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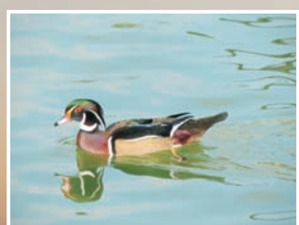


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WATER & FISH

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IN THE FRAME OF THE EU FP7 PROJECT - AREA

7th INTERNATIONAL CONFERENCE "WATER & FISH"

CONFERENCE PROCEEDINGS

Faculty of Agriculture, Belgrade-Zemun, Serbia
June, 10 – 12. 2015.

PUBLISHER
University of Belgrade, Faculty of Agriculture

FOR THE PUBLISHER
Prof. Dr. Milica Petrović

EDITORS IN CHIEF
Prof. Dr. Vesna Poleksić and Prof. Dr.Zoran Marković

COMPUTER DESIGN
Božidar Rašković

TECHNICAL PREPARATION OF A COVER
Božidar Rašković

PRINTED BY
GRAPHIC, Novi Sad

NUMBER OF COPIES PRINTED
400 COPIES

ORGANIZATION OF THE CONFERENCE SUPPORTED BY:
Ministry of Education, Science and Technological Development Republic of Serbia
Ministry of Agriculture and Environmental Protection
Provincial Secretariat for Agriculture, Water and Forestry AP Vojvodina

GENERAL DONOR
Veterinary Institute "Subotica"

UNIVERZITET U BEOGRADU, POLJOPRIVREDNI FAKULTET - SRBIJA

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U OKVIRU PROGRAMA
EU FP7 PROJEKTA AREA

VII MEĐUNARODNA KONFERENCIJA ”VODA I RIBA”

ZBORNIK PREDAVANJA

Poljoprivredni fakultet, Beograd– Zemun, Srbija
10. – 12. jun, 2015. godine

IZDAVAČ
Univerzitet u Beogradu, Poljoprivredni fakultet

ZA IZDAVAČA:
Prof. dr Milica Petrović

GLAVNI I ODGOVORNI UREDNICI
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RAČUNARSKA OBRADA
Božidar Rašković

TEHNIČKO UREĐENJE KORICA
Božidar Rašković

ŠTAMPA
GRAPHIC, Novi Sad

TIRAŽ
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Održavanje skupa pomoglo:
Ministarstvo prosvete, nauke i tehnološkog razvoja Republike Srbije
Ministarstvo poljoprivrede i zaštite životne sredine Republike Srbije
Pokrajinski sekretarijat za poljoprivredu, vodoprivredu i šumarstvo AP Vojvodine

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MICHAEL CHATZIEFSTATHIOU[1, 2], STERIANI MATSIORI[3]
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NOVA ULOGA REGIONALNE SARADNJE U ODRŽIVOM RAZVOJU AKVAKULTURE I RIBARSTVA: MAKROREGIONALNE STRATEGIJE (DUNAVSKA, JADRANSKO - JONSKA) I REFORMISANA UREDBA EVROPSKE UNIJE O RIBARSTVU

Apstrakt

Akvakultura i ribarstvo mogu da doprinesu održivom razvoju, međutim da bi se ovo postiglo, potrebno je da postoji određenae politika. Razvojni put može da bude deo strateške politike, a pomak ka održivijim putevima zahteva strateške odgovore kroz javne uredbe i kompromise, koje takođe doprinose rešavanju nekih konflikata. Uključivanje širokog spektra interesnih strana u ove procedure - na globalnom, regionalnom, nacionalnom i lokalnom nivou – je neophodno da bi se osiguralo da su predložene politike efikasne, koherentne i održive i da doprinose dugoročnoj održivosti životne sredine, ekonomskoj i socijalnoj održivosti sektora i celog regiona.

Osnovni činilac razvojnog programa za unapređenje održivosti je stepen u kome se on bavi neophodnim kompromisima specifičnim za region. Imajući ovo u vidu, saradnja na regionalnom nivou sada ima još veću ulogu kada je reč o Evropskim politikama. Dobri primeri su Makroregionalne Strategije Evropske Unije (EUS) i reformisana Zajednička Ribarstvena Politika (Common Fisheries Policy - CPF), koje uzimaju u obzir činjenice da je Evropska Unija pozitivno orijentisana ka životnoj sredini, posvećena principu održivog razvoja, takođe najveće jedinstveno tržište riba u svetu, ali i uvoznik ribe i proizvoda od ribe. Ovaj rad u kratkim crtama predstavlja glavne principe koji stoje iza pomenutih strategija i politika i ishode koji će iz njih proizići i koji će uticati na upravljanje sektorom, kao i na njegovu održivost.

„Makroregionalna Strategija” je integrisani radni okvir, preporučen od strane Saveta Evrope, čiji je cilj bavljenje izazovima u određenim geografskim oblastima gde se nalaze i države članice Evropske Unije kao i treće zemlje. Cilj ove strategije jeste da pojača saradnju radi postizanja ekonomske, društvene i teritorijalne povezanosti. Trenutno postoje tri makro regionai strategije za njih: Strategija Evropske Unije za Dunavski region (EUSDR) i Evropska Strategija za Jadranski i Jonski region (EUSAIR) koji imaju određeni značaj za naš region dok se treća strategija (EUSBSR) bavi Baltičkim morem. Akvakultura i ribarstvo su važni sektori za Plavi Rast u Jadransko-Jonskom regionu, a zaštita životne sredine koja je obuhvaćena Dunavskom Strategijom je neophodna za održivi razvoj u ovom krhkom region, ali istovremeno i ekološki bogatom.

Cilj Uredbe o ribarstvu Evropske Unije - Zajedničke Ribarstvene Politike (Common Fisheries Policy - CFP) je da osigura da su akvakultura i ribarstvo održivi za životnu sredinu, kao i ekonomski i društveno i da obezbeđuju zdravu hranu za stanovnike Evropske Unije. Reformisana uredba Zajednička Ribarstvena Politika Evropske Unije podrazumeva novi način upravljanja, koji daje zemljama Evropske Unije bolju kontrolu na državnom i međunarodnom nivou nad četiri glavne oblasti ove uredbe: upravljanje u ribartsvu; međunarodne uredbe; uredbe o tržištu i trgovini; politike finansiranja uključujući i pravila o učešću interesnih strana iz oblasti akvakulture. Države članice Evropske Unije treba da sarađuju na regionalnom nivou da bi usvojile zajedničke preporuke i druge načine za razvoj i implementaciju mera očuvanja životne sredine i mera koje utiču na ribarske aktivnosti u oblastima koje su značajne za vodene resurse i životnu sredinu.

Ključne reči: održivi razvoj, akvakultura, ribarstvo, makro region, uredba o ribarstvu - Zajednička Ribarstvena Politika
Keywords: sustainable development, aquaculture, fisheries, macro-region, common fisheries policy

INTRODUCTION

For many people looking at the role of fisheries in development, the debate is between two possible paths: a robust and growing artisanal sector that is rooted in rural communities, and a modern industrial fishery that delivers maximum value from the resource. Alternatively, this choice can be seen as being between preserving a livelihood and food source benefitting the rural poor on the one hand, and on the other as economic growth. Rather than take sides in this debate, it is better to focus on the central role of good resource management as the fundamental basis for development in any form. Promoting growth in fisheries without first resolving weaknesses in fisheries governance is unlikely to lead to sustainable progress (OECD, 2013).

The development of aquaculture also fundamentally depends on the priorities and decisions taken at national and/or regional level. Aquaculture is a key component of the Blue Growth Initiative of the Food and Agriculture Organization (FAO) of the United Nations, due to its potential for sustainable socioeconomic growth, food security and employment. The 31st session of the Sub-Committee on Aquaculture of the FAO Committee on Fisheries

(COFI) (St. Petersburg, Russian Federation, 2013), outlined priorities including the need for bilateral, multilateral and regional cooperation to support the global advancement of aquaculture and the implementation of an ecosystem approach to aquaculture.

For both fisheries and aquaculture, the fundamental message is the same: sustainable growth in these sectors depends on respecting natural limits. Temporary surges in growth often come at the cost of worsened future opportunities. These must be replaced by development efforts that focus on getting the most out of the resource with an emphasis on long-term planning and a well-supported and competent system of governance (OECD, 2013).

National and transnational cooperation, at every level, is a powerful asset and should be further nurtured in order to promote sustainable stock exploitation and sound aquaculture growth in our region, the Mediterranean and Black Sea. In light of the need to drive forward this process, all interested parties at the regional level must involved and should provide general agreed policy directions and adjusted technical guidelines while ensuring enhanced transparency and participation.

REGIONAL COLLABORATION

Aquaculture and fisheries can contribute to growth and development, and can support rural communities and better nutrition (OECD, 2013). Policies to support fisheries and aquaculture’s role in development can be effective, but coherence for development demands an inclusive approach encompassing all aspects of managing a sector based on a complex and renewable natural resource. Competing policy interests combined with governance failures, administrative capacity constraints and changing global fish production and consumption patterns have led to mismanagement, degradation and overexploitation of fisheries in many cases. In order to reverse these trends, short-term questions of employment and profits of fishers must be carefully balanced against longer term sustainability.

Involving an extensive range of stakeholders at global, regional, national and local levels - such as governments, multilateral institutions, the private sector, regional fisheries organisations and regional banks - has to be seen as a key factor in ensuring that policies are effective, coherent and sustainable (OECD, 2013). It also enables stakeholders to ask the question of whether there are alternative and more sustainable development paths for the region and what they might look like, and to consider explicitly the types of policy response that would secure a change from existing to alternative paths.

In doing so it allows a challenge to prevailing trends and to inertia in policy making caused by an acceptance that the only way to develop is by continuing on the present path. Key issue for a development programme concerned with enhancing its sustainability is the degree to which it addresses regionally specific and significant trade-offs. The key questions (GHK *et al*, 2002) are:

- Whether the trade-offs are considered important to the achievement of regional aspirations for social welfare? and if so,
- Whether additional measures, explicitly framed as a response to the trade-offs, and designed to minimise the negative consequences (particularly in relation to perceived critical thresholds) and enhance the positive effects, should be integrated within the programme?

This implies that the development path can be the subject of or described by a vision and strategic policy goals; and that the move towards more sustainable paths requires strategic responses to trade-offs, requiring some conflict resolution, through public policy and public policy agencies. This would note that there are some regional level trade-offs that are outside the direct influence of regional stakeholders, and which require policy responses at higher levels; with the attendant need to integrate across policy levels (GHK et al, 2002).

MACROREGIONAL STRATEGY (EUS)

Since 2009 the Directorate General for Regional and Urban Policy has been engaged in a new approach to tackle problems of a cross-regional nature, the “Macroregions”. The “Macroregional Strategy” is an integrated framework, endorsed by the European Council, to address common challenges in a given geographical area where both Member States and third countries are located. The objective is to strengthen cooperation in order to achieve economic, social and territorial cohesion.

Macroregional strategies have to concentrate their actions within a specific geographic area, but without creating new-barriers in the EU and stressing the importance of due involvement of all EU Member States and all interested stakeholders on transnational, regional and local level, as appropriate, in the implementation of the Strategies. At this stage, the Union has initiated three Macro-regional Strategies: the European Council endorsed the EU strategy for the Baltic Sea Region (EUSBSR) in October 2009, the EU Strategy for the Danube Region (EUSDR) in June 2011, and in addition, the newest EU Strategy for the Adriatic and Ionian Region (EUSAIR) on 23-24 October 2014.

The EU’s Baltic and Danube macro-regional strategies, involving over 20 EU and non-EU countries, have pioneered a unique kind of cooperation. This is based on the idea that common challenges facing specific regions - whether environmental, economic, territorial or security-related, are best tackled collectively. Furthermore, it makes sense to plan together for the most effective deployment of the resources available. The approach provides an integrated framework bringing together Member States and non-EU countries in the same geographical area to address common challenges. The overriding aim of a macro-regional strategy is to mobilise new projects and initiatives. The approach offers many potential benefits in terms of strengthened cooperation for economic, social and territorial cohesion.

A specific pillar dedicated to the environment in the Danube Strategy is essential for this ecologically rich and often fragile Region, to ensure that progress on environmental actions and projects can be closely monitored.

The pillar focuses on three Priority Areas: (1) To restore and maintain the quality of waters; (2) To manage environmental risks and (3) To preserve biodiversity, landscapes and the quality of air and soil.

On 14 December 2012, the Heads of State or Government of the EU called on the European Commission to bring forward an EU Strategy for the Adriatic and Ionian Region (EUSAIR) before the end of 2014. The proposed Strategy will build on the experience gained in the existing ones for the Baltic Sea macro-region and the macro-region along the Danube River. It will also incorporate the Maritime Strategy for the Adriatic and Ionian Seas, which was adopted by the European Commission on 30 November 2012.

The EU Strategy for the Adriatic and Ionian Region aims to consolidate the already extensive cooperation among the 8 countries of the region, thanks to existing European cooperation programmes and other regional schemes, such as the Adriatic Ionian Initiative.

A macro-regional strategy for the Adriatic and Ionian Region aims to give a new impetus to the cooperation, and to find joint solutions to common challenges.

Eight (8) countries in the Adriatic and Ionian Region are covered by the Strategy: four EU Member States (Croatia, Greece, Italy and Slovenia) and four non-EU countries (Albania, Bosnia and Herzegovina, Montenegro and Serbia). The 8 countries of the Region are not only diverse in terms of socio-economic development and geographic size, but also have different international status as only 4 of the partner countries are EU members.

The biggest issues which need to be tackled in cooperation are related to environment, transport and energy. The main opportunities for development are linked to maritime and marine innovation, blue growth and tourism. The main objective of the proposed Strategy is to promote sustainable economic and social prosperity in the Region, by improving its attractiveness, competitiveness and connectivity, while at the same time preserving the environment and ensuring healthy, balanced marine and coastal ecosystems. The Strategy is also expected to contribute to the EU integration of the candidate and potential candidate countries in the region.

EUSAIR has the capacity to contribute to the achievements of the objectives of the “EUROPE 2020” Strategy, to ensure smart, sustainable and inclusive growth, to increase the competitiveness of the European Union, and to contribute to the reinforcement of existing EU horizontal policies. In its initial phase, it is envisaged that the Strategy will be concentrated on the following four pillars: 1) Marine and Maritime Growth (Blue Growth), 2) Connecting the Region, 3) Environmental quality, and, 4) Sustainable Tourism.

Moreover, there will be two cross-cutting issues: a) Capacity building, including communication, and, b) Research, innovation and SMEs.

Fisheries and aquaculture are important sectors for Blue Growth in the Adriatic-Ionian Region. In particular, the social, cultural and economic contribution of fisheries is crucial at local and regional level, especially on islands and in remote regions (Chatziefstathiou *et al*, 2013). Coordinators of the Pillar 1 are Greece and Montenegro.

The **overall objective** of Pillar 1 “Blue Growth” is about driving innovative maritime and marine growth in the Adriatic-Ionian Region by promoting sustainable economic growth and jobs as well as business opportunities in the blue economy sectors. This requires building on the regional diversity in the Adriatic-Ionian Region, and taking into account that there are various pathways to innovative maritime and marine growth. At the same time a number of challenges and development opportunities need to be approached through cooperation among the countries, regions and cities.

*The **specific objectives** for this pillar are:*

1. To promote research, innovation and business opportunities in blue economy sectors, by facilitating the brain circulation between research and business communities and increasing their networking and clustering capacity.
2. To adapt to sustainable seafood production and consumption, by developing common standards and approaches for strengthening these two sectors and providing a level playing field in the macro-region.
3. To improve sea basin governance, by enhancing administrative and institutional capacities in the area of maritime governance and services.

To achieve the abovementioned objectives Pillar 1 will focus on three ***topics****:*
Topic 1 - **Blue technologies**
Topic 2 - **Fisheries and aquaculture**
Topic 3 - **Maritime and marine governance and services**

Fisheries cooperation is well advanced in the Adriatic - Ionian Sea basins. Networking and synergies among different initiatives is useful to strengthen dialogue and opportunities, to coordinate and establish our priorities. There is a need for stronger cooperation in order to protect our seas, particularly through sustainable fisheries methods within the framework of EU legislation. This is the best way to protect many fisheries enterprises that are facing strong crisis. We must take into account fisheries legislations of EU members and non-member countries and the different level of their development in the field of fisheries.

We also should consider and respect before any new action the existing complex legal framework for fisheries in the Mediterranean, in particular, the EU legislation, the rules issued by GFCM, ICCAT, etc. EU member states could support non-member states in the area on the implementation of the technical measures needed to harmonize their governmental legislation with the EU regulations.

EU COMMON FISHERIES POLICY (CFP)

The CFP is a set of rules for managing European fishing fleets and for conserving fish stocks. Designed to manage a common resource, it gives all European fishing fleets equal access to EU waters and fishing grounds and allows fishermen to compete fairly. Stocks may be renewable, but they are finite. Some of these fishing stocks, however, are being overfished. As a result, EU countries have taken action to ensure the European fishing industry is sustainable and does not threaten the fish population size and productivity over the long term.

The CFP was first introduced in the 1970s and went through successive updates, the most recent of which took effect on 1 January 2014. The new Common Fisheries Policy (CFP) has been agreed by Council and Parliament and is effective from 1 January 2014. On 13 July 2011, European Commission presented its proposals for the reform of EU common fisheries policy. On 2 December 2011, it proposed a new fund for EU's maritime & fisheries policies for the period 2014-2020: the European maritime and fisheries fund (EMFF).

The new CFP aims to ensure that fishing and aquaculture are environmentally, economically and socially sustainable and that they provide a source of healthy food for EU citizens. Its goal is to foster a dynamic fishing industry and ensure a fair standard of living for fishing communities. Although it is important to maximise catches, there must be limits. We need to make sure that fishing practices do not harm the ability of fish populations to reproduce. The current policy stipulates that between 2015 and 2020 catch limits should be set that are sustainable and maintain fish stocks in the long term.

To this day, the impact of fishing on the fragile marine environment is not fully understood. For this reason, the CFP adopts a cautious approach which recognises the impact of human activity on all components of the ecosystem. It seeks to make fishing fleets more selective in what they catch, and to phase out the practice of discarding unwanted fish. The reform also changes the way in which the CFP is managed, giving EU countries greater control at national and regional level. The CFP has 4 main policy areas: 1) Fisheries management, 2) International policy, 3) Market and trade policy, 4) Funding of the policy. The CFP also includes rules on aquaculture and stakeholder involvement

The EU is the largest single fisheries market in the world and a net importer of fish and fish products. More than a quarter of the fish caught by European fishing boats are actually taken outside EU waters. Around 8 % of EU catches (2004-06) are made under fishing agreements with countries outside the EU, while another 20 % are taken on the high seas, mainly in regions under the care of regional fisheries management organisations.

As a major fishing power, and the largest single market for fisheries products in the world, the EU also plays an important role in promoting better governance through a number of international organisations. This involves developing and implementing policy on fisheries management and – more generally – the Law of the Sea. The EU works closely with its partners from around the globe through the United Nations system, including the Food and Agriculture Organisation (FAO), as well as in other bodies, such as the Organisation for Economic Co-operation and Development (OECD).

What is “Regionalisation” and how it works

It is useful and much easier to cooperate through common projects on subsidiary activities to fisheries that can give an important help to fishing enterprises in today crisis. Transborder projects on Aquaculture, Fish-tourism, promotion of marine culture, training were very welcome already by all parts.

Management of fishing stocks until now is EU exclusive competence, and regulation of fishing activities at Member States territorial waters could be performed only at EU level. In international waters prevailing legal instruments already exist within the General Fisheries Commission for the Mediterranean (GFCM), and within the International Commission for the Conservation of Atlantic Tuna (ICCAT). Hence, establishment of management plans at sub-regional level could not be envisaged until now, unless carried out in full respect of the existing EU and multilateral frameworks. Other initiatives can be undertaken at this level for sustainable fisheries management, such as contribution to the scientific basis.

Following the reform of CFP, from now-on according to Article 18 of the regulation where the Commission has been granted powers (e.g. in multiannual plans) to adopt measures by means of delegated or implementing acts in respect of a Union conservation measure applying to a relevant geographical area, Member States having a direct management interest affected by those measures may, and within a deadline to be stipulated in the relevant conservation measure and/or multiannual plan, agree to submit joint recommendations for achieving the objectives of the relevant Union conservation measures, the multiannual plans or the specific discard plans. The Commission shall not adopt any such delegated or implementing acts before the expiry of the deadline for submission of joint recommendations by the Member States.

Member States (MS) should cooperate at regional level in order to adopt joint recommendations and other instruments for development and implementation of conservation measures and measures affecting fishing activity in areas protected by environmental law.

In the framework of regional cooperation, the Commission should only adopt conservation measures through implementing acts or delegated acts where all Member States concerned in a region agree on a joint recommendation.

The Union should cooperate with third countries and international organisations for the purpose of improving compliance with international measures. The position of the Union should be based on the best available scientific advice. The Union should promote the objectives of the CFP internationally, ensuring that Union fishing activities outside Union waters are based on the same principles and standards as those applicable under Union law, and promoting a level–playing field for Union operators and third-country operators.

To this end, European Union should seek to lead the process of strengthening the performance of regional and international organisations in order to better enable them to conserve and manage marine living resources under their purview, including combating illegal, unreported and unregulated (IUU) fishing.

Aquaculture

Farming finfish, shellfish and aquatic plants is one of the world’s fastest growing food sectors; it already provides the planet with about half of all the fish we eat. Mediterranean Aquaculture has a long history and there is evidence of existence dated back to more than 2.000 years ago. Intensive marine aquaculture made possible at 1980s, when technical difficulties in reproduction, feeds and cage technology were overcome. In Europe, aquaculture accounts for about 20% of fish production and directly employs some 80.000 people. EU aquaculture is renowned for its high quality, sustainability and consumer protection standards. EU overall output has been more or less constant in volume since 2000 whereas global production has been growing at nearly 7% per year.

The Commission intends to boost aquaculture through the Common Fisheries Policy reform, and has published Strategic Guidelines presenting common priorities and general objectives at EU level. One of the main goals of CFP is also to promote the development of sustainable Union aquaculture activities to contribute to food supplies and security and employment. Four (4) priority areas have been identified in consultation with all relevant stakeholders: reducing administrative burdens; improving access to space and water; increasing competitiveness; exploiting competitive advantages due to high quality, health and environmental standards.

Aquaculture should contribute to the preservation of the food production potential on a sustainable basis throughout the Union so as to guarantee long-term food security, including food supplies, as well as growth and employment for Union citizens, and to contribute to meeting the growing world demand for aquatic food.

On the above basis, the Commission and EU countries will collaborate to help increasing the sector’s production and competitiveness. EU countries are asked to set up multiannual plans to promote aquaculture. The Commission will help with the coordination and exchange of best practices. Farmed in the EU - a spinoff of the Inseparable campaign - promotes sustainable seafood and highlights the importance of aquaculture, as one of the world’s fastest growing food sectors.

Market organisation

The Common Organisation of the Markets, the EU policy for managing the market in fishery and aquaculture products, is one of the pillars of the Common Fisheries Policy.

The Common Organisation of the Markets strengthens the role of the actors on the ground: producers are responsible for ensuring the sustainable exploitation of natural resources and equipped with instrument to better market their products. Consumers receive more and better information on the products sold on the EU market, which, regardless of their origin, must comply with the same rules. Thanks to dedicated tools, it is now possible to have a better understanding of how the EU market functions.

Today, the Common Organisation of the Markets has come a long way from its beginnings and is a flexible instrument that ensures the environmental sustainability and economic viability of the market for fishery and aquaculture products. The five main areas covered by the scheme are: Organisation of the Sector, Marketing standards, Consumer information, Competition rules, and Market intelligence.

European Maritime and Fisheries Fund (EMFF)

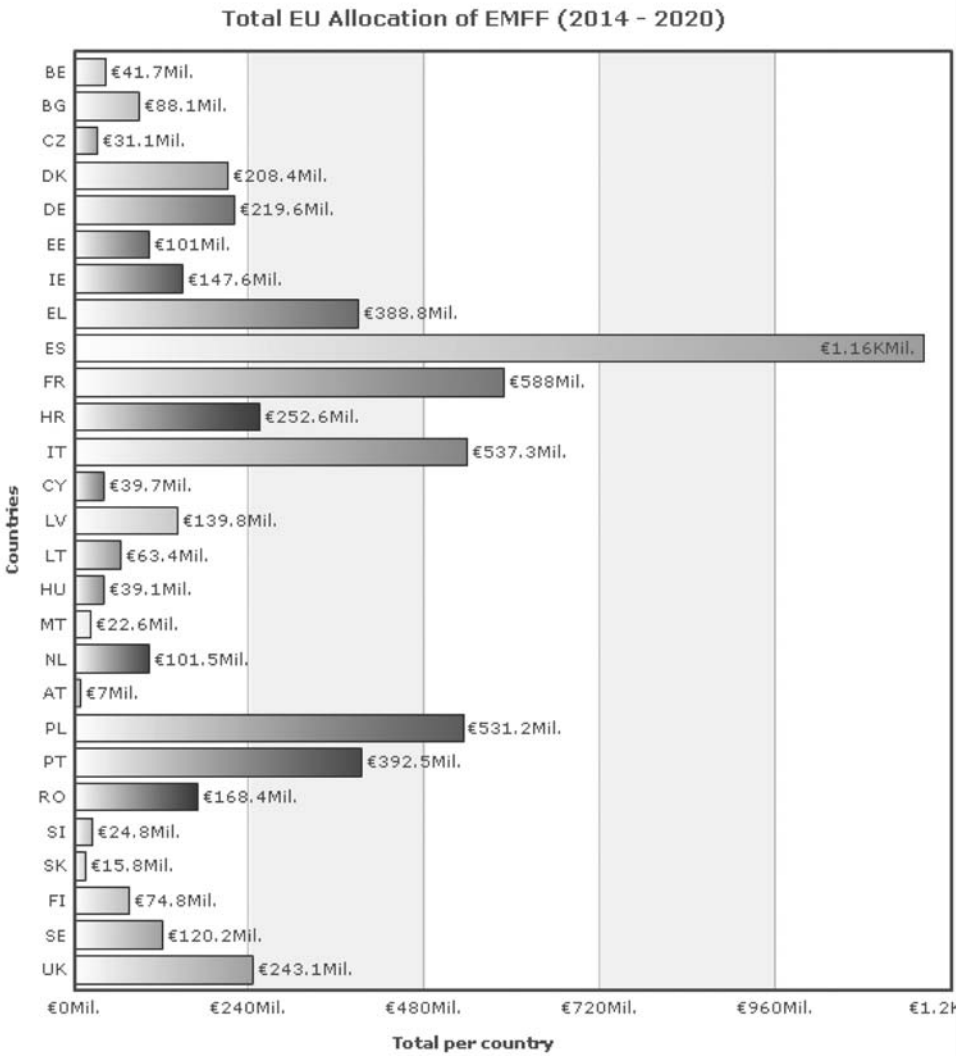
The EMFF is the fund for the EU’s maritime and fisheries policies for 2014-2020. It is one of the five European Structural and Investment (ESI) Funds which complement each other and seek to promote a growth and job based recovery in Europe. The fund helps fishermen in the transition to sustainable fishing, supports coastal communities in diversifying their economies, finances projects that create new jobs and improve quality of life along European coasts, makes it easier for applicants to access financing. The following table presents the financial allocation from EMFF per Member State.

The Fund is used to co-finance projects, along with national funding. Each country is allocated a share of the total Fund budget, based on the size of its fishing industry. Each country then draws up an operational programme, saying how it intends to spend the money. Once the Commission approves this programme, it is up to the national authorities to decide which projects will be funded. The national authorities and the Commission are jointly responsible for the implementation of the programme.

CLOSING REMARKS

This work, a policy paper, based on original EU texts, attempts to presents briefly the main principles behind these new strategies & policies - their differences from the older, and the possible outcome that will have to our region’s aquaculture & fisheries sector’s governance and sustainability - to colleagues that are not accustomed to European Union’s legislation and terminology, as the original documents are long, detailed, and complex, technical regulations. Their numbers are given below, at the references, for those seeking more information.

The main outcome from the synthesis of these policies is that regional collaboration will have a continuously increasing and enhanced role, and we must be prepared as a sector to work together, and implement swiftly these provisions, to gain the maximum benefit.



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GOVERNANCE IN AQUACULTURE IN CENTRAL AND EASTERN EUROPE AND THE ROLE OF PRODUCERS ASSOCIATIONS

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UPRAVLJANJE U AKVAKULTURI U CENTRALNOJ I ISTOČNOJ EVROPI I ULOGA UDRUŽENJA PROIZVOĐAČA

Apstrakt

Prepoznato je da je upravljanje važan element održivog razvoja akvakulture. Primena četiri principa upravljanja – odgovornost, učinkovitost i efikasnost vlada, pravičnost i predvidljivost vladavine prava – ima ključni značaj za održivi razvoj sektora akvakulture. Pored vlada, postoje i drugi učesnici koji upravljaju akvakulturom kao što su ljudske zajednice, nevladine organizacije i proizvođači (Hishamunda et al., 2014). Evropska Komisija je lansirala široku reformu upravljanja za unapređenje demokratskih procesa u EU, predložena su 4 osnovna pravca promena: veće učešće građana, efikasnije definisanje pravila i zakona, angažovanje u debati o globalnom upravljanju i na kraju, ponovno usredsređivanje zakona i institucija na jasne ciljeve. 2001. godine objavljen je document “Evropsko upravljanje – bela knjiga” (EC, 2001) koji identifikuje 5 principa koji su osnov dobrog upravljanja: (1) otvorenost (transparentnost i komunikacija prilikom donošenja odluka); (2) učešće/participacija; (3) odgovornost; (4) efikasnost; (5) koherentnost (uzimajući u obzir krajnje različite politike koje postoje u EU). U EU je Zajednička Politika Ribarstva, “Common Fisheries Policy (CFP)” osnovni okvir rukovođenja u ribarstvu i akvakulturi i osnovni finansijski instrument za podršku implementaciji Evropskog Maritimnog i Fonda Ribarstva, “European Maritime and Fisheries Fund (EMFF)”. Akvakultura je važan stub CFP od kako morska i slatkovodna akvakultura značajno doprinose radnim mestima i lokalnom razvoju, kao i tržištu namirnica - plodova mora i uključuje veliki broj pod-sektora, deo je Evropskog kulturnog nasleđa i dinamičan je i inovativan sector.

Komisija je 2013 objavila “Strateško uputstvo za održivi razvoj akvakulture u EU” (EC, 2013). Poglavlje 4 Uputstva objedinjuje osnovne činioce novog načina upravljanja koji podržava EU akvakulturu, na osnovu sledećeg: (1) priprema nacionalnog strateškog plana za nekoliko godina za promociju održive akvakulture od strane država članica; (2) komplementarnost sa Evropskim Maritimnim i Fondom za Ribarstvo (EMFF); (3) razmena dobrih praksi; (4) uspostavljanje Saveta za akvakulturu “Aquaculture Advisory Council

(AAC)". U okviru evropskog FP7 projekta "Aquainnova – Podrška upravljanju i učešću više zainteresovanih strana istraživanju i inovaciji u akvakulturi, Supporting governance and multi-stakeholder participation in aquaculture research and innovation" razvijen je dokument Budućnost Evropske akvakulture "The Future of European Aquaculture" (EATiP, 2012) koji uključuje documente Vizija akvakulture i Strateško istraživanje i inovacije. Evropska federacija proizvođača u akvakulturi (FEAP) je dobar primer aktivnog učešća proizvođača u upravljanju u akvakulturi kroz konsultacije interesnih grupa, stručne sastanke, projekte i publikacije. Iako su udruženja proizvođača iz samo 5 zemalja Centralne i Istočne Evrope (CEE) predstavljene u FEAP (Hrvatska, Češka Republika, Mađarska, Litvanija, Poljska), aktivnosti FEAP takođe doprinose upravljanju u slatkovodnoj akvakulturi koja dominira u CEE regionu. Vlade igraju odlučujuću ulogu u upravljanju u akvakulturi u nekim CEE zemljama i dalji napori su neophodni za ojačavanje organizacije proizvođača i njihove sposobnosti da im se glas čuje. Međunarodna saradnja može da pomogne ovim naporima naročito ona među organizacijama proizvođača u EU i ne-EU zemljama.

Ključne reči: slatkovodna akvakultura, upravljanje, udruženja proizvođača
Key words: freshwater aquaculture, governance, producers associations

Abstract

It has been well recognised that governance is an important element of sustainable aquaculture development. The application of the four governance principles – accountability, effectiveness and efficiency of governments, equity and predictability of the rule of law – has a vital importance for sustainable development of the aquaculture sector. In addition to governments, there are other participants in aquaculture governance such as communities, non-governmental organizations and producers (Hishamunda et al., 2014). In the European Union the Commission has launched a vast reform of governance in order to drive forward a wide-ranging democratic process in the EU, and proposes four major changes: more involvement of citizens, more effective definition of policies and legislation, engagement in the debate on global governance, and finally the refocusing of policies and institutions on clear objectives. In 2001 the document "European governance - A white paper" was published (EC, 2001) that identifies five principles that are the basis of good governance according to the followings: (1) openness (transparency and communication in decision making); (2) participation; (3) accountability; (4) effectiveness; and (5) coherence (taking into account the extremely diverse policies in the EU). In the European Union the Common Fisheries Policy (CFP) is main governing framework of fisheries and aquaculture and the financial instrument to support its implementation is the European Maritime and Fisheries Fund (EMFF). Aquaculture is an important pillar of the CPF since the marine and freshwater aquaculture is a significant contributor to job and local development, substantial contributor to market for aquatic food, it includes wide range of sub-sectors, part of European cultural heritage and a dynamic and innovative sector. A "Strategic Guidelines for the sustainable development of EU aquaculture" was published by the Commission in 2013 (EC, 2013). The 4th Chapter of the guideline summarizes the main issues of a new governance to support EU aquaculture according to the followings: (1) preparation of multiannual national strategic plan for the promotion of sustainable aquaculture by the Members States; (2) complementarity with the European Maritime and Fisheries Fund (EMFF); (3) exchange of best practices; (4) establishment of an "Aquaculture Advisory Council (AAC). In the frame

of an EU funded FP7 project "Aquainnova - Supporting governance and multi-stakeholder participation in aquaculture research and innovation" the document "The Future of European Aquaculture" was elaborated (EATiP, 2012) that includes the "Vision document" and the "Strategic Research and Innovation Agenda". The Federation of European Aquaculture Producers (FEAP) is a good example of the active participation of producers in aquaculture governance through stakeholder consultations, professional meetings, projects and publications. Although producers associations of only five countries are represented in FEAP from Central and Eastern Europe (Croatia, Czech R., Hungary, Lithuania, Poland), FEAP activities also contribute to the support of governance related to freshwater aquaculture that is dominant in the CEE region. Governments play decisive role in aquaculture governance in some CEE countries and further efforts are needed to strengthen the organisation level of producers and their capacity to make their voice heard. International collaboration may assist these effort especially between producers organisation in EU and non-EU countries.

1. GOVERNANCE IN AQUACULTURE

Since governance has been well recognised as an important element of sustainable aquaculture development the FAO Committee on Fisheries (COFI) Sub-Committee on Aquaculture requested FAO to prepare Guidelines for Improving Governance in Aquaculture. The report "Policy and Governance in Aquaculture – Lessons learned and way forward" (Hishamunda et al., 2014) summarizes the main issues facing general aquaculture governance, current best practices and potential challenges for the future. The application of the four governance principles – accountability, effectiveness and efficiency of governments, equity and predictability of the rule of law – are suggested as necessary for sustainable development of the aquaculture industry. Accountability and predictability provide assurances to entrepreneurs that property rights and contracts will be honoured, while intergenerational equity suggests ecological conservation. The principle of effectiveness and efficiency implies that regulation of aquaculture will be sufficient without being too troublesome, and also perhaps decentralization and public participation. Based on the four principles, administrative and legislative frameworks can assist aquaculture development to develop sustainably. In addition to governments, there are other participants in aquaculture governance such as communities, non-governmental organizations and producers.

In the European Union the Commission has launched a vast reform of governance in order to drive forward a wide-ranging democratic process in the EU, and proposes four major changes: more involvement of citizens, more effective definition of policies and legislation, engagement in the debate on global governance, and finally the refocusing of policies and institutions on clear objectives. In 2001 the document "European governance - A white paper" was published (EC, 2001) that identifies five principles that are the basis of good governance according to the followings: (1) openness (transparency and communication in decision making); (2) participation; (3) accountability; (4) effectiveness; and (5) coherence (taking into account the extremely diverse policies in the EU).

2. FISHERIES AND AQUACULTURE MANAGEMENT IN THE EU

The main governing framework of fisheries and aquaculture in the EU is the Common Fisheries Policy (CFP) that was first introduced in the 1970s and went through successive updates, the most recent of which took effect on 1 January 2014. The CFP has four main policy areas: (1) fisheries management; (2) international policy; (3) market and trade policy; and (4) funding of the policy (CPF, 2013). Funding is provided through the European Maritime and Fisheries Fund (EMFF). The amount allocated for the planning period of 2014-2020 is 6400 million €, however direct support for aquaculture development is less than 10 % of the total fund. Although fisheries management is a priority area of the Common Fisheries Policy, aquaculture is an important pillar of the CPF since the marine and freshwater aquaculture is a significant contributor to job and local development, substantial contributor to market for aquatic food, it includes wide range of sub-sectors, part of European cultural heritage and a dynamic and innovative sector. The European Commission elaborated the "Communication to give new impetus to the sustainable development of European aquaculture" in 2009 (EC, 2009), and in 2013 a "Strategic Guidelines for the sustainable development of EU aquaculture" was published by the Commission (EC, 2013). The 4th Chapter of the guideline summarizes the main issues of a new governance to support EU aquaculture according to the followings: (1) preparation of multiannual national strategic plan for the promotion of sustainable aquaculture by the Members States; (2) complementarity with the European Maritime and Fisheries Fund (EMFF); (3) exchange of best practices; (4) establishment of an "Aquaculture Advisory Council (AAC). The sector has been actively involved in the preparation of the "Strategic guidelines" through stakeholder consultations and a document "The Future of European Aquaculture" was also published in 2012 as a result of the EU funded FP7 project "Aquainnova" (EATiP, 2012). The title of the 'Aquainnova' project was "Supporting governance and multi-stakeholder participation in aquaculture research and innovation". In the frame of the project a "Vision document" and "Strategic Research and Innovation Agenda" has been elaborated. The coordinator of the project was the European Aquaculture Technology and Innovation Platform (EATiP) that has about 60 members from European commercial companies, research institutions national and international organisations. It should be mentioned however, that Eastern European stakeholders are extremely underrepresented in this important European aquaculture platform. Freshwater issues however are properly addressed in the "Vision" and "SRIA" of the document "Future of European Aquaculture" mainly through the active work of experts from Czech Republic, Hungary and Poland. The Freshwater Session of the Aquainnova project for example was organised in Warsaw, Poland.

