should be conducted in the following years, since the best performance is expected during the third year. Although there has been constant improvement in wetland construction over past decades, there are still obstacles to overcome in achieving sustainable, water quality regarding pathogen removal. Second year study, as well as long term study should be undertaken with possible changes in vegetation and other wetland parameters to examine the suitability of constructed wetland for treating domestic wastewater.

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FISH WELFARE FROM PERSPECTIVE OF CONSUMERS IN SERBIA (PRELIMINARY RESULTS)

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DOBROBIT RIBA IZ UGLA POTROŠAČA U SRBIJI (PRELIMINARNI REZULTATI)

Apstrakt

Dobrobit farmskih životinja, među kojima su i ribe, u svetu je poseban značaj dobila u toku poslednje decenije, uz podršku odgovarajućih zakona i propisa kojima su definisani standardi u vezi uslova gajenja, transporta i klanja. Napori koji se ulažu u razvoj i unapređenje proizvodnje i poboljšanje kvaliteta ribljeg mesa u Srbiji takođe nameću potrebu da se obezbedi i visok nivo dobrobiti farmski gajenih riba. Ovo preliminarno istraživanje imalo je za cilj prikupljanje i analizu podataka o informisanosti i zainteresovanosti javnosti u Srbiji za zaštitu dobrobiti riba. U tu svrhu, upitnike je anonimno popunilo 235 punoletnih lica sa teritorije Republike Srbije. Dobijene informacije mogu da posluže i za određivanje optimalnog obima uzorka za buduća ispitivanja.

Statistička analiza strukture odgovora ispitanika u odnosu na njihove demografske, ekonomske i druge karakteristike izvršena je uz pomoć hi-kvadrat testa. Rezultati testiranja ukazuju da se odgovori značajno razlikuju u zavisnosti od nivoa obrazovanja, visine mesečnih primanja i mesta stanovanja ispitanika. Osim na pitanje „da li su upoznati da u svetu postoje propisi u vezi dobrobiti riba u toku uzgoja, transporta i klanja“ (pitanje 4), anketirani su na ostala pitanja u vezi dobrobiti (2,3,5 i 6) najčešće odgovorili potvrdno. Prema rezultatima, najčešće ispitanici sa višim i visokim obrazovanjem poznaju i razumeju termin "dobrobit životinja" (79,07%), kao i postojanje propisa u svetu o zaštiti dobrobiti životinja (49,61%). S druge strane, nezavisno od nivoa obrazovanja, najveći deo anketiranih češće bi kupovao ribu ili proizvode od ribe proizvedenu u uslovima u kojima se maksimalno poštuju životne potrebe riba i štiti njihova dobrobit (63,83%), a spreman je i da za to plati i više ukoliko je potrebno (57,87%).

O dobrobiti životinja bili su bolje informisani ispitanici sa mesečnim neto ličnim prihodom većim od 40000 dinara (tj. primanjima iznad republičkog proseka) u odnosu na one sa manjim prihodima (76,98% : 56,88%). Ispitanici sa većim primanjima pokazali su i veću spremnost da češće kupuju ribu i proizvode od ribe uz saznanje da su proizvedeni u uslovima poštovanja dobrobiti (68,25% : 58,72%). Većina ispitanika iz gradskih i seoskih naselja (69,38% iz gradova sa više od 100.000 stanovnika – Beograd, Novi Sad, Niš i Kragujevac, i 66,67% iz manjih gradova; 59,26% iz seoskih naselja) smatra da je potrebno zaštititi dobrobit farmski gajenih riba, a spremni su i da plate više za bolji kvalitet, koji je omogućen gajenjem ribe uz poštovanje principa dobrobiti (57,50% iz velikih gradova; 68,75% iz manjih gradova i 40,74% iz seoskih naselja).