3. PRODUCERS ASSOCIATIONS IN EUROPEAN AQUACULTURE GOVERNANCE

The Federation of European Aquaculture Producers (FEAP) is a good example of the active participation of producers in aquaculture governance through stakeholder consultations, professional meetings, projects and publications. The FEAP Code of Conduct is also good example of self-regulation. The code of conduct has nine themes that cover environment issues, consumer issues, husbandry, socio-economic issues, and the public image of the industry. Although producers associations of only five countries are represented in FEAP from Central and Eastern Europe (Croatia, Czech R., Hungary, Lithuania, Poland), FEAP activities also contribute to the support of governance related to freshwater aquaculture that is dominant in the CEE region. The Freshwater Commission of FEAP that has a Chair from Hungary regularly addresses freshwater issues (e.g. predation of wild animals, ecological services of fish ponds) and FEAP assisted the organisation of the Workshop of European Fishpond Producers that was held in Hungary in 2013. The draft resolution paper of the workshop was accepted later during the 2nd, International Carp Conference in Wroclaw. There are great differences in organisational level and activity of producers associations even in EU countries and their involvement in governance also show great diversity, however producers' organisations and their activity in non-EU Central and Eastern Europe (CEE) countries are hardly visible. In a survey in 2004 only few active producers' organisations have been identified in the CEE region (Bekefi et al., 2004) and limited information was available about their structure and function. The situation has not been changed much in the past ten years and level of the organisation of producers is still very low in some countries. Governments play decisive role in aquaculture governance in some CEE countries and further efforts are needed to strengthen the organisation level of producers and their capacity to make their voice heard. International collaboration may assist these efforts especially between producers organisation in EU and non-EU countries. The Network of Aquaculture Centers in Central and Eastern Europe (NACEE) having members from the academic sector and the industry in both EU and non-EU countries can also contribute to strengthening producers associations in member countries since the involvement of strong producers' association is a basic precondition of good governance.

CONCLUSIONS

Governance has been well recognised as an important element of sustainable aquaculture development. There have been efforts to improve governance both by international organisations (e.g. FAO, EU) and national governments. Although governments play primary role in governance, producers through their producers' organisation should play important role in aquaculture governance besides other participants such as communities and non-governmental organizations. The Federation of European Aquaculture Producers (FEAP) is a good example of the active participation of producers in aquaculture governance through stakeholder consultations, professional meetings, projects and publications. There are great differences in organisational level and activity of producers associations even in EU countries and their involvement in governance also show great diversity, however producers' organisations and their activity in non-EU Central and Eastern Europe (CEE) countries are hardly visible. Further efforts are needed to strengthen the organisation level of producers and their capacity to make their voice heard in some CEE countries. International collaboration may assist these efforts especially between producers organisation in EU and non-EU countries, since the involvement of strong producers' association is a basic precondition of good governance.

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FRESHWATER AQUACULTURE IN CENTRAL AND EASTERN EUROPE: NEEDS FOR RESEARCH AND DEVELOPMENT

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GAJENJE SLATKOVODNIH RIBA U CENTRALNOJ I ISTOČNOJ EVROPI: POTREBE ZA ISTRAŽIVANJEM I RAZVOJEM

Apstrakt

Gajenje slatkovodinih riba ima veoma važnu ulogu za stabilizaciju biodiversiteta, resursa podzemnih voda, klime regiona kao i za snabdevenost hranom u Centralnoj i Istočnoj Evropi. Velika konkurencija cena ribe na međunarodnom tržištu i promene u navikama ishrane ljudi pojačavaju pritisak na mala i srednja preduzeća u Centralnoj i Istočnoj Evropi koja se bave gajenjem ribe i mogu da ugroze predeo oko uzgojnih jezera. Da bi povećali vrednost svih karika u lancu proizvodnje slatkovodnih riba, naučnici i proizvođači su identifikovali uobičajene i potencijalne izazove. Da bi se procenile potrebe sektora akvakulture u centralnoj i istočnoj Evropi, sprovedeni su polu strukturirani intervjui sa proizvođačima i prerađivačima ribe i udruženjima u Poljskoj i Češkoj, u okviru projekta SIAD i FP7 EU projekta TRAFOON. Takođe su održane radionice sa istraživačima u sektoru akvakulture duž svake karike lanca proizvodnje riba u Centralnoj i Istočnoj Evropi. Različite interesne strane su identifikovale nekoliko izazova koji ometaju razvoj slatkovodne akvakulture u Centralnoj i Istočnoj Evropi. Razvoj akvakulture zahteva postojanje harmonizovanog regulativnog/pravnog okvira. Razmena znanja bi imala pozitivan uticaj na razvoj uniformnih standarda za održivost, i pomogla bi stvaranju pravnih procedura za izdavanje dozvola i licenci. Potrebno je izvršiti ekonomsku procenu usluga koje ekosistem ribnjaka pruža. Poboljšana tehnoligija koja je povoljna po životnu sredinu i sistemi za upravljanje kvalitetom u proizvodnji i preradi su neophodni da bi se obezbedila ustaljenija ponuda proizvoda od ribe viskog kvaliteta. Potrošači treba da budu bolje informisani o funkciji koju slatkovodna akvakultura i njeni proizvodi imaju za životnu sredinu.

Da bi došlo do održivog razvoja sektora akvakulture i da bi se izgradilo poverenje javnosti, veoma je važno podstaći društvene inovacije koje su primenljive na pojedinačni sektor ili pojedinačnu teritoriju. Da bi došlo do održivog razvoja sektora slatkovodne akvakulture potrebno je razviti strategije makro regionalnog razvoja koje su prilagođene određenim tržištima, uslovima životne sredine, ponašanju potrošača, kulturi i tradiciji i koje nude potencijal za razvoj izvan granica jedne zemlje. Razvoj i adaptacija ključnih tehnologija potrebni su da bi se stabilizovala konkurentna i održiva proizvodnja i prerada ribe u čijoj su proizvodnji resursi efikasno iskorišćeni.

Ključne reči: akvakultura slatkih voda, buduće teme za istraživanje, agenda za strateško istarživanje i inovacije, Evropska akvakultura

Key words: freshwater aquaculture, future research topics, strategic research and innovation agenda, European aquaculture

INTRODUCTION

The “Blue Economy” in Europe not only offers possibilities for aquaculture in coastal and marine areas but also in freshwater aquaculture. Especially in Central and Eastern Europe with the abundant water resources, freshwater aquaculture has a great potential to increase European fish production and generate income in rural areas as targeted in the (EATiP 2012).

Fish and seafood play an important role in human nutrition by providing essential fatty and amino acids, minerals and vitamins (FAO 2014). However, fish consumption in Central and Eastern European regions is the lowest in Europe (FAO 2014) and is below the level recommended by nutritionists. The demand of European consumers for sustainably produced and high quality fish products can only be covered if aquaculture makes a substantial contribution (FAO 2014). European markets depend to a large extent on imported fishery and aquaculture products mainly from Asia (FAO 2014). However, China may change from the world’s largest exporter to a net-importer of fish products in the next two decades as their own domestic market expands (World Bank 2014). Utilizing the potential of freshwater aquaculture production in Europe could be part the EU’s Blue Economy Strategy to increase food security in the Europe and reduce dependence on imports in the long-term.

Ponds are one of the most important sites for aquaculture and have several functions in addition to food production. They play an important role in the landscape by maintaining biodiversity, fostering the regeneration of groundwater resources, stabilizing the regional climate, diversifying land utilization and food supply, and increasing income potential (Kerepeczki et al., 2011). Ponds have been an element of the landscape for centuries with pond fish as an essential feature of traditional and religious cuisine. However, aquaculture can only fulfil the above functions if production is economically, socially and environmentally sustainable. In Central and Eastern Europe, consisting also of land-locked countries, pond aquaculture has been more important and more prominent than in other European regions. In the current stressful economic environment, production from pond aquaculture in these countries has already decreased in the past 20 years or is at risk to decrease in future. This poses a threat to the ecological and cultural functions of ponds in the region. In addition, the current severe price competition in international markets for fish and the associated changes in dietary habits of the population increase the pressure on small and medium-sized enterprises (SMEs) that produce and process fish in Central and Eastern Europe, and threaten the existence of the local pond aquaculture landscape. The Central and Eastern European region is undergoing political and economic transformation and so this is a perfect time to implement strategies for sustainable aquaculture development in order to maintain the ecological function of pond aquaculture and increase food quality and rural development.

MATERIALS AND METHODS

To assess the needs, challenges and potentials of the freshwater aquaculture sector in Central and Eastern Europe, several stakeholders from the aquaculture sector were included into the analysis. To assess the needs of small and medium fish producers, semi-structured interviews with aquaculture producers, processors and associations were performed in two Central European countries (Poland and Czech Republic) within the EU FP7 project TRAFOON. The findings were quantitatively and qualitatively analysed by a multi-stakeholder workshop (7-8/10/2014, Vodnany, Czech Republic). Within the SIAD project, two workshops of researchers of different aquaculture disciplines were organized (4-5/6/2014, Vodnany, Czech Republic, and 1-3/12/2014, Stuttgart, Germany) to identify common challenges of and potential solutions for the freshwater aquaculture sector specifically in the Danube region. The Central European Aquaculture Conference (27-28/11/2014, Budapest, Hungary) was performed to identify common challenges and potentials of the aquaculture sector in Central and Eastern Europe (and associated countries). The results of the inventories and the four workshops were used to collectively develop this paper.

RESULTS AND DISCUSSION

Within the multi-stakeholder workshops the following challenges and potentials of boosting freshwater aquaculture in Central and Eastern Europe were identified. The development of aquaculture is not only influenced by the legislation specific for the aquaculture sector but also by numerous other laws such as the water framework directive, other environmental and natural conservation legislation, and regulations governing regional planning and construction, animal welfare, food safety etc. Some of these regulations from different sectors have conflicting effects on the aquaculture sector and need to be harmonized in order not to impede its development. Many of the challenges facing Central and Eastern European freshwater aquaculture are common to all parts of the region. Sharing knowledge and best practices will help to develop uniform standards for sustainability, and facilitate the legal procedures for the granting of permits and licences.

The prerequisite for aquaculture and its development is the achievement/ conservation of good water quality in the rivers and water sources. Sustainable aquaculture development depends on the enforcement of water quality regulations at regional and local levels. An economic valuation is urgently needed to quantify the ecosystem services of pond aquaculture. A specific compensation payment system for fish ponds should be elaborated based on the value of ecosystem services achieved by pond aquaculture and should apply the principle “public money for public goods”. There is a need for farmers to improve environmental-friendly technologies and quality management systems in order to ensure a steadier supply of high quality fish. This can be achieved by combining extensive and intensive production technologies and polyculture systems which will improve the efficiency of resource use, animal welfare, and net economic returns and place greater emphasis on the ecological function of aquaculture systems. Adaptation strategies must be developed so the region can be resilient in the face of climate change and the threat of fish diseases as well as development of new food-borne diseases.

Higher consumption of freshwater fish as a healthy food must be promoted. Consumer attitudes and behaviour must be evaluated at regional and trans-regional levels to identify consumer groups that can be targeted specifically by a differentiation of fish products and improvement of product presentation to meet the needs of consumers nutritionally and culturally. The development of technologies and standards in production and processing is essential to increase all aspects of fish product quality including nutritional value, sensory attributes and product safety. Consumers need to be informed more intense about the environmental function of freshwater aquaculture and its products by means of awareness campaigns that target the different consumer groups with appropriate information. Suitable use of labelling, certification and quality standardization are essential so that consumers will trust aquaculture products and include them in their diets on the long term. In order to allow sustainable development of the aquaculture sector and build public confidence, it is important to encourage the formation of producer and consumer associations, and communication platforms for stakeholders at all points along the production chain.

Worldwide, freshwater aquaculture is the predominant production system for fish. The leading countries outside the EU in terms of quantity of production are China, India and the ASEAN countries. The country with the most intensive systems is Israel. Fostering cooperation with these regions will be beneficial to the development of European aquaculture.

CONCLUSIONS

The sustainable development of the freshwater aquaculture sector requires macro-regional development strategies that are tailored to specific markets, environmental circumstances, consumer behaviour, cultures and traditions and offer the potential for cross-border development. This development and adaptation of key technologies is needed to stabilise a competitive, sustainable and resource-efficient fish production and processing. The development of the aquaculture sector in this region should be guided by principles of sustainability (environmental, social, and economic) and by the potential for increasing human and animal welfare.

ACKNOWLEDGEMENTS

We thank the EU FP7 project TRAFOON (grant no. 613912), the SIAD project (German BMBF, grant no 01DS13022), and the European Fishery Fund for financially supporting these activities. We especially thank the fish producing farmers for sharing their perspectives.

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THE SPAWNING EFFICIENCY INDEX AS A TOOL IN AQUACULTURE RESEARCH AND PRODUCTION

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INDEKS EFIKASNOSTI MRESTA KAO ALATKA ZA ISTRAŽIVANJE I PROIZVODNJU U AKVAKULTURI

Apstrakt

Intenzivno gajenje u akvakulturi je vrsta proizvodnje koja zahteva strogu kontrolu celog proizvodnog procesa. Ovo takođe važi za protokol reprodukcije, koji predstavlja prvi korak u intenzivnoj akvakulturi.

Efikasan protokol za reprodukciju je obično zasnovan na jednom broju veoma detaljnih eksperimenata, gde se svi faktori odgovorni za efikasnost mresta proveravaju. Međutim, glavna slaba tačka ovog procesa je ta što su zaključci o efikasnosti pojedinačnih protokola zasnovani na kratkoročnim ekperimentima, čiji su glavni indikatori preživljavanje embriona, stopa izleganja ili uopšteni kvalitativni parametri ranih stupnjeva larvi. Cilj ove studije je da proveri da li specijalno dizajniran indeks efikasnosti mresta (koji predstavlja broj mlađi proizveden u odnosu na jedinicu težine ženki) može da pokaže značajne razlike i slabosti najčešće korišćenih markera za kvalitet jaja, koji su indikatori efikasnosti mresta. Izabrana vrsta za model bio je Evroazijski grgeč, koji je danas jedan od najboljih kandidata za gajenje u intenzivnom sistemu u slatkim vodama.

Ribe su, u toku jeseni uzete iz zemljanog ribnjaka. Riba je bila izložena hladnim vremenskim uslovima u periodu od 40 dana. Ženke su zatim, kada je temperatura vode ponovo dostigla 10°C, nasumično raspodeljene u 6 tretmana (n=10 za svaku grupu). Svaka grupa bila je podvrgnuta različitim protokolima stimulacije hormona sa lososovim GnRHa (10, 25, 50 i 100 µg kg^{-1} u toku prvog ubrizgavanja i sa100 µg kg^{-1} u toku drugog, sedam dana posle prve aplikacije). Nakon toga, ribe su stavljene u odvojene tankove od 300 l.

Posle drugog ubrizgavanja, temperatura je podignuta na 12°C a svakom tanku dodato je 10 muških jedinki (koje su 4 dana ranije primile injekciju hCG u dozi od 500 IU kg^{-1}).

Riba je ostavljena da se spontano mresti. Jaja su sakupljana u narednih 10 dana i inkubirana na 14°C. Stopa preživljavanja embriona ustanovljena je u stadijumu očnog mehura. Posle izvaljivanja larve grgeča su gajene (iz svake grupe odvojeno) prateći isti protokol u toku od 48 dana. Larve su prvo hranjene sveže izvaljenim *Artemia* nauplii. 28og dana, gajene larve su nasilno odviknute i prebačene (bez uporedog hranjenja) na komercijalnu hranu.

Ustanovljena je stopa preživljavanja mlađi iz svake grupe posle odvikavanja i ukupna dužina tela (TL, mm). Izračunat je i indeks efikasnosti mresta (SEI) za svaku grupu, i on predstavlja broj odviknutih riba dobijenih od 1 kg ženki.

U kontrolnoj grupi 1 (koja je dva puta tretirana placebom) ovulacija se nije desila. U kontrolnoj grupi 2, dve ribe su ovulirale. Primena procesa u nekoliko ponavljanja izazvala je ovulaciju u 40 i 60%. Stopa preživljavanja embriona kod sakupljenih jaja bila je najniža u kontrolnoj grupi II. Među ostalim grupama zabeležena je slična stopa preživljavanja embriona. Nije bilo razlika među izlegnutim larvama, ako se uzme u obzir efikasnost punjenjaribljeg mehura kao i krajnja TL proizvedene ribe. Najniže SEI vrednosti zabeležene su u kontrolnoj grupi II. Najviše SEI vrednosti zabeležene su kod ribe koja je inicijalno tretirana sa 10 i 25 μg kg^{-1}.

Rezultati ove studije pokazuju da je SEI vrednost jako dobar i siguran indicator koji dozvoljava pouzdanu verifikaciju reproduktivnih protokola. Međutim, čini se da broj proizvedenih larvi sa napunjenim mehurom takođe može da posluži kao dobar indikator kvaliteta mresta. Ovakav indikator bi načinio process verifikacije efikasnosti reproduktivnih protokola mnogo kraćim i manje napornim, nego što se to ranije mislilo.

Takođe, rezultati ove studije pokazuju da ponavljana primena GnRHa može da poboljša mrest izvan sezone. Međutim, potrebno je raditi na optimizaciji doza i intervalima u kojima se daju injekcije da bi se proverila korisnost ovog procesa.

Ključne reči: hormonski tretman, efikasnost reprodukcije, gajenje percida, kvalitet jaja
Key words: hormonal treatment, reproduction effectiveness, percid culture, egg quality

INTRODUCTION

Intensive aquaculture is a type of production which requires strict control over the entire production process. This applies also to the reproductive protocol, which is the first step of intensive aquaculture (Mylonas et al. 2010, Żarski et al. 2011a).

Effective reproductive protocol is usually based on a number of very detailed experiments. However, the main weakness is that the conclusions are very often drawn on the base of short-term experiments where main indicators were embryonic survival, hatching rate or general qualitative parameters of early larval stages (Ronyai and Lengyel 2010, Żarski et al. 2011a, 2011b). Up to now there is no data clearly showing how different reproductive protocols affecting larvae quality from the perspective of further rearing procedures.

Eurasian perch, *Perca fluviatilis* L., is one of the most perspective freshwater fish species dedicated to the intensive aquaculture. Although huge progress has been made the seasonality of production is still one of the main problems in intensive production. To this end, many efforts have been undertaken at the development of out-of season reproduction which would allow production of stocking material year round. However, variable spawning effectiveness is usually observed. This probably stem from the fact that the research on reproduction very rarely consider the suitability of the produced larvae for further production process. In effect, many protocols considered survival rate of embryos and general quality of freshly hatched larvae (Kucharczyk et al. 1996, 1998, Kouril et al. 1997, Ronyai and Lengyel 2010, Żarski et al. 2011a, 2011b) as an egg quality indicators. It should be, however, emphasized that in the case of Eurasian perch even very low quality egg may exhibit developmental competence until hatching (Żarski et al. 2011b). Therefore, the aim of the study was to verify whether the specifically designed spawning efficiency index (which represents the number of weaned juveniles produced from a weight unit of females) may reveal the significant differences and weaknesses of typically used markers of egg quality being the indicators of spawning effectiveness.

MATERIALS AND METHODS

The fish (100 females and 75 males with an average weight of ~120 g) were obtained from the earthen ponds during autumn. Fish were kept in a recirculating aquaculture system (RAS) in 1000 l tank where they were subjected to 40 day-long vernalization period during which fish were kept in constant dimness (<5 lx). Next, when temperature was raised back to 10°C the females were divided randomly into 6 treatment groups (n=10 for each group). Each group were subjected to a various hormonal stimulation protocols (Tab. 1) and next they were placed separately to a 300 l tanks. After the second injection temperature was raised to 12°C and to each tank 10 males (injected 4 days in advance with the hCG at a dose of 500 IU kg^{-1}) were placed. The fish were left to spawn spontaneously. The eggs were collected for the next 10 days and incubated at 14°C. At the eyed-egg stage the survival rate of embryos was determined. After hatching, larvae were reared (from each group separately, in triplicates) with the same protocol for 48 days. Briefly, larvae were reared for the first 12 days at 15°C. At that time the swim bladder inflation effectiveness (SBIE - representing percentage of larvae with inflated swim bladder) was determined under the stereoscopic microscope. Next the temperature was gradually increased up to 25°C. Larvae were exposed to constant light conditions (24L:0D) for the entire rearing period. Larvae were fed first with freshly hatched *Artemia* nauplii twice a day *ad libitum* (the food was available for the larvae all the time). On day 28 of rearing larvae were transferred to the other RAS, were they were placed in 50 l tanks with the same stocking density (20 ind. l^{-1}). Then, fish were then weaned sharply (without co-feeding) into commercial diet. The tanks were cleaned twice a day. All the dead fish were counted in order to estimate the final survival rate of larvae. At the last day of rearing the number as well as total length (TL, mm) of juveniles produced from each group was determined. The spawning efficiency index (SEI), representing the number of weaned fish obtained from 1 kg of females, was calculated for each group.

The results obtained for different groups were analysed with one-way analysis of variance (ANOVA) and Tukey post-hoc test ($p<0.05$) was applied.

Table. 1. The hormonal treatment protocol applied during the experimental out-off season spawning of Eurasian perch. As a spawning agent pure salmon gonadoliberine analog was used (sGnRHa). Placebo treatment refers to the injection with 0.9% NaCl solution.

Group	1st injection	2nd injection
Control I	Placebo	Placebo
Control II	Placebo	100 µg kg^{-1}
E1	10 µg kg^{-1}	100 µg kg^{-1}
E2	25 µg kg^{-1}	100 µg kg^{-1}
E3	50 µg kg^{-1}	100 µg kg^{-1}
E4	100 µg kg^{-1}	100 µg kg^{-1}

RESULTS

In control group I (which was twice treated with placebo) no ovulation was certified. In control group II 2 fish were found to ovulate. The application of repeated administration caused ovulation in 40 and 60% of ovulation (Tab. 2). Embryonic survival rate of the collected eggs was the lowest ($p<0.05$) in control group II. Among the remaining groups similar ($p>0.05$) survival rate of embryos was noted (Tab. 2). There was no differences ($P>0.05$) among all the hatched larvae as considering the swim bladder inflation effectiveness as well as the final TL of the fish produced (Tab. 2).

The lowest SEI values were recorded for the control group II. The highest ($P<0.05$) were in groups E1 (Tab. 2).

Table 2. The results of the out-of season spawning of Eurasian perch after the application of different hormonal treatments and larvae characteristic obtained during the rearing period. SBIE – swim bladder inflation effectiveness observed in larvae obtained from different treatment groups, TL – total length of the body of juveniles at the end of the rearing period, SEI – spawning effectiveness index. Survival rate of larvae refer to the final survival of larvae from the start of weaning period until the end of experiment. Data in rows marked with different letter superscript were statistically different ($p<0.05$).

Group	Control II	E1	E2	E3	E4
Ovulation rate (%)	20	60	60	40	40
Embryonic survival rate (%)	22.6 ±16.0^{b}	63.0 ±39.2^{a}	46.4 ±51.1^{a}	60.9 ±40.8^{a}	67.8 ±26.6^{a}
SBIE (%)	31.3 ±9.1	38.6 ±6.1	36.2 ±6.4	32.9 ±7.8	35.4 ±9.2
Survival rate of larvae (%)	26.5 ±5.1	34.6 ±4.8	25.8 ±6.2	31.0 ±6.2	26.5 ±4.3
Final TL (mm)	132.5 ±11.6	131.9 ±10.7	134.3 ±11.0	131.1 ±15.4	129.0 ±9.5
SEI (No. of fish per kg of females)	29 ±6^{c}	265 ±23^{a}	194 ±41^{b}	173 ±46^{b}	181 ±43^{b}

DISCUSSION

Hormonal induction of ovulation in percids was usually conducted by a single injection of the spawning agent what usually allowed to obtain high ovulation rate and embryonic survival (Kucharczyk et al. 1996, 1998, Ronyai and Lengyel 2010, Żąrski et al. 2011a). However, in the present study single injection (applied in control group II) was not enough to obtain satisfactory results. This could probably stem from too short vernalization period. Szczerbowski et al. (2009) reported that at least two months of wintering should be applied. However, shorter period of vernalization allowed to exhibit very clear differences between the treatment groups in terms of ovulation and embryonic survival rate. It allowed also to proof that repeated administration of GnRHa alone may improve the ovulation rate and enhance the egg quality obtained. Until now, the administration of the GnRHa was applied only in a single injection and very often together with dopamine antagonists (Szczerbowski et al. 2009, Targońska et al. 2014). To the best of our knowledge only Kouril et al. (1997) reported that application of GnRHa alone can be successfully applied in Eurasian perch. These authors reported that high dose (125 µg kg^{-1}) resulted in high ovulation rate. In the present study hormone dose applied in a single injection was also very high (100 µg kg^{-1}). However, the reaction of the fish organism was significantly weaker. But it stem probably from the much less advanced maturation stage of the fish in this study than those used by Kouril et al. (1997).

In the present study the efficiency of spawning, when ovulation and embryonic survival rates were reconsidered, was similar to the other studies on out-of season propagation of this species (Szczerbowski et al. 2010, Żarski et al. 2011a, Targońska et al. 2014). However, high standard deviation of the embryonic survival data made impossible to find any differences between the groups as considering egg quality. Finally, the calculated SEI indicated that groups treated with the initial dose of 10 and 25 µg kg^{-1} exhibit the highest efficiency of spawning. It is worth mentioning that the larval performance was quite similar among the groups after the inflation of the swim bladder. This suggests, that the larvae with inflated swim bladder can be considered as a fish with quality high enough to be suitable for further rearing procedure. The similar conclusion was also drawn by Żarski et al. (2011a) who also reported that SBIE was a good quality indicator of larvae in Eurasian perch. Therefore, it could be expected that the number of larvae with inflated swim bladder, not a weaned juveniles, can be reliable indicator of spawning efficiency.

CONCLUSIONS

The result of the present study suggests, that the SEI is a very good and reliable indicator allowing credible verification of the reproductive protocols. However, it seems that the number of produced larvae with inflated swim bladder can also constitute reliable indicator of spawning quality. Such an indicator would allow making the process of verification of effectiveness of reproductive protocols much shorter and less laborious, than initially expected.

Additionally, the results of the present study indicates that the repeated administration of GnRHa may improve the out-off season spawning. However, more work is needed at optimization of the doses and injection intervals in order to verify its usefulness.

ACKNOWLEDGEMENTS

This study was financed by the EUREKA project “Development of an induced spawning technology and hatchery manual for the Eurasian perch (*Perca fluviatilis*)” (E! 8028 PERCAHATCH).

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IMPLEMENTATION OF FISHING TECHNOLOGIES AND SUSTAINABLE DEVELOPMENT IN SLAUGHTERHOUSE SYSTEMS

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PRIMENA RIBARSKIH TEHNOLOGIJA U ODRŽIVOM RAZVOJU KLANIČNIH SISTEMA

Apstrakt

Primenom ribarskih tehnologija i sistema za prečišćavanje se na bezbedan, proveren i renomiran način uklanja otpad iz klanične industrije vodeći računa o zaštiti životne sredine. Sa otpadom dobijenim iz klanične industrije se postupa na način koji aktivno doprinosi ekonomskim, socijalnim i ekološkim ciljevima održivog razvoja. Sam spoj klanične industrije i ribarskih tehnika predstvalja novinu kako u svetu tako i kod nas (Ćirković, 2014). Upravljanje otpadom je jedan od najsloženijih problema u sistemu upravljanja zaštitom životne sredine. Uzimajući u obzir prirodu sirovina i nastalih proizvoda, otpadne vode koje su nastale u toku proizvodnje i prerade prehrambenih proizvoda imaju po prirodi biorazgradivi karakter. Imajući u vidu da je štednja vode korisna, a prečišćavanje otpadnih voda zakonska obaveza trebalo bi imati na umu da će vode biti sve manje, da će voda biti sve skuplja i da se mora štedeti jer se dva puta plaća - prvi put kada se dovodi u pogon i drugi put kada se kao zagađena mora prečistiti. Činjenica da smo evropska i podunavska zemlja obavezuje nas da se u pogledu zaštite životne sredine ponašamo u istoj meri u kojoj su se obavezale i ostale evropske zemlje. Zahtevi EU podrazumevaju maksimalno smanjenje zagađenja svih vrsta koje se postiže izborom tehnologija koje proizvode manja zagađenja, kao i efikasnijim korišćenjem sistema za tretman produkovanih zagađenja. Prečišćavanje industrijskih otpadnih voda je trenutno apsolutno neophodno. Izlazna otpadna voda iz industrijskih pogona može imati veliki uticaj na kvalitet podzemnih voda i vodenih tokova.

Iz tog razloga povećava se broj institucija i industrijskih kompanija koje odgovorno vode računa o ovom problemu. Sve klanične industrije su obavezne da na bezbedan način izvrše prečišćavanje klanične vode pre puštanja u prirodne recipijente ili kanalizaciju uz obavezno plaćanje nadoknade za ispuštene otpadne vode koja je regulisana zakonom. Primena ove ideje je neophodna jer je ugrožavanje životne sredine jednosmerni problem današnjice, a i problem našeg ulaska u Evropsku zajednicu. Pored toga, nije zanemarljiv ni ekonomski benefit. Naše rešenje uklanja problem otpadnih voda iz klanične industrije, a izgradnjom ribnjaka u sklopu klanice se omogućava proizvodnja ribe u prečišćenoj vodi poreklom iz klanične industrije uz dodatak vode iz bunara. Sam spoj klanične industrije i ribarskih tehnika predstvalja novinu kako u svetu tako i kod nas. Tokom naših ogleda ukazali smo na: održivost klanične industrije korišćenjem ribarskih tehnologija i ekološko rešenje problema otpadnih voda, ribu kao bioindikatora zagađenja životne sredine i kontrole uspešnosti prečišćavanja otpadnih voda klanične industrije; prevođenje dela organskih materija putem prirodne hrane u meso ribe; uravnoteženje i zadovoljavajući kvalitet vode na završetku procesa prečišćavanja, s obzirom da kvalitetna voda danas predstavlja nedostajući resurs; konstantan uvid u zdravstveno stanje riba; proizvodnju ribe kao kvalitetne namirnice sa aspekta mikrobiologije i nutritivne vrednosti ribljeg mesa; korišćenje vode u zalivnom sistemu ribnjaka kao zaokruženog procesa od otpadne vode do vode zadovoljavajućeg kvaliteta za ratarske kulture; sistemi za energetsku održivost: drvenasti ostatak biljnih kultura se loži u energani klanice sa kostima koje su neiskorišćene u mesarskoj industriji čime se postiže veća energetska efikasnost.

Ključne reči: održivi razvoj, ribnjak, otpadna voda, riba kao bioindikator
Keywords: sustainable development, pond, wastewater, fish as bioindicators

INTRODUCTION

Our work provides a compound of the slaughter industry and fishing technologies aimed at improving environmental protection. The application of fishing technology and treatment systems are in a safe, secure, reputable and cost-effective way removes wastewater from the slaughterhouse (Ćirković, 2015a).

So far, the application of the filters system is to thrive after a series of purification methods to obtain the exit of water of appropriate quality, which as such is discharged into drains and sewage system. The fact is that the slaughter industries often do not adhere to the defined capacity, which results in the output of poor quality water. In addition, it should be noted that purified water has no practical value, even when it is of satisfactory quality, when it is discharged into the surrounding channels without exploitation, it is an economic loss (Ćirković, 2015). The aim of our work is to improve the quality of treated water from slaughterhouses and to return to its utility value. The result of our work is to ensure the rational use of water from slaughter houses, with use of fisheries technologies.

MATERIAL AND METHODS

During our research, which was carried out in the meat industry, „Djurdjevic” in Pećinci, the designed system with first pond, ponds with aeration and irrigation canal. Our work ensured that the use of the aerator and the addition of well water provide suitable environmental conditions for the cultivation of carp fish (Cirkovic, 2015). During our tests, we have provided that the resulting waste water going through the system for a rough separation, which separates the first stage of the physical separation of solid materials by passing through a spiral press the built-in channel. After that, with the help of cells to suppress liquid, water is pumped into the pools. On first pool comes to semi-automatic gravity that rise turbidity particles to the surface of the water carried by gas bubbles, usually air. Raw water is then subject to gross and fine purification - ie equalizing raw sewage comes to the treatment device through a channel with a rough mechanical grates, where larger solid waste is removed. Thus purified water is transferred into the pool for the biological oxidation and nitrification aeration assisted with microbubbles at the bottom of the pool, and the removal of ammonia nitrogen in the middle of the pool with no dissolved oxygen. Finally, we have the final sedimentation static separation of sludge from treated water, return activated sludge in the oxidation stage and equalization, the disposal of excess sludge to the landfill or the required space. A final disinfection of treated water is performed by adding sodium hypochlorite, disinfected water through a pad of silica sand with the help of pumps due to first pond. The surface of the first pond 50x3m is equipped with two aerators that enrich the water with oxygen. Two of the aerator allows reaching of good contact between the water and air that is to reach the intensive transporting gases or volatile substances in the water and out of it. These gases are passed are usually O_2, CO_2, N_2, H_2S, CH_4, NH_3 and numerous identified and unidentified volatile organic compounds that pollute the waste water. Aeration is necessary when wastewater shows a deficit of dissolved oxygen or is loaded with hydrogen sulphide. Water quality is improved with the system of pumps. In addition to water from slaughterhouses fresh well water that is rich in oxygen is added in ponds. The quality of fishery water corresponds to 3rd class water according to the current Regulation and favors the cultivation of carp fish. In 2013 pond is stocked with fry in an amount of 500kg / ha. Suitable physical and chemical conditions of water, which is rich in oxygen and nutrients, have contributed to achieve yields of fish three times higher than the average, 3500kg / ha, during 2013. On the basis of health surveys of fish and meat quality was found that the health of fish was beneficial, while the flesh demonstrated high nutritional value with 4% fat and 18% protein. Excess water from the pond to overflow system is dirrected in irrigation canal whose water quality is 2/3 class water by the current Regulation, which favors the use of the same water for irrigation. Using water from the irrigation system that is warm and that the appropriate quality favors arable crops and affects their yield (Ćirković, 2009). During 2013 an area of 100 ha was irrigated with water from the irrigation system and the yield reached 50 t /ha silage maize. Unused woody parts of corn were used for the production of hot water in power plant.

RESULTS AND DISCUSSSION

During the conducted tests the following results were obtained: the pond water which corresponds to 3rd class according to the Regulation on limit values of pollutants in surface and ground waters and sediments („Official Gazette" no. 50/2012), which is suitable for growing cyprinid fish production was 3500 kg / ha of fish (3 times higher than the average national production according to the Statistical Yearbook), (Ćirković, 2015); complete nutrient consumption amounted to 1.5 kg of feed for 1kg of gain; health status of the fish during the growing season was favorable (Bogut, 2006). Survival amounted to 95%. The quality of the meat showed a high nutritional value (18% protein and 4% fat).

Water from the irrigation canal which corresponds to class 2nd / 3rd class according to the Regulation on limit values is used to irrigate crops. Irrigation of 100 hectares of land produced corn silage of 50 t / ha. Unused woody part of corn is used in power plants.

CONCLUSIONS

The application of fishing technology enables security during water purification systems in slaughterhouses, because it represents a shock absorber when the technologists do greater than anticipated number of slaughter and undermine the capacity of filters. Our system provides production in all seasons because aeration reduces the amount of organic matter.

Incorporating organic matter from first pond water in fish meat through natural food, we achieved savings in the complete feed mixture and provide a high yield of fish. Water from the canal system of the pond is used optimally for watering the surrounding crops, given its chemical composition and temperature.

Our technology has enabled us to achieve good quality in addition to waste water and achieve good economic sense in the cultivation of fish and agricultural crops.

ACKNOWLEDGEMENTS

The paper is part of the project TR 31011, funded by the Ministry of Science and Technology of the Republic of Serbia.

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SUSTAINABLE INTENSIFICATION IN FRESHWATER FISH FARMING IN HUNGARY

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ODRŽIVA INTENZIFIKACIJA GAJENJA SLATKOVODNIH RIBA U MAĐARSKOJ

Apstrakt

Termin "održiva intenzifikacija" je relativno nov i koncept koji se razvija iz akvakulture koja je definisana kao oblik proizvodnje u kome se "prinos povećava bez nepovoljnog uticaja na životnu sredinu i bez dodatnog kultivisanja zemljišta". Kako postoje ograničenja u konvencionalnoj intenzifikaciji slatkovodnog ribarstva, povećanje proizvodnje zahteva inovativne pristupei korišćenje novih sistema i tehnologija kao što su Kombinovana intenzivno ekstenzivna (CIE) proizvodnja ili različitih recirkulacionih sistema (RAS) uključujući i RAS na otvorenom na ribnjacima i protočni sistemi.

"Jászkiséri Halas" Kft. u Mađarskoj izgrađen je i radi kao novi tip CIE sistema koji se naziva sistemom "ribnjak u ribnjaku", iako je mali intenzivni system nije jezero već plutajući tank. Intenzivna komponenta "ribnjaka u ribnjaku" se sastoji od 4 uzgojna tanka (30 m^3 svaki) koji su uklopljeni u plutajuću jedinicu ukupne zapremine 120 m^3. Intenzivna jedinica je bila smeštena u jednom uglu od 20 ha ekstenzivnog ribnjaka u kome se praktikuje konvvencionalna polikultura, a ribnjak koristi nutrijente izintenzivne jedinice. Voda cirkuliše između intenzivne i ekstenzivne komponente sistema putem pumpi. U intenzivnoj jedinici proizvodi se hibridni prugasti grgeč u maksimalnoj gustini nasada od 40 kg/m^3. Prinos u ekstenzivnom ribnjaku gde je takođe dodavana hrana dostigao je 1050 kg/ha. Još jedan tip CIE sistema nazvan "kavez u ribnjaku" koristi se u "Aranyponty Zrt." Za intenzivnu proizvodnju Evropskog soma u intenzivnoj jedinici i ekstenzivnu proizvodnju šarana, belog tolstolobika, belog amura i veslonosa u konvencionalnom ribnjaku. Jedan kavez od 50 m^3 postavljen je u ribnjak od 1 ha površine u kome se proizvodi 500 kg evropskog soma (1350 g). Organski otpad iz jednog kaveza donosi dovoljno nutrijenata za 1 ha površine ribnjaka gde su šaran i veslonos osnovne vrste. Nema dodavanja đubriva i dodatne hrane i ekstenzivnom delu gde je prinos bio 700 kg/ha.

"Spoljašnji RAS na ribnjaku" nastao je u "Jászkiséri Halas" Kft. konvertovanjem jednog zimovnika (2000 m^2) iza velikog (20 ha) ekstenzivnog ribnjaka u intenzivni bazen za proizvodnju šarana. Voda između malog intenzivnog i velikog ekstenzivnog jezera,

koje je takođe služilo kao jedinica za prečišžavanje, cirkulisala je pomoću male pumpe sa propelerom. U malom jezeru se intenzivno proizvodi šaran (7000 fish/ha) uz dodavanje peleta. Finalna prosečna telesna masa bila je 1.5 kg i bruto prinos je bio 10,000 kg/ha. U ekstenzivnom jezeru prinos je bio oko 1 t/ha uz korišćenje dodatne hrane.

”Spoljašnji RAS na pastrmskoj farmi” sagrađen je i funkcioniše u “Hoitsy and Rieger Kft.” gde se proizvodi kalifornijska pastrmka, potočna i jezerska pastrmka u 18 betonskih bazena ukupne površine 3700 m^2. Da bi se pastrmke proizvodile intenzivno u zaštićenoj životnoj sredini koja je i turističko područje, farma je izgtadila poseban sistem za tretman vode koji uključuje RAS na otvorenom (bubanj filteri i plutajući biofilter) i veštački izgrađena močvara (sa 7 različitih vodenih biljaka). Kao rezultat upotrebe novog sistema za tretman vode farma može da održava nivo proizvodnje bez ikakvog negativnog uticaja na vodu obližnjeg potoka i okolne ekosisteme.

Ključne reči: slatkovodna akvakultura, kombinovanje intenzivno-ekstenzivnog sistema, održivost, intenzifikacija
Keywords: freshwater aquaculture; combined intensive-extensive system; sustainability; intensification

INTRODUCTION

The term “sustainable intensification” is a relatively new and evolving concept emerged in agriculture that has been defined as a form of production wherein “yields are increased without adverse environmental impact and without the cultivation of more land”. Although marine aquaculture has future potential to explore off shore areas for increasing production, freshwater aquaculture will remain an important food production sector in some regions like Central and Eastern Europe. There are limitations in conventional intensification of freshwater fish farming by increasing stocking density and applying formulated feed due to various reasons such as environmental and animal welfare regulations, social concerns and the increasing competition for freshwater resources. Therefore the increase of freshwater aquaculture production requires innovative approaches and the use of new systems and technologies such as Combined Intensive Extensive (CIE) production systems or various recirculating aquaculture systems (RAS) including open air RAS for fish ponds and flow through systems. The potential in practical application of CIE systems however, is demonstrated by some innovative farmers in Hungary that may give a boost to the wider application of such systems in Central and Eastern Europe. Although the introduction of such systems to the practice is rather slow, the positive examples of the practical use of these systems may give a boost to sustainable intensification in freshwater aquaculture.

RESULTS AND EXPERIENCES WITH THE PRACTICAL APPLICATION OF “POND-IN-POND” AND “CAGE-IN-POND” CIE SYSTEMS

The “Jászkiséri Halas” Kft. was the first fish farm of Hungary that built and operated a new type of CIE system called “pond-in-pond” system although the small intensive unit is not a pond but a floating tank. The intensive component of the “pond-in-pond” system consists of four fish rearing tanks (30 m^3 each) that are arranged in a floating unit with a total volume of 120 m^3. The intensive unit was placed in one corner of a 20 ha extensive fish pond where conventional polyculture production was carried out (Figure 1.).

Figure 1. The intensive unit of the “Pond-in-pond” system in operation in a 20 ha extensive fish pond of the “Jaszkiseri “Halas” Kft”

There was a continuous water flow through the tanks of the intensive unit that was ensured by low head high capacity air lift pumps. The air was provided by an air blower that was driven by an electric motor of 2 kW. The formulated feed was distributed to the fish rearing tank by automatic feeder. Hybrid striped bass (*Morone saxatilis x M. Chrysosps*) was raised in the tanks intensively during the natural growing season fed with floating pellet, while the extensive pond also served as a natural treatment unit to remove the nutrients from the effluent of the floating rearing tanks. The total yield in the intensive system was 4800 kg (40 kg/m^3) and the total income was 28,800 € when the fish was sold on a price of 6 €/kg. In the large (20 ha) extensive pond large size two summer old carp of 300 g was stocked with a stocking density of 1000 fish/ha. The production was based on the use of natural food and supplementary feeding by cereals. At the end of the growing season the gross yield was 1050 kg/ha (700 fish/ha with an average weight of 1.5 kg). The survival rate was only 70 % due to the massive bird predation. The net result of the extensive fish rearing was 656 €/ha.

This special type of Combined Intensive Extensive (CIE) system called “Cage in pond” system was applied at “Aranyponty Zrt.” for the intensive production of European catfish (*Silurus glanis*) in the intensive unit and the extensive production of common carp (*Cyprinus carpio*), silver carp (*Hypophthalmicthys molitrix*), grass carp (*Ctenopharyngodon idella*) and paddle fish (*Polyodon spatula*) in the conventional earthen pond. One summer old European catfish was stocked into the floating cages (5x5x2m) that were placed in a fish pond (Figure 2). Floating pellet was distributed to the cages by automatic feeder driven by solar panels. The fish meal was substituted completely by soybean meal and meat meal. The F.C.R. was 1.95 kg. When the dissolved oxygen level in the pond decreased to low level paddle wheel aerator supplied air to the water body.

Figure 2. Floating cages in a fish pond at the “Aranyponty Zrt.” for the intensive rearing of European catfish

The catfish reached 1350 g individual weight by the end of the growing season. No any disease problem occurred during the intensive rearing of the catfish. One cage of 50 m3 was put in a one hectare pond area, in which 500 kg European catfish was produced. The organic wastes from one cage provided sufficient nutrients for one hectare pond area where common carp and paddle fish were the main species. No fertilizer and supplementary feed were applied in the extensive pond, where the yield was 700 kg/ha (500 kg/ha common carp and 200 kg/ha paddle fish). The grass carp and silver carp as complementary species contributed to the improvement of the nutrient utilization in the system.

RESULTS AND EXPERIENCES WITH THE PRACTICAL APPLICATION OF “POND WATER RECYCLING” OR OUTDOOR RECYCLING AQUACULTURE SYSTEM (RAS)

Outdoor RAS in a pond fish farm was created in the “Jászkiséri Halas” Kft. through converting one of the wintering ponds (2000 m^2) beside a large (20 ha) extensive pond into an intensive pond for carp production. The water between the small intensive pond and the large extensive pond that was also served as water treatment unit was circulated by a small head high capacity propeller pump. The view of the intensive pond equipped with automatic feeder and surface aerator is shown in Figure 3.

Figure 3. View of the intensive carp rearing pond as a component of a fish pond RAS in the "Jaszkiseri Halas" Kft.

In the small (2000 m^2) intensive pond two summer old carp of 300 g was stocked with a stocking density of 7000 fish/ha. The fish was fed on pelleted feed with an F.R.C. of 1:1.6. The gross yield was 10,000 kg/ha (6600 fish/ha with an average weight of 1.5 kg). The survival rate was 94% due to effective protection against bird predation. The net result of the intensive production was 4,293 €/ha. Main data of the extensive pond production are shown in Chapter 3.1.

<u>Outdoor RAS in a trout farm</u> was built and operated by "Hoitsy and Rieger Kft." that is a leading trout farm in Hungary producing Rainbow trout (*Onchorhynchus mykiss Walbaum, 1792.*), brown trout (*Salmo trutta m. fario Linné, 1758.*), and brook trout (*Salvelinus fontinalis Mitchill 1815.*) in 18 raceways with a total area of 3700 m^2. The annual production of the farm is 36-38 tons of market size fish according to the availability of the supply water. In order to produce trout intensively in a protected environment that is also a touristic area the farm built a special water treatment system that includes open air RAS and a constructed wetland. The open air RAS also contribute to decrease the dependence on the natural water supply since the flow rate of the creek is varying greatly. The water treatment system comprises the following main units: (1) drum filter; (2) biological filter; (3) constructed wetland. The water is circulated by air lift pumps. In order to ensure safe operation there are two independent (but connectable) water circles in the RAS. The effluent water from the raceways flows to the drum filters (with a mesh size of 70 µ) by gravity. The sludge from the drum filter is collected in a pit where it is mixed with sawdust and removed once a year. The biological filter is a "floating bed" biofilter with a 800m^2/m^3 effective filter area (Figure 4.). The plastic media in the biological filter is kept floating by air that is blown into the water

through perforated pipes placed in the bottom of the filter tank. The air also provide oxygen to the nitrifying bacteria and helps the removal of harmful gases first of all CO_2.

Figure 4. The view of the biological filter at the “Hoitsy and Rieger” intensive trout farm

The water is circulated through the fish tanks, the drum filter and the biological filter by air lift pumps with low head (max 25cm) and high capacity. The water recycling in the system is partial, therefore a part of the water is released regularly to the creek Garadna that is flowing along the farm area. However the effluent water from the farm is treated on a constructed wetland where 7 different water plants remove efficiently the N and P from the water, thus the intensive trout farm doesn’t have any negative impact on the water of the creek and the surrounding ecosystem.

ACKNOWLEDGEMENTS

The authors thank the Hungarian Fisheries Operational Program (“HOP”) and the Hungarian Economic Development Operational Program “GOP” -2011-1.1.1 for financially supporting activities of the development and testing the new type of fish production systems in farming conditions.

POSSIBILITIES OF THE USE OF MICROENCAPSULATED DIETS FOR COMMON CARP (*CYPRINUS CARPIO*) LARVAE

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MOGUĆNOSTI KORIŠĆENJA MIKROINKAPSULIRANE HRANE ZA LARVE ŠARANA (*CYPRINUS CARPIO*)

Apstrakt

Glavni cilj trenutnih istraživanja u oblasti ishrane larvi vezano je za potencijale novih tehnologija i strategija ishrane. Formulisana je suva hrana sa hidrolizovanim proteinima mora i mikroinkapsuliranim vitaminima (C i E). .U toku 24 dana eksperimenta na larvama šarana, počev od 3 dana posle izleganja, testirano je 5 različitih strategija ishrane sa *Artemia nauplii* i suvom hranom. Na kraju perioda u kome su larve bile izložene ograničenom prostoru i uslovima stresa usled hladnoće smeštene su u odgovarajući prostor na jedan sat. Pre i posle izlaganja stresu izmereni su nivoi prethodno pomenutih vitamina HPLC metodom. Statistički značajne razlike na nivo preživljavanja pokazale su se posle 15 Dph, kada su mali prirast i preživljavanje utvrđeni u oglednim grupama, osim u kontrolnoj grupi. Za vreme delovanja stresora sadržaj vitamina C opadao je ka niskim vrednostima slično u svim grupama, međutim potrošnja vitamina E je bila manja. Nivo vitamina E bio je viši u grupama koje su duže vreme hranjene suvom hranom obogaćenom inkapsuliranim vitaminima.. U pozadini slabih rezultata prirasta pri korišćenju suve hrane možemo reći da je sposobnost suočavanja sa stresorom bila dobra kod larvi šarana starih 24 dana. Korišćenje formulisanih mikroinkapsuliranih smeša kao početne hrane za larve obećava, ali moraju da se razviju tehnike ishrane i bolja strategija, na primer modifikovanjem učestalosti hranjenja.

Ključne reči:mikroinkapsulirana hrana, larve šarana, antioksidativni vitamini
Keywords: microencapsulated diet, Common carp larvae, antioxidant vitamins

INTRODUCTION

Larval rearing of the intensive cultured freshwater species still depends mainly on live food *(*i.e algae, rotifers and *Artemia).* Nutritional value and compositions of live foods is not stable and they have a need for supplementation with different macro and micro-nutrients like vitamins or minerals. Also, limited amount of data is available for vitamin utilization before the onset of the first feeding. The modern agro-feed industry is already exploiting the potential of novel nutrient delivery vectors (Hamre et. al., 2013). The aim of our trial was to develop a weaning strategy for better utilization of microencapsulated diet enriched with vitamins for carp larvae.

MATERIALS AND METHODS

The 24-day experiment was started with 3-day old common carp larvae. The same feed was distributed to the 5 groups, in triplicates. The diet was prepared by low-shear extrusion at low temperature (40 ºC). Some of the marine proteins were hydrolysates containing 100 $mgkg^{-1}$ ascorbate phosphate and 250 $mgkg^{-1}$ Vitamin E which have been previously encapsulated to avoid leaching. The larvae were reared in 45 dm^3 tanks in a recirculating system. The water temperature was between 21-23 °C. The stocking density was 1000 fish/tank.

The feeding was carried out by hands following the feeding protocol (Table 1.) four times per day to visual satiation. Co-feeding means that the fish were fed each day two times with *Artemia*, two times with dry feed.

Table 1. Feeding protocol

	Artemia	Co-feeding	Dry feed
	Days of feeding		
Treatment 1. (T1)	0	4	20
Treatment 2. (T2)	12	12	0
Treatment 3. (T3)	8	4	12
Treatment 4. (T4)	6	4	14
Treatment 5. (T5)	6	2	16

During the nutritional trial 4 times weight and length measurements were done. At the end of the 24 days trial, samples have been taken for body composition and biochemical measurements. Stress experiment was implemented using all of the groups, except the T1. Larvae from the first group did not take part of the stress experiment because of the low growth and survival rate parameters. First of all, confinement circumstances were established by reducing the water depth from 22 cm to 2 cm for 1 hour. During this the temperature was decreased from 22 °C to 13.5 °C due to the suspension of the heating. After the stress experiment the larvae was kept further four days with the same conditions as before to check the antioxidant vitamin levels after a recovery session.

Vitamin C and B from carp larvae was determined by an isocratic reverse-phase HPLC method (Papp et al., 1998). The assessment of Vitamin E was performed by method of Ochoa et al (1992).

RESULTS AND DISCUSSION

Observing the body mass data, after 2 days the difference was shown between the treatment 1 and the other groups, the scissor is opened forward from day 4 (Figure 1). Visible and statistically significance appears from day 16 between the treatment 2 and the other groups. In this time under 8 days the larvae grew more than double. That result shows that strategic change needs after that time.

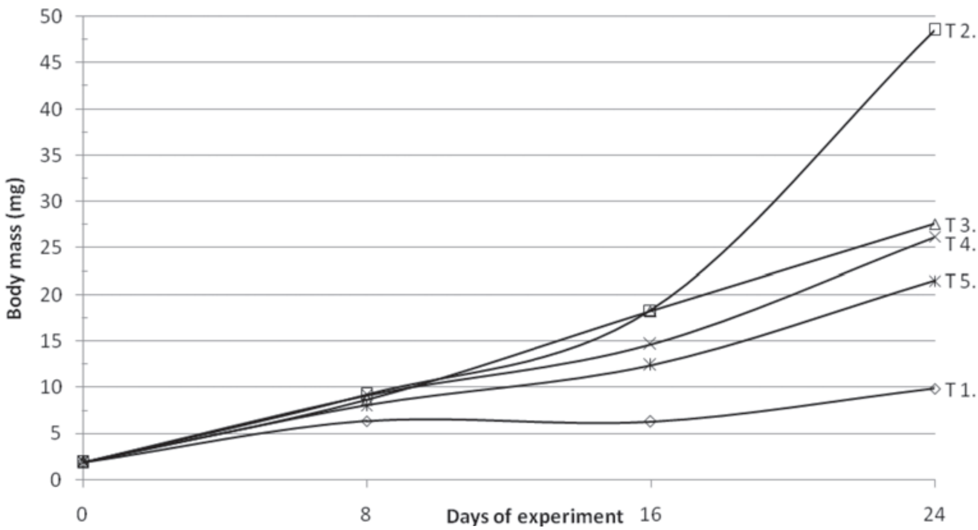

Figure 1. Growth parameters

Studying the survival rate (Figure 2), differences were shown only after the twelfth day of feeding. The best survival rate (89.9 %) had that group (T 2) which was fed the longest time with live prey. The worst is the "dry feed group" (T 1) with 58.1 % survival at the end of experiment. In the case of T3-T4-T5 group the survival has not correlated with weight gain. The fifth group shows better survival rate, but the body mass results shows the opposite of this.

From the confinement and slightly cold stress experiment the next observation could be taken. The highest Vitamin C level (Figure 3) was determined in the control group, which were fed mostly with A*rtemia* (420 µg/g d.w.), but in the other groups presenting appreciable the same state of supply. In case of the Vitamin E (Figure 4) the levels are different; probably more available vitamin E has been taken up by fish from dry feed than from *Artemia*. In the stress situation the antioxidant vitamins, like Vitamin C and E, are used in different way. Vitamin C content decreases similarly to low level in all groups, meanwhile Vitamin E was less consumed. The Vitamin E levels were not decreased as much as Vitamin C. In group 2, 3 and 5 the larvae utilized approx 50 µg/g (d.w) Vitamin E to handle the stress conditions.

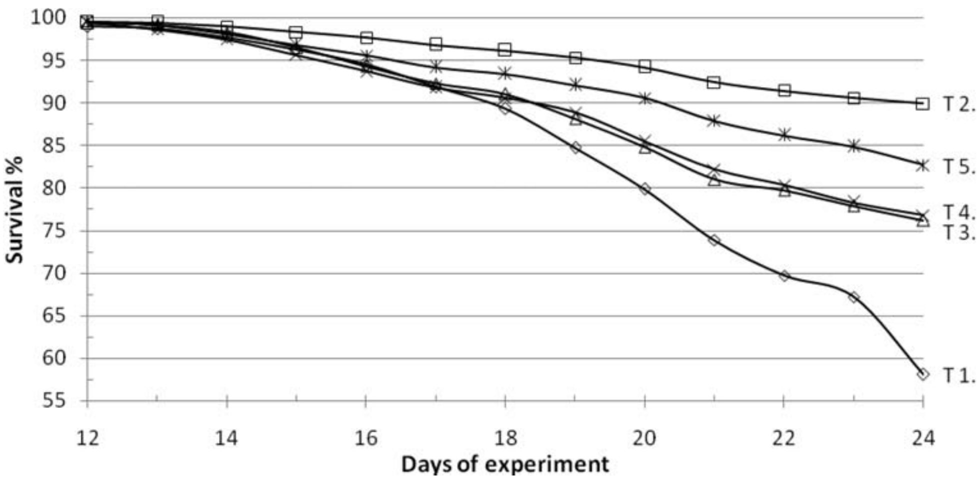

Figure 2. Survival rate

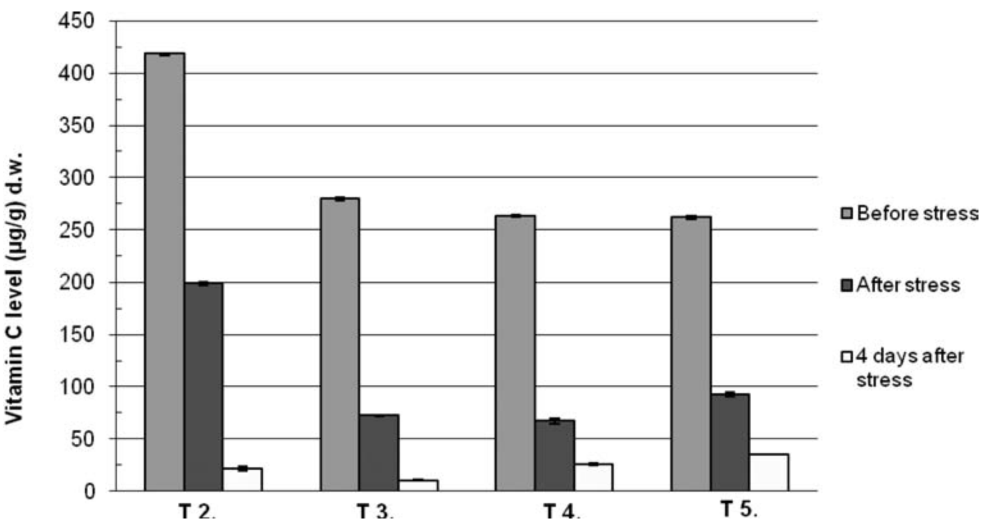

Figure 3. Vitamin C levels

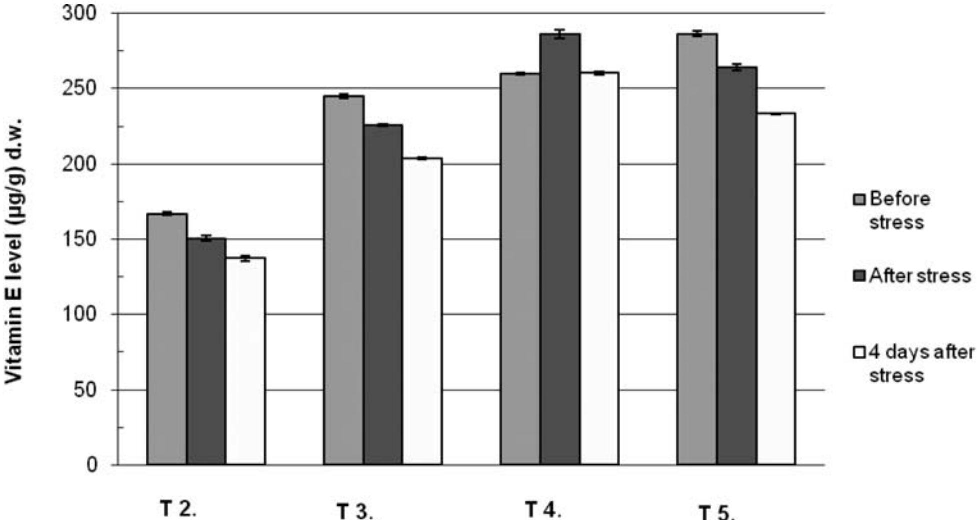

Figure 4. Vitamin E levels

CONCLUSIONS

Behind the weak growth resulted with dry feeding we can say that stress handling ability was good in the case of 24 days old carp larvae. Using formulated microencapsulated diets as starter feed for carp larvae is promising, but have to develop the feeding techniques and a better strategy, for example modifying the feeding frequency.

ACKNOWLEDGEMENTS

This study was supported in part of ARRAINA (FP7 288895) and AQUAREDPOT projects (FP7-316266).

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THE CARP, FROM THE AQUATIC FIELD TO THE DISH: KEY POINTS IN THE SEMI INTENSIVE PRODUCTION AND PLACEMENT

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ŠARAN, OD VODENE NJIVE DO TANJIRA: KLJUČNE TAČKE U POLUINTENZIVNOJ PROIZVODNJI I PLASMANU

Apstrakt

Jedna od najviše gajenih ciprinidnih vrsta riba u svetu je šaran. Gaji se u različitim sistemima, od ekstenzivnih do intenzivnih. Među brojnim načinima gajenja, poluintenzivna proizvodnja je apsolutno dominantna. Ovakva proizvodnja se bazira na kombinaciji prirodne i dodatne hrane. Prirodnom hranom se obezbeđuje proteini, ali i ostali potrebni sastojci neophodni za pravilan rast i razvoj ribljeg organizma, dok se dodatnom prihranom podmiruju energetske potrebe, ali i nedostajući proteini, minerali, vitamini, pre svega u periodima depresije prirodne hrane. Hrana za gajenog šarana u velikoj meri utiče na proizvodne rezultate. Međutim hrana, iako veoma važna, samo je jedna karika u lancu bitnih ključnih tačaka od vodene njive do tanjira. **Priprema ribnjačkih jezera** pre nasada predstavlja važnu agrotehničku meru, a koja se često izostavlja, pre svega pri gajenju konzumnog šarana. Prezimljavanje ribnjačkog dna na suvom, tanjiranje, zakrečavanje, a potom đubrenje su od velikog uticaja na razvoj prirodne hrane, ali i na proces mineralizacije organskih materija u ribnjačkoj podlozi, kao i na dezinfekciju ribnjaka, čime se obezbeđuje povoljni ambijentalni uslovi za gajenje šarana. **Izbor monokulture ili polikulture** u velikoj meri može uticati na ostvarenje profita u poluintenzivnoj proizvodnji šarana, ne samo zahvaljujući maksimalnom iskorišćavanju prirodnih potencijala ribnjaka, već i zahvaljujući obezbeđivanju stabilnosti ribnjačkog ekosistema. **Kvalitet šaranske mlađi za nasad** jedna je od najvažnijih ključnih tačaka. Izbor mlađi dobijene od selekcionisanih matica predstavlja najbolji, a izbor mlađi iz divljeg mresta najlošiji izbor sa nepredvidivim rezultatima. Jedna od dilema je i da li u ribnjačkim objektima gajiti samo **jednu uzrasnu kategoriju ili**

primeniti mešani nasad? Nasad dvogodišnje mlađi za gajenje konzumnog šarana koji je u toku prethodnog perioda bila bolesna zajedno sa jednomesečnom ili jednogodišnjom mlađi predstavljaju loš tehnološki potez. Međutim, mešani nasad, predstavlja dobar izbor ukoliko želimo da maksimalno iskoristimo prirodnu hranu, kao i u ribnjačkim uslovima u kojima prihranu riba obavljamo peletiranom hranom. Nakon dobre pripreme ribnjačkog objekta, kao i izbora mlađi za nasad predstoji nam **odluka kojom vrstom hrane prihranjivati šarana**. Izbor žitarica u poluintenzivnoj proizvodnji je opravdan u uslovima dobre razvijenosti prirodne hrane, želje da se proizvodi od 1 do 1,5 t/ha, kao i nemogućnosti plasmana veće količine ribe na tržište. Koncentrovane smeše su u prednosti kada se želi proizvesti više od 1,5 t/ha i šaran boljih nutritivnih svojstava. Od koncentrovanih, ekstrudinara hrana je po najvećem broju kriterijuma u prednosti u odnosu na peletiranu.Veoma je važno voditi računa i o činjenici da u različitim periodima proizvodne sezone (pre svega u zavisnosti od temperature i razvijenosti prirodne hrane) gajenom šaranu odgovora hrana različitog sastava (različitog sadržaja proteina, ugljenih hidrata, vitamina, minerala). Jedan od ključnih faktora za dobre proizvodne rezultate je i potreba **da se ribe sačuvaju od ihtiofagnih ptica, sisara, pa i čoveka**. Pored odabira pravih tehnoloških postupaka **stalno praćenje zdravstvenog stanja gajenog šarana, kao i preventivno delovanje** su najbolji načini održavanja stabilnosti zdravstvenog stanja gajenih riba. Iako poluintenzivno gajenje šarana u pogledu **dobrobiti riba**, predstavlja jedan od oblika proizvodnje koji je najbliži idealnom, sam izlov, transport i skladištenje u prodavnicama živog šarana je period „pakla“ za ovu vrstu. Otuda je neophodno posebnu pažnju posvetiti tehnologiji izlova i transporta šarana u cilju unapređenja dobrobiti riba, a sa ciljem smanjenja stresa kod izlovljene šaranske mlađi, što će imati pozitivan efekat na smanjenje mortaliteta, održavanje dobrog zdravstvenog stanja u narednim periodima gajenja šarana, kao i samog kvaliteta mesa konzumnog šarana. Među poslednjim, ali za dalji razvoj šaranskog ribarstva jednim od ključnih faktora je **način prometa šaranom**. Promet živog šarana sa aspekta ekonomije predstavlja skup način, sa aspekta dobrobiti riba nehuman, a sa aspekta praćenja trendova plasmana ribe zastareli i neusklađen način prodaje sa zahtevima savremenog kupca.

Ključne reči: šaran, ključne tačke, poluintenzivna proizvodnja, promet
Keywords:carp; key points; semi-intensive production; trade

INTRODUCTION

One of the globally most commonly cultured fish species is the Carp (Takeuchi et al., 2002). It is cultured in different systems, from extensive production forms to intensive systems. Among numerous culture systems, a semi intensive production is more dominant than the others. Such production is based on combining natural with added feed. With natural food body building blocks are provided – proteins and other essential constituents (vitamins, minerals, carbohydrates…) needed for proper growth and development of the fish organism. Added carbohydrates are primarily supplied for energy needs, but also missing proteins, minerals, vitamins, mainly during periods of natural food depression. The right choice of feed for cultured carp influences production results to a great extent. Although added feed is of utmost importance, it is still just a single link in the chain of key points from aquatic field to consumer’s plate.

PREPARATION OF FARM INFRASTRUCTURE

Pond preparation before stocking is an important agrotechnical procedure, often omitted, primarily in ponds where 2 summer old and consumable size carp is stocked. Dry pond bottom during wintering, disking, liming, and subsequent fertilization are of utmost importance for natural food development and also for bottom organic matter mineralization and natural pond disinfection. Preparation of farm infrastructure in the cold season (from November till March) provides better environmental conditions for the next season of carp culture (Marković, 2010).

CHOICE BETWEEN MONOCULTURE AND POLYCULTURE

The decision whether to produce in mono or polyculture influences the final profitability in the semi intensive carp culture to a great extent. This is not only because of maximal use of pond natural potential (natural food for accompanying species), but also because of the maintenance of the pond ecosystem stability. In cases of higher development of macrophytes, that is white grass carp (*Ctenopharyngodon idella*) food, it is necessary to stock with one summer old fry of this species in cases when common duckweed (*Lemna minor*) is present, and with bigger specimens in cases when larger macrophytes (common reed – *Phragmites communis*, bulrush – *Typha latifolia*) are developed. It has to be kept in mind that some macrophytes, such as Yellow Floating-heart (*Nymphoides peltata)* will not be consumed by the white grass carp. White bighead (*Hypophthalmichthys molitrix*) is stocked when phytoplankton (primarily green algae) dominates, and gray bighead (*Aristichthys nobilis*) when there is more zooplankton than common carp can consume. Stocking of predator species (catfish – *Silurus glanis*; pikeperch – *Stizostedion lucioperca*) is carried out by building pikeperch nests, for pikeperch stocking, or restocking with small catfish, mainly in ponds where larger carp categories are reared. By this procedure wild fish that enters the pond through the water supply system is controlled, as well as weak individuals and those that are not healthy. The choice of monoculture is a better option in cases of semi intensive production intensification, with the use of extruded feed, and when natural food is largely neglected, as well as in cases when natural food is still not fully developed. In cases when accompanying species are stocked in the pond where natural food is insufficient, this fish will be directed towards carp feeding points and eat extruded feed. This will have a negative effect on production profitability.

CHOICE OF THE CARP FRY FOR STOCKING

Carp fry quality is one of the most important key points. The choice of fry obtained from selected broodstock is the best decision, while taking fry from wild spawning is the worst due to unpredictable production results. One of the dilemmas is whether to culture only one age category or combine culturing fry and consumable carp. Fish ponds in which diseases appear are not suitable for mixed sized stocking. For consumable carp production stocking with fry that has been in poor health, together with one month old or one summer old fry, represents a faulty technological step, since disease-causing agents might be in a silent/latent state. However mixed sized stocking is a good choice in ponds where natural food is developed during most of the growing season, as well as when pelleted feed is used as supplemental.

CHOICE OF FEED AND FEEDING DYNAMICS

After a proper preparation of fish ponds, the decision needs to be made on the type of culture and fry of good quality, and about the type of carp supplemental feed. The option of using cereals in the semi intensive system is justifiable if natural food is developed and if the goal is 1 to 1.5 tons fish per hectare, as well as the lack of opportunities for appropriate market placement. Concentrated formulated feed is an advantage when higher production than 1.5 t/ha is envisaged (Marković, 2010), and when the goal is to produce carp with better nutritive characteristics: higher protein content, less lipids, better fatty acid profile (Trbović et al. 2013). Among concentrated formulated feeds the extruded type compared to pelleted has many advantages when it comes to most criteria such as physical, chemical, or microbiological feed properties. However, in conditions of mixed sized stocking as well as carp fry monoculture in ponds, with good water quality, due to indirect positive effects on natural food development, the use of pelleted feed could result in higher production (Ćirić et al., 2015). Besides the right choice for supplementary feed for carp production, it is important to take care about different periods during the season, (primarily in terms of temperature and level of natural food development), since cultured carp needs different feed content/quality in terms of protein, carbohydrates, vitamins, and minerals.

When it is about feeding dynamics, more frequent feeding (several times a day), harmonized with

Highest utilization of natural food gives the best results.

PROTECTING THE CARP FROM ICHTHYOPHAGOUS BIRDS, MAMMALS, POACHING AND MONITORING HEALTH STATUS OF CULTURED FISH

One of the key factors for good production results is the need to protect cultured fish from ichthyophagous birds, mammals, and poaching. Besides choosing best production procedures, monitoring carp health, prevention of diseases is the best way of keeping fish in good health conditions. When optimal technological procedures and maintenance of fish health are applied, the end of the growing season and harvest should not represent any problem and justify all the efforts invested. Although semi intensive carp production is the system close to the ideal with regards to fish welfare, harvest, transportation and stocking in fishmonger where live carp is sold represents the period of hell for the fish. Thus, special attention should be devoted to harvest technology and transportation in order to reduce stress and contribute to carp welfare. This will also have positive effects on mortality reduction and maintenance of good health status, as well as on flesh quality of consumable carp.

CARP TRADE

One of the last but not the least key factors in carp culture development in the future is carp trade. Trade of live carp that is dominant in Europe, from economical point of view is an expensive method, particularly from the welfare aspect, as well as obsolete and not harmonized with consumers’ demand. Selling live carp requires possession of appropriate transportation means by the producer and/or costumer; transport of water with the fish;

providing a pool for keeping live fish in a fishmonger; and finally gutting the fish. All this increases costs and decreases demand for this species. Trade of carp meat and more attractive products such as smoked carp with dried plums, currants or cranberries that are prepared “closer to the dish” will result in elimination or decrease of mentioned negative properties of carp trade, as well as more places and possibilities for offering new products of carp meat and increase its consumption.

ACKNOWLEDGMENT

The study was supported by Serbian Ministry of Science and Technological Development (project TR 31075) and by EC (project AREA – FP7-REGPOT-2012-2013-1, Grant agreement no 316004).

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DO OXALATES FROM PLANT-BASED AQUAFEEDS IMPEDE GROWTH OF COMMON CARP *CYPRINUS CARPIO*?

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DA LI OKSALATI IZ HRANE ZA RIBE BILJNOG POREKLA OMETAJU RAST ŠARANA, *CYPRINUS CARPIO*?

Apstrakt

Jedan od najvažnijih izazova održivog razvoja u akvakulturi je održivo obezbeđenje hrane, koje zahteva upotrebu manje konvencionalnih sastojaka za hranu za ribe kao što su presovane pogače i uljarice. Neke od ovih sirovina imaju visok sadržaj oksalata, koji su poznati kao antinutricioni faktor za neke domaće životinje. Oksalat je proizvod metabolizma biljaka, ali se takođe pojavljuje u metabolizmu životinja. Zavisno od vrste biljaka oksalati se nakuplja: kao rastvorljiva so vezana za natrijum, kalijum ili amonijum; kao nerastvorljiva so vezana sa dvovalentnim jonima kalcijuma, magnezijuma ili gvožđa; i kao kombinacija oba navedena sliučaja. Negativni efekti oksalata kod sisara uključuju smanjenje raspoloživosti minerala iz hrane što izaziva njihov nedostatak, zatim formiranje kamenčića oksalata kalcijuma ili magnezijuma u bubregu i drugim tkivima, što rezultira artritisom ili simptomima gihta. Za nepreživare preporučen je maksimalni sadržaj od 0.5% u hrani. Hrana za ribe koja sadrži lišće, presovane pogače ili proizvode od pirinča može lako da dostigne ili pređe navedeni sadržaj oksalata, međutim, koliko je nama poznato, ova problematika nije do sada nikada bila ispitivana.

Da bi se ispitali efekti na ribe, šarani (*Cyprinus carpio*) su hranjeni hranom koja sadrži oksalate u količinama od 0%, 0.5%, 1.5% i 2.5% tokom 8 nedelja. Sva hrana je bila redovno konzumirana, a preživljavanje riba je bilo 100%. Prirast ribe koja je hranjena hranama koje su sadržale oksalate je bio bolji nego u kontroli (0%), sa značajnim razlikama između tretmana 1.5% i 2.5% ($p \leq 0.05$, Tab.1). Isto je važilo i za vrednosti SGR, MGR i PER (Tab.2). FCR je bio značajno niži za tretmane 1.5% i 2.5% u odnosu na kontrolu, dok u slučaju tretmana 0.5% to nije bio slučaj. PPV je bio viši za tretmane 1.5% i 2.5% u odnosu na tretmane sa nižim sadržajem oksalata. LPV je bio značajno viši u kontroli u odnosu na hrane sa 1.5% i 2.5% oksalata, dok za vrednosti EPV nije bilo razlike.

Utvrđene su statistički značajne razlike u telesnom hemijskom sastavu eksperimentalnih riba: kontrola i tretman 0.5% su imali više lipida i energije, kao i niži sadržaj vlage i pepela u odnosu na tretmane 1.5% i 2.5%. Nije bilo razlike u proteinskom sastavu (Tab.3). Retencija kalcijuma, magnezijuma i fosfora je bila statistički značajno niža u kontroli i hrani sa 0.5% oksalata u odnosu na hrane koje su sadržale 1.5% i 2.5% oksalata (Tab.4).

Oksalati kod šarana imaju pozitivne efekte na rast i pretpostavlja se da bi to moglo biti zbog njihovog antimikrobijalnog delovanja u crevima. Efekti na telesni sastav su bili izraženi: više oksalata rezultiralo je višim sadržajem minerala i nižim sadržajem lipida. Tokom trajanja ogleda nije bilo antinutricionih efekata, naročito nedostatka minerala, kao što je to opisano u literature; ipak, bilo bi neophodno dugoročno ispitivanje koje bi uključilo i histopatologiju da bi se doneo zaključak da li su oksalati blagotvorni u hrani za šarana, kao i da bi se osvetlili potencijalni mehanizmi poboljšanog rasta.

INTRODUCTION

In the continuing drive to replace animal protein (mostly fishmeal) in fish feeds with plant protein, research has focused on identifying and minimizing the influence of anti-nutritional components present in most plant-based feedstuffs. Anti-nutritional components are defined as substances that interfere negatively with food utilization or adversely affect the health status of the feeding animals (Francis et al., 2001). Despite a range of treatment methods employed to minimize anti-nutritional factors in plant feedstuffs, such as dry heat treatment ("toasting"), moist heat treatment, aqueous or alcoholic extractions, some of these substances cannot be removed and end up in the feed (Francis et al., 2001).

A good example for these is oxalic acid and its salts (oxalates). Oxalic acid is a metabolic product formed through several pathways in plants and animals.Oxalatesof monovalent ions, such as sodium, potassium or ammonium are well soluble in water while those oxalates formed with divalent ions, such as calcium, magnesium and iron are almost insoluble (Libert&Franceschi, 1987; Savage et al., 2000).