Uzimajući u obzir sve rezultate, može se reći da među potrošačima u Srbiji postoji zainteresovanost za zaštitu dobrobiti farmski gajenih riba, što ukazuje na značaj ove teme. Takođe, bolja informisanost javnosti može da doprinese intenzivnijim zahtevima za ribom boljeg kvaliteta i stvaranju realne potrebe za unapređenje uslova gajenja, čime se pruža mogućnost za dalja istraživanja u ovoj oblasti.

Ključne reči: dobrobit, ribe, upitnik, potrošači, Srbija
Keywords: welfare, fish, questionnaire, consumers, Serbia

INTRODUCTION

Fish welfare is a topic developed particularly in the last decade in the world, simultaneously with intensification of fish production. The need to protect welfare of farmed fish resulted from the same reasons as for other farm animals. It is greatly influenced by experts' opinion and demands of consumers. Thus, in the European Union minimum standards for the protection of animals bred for farming purposes (including fish) have been laid down in an EU Council Directive from 1998. In 2005 the Council of Europe adopted a recommendation on the welfare of farmed fish and in 2008 the World Organization for Animal Health adopted guiding principles for fish welfare; also the industry has adopted various measures to safeguard fish welfare (EFSA, 2009; Solgaard and Yang, 2011).

In recent years, animal rights and welfare issues also become important in Serbia. The most discussed problems are related to human-cohabitating animals e.g. stray dogs (Vucinic et al., 2012) and to animals whose products are mostly used i.e. cows, pigs and poultry (Hristov et al., 2009; Relic et al., 2010) while fish welfare still does not represent such important topic as in other countries. However, Serbia's potentials in fishery and efforts directed to fish production improvement (Marković et al., 2011) indicate the need to ensure a high level of welfare of farmed fish. This is supported by the fact that diet, rearing conditions, transport, and slaughter procedures have a proven impact on raw meat's quality and possibility for its processing (Relić et al., 2010). Serbian legislative ("Animal welfare law", Anon., 2009) provides the possibility of protecting fish welfare. However, appropriate regulations are still required.

In this paper the results of preliminary research related to Serbian consumers' awareness and interest in welfare of farmed fish are presented.

MATERIALS AND METHODS

The research was conducted in the spring of 2013 by an anonymous survey of 235 adult persons from the territory of the Republic of Serbia. The questionnaire with four groups of questions was distributed to the respondents. The first group contained questions related to the structure of respondents: gender and age, level of education, amount of monthly net personal income, number of household members, presence of juveniles in the household and place of residence. In the second group were questions about frequency of fish consumption and habits of Serbian consumers. The third group was questions about terms related to rearing conditions and stress in fish, and the fourth group was questions about welfare issues.

In this paper, a part of results related to the answers which are significant to obtain information about consumer awareness and interest in the welfare of fish is presented. Beside the answers on the first set of questions, the responses to the questions from other groups were analyzed: question concerning the frequency of consumption of fresh fish and/or fish meat products consumption in the diet (Question 1), as well as answers of all questions from the fourth group, which considers knowledge of terms "animal welfare" (Question 2), the need to protect the welfare farmed fish (Question 3), the notion of the presence of regulations in the world on fish welfare during rearing, transport and slaughter (Question 4), the frequency of buying fish and/or fish meat products depending on the rearing conditions (Question 5) and readiness of the consumers to pay more for fish and/or fish products if they are produced under conditions of maximum respect of basic needs of fish and protection of their welfare (Question 6).

Data analysis was performed by using Stat Soft 6.1., and Microsoft Excel 2007 softwares. Distributions of responses are presented graphically through the structural circle or rectangle. For statistical analysis of the uniformity of answers through different categories and independence of the responses with respect to their demographic and other characteristics the chi-square test (x^2) was used.

RESULTS AND DISCUSSION

Demographic structure (gender and age), economic (personal net monthly income) and other structures in the sample (educational structure, the structure of households by number of members and presence of juveniles, and categorization by the type of residence) correspond to the same structures in Serbia and the selected sample can be considered representative despite the random method of selection.