Oxalate has long been considered an anti-nutritional factor in humans and consumption in the form of plants containing high amounts of oxalate, such as spinach, beet or rhubarb, has been recommended not to exceed an upper limit (Noonan & Savage, 1999). Documented adverse effects of dietary oxalate in mammals include the binding of oxalate with calcium in the intestinal lumen to form insoluble calcium oxalate making calcium unavailable for absorption and excreting it with the faeces (Noonan & Savage, 1999). This may lead to low blood calcium levels (Blaney et al., 1982) and in cases of long-term exposure, bone material may be excessively mobilized to compensate for the shortages of minerals (Rahman et al., 2013). Further, long-term exposure to a high-oxalate diet may lead to formation of Ca- or Mg-oxalate stones in the kidney, which can cause urine flow problems or kidney failure (Noonan & Savage, 1999). Rahman et al. (2013) recommended that for ruminants dietary oxalate consumption should be less than 2% in order to avoid oxalate poisoning and less than 0.5% for non-ruminants. Soluble oxalate content in some potential fodder crops used in aquaculture diets are within these critical ranges: saltbush *Atriplexhalimus* 1.0 – 3.0%, rice *Oryza sativa* 1.0 – 2.5%, alfalfa *Medicago sativa* 0.9-1.1%; *Jatropha curcas* 2.4%. Some authors have postulated potential anti-nutritional effects of oxalate in fish nutrition (Yousif et al., 1994; Francis et al., 2001), but the effects of dietary oxalate on aquaculture species remain to be investigated. In this trial, the effect of four different concentrations (0%, 0.5%, 1.5% and 2.5%) of soluble oxalate in a standard diet on the growth performance, body composition and mineral availability of common carp (*Cyprinus carpio*) were investigated.

MATERIAL AND METHODS

Four isonitrogeneous and isoenergetic diets with fishmeal and wheatmeal as main ingredients were produced containing 0% (Control), 0.5%, 1.5% and 2.5% of di-sodium-oxalate. Protein content was formulated to be 32% and lipid content 12% (50% fish oil, 50% sunflower oil). Vitamins and minerals were supplied in the form of a premix. Essential amino acids were added to meet the requirements of carp (NRC, 2011).

Cyprinus carpio were hatched and reared to the initial experimental weight of 9.85 ± 0.23 g at the Thuenen Institute of Fisheries Ecology, Ahrensburg, Germany. A total of 100 experimental fish (five replicates for each treatment, each replicate containing 5 fish) were distributed into a total of twenty 40 L-aquaria connected to a recirculation system. Water temperature was 26°C, with 1.5 L / min/tank flow rate. The experimental duration was 8 weeks.

Fish were fed according to multiples of their basic metabolic maintenance ration (Richter et al., 2002). Fish were fed 5 times the maintenance ration on a daily basis throughout the experiments. The ration was given in three installments per day. Fish were weighed every two weeks individually and without anesthesia in a bowl containing water from the respective tank. Feed amount was adjusted accordingly after every weighing.

Before the experiment, 8 fish were sacrificed with an overdose of ethyleneglycolmonophenylether (Liasko et al., 2010) and stored in polyethylene bags at -20°C. At the end of the study, all experimental fish were sacrificed in the same manner. For further processing, fish were autoclaved. Subsequently, fish were homogenized using an ultra turrax blending device and transferred to a pre-weighed plastic container. The homogenized material was frozen and freeze-dried. After samples were completely dry, they were weighed and again homogenized in a standard electric coffee grinder to obtain a fine powder with which subsequent analyses were conducted.

For chemical analysis dried material from all 5 fish per replicate was pooled. Nitrogen analysis of feed and whole body fish was conducted with a TrueSpec N Macro (LecoInstrumente GmbH). Protein content was calculated as nitrogen x 6.25. Lipids were analyzed through accelerated solvent extraction (Dionex, ASE200). Ash content was measured after combusting the samples at 550°C for one hour in a muffle furnace (Nabertherm). Gross energy was measured with a bomb calorimeter (Parr 6100). Mineral analyses of feed, whole body and faeces were conducted with a Thermo Scientific Series 2 Inductively Coupled Plasma Mass Spectrometer (ICP-MS) after digesting the material in concentrated salpetric acid for 20 minutes at 190°C in a CEM Mars Xpress microwave digester.

Growth of the fish was expressed as specific growth rate (SGR), feed conversion ratio (FCR), body mass gain (BMG), metabolic growth rate (MGR), protein efficiency ratio (PER), protein productive value (PPV), lipid productive value (LPV) and energy productive value (EPV).

Data was tested for normal distribution with the Shapiro-Wilk test. Percentages were arcsine transformed before analysis. One-way ANOVA ($p \leq 0.05$) was used to analyze treatments. Tukey’s test was applied as post-hoc test. Statistics were conducted with Statistica 8 software.

RESULTS

All diets were consumed at all times and fish survival was 100%. Body mass gain of fish was higher in all diets containing oxalate than in the control (0%) diet, with differences between treatments 1.5% and 2.5% being significant ($p \leq 0.05$, Table1). The same was true for SGR, MGR and PER (Table 2). FCR was significantly lower for treatments 1.5% and 2.5% than the control diet, while treatment 0.5% was not. PPV was higher for treatments 1.5% and 2.5% than for lower oxalate treatments. LPV was significantly higher in the control diet than in diets 1.5% and 2.5%, while there was no difference in EPV.

Table 1: Body mass gain of common carp fed different concentrations of soluble oxalate for 8 weeks.

Treatment	Control				0.5%				1.5%				2.5%			
IW (g)	9.87	±	0.20		9.90	±	0.29		9.83	±	0.27		9.81	±	0.23	
FW (g)	26.2	±	1.09	b	28.4	±	2.61	a,b	31.5	±	2.23	a	32.1	±	2.32	a
BMG (g)	16.3	±	1.26	b	18.5	±	2.48	a,b	21.6	±	2.01	a	22.3	±	2.28	a
BMG (%)	165.1	±	15.9	c	186.9	±	23.2	b,c	219.8	±	16.0	a,b	225.9	±	23.4	a

Values are mean +/- standard deviation, n = 5. Values with different superscripts are significantly different from each other ($p \leq 0.05$). IW: Initial weight; FW: Final weight; BMG: body mass gain.

There were significant differences in the body composition of experimental fish, the control treatment and treatment 0.5% being significantly higher than 1.5% and 2.5% in body lipid and energy and significantly lower in body moisture and ash contents.There was no difference in body protein content (Table 3)

Table 2. Nutrient utilization of common carp fed different concentrations of oxalate for 8 weeks.

Treatment	Control				0.5%				1.5%				2.5%			
SGR	1.74	±	0.11	b	1.88	±	0.15	a,b	2.07	±	0.09	a	2.11	±	0.13	a
FCR	1.74	±	0.10	a	1.61	±	0.15	a,b	1.45	±	0.07	b	1.47	±	0.11	b
MGR	7.35	±	0.43	b	7.96	±	0.65	a,b	8.80	±	0.42	a	8.95	±	0.54	a
PER	1.72	±	0.11	a	1.90	±	0.18	a,b	2.14	±	0.11	b	2.12	±	0.16	b
PPV	22.9	±	1.34	b	24.8	±	2.51	b	28.2	±	1.51	a	28.2	±	2.64	a
LPV	43.5	±	1.14	a	44.0	±	6.26	a	36.8	±	5.66	a,b	33.4	±	2.71	b
EPV	20.6	±	0.53		21.4	±	2.23		21.0	±	1.55		20.8	±	1.55	

Values are mean +/- standard deviation, n = 5. SGR: specific growth rate (% / day); FCR: feed conversion ratio; MGR: metabolic growth rate (g * $kg^{0.8}$ / day); PER: protein efficiency ratio; PPV: protein productive value (%); LPV: lipid productive value (%); EPV: energy productive value (%). Values with different superscripts are significantly different from each other ($p \leq 0.05$)

Table 3. Body composition of common carp fed different concentrations of oxalate for 8 weeks.

Treatment	**Initial Fish**	**Control**		**0.5%**		**1.5%**		**2.5%**	
Moisture (%)	77.5 ± 0.67	75.1 ± 0.53	b	75.6 ± 0.34	b	76.8 ± 0.74	a	77.0 ± 0.59	a
Protein (%)	14.6 ± 0.33	13.8 ± 0.14		13.6 ± 0.16		13.6 ± 0.21		13.7 ± 0.31	
Lipid (%)	3.82 ± 0.38	7.27 ± 0.36	a	7.13 ± 0.48	a	5.67 ± 0.67	b	5.29 ± 0.27	b
Ash (%)	3.35 ± 0.18	2.38 ± 0.08	b	2.38 ± 0.07	b	2.65 ± 0.09	a	2.74 ± 0.09	a
GE (kJ/g)	4.89 ± 0.27	6.13 ± 0.17	a	5.94 ± 0.12	a	5.44 ± 0.25	b	5.31 ± 0.16	b

Values based in wet weight. Values are mean +/- standard deviation, n = 5. Values with different superscripts are significantly different from each other ($p \leq 0.05$)

Calcium, magnesium and phosphorus retention was significantly lower in low oxalate treatments (0% and 0.5%) compared to higher oxalate treatments (1.5% and 2.5%, Table 4).

Table 4. Mineral retention of selected minerals of common carp fed different concentration of oxalate for 8 weeks.

Treatment	0%		**0.50%**		**1.50%**		**2.50%**	
Mg	6.15 ± 0.53	a	6.51 ± 0.91	a	9.22 ± 0.45	b	9.88 ± 0.37	b
P	12.7 ± 1.01	a	14.1 ± 2.08	a	21.4 ± 2.26	b	22.6 ± 0.82	b
Ca	9.83 ± 0.99	a	11.0 ± 1.82	a	18.2 ± 1.98	b	20.2 ± 0.64	b
Zn	30.8 ± 5.10		28.7 ± 6.74		31.5 ± 5.47		29.3 ± 2.92	

Values in %. Values are mean +/- standard deviation, n = 5. Values with different superscripts are significantly different from each other ($p \leq 0.05$)

DISCUSSION

To our knowledge the present study is the first to deal with the influence of oxalate as an anti-nutritional factor in carp. Di-sodium oxalate was chosen because of its high solubility at neutral pH values compared to calcium or magnesium oxalate. The maximum treatment level of 2.5% was chosen to represent a theoretical maximum oxalate content in a fish diet consisting largely of high-oxalate plant ingredients, such as detoxified *Jatropha curcas* kernel meal. The present study shows significant impacts of oxalate on growth development, body nutrient and mineral composition of the experimental fish. There was a tendency for treatments 0% and 0.5% to show similar results, while diets 1.5% and 2.5% were different from the lower oxalate treatments, but similar to each other.

Growth

There was a significantly positive effect of oxalate on body mass gain, specific growth rate and feed conversion ratio of experimental fish. This effect was unexpected as a decrease in performance, possibly through mineral deficiencies or direct oxalate poisoning of fish in the high-oxalate treatments, might have been expected.

Oxalate is the salt of oxalic acid, a strong, organic acid (Francesch i& Horner, 1980). Organic acids or their salts have received considerable attention as growth promoting agents (Lueckstädt et al., 2008).

Body Composition

There were significant differences in body composition of experimental fish for treatments 0% and 0.5% compared to 1.5% and 2.5%. Higher ash values in treatments 1.5% and 2.5% reflect improved mineral retention of these treatments. There was no impact of oxalate concentration on body protein content.

Mineral composition of carcass and mineral retention

Most existing literature regarding the effects of oxalate in the digestive tract of humans or livestock states that oxalate binds to divalent cations making these unavailable for absorption. This is shown by increased cation content in the faeces and concomitant lower digestibility and retention values for these cations (Noonan & Savage, 1999; Rahman et al., 2013). However, our results show the opposite trend with treatments 1.5% and 2.5% showing clear increases in calcium, magnesium and phosphorus retention compared to treatments 0% and 0.5%.

CONCLUSION

The effects of different concentrations of dietary oxalate on common carp have not been previously investigated despite high contents of this anti-nutritional factor in popular feedstuffs. Oxalate in carp had a positive effect on growth and it is hypothesized that this may be due to antimicrobial effects exerted in the intestine. The effects on body nutrient composition were distinct, higher oxalate promoted higher mineral and lower lipid content. No anti-nutritional effects, predominantly mineral deficiencies, as described in the literature could be detected over the trial period; however, long-term studies including histopathology are required in order to conclude whether oxalate is beneficial in carp feeds and to elucidate potential mechanisms.

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CURRENT ISSUES AND PRINCIPLES OF COMMON CARP (*CYPRINUS CARPIO*) ORGANIC POND FARMING IN EUROPE. AN OVERVIEW

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PREGLED TRENUTNIH IZAZOVA I PRINCIPA ORGANSKOG UZGOJA ŠARANA (*CYPRINUS CARPIO*) U JEZERIMA U EVROPI

Apstrakt

Šaran (*Cyprinus carpio*) je jedna od najznačajnijih vrsta ribe u akvakulturi u slatkovodnim jezerima. Akvakultura u slatkovodnim jezerima se često karakteriše kao sistem sa malim novčanim i ulaganjima radne snage, i najčešće služi lokalnom tržištu. Šaran je nedavno klasifikovan: kada je reč o svetkoj proizvodnji zauzima treće mesto, a kada je reč o vrednosti, sedmo (Tabela 1). U 2013. godini, ukupna EU27 proizvodnja šarana iznosila je 57,254 tone (FEAP 2014). Zemlja sa najvećom proizvodnjom šarana bila je Poljska (31% ukupne EU27 proizvodnje šarana), Češka (29%), Mađarska (17%), Nemačka (10%) i Francuska (6%).

Ipak, izveštaji o organskom gajenju šarana dolaze samo iz Mađarske (~ 700 t), Nemačke (~ 200 t) i Austrije (~ 150 t). Jezerske oblasti gde se gaji šaran na organski način zauzimaju 4700 ha u Mađarskoj i 550 ha u Austriji, što predstavlja približno 20% ukupne oblasti u kojima se gaji riba u obe zemlje (Varadi, Phuong 2007).

Šaran je idealan kandidat za organsko gajenje jer zauzima nisko mesto u lancu ishrane, hrani se prirodnom hranom, u jezerima, i ima minimalan uticaj na životnu sredinu. Iako je način na koji se šaran gaji u jezerima kvazi organski i prelaz na sertifikovano organsko gajenje nije zahtevan kao kod drugih vrsta, prisustvo šarana na organskom tržištu ribe je još uvek jako nisko. Takođe, neke druge, dodatne vrste riba koje se mogu gajiti zajedno sa šaranom pokazuju visok potencijal za organsko gajenje, zbog minimalnih potreba u smislu upravljanja jezerom i sertifikacije.

Trenutni standardi za organsko gajenje šarana ipak nisu sasvim usklađeni kada je reč o prihvatljivim načinima za reprodukciju. Takođe, postoje glavna ograničenja i problemi kao što je nedostatak organske hrane, predatori, razlikovanje od neorganskih proizvoda, nedostatak saradnje kada nastanu tehnički problem i marketing, pojava kostiju i stav potrošača da je šaran jevtina hrana.

Marža za organskog šarana može da bude dobra ukoliko je prodajna cena znatno viša od troškova proizvodnje. Potrebno je harmonizovati standarde, poboljšati preradu i marketing (kada je reč o filetima bez kostiju) i podržati bolju sardanju među organskim uzgajivačima. Budućnost je svetla, što se tiče svih gore navedenih stavki, međutim trenutna potrošnja organskog šarana opada. Potrebno je repozicionirati organskog šarana na tržištu i proizvoditi više filetirane nego cele ribe.

Ključne reči: potrošač šarana, tržište za šarana, šaransko jezero, organska akvakultura, akvakultura u jezeru

Tabela 1 Svetska proizvodnja najvažnijih 10 vrsta u akvakulturi (ribe, rakovi i školjke) u 2012. godini (FAO 2014). Napomena: % - procenat ukupne svetske proizvodnje/vrednosti

Vrste	Proizvodnja		Vrednost	
	tona	%	10^3 USD	%
Beli amur *Ctenopharyngodon idella*	5 028 661	7,55	6 464 586	4.69
Beli tolstolobik *Hypophthalmichthys molitrix*	4 189 578	6.29	5 540 946	4.02
Šaran ***Cyprinus carpio***	3 791 913	5.69	5 207 971	3.78
Morska školjka *Ruditapes philippinarum*	3 785 311	5.68	3 546 979	2.58
Nilaska tilapija *Oreochromis niloticus*	3 197 330	4.80	5 260 695	3.82
Pacifički beli škamp *Penaeus vannamei*	3 178 721	4.78	13 592 534	9.87
Sivi tolstolobik *Hypophthalmichthys nobilis*	2 898 816	4.35	3 723 608	2.70
Indijski šaran *Catla catla*	2 761 022	4.14	5 488 405	3.98
Karaš *Carassius carassius*	2 451 845	3.68	2 674 406	1.94
Losos *Salmo salar*	2 066 561	3.10	10 095 957	7.33
Ukupno u svetu	66 633 253		137 731 508	

ZAHVALNICA

Ovo istraživanje je finansijski podržano od strane Ministarstva za obrazovanje, mlade i sport u Češkoj – projekti CENAKVA (No. CZ.1.05/2.1.00/01.0024) i CENAKVA II (No. LO1205 pod NPU I programom), i od strane projekta OrAqua (KBBE.2013.1.2-11).

Napomena: Celokupan tekst predat je za publikaciju u naučnom časopisu ‘Reviews in Aquaculture’.

Abstract

Common carp (*Cyprinus carpio*) is one of the most important fish species in freshwater pond aquaculture which is often characterized as a low input system with low labour productivity and low capital intensity, serving mainly local markets. Recently, common carp is classified on 3rd and 7th rank regarding world production and its value, respectively (Table 1). In 2013, the total EU27 aquaculture carp production was 57,254 tonnes (FEAP 2014) being mostly produced in Poland (31 % of total EU27 carp production), Czech Republic (29 %), Hungary (17 %), Germany (10 %) and France (6 %).

Nevertheless, organic carp production is currently reported just from Hungary (~ 700 t), Germany (~ 200 t) and Austria (~ 150 t). Pond areas with organic carp production occupy 4700 ha and 550 ha in Hungary and Austria respectively, which represents approximately 20% of the total fishpond area in both countries (Varadi, Phuong 2007).

Carp represents an ideal candidate for organic status since it is low in the food chain, feeds naturally and in pond farming, it has a minimum impact on the environment. Despite the way carp are farmed in ponds is already quasi-organic and the shift to their certified organic farming is not as demanding as it is for some other species, the proportion of carp on the organic fish market is still very low. Obviously, also the other supplementary carp pond fish species prove a high potential to be considered as organic products with minimum requirements in sense of pond management and certification. Current organic standards for common carp are, however, facing some inconsistencies such as acceptable ways of reproduction, as well as the main constraints and problems such as shortage of organic feed, predation of wild animals, differentiation from non-organic product, lack of cooperation on technical issues and marketing, bones appearance and consumer perception of carp as a cheap food. The margins for organic carp can be good with sales price being significantly higher than production costs. Organic carp culture needs help to harmonise standards, to improve processing and marketing (boneless fillets) and to support greater cooperation among organic farmers. There are good long term prospects with this respect, though current consumption is on the wane. There is a need to reposition organic carp in the market and produce more fillets rather than just live and/or whole fish.

*Keywords**:** carp customer, carp market, carp pond, organic aquaculture, pond aquaculture*

Table 1. World aquaculture production of top 10 aquaculture (fish, crustacean and mollusc) species in 2012 (FAO 2014). Note: % percentage world total

Species	Production		Value	
	tonnes	%	10^3 USD	%
Grass carp *Ctenopharyngodon idella*	5 028 661	7,55	6 464 586	4.69
Silver carp *Hypophthalmichthys molitrix*	4 189 578	6.29	5 540 946	4.02
Common carp *Cyprinus carpio*	3 791 913	5.69	5 207 971	3.78
Manila clam *Ruditapes philippinarum*	3 785 311	5.68	3 546 979	2.58
Nile tilapia *Oreochromis niloticus*	3 197 330	4.80	5 260 695	3.82
Pacific white shrimp *Penaeus vannamei*	3 178 721	4.78	13 592 534	9.87
Bighead carp *Hypophthalmichthys nobilis*	2 898 816	4.35	3 723 608	2.70
Catla *Catla catla*	2 761 022	4.14	5 488 405	3.98
Crucian carp *Carassius carassius*	2 451 845	3.68	2 674 406	1.94
Salmon *Salmo salar*	2 066 561	3.10	10 095 957	7.33
World total	66 633 253		137 731 508	

ACKNOWLEDGEMENTS

The study was financially supported by the Ministry of Education, Youth and Sports of the Czech Republic – projects CENAKVA (No. CZ.1.05/2.1.00/01.0024) and CENAKVA II (No. LO1205 under the NPU I program), and by the project OrAqua (KBBE.2013.1.2-11).

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Note: Full text was submitted as manuscript for publication in Reviews in Aquaculture.

COMPARISON OF GROWTH, SURVIVAL RATE, AND NUMBER OF MARKETABLE FISH PRODUCED OF KOI CARP, *CYPRINUS CARPIO* L., IN OUTDOOR EARTHEN PONDS WITH ENDOGENOUS CULTURE OF *MOINA* SP. OR *DAPHNIA* SP. AND EXOGENOUS SUPPLY OF MIXED PLANKTON

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POREĐENJE RASTA, STOPE PREŽIVLJAVANJA I KOLIČINE KOI ŠARANA, *CYPRINUS CARPIO* L., PROIZVEDENOG ZA TRŽIŠTE, U SPOLJNIM ZEMLJANIM JEZERIMA SA ENDOGENOM KULTUROM *MOINA* SP., ILI *DAPHNIA* SP. I EKZOGENOM SNABDEVANJEM MEŠAVINOM PLANKTONA

Apstrakt

U ovom radu ispitan je efekat različitih sistema upravljanja na rast i preživljavanje koi šarana, *Cyprinus carpio* L. u bazenima. Riblje larve (0.14 ± 0.015 g) gajene su 3 meseca (od 5. jula do 3. septembra 2011.). Postojala su četiri tretmana/načina gajenja: ribe su nasađene u spoljnim bazenima i gajene sa: endogenom kulturom *Moina* sp. (P1), *Daphnia* sp. (P2), egzogeno dodatom mešavinom planktona (P3) i kontrolom u kojoj je korišćena komercijalna peletirana hrana (Tokyu Corp., Japan; koja sadrži 32% sirovog proteina) (P4). Svaki tretman rađen je u triplikatu. Riba je hranjena dnevno, sa nešto više hrane u grupi P3 i P4, da bi se eliminisala mogućnost da nedostatak hrane bude ograničavajući faktor za rast. Bazeni su pokriveni jednim slojem plastike za zaštitu od ptica. Stalni nivo vode u bazenima je održavan periodičnim dodavanjem podzemne vode, da bi se nadoknadio gubitak usled isparavanja. Oko 1000 litara viška vode otpušteno je iz bazena P3 svakog dana dok je trajalo uvođenje vode sa živim planktonom.

Vrednost rastvorenog kiseonika bila je najviša u P3 bazenu ($p<0.05$). Tretman P4 pokazao je najvišu provodljivost, NH_4^-N, NO_2^-N, NO_3^-N, PO_4^-P, i bikaronatnu alkalnost, koje su bile znatno više ($p<0.05$) nego kod drugih tretmana. Krajnja telesna težina koi šarana varirala je od 4.25 do 8.52 g kod različitih tretmana. Pri izlovu, najveći prirast dostignut je u grupi P3, zatim P2, P1 i P4 ($p<0.05$). Značajna razlika ($p < 0.05$) se pokazala u preživljavanju među tretmanima, od 71.12% (P4) do 94.12% (P3). Da bi se ustanovila količina ribe za tržište, procenjen je procenat i broj ribe koja je prevazilazila ukupnu težinu od 5g. Procena je izvršena raspodelom frekvencije veličine na kraju ispitivanja. Količina ribe koja se mogla plasirati na tržište bila je znatno veća u grupi P3 ($p < 0.05$) neko kod drugih tretmana. Iz ovog istraživanja može se zaključiti da egzogeno snabdevanje mešavinom planktona predstavlja bolju alternativu od gajenja sa endogeno gajenim *Moina* sp. ili *Daphnia* sp.

Abstract

The effect of different management systems on the growth and survival of koi carp, *Cyprinus carpio* L. in ponds was investigated. Fish larvae (0.14 ± 0.015 g) were cultured for three months (5 July to 3 September' 2011). There were four treatments: fish were stocked in outdoor ponds under endogenous culture of *Moina* sp. (P1), *Daphnia* sp. (P2), exogenous supply of mixed plankton (P3) and a control treatment where a commercial pellet (Tokyu Corp., Japan; containing 32% crude protein) was applied as food (P4). There were three replicates for each treatment. The fish were fed daily slightly in excess of satiation in P3 and P4 to eliminate the possibility of food supply being a limiting factor to growth. A single layer of plastic bird netting was used to cover the ponds. Constant water levels were maintained in the ponds by supplying ground water periodically to compensate for loss due to evaporation. Approximately 1000 l of excess water was discharged from the P3 ponds every day during the introduction of live plankton-water.

Values of dissolved oxygen were highest in the P3 ($p<0.05$). The P4 treatment showed the highest concentrations of conductivity, NH_4^--N, NO_2^--N, NO_3^--N, PO_4^--P, and bicarbonate alkalinity, which were significantly higher ($p<0.05$) than the other treatments. The final body weight of the koi carp ranged from 4.25 to 8.52 g in the different treatments. At harvest, maximum weight gain was achieved in the P3, followed by P2, P1 and P4 in decending order ($p<0.05$). There was a significant difference ($p < 0.05$) in the survival of koi carp among the treatments, ranging from 71.12% (P4) to 94.12% (P3). To determine the output of marketable fish, the percentage and number of fish exceeding a total weight of 5 g was estimated from the size-frequency distribution at the end of the study. The number of marketable fish was significantly higher in P3 ($p < 0.05$) than other treatments. From the present investigation, exogenous supply of mixed plankton appeared to be a better alternative to culturing koi carp in ponds under endogenous culture of *Moina* sp. or *Daphnia* sp.

APPLICATION OF SPERM CRYOPRESERVATION TO THE CULTURE AND CONSERVATION OF SALMONID SPECIES: A SLOVENIAN-HUNGARIAN COLLABORATION

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PRIMENA KRIOPREZERVACIJE SPERME NA GAJENJE I OČUVANJE SALMONIDNIH VRSTA: SARADNJA SLOVENIJE I MAĐARSKE

Apstrakt

U svrhu očuvanja dve salmonidne vrste koje su autohtone u slivu reke Soče u Sloveniji, pastrmske glavatice (*Salmo marmoratus*) i lipljena Jadranskog porekla (*Thymallus thymallus*) primenjena je krioprezervacija sperme. Populacije ovih vrsta ozbiljno su ugrožene hibridizacijom i introgresijom sa alohtonim vrstama: potočnom pastrmkom (*Salmo trutta m. fario*) i lipljenom Dunavskog porekla koji su unešeni u sliv reke Soče u dvadesetom veku. Ribolovački klub Tolmin, koji upravlja jednim delom reke Soče, razvio je akcioni plan za očuvanje genetskih resursa i restauraciju autohtonih vrsta u njihova primarna staništa. Ovaj akcioni plan podrazumeva prestanak poribljavanja alohtonim salmonidnim vrstama koje mogu da naprave hibride sa lokalnim vrstama, identifikaciju postojećih "čistih" vrsta, stvaranje matica od onih jedinki koji pripadaju čistim vrstama, poribljavanje potomcima čistih vrsta, stvaranje "utočišta" za postojeće čiste vrste i stalni monitoring populacija riba u njihovim vodotokovima.

Genetske analize ovih populacija izvršili su naučnici sa Odeljenja za Nauke o životinjama Univerziteta u Ljubljani. Krioprezervacija sperme Jadranskog lipljana i pastrmske glavatice čini sastavni deo akcionog plana za očuvanje od 2009. godine. Kada je reč o lipljanu, ne postoji više ni jedna čista populacija, stoga je cilj programa očuvanja povećanje udela Jadranskog genotipa kod matica. Sperma i uzorak peraja uzorkovani su od divljih mužjaka na mestu mresta. Sperma je krioprezervirana i sačuvana dok genetske analize nisu završene za svaki od uzoraka (2-3 nedelje). Krioprezervirana sperma koja je sadržala veću

količinu Jadranskog genotipa nego što je to na početku definisano je otopljena i iskorišćena za fertilizaciju jaja takozvanih ‘Jadranskih’ ženki.

Potomstvo iz jaja oplođenih krioprezerviranom spermom je odgajeno do matica i trenutno 70-80% matica lokalnog lipljana potiče iz krioprezervirane sperme. Kada je reč o pastrmskoj glavatici, krioprezervirana sperma se koristi za stvaranje ‘utočišta’. Sperma se sakuplja od divljih mužjaka čiste populacije pre sezone mresta (rani novembar) a zatim se krioprezervira. Sperma se čuva u tečnom azotu do sezone mresta (decembar-januar) kada dolazi do oplodnje jaja ženki koje pripadaju identičnoj populaciji. Oplodnja se obavlja krioprezerviranom supermom.

Jaja u stadijumu očne mrlje se zatim nasade u veštačka gnezda u pripremljenom potoku koji predstavlja ‘utočište’. Stoga veliki broj mužjaka iz čiste populacije učestvuje u stvaranju novih populacija, dok u isto vreme nije potrebno ukloniti mužjake iz originalne populacije, a nasađivanje jaja u stadijumu očne mrlje obezbeđuje obeležavanje nove teritorije. Isti protokol se koristi za krioprezervaciju obe vrste: sperma se meša u odnosu 1:1 sa ekstenderom koji sadrži 200 mM glukoze, 40 mM KCl, 30 mM trisa (pH iznosi 8.0 sa cc. HCl) a metanol se koristi kao krioprotektant u finalnoj koncentraciji od 10% v/v . Cevčice od 0.5-ml se pune rastvorenom spermom koja se zatim zamrzava u pari tečnog azota na 3cm iznad nivoa azota u trajanju od 3 minuta. Pošto se sačuva u tečnom azotu, uzorci se tope u vodenoj kupatilu na 40 °C u trajanju od 13 sekundi. Ovako razvijeni protokol rezultira u 50-70% izmrešćenih jedinki kod obe vrste.

Abstract

Sperm cryopreservation was applied to the conservation efforts of two salmonid species autochthonous to the drainage of the Soča river in Slovenia, the marble trout (*Salmo marmoratus*) and the Adriatic lineage of the grayling (*Thymallus thymallus*). Populations of these species were seriously compromised by hybridization and introgression with allochthonous species: the brown trout (*Salmo trutta m. fario*) and the Danubian lineage of the grayling that were introduced to the Soča drainage during the 20th century. The Angling club of Tolmin, that manages a part of the Soča system has developed an action plan for the conservation of the genetic resources and restoration of the autochthonous species in their original habitats. This action plan includes the cessation of the stocking of any allochthonous salmonids that could hybridize with the local species, identification of existing pure populations, establishment of broodstocks of non-introgressed individuals, stocking the rivers with the progeny of the broodstocks, creation of „sanctuary” streams for the existing pure populations and continuous monitoring of fish populations in their watercourses. Genetic analyses of the populations are conducted by scientists from the Department of Animal Science of the University of Ljubljana. Cryopreservation of sperm from the Adriatic grayling and the marble trout has constituted an integral part of the conservation activities since 2009. In case of the grayling, no pure populations remain, thus, the objective of the conservation program is to increase the proportion of Adriatic genotype in the broodstock. Sperm and fin clips were collected from wild males on the spawning grounds. Sperm was cryopreserved and stored until the genetic analysis was completed on each sample (2-3 weeks). Cryopreserved sperm of individuals containing higher than a pre-defined proportion of Adriatic genotype was thawed and used for fertilization of eggs known „Adriatic” females. Progeny hatching from eggs fertilized with cryopreserved sperm was grown to broodstock and currently 70-80% of the local grayling broodstock originates from cryopre-

served sperm. In case of the marble trout, sperm cryopreservation is used in the creation of „sanctuary” streams. Sperm is collected from wild males of a given pure population prior to the spawning season (early November) and cryopreserved. Sperm is stored in liquid nitrogen until the spawning season (December-January) when eggs of females from the identical population are fertilized with the cryopreserved sperm. Eyed eggs are then stocked into artificially created nests in the prepared „sanctuary” stream. Thus, a high number of males of the given pure population participates in the creation of the new population, removal of males from the original population is unnecessary, and stocking of eyed eggs ensures imprinting at the new location. The same protocol is used for cryopreservation of both species: sperm is mixed at a ratio of 1:1 with an extender containing 200 mM glucose, 40 mM KCl, 30 mM Tris (pH set to 8.0 with cc. HCl) and methanol is used as a cryoprotectant at 10% v/v final concentration. Diluted sperm is loaded into 0.5-ml straws and frozen in the vapor of liquid nitrogen at 3 cm above the level of nitrogen for 3 minutes. Following storage in liquid nitrogen, samples are thawed at a 40 °C water bath for 13 sec. The developed protocol results in 50-70% hatch in both species.

THE PRESENT STATUS OF RAINBOW TROUT AQUACULTURE IN TURKEY

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TRENUTNI STATUS GAJENJA KALIFORNIJSKE PASTRMKE U TURSKOJ

Apstrakt

U Turskoj, ukupna proizvodnja ribe i školjki bila je 607.515 tona u 2013. Udeo ulova iz slakih voda predstavljao je 5.8%, što je činilo 35.074 tone ukupne proizvedene ribe, i ta vrednost je zaista bila niska. Dominantne vrste u ukupnoj proizvodnji su pastrmka, šaran, brancin, orada, iverak, školjke, slatkovdni rak, itd. Ukupna proizvodnja kalifornijske pastrmke dostigla je 122.873 tone godišnje a ta vrednost je predstavljala je 20.23% ukupno proizvedene ribe u 2013 godini. Danas se u Turskoj, još uvek, najviše investira u gajenje kalifornijske pastrmke u industriji akvakulture.

Cilj ovog istraživanja je da pruži informacije o gajenju kalifornijske pastmke (*Oncorhynchus mykiss*) i da podigne svest o njenim razvojnim trendovima gajenja u Turskoj.

Ključne reči: Kalifornijska pastrmka, akvakultura, predviđanje o proizvodnji, Turska.

Abstract

In Turkey, the total production of fish and shellfish was 607.515 tons in 2013. The contribution of freshwater catch was established 5.8% by 35.074 tons in the total fishery production and its value was really very small. The dominant species in total production were trout, carp, sea bass, sea bream, turbot, mussel, crayfish and etc. Total production of rainbow trout reached to 122.873 tons/year and its value was 20.23% in total fish production in 2013. Today, rainbow trout aquaculture is still favor investment in fishery industry in Turkey.

This study aimed to inform about rainbow trout (*Oncorhynchus mykiss*) aquaculture and to raise awareness for its development trends in Turkey.

Keywords: Rainbow trout, Aquaculture, Production projection, Turkey.

SPERMATOGONIAL TRANSPLANTATION AS A NOVEL TECHNIQUE IN AQUACULTURE AND FISH CONSERVATION

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TRANSPLANTACIJA SPERMATOGONIJA KAO NOVA METODA U AKVAKULTURI I KONZERVACIJI RIBA

Apstrakt

Poslednjih godina, primena primordijalnih germinativnih ćelija (primordial germ cells - PGCs) i spermatogonijalnih stem ćelija (spermatogonial stem cells - SSCs) riba je postala veoma značajna zbog razvoja metode transplantacije ovih ćelija. Od kada su Brinster i Avarbock (1994) razvili ovu metodu, ona se uspešno koristi za čuvanje genetskog materijala ugroženih vrsta i u stvaranju novih transgenih linija kod miševa i domaćih životinja. Uvođenje ove metode kod riba predstavlja značajan napredak u oblasti reproduktivne biotehnologije, akvakulture, konzervacione biologije, kao i u razvoju novih transgenih linija različitih vrsta riba.

Osnovu ove metode predstavlja transplantacija germinativnih ćelija (PGC, SSC) iz donorskog organizma u organizam primaoca. Najinteresantnija u tom smislu je upotreba nediferenciranih spermatogonija A tipa (A_{und}) koje imaju sposobnost samoobnavljanja ali i proizvodnje ćelija kasnijih faza spermatogeneze. Postoji takođe nekoliko specifičnih osobina SSC koje ih čine pogodnim za transplantaciju: (1) sposobnost da kolonizuju testis primaoca odakle produkuju donorsku spermu, (2) mogućnost da se nakon transplantacije u primaocu muškog pola razviju u spermatogonije, a u primaocu ženskog pola u oogonije i (3) mogućnost genske manipulacije sa ciljem produkcije transgenih riba (Lacerda i sar., 2010).

Prilikom transplantacije SSC, posebna pažnja mora biti usmerena ka izboru vrste donora i primaoca. Najbolje bi bilo da donor i primalac ne budu filogenetski previše udaljeni, kao i da primalac ima kratak reproduktivni ciklus i manje dimenzije tela kako bi ekonomski bio pogodniji za gajenje. Donorska vrsta je obično vrsta za koju postoji određneni interes, bilo ekonomski, naučni ili konzervacioni.

Tokom transplantacije, kompatibilnost između primaoca i donora može biti ograničavajući faktor u uspehu samog procesa. U najgorem slučaju, primalac, usled imune reakcije, može u potpunosti odbaciti transplantirano tkivo ili ćelije. To je najčešće slučaj ukoliko se vrši transplantacija SSC iz odraslog donora u odraslog primaoca.

Kako bi se izbegao problem izazvan transplantacijom između dve odrasle jedinke, koristi se prednost ontogenije primaoca, posebno ontogenije njegovog imunog sistema, tako što se za primaoca koriste embrioni ili larve. Ovi stadijumi kod riba nemaju razvijen imuni sistem niti diferencirane T-ćelije (Takeuchi et al., 2003; Yoshizaki et al., 2011) te s toga nemaju mehanizam pomoću kojeg bi odbacili donorsko tkivo. Takođe, lakše je blokirati razvoj endogenih primordijalnih germinativnih ćelija kod larvi, nego ukloniti SSC iz već razvijenih gonada kod odraslog donora.

Pored odabira odgovarajuće vrste donora i primaoca, neophodno je na pravi način izolovati specifične ćelije koje treba da budu transplantirane. Ovaj proces je donekle jednostavniji kada je u pitanju tansplantacija PGC s obzirom na njihov daleko manji broj u odnosu na spermatogonije i na to da one još uvek nisu potpuno razvijene u gonadama. S druge strane, SSC su dobro razvijene u gonadama i najčešće zauzimaju karakteristično mesto unutar pojedinačnih niša u testisu specifičnih za tu vrstu ćelija. Prilikom izolacije nediferenciranih spermatogonija A tipa iz testisa odrasle jedinke, veome je bitno voditi računa od morfologiji tih ćelija kao i specifičnim markerima pomoću kojih ih je moguće razlikovati od ostalih tipova spermatogonija (A_{diff}, B), spermatocita i spermatida. Osnovne histološke metode u kombinaciji sa imunohistohemijom, *in situ* hibridizacijom ili *in situ* PCR metodom se mogu koristiti za identifikaciju spefifičnih molekularnih markera (proteina ili RNK) u ćelijama unutar ćelijskih niša i koji se u daljem toku rada mogu koristiti za izolaciju određenih ćelija.

Pre transplantacije PGC ili SSC, neophodno je izolovati željene ćelije iz donorskog tkiva. Nakon multienzimske razgradnje tkiva testisa, ćelije se izoluju na osnovu njihove morfologije i veličine i/ili specifičnih molekularnih markera zbog kojih čitav proces može biti species-specifičan.

Kombinacija transplantacije PGS i SSC sa krioprezervacijom daje dodatni značaj ovoj metodi s obzirom da još uvek ne postoji optimizovan protokol za uspešnu krioprezervaciju jaja i embriona riba, pre svega zbog prisustva velike količine žumanceta i masti.

Krioprezervacija ćelija kao što su PGS i SSC, koje imaju mogućnost da produkuju spermatozoide ili oocite u zavisnosti od pola jedinke primaoca, ima izuzetno veliku perspektivu primene u konzervacionoj biologiji i akvakulturi. Istraživanja su pokazala da krioprezervirane SSC nakon odmrzavanja i transplantacije u telo primaoca mogu proizvesti spermatozoide i oocite donorske vrste (Kobayashi et al., 2007). Na taj način, čuvanje gameta nije neophodno jer krioprezervacijom germinativnih ćelija i njihovom transplantacijom, moguće je dobiti gamete oba pola.

Kljčne reči: spermatogonija, izolacija, primalac, donor

Abstract

In recent years, the importance of manipulations of primordial germ cells (PGCs) and spermatogonial stem cells (SSCs) in fish has drastically increased due to development of transplantation method of these cells. Since its development by Brinster and Avarbock (1994), this method has been successfully used in the preservation of genetic material of

endangered species and in the creation of new transgenic lines of mice and farm animals. Introduction of this method in fish leads to advances in reproductive biotechnology, aquaculture, development of new transgenic lines and conservation biology of fish.

The base of this method lies in the transplantation of the germinative cells (PGCs, SSCs) from donor organism into recipient organism. Undifferentiated spermatogonia type A (A_{und}) which have the ability of self-renewal are the most interesting for transplantation since they have the ability of self-renewal, but can also produce later stage cells.

There are several advantages of using SSCs in transplantation process: (1) the capability of SSCs to colonize the testis of the recipients where they are able to produce donor-derived sperm, (2) plasticity in development since SSCs can develop into spermatogonia in male recipients and oogonia in female recipients and (3) the possibility of genetic manipulation in SSCs in order to produce transgenic fish (Lacerda et al., 2010).

When transplanting SSCs, special attention must be given to the choice of donors and recipients species. It is best that donor and recipient organisms are phylogenetically not too distant, that recipient organisms have a short reproductive cycle and that they are small for a more economic rearing. Donor species are usually species which attract certain interest, whether its an economic, scientific or conservation interest.

During transplantation, compatibility between recipient and donor may be a very limiting factor in transplantation success. In the worst-case scenario, recipients may completely reject the transplanted tissue or cells due to immunological reaction. This is especially the case when transplanting SSCs isolated from adult donors into adult recipients. In order to evade the problems caused by adult-adult transplantations, scientists have taken advantage of the ontogeny of recipients, mainly the ontogeny of their immune system, and used embryos and larvae as recipients. Embryos and larvae do not have a developed immune system nor differentiated T-cells (Takeuchi et al., 2003; Yoshizaki et al., 2011), therefore they do not have mechanisms to reject the donor tissue. Furthermore, it is easier to knock-out larval endogenous PGCs than to deplete SSCs from already developed gonad.

Apart from choosing the right donor and recipient organisms, it is necessary to isolate specific cells that need to be transplanted. This is to some extent easier when transplanting PGCs, since there are fewer of them than SSCs, and they have not yet fully developed inside the gonads. On the other hand, SSCs are well developed inside the gonads and usually take their specific place within the spermatogonial stem niche. When isolating undifferentiated spermatogonia type A from adult testis, special attention must be given to their morphology and specific markers that distinguish them from other types of spermatogonia (A_{diff}, B), spermatocytes and spermatids. Basic histology may be coupled with immunohistochemistry, in situ hybridization or in situ PCR which would enable the identification of specific molecular markers within the cells of the niche (proteins or RNA). All this data can be further used in isolation of particular cells.

Prior to transplantation, PGCs and spermatogonia need to be isolated from the donor tissue. After multi-enzymatic digestion it is possible to isolate cells based on their morphology and size, and/or specific molecular markers and the whole process can be species-specific.

A great advantage of transplantation of PGCs and SSCs is that this method can be very well combined with cryopreservation. There are still no optimized protocols for cryopreservation of fish eggs and embryos, mostly due to presence of large amount of yolk and fat. Since PGCs and SSCs can develop into both sperm and eggs, cryopreservation of these cells could have a great perspective in conservation biology but also in aquaculture. Studies have shown that frozen/thawed SSCs transplanted into recipients give rise to potent donor sperm and eggs in the recipients (Kobayashi et al., 2007). In this way, there is no need to conserve both sperm and eggs since successful cryopreservation of germ cells can give rise to both sperm and eggs after transplantation.

Keywords: spermatogonia, isolation, recipients, donor

ACKNOWLEDGEMENTS

The work was supported by the COST Action FA1205 AQUAGAMETE and the project 8526-5/2014/TUDPOL of the Ministry of Human Resources of Hungary awarded to Szent István University.

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NUTRITIONAL REQUIREMENTS OF JUVENILE PIKE (*ESOX LUCIUS*) REARED IN RECIRCULATING AQUACULTURE SYSTEM

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POTREBE U ISHRANI MLAĐI ŠTUKE (*ESOX LUCIUS*) GAJENE U RECIRKULACIONOM SISTEMU

Apstrakt

Zahvaljujući napretku tehnologije, danas je moguće intenzivno gajenje juvenilne štuke u recirkulacionom sistemu korišćenjem formulisane komercijalne hrane (Wolnicki i Górny 1997). Komercijalna hrana koja se trenutno koristi za ishranu štuke je formulisana za druge vrste kao što su pastrmka, som i jesetra. Ova hrana se veoma razlikuje po sastavu proteina i sadržaju energije, što može da utiče na parametre proizvodnje. Smanjivanje odnosa svrarljivosti proteina/energetska efikasnost (DP/DE) u smešama može dovesti do većeg zadržavanja proteina, međutim može imati efekte na zdravlje riba i kvalitet proizvoda. Prvi cilj ove studije je bio da se istraži efekat komercijalnih smeša sa različitom koncentracijom proteina i lipida na juvenilne štuke manje od 20g (eksperiment I) i preko 70g (eksperiment II). Današnji trendovi u proizvodnji hrane za ribe su usmereni u pravcu zamene ribljeg brašna alternativnim izvorima proteina kao što su biljke, suvozemne životinje i nus-proizvodi. Ovi trendovi su dirigovani kako ekonomskim tako i etičkim pitanjima (Brinker and Reiter 2011). Drugi cilj ovog rada je bio da se istraži delimična zamena ribljeg brašna u smešama. U trećem eksperimentu, ispitivana je delimična zamena ribljeg brašna sa pšeničnim glutenom i živinskim brašnom.

INTRODUCTION

Thanks to advancements in rearing technologies, it is now possible to conduct intensive rearing of juvenile pike in RAS using commercial, formulated feed (Wolnicki andGórny 1997). Commercial diets which are currently used for pike are developed for other species including trout, catfish, sturgeon etc. These diets are greatly differing in protein and energy content, which can influence the production parameters. Decreasing dietary DP/DE ratio the diet can results in an increase of protein conservation, however it could have many implications on fish health and product quality. The first aim of this study was to investigate the effects of commercial diets containing different protein and lipid concentration in case of rearing pike juveniles less than 20g (trial 1) and over 70g bodyweight (trial 2). Current trends in fish feed production are seeking for the replacement of fishmeal by alternative protein sources such as plant, terrestrial animal and by-products. These trends are being driven by both economic and ethical concern (Brinker and Reiter 2011). The second aim of this study was to investigate the partial substitution of fishmeal in the diet. In trial 3 the effects of partial replacement of fish meal with wheat gluten and poultry meal were studied.

MATERIALS AND METHODS

Trial 1

Four different commercial diet (Aller Aqua Poland) with different protein and lipid concentration were compared: 488 EX with CP (Crude protein) 54%, CF (crude fat) 12%; 505 EX CP 50%, CF 16%; SAFIRCP 45%, CF 20% and AVANTCP 42%,CF 24%. 12 sets of 350 fish (w0=3.01±0.1) were distributed into 250L tanks linked to recirculating system. The daily feed portion were determined as 3.5% of biomass until day 24; 3% until day 42; 2% until day 50 and 1.5% until day 71 when the trial was finished.

Trial 2

Three different commercial diet (Aller Aqua Poland) with different protein and lipid ratio were compared: STURGEON REP EX with CP (Crude protein) 52%, CF (crude fat) 12%; METABOLICACP 52%, CF 15% andPRIMO CP 37%, CF 12%. 9 sets of 23 fish (76.1±13.4g) were distributed into 250L tanks linked to recirculating system. Fish were fed two to three times a day by hand until visual satiation was achieved. The duration of the trial was 9 weeks.

Trial 3

Three different protein content experimental diets were compared. The diet C contained 100% fishmeal as the protein source, diet P 25% of fishmeal was replaced with wheat gluten, and diet A 25% of fishmeal was replaced with poultry meal. All diets were isonitrogenous and isoenergetic. The 9 sets of 25 fish (w0: 11.3±1.6g) were distributed into 80L tanks linked to recirculating system. Fish were fed once a day by hand until visual satiation was achieved. The duration of the trial was 4 weeks.

RESULTS AND DISCUSSION

Comparison of different protein and energy content of commercial diets

In both cases, in trial 1 and trial 2, where fish were fed with high protein and low fat content diet had better SGR (Specific Growth Rate) and FCR (Fed Conversion Rate) values (see Table 1). The low protein concentration can decrease the growth and feed utilization. The increased energy intake through increasing the dietary lipid concentration from CL16% to

CL 20% in trial 1 could improve the PER (protein efficiency ratio). However, in trial 2 the increased lipid concentration does not provide significantly better protein utilization with bigger size pike.

Table 1. Summary of growth and feed utilization in Trial 1 and Trial 2

	488 EX	505 EX	Safir	Avant	Primo	Stur.Rep. Ex.	Metabolica
	Trial 1				Trial 2		
SGR	3.01±0.04 [a]	2.87±0.12 [ab]	2.83±0.1 [b]	2.39±0.06 [c]	0.37±0.11 [a]	0.88±0.06 [b]	0.86±0.08 [b]
FCR	0.69±0.01 [a]	0.75±0.01 [b]	0.78±0.04 [bc]	0.93±0.04 [c]	2.53±0.45 [a]	0.97±0.01 [b]	0.97±0.01 [b]
PER	2.67±0.6 [a]	2.86±0.14 [b]	2.66±0.02 [a]	2.57±0.11 [a]	1.08±0.17 [a]	2.02±0.34 [b]	1.97±0.03 [b]

Where SGR: specific growth Rate (%/day), FCR: Feed conversion rate (g/g), PER protein efficiency ratio (g/g). The different letters indicate significant differences between the groups (ANOVA $p < 0.05$)

Effects of the partial fishmeal replacement

The 25% replacement of the fishmeal with alternative protein sources did not have a significant effect (ANOVA $p<0.01$) on growth and feed utilization (see Table 2). The slightly lower growth in case of fish fed with diet P could be caused by the lower feed intake which may results in decreasing growth in longer term period.

Table 2.

	diet C	diet P	diet A
SGR (%/day)	2.78±0.17	2.53±0.13	2.75±0.18
FCR (g/g)	0.83±0.04	0.80±0.01	0.77±0,08
Feedintake/group (g)	215.3±17.2	181.4±5.3	215.4±8.4

Where SGR: specific growth Rate (%/day), FCR: Feed conversion rate (g/g), Feed intake / group: total amount feed fed in each aquaria (g) – uneaten feed (g).

AKNOWLEDGEMENT

This work was supported by the Hungarian Scientific Research Fund (OTKA pd84289). We would like to express our thanks to Dr. Carsten Schulz and Arndt von Dawitz - GMA Büsumfor the experimental feed. Our participation was supported by TÁMOP-4.2.1.C-14/1/KONV-2015-0008.

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INTENSIVE AQUACULTURE OF THE PIKEPERCH (*SANDER LUCIOPERCA*) IN ROMANIA: CURRENT STATUS AND PERSPECTIVES

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INTENZIVNI UZGOJ SMUĐA (*SANDER LUCIOPERCA*) U RUMUNIJI: STANJE I PERSPEKTIVE

Apstrakt

Smuđ je jedna od najvrednijih slatkovodnih vrsta riba sa dobrom tržišnom perspektivom u Evropskim zemljama. Na osnovu Rumunskog Operativnog Programa za ribarstvo i maritimne poslove 2014-2020, u narednim godinama će biti podržano uvođenje u Rumunsku akvakulturu novih vrsta visoke ekonomske vrednosti, naročito domaćih vrsta kao što je smuđ i novih tehnologija gajenja za ovu vrstu. Tako će nove farme koje koriste intenzivnu tehnologiju gajenja smuđa imati bolje šanse nego u prošlosti da dobiju finansijsku pomoć. U svakom slučaju 5 novih ribnjaka sa recirkulacionim sistemom za gajenje smuđa su projektovani i izgrađeni u poslednje 2-3 godine. Ovi ribnjaci imaju, bar delimično, aktivnosti usmerene na intenzivno gajenje smuđa. U svakom slučaju, prisutni su različiti stadijumi implementacije projekta (od projekta do pune aktivnosti), ali nijedan nije zaokrižio proizvodni process gajenja smuđa u intenzivnom sistemu.

Abstract

Pikeperch is one of the most valuable freshwater fish species having a good market perspective in European countries. According to Romanian Operational Program for Fisheries and Maritime Affairs 2014-2020, the introduction in Romanian aquaculture of new species with high economical value, especially indigenous species like pikeperch, and new technologies for these species, will be supported in the next years. Therefore, new fish farms applying intensive technology for pikeperch culture could have a better chance to get financial support than in the past. Anyway, five new fish farms with units intended for pikeperch

aquaculture in recirculating aquaculture system (RAS) already started to be designed and built in the last 2-3 years. These farms have at least partially activities in the direction of intensive pikeperch farming. Anyway, different stages of implementation of the projects (from design to running activity) were found at this moment, none of them completing a production cycle for pikeperch reared in intensive system.

THE POTENTIAL OF MICRO ALGAE AS FEED INGREDIENT FOR ATLANTIC SALMON

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MOGUĆNOST KORIŠĆENJA MIKROALGI U HRANI ZA KARNIVORNE VRSTE RIBA

Apstrakt

Potreba za sastojcima visokog kvaliteta u proizvodnji hrane za ribe raste zajedno sa razvojem proizvodnje u akvakulturi u svetu. Riblje brašno i riblje ulje su najčešće birani sastojci za hranu za ribe zbog njihove visoke nutritivne vrednosti i ukusa. Riblje brašno sadrži onu količinu amino kiselina koja je ribi potrebna. Riblje ulje je odličan izvor esencijalnih n-3 masnih kiselina dugog lanca, koje su veoma važne za unapređenje zdravlja, kako kod riba tako i kod onih koji konzumiraju ribu. Riblje brašno i ulje su zamenjivani biljnim sastojcima u proteklih 25 godina. Međutim, biljn sastojci često sadrže širok spektar antinutririvnih sastojaka koji imaju negativan uticaj na zdravlje ribe kao i korišćenje hrane za ribe. Ulje biljnog porekla u hrani za ribe menja sastav masnih kiselina u tkivu ribe, umanjuje povoljan profil masnih kiselina pa dominiraju manje pogodne n-6 masne kiseline. Postoji sve veća zainteresovanost za pronalaženjem izvora hrane za ribe u nižim trofičkim nivoima morskih ekosistema, kao što su mikroalge. Morske mikroalge su primarni proizvođači n-3 masnih kiselina i zbog toga su verovatno bolja alternativa za riblje ulje od biljnog. Neke mikroalge imaju odgovarajući sastav proteina kao i profil amino kiselina. Cilj ovog eksperimenta je da istraži nutritivnu svarljivost (ADC) suve materije (DM), proteina i pepela mikroalgi *Nanofrustulum* (C3)*, Desmodesmus* (C4) i *Nannochloropsis* (C1) koji su inkorporirani u hranu za Atlantskog lososa, *Salmo salar.*

Dva testa svarljivosti su urađena sa Atlantskim lososom. Cilj prvog ekperimenta (inicijalni test, P, prosečna inicijalna težina ribe 1000g) bio je da istraži svarljivost sastojaka 3 alge tako što je kontrolna hrana bazirana na ribljem brašnu rastvorena sa 30% test sastojka (u odnosu 70:30). Ove tri vrste hrane su proizvedene hladnim procesom peletiranja. Drugi eksperiment (ekperiment provere, V; prosečna inicijalna težina ribe 436g), urađen je da bi potvrdio rezultate C1 i C4 korišćenjem ekstrudirane hrane. Feces je sakupljan metodom ceđenja.

U oba eksperimenta, primećene su značajne razlike u svarljivosti ADC mikroalgi. Sveukupno, najviša ADC za proteine, DM i pepeo ($P<0.05$) utvrđena je kod hrane C3, dok nije bilo značajnih razlika između C1 i C4. Kada je reč o eksperimentu V, najviša ADC suve materije i proteina utvrđena je za hranu C1 ($P<0.05$), dok se razlika ADC pepela nije pokazala među različitim tipovima hrane. ADC proteina i suve materije je bio u istom opsegu kao i vrednosti eksperimenta P, dok je ADC pepela bio viši.

Nutritivna svarljivost varira među različitim vrstama mikroalgi. Ona verovatno takođe zavisi od tehnoloških uslova procesuiranja hrane. Zasnovano na ADC vrednostima, iako je C3 pokazala bolji potencijal, njen visok sastav pepela i nizak sastav proteina, mogu biti ograničavajući factor za korišćenje ove mikroalge u ishrani za ribe. Velike razlike nisu pronađene u vrednostima svarljivosti između algi C1 i C4. Ova zapažanja treba dalje potvrditi dugoročnim eksperimentima hranjenja Atlantskog lososa da bi se u potpunosti definisao potencijal sva tri kandidata.

Ova studija je deo projekta "Proizvodnja velikih dimenzija goriva i hrane iz mikroalgi". Projekat je finansiran od strane Odeljenja za Energiju Sjedinjenih Američkih Država.

INTRODUCTION

The demand for high quality ingredients to produce aquafeeds is increasing with the growth in world aquaculture production. Fishmeal and fish oil have been preferred ingredients in aquafeeds because of their high nutritional quality and palatability. The amino acid content in fishmeal matches the requirement of fish. Fish oil is an excellent source of the long-chain essential n-3 fatty acids, which are important to promote good health both for the fish and the consumer of the fish. Fishmeal and oil have been replaced by plant ingredients during the course of the past 25 years. However, plant ingredients often contain a wide range of anti-nutritional factors that have a negative impact on fish health as well as feed utilization. Use of plant oils in aqua diets also changes the fatty acid composition of fish tissue to a less favorable n-6 dominated profile. There is an increasing interest for exploring feed resources from a lower trophic level in the marine ecosystem, such as microalgae, in aqua diets. Marine microalgae are primary producers of n-3 fatty acids and therefore may be a more promising alternative to fish oil compared to plant oils. Some of the microalgae also have a favorable protein content as well as amino acid profile. The aim of the present experiment was to investigate apparent nutrient digestibility (ADC) of dry matter (DM), protein and ash from *Nanofrustulum* (C3), *Desmodesmus* (C4) and *Nannochloropsis* (C1) that were incorporated in feeds for Atlantic salmon, *Salmo salar.*

MATERIALS AND METHODS

Two digestibility trials were carried out with Atlantic salmon. The first experiment (Pre-study, P; initial fish av. wt. 1000 g), aimed to investigate ingredient digestibility of the three algae by diluting a fishmeal based control diet with 30% test ingredient (70:30 ratios). These three feeds were produced by cold pelleting process. The second experiment (verification experiment, V; initial fish av. wt. 436 g), was carried out to confirm the results of C1 and C4 using extruded diets. Stripping method was used to collect the feces.

RESULTS

Significant differences were noted in ADC of the microalgae, in both experiments. Overall, highest ADC for protein, DM and ash ($P<0.05$) was observed for C3, while no significant differences were noted between C1 and C4. For experiment V, highest ADC of DM and protein was observed for C1 ($P<0.05$), while ADC of ash showed no differences between the feeds. ADC’s of protein and DM were in the same range as values in experiment P, while ADC of ash was higher.

CONCLUSION

The nutrient digestibility varies among different strains of microalgae, perhaps also depending on the feed processing condition. Based on ADC values, though C3 revealed a better potential, its high ash and low protein content, may be a limiting factor in its application as a feed ingredient. No large differences were noted in the digestibility values between the algae C1 and C4. The present observations have to be further confirmed through long-term feeding experiments with Atlantic salmon to fully define the potential of the three candidates.

ACKNOWLEDGEMENT

This study is part of the project “Large-scale production of fuels and feed from microalgae” funded by the US Department of Energy.

SUSTAINABLE MEASURES FOR IMPROVEMENT OF RHEOPHILIC FISH PRODUCTION – PRELIMINARY RESULTS WITH CHUB (*SQUALIUS CEPHALUS*) POND CULTURE

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ODRŽIVE MERE ZA UNAPREĐENJE GAJENJA REOFILNIH VRSTA RIBA – PRELIMINARNI REZULTATI GAJENJA KLENA (*SQUALIUS CEPHALUS*)

Apstrakt

U jednom delu životnog ciklusa, posebno tokom ranih stadijuma razvoja većina reofilnih riba nastanjuje visoko produktivna obalska i lentična staništa reka. Ovakva staništa su često degradirana usled regulacije rečnih obala i toka reka kao i izgradnje brana. Nekada jedna od najrasprostranjenijih slatkovodnih ciprinida Evrope, klen (Squalius cephalus L.), tokom poslednje decenije pokazuje znake opadanja brojnosti u nekim rekama. Iako nema neku posebnu ekonomsku vrednost, ova riba je cenjena u sportskom ribolovu, ali pre svega ona je autohtona vrsta Evrope koja ukazuje na ekološki status reka, pa se njeno prisustvo u vodenim ekosistemima ne sme zanemariti. Osim mera za očuvanja prirodnih staništa, brojnost populacije riba se održava i poribljavanjem.

Produkcija mlađi je relativno zahtevna faza u gajenju ove vrste riba, posebno u segmentu obezbeđivanja dovoljno kvalitetne hrane. U cilju smanjivanja cene ove faze gajenja od velikog značaja može biti primena održivih mera koje bi poboljšale iskoristljivost prirodnih resursa.

Shodno tome, cilj ovog rada je bio da se istraži efikasnost održivih mera u gajenju mlađih uzrasnih kategorija klena (1+) radi poribljavanja prirodnih staništa.

Istraživanje je obavljeno tokom 7 meseci, od aprila do oktobra 2014. godine u 8 identičnih eksperimentalnih ribnjačkih bazena Fakulteta za ribarstvo i zaštitu voda (Univerzitet Južna Bohemija), u okviru AQUAECXEL FP7 projekta, „Transnational Access - TNA“. Jezera veličine 0,08 ha su nasađena sa po 1250 jedinki jednogodišnje mlađi klena. Primenjena su dva tretmana, žute zamke za insekte i sveža biljna biomasa, u triplikatima i dve kontrole. Tokom trajanja eksperimenta, ni u jednom od jezera ribe nisu hranjene dodatnom hranom.

Osnovni parametri vode su mereni jednom mesečno. Standardna dužina i masa tela riba je merena na početku i na kraju eksperimenta. Od parametara rasta riba, određivani su prirast, specifična stopa rasta (SGR) i kondicioni faktor (CF).

Rezultati su pokazali da nije bilo stastističkih razlika u parametrima kvaliteta vode između tretmana i da su izmerene vrednosti bili u opsegu, koji je karakterističan za prirodna staništa juvenila klena. Na kraju eksperimenta (u oktobru) prosečna masa i dužina tela riba iz različitih tretmana su se značajno razlikovale ($p<0.001$), sa najvećim vrednostima u tretmanu sa žutim zamkama, osrednjim razlikama u tretmanu sa biljnom masom i najnižim u kontroli (35.78 ± 4.29 g, 29.74 ± 5.12 g, 26.40 ± 3.63 g i 130.7 ± 4.2 mm, 123.5 ± 5.8 mm, 116.8 ± 4.9 mm). Vrednosti prirasta riba su se takođe statistički veoma značajno razlikovale između tretmana ($p<0.001$), pri čemu je bolji prirast ostvaren u tretmanu sa žutim zamkama u odnosu na biljnu biomasu i kontrolu. Kod specifičnog prirasta riba nisu nađene značajne razlike između tretmana, ali su njihove vrednosti bile značajno veće od kontrole ($p<0.001$). Međutim, kondicioni faktor (FC) je pokazao statistički značajne više vrednosti u tretmanu sa žutim zamkama i kontroli u odnosu na tretman sa biljnom masom ($p<0.005$).

S obzirom na dobre rezultate u prirastu riba, ova jednostavna, ekonomski isplativa, a ekološki održiva mera za poboljšanje iskoristljivosti prirodnih resursa se može primeniti u gajenju drugih reofilnih vrsta riba. Osim u konzervacione svrhe, ova mera bi mogla naći primenu i u ekstenzivnom i poluintenzivnom sistemu gajenja riba, posebno onih vrsta koje žive u sličnim ambijentalnim uslovima i imaju sličnu ishranu, kao što je šaran i druge omnivorne vrste riba ili pak predatorske ribe koje konzumiraju insekte tokom mlađih životnih stadijuma.

Ključne reči: reofilne vrste riba, Squalius cephalus, klen, poluintenzivan sistem, održive mere
Keywords: reophilic fish, Squalius cephalus, chub, semiintensive system, sustainable measures

INTRODUCTION

Currently, populations of some rheophilic fish species in Europe are under threat due to continuous pollution, loss of habitats, overfishing and other anthropogenic influences (e.g. deterioration of physical habitat). In certain parts of their life cycle, especially during embryonic and early life stages (larvae and juveniles), most rheophilic fish inhabit highly productive inshore or slow-flow river zones called “nursing areas”. These habitats are particularly affected by river channelizations, flow regulations and dam constructions. The chub, *Squalius cephalus* (L.) used to be one of the most common fish species in European freshwaters, but now local populations in some rivers are becoming decimated (Kupren et al., 2008). Although without special economic value, this fish is appreciated in recreational fisheries, but most important, it is an indigenous European fish indicating the river health, thus it should not be overlooked.

Production of fish fry in ponds is a quite demanding phase in fish farming, especially in providing fish with good quality feed. In order to decrease the cost of this phase, implementation of sustainable supplemental measures that will improve the usability of natural resources can be a valuable option.

Adults and larvae of aquatic and terrestrial insects are a significant part of the diet for most rheophilic fish species. Due to their high protein content, insects are seriously considered as an economically feasible and sustainable substitution of, or addition to expensive commercial feeds, particularly for fish fry rearing. Studies from natural habitats of chub show that these fish highly rely on inputs from terrestrial ecosystems. Insects that appear on the water surface mostly emerge from the water body or its riparian habitats and to a smaller degree directly from the air (“aerial plankton”) (Sanzone et a., 2003). Due to immediate contact with the shorelines, small water bodies are likely to provide higher insect biomass for fish feeding compared to large ones. Additional improvement of natural resources utilization in fish culture can be done by introducing insect traps and plant biomass on the water surface. Hence, the aim of this study was to investigate their efficiency as sustainable supplemental measures in rearing young chub (1+) for restocking natural waters.

MATERIAL AND METHODS

The study was carried out during 7 months, from April to October 2014 in 8 uniform experimental fish ponds of the Faculty of Fisheries and Protection of Waters (University of South Bohemia) as a part of the AQUAEXCEL project - „Transnational Access - TNA“. The 0.08 ha ponds were stocked with 1250 individuals (mean total biomass 3294 ± 120.44 g) of one-year-old chub fingerlings per pond. Two experimental treatments (installation of yellow insect traps and regular fresh plant biomass application) and the control were tested in triplicates, and duplicate, respectively. The yellow insect traps installation consisted of 8 yellow plates (0.7 x 0.7 m) evenly distributed and submerged (1 - 2 cm) under water surface, nearby (~3 m) the shore-line. The treatment with plant biomass (a mixture of fresh grass and meadow plants) application consisted in its distribution at a rate of 150 $kgha^{-1}$ to ponds weekly providing a suitable substratum for colonization by phytophilous water invertebrates. No treatments were applied in the control ponds. During the experiment, fish in any of the ponds were not provided with supplementary feed. The essential environmental parameters (oxygen concentration, oxygen saturation, temperature, pH, turbidity, conductivity and transparency) were measured monthly. Standard length and weight of body fish were measured at the beginning and end of the experiment. Weight increment (ΔW), specific growth rate (SGR) and condition factor (CF) were calculated using the following equations: $\Delta W = Wt - Wo$; specific growth rate, $SGR = (100 \times (\ln Wt - \ln Wo) \times days^{-1})$; $CF = (W * L^{-3}) \times 100$, where W, Wo and Wt is the current, initial and final body weight (g), respectively and L is the standard body length (cm).

Results of environmental variables, standard length, weight and growth parameters of fish in the different treatments were tested using analysis of variances (one-way ANOVA) with treatment as the main factor. Differences between treatments were tested using Tukey’s post hoc test. The analyses were performed using PAST 3.06. (Hammer et al., 2001).

RESULTS AND DISCUSSION

There was no difference in the environmental parameters among treatments, and the values were mostly within the range of conditions as in natural habitats of juvenile chub. At the end of the study period (October) the average weight and standard length of chub from experimental treatments were significantly different ($p<0.001$), being the highest in fish from insect trap treatment, intermediate in treatment with plant biomass and the lowest in control (35.78 ± 4.29 g, 29.74 ± 5.12 g, 26.40 ± 3.63 g respectively and 130.7 ± 4.2 mm, 123.5 ± 5.8 mm, 116.8 ± 4.9 mm respectively). Accordingly, body increment differed among the treatments being significantly higher in the treatment with insect traps compared to the one with plant biomass and control ($p<0.001$). No significant differences were found for specific growth rates between the treatments but their values were significantly higher than in the control ($p<0.001$). However, the condition factors of fish from the control were significantly higher than those from treatment with plant biomass ($p<0.001$), while no difference was found between control and treatment with insect traps.

Insect traps have been widely used as a standard tool in faunistic and agricultural surveys for collecting insects. Especially the yellow water traps have been preferred for these purposes because they attract high diversity and amounts of insects. Their application as a food support in fish ponds is scarce in literature. Erhard et al. (1993) measured the insect biomass and its application in fish farming. However, this measure is quite well known to aquaculturists particularly in low-intensity (hobby) salmonid culture.

CONCLUSIONS

Contributing considerably to fish growth performances, these simple, economically feasible and ecologically sustainable measures for the improvement of utilization of natural resources can be applied to the culture of different coarse and reophilic fish species. In addition to conservation issues, this supplemental measure can be also applied in extensive and semi-intensive production of fish species having similar habitat and feeding requirements, as common carp and other omnivorous or even predatory species which are able to ingest insects during earlier life stages.

ACKNOWLEDGEMENT

This study was supported by AQUAEXCEL (Aquaculture Infrastructures for Excellence in European Fish Research) No. 262336, in the frame of Transnational Access – TNA and by the Ministry of Education, Youth and Sports of the Czech Republic through Projects “CENAKVA” (No. CZ.1.05/2.1.00/01.0024) and “CENAKVA II” (No. LO1205 under the NPU I program); Project CZ.1.07/2.3.00/30.0006 - for the Creation of Post-doc Positions at the University of South Bohemia - Inter-sectional Mobility through Expert Stays at Leading Foreign R&D Institutions; and Project No. 074/2013/Z of the Grant Agency of the University of South Bohemia in Ceske Budejovice.

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IMPLEMENTING EFFECTIVE VETERINARY BIOSECURITY PROGRAMS IN AQUACULTURE THAT MEET INTERNATIONAL STANDARDS AND NATIONAL REGULATIONS

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IMPLEMENTACIJA PROGRAMA ZA VETERINARSKU BIOSIGURNOST U AKVAKULTURI U SKLADU SA MEĐUNARODNIM STANDARDIMA I NACIONALNOM REGULATIVOM

Apstrakt

Progresivno povećanje rizika od izbijanja, kao i sve veći uticaj zaraznih bolesti na proizvodnju u akvakulturi širom sveta je u poslednjih 10 godina pokrenulo rasprave velikog broja učesnika na mnogobrojnim konferencijama, simpozijumima i radionicama o tome kakve procedure treba ugraditi u biosigurnosne planove i programe. Ključni zadatak se sastojao u određivanju koji bi proceduralni elementi bili u saglasnosti sa međunarodnim standardima, npr. procesima i procedurama opisanim u Kodu i Priručniku OIE (Svetske Organizacije za Životinjsko Zdravlje) kao i u nacionalnim propisima. U pokušaju da se nađe ravnoteža između regulatornih zahteva i praktičnih pristupa koji bi bili korisni i upotrebljivi za sve zainteresovane strane (od proizvođača do državnih organa) identifikovani su sledeći prioriteti:

Svaki biosigurnosni program treba da bude

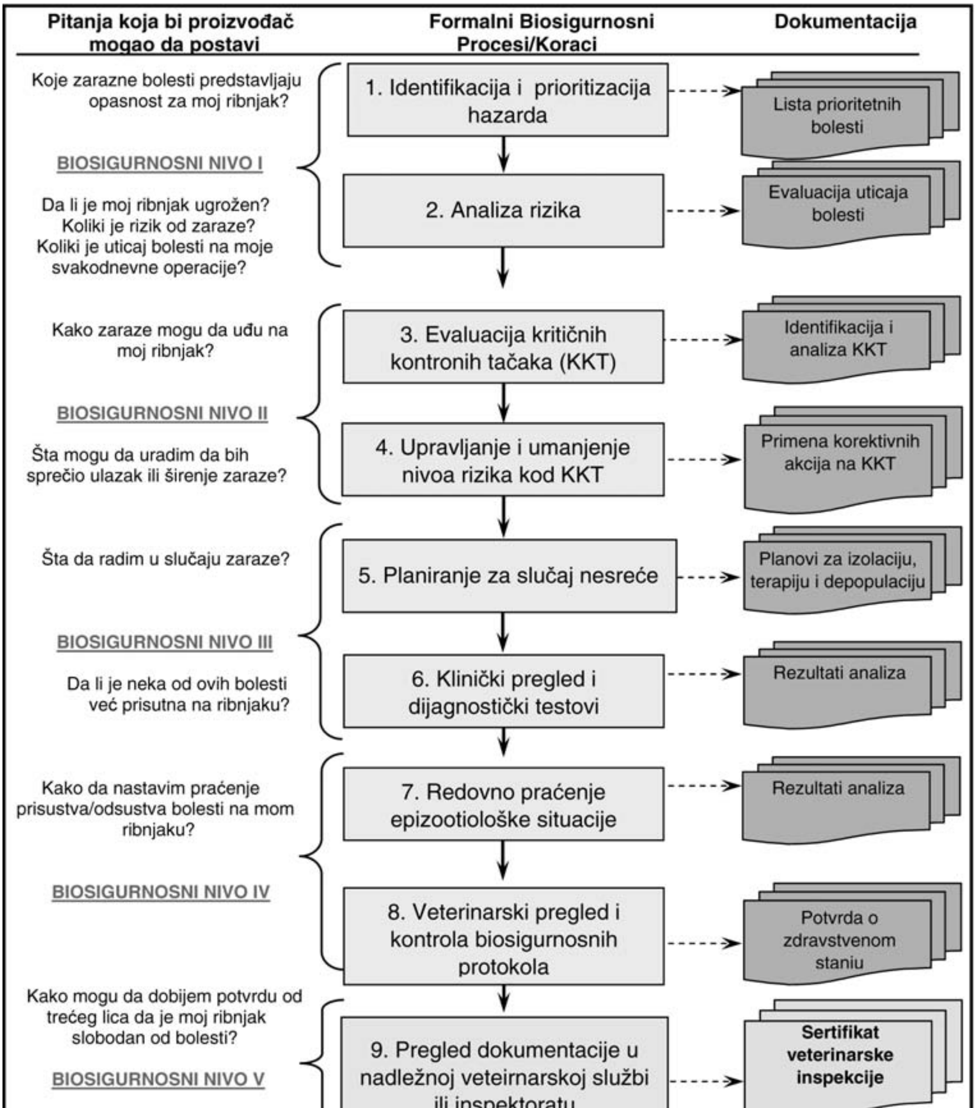
a) praktičan i ekonomičan;
b) fokusiran na infektivne i zarazne bolesti;
c) uključuje procedure preventive, kontole i eradikacije bolesti u tačno određenim epizootiološkim jedinicama
d) baziran na naučno potvrđenim i opravdanim veterinarskim procedurama;
e) ugradi međunarodno priznate standarde iz OIE Koda i Priručnika; i,

f) zasnovan na javno-privatnom partnerstvu i saradnji između proizvođača, veterinara i paraveterinarskih službi, i državnih organa.

Sa fokusom na gore navedene prioritetne principe, Međunarodni Konzorcijum za Veterinarsku Biosigurnost u Akvakulturi (IAVBC) je testirao procedure iz Slike 1, sa učesnicima nekoliko konferencija i radionica u više zemalja (Norveška, Južna Afrika, Čile, itd.), u pokušaju da se definiše sveobuhvatni pristup razvoja, primene, provere i sertifikacije efektivnih programa biosigurnosti u akvakulturi. Osnova za biosigurnosni program je pravilno definisanje epizootiološke jedinice (Epi-jedinica), geografski određene populacije životinja na kojoj su primenljivi svi koraci i/ili procesi predviđeni u biosigurnosnom planu. Epi-jedinica može na primer biti jedan ribnjak, ili više ribnjaka u jednom odeljku (OIE “kompartmentu”) koji se nalaze na različitim lokacijama ali pod jedinstvenom upravom; ali može takođe biti i zona (region u okviru države), ili čak cela država. Svaka Epi-jedinica je donekle odvojena od ostalih populacija, na taj način olakšavajući kontrolu nad širenjem zaraze, međutim unutar Epi-jedinice širenje zaraznih bolesti među populacijom se odvija relativno lako.