Number of males (110) did not significantly differ ($x^2 = 0.96$, $P = 0.328$) from the number of female respondents (125). Respondents were not equally distributed by age contingents ($x^2 = 25.66$, $P < 0.001$), and the majority were in the group of 40 to 49 years (71). Persons under the age of 40 (101) were more prevalent than people older than 49 years (63). Number of respondents with lower (106) and higher education (129) was not significantly different ($x^2 = 2.25$, $P = 0.134$). According to the monthly net personal income respondents were equally distributed in the group with incomes lower and higher than the national average ($x^2 = 1.23$, $P = 0.267$) of about 40,000 dinars (Anon, 2013). Predominant respondents were from households with three (67) and four (65) household members, and the presence of respondents from households with different number of members was statistically very significant ($x^2 = 36.13$, $P < 0.001$). Persons under the survey were in significantly larger number (153) in households with no person under the age of eighteen years ($x^2 = 21.45$, $P < 0.001$), as well as in four cities with over 100,000 residents i.e. Belgrade, Novi Sad, Niš and Kragujevac (160) ($x^2 = 130.53$, $P < 0.001$).

Based on the responses to the Question 1, consumers in Serbia eat fish and/or fish products several times a month (Fig. 1).

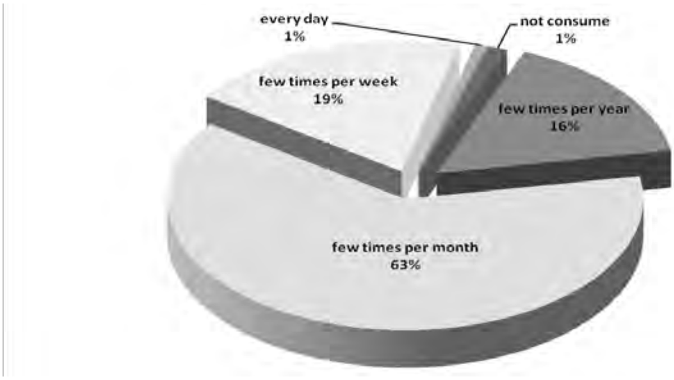

Figure 1. Layout of the respondents according to the frequency of fish and/or fish meat products consumption

Except to the Question 4 (about awareness regarding the existence of the fish welfare regulations in the world) to the rest of the questions (2, 3, 5 and 6) the respondents answered mainly affirmatively (Fig. 2).

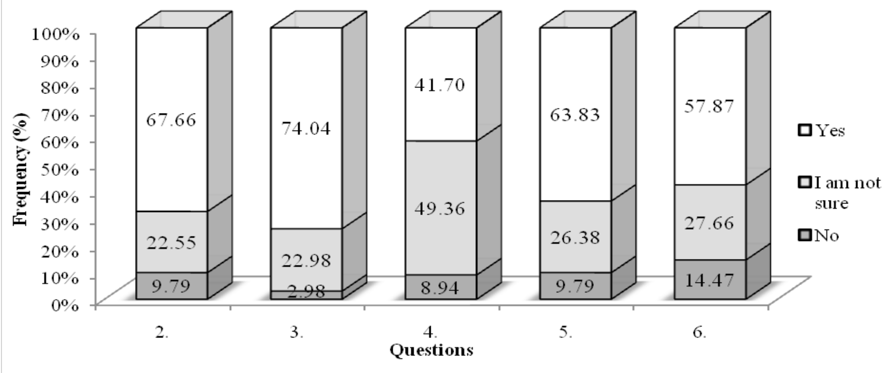

Figure 2. Frequency of answers to the questions related to welfare

According to the results in Table 1 frequency of fish and/or fish products consumption (Question 1) did not depend on gender and age, number of household members and the presence of juveniles in the household. Awareness of the respondents about meaning of the term "animal welfare" (Question 2) depended on their education and personal income. Residents of different types of settlements (big city, city, and village) have had a different opinion about the need to protect the welfare of farmed fish Question 3). The structure of the answers regarding information about fish welfare regulations in the world (Question 4) is not the same for respondents with different levels of education.