Sledeći princip od velike važnosti je da sve procedure primenjlive na odabranu Epi-jedinicu moraju biti osmišljene unapred i dobro dokumentovane. Ovaj princip zahteva *a priori* evaluaciju Epi-jedinice zajedno sa napisanim biosigurnosnim planom koji opisuje sve korake i procedure koje će biti uvedene na Epi-jedinici (ribnjaku), kao i dokumentaciju o svim procedurama koje su već primenjene (npr. zapisnici o primenjenim biosigurnosnim merama). Uz periodične terenske evaluacije biosigurnosnih aktivnosti i pregleda ribljih populacija u okviru Epi-jedinice, napisani planovi i dokumentacija o primenjenim procedurama postaju fokus za audite i sertifikacije. Takođe je važno napomenuti da je biosigurnosni plan specifičan za individualnu Epi-jedinicu.

Slika 1. Koraci za pripremu, upotrebu, kontrolu i sertifikaciju biosigurnosnog programa namenjenog prevenciji, kontroli i mogućoj eradikaciji bolesti u bilo kojoj Epizootiološkoj jedinici (ribnjaku, gazdinstvu, okrugu, zoni, regionu ili državi).



Upotrebljivost i opravdanost predloženih biosigurnosnh koraka i procedura se zasniva na sledećim formalnim procesima: analiza rizika i hazarda (identifikacija i prioritizacija hazarda, procena rizika, upravljanje i ublažavanje rizika, i komunikacija rizika); analiza i korekcija kritičnih kontrolnih tačaka (uključujući procenu i planove za korekciju aktivnosti u toku kojih zarazna bolest može ući ili izaći iz Epi-jedinice); epidemiološka analiza (uključujući neophodnu dijagnostiku, praćenje epizootiološke situacije na terenu,

i utvrđivanje epizotiološkog statusa zaraznih bolesti u Epi-jedinici); priprema za slučaj nesreće (priprema protokola o hitnoj kontroli i eradikaciji bolesti u slučaju izbijanja zaraze); kao i formalne procedure pregleda/audita i sertifikacije Epi-jedinice u cilju dobijanja statusa "slobodno od bolesti" koji može pomoći prilikom npr izvoza ribe u EU. Plan ove prezentacije je da predstavi i ukratko opiše važnost svake procedure, kao i načine na koji se one mogu uklopiti u jedinstven biosigurnosni program (Slika 1.). Ovaj pregled će biti od naročite koristi privatnim ili državnim veterinarima, kao i državnim službenicima odgovornim za pomoć proizvođačima koji razvijaju biosigurnosne programe na pojedinačnim ribnjacima ili većim Epi-jedinicama kao što su kompletna ribarska gazdinstva.

Ključne reči: biosigurnost u akvakulturi, epizootiološka jedinica, OIE standardi, nacionalni propisi.

Abstract

Facing progressively increasing risks and impacts of disease on aquaculture productions in all countries, over more than a decade at numerous conferences, symposia and workshops, a large number of individuals have discussed and debated what procedure that should be incorporated into biosecurity programs. A key feature has been determining which procedures will meet International Standards (i.e. processes and procedures in OIE Codes and Manuals) and National regulations. In balancing these requirements with practical approaches that aquaculture producers can implement, and are effective and useful for all stakeholders around the world (from producers to governmental regulators), the following were recognized as priorities for all biosecurity programs:

a) Be practical and economic;
b) Focus only on infectious and contagious diseases;
c) Include procedures that address disease prevention, control and eradication in definable epidemiological units;
d) Be based on well established, sound scientific justifiable veterinary procedures;
e) Incorporate internationally accepted standards in the OIE Code and Manual; and,
f) Involve public-private partnerships and collaboration between producers, aquatic veterinarians and paraveterinary professionals, and governmental regulators.

In focusing on these principles, the International Aquatic Veterinary Biosecurity Consortium (IAVBC) has tested the procedures in Figure 1, with stakeholders at several conferences and workshops in Norway, South Africa, Chile, and elsewhere, that involve an integrated approach for developing, implementing, auditing and certifying effective aquaculture biosecurity program. At the core of a biosecurity program is defining an *epidemiologic unit* (EpiUnit), a well-defined geographical population of animals, on which all biosecurity steps or processes will be implemented. An EpiUnit might be an establishment (farm), a compartment (different locations that are all managed as an integrated operation, usually under one ownership), a zone (typically a region of a country), or a whole country. To some degree, each EpiUnit population is separated from other populations, allowing control over the spread of disease. However, within the EpiUnit, infectious and contagious diseases transmission between individuals is relatively easy.

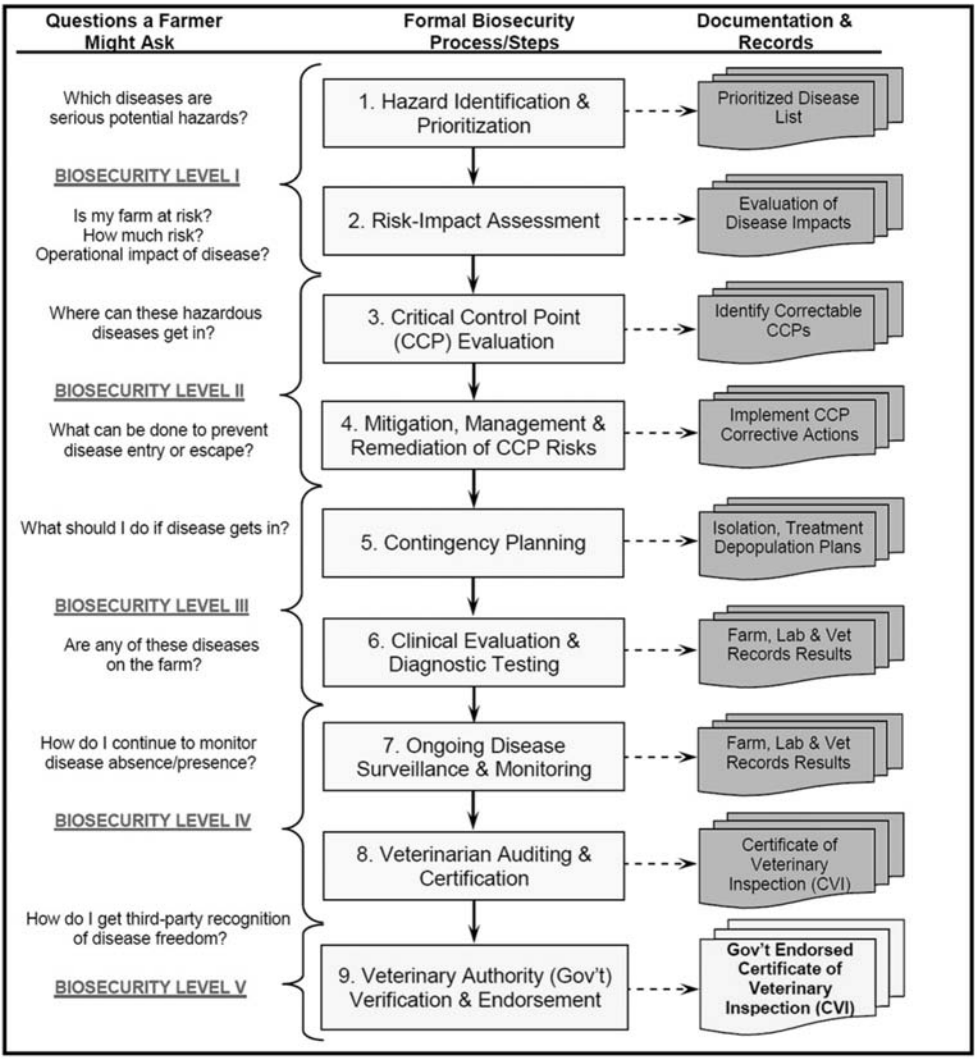
A second important principle is that all procedures implemented for a selected EpiUnit must be thought out ahead of time, and well documented. This requires both an *a priori*

evaluation of the EpiUnit, and a written biosecurity plan that addresses all steps and processes to be implemented in the EpiUnit, and documentation of all procedures that are implemented over time (i.e. a biosecurity implementation record). Along with periodic onsite evaluation of operations and animals on the EpiUnit, the written plan and the documentation of implemented procedures become the focus for auditing and certification. Every biosecurity plan will be specific for an individual EpiUnit.

To be effective and justifiable the processes and procedures need to involve several formal processes, including: hazard and risk analysis (hazard identification and prioritization, risk assessment/evaluation, risk management/mitigation and risk communication); analysis and remediation of critical control points (including evaluation and mitigation plans for correcting practices where disease could enter or leave the epidemiological unit); epidemiological principles (including necessary diagnostics, surveillance, monitoring and determining the status or freedom of diseases in the epidemiological unit); emergency preparedness (contingency protocols for disease control and eradication); and, auditing of procedures and records, and certification (providing assurance of disease freedom and useful as compliance incentives). This presentation will outline and provide an overview of the importance of each procedure, and how these can be implemented and integrated (Figure 1). This outline will be useful for other veterinarians or government officials to assist producers in developing effective and efficient biosecurity programs in aquaculture operations and larger EpiUnits.

Key words: aquaculture biosecurity, epidemiological units, OIE Standards, National Regulations

Figure 1. Steps for developing, implementing, auditing and certifying an effective biosecurity program intended to prevent, control and possibly eradicate disease in any epidemiological unit (a tank/pond, farm, state/province, zone, region or country).



ALLERGENIC PROTEINS IN FISH

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ALERGENSKI PROTEINI U RIBI

Apstrakt

Riba predstavlja znatan deo ishrane ljudi u svetu. Pre svega, riba je značajan izvor proteina (15-24%) visoke biološke vrednosti, bogata je mineralima, vitaminima, a posebno esencijalnim masnim kiselinama za koje je dokazano da pogoduju u prevenciji mnogobrojnih oboljenja. Zbog velikog značaja polinezasićenih masnih kiselina n-3 klase, u Evropi su date i preporuke o optimalnom dnevnom unosu. Međutim, pored hranljivih svojstava koje ima, riba može biti i izvor različitih bioloških i hemijskih opasnosti. Od bioloških opasnosti posebno su značajni paraziti (*Trematodae, Nematodae, Cestodae*), bakterije (*Salmonella spp, E. coli, Vibrio parahemolyticus, Vibrio vulnificus, Listeria monocytogenes, Clostridium botulinum, Staphyloccocus aureus*), virusi (Norwalk virus, Entero virusi, Hepatitis A, Rotavirus) i biotoksini. Najznačajnije hemijske opasnosti su policiklična aromatična jedinjenja, histamin i teški metali (živa, olovo, kadmijum, arsen, gvožđe). Alergije usled konzumiranja pojedinih vrsta namirnica su u porastu poslednjih godina. Veliki pokret oko pravilnog načina ishrane je doveo do toga da ljudi sve češće konzumiraju ribu, proizvode od ribe kao i različite plodove mora. Pored različitih opasnosti koje mogu poticati iz ribe, posebni značaj poslednjih godina se daje ribi kao potencijalnom alergenu. Naime, veliki broj alergija koje se javljaju u svetu pripisuju se alergenima koji potiču iz mesa ribe, pre svega proteinima mesa ribe. Učestalost alergija koje se povezuju za unosom mesa ribe varira u Evropskim zemljama (Norveška 1,5%; 2,3% Turska, 2,3% Grčka; Švedska 1.2-3.2 %). Kao najznačajniji proteinski alergen iz mesa ribe navodi se parvalbumin (ß tip), koji je izolovan kod velikog broja vrsta. Smatra se da su šaran i bakalar najčešći izvori parvalbumina koji se dovodi u vezu sa različitim vidovima alergijskih reakcija. Potencijalni alergeni su takođe kolagen i želatin koji su izolovani iz kože i pojedinih organa riba. Takođe, značajan alergen iz plodova voda je i tropomiozin, arginin kinaza, aldolaza. Pored ovih alergena, značajni alergeni mogu da potiču iz ikre, pojedih vrsta kavijara, a opisani su slučajevi gde su alergijske reakcije povezane sa kolagenom koji se nalazi u ekstracelularnom matriksu

proteina. Alergeni koji dovode do različitih alergijskih reakcija, pored proteina mesa ribe, mogu poticati i od gotovih proizvoda od ribe. Tu spadaju različiti panirani proizvodi od ribe koji sadrže celer, gluten i druge dodatke koji mogu biti potencijalni alergeni. Zbog značaja koji imaju na zdravlje ljude, tehnologija je omogućila različite metode za detekciju ovih alergena. Kao neke od njih navode se ELISA (Enzyme-linked immunosorbent assay), RAST (Radioallergosorbent test) i RIE (Rocket Immuno-electrophoresis). Koja će se metoda detekcije primeniti, prvenstveno zavisi od dostupnosti alergena i praga njegove detekcije. Industija mesa je razvojem tehnologije uvela pojedine tehnološke prosece koji imaju mogućnost inaktivacije pojedinih alergena, pre svega proteina mesa ribe. Visoke temperature koje se primenjuju u obradi mesa ribe mogu uticati na ove alergene, tako što će smanjiti alergeni potencijal, dok neki tehnološki postipci nemaju tu mogućnost.

Ključne reči: riba, alergeni, protein, identifikacija alergena
Key words: fish, allergens, protein, identification of allergen

INTRODUCTION

It is common knowledge that fish are a nutritious component of a human diet, as they constitute a valuable and desired source of protein and polyunsaturated fatty acids (Baltić i Teodorović, 1997). However, they are likely to pose a risk to consumer health. The hazards of fish are associated with biological and chemical contaminations (Baltić et. al., 2013a). The biological contaminations include pathogenic bacteria such as *Listeria monocytogenes*, *Pseudomonas spp*., *Aeromonas spp*., *Clostridium botulinum* and parasites (Baltić et. al., 2013b). Chemical contamination concerns mainly polycyclic aromatic hydrocarbons, heavy metals such as cadmium, mercury, lead, iron (Ivanović et al., 2014) and histamine (Baltić et al., 2009).

The prevalence of food allergy in Europe is uncertain. Using food challenges as a criterion for diagnoses, the prevalence of food allergy in Europe has been estimated to be between 3 and 4%, both in children and adults. About 75% of allergic reactions among children in Europe are due to eggs, peanut, cows‘milk, fish and various nuts (EFSA, 2014).

Food allergy is increasing at a faster rate than any other allergic disorder. In the last few decades, a large movement toward healthier eating makes seafood one of the major foods consumed worldwide (Wild and Lehrer, 2005). Exposure to seafood can cause a variety of health problems, including gastrointestinal disorders, urticaria, immunoglobulin E (IgE)-mediated asthma and anaphylaxis. A true allergy is known as type-one hypersensitivity that activates the human mast cells, a type of white blood cells, producing an IgE and other inflammatory mediators such as cytokines.

Allergic reactions are directed to two major groups: fish and shellfish. The prevalence of “food allergy” as perceived by the general population is several times larger than the prevalence that can be verified by standard diagnostic procedures. Prevalence of self-reported allergy to fish in children was lower in other Northern European countries with high fish consumption, like Iceland (1.5-2.2%), Norway (1.5%) or Sweden (1.2-3.2%). Fish was one of six foods found in DBPCFC (Double-blind, placebo-controlled food challenge) to be the most common allergens (Bock and Atkins, 1990).

FISH ALLERGENS

The Atlantic cod (*Gadus morhua*) was the first model for studying fish allergens, *Gad c1* (12 kDa). This glycoprotein is identified later as parvalbumin, which buffers calcium during muscle relaxation. In the case of fish, more than 20 proteins, mainly parvalbumins, have been classified as the major allergens (Barros and Cosme, 2013). Parvalbumin represents the major clinical cross-reactive fish allergen with sequence homology ranging from 60-80%. This feature was comprehensively applied to exploit the closeness between fish allergens and their human homologs. The allergenicity of the parvalbumin was studied in purified forms from different types of fish along with two other high molecular weight allergens: 29 and 54 kDa. In addition, other fish allergens are characterized such as collagen and gelatin isolated from skin and muscle tissues (Taylor, 2008), fish hormones like vitellogenin in caviar and many other allergens.

The literature reports that there is no cross-reactivity between fish allergens and shellfish (Lopata and Lehrer, 2009). Codfish allergens were the first food allergens to be purified and characterized. Codfish contains one major allergen contained in the sarcoplasmic proteins of fish muscle, *Gad c 1*. *Gad c 1* is a parvalbumin. Parvalbumin allergen in Atlantic cod, *Gad m 1*, encoded by a gene distinct from that of *Gad c 1*, has been identified. Parvalbumins from fish represent extremely abundant and stable allergens. They are considered by some authors to be the major and sole fish allergens for 95% of patients suffering from IgE-mediated fish allergy. Parvalbumins are small (12 kDa; 108-109 amino acid residues) calcium-binding muscle proteins, and are present in high amounts in the white muscles of lower vertebrates and in lower amounts in fast twitch muscles of higher vertebrates, and have a function in calcium buffering and possibly in muscle relaxation.

Parvalbumin has been found to be a major allergen in various other fish species (Table 1). Hamada et al. (2003) demonstrate that parvalbumin is a major allergen in three species of mackerels (*Scomber japonicus*, *S. australasicus* and *S. scombrus*) said to be the fish most frequently involved in IgE-mediated food allergy in Japan.

Table 1. Fish allergens (EFSA, 2014)

Biochemical name	Allergen	Common name	Scientific name	Source	Molecular weight[a]
β-Parvalbumin	Clu h 1	Atlantic herring	*Clupea harengus*	Fish meat	12[1]
	Cyp c 1	Carp	*Cyprinus carpio*		
	Gad c 1	Codfish	*Gadus callarias*		
	Gad m 1	Atlantic cod	*Gadus morhua*		
	Lat c 1	Barramundi	*Lates calcarifer*		
	Lep w 1	Whiff	*Lepidorhombus whiffiagonis*		
	Onc m 1	Rainbow trout	*Oncorhynchus mykiss*		
	Sal s 1	Atlantic salmon	*Salmo salar*		
	Sar s 1	Pacific pilchard	*Sardinops sagax*		
	Seb m 1	Ocean perch	*Sebastes marinus*		
	Thu a 1	Yellowfin tuna	*Thunnus albacares*		
	Xip g 1	Swordfish	*Xiphias gladius*		
Tropomyosin	Ore m 4	Mozambique tilapia	*Oreochromis mossambicus*	Fish meat	33[b]
β-Enolase	Gad m 2	Atlantic cod	*Gadus morhua*	Fish meat	47.3[b]
	Sal s 2	Atlantic salmon	*Salmo salar*		47.3[b]
	Thu a 2	Yellowfin tuna	*Thunnus albacares*		50
Aldolase A	Gad m 3	Atlantic cod	*Gadus morhua*	Fish meat	40
	Sal s 3	Atlantic salmon	*Salmo salar*		40
	Thu a 3	Yellowfin tuna	*Thunnus albacares*		40
Vitellogenin (β' component)	Onc k 5	Chum salmon	*Oncorhynchus keta*	Fish roe	18[b]

[1] Approximate – slight variation exists between species; [a] MW (SDS-PAGE); [b] kDa

Collagen has been recently proposed as an important fish allergen (Hamada et al., 2003), although further verification is needed. A high-molecular weight allergen from tuna (*Thunnus albacares*) muscle was found to bind specific IgE from 5 out of 8 allergic patient sera, but none of the normal control sera. The authors concluded that the allergen was collagen, probably type 1 collagen, which is the representative collagen in fish muscle. Mammalian and fish gelatins have some similarities, and the possibility of allergic cross-reactivity must be considered. Hamada et al. (2003) concluded that collagen is commonly allergenic and cross-reactive regardless of fish species. Thus, there appears to be little or no cross-reactivity between mammalian and fish collagens, whereas fish collagens from different species appear to be broadly cross-reactive. It seems reasonable to treat fish collagens from different species as one entity. No data have been found regarding cross-reactivity between collagens from different organs (e.g. skin and muscle) from the same species of fish (EFSA, 2014). A report on repeated anaphylactic reactions after intake of Russian Beluga caviar has recently been published (Untersmayr et al., 2002). The patient had no clinical allergy to fish, and was skin test and specific IgE negative to fish (test fish species not specified). Serum contained specific IgE to several proteins in Beluga caviar (derived from *Hiso huso*) and Sevruga caviar (derived from *Acipenser stellatus*) and also to a lesser degree to proteins in "false" caviar (collected from lumpsucker- *Cyclopterus lumpus*, or salmon and trout), but there was skin test positivity only to Beluga and Sevruga caviars. Considering the near absence of other reports on allergy to fish roe, this allergy appears to be rare (EFSA, 2014). In addition to fish and seafood, potential allergens can be fish products, which contain gluten, celery and other allergens. This is especially true of breaded fish products (Janjić et al., 2015).

SHELLFISH ALLERGENS

In shellfish, crustaceans and mollusks, the protein tropomyosin (TM) seems to be the major allergen responsible for ingestion-related allergic reactions. Tropomyosin belongs to the family of actin filament-binding proteins with different isoforms that can be expressed in muscle, and non-muscle tissues. Complex of TM and troponin regulates the calcium sensitive interaction of actin and myosin. In addition, the allergenicity of TM was confirmed in six species of crustaceans: black tiger prawn (*Penaeus monodon*), kuruma prawn (*Penaeus japonicas*), pink shrimp (*Metapenaeus Monocerus*), king crab (*Lopholithodes Mandtii*), snow crab (*Chionoecetes opilio*), and horsehair crab (*Limulus polyphemus*) by immunoblotting and the overall sequence identity showed more than 90% homology (Motoyama et al., 2007).

Many other allergens have been identified in crustaceans. Yu et al. (2003) identified arginine kinase (AK) (40 kDa) as a novel shrimp allergen. The amino acid sequence of this protein showed 60% similarity to AK of the crustacean, kuruma prawn (*Penaeus japonicas*) (Yu et al., 2003). AK was recently reported as an allergen in different crustacean species which was identified in white shrimp (*Litopenaeus vannamei*), gulf shrimp (*Penaeus aztecus*), chinese shrimp (*Fenneropenaeus chinensis*), black tiger prawn (*Penaeus monodon*) and other shrimp species using a proteomics approach. Moreover, AK has been identified in other crab species: mud crab (*Scylla serrata*), and by group in snow crab (*Chionoecetes opilio*) (Rahman et al., 2011), where 49% of the participant patient’s sera have a reactivity with AK. Arginine kinase also has been reported is allergen in some other invertebrates, such as the house dust mite (*Dermatophagoides farinae*), Indian-meal moth (*Plodia interpunctella*), and silkworm larvae (*Bombyx mori*). Recent studies have reported other novel crustacean allergens. Shiomi et al. (2008) identified the immunoreactive band (20 kDa) as sarcoplasmic reticulum Ca-binding protein, which was consequently extracted from black tiger shrimp (*Penaeus monodon*). Recently, this allergen was also identified in white shrimp (*Litopenaeus vannamei*), and in snow crab (*Chionoecetes opilio*). Sarcoplasmic calcium binding protein (SCP) is an invertebrate EF-hand calcium buffering protein that fulfills a similar function in muscle relaxation as vertebrate major allergen parvalbumin. Myosin light chain was identified as an allergen in white shrimp (*Litopenaeus vannamei*) and also identified in black tiger prawn (*Penaeus monodon*). Since TM is a common allergen in both crustaceans and mollusks other potent allergens such as myosin heavy chain, troponin, actine, hemocyanin, and amylase are reported also in molluscan shellfish (Rahman et al., 2012).

ACKNOWLEDGEMENTS

This paper was supported by the Ministry of Education and Science, Republic of Serbia, Project TR 31011.

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ASSESSMENT OF THE METAL AND TRACE ELEMENT CONTENTS IN TISSUES OF FOUR COMMERCIAL FISH SPECIES FROM THE DANUBE RIVER, BELGRADE

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PROCENA SADRŽAJA METALA I ELEMENATA U TKIVIMA 4 KOMERCIJALNE VRSTE RIBA IZ DUNAVA KOD BEOGRADA

Apstrakt

Cilj ovog istraživanja je bio da se proceni akumulacija elemenata u četiri komercijalne vrste riba na dva lokaliteta na Dunavu, u Beogradu. U ovom istraživanju, određene su koncentracije 11 elemenata (As, Cd, Cr, Co, Cu, Fe, Hg, Mn, Ni, Se, i Zn) u tkivima mišića, jetre i škrga sledećih vrsta: mrena (*Barbus barbus*), deverika (*Abramis brama*), štuka (*Esox lucius*) i smuđ (*Sander lucioperca*). Rezultati su ukazali da je distribucija metala i elemenata u tragovima u različitim tkivima specifična za vrstu. Koncentracije As, Cd, Fe, Hg, i Zn u mišićima riba su bile ispod maksimalno dozvoljenih koncentracija MDK, utvrđenih od strane EU i Republike Srbije. Na osnovu dobijenih rezultata, neophodno je uspostaviti program stalnog monitoringa na Dunavu u Beogradskom regionu.

Ključne reči: Dunav, riba, metal, teški metali, ICP-MS
Keywords: Danube River, fish, metal, trace element, ICP-MS.

INTRODUCTION

The Danube River is polluted from various sources, such as industry, agriculture and energetics (Milanović et al. 2010). Metals and trace elements are considered to be among major pollutants in the Danube Basin (Teodorović 2009). Fish, due to their position at the top of the aquatic food chains, are able to accumulate metals and trace elements at such levels that could pose a threat not only to the fish but also to the human population (Yilmaz et al.2007). Therefore, fish are considered as one of the best pollution indicators of aquatic ecosystems. Accumulation levels and distribution patterns of metals and trace elements differ depending on fish species as well as on fish tissue (Višnjić-Jeftić et al. 2010, Jarić et al. 2011). Previous studies of metal and trace element concentrations (Jovičić et al. 2014; Lenhardt et al. 2012) indicated great importance of fish tissue contamination monitoring, given that toxic chemicals can produce adverse effects on consumers.

In the present study, 11 elements were analyzed in the muscle, liver and gills of the four fish species from the Danube River. The main aim of the present study was to assess potential impacts of accumulation patterns in fish tissues in the Danube River.

MATERIAL AND METHODS

Sample collection

Fish specimens were collected from the two localities on the Danube River on the teritory of the city of Belgrade, Serbia (downstream Danube section 44° 52' 29'' N, 20° 22'01'' E; upstream Danube section 44° 49' 57'' N, 20° 27' 47''E). Samples were collected between November 2012 and September 2013. Samples of the muscle, gills and liver of the following fish species were collected: barbel (*Barbus barbus*), freshwater bream (*Abramis brama*), northern pike (*Esox lucius*) and pike-perch (*Sander lucioperca*).

Sample preparation and analysis

Samples were dried by Freeze Dryers Rotational-Vacuum-Concentrator, GAMMA 1-16 LSC, Germany, and sample portions of approximately 0.3 g (dry weight) were afterwards processed in a microwave digester (speedwave™ MWS-3$^+$; Bergof Products + Instruments 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°C). Potential presence of trace elements in chemicals used in digestion was resolved by using a number of blank samples. Following a cooling to 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 mass spectrometry (ICP-MS). Instrument was Thermo Scientific (Bremen, Germany), model iCAP Q. The following isotopes were measured: ^{52}Cr, ^{55}Mn, ^{57}Fe, ^{59}Co, ^{60}Ni, ^{63}Cu, ^{66}Zn, ^{75}As, ^{77}Se, and ^{111}Cd. The quality of the analytical process was controlled by the analysis of reference material in each sample batch (NIST CRM 1577c). All concentrations were expressed as μg g^{-1} dry weight (dw).

Mercury (Hg) was measured using cold vapor technique by atomic absorption spectrometer Varian "SpectrAA 220" with VGA 77 hydride system. The wavelenght line for Hg was 257.3 nm. The quality of the analytical process was controlled by the analysis of reference material in each sample batch (BCR-186R).

Concentrations in fish meat (i.e., muscle samples) were compared with the maximum allowed concentrations (MAC) for the utilization in human diet, established by the European Union (EU) and the national legislation (European Commission Regulation, 2006, Official Gazette of RS 2011).

Normality of distribution of analyzed data samples was tested by Kolmogorov-Smirnov test. Assessment of the differences among the groups was performed by means of the Kruskal-Wallis H test, which was followed by comparisons of particular pairs of samples by Mann-Whitney U test ($p<0.05$ was used as a threshold value).

RESULTS

Metal and trace element concentrations in analyzed tissues of the four fish species on the two studied sites are presented in Table 1 and Table 2. There were only a few differences between elemental concentrations among the two studied localities.

In the muscle of the barbel, As and Ni concentrations were higher in the upstream Danube section, while Se concentrations were higher in the downstream section. Concentrations of these elements in the liver were higher in the downstream Danube section. Gills had a higher As concentrations in downstream section, while Ni and Se concentrations were lower (Table 1 and 2).

Table 1. Heavy metal and trace element concentrations in muscle, liver and gills of the four fish species at the upstream Danube section (means ± standard deviation). Concentrations are expressed as $\mu g\ g^{-1}$ dry weight, nd indicates the values below the detect on threshold.

Tissue	**Element**	**Barbel**	**Freshwater Bream**	**Northern Pike**	**Pike-Perch**
Muscle	As	0.289±0.151	0.325±0.145	0.263±0.090	0.548±0.282
	Cd	nd	nd	0.010±0.011	0.010±0.367
	Cr	0.165±0.056	0.183±0.103	0.416±0.786	0.152±0.079
	Co	nd	0.030±0.023	nd	0.010±0.022
	Cu	1.579±0.365	1.973±1.055	2.477±1.054	1.237±0.439
	Fe	16.193±8.552	25.784±25.788	23.307±13.914	59.059±91.085
	Hg	0.962±0.359	0.447±0.391	1.351±0.644	1.790±0.367
	Mn	1.131±0.479	1.665±0.845	1.116±0.604	4.174±7.435
	Ni	0.410±0.371	0.220±0.145	0.340±0.315	0.210±0.234
	Se	1.118±0.624	1.884±0.881	1.089±0.237	1.604±0.907
	Zn	19.430±5.503	36.675±9.827	48.429±22.487	47.312±19.871
Liver	As	3.043±0.397	7.522±4.008	2.338±1.526	7.617±5.319
	Cd	0.060±0.031	0.180±0.216	0.040±0.050	0.340±0.470
	Cr	0.111±0.061	0.094±0.106	0.175±0.048	0.230±0.255
	Co	0.050±0.019	0.170±0.063	0.060±0.010	0.430±0.334
	Cu	22.069±8.607	69.331±51.031	27.451±10.203	17.066±7.012

	Fe	139.626±26.007	258.193±165.011	126.522±64.167	425.626±421.694
	Hg	0.246±0.119	0.156±0.149	0.350±0.358	0.901±0.435
	Mn	2.052±0.473	16.195±26.174	11.431±13.850	6.473±4.671
	Ni	0.350±0.611	0.460±0.698	0.530±0.168	0.520±0.565
	Se	0.325±0.105	0.310±0.141	0.491±0.298	3.442±1.591
	Zn	72.689±19.463	133.630±70.709	151.546±31.563	120.439±74.104
Gills	As	2.566±0.746	3.197±0.933	1.786±0.855	1.665±0.420
	Cd	0.030±0.011	0.020±0.002	0.020±0.007	0.030±0.024
	Cr	0.326±0.088	0.332±0.122	0.267±0.068	0.385±0.343
	Co	0.060±0.010	0.220±0.080	0.040±0.011	0.050±0.041
	Cu	2.590±0.161	2.550±0.668	2.053±0.293	1.708±0.654
	Fe	279.985±74.612	353.657±109.036	194.362±49.913	136.979±102.696
	Hg	0.161±0.048	0.070±0.045	0.143±0.137	0.413±0.245
	Mn	12.627±7.102	37.221±12.816	28.383±13.795	11.372±7.463
	Ni	0.370±0.244	0.820±0.768	0.520±0.303	0.580±0.474
	Se	0.253±0.067	0.267±0.095	0.179±0.075	0.936±0.640
	Zn	73.368±10.987	107.995±36.395	494.037±79.150	76.629±36.325

Higher Ni concentrations in the muscle of the freshwater bream were observed in the downstream section, while concentrations of Se were higher in the upstream section. Concentrations of As and Mn in the liver were higher in the downstream section, while concentrations of the Ni and Se were lower at this locality. Gills had higher As and Mn concentrations in the downstream section, while Cr, Ni and Se concentrations were higher in the upstream Danube section (Table 1 and 2).

Northern pike muscle had higher As concentrations at the downstream section. Liver had higher As, Cu and Mn concentrations and lower Se concentrations were also observed in the downstream section. Concentrations of As and Mn in gills were higher in specimens from downstream section, while Se concentrations were lower (Table 1 and 2).

Table 2. Heavy metal and trace element concentrations in muscle, liver and gills of the four fish species at the downstream Danube section (means ± standard deviation). Concentrations are expressed as μg g^{-1} dry weight, nd indicates the values below the detection threshold.

Tissue	Element	Barbel	Freshwater Bream	Northern Pike	Pike-Perch
Muscle	As	0.289±0.151	0.325±0.145	0.263±0.090	0.548±0.282
	Cd	nd	nd	0.010±0.011	0.010±0.367
	Cr	0.165±0.056	0.183±0.103	0.416±0.786	0.152±0.079
	Co	nd	0.030±0.023	nd	0.010±0.022
	Cu	1.579±0.365	1.973±1.055	2.477±1.054	1.237±0.439

	Fe	16.193±8.552	25.784±25.788	23.307±13.914	59.059±91.085
	Hg	0.962±0.359	0.447±0.391	1.351±0.644	1.790±0.367
	Mn	1.131±0.479	1.665±0.845	1.116±0.604	4.174±7.435
	Ni	0.410±0.371	0.220±0.145	0.340±0.315	0.210±0.234
	Se	1.118±0.624	1.884±0.881	1.089±0.237	1.604±0.907
	Zn	19.430±5.503	36.675±9.827	48.429±22.487	47.312±19.871
Liver	As	3.043±0.397	7.522±4.008	2.338±1.526	7.617±5.319
	Cd	0.060±0.031	0.180±0.216	0.040±0.050	0.340±0.470
	Cr	0.111±0.061	0.094±0.106	0.175±0.048	0.230±0.255
	Co	0.050±0.019	0.170±0.063	0.060±0.010	0.430±0.334
	Cu	22.069±8.607	69.331±51.031	27.451±10.203	17.066±7.012
	Fe	139.626±26.007	258.193±165.011	126.522±64.167	425.626±421.694
	Hg	0.246±0.119	0.156±0.149	0.350±0.358	0.901±0.435
	Mn	2.052±0.473	16.195±26.174	11.431±13.850	6.473±4.671
	Ni	0.350±0.611	0.460±0.698	0.530±0.168	0.520±0.565
	Se	0.325±0.105	0.310±0.141	0.491±0.298	3.442±1.591
	Zn	72.689±19.463	133.630±70.709	151.546±31.563	120.439±74.104
Gills	As	2.566±0.746	3.197±0.933	1.786±0.855	1.665±0.420
	Cd	0.030±0.011	0.020±0.002	0.020±0.007	0.030±0.024
	Cr	0.326±0.088	0.332±0.122	0.267±0.068	0.385±0.343
	Co	0.060±0.010	0.220±0.080	0.040±0.011	0.050±0.041
	Cu	2.590±0.161	2.550±0.668	2.053±0.293	1.708±0.654
	Fe	279.985±74.612	353.657±109.036	194.362±49.913	136.979±102.696
	Hg	0.161±0.048	0.070±0.045	0.143±0.137	0.413±0.245
	Mn	12.627±7.102	37.221±12.816	28.383±13.795	11.372±7.463
	Ni	0.370±0.244	0.820±0.768	0.520±0.303	0.580±0.474
	Se	0.253±0.067	0.267±0.095	0.179±0.075	0.936±0.640
	Zn	73.368±10.987	107.995±36.395	494.037±79.150	76.629±36.325

Muscle of the pike-perch had Fe, Mn and Se concentrations higher in specimens from the downstream section, while As and Ni concentrations were lower. Concentrations of As and Cu were higher in the liver tissue from the downstream section specimens, while Ni concentrations were higher in specimens from the upstream section. In gills, concentrations of As were higher in specimens inhabiting the downstream Danube, but they had lower Cr concentrations (Table 1 and 2). Concentrations of As, Cd, Fe, Hg, and Zn in the fish muscle were below the MAC established by both the EU and the Republic of Serbia.

DISCUSSION

Distribution of metal and trace elements in different tissues showed species-specific patterns. In the present study, the species with the highest overall accumulation in the muscle tissue in the downstream section was the pike-perch. Barbel had the highest concentrations of the metal and trace elements in the muscle at the upstream section. However, concentrations of the As, Cd, Cu, Fe and Zn were below MAC in all analyzed muscle samples, which indicates that the meat of the studied fish species can be utilized in the human diet. The gills, due to their direct contact with the water, are considered to be the main site of the metal uptake and can indicate the water as a main source of the contamination (Storelli et al. 2007). In the current study, gills had the highest Cr, Zn and Fe concentrations.

Previous research (Lenhardt et al. 2012) in the Belgrade Danube section, showed some differences from our findings. Since freshwater bream was analyzed in both studies, we observed that overall metal and trace element concentration in liver and muscle was higher in the current study, which can be potentially explained by recent pollution emissions. Additionally, there were no significant differences in the overall gill metal and trace element concentrations.

Since liver is considered as a tissue with the highest accumulation ability (Uysal et al. 2009) and our study showed increase in metal and trace element concentrations, we strongly emphasize the necessity to establish continuous monitoring activities in the future, due to possible environmental deterioration. It is important to establish permanent monitoring of metals and trace elements in these localities, as well as to establish a system of suitable indicator species.

ACKNOWLEDGEMENTS

This study was supported by the Ministry of Education, Science, and Technological Development of the Republic of Serbia, Project No. TR37009 and Project No. 173045.

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PESTICIDE CONTAMINATION IN FARMED FISH - EXPOSURE PATHWAYS, BIOACCUMULATION AND CONSUMER RISK

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ZAGAĐENJE PESTICIDIMA GAJENIH RIBA – PUTEVI IZLOŽENOSTI, BIOAKUMULACIJA I RIZIK ZA POTROŠAČE

Apstrakt

U današnje vreme, globalno, proizvodnja u akvakulturi pokriva oko 50 % konzumirane ribe. Sa porastom obima proizvodnje u akvakulturi i porastom korišćenja biljnih sirovina u proizvodnji hrane za ribe, postoji potencijalni rizik od ostataka pesticida u jestivim proizvodima od ribe. Međutim podaci o monitoringu ostataka pesticide u komercijalnoj hrani za ribe uglavnom nedostaju, a potencijal prenosa ostataka pesticida sa useva na jestiva tkiva riba bi trebalo da bude bolje osvatljen.