Readiness of the respondents to buy more fish and/or fish products (Question 5) depends on the level of their education, the level of personal net monthly income, and the place of residence, while willingness to pay more for these (question 6) depends only on the level of their education and the place of residence. The frequency of fish and/

or fish products consumption (Question 1) has been associated with: knowledge of the term "animal welfare" (Question 2), the decision of consumers to buy more fish and fish products if they are produced with maximum respect to welfare principles (Question 5), and their readiness to pay more for such products (Question 6).

Table 1. Significance levels (P – values)* of tested independence of respondents layout on the modalities of their characteristics, and their responses to the question

Characteristics	Number of the question					
	1.	2.	3.	4.	5.	6.
Gender	0.104	0.187	0.807	0.358	0.231	0.106
Age	0.329	0.504	0.210	0.431	0.827	0.750
Level of education	0.243	**<0.001**	0.070	**0.008**	**<0.001**	**0.004**
Monthly income	**0.008**	**0.004**	0.367	0.316	**0.019**	0.064
Number of household members	0.900	0.687	0.823	0.072	0.699	0.891
Juveniles in the household	0.140	0.158	0.509	0.668	0.581	0.404
Place of residence	0.110	0.814	**<0.001**	0.726	**<0.001**	**0.004**
Frequency of fish consumption	-	**0.008**	0.366	0.162	**0.030**	**0.011**

* Bolded values are significant

Further analysis showed that most of the people with higher (79.07%) and lower (53.77%) **level of education** know the term "animal welfare" and its meaning, and the lowest proportion (2.13% with higher and 16.98% with lower level of education) had never heard of it. Structure of the responses was favorable in both categories of respondents. The similar proportion of the respondents with lower (49.06%) and higher education (49.61%) presumed the presence of fish welfare regulations in the world. However, more consumers from the group of less-educated did not know about foreign welfare regulations (15.09% : 3.88%), and the bigger part of educated was familiar with it (46.51% : 35.85%). Regardless of the level of education, majority of the respondents (56.60% and 64.34%) more likely would buy fish produced in the compliance with welfare requirements; for the smaller number (17.92% and 3.10%) production conditions are not significant, and the rest of respondents were undecided (25.47% and 27.13%). Among respondents with lower and higher education, the smallest proportion would not pay more for fish grown in welfare protected conditions (22.64% and 7.75%, respectively). Similar results were obtained by Solgaard and Yang (2011) where higher educated persons appeared to be more willing to pay extra for welfare.

The structure of respondents according to their knowledge about animal welfare in groups with **different monthly net personal income** very significantly differed (Table 1). The term and meaning of "animal welfare" have been known to the majority of the respondents with lower (56.88%) and higher income (76.98%). In the subgroup with monthly income up to 40,000 RSD 12.84% and in the second subgroup (over 40,000 RSD) 7.14% of respondents never heard of "animal welfare". Furthermore, income level significantly affected the decision to purchase more fish and/or fish products (Table 1). For most of the consumers in both groups (58.72% and 68.25%), information about better fish rearing conditions was important, and it was not significant in 15.60% (lower income) and 4.76% (higher income).

The structure of the answers on the third, fifth and sixth question differed in the respondents from **different types of settlements** (Table 1). In the cities 75.00% or more and in the villages 51.85% of the respondents protection of the welfare of farmed fish consider as necessary. The opposite opinion expressed 1.88% of respondents from the largest cities, 0.00% from other cities and 14.81% from rural areas. More fish and/or fish products would like to buy 62.50% of the respondents from the four largest cities, 81.25% from other cities and 40.74% from villages, if they for certainly know it was produced with maximum fish welfare protection. Furthermore, in the cities 31.25% and 18.70%, and in the villages (22.22%) of the consumers were undecided. Respondents which would not change the frequency of purchasing after being informed that the production takes into account fish welfare were from rural areas (37.04%). In the large cities 57.50% of responders, and 68.75% from other cities and 40.74% in villages was ready to pay more for fish welfare. In negative answers the opposite tendency was registered: 11.25% of respondents from large cities, 12:50% from other cities and even 37.04% from the villages are not ready to pay more.