Predstavljen je simpleks pristup za procenu maksimalnog opterećenja pesticidima u hrani za ribe. Dat je pregled aktuelnih istraživanja o obimu i tipu rezidua koji se mogu pojaviti u jestivim proizvodima od riba izloženim pesticidima.

Abstract

Today, aquaculture production provides roughly 50% of the worldwide consumed fish. Due to the increase in fish farming and the increase in use of plant commodities as a source of feed, there is a potential risk for pesticide residues in edible products in fish. However, monitoring data on pesticide residues in commercial aquaculture diets are mostly missing and the potential for transfer of pesticide residues from crops into edible tissues in fish needs to be further elucidated. A simplex approach for the estimation of the maximum burden of pesticides in compound feeds for fish is presented. An overview of current research activities on the extent and nature of residues that may occur in edible commodities of fish exposed to pesticides is provided.

SURVEILLANCE OF FISH DISEASES IN SERBIA

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NADZOR BOLESTI RIBA U SRBIJI

Apstrakt

U Srbiji se vrši program nadzora virusnih i bakterijskih bolesti na osnovu Programa mera zdravstvene zaštite životinja, u skladu sa propisima Evropske Unije, i to: virusne hemoragične septikemije (VHS) i zarazna hematopoezne nekroze (IHN), zarazne nekroze pankreasa (IPN), prolećne viremije šarana (SVC) i renibakterioze (BKD). Cilj programa praćenja i nadzora bolesti riba je dobijanje i održavanje statusa slobode od bolesti, iskornjivanje ili sprečavanje širenja bolesti. Primarna ciljna populacija u programu nadzora su kalifornijska pastrmka i šaran.

U Srbiji postoji nacionalno zakonodavstvo kao osnov za nadzora i kontrolu bolesti riba, kao i lista bolesti obaveznih za prijavljivanje. Pored nacionalnog zakonodavstva, prihvaćeni su i principi navedeni u Direktivi 2006/88/EC, koji se odnose na zahteve za kontrolu zdravlje riba u akvakulturi i njihovih proizvoda. Praćenje i nadzor virusnih bolesti vrše se na osnovu postupaka ispitivanja datih u Odluci Komisije 2001/183/EC, a za bakterijske bolesti, koriste se standardne dijagnostičke procedure.

Klinički pregledi na ribnjacima vrše se dva puta godišnje i uzimaju se uzorci za virološka i bakteriološka ispitivanja, u svrhu dokumentovanja odsustva bolesti. Postupak ispitivanja, dat u Priručniku OIE za dijagnostiku bolesti riba, osnova je za ispitivanja. Uzorci svih kategorija riba iz 56 šaranskih i 52 pastrmska ribnjaka se godišnje ispituju na prisustvo bolesti. 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 su 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. 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). Na osnovu rezultata nadzora, Srbija se smatra slobodnom od VHS, IHN i KHV. Najveći problem predstavlja zarazna nekroza pankreasa. Renibakterioza je prisutna na određenom broju ribnjaka.

Ključne reči: nadzor, bolesti riba
Keywords: surveillance, fish diseases

INTRODUCTION

On a global scale, fish and fishery products are the main food supply for human beings. It is widely known that the supplies of fish from traditional fisheries are more or less constant and that the shortage in fish and fish products has to be met by aquaculture. 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 (Jeremić, 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).

MATERIAL AND METODS

Samples of all fish categories from 56 carp and 52 trout farms were examined annually for the presence of certain viral and bacterial diseases. Clinical examination and selection of samples for laboratory was done on the fish farms. For virus isolation, 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. Supernatants were inoculated at 24 hours old culture of EPC, CCB 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 homogenates 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 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

The fish farming industry is based on cold water species, rainbow trout (*Onchorhynchus mykiss*), brown trout (*Salmo trutta* m. *fario*) and warm water species, common carp (*Cyprinus carpio*), grass carp (*Ctenopharingodon idella*), silver carp (*Aristichthys nobilis*), bighead carp (*Hypophthalmichtys molitrix*), catfish (*Silurus glanis*), pike (*Esox lucius*), and pike perch (*Stizostedion lucioperca*). In Table 1 an overview over the farmed fish species as well as the size and production of these species are given.

Over the last decade, several emerging or serious diseases in fish have been diagnosed in farmed and feral populations, creating large problems in the fish farming industry and thus being the subject of surveillance and monitoring programs. Differences between infectious diseases in fish and those of terrestrial animals mean that the approach to the problems and the eradication efforts differ as the diseases may spread effectively through the flowing water (Håstein et al., 1999).

Table 1. Number of fish farms, species and production in Serbia

No. of farms	Species	Total production
108	rainbow trout (*Onchorhynchus mykiss*) brown trout (*Salmotrutta* m. *fario*) common carp (*Cyprinus carpio*) grass carp (*Ctenopharingodon idella*) silver carp (*Aristichthys nobilis*) bighead carp (*Hypophthalmichtys molitrix*) catfish (*Silurus glanis*) pike (*Esox lucius*) pike perch (*Stizostedion lucioperca*)	14.000 tons

In the following, the basis for and the results of monitoring and surveillance programs were described (Tab.2), as well as measures established for some of the diseases considered to be of major concern.

Table 2. Diseases and target species under surveillance

Disease	Susceptible species
IPN	Salmonids
VHS	Salmonids
IHN	Salmonids
SVC	Cyprinids
KHV	Carp & Koi carp
BKD	Salmonids

Serbia has a national legislation as a basis for aquatic animals‘ disease surveillance and control, as well as regulations listing notifiable diseases of concern. In addition to the national legislation, the principles laid down in the Council Directive 2006/88/EC in regards to animal health requirements for aquaculture animals and products thereof were accepted. Monitoring and surveillance of fish viral diseases have mainly been based on the testing procedures given in the Commission Decision 2001/183/EC while for the bacterial diseases, standard diagnostic procedures has been used for screening purposes.

Serbia runs a surveillance program for viral haemorrhagic septicaemia (VHS) and infectious haematopoietic necrosis (IHN) based on EU regulations. Monitoring program for infectious pancreatic necrosis (IPN), spring viraemia of carp (SVC) and bacterial kidney disease (BKD) takes place in Serbia on a national level. The aim of the monitoring and surveillance programs for fish diseases is to document and maintain freedom of disease, either to eradicate a disease or to keep it under control within certain bounds.. The main target population for the monitoring and surveillance programs are rainbow trout and common carp. The size of the target populations is given in Table 1. All types of farms are included in the survey such as hatcheries, brood stock farms as well as grow out farms. The diseases under surveillance and the target species for the disease in question are shown in Table 2.

The basis for the surveillance and monitoring programs is partly based on EU regulations, OIE criteria or criteria derived from the national legislation. The participation is compulsory as regards approval and maintenance of disease free status. For the sampling of fish for surveillance the responsible authority is the district veterinary inspector in coordination with the local veterinary institute and the national reference laboratory.

Fish farms are inspected clinically biannually and samples for virological examinations are collected from the fish farms each year to document freedom for IHN, IPN and VHS. The examination procedures given in the OIE Diagnostic Manual for Aquatic Animal Diseases are the basis for examinations as regards KHV, BKD, and SVC.

The veterinary authorities are responsible for the implementation of measures that will be used in order to control a given notifiable disease. The implementation involves both central and regional veterinary officers. If disease is diagnosed, stamping out procedures followed by cleaning, disinfection and fallowing will be carried out. Prevention may be achieved by avoiding introduction of disease free eggs and/or fish into disease free farms, as well as using protected water supply (e.g. spring-, borehole water). An infected farm may restock after fallowing if no signs of infection appear after a sanitation program has been carried out. In Serbia affected farm will have to pay themselves for any measures imposed by the authorities for the time being, because no compensation is granted. Health certificates and/or transportation documents is needed in connection with deliveries of live fish for stocking into grow out farms and restocking into rivers. The record of findings are kept by the responsible authorities, both regionally and centrally. The diagnostic laboratories also keep the necessary documentation on the examinations carried out. Furthermore, all farms have to keep records on events in the farms that can be requested by Competent Authority.

CONCLUSIONS

Serbia is considered to be free from VHS, IHN and KHV. Infectious pancreatic necrosis is considered to be the main viral disease problem. BKD has been reported but the level appear to be relatively low. Serbia have established appropriate surveillance and monitoring for fish diseases of concern to the fish farming industry. Due to these systems and good management practices, the fish disease situation is generally good compared to other countries.

ACKNOWLEDGMENT

This research was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia as part of projects number 31011 and 31075.

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FISH EMBRYOS AS ALTERNATIVE MODELS IN TOXICOLOGY: A REVIEW

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EMBRIONI RIBA KAO ALTERNATIVNI MODEL U TOKSIKOLOGIJI: REVIJALNI PREGLED

Apstrakt

Povećan broj ksenobiotika ugrožava životnu sredinu. Ova bioaktivna jedinjenja dospevaju u vodenu sredinu na različite načine i imaju značajan uticaj na akvatični živi svet. Za procenu opasnosti i rizika industrijskih hemikalija, proizvoda zaštite bilja, biocida, dodataka u hrani i lekova koriste se testovi na životinjama. Akutni test toksičnosti za ribe je obavezni korak u procesu ispitivanja toksičnosti hemikalija. Međutim njihova ekotoksikološka relevantnost je pod znakom pitanja. Povrh toga ribe se u ovim testovima podvrgavaju značajnom bolu i patnji. U skladu sa filozofijom 3r (redukcija/smanjenje, refinement/prečišćavanje, replacement/zamena) embrioni riba se smatraju metodom zamene ili prečišćavanja budući da ovi razvojni stadijumi nisu zakonski zaštićeni (Direktiva 2010/63/EU) i izgleda da osećaju manju bol i patnju.

Cilj ovog pregleda bio je da se sumiraju dostupni podaci o testovima toksičnosti na embrionima riba i ukaže na moguće perspektive korišćenja ovog modela u savremenoj toksikologiji. Princip testa toksičnosti sa embrionima riba zasniva se na izloženosti vijabilnih embriona ksenobioticima, od oplođenja do kraja embriogeneze. Test toksičnosti sa embrionima riba validiran je u OECD studiji i prihvaćen kao OECD TG 236 96-h test za procenu toksičnosti za embrione riba. Određuje se letalnost koja je označena koagulacijom embriona, nedostatkom somita, ispravljanja repa ili otkucaja srca. Povrh smrtnosti, složeniji pokazatelji kao što je razvoj očiju, prisustvo krvotoka ili razvojne malformacije mogu se uočiti na stadijumima izvaljenih eleutero embriona. Mogućnost praćenja različitih pokazatelja čini embrione riba odličnim modelom za ispitivanje i razumevanje mehanizama toksičnosti i indikatorom mogućih nepovoljnih i dugotrajnih efekata.

Na osnovu izvedenih istraživanja, test toksičnosti sa embrionima riba je razumna alternativa akutnom testu toksičnosti na ribama. Raspon mogućih aplikacija je značajan i ima mnogo perspektiva u budućnosti: kao što je dalja identifikacija molekularnih markera – indikatora načina delovanja ili uspostavljanje veze među efektima u kratkotrajnim testovima sa embrionima i dugotrajnih efekata na individue. Test toksičnosti sa embrionima riba takođe može da doprinese smanjenju broja eksperimenata na životinjama što je jedan od osnovnih principa EU legislative u oblasti dobrobiti.

Ključne reči: testovi toksičnosti sa životinjama, REACH, Danio rerio. Test akutne toksičnosti na ribama, vodena sredina
Keywords: Animal toxicity test, REACH, Danio rerio, fish acute toxicity test, aquatic environment

INTRODUCTION

In EU, most industrial chemicals are regulated by the Regulation (EC) No 1907/2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals, known as REACH (EU, 2006). Animal tests are used to assess the environmental hazard identification and risk assessment of industrial chemicals, plant protection products, biocides, feed additives and pharmaceuticals (Scholz et al., 2013). Bioactive compounds can reach the aquatic environment in many ways and have considerable effects on aquatic biota. Fish play a critical role in aquatic food webs and are an important food source for humans. Fish have been accepted as vertebrate representatives for the aquatic environment in acute as well as chronic toxicity tests and are also used to monitor the quality of effluents and surface waters (Lammer et al., 2009). The fish acute toxicity test (FAT) is a mandatory step in environmental hazard and risk assessment of chemicals. However, its ecotoxicological relevance is questionable. Value of LC_{50} may vary among different species and short-term exposure to high concentrations of toxicant in nature is not expected. The only exception may represent accidental spills. Moreover, fish are subjected to considerable pain and suffering (Nagel, 2002). Growing number of chemicals consumption brings request for novel and reliable methods identifying possible hazard of chemical compounds (Oxendine et al, 2006).

The aim of this review was to summarize available data on fish embryo toxicity tests (FET) and introduce their possible perspectives in modern toxicology.

MATERIALS AND METHODS

The review summarizes available data on fish embryo toxicity tests and introduce their possible perspectives in modern toxicology.

Available research articles dealing with fish embryo toxicity tests were collected and studied. The research findings were summarized and the review was completed.

RESULTS AND DISCUSSION

In accordance with reduction, refinement and replacement philosophy (the 3Rs; Russell and Burch, 1959) fish embryos are considered as replacement or refinement method since these developmental stages are not legislatively protected (Directive 2010/63/EU) and are likely to experience less pain or suffering. (EFSA, 2005). The idea of embryo assay has been suggested by Schulte and Nagel (1994). The principle insists in the exposition of viable embryos, from fertilization until the completion of embryogenesis (48 h), to xenobiotics when various responses are recorded. Coagulation of eggs and embryos, lack of somite formation, non-detachment of the tail and lack of heartbeat are assessed to be lethal and are observed in order to determine LC_{50} (Nagel, 2002). Additionaly, sub-lethal endpoints such as completion of gastrula, formation of somites, development of eyes, spontaneous movement, hearbeat/ blood circulation, pigmentation and oedema may be recorded to indicate the mode of action of toxic coumpound. The analysis can also include the screening for developmental disorders to indicate teratogenic effects (Nagel, 2002): malformations of the head, otoliths, tail and heart, modified structure of the corda, scoliosis, rachischisis, deformity of yolk, growth-retardation and length of tail. Although the FET was originally designed as an alternative to the FAT, the FET was internationaly standardized. In Germany, previous acute fish toxicity testing of effluents was replaced by a standardized 48-h wastewater test with zebrafish (*Danio rerio*) embryos (DIN 2001, ISO 2007) in 2005. The FET was also validated during an OECD validation study and adopted as OECD TG 236 as a 96-h test to assess toxicity using zebrafish embryonic stages (Braunbeck et al., 2014). In some studies the extended version of 96-h FET has been prioritized to cover the critic phase of hatching and eleutheroembryo (the free embryo) development (e.g. Carlsson et al., 2013 or Selderslaghs et al., 2009). In accordance with Directive 2010/63/EU, the earliest life stages of fish are not legislatively protected until they feed independently. The way of feeding (endogenous or exogenous) also distinguishes the developmental phases of embryo (egg), eleutheroembryo (feeding off the yolk sac) and larvae (exogenous feeding) (Belanger et al., 2010). There is a strong need to distinguish between non-protected and protected life intervals among different fish species. According to Strähle et al. (2012) zebrafish are capable of independent feeding at 120 h post-fertilization. Belanger et al. (2010) suggest that tests conducted on zebrafish embryo should be terminated between 24 and 48 h after hatching. However, at 144 hpf active food uptake was not documented in all individuals (Belanger et al., 2010) and the non-feeding eleutheroembryo stage can be interpreted as an extension phase of embryonic development (Strähle et al., 2012; Brauenbeck et al., 2014).

Zebrafish (*Danio rerio*) belongs among prominently used toxicological models . Main benefits of zebrafish usage are small size and optimum breeding and maintenance conditions. The zebrafish mature rapidly (within 3 month at 26 °C) and are capable of laying 50–200 eggs every day. The embryos are high in fecundity and their transparency as well as well described development (e.g. by Kimmel el al., 1995) are big advantages of using zebrafish embryos in fish embryo toxicity tests (Hill et al., 2005; Nagel, 1993). Even though the zebrafish is the most used species in this context, additional species such as fathead minnow (*Pimephales promelas*) and medaka (*Oryzias latipes*) should be pursued in the future (Belanger et al., 2013).

In accordance with study comparing results from FAT and FET conducted by Nagel (2002) the fish embryo test is a reasonable alternative to fish acute toxicity test. Recently, Lammer et al. (2009) and Belanger et al. (2013) summarized the FET and FAT studies in order to understand their potentional relationships and the FET applicability in chemical testing. Their results were in accordance with those from Nagel (2002) and provided scientific support for the FET as an alternative to fish acute toxicity test.

CONCLUSIONS

The fish embryo toxicity test is a reasonable alternative to fish acute toxicity test with wide range of future perspectives. The fish embryo toxicity tests could also contribute to the reduction of animal experiments, which is one of the core principles of EU welfare legislation.

ACKNOWLEDGEMENTS

This study was supported by IGA VFU 226/2015/FVHE.

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ANALYSIS OF THE DISTRIBUTION OF FRESHWATER FISH IN SERBIA

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ANALIZA DISTRIBUCIJE SVEŽE SLATKOVODNE RIBE U SRBIJI

Apstrakt

Dosadašnja istraživanja marketinga ribe uglavnom su bila posvećena opštim informacijama koje se odnose na potrošnju i globalne tendencije, a manje na distribuciju sveže rečne ribe, koja se u Srbiji najčešće vrši preko trgovine na malo. Stoga je u ovom radu posvećena pažnja razmatranju kanala distribucije od ulova ribe do potrošača. Podaci za ovo istraživanje su prikupljeni anketiranjem lokalnih ribara. Intervju je lično vršio istraživač. Podaci su obrađeni u programskom paketu SPSS. Na osnovu analize došlo se do sledećih zaključaka: kod većine anketiranih osoba koje se bave ribolovom, ova delatnost predstavlja jedino zanimanje, tako da osobe koje se profesionalno bave ovom aktivnošću imaju već uređene sopstvene kanale distribucije. Pokazano je da u rečnom ribarstvu faktor udaljenosti ima značajan efekat kroz dva aspekta: udaljenost od mesta stanovanja do mesta ukotvljenja, i udaljenost od mesta ukotvljenja do mesta ribarenja. Najveći deo ulovljene ribe prodaje se u svežem i smrznutom stanju. Očišćena riba se znatno ređe distribuira između ribara i nakupaca. Distribucija ribe između ovih subjekata u obliku viših nivoa prerade, poput fileta, predstavlja retkost. Takođe je utvrđeno da se tražnja za ribom značajno menja u toku jedne godine, a da vodeće mesto u tražnji za slatkovodnom ribom ima pastrmka, čiji transport do tržišta Beograda nije lokalnog tipa, za razliku od šarana. Na osnovu intrevjua prodavaca ribe na malo, smatra se da je cena najvažniji faktor prilikom prodaje ribe.

Ključne reči: distribucija, riba, Srbija

Abstract

Previous studies of fish market were mainly related to the consumption and global tendencies, and less to distribution of freshwater fish, which in Serbia is mostly carried out via the retail trade. Therefore, this paper consider distribution channels from catching fish to consumers. Data were collected by interviewing local fishermen. The interview was directly conducted by researcher. The data were analyzed using statistical package SPSS. Based on the analysis, following conclusions have been made: For the majority of respondents fishing is their job, the only source of income, so they are professionally engaged in this activity, thus they already have their own distribution channels. It is shown that factor of distance has a significant effect in two aspects: the distance from place of residence to the place of anchoraging, and the distance from the place of anchorage to the place of fishing. The majority of fish caught is sold fresh or frozen. Cleaned fish is considerably less distributed between fishermen and dealers. Distribution of more processed fish, such as fillets, is rare between these marketing levels. It was also found that the demand for fish changes significantly during the year. But, trout has the leading position in the demand for freshwater fish in Belgrade, even despite the fact that it does not have a local character, as opposed to carp. According to results of interview given by fishmongers, the price of fish is the most important factor when selling fish.

Keywords: distribution, fish, Serbia

COMPARATIVE SHAPE ANALYSIS OF WILD AND REARED STERLET (*ACIPENSER RUTHENUS* L.)

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UPOREDNA ANALIZA OBLIKA PRIRODNIH I UZGAJANIH KEČIGA
(ACIPENSER RUTHENUS L.)

Apstrakt

Usled ugroženosti kečige, brojni programi poribljavanja postoje, pa je od velikog značaja procena odgovarajućih primeraka za te programe. Metodom geometrijske morfometrije su analizirane promen oblika jedinki iz prirodnih populacija i iz uzgoja. ANOVA varijable veličine je pokazala da su jedinke iz uzgoja krupnije u odnosu na jedinke iz prirodnih populacija. Takođe, CVA varijable oblika je pokazala promene u predelu glave i repa kod jedinki iz prirodnih populacija. Ovi rezultati mogu predstavljati dobru osnovu kod procene odgovarajućih jedinki za poribljavanje otvorenih voda.

Ključne reči: geometrijska morfometrija, poribljavanje, recirkularni sistem uzgoja,
Keywords: geometric morphometrics, stocking, recirculating aquaculture system

INTRODUCTION

Sterlet (*Acipenser ruthenus* L.) populations have experienced a decline during 20th century, throughout Danube River basin, and their presence in Germany and Austrian sections is dependent on stocking efforts (Reinartz, 2002). To compensate decline of wild sterlet populations and boost their commercial harvest, countries in the Middle and Lower Danube also carried out stocking with larvae, fingerlings and juveniles (Smederevac-Lalić *et al*., 2011; Lenhardt *et al*., 2012). However, according to Neff *et al.* (2011), current breeding programs are too focused on genetic diversity and do not take into account complexities of the genetic architecture of wild populations fitness. Additionally, risk associated with (re) stocking include: increase competition and/or predation (Aprahamian *et al*., 2003), inbreeding or outbreeding depressions (Ludwig *et al*., 2009), while adaptation ability of stock specimens can be jeopardized if they are introduced in non-native areal (Ludwig, 2006). Some meristic and morphological differences between wild and reared sterlet were reported by Lenhardt *et al.* (2012), while various authors used shape analysis to asses body variations between wild and hatchery-reared specimens (von Cramon-Taubadel *et al*., 2006; Cvijanović *et al*., 2011; Arechavala-Lopez *et al*., 2012). Bearing all this in mind, careful selection of proper specimens for stocking programs should be mandatory.

The objective of this study is to assess the body variations between wild and farmed sterlet, as well as to assess differences in body shape between two different rearing systems.

MATERIAL AND METHODS

During July 2011, 68 specimens of sterlet were caught with gill nets by professional fishermen at the Tisza River (26 specimens near Bečej, N 45°37'39.22" E 20°05'40.01") and the Danube River (42 specimens near Bačka Palanka, N 45°14'05.22" E 19°22'20.64") sites. Acquiring samples from the aquaculture facility „FISH FARM" (Vršac, N 45°07'17.84" E 21°18'08.25") and Faculty of Fisheries and Protection of Waters (Vodňany, N 49°09'38.15" E 14°10'25.84"), was during January and February 2015, with collection of 35 and 32 specimens respectively. Both aquaculture facility represent recirculating aquaculture systems (RAS), with similar water flow regime (7m^3/h and 6.5-7m^3/h, respectively). Each fish was photographed from lateral (left side) and ventral aspect, with a Panasonic DMC-FZ40 digital camera mounted on tripod, at the same resloution and with ruler placed next to each specimen. In this study 11 landmarks defining the body outline were chosen (Fig. 1) for both landmark configuration (lateral and ventral). TpsDig software (Rohlf, 2005) was used to aquire morphometric data. Generalized Least Squares Procrustes superimposition (GLS) was applied for shape analyses, because it preserves all information about shape variability among the specimens and remove only information unrelated to shape (scale, position and orientation; Rohlf and Slice, 1990). For the analyses of size difference, we used the centroid size (CS, which represents the square root of the sum of the squared distances of a set of landmarks from the centre of gravity). Cordgen6 software (IMP series; Sheets, 2003) was used for calculation of the CS and GLS. An analysis of variance (ANOVA) was used to determine if CS differed significantly depending on location or age. Differences in shape between groups based on locations and between age classes were determen with the Canonical Variate Analysis (CVA). The purpose of CVA is to simplify the description of difference among groups (Zelditch et al., 2004), and analysis was carried out in CVAGen6 software (IMP series, Sheets, 2003). Age data were obtained from aquaculture, with sterlet from „FISH FARM" (35 specimens) and Vodňany (32 specimens) beloging to 0+ age classe. All assessed indivduals from wild were immature, and based on the estimate age at first maturity in the Danube sterlet populations (Kolarević, 2004), they were 0+ (68 specimens).

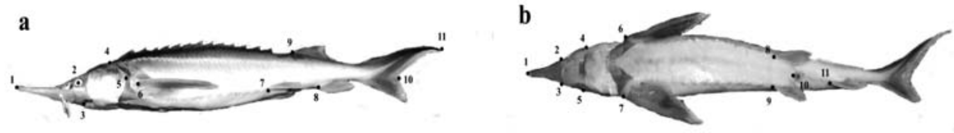

Figure 1. Position of the landmarks: a) lateral aspect: (1) snout tip, (2) eye, (3) front of mouth opening, (4) anterior insertion of the first dorsal scutum, (5) apex of the opercular spine, (6) dorsal insertion of pectoral fin, (7) anterior insertion of pelvic fin, (8) anterior insertion of anal fin, (9) anterior insertion of dorsal fin, (10) fork, (11) tip of caudal fin ; b) ventral aspect: (1) snout tip, (2)-(3) points where the line (connecting insertions of barbell) crosses the profile, (4)-(5) point where the line (connecting side of mouth opening) crosses the profile, (6)-(7) insertion of pectoral fins, (8)-(9) insertion of pelvic fins, (10) tip of anal opening, (11) insertion of anal fin.

RESULTS

All samples (n=135) in terms of size (expressed as CS) showed significant differences between groups based on locality. ANOVA of CS values for grouping based on locality show significant differences for both landmark configuration (F=272.87, $p<0.001$ and F=131.75, $p<0.001$ respectfully). A box-plot representing the distribution of CS show differences in CS values (Fig. 2). Tukey HSD post-hoc test show that differences of CS (lateral configuration) were higher between Vodňany and wild sterlet (p=0.000008 for both sampling sites), while differences between two rearing systems were also significant (p= 0.0377). Same differences for ventral landmark configuration was observed between Vodňany and wild sterlet (p=0.000008 for both sampling sites), while differences between two rearing systems were somewhere higher (p=0.000024).

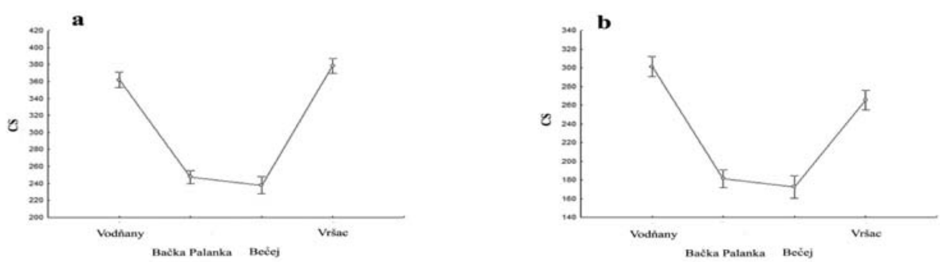

Figure 2. Box-plot of centroid size: a) grouping based on locality (lateral landmarks configuration); b) grouping based on locality (ventral landmarks configuration)

Canonical Variate Analysis (CVA) using partial warp scores show significant ($p<0.001$) differences between groups from different localities for both sets of landmarks configurations. For lateral landmarks configuration (Fig. 3), first CVA axes (λ=0.038, χ^2=400.61, d.f.=54) separated all sterlet from Vodňany specimens, while second CVA axes (λ=0.31, χ^2=145.46, d.f.=34) separated wild from „FISH FARM". For ventral configuration (Fig. 4), first CVA axes (λ=0.015, χ^2=513.33, d.f.=54) separated wild and reared specimens, while second CVA axes (λ=0.16, χ^2=226.87, d.f.=34) seperated speciemns from different rearing conditions. Eigenvalue for first two canonical axes (at lateral landmarks configurations) were 9.27 and 3.47 respectively, while the Eigenvalue for first two canonical axes (at ventral landmarks configurations) were 6.96 and 1.35 respectively.

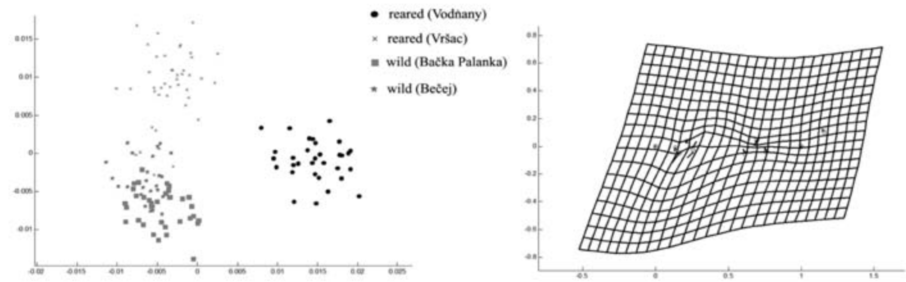

Figure 3. CVA score (lateral landmarks configuration) of first and second canonical variate analyses based on partial warps and uniform scores for all specimens (wild and reared) and partial warp grid for comparison between locality over first canonical variates.

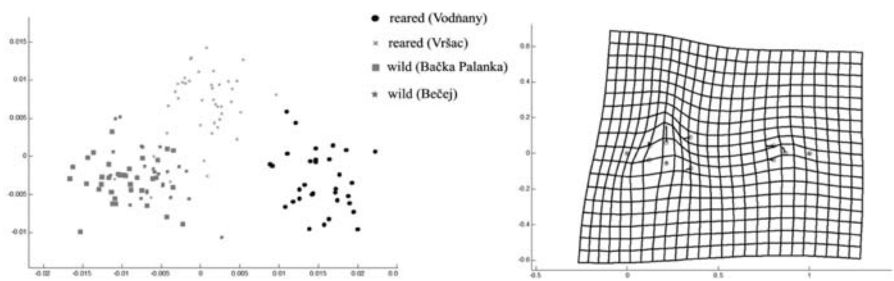

Figure 4. CVA score (ventral landmarks configuration) of first and second canonical variate analyses based on partial warps and uniform scores for all specimens (wild and reared) and partial warp grid for comparison between locality over first canonical variates.

DISCUSION AND CONCLUSION

Shape analysis performed in the present study indicated that there were differences between reared and wild sterlet, as well as body variations between both RAS systems.

In terms of centroid size, there was a significant difference between wild and reared sterlets. Also, difference was observed among both rearing systems and also among separate wild populations of sterlet. Fish from aquaculture have bigger body size, which can be attributed to better feeding conditions. Also, differences in CS value for both RAS systems can be attributed to different broodstock material.

Reinartz *et al.* (2011) produced clear evidence for a panmictic sterlet population across the entire Danube and its tributaries, so the question of whether local adaptations exist is something that has to be address in restocking programs. Shape analysis suggests that there is a significant difference between reared and wild starlet for both landmarks configurations. CVA show that tail region of Vodňany sterlets is narrowed if compared with other samples, when lateral landmarks configuration is applied. Since the conditions of rearing are similar in both Vodňany and Vršac RAS, this may be indicator of different broodstock material. In ventral landmarks configuration, both tail and head region of wild sterlets are thinned. Changes in head and tail region can be attributed to different flow conditions in wild and/or different food regime of wild sterlets. Still, future researches are needed, and especially detail evaluation of head and tail regions with additional landmarks and/or semi-lendmarks, defining body shape changes.

As Loy *et al*. (1999) stated, geometric morphometrics is a highly powerful tool and it's recommended in wild sturgeons populations, characterization of hybrids, and in quantifying within population variability and morphological plasticity. Also, this method can be good tool for optimization of breeding conditions for stocking specimens, but further research is needed.

ACKNOWLEDGEMENT

This study represents activities within the Project No. 173045, funded by the Ministry of Education, Science and Technological Development of the Republic Serbia, and also supported by AQUAEXCEL TNA project 0134/09/09/24b/B.

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ECOLOGICAL, ECONOMIC AND SOCIAL PARAMETERS OF RECREATIONAL FISHING ON THE RESERVOIR OF AOOS RIVER

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EKOLOŠKI, EKONOMSKI I SOCIJALNI PARAMETRI REKREATIVNOG RIBOLOVA NA AKUMULACIJI REKE AOOS

Apstrakt

U cilju prikupljanja podataka o rekreativnom ribolovu sprovedeno je anketiranje ribolovaca na akumulaciji reke Aoos u severozapadnoj Grčkoj. Upitnik je obuhvatio pitanja o demografiji, alatima koje koriste za pecanje, uslovima za ribolov, opremi, ciljnim vrstama, intenzitetu odlaska u ribolov, ribolovnom naporu, sezonama, podacima o ulovu. Prikazani rezultati ukazuju da rekreativni ribolov na tom području ima potencijal za razvoj turizma i poboljšanje socioekonomskog uticaja u čitavom regionu. Ribolovci imaju sve izraženiju svest o životoj sredini. Šaran je trenutno vrsta koja se najviše lovi i koja održava ovu aktivnost, a ukazuje da će ova vrsta moći opstati i na duže ukoliko dodje do promene klimatskih uslova i ukoliko protok bude smanjen kao posledica globalnog otopljavanja i kompeticije sa drugim korisnicima. Rukovodeći se rezultatima u ovom radu koji ukazuju na potencijal za razvoj ribolovnog turizma i povećanje ekološke vrednosti ovog područja, upravljači područjem bi trebalo da razmotre mogućnosti poribljavanja na području akumulacije koju formira brana na reci Aoos. Konačna preporuka za uspešno upravljanje ribolovom na području hidroelektrane treba da bude bazirana na kombinovanju parametara koji uključuju ekonomske, ekološke i ribarstvene podatke.

Ključne reči: reka Aoos, hidroelektrana, rekreativni ribolov, anketiranje
Keywords: Aoos River, hydroelectric dam, recreational fisheries, survey

INTRODUCTION

Freshwater ecosystems, including artificial dams provide a range of services including recreational fisheries. In fact, recreational fishing in freshwaters is a major economic activity in developed countries (Arlinghaus et al., 2014; Lothrop et al., 2014). Recreational fishing is popular in the countries of EU, with participation levels varying from 1% of the population in the south to over 30% in northern ones (Rangel & Erzini, 2007). Hydroelectric dams provide a range of potential usages and economic developments, including fisheries (Jackson & Marmulla, 2001). The management of reservoirs involves the construction of fish passage and the restocking measures (Baumann & Stevanella, 2012; McGinnis, 1994), creating opportunities for recreational fishing activity and economy. The motivation of fishermen and public may be influenced by several parameters, including considerations of ecological restoration and protection of native fish species (Novaes & Carvalho, 2011). The Aoos River is 260 km long, 70 km length, located within the Greek borders. The river has a large hydroelectric dam near its springs, producing 103 MWh annually. The regional economy is characterised by agricultural production, but exhibits prospects for tourism (Andreopoulos et al., 2015). The aim of the study was to collect data on fish catch, fishing effort, species composition and preferences, demographic data of the fishermen and their attitudes and awareness of fisheries management parameters of the aquatic ecosystem of the river Aoos reservoir.

MATERIALS AND METHODS

A survey was carried out on a weekly basis over a period of one month in order to collect fisheries data about recreational fishing on the reservoir of Aoos hydroelectric dam in NW Greece. The questionnaire was distributed, completed and collected at the site (n=22). Fishing effort was estimated on the basis of the number of fishermen and hours spent in fishing. The survey included questions regarding preferences for target species, frequency of fishing trips, fishing effort, season and catch. Data were analysed with SPSS version 10.1.

RESULTS AND DISCUSSION

The majority of the surveyed recreational fishermen on Aoos reservoir were between 30-40 years old, were educated up to secondary education level and were visiting the reservoir at least every fortnight. They reported that they did not fish during the winter and spring at the reproductive period of trouts and carps respectively. There are several factors which can motivate and satisfy recreational fishermen, may have goals for specific species and size of fishes, but also may get a significant level of satisfaction from the whole fishing experience including the travelling, socializing and catch (Beardmore et al., 2011; Carlin et al., 2012). Surveys can provide useful information about fishing effort, fish catch and target species. The fishing effort of recreational fishermen can be estimated by the length of time (in the present work measured in hours) spent for fishing (Jenkins & Morais, 1971).

Fish catch can be measured according to total weight of fish captured (Deines et al., 2013). These two parameters and other fisheries data can be used for fisheries statistics and fisheries management (Deines et al., 2013). In the present study, all of the surveyed recreational fishermen were using fishing rods and their target species was carp (*Cyprinus carpio*). They were fishing for more than eight hours per day (mean 7.63 ±1.12 hrs), 2.4 times per month, capturing more than five carps per fishing session with frequently reporting size above 3 kg (Fig. 1). The Ratio of means estimator (R1, mean catch per angler divided by mean effort per angler) was 0.38±0.21 (Table 1). They presented increased environmental awareness, as all reported that pollution and illegal fishing is currently compromising the aquatic ecosystem of Aoos reservoir. As the carp is considered as neither a non native species nor endangered, the fishermen did not hesitate to target this species for their recreational fishing activity. The size of carp captured ranged between 1 and 5 kg. Increasing fishing effort resulted in increased total amount of catch (Fig. 2). The age of the carp caught ranged from 1 to more than 6 years. Regression analysis between the fishing effort and total catch indicated that catch increased with the fishing effort ($r^2=0.801$, $p<0.05$). There was no significant effect of fishing effort on the fish size caught. The results reveal that the carp is the main target species, while catch rate of trout and other species was extremely low, indicating the relative high abundance of the carp in the reservoir of Aoos River.

Table 1. Descriptive statistics of fishing effort and fish catch of carp in Aoos reservoir. R1: Ratio of means estimator (monthly mean catch per fisherman divided by mean effort per fisherman). Fish caught: Average fish catch (kg), length of fishing session (hours per month and day), and catch per unit effort (CPUE) of the surveyed recreational fisherman.