Significant relation between the **frequency of fish consumption** and knowledge of the term welfare is interesting: consumers who eat fish and/or fish products several times a week or every day (Question 1) in 91.11% i.e. 100% knew the term "animal welfare" and its meaning (Question 2). Minimum involvement of welfare acquainted (57.89%) was among consumers which use fish and/or fish products several times a year. Among respondents who consume fish few times a month (what is the most often according to figure 1) 62.59% knew the term "animal welfare" and what it means, and 66.67% of non consumers knew about welfare. The proportion of respondents who have never heard the term "animal welfare" is moving in the opposite direction of the frequency of fish consumption. All of the respondents who eat fish every day were more likely to buy and willing to pay more for fish cultured with respect of the welfare principles. Approximately the same percentage of consumers of fish a few times a year was not ready (34.21%) and was ready (36.84%) to pay for more expensive fish or products.

$x^2 x^2$-test also showed that there is a dependency in the distribution of respondents by answer to two questions from the group about welfare (Table 2). Only consumers' decision about frequent purchasing of fish or its products (Question 5) does not depend on their information about welfare regulations in the world.

Table 2. Significance level (P – values)* of the independence of respondents distribution by their answers to the two questions about welfare

Number of the question	3.	4.	5.	6.
2.	**<0.001**	**<0.001**	**<0.001**	**<0.001**
3.		**<0.001**	**<0.001**	**<0.001**
4.			0.166	**0.003**
5.				**<0.001**

* Bolded values are significant

Data obtained in this survey are comparable to the results from the paper by Honkanen and Olsen (2009), and Solgaard and Yang (2011). Their surveys were conducted in countries where the welfare of farmed fish is more important issue then currently in

Serbia, and their experiences can significantly contribute to the adoption of appropriate standards of fish breeding in our country. The importance of surveys in endorsement of welfare regulations confirmed the paper by Stafford and Mellor (2009).

CONCLUSSIONS

Based on the results, city residents are more interested in fish welfare protection than those living in villages. They showed more willingness to frequently purchase fish produced according to the welfare standards and to pay more for that. Besides, more educated consumers and those with higher personal income are better informed about animal welfare and more likely to buy fish and fish products if they were confident that during the production attention was paid to fish welfare. More educated consumers are also better informed about presence of regulations regarding fish welfare in the world, and they are willing to pay more for better quality of fish.

Considering all results of this preliminary study, there is an interest among consumers in Serbia to protect the welfare of farmed fish, which indicates the importance of this issue. By Better informing of consumers about fish welfare can contribute to a real need for improvement of fish rearing conditions through appropriate regulations. This provides an opportunity for further research in this area.

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IDENTIFICATION AND DISTRIBUTION OF SEA CUCUMBERS IN THE CHABAHAR BAY, IRAN

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IDENTIFIKACIJA I DISTRIBUCIJA MORSKOG KRASTAVCA IZ CHABAHAR ZALIVA, IRAN

Apstrakt
Morski krastavci su najvažniji bodljokošci koji predstavljaju veliki resurs Severne obale Persijskog zaliva i Omanskog mora. Uprkos koristi u nutritivnom i farmaceutskom pogledu rađeno je samo nekoliko studija o njihovoj identifikaciji i distribuciji. Predstavljeno istraživanje je obavljeno radi identifikacije morskih krastavaca u zalivu Chabahar i procene uticaja aktivnosti čoveka na njih. Uzorkovanje je obavljeno podelom ispitivanog terena na 4 regiona sa najvećom verovatnoćom nalaženja morskih krastavaca. Rezultati su pokazali prisustvo 7 vrsta: *Stichopus variegates, Holothuria leucopspilota, H. pervicax, H. atra, H. arenicola, H. hilla* i *H. parva* koje pripadaju familijama Holoturia i Stichopus. *Stichopus variegates* je imao najveću masu i dužinu tela, zatim su po veličini sledili *Holothuria leocospilota* i *Holothuria pervicax.* Istraživanje je pokazalo da je zaliv Chabahar bogat morskim krastavcima visoke ekonomske vrednosti, ali su dalja istraživanja neophodna o nutritivnim i drugim osobinama u cilju njihove reprodukcije i gajenja u Južnom Iranu.

Ključne reči: morski krastavci, identifikacija vrsta, zaliv Chabahar
Keywords: Sea cucumber, identification of species, Chabahar Bay

INTRODUCTION

Sea cucumbers are the main members of the food chain in moderate and coral reef ecosystems and play an important role as saprophage and planktonphage in aquatic ecosystems. In addition, the eggs and larvae of these species are considered to be aimportant sources of food for other marine species (Bruckner et.al. 2003).

Most of the sea cucumbers have habitats in the intertidal zone while lesser numbers of them live deep in the oceans (Sminrov et al., 2000).Size of Echinodermata can vary between a few millimeters to more than 2 meters and have various colors. Until now, 1400 species of sea cucumbers have been identified and reported throughout the world.

Sea cucumbers feed on the residues of organic materials and aquatic microorganisms using their tentacles (Castro and Huber, 2005). Saprophagous sea cucumbers play an important role in coral reefs recycling process (Bakus, 1973). Their diet can be diverse, blue-green filamentous algae, red algae, live microorganisms, coral fragments, diatoms, and foraminifers (Des Rocher, 1999).

The first report on the sea cucumbers of the Persian Gulf was recorded in 1940. He studied the Echinodermata in the eastern part of the Persian Gulf that has a more suitable environment and richer fauna than other parts (Semper, 1940). More Echinodermata species were also identified during an extensive research carried out by Farskal (1938) on marine organisms in the southern seashore of the Persian Gulf. However, no research was done on the sea cucumbers of the north Oman Sea. Accordingly, the present study was designed to study sea cucumber population of the Chabahar Bay in the north of the Oman Sea.

MATERIAL AND METHODS

Chabahar Bay, the biggest gulf of Iran, is located on the north of Oman Sea and the furthest end of southeast borders of Iran. This study was done in the eastern side of the Chabahar Bay which is the most influenced ecosystem by human activities of the Chabahar Port. In the first step, 4 regions with the highest probability of having sea cucumbers were chosen. In each station, 4 squares measuring 10×20 m^2 were searched thoroughly by deep diving and sea cucumbers found were counted and identified. According to the Leske key (Leske, 1778) considering all of the 16 squares in each sampling, an area of 3200 m^2 was studied. Base on the variability of sea cucumbers length to stress, all samples were measured and the send to the laboratory.

RESULTS

During one year of investigation, 7 species of sea cucumbers were observed which are presented in Table 1. Overall, individuals of species *Stichopus variegatus* had longer length and bigger weight compared to the other species followed by *Holothuria leucospilota* and *H. hilla.*

Table 1. Species of the sea cucumbers in the Chabahar Bay.

Family	**Scientific name**	**English name**	
Holothuria	*Holothurialeucospilota*	Leucospilota	Brandt, 1835
	H. hilla	Hilla	Lesson, 1830
	H. atra	Atra	Jaeger, 1833
	H. arenicola	Arenicola	Simper, 1868
	H. parva	Parva	Krauss in Lampert
	H. pervicax	Pervicax	Selenka, 1867
Stichopodidae	*Stichopus variegaes*	Variegates	Simper, 1868

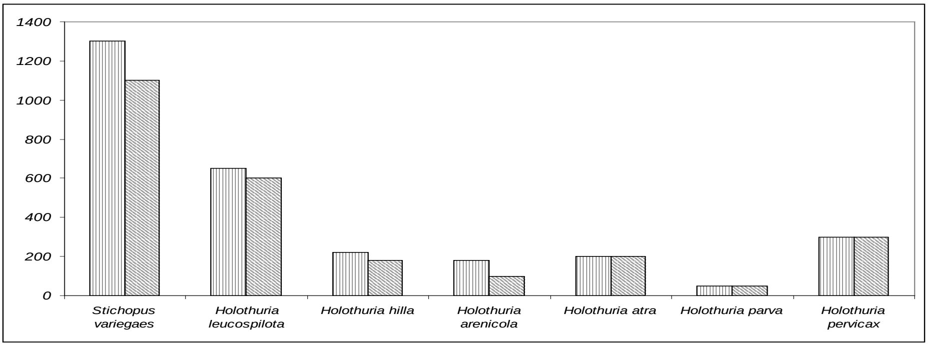

Figure 1. Mean weight of different species of sea cucumbers in the Chabahar Bay.

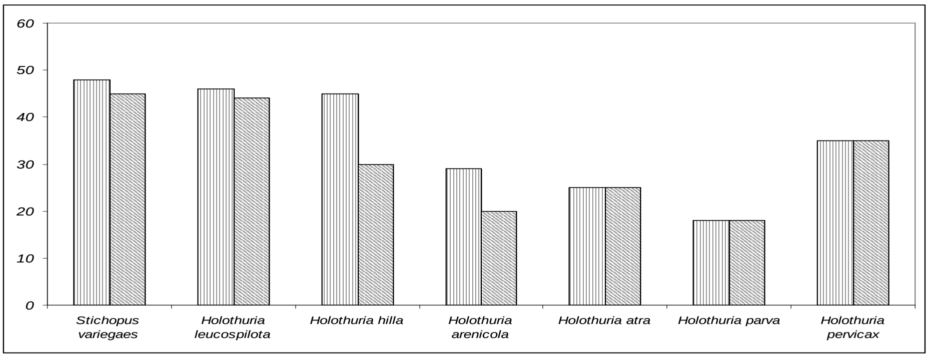

Figure 2. Mean length of different species of sea cucumbers in the Chabahar Bay.

DISCUSSION

All seven species observed in Chabahar Bay were from *Aspidochirotida* order, belonging to *Holothoriidea* (6 species) and *Stichopodidea* (1 species) families. Previous investigations show that members of *Aspidochirotidea* order, *Holothuriidea* and *Stichopodidea* families predominate in tropical shallow seas (Zenkowich, 1978). Apodida order diversity generally increases by moving away from the equator and Molpadiida order is found in higher latitudes with higher depth. The highest species richness of the families is seen in coral cliffs reefs, reaching up to 20 species per hectare.

Lack of other families' species may be related to habitat conditions. In this investigation, due to low depth of sampling regions, species of other families of *Aspidochiroidea* order were not seen since they live in deep waters (Mortensen, 1980). The observed species are epibenthic species which live on hard or soft sea beds.

In this research, length and weight of the species were measured only once. However, there was no relation between length and weight of sea cucumbers. *H. leocospilota* with the earthworm body shape may have different length depending on status of sampling time. This feature is seen in other sea cucumbers as well. The flexibility of sea cucumber body is attributed to their tiny and dispersed ossicles (Mitchell, 1988). Therefore sea cucumbers are unique from biological, behavioral and morphologic aspects compared to other aquatic animals. For this reason, biometry methods which are used for other marine species may fail in sea cucumbers (Chen, 2003). Considering high diversity of species in intertidal zone there is a great potential for sea cucumber harvesting from of Chabahar Bay and application in the fields of nutrition, pharmaceutics, cosmetics, and health products.

Chabahar Bay as a commercial port has all infrastructural and environmental requirements for breeding and reproduction of sea cucumbers. Considering the earlier studies on the region's unique species (Rocher, 1999) we can be confidentl about the future of breeding and reproduction of sea cucumbers.

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