	(R1)	Average fish catch (kg) per month	Average length of time spent in fishing per month	Average fishing effort (hrs/day)	Average CPUE (Catch per unit effort: fish catch/hour)
MEAN	0,38	19,39	17,74	7,63	2,55
SD	0,21	10,41	9,86	1,12	1,37
MIN	0,21	10,41	9,86	1,12	1,37
MAX	0,38	19,39	17,74	7,63	2,55

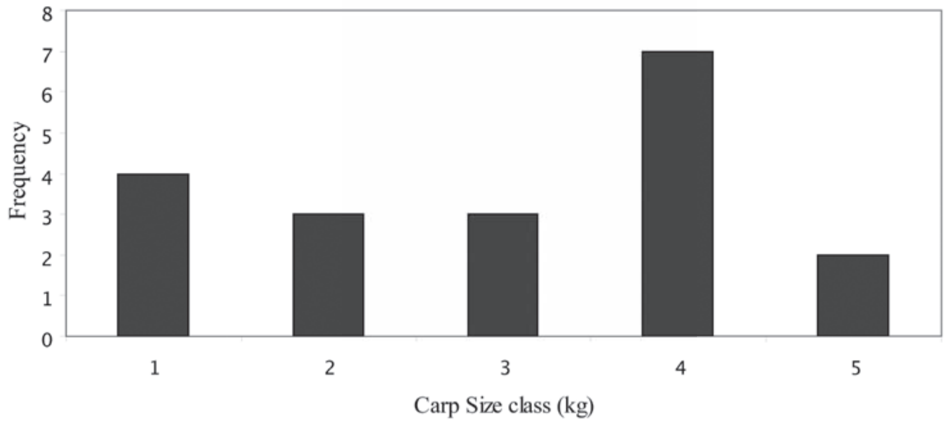

Figure 1. Frequency distribution of caught carp size (kg) in Aoos reservoir.

Carp is extremely tolerant and could sustain fisheries even with reduced water flow rates as expected (Leontaritis & Baltas, 2014) and could be a candidate for possible future restocking plans. Recreational fishing activity may not necessarily rely on a particular species. They may be equally satisfied if other fish species are available. Catch rate per effort is also an important parameter which can influence the motivation and satisfaction of recreational fishermen (Beardmore et al., 2011; Carlin et al., 2012).

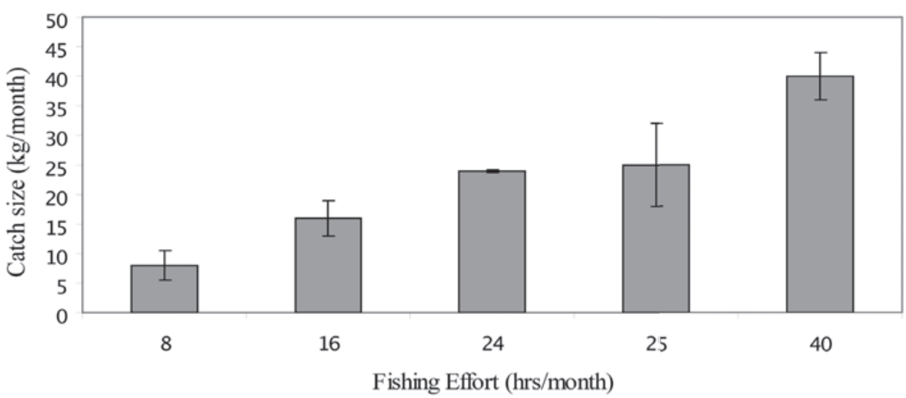

Figure 2. Fishing effort (based on carp) per month per fishermen in Aoos reservoir.

CONCLUSIONS

The data presented herein, indicate that the recreational fishing provides a potential for tourism development increasing the socio-economics impacts of the region. This view should be considered by the local stakeholders as sustainable method of development in the region. The reservoir of Aoos constitutes a significant element of the ecological value of the region (Andreopoulos et al., 2015). The result of the survey indicates that recreational fishermen have increased environmental awareness, consequently they constitute a significant group of consumers of the ecological value which characterises the region. Although the carp is a non native species, it is currently main prey for fishing in the reservoir. Planners should take into account the possibility of restocking fish in Aoos reservoir, based on the present results which support the increasing socio-economic impacts of recreational fishing and the ecological value of the region. The final decision for management of Aoos reservoir should be based on a combination of economic, ecological and fisheries data.

ACKNOWLEDGMENTS

The authors are grateful for support provided by the local association Ioannina Fishing Club.

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DAPHNIA HYBRIDIZATION IN PERI-ALPINE LAKES OVER SPACE AND TIME

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VREMENSKA I PROSTORNA HIBRIDIZACIJA *DAPHNIA* U PERI-ALPSKIH JEZERIMA

Apstrakt

Tri vrste dafnija *D. longispina* kompleksa (*D. galeata, D. longispina* and *D. cucullata*) i njihovi hibridi se mogu naći u jezerima širom Evrope. Mnogi faktori (npr. kvalitet i količina hrane, predacija, bolesti, kompeticija) su analizirani u cilju ispitivanja koegzistencije parentala i hibrida. Analizom uzoraka sedimenata rekonstruisali smo neke od populacija *Daphnia* severno od Alpa i pokazali da je eutrofikacija važan faktor koji utiče na sastava populacija *Daphnia* tokom vremena. U cilju prostornog analiziranja sastava, ispitivali smo populacije *Daphnia* severno i južno od Švajcarskih Alpa. Utvrdili smo da su jezera severno od Alpa invadirana jednom vrstom dafnija, *D. galatea*, i postavili hipotezu da su jezera južno od Alpa invadirana drugom vrstom dafija, *D. longispina*. Prvi eksperiment koji se bavio životnim ciklusom je pokazao izvesnu potvrdu ove hipoteze. Za dalje ispitivanje ove hipoteze potrebno je analizirati uzorke sedimenta iz jezera na južnoj strani Alpa. Predstavićü nove rezultate ovih istraživanja.

Ključne reči: Daphnia longispina kompleks, Švajcarski alpi, analiza sedimenata

Abstract

The three *Daphnia* species of the *D. longispina* complex (*D. galeata, D. longispina* and *D. cucullata*) and their hybrids can be found in lakes all over Europe. Many factors (e.g. food quality and quantity, predation, diseases, competition) have been studied to explain why parentals and hybrids co-occur. We reconstructed some of the Daphnia populations North of the Alps over time, using sediment cores, and showed that eutrophication is an important factor in determining the composition of *Daphnia* populations over time. To study on a spatial scale the composition of *Daphnia* populations we surveyed *Daphnia* populati-

ons north and south of the Swiss Alps. We found that Lakes North of the Alps were invaded with one species (*D. galeata*) and hypothesize that lakes south of the Alps were invaded with *D. longispina.* A first life history experiment shows some evidence for this hypothesis. Further, testing of this hypothesis needs to come from sediment cores from lakes from the south side of the Alps. I will present recent data about these studies.

Keywords: Daphnia longispina complex, Swiss Alps, sediment cores

SCIENTIFIC MONITORING OF THE ALIEN FISH AND CRUSTACEANS SPECIES IN THE ADRIATIC SEA (MONTENEGRIN COAST)

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NAUČNO PRAĆENJE ULASKA INVAZIVNIH VRSTA RIBA I RAKOVA U JADRANSKO MORE (CRNOGORSKO PRIMORJE)

Apstrakt

Ljudske aktivnosti, posebno prokopavanje Sueckog kanala, 1869. godine imale su za posledicu prodor, tim novim koridorom vrsta iz Indijskog okeana preko Crvenog mora.. Osim toga, poslednju deceniju obeležile su značajne promjene u klimi koju karakterišu vruća i suva ljeta i hladne zime, kao i česte oluje, poplave i šumski požari. Takođe su evidentne temperaturne promjene u morskim ekosistemima, s povećanjem temperature (tropikalizacija) u sjevernom Mediteranu, uključujući i Jadran. To je izazvalo širenje toplovodnih vrsta prema sjeveru Mediterana u područja koja nisu naseljavali prije. Hrvatski i crnogorskih ihtiolozi su kroz naučno-istraživačku saradnju identifikovali i opisali 46 novih vrsta u Jadranu. To su najčešće vrste iz Indo-Pacifika koji su došli na Mediteranu kroz Suecki kanal. Neke vrste stigle su iz istočnog Atlantika kroz Gibraltarski proloz. Osim migrantskih vrsta, nekoliko novih vrsta iz porodice Gobiidae su otkrivene u Jadranu, uglavnom zbog novih ribolovnih alata i tehnika, a nove dubokomorske vrste otkrivene su u ekspedicijama u južnojadranskoj kotlini. Osim riba potvrđeni su i nalazi nekih vrsta dekapodnih rakova, naročito na ušću jadranskih rijeka. Takođe je uočeno širenje areala na sjever Jadrana vrsta koje su nekada dominantno bile prisutne u južnom, toplijem Jadranu, kao sto su *Pomatomus saltatrix*. Ove pridošlice, osim što remete ustaljene ekološke odnose, donose i socioekonomske promjene, naročito u mjestima gdje je ribolov tradicionalna djelatnost, kao što su ušća rijeka Bojane i Neretve.

Ključne reči: Jadransko more, alohtone vrste, novi nalaz, dodatni nalaz, klimatske promene
Key words: Adriatic Sea, alien species, new record, additional record, climate changes

INTRODUCTION

So far, 60 fish species from the Red Sea have been registered in the Mediterranean, and those species have been named “lessepsian migrants”, after the French developer of the Suez Channel, Ferdinand Lesseps (CIESM 2013). Apart from these, several migrants from the east Atlantic have also been identified in the Mediterranean.

The past decade has been marked by significant changes in climate which caused hot, dry summers, cold winters, storms, floods and forest fires. Those changes have influenced the world’s fauna, notably on distribution and spread of certain populations. Temperature changes have also been identified in the marine ecosystems, with an increase in temperature (tropicalization) in the northern Mediterranean, including the Adriatic. This caused the spread of warm–water species northwards, to the areas of the Mediterranean they haven’t inhabited before.

Croatian and Montenegrian ichthyologists identified and described 46 new species in the Adriatic, (Lipej and Dulčić, 2010; Dulčić and Dragićević, 2011; Vesna Mačić *et.al.,* 2014). These are mostly species from the Indo–Pacific that came to Mediterranean through the Suez Channel. Some species arrived from the eastern Atlantic through the Gibraltar Straits. The species in question have already been identified in other parts of the Mediterranean, and their presence was now confirmed in the Adriatic, as the northernmost area of the Mediterranean Sea. Beside the migrant species, several new species from the family Gobiidae have been discovered in the Adriatic, mostly due to new fishing gears and techniques used (Pallaoro and Kovačić, 2000). New deep–water species were found and identified in the expeditions to the South Adriatic Pit (Ungaro *et.al.,* 2001).

The aim of the present work is to report additional records of fishes which have until now been regarded as rare or less known and those which have only recently been recorded in the Adriatic Sea for the first time.

MATERIAL AND METHODS

Several species have been registered in Montenegrin waters which were previously unknown to fishermen. They have informed the Institute of Marine Biology about them, and the specimens were brought to the Laboratory where the identification as well as morphometric characteristics analysis were performed in accordance with the certain identification keys. Total lengths (TL) were measured using fish measuring board (precision of 1 mm), measuring tape (precision 1 mm) and digital caliper (precision 0.01 mm). Weight (W) was measured using a high precision (0.01 g) electronic balance, Sartorius Extend ED 4202S.

The first catch of the previously unknown species, occurred on 5 January, 2008, in front of Budva, at a depth of 80 meters, when Blunthead puffer, *Sphoeroides pachygaster* (Müller & Troschel, 1848) was caught with the trammel net (Fig. 1). The analysis of specimens in the Laboratory showed that it was a female, with length of TL = 45 cm and weight of 1480 g. In February of that year at the Great Sand beach, Bluespotted cornethfish, *Fistularia commersonii*, (Ruppel, 1838), was found (TL = 71 cm, W= 350 g). (Fig 1). Two subsequent repeated findings of this species were recorded in November and December 2013. One specimen was caught in a gillnet called polandara near Tivat in the Boka Kotorska Bay, while second was caught with a spear gun near Budva (Joksimovic, et. al., 2008; Dulčić, et. al., 2014).

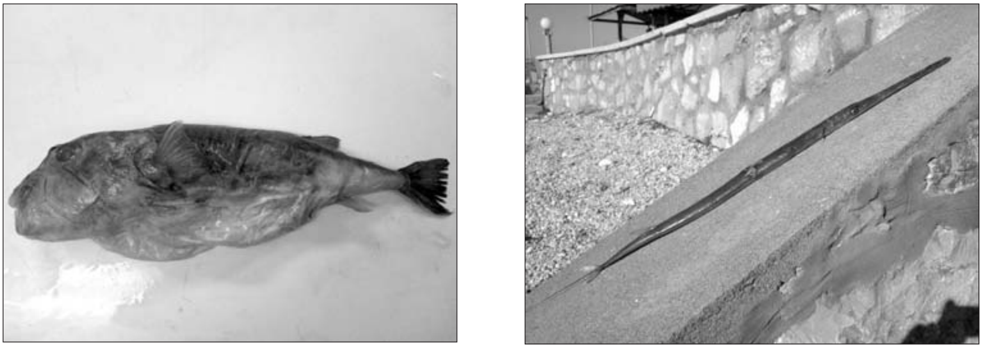

Figure 1. Species *Sphoeroides pachygaster* and *Fistularia commersonii* caught in the Montenegrin coast.

On 9th June 2011 a specimen of *Tylosurus. acus imperialis* (Rafinesque, 1810), Agujon needlefish (Fig. 2) , was caught in front of St. Nikola Island, Budva (Montenegro) in shallow waters (6.5 m depth). The fish was found still alive, entangled in a gillnet for bonito (“polandara”) with a 42 mm diamond mesh size. On 26 June 2014, a specimen of *T. acus imperialis* (Fig. 2) was caught in front of Platamuni, Budva (Montenegro) in shallow waters (6.5 m depth) (Dulčić, et al., 2014b). One specimen of the Dusky spinefoot, *Syganus luridus* (Rüppell, 1829), (TL = 17.4 cm, W = 83.75 g) was captured in Bigova Bay on 7 September 2014 with a trammel net (Fig. 3). In the Adriatic Sea, this species was caught and recorded three times previously, in Piran Bay and Mljet Channel in 2010 and in Konavle in 2011 (Đurović *et. al*., 2014). The first finding of Blue runner, *Caranx crysos* (Mitchill, 1815) in Montenegrin waters was provided by a fisherman from Ulcinj on 1 March 2013 (Dulčić *et al*., 2014a) (Fig. 3.). The new specimen was caught by beach seine called srdelara on 9 December 2013 near settlement Orahovac near Kotor in Boka Kotorska Bay. Considering the information that juveniles and adults have been recorded in the area of the whole eastern Adriatic, it seems that *C. crysos* established its population in the Adriatic (Dulčić *et.al*., 2014b).

Figure 2. Species *Tylosurus acus imperialis*, caught in the Montenegrin coast.

Figure 3. Species *Siganus luridus i Caranx crysos* caught in the Montenegrin coast.

First documented record of the *Callinectes sapidus* (Rathbun, 1896), American blue crab, occurence was in December 2013 in the Boka Kotorska Bay, where two adult male specimens were caught by gillnet called polandara at a depth of 15 m on sandy-mud bottom in Tivat Bay (Kapiris *et al*., 2014), (Figure 4). A single adult female specimen of the Northern brown shrimp, *Farfantepenaeus aztecus*, a species native to the western Atlantic coasts, was caught by a "bukvara" gillnet at a depth of 20-25 m on sandy-mud bottom in Boka Kotorska Bay (Southern Adriatic Sea) on 19 September 2013 (Marković *et al*., 2014). Total length of carapace was 48 mm CL and total length was 200 mm TL.

RESULTS AND DISCUSION

Studies that have been carried out in the last decade have shown that 46 new fish species have been recorded in the Adriatic Sea, so the total number of species in the Adriatic is now above the 450, compared to the previous checklist which listed 407 species (Dulčić and Dragicevic, 2011, Jardas 1996).

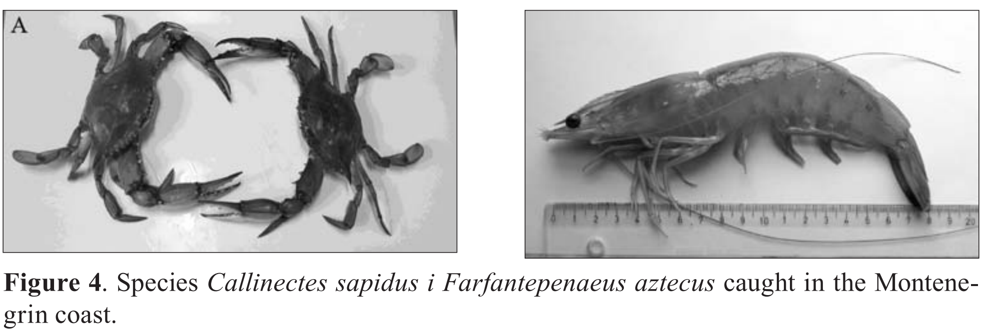

Figure 4. Species *Callinectes sapidus i Farfantepenaeus aztecus* caught in the Montenegrin coast.

Active migrations in the Mediterranean Sea through the Suez Canal make the entrance of species from the Red Sea possible, resulting in a total of 13 Lessepsian migrants species, of which nine in the Adriatic. Current global climate changes have an impact on changes in the marine ecosystem. As a signal of these changes, a spreading of thermophilic species in the northern parts of the Mediterranean was observed, as well as in the Adriatic Sea as its northern part. Expansion of the southern Adriatic species, such as *Pomatomus saltatrix* into the northern part of Adriatic was also observed. Their arrival and occupation of ecological niches, certainly affects the local, indigenous species. These newcomers, in addition to disrupting the established ecological relationships, may also cause socio-economic changes, especially in areas where fishing is a traditional activity. Increased biomass of *P. saltatrix* threatens the catches of mullet species at the mouth of the Neretva River.

The presence of adult males of *C. sapidus* in Boka Kotorska Bay suggests that this species may have established a population like in the neighbouring areas of Albania (Beqiraj & Kashta, 2010) and Croatia (Dulčić *et al*., 2011), but the presence of ovigerous females and juveniles are also necessary to prove this theory, so further research should be undertaken.

This is the first record of *Farfantepenaeus aztecus* in the Adriatic Sea (Olivera Marković *et al*., 2014) but second in the Mediterranean (Deval *et al*. 2010) where few individuals from the Gulf of Antalya were collected. These authors considered ship ballast waters as the most likely vector for its introduction in the Mediterranean. The caught individuals would have entered the spawning period (and the theoretical individuals in the rest of the population might have spawned), and it can be expected that the populations will spread and find their specific niche in the ecosystem of the Adriatic.

CONCLUSIONS

The dynamics of living organisms and complex mutual relationship of nature and living creatures once again demonstrate the unpredictability of the natural processes. Caution is required when attempting to interpret these situations and their relations to the global climate changes, which will undoubtedly influence the life on Earth.

These and similar findings require cooperation of the scientists from all countries of the Adriatic, so that potential new findings would be promptly identified and information regarding them exchanged. It is also necessary to continually inform the fishermen about the species whose spread to the Adriatic is expected in the near future, as they are the first to have the opportunity to come into contact with new species, as was the case with the species discussed.

ACKNOWLEDGEMENTS

The authors would like to thank Mr. Ilija Rafailović, Miko Boljević, Slavko Petroviću, Dejan Đurović, Nikola Lazarević, who provided the alien specimens for study. The authors would like to give special thanks to prof. Bayram Öztürk, University of Istanbul, for his useful comments on the manuscript. This work has been supported by the Ministry of Science of Montenegro and HERIC project trough the BIO-ICT Centre of Excellence (Contract No. 01-1001). Authors also thank to the Ministry of Science of Croatia and Ministry of Science of Montenegro which helped the realization of this research program through bilateral scientific cooperation between the Croatian and Montenegrin part of the project ADRIA.

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ECONOMIC EFFICIENCY OF FILLETING YIELD OF FARMED MEDITERRANEAN MARINE FISH SPECIES

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EKONOMSKA EFIKASNOST FILETIRANOG PRINOSA RIBLJIH VRSTA GAJENIH U MEDITERANU

Apstrakt

Filetirani prinos (FY%) i koeficijent konverzije hrane (FCR) gajenog brancina *(Dicentrarchus labrax);* orade (*Sparus aurata*); kalifornijske pastrmke *(Oncorynchus mykiss)* i hame *(Argyrosomus regius)* ispitani su kod riba gajenih na severo zapadu Grčke. Rezulati predstavljaju procenjenu količinu ribe koje je potrebna da se proizvede da bi se dobio kilogram filetirane ribe. Ova procena je zahtevana u svrhe operativnog menadžmentaza kompanija koje se bave preradom ribe.

Najniži FY i FCR bio je kod hame, dok su više vrednosti zabeležene kod gajene kalifornijske pastrmke. Dobijeni rezultati ukazuju da se FCR, FY i tržišna cena moraju uzimati u obzir za procenu ekonomske efikasnosti gajene ribe. U stvari FY ne mora da utiče na ekonomsku efikasnost proizvodnje hame, vrste koja ima mnogo veću tržišnu vrednost u poređenju sa brancinom, oradom i kalifornijskom pastrmkom

Ključne reči: akvakultura, ekonomija, prinos fileta
Keywords: aquaculture, economics, filleting yield

INTRODUCTION

Somatometric parameters of farmed fish may vary according to endogenous and exogenous parameters including genetic (Taylor and McPhail 1985), food availability (Park et al., 2001; 2007) and overall environmental conditions to which farmed fish are exposed (Sara, et al., 1999). Farmed fish may therefore exhibit between and within species variability in morphological parameters (Sara, et al., 1999; Mairesse et al., 2005). The economics of aquaculture can be influenced by a combination of parameters, nevertheless, feed cost is a major component of total production cost in fish farms and feed conversion rate (FCR) is improved when lees amount of aquaculture feed is required for producing a unit of farmed fish body mass. Furthermore, the edible part of farmed fish constitutes less than 50% of the total body weight of farmed fish. In fact filleting yield (FY%) is much lower in all farmed fish and fillet weight represent the commercial important portion of total body weight of produced fish. For this reason both FCR and FY% are significant economic parameters for aquaculture. The purpose of the present work was to compare filleting yield and FCR of farmed sea bass (*Dicentrarchus labrax*); gilthead sea bream (*Sparus aurata*); rainbow trout (*Oncorynchus mykiss*); and meagre (*Argyrosomus regius*). This was investigated in farmed fish in NW Greece.

MATERIALS AND METHODS

Except trout which was reared in fresh water pond, the other species were reared in sea cages. After harvesting, samples were transferred to the laboratory, where somatometric measurements and filleting took place. Fish were manually gutted, skinned and beheaded before filleting. Data of FCR were collected from the fish farm logs.

RESULTS AND DISCUSSION

The filleting yield and FCR of farmed sea bass, sea bream, rainbow trout and meagre are presented in Table 1. The filleting yield and FCR of meagre was lower from the sea bass and gilthead sea bream, indicating that meagre exhibits improved feed conversion ration but lower percentage of fillets present in the final product. These two parameters have a significant effect on economic parameters of aquaculture. Feed cost represents a major element of the cost of producing farmed fish, frequently reaching levels more than 40% of the total cost. A lower FCR value indicates that less feed is required to produce 1 kg of farmed fish. The economics of aquaculture is even better when a lower FCR is combined with a higher filleting yield, as is the case for farmed meagre when compared to farmed sea bass and gilthead sea bream in the present work. On the contrary, farmed trout exhibited much lower efficiency in converting feed to body mass, nevertheless, trout exhibited higher filleting yields than the other three marine fish species used in the present work.

Table I. Feed conversion ration (FCR) and fillet yield (FY) of farmed Sea bass, Sea bream, Rainbow trout and Meagre (Average values of 25 fish ±SD)

	Sea bass	Sea bream	Rainbow trout	Meagre
FCR	1.18	1.11	1.43	1.08
FY%	42.15(2.19)	44.93(3.62)	51.06(4.18)	39.58(2.94)

The meagre is a potential new species for Mari-culture with very good prospects for rapid growth, low mortality (Mittakos et al 2012) and high nutritional content of lipids (Grigorakis et al 2011), nevertheless, the results indicate that trout is better in producing fillets but sea bream, sea bass and meagre cultivated in sea water cages exhibited better feed conversion ratios than trout reared in fresh water ponds.

CONCLUSIONS

The results indicate that rainbow trout exhibited better filleting yield when compared to farmed sea bream, sea bass and meagre cultivated in sea water cages exhibited better feed conversion ratios than trout reared in fresh water ponds. The results indicate that both FCR and FY should be used in order to evaluate the economic efficiency of farmed fish. Meagre and trout have a much grater market value when both are sold as fillets but the economic consequences of lower FY of farmed meagre may have limited consequences for the economic efficiency of producing this species. In fact, even a lower filleting yield may not influence the economic efficiency of producing meagre which has a much greater market value compared to farmed sea bas, sea bream and rainbow trout.

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AKVARIJUM PULA

Apstrakt

Akvarium Pula je najveći javni akvarijum u Hrvatskoj (u privatnom vlasništvu), otvoren 2002.god. Smešten je u austro-ugarskoj tvrđavi Verudela (zaštićeni spomenik kulture, deo fortifikacijskog prstena oko Pule) na površini većoj od 2000 m^2. Izložba je fokusirana na jadranske i sredozemne vrste, uz veliki morski i slatkovodni dio, ali i slatkovodni hladnovodni. Akvarium trenutno sadrži oko 130 000 litara vode u velikom broju bazena, zapremnine od 50 do 36 000 litara. Naši budući projekti su izgradnja novih bazena na tri nivoa u središnjem dijelu utvđenja i, ukoliko se ostvari saradnja sa firmom *La Rochelle Aquarium and Coutan groupe,* veliki tunelski akvarij u rovu za veće vrste morskih pasa. Od svog otvaranja Aquarium je u procesu konstantnog rasta i razvoja, tako da je od skromnih početaka narastao u respektabilnu instituciju, fokusirajući se ne samo na izlaganje životinja nego i na edukaciju, brigu o morskim kornjačama, istraživanje i razmnožavanje životinja. Do sada smo imali uspjeha s razmnožavanjem morskih konjića, meduza i sipa, s budućim planovima uspostavljanja labosa za razmnožavanje. Centar za oporavak morskih kornjača, prvi ove vrste u Hrvatskoj, počeo je delovati unutar Akvariuma 2005. god., pružajući medicinsku pomoć bolesnim i ozleđenim morskim kornjačama i educirajući javnost o ugroženim vrstama. Rad Centra je prepoznat i u širim okvirima, tako da je Centar deo velikog transnacionalnog projekta koji se bavi morskim kornjačama, kitovima i dupinima (NETCET). Pošto je edukacija važan dio našeg posla, Akvarium Pula nudi inovativne i interesantne edukativne teme kroz program „Plava škola". Većina polaznika su srednjoškolci iz Nemačke i Austrije, a program postaje svake sezone sve popularniji.

Abstract

Aquarium Pula is the biggest and privately owned public aquarium in Croatia, opened in 2002. It is situated in an Austro-Hungarian fortress Verudela (protected historical monument, part of a large fortification ring encircling Pula) on more than 2000 m^2. Exhibition focuses mostly on Adriatic and Mediterranean species with a significant marine and freshwater tropical display, as well as a cold freshwater part. Currently, Aquarium has about 130

000 liters of water in a large number of tanks, sizing from 50 to 36 000 liters. Our future projects are building new tanks on three levels in the central part of the fort and hopefully, in collaboration with *La Rochelle Aquarium and Coutan groupe,* a big tunnel aquarium in the moat for larger shark species. Since its opening the Aquarium is in a state of constant growth and development, so from humble beginnings it has grown to a respectable institution, focusing not only on exhibiting animals but also on education, marine turtle care, research and animal breeding. We have had success in breeding sea horses, jellyfish and cuttlefish, with future plans of setting up a breeding lab. The Marine Turtle Rescue Centre, first of its kind in Croatia, began operating within the Aquarium in 2005, providing medical treatment for sick and injured turtles and raising public awareness on endangered species. The work of the Centre has been widely recognized, so it is a part of a large transnational project concerning the protection of marine turtles and cetaceans (NETCET). With education being an important part of our work, Aquarium Pula offers innovative and interesting educational themes through our „Blue school" program. The majority of participants are high school students from Germany and Austria and the program is becoming more and more popular every season.

FLUCTUATIONS OF THE CATCH OF SOME PELAGIC SPECIES OF THE MEDITERRANEAN SEA

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FLUKTUACIJE ULOVA NEKIH PELAGIČNIH VRSTA RIBA U MEDITERANU

Apstrakt

U radu su analizirani 63 godine dugački podaci o ulovu tune (*Thunnus thynnus*) i srdele (*Sardina pilchardus*) u Mediteranu, metodom spektralne (Fourierove) analize i kros korelacjom. Analiza je pokazala da ulov tune sadrži ciklične komponente od 2.6, 3.65, 5.64, 7.75, 10.34 and 15.5 i verovatno 31 godinu, dok su u ulovu srdele najizraženije bile amplitude od 2.44, 3.65, 6.2, 7.75 i 10.34 godina. Prema tome, oba su vremenska niza bila koherentna u ciklusima od 3.65, 7.75 i 10.34 godina. Takođe je ustanovljeno da je ulov tune koherentan sa indeksom severoatlantske oscilacije (NAO) i to u periodima od 2, 3.35, 4.43, 6.89, 10.34 i verovtno 31 godina. Ovo ukazuje da klimatski ciklusi utiču na fluktuacije populacije ove ribe. Nadalje, upoređenje fluktuacija ulova tune u zapadnom i istočnom Mediteranu je pokazalo da su one potpuno sinhrone.

Ključne reči: Thunnus thynnus, Sardina pilchardus,ulov, fluktuacija, Mediteran
Key words: Thunnus thynnus, Sardina pilchardus, catch, fluctuations, Mediterranean

INTRODUCTION

In the Mediterranean Sea sardine (*Sardina pilchardus*) are one of the most important species in terms of biomass and commercial interest (Palomera et al. 2007). Small pelagic fish have important role in the food web and connecting lower and upper trophic levels, getting the whole ecosystem functioning (Palomera et al. 2007). In general small pelagic populations are subject to considerable fluctuations caused by environmental variability and relatively short life cycle (2–3 years) (Palomera et al. 2007).

Bluefin tuna (*Thunnus thynnus*) was exploited in the Mediterranean Sea since antiquity, but recently it become highly profitable. Their stock is likely overexploited and threatened by the highest fishing pressure of its entire history (Fromentin and Ravier, 2005). Implementation of a Total Allowable Catch – quota (TAC), induced in 2007, increased uncertainties in the catch statistics data and made the standard stock assessment inoperative. Oscillation in the tuna catch recorded from 1950s up to nowadays has been considered by some authors as a change in the migration patterns influenced by environmental and trophic origins in relation to the North Atlantic Oscillation. NAO index is found to be important for the atmospheric circulation in the north Atlantic and Mediterranean (Grbec et al. 2002). The aim of this paper was to analyze existing data on long-term fluctuations of the two pelagic species present in the Mediterranean Sea, and to assess whether there is a relationship between them, and to connect climatic fluctuations to fish-catch fluctuations.

MATERIAL AND METHODS

The fish landing data used in the analysis correspond to the fish caught in the coastal and open seas of Mediterranean. Data on the total annual catches of sardine and bluefin tuna, from 1950 to 2013 (63 years) were taken from the FAO FishStat Plus data basis. Fisheries statistics for the period presented the catch data by species. We decided to analyze just two representatives from the trophic food chain, prey and predator species, both very commercially important and threatened by overexploitation in the recent decades.

Catch statistices of sardine and bluefin tuna from all Mediterranean countries were taken and analysed. Some of the countries gave the aproximated catch data, for example Malta, some countries had very small, not comparative catch data, so we decided to make the analises of the cumulative catch from all Mediterranean countries.

RESULTS

Analysed catches of bluefin tuna and sardine in the period from 1950 to 2013 have similar fluctuations. Bluefin tuna schools migrate each spring in Mediterranean for the spawning and leaves in autumn (Ravier and Fromentin, 2001). According to available statistical data, catch increased from 1950 and reached peak in 1995 when highest bluefin tuna catch was 33975 t. From 2007, when quotas were established catch statistics show downward trend and achives the same values like in 1950s.

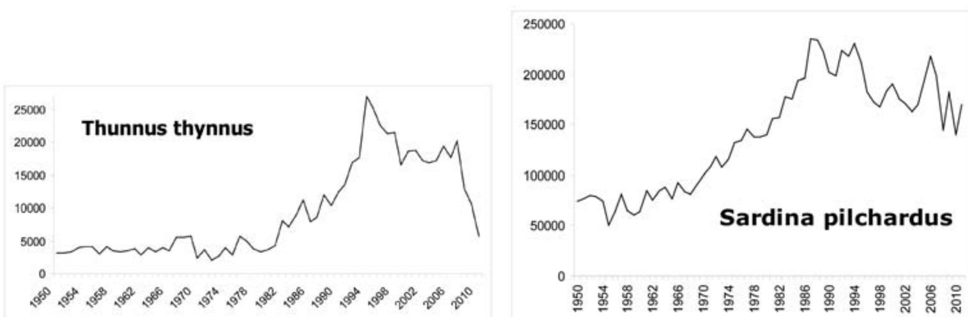


Figure 1. Total bluefin tuna and sardine catch in Mediterranean Sea

On the other side sardine catch, which has no quotas, also increased until 1987, when it reached maximal value of 289317 t. After that time catch gradually decreased, but it still was higher then in 1950s.

Analysis of bluefin tuna catch time series performed in this paper, showed amplitudes at the periods of 2.6, 3.65, 5.64, 7.75, 10.34, 15.5 and probably 31years. Sardine catch showed amplitudes at the periods of 2.44, 3.65, 6.2, 7.75 and 10.34 years. Consequently, both species experienced common periods of 3.65, 7.75 and 10.34 years (Figure 2).

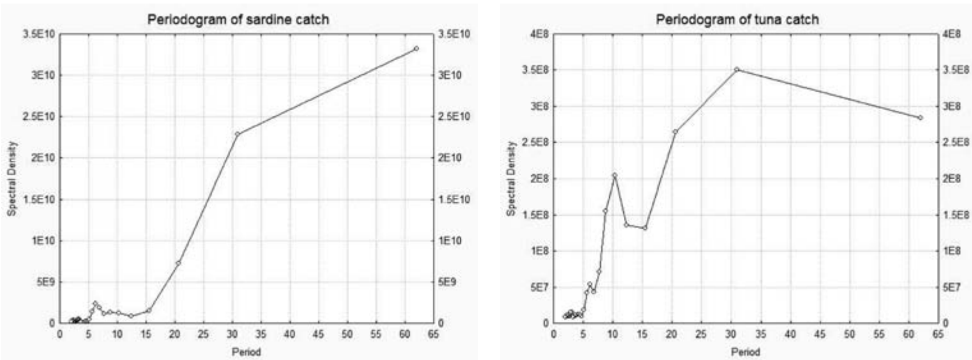


Figure 2. Periodograms of sardine and bluefin tuna catch in Mediterranean Sea

We found that there are no significant differences between West and East Mediterranean catches of both species (Figure 3).

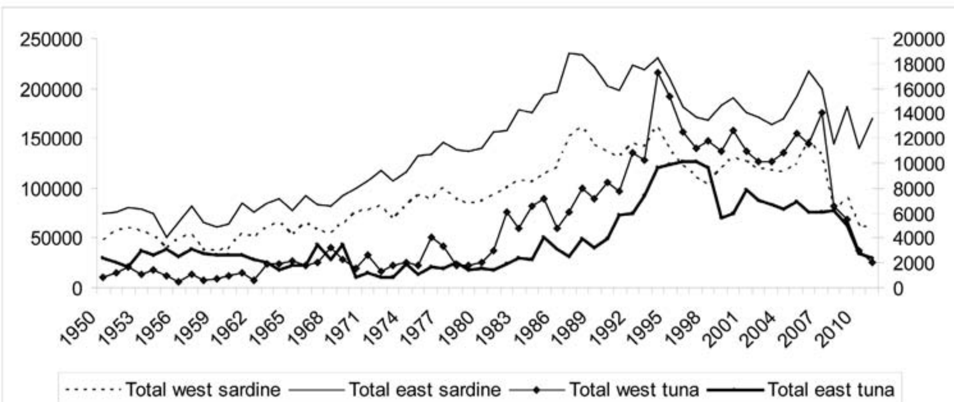
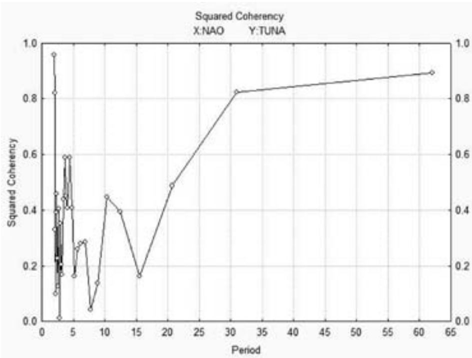


Figure 3. West and East Mediterranean catches of bluefin tuna and sardine species.

The squared coherency analysis showed that NAO and bluefin tuna catch were coherent at the periods of 2, 3.35, 4.43, 6.89, 10.34 and probably 31 years (Figure.4a), which confirms their functional relationship, while for sardine it is already known that it is related with NAO (Grbec et al. 2002). We also found that fluctuation of bluefin tuna catch follows NAO fluctuations with the four years delay (Figure. 4b).



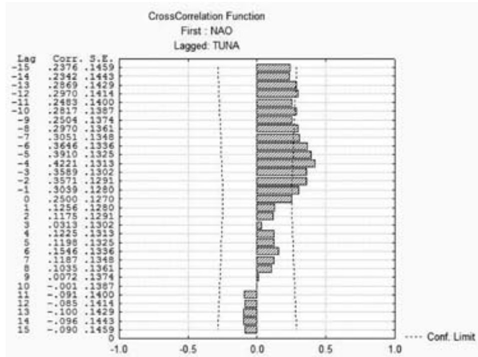


Figure 4. a) Squared coherency between bluefin tuna catch and NAO, b) Crosscorrelation function between bluefin tuna and NAO

DISCUSSION

Fluctuations of fish catch are the result of cumulative effects. In addition to cyclical natural processes in the environment, anthropogenic factors significantly affect environmental characteristics and catch. Fishing commonly changes the relative abundance of fish species but may also change the structure and functioning of the ecosystem (Cury et al. 2000).

The decline in the catches is suspected to be primarily due to under-reporting, following the implementation of quotas particularly for the tuna species.

The quality of catch statistics depends on the methodology of collecting data, which affects their reliability (Welcomme et al. 2010; Baigún et al. 2013).

The dynamics of pelagic fish species is strongly influenced by environmental factors, determining food availability both in time and space for larvae and juveniles (Grbec et al. 2002). Fluctuation of the fish resources in Mediterranean are influenced by the geostrophic current, front system which brings nutrients to Mediterranean Sea. These fronts are usually characterised by high levels of biological activity, and particulary, of primary production. Geostrophic fronts exhibit complex current and hydrological structures (Videau et al. 1994).

There is evidence about connection between the hydroclimate variables and pelagic species. Grbec et al. (2002) compared year-to-year fluctuations of small pelagic fish landings in the eastern Adriatic coast with climatic fluctuations over the Northern Hemisphere and salinity fluctuations in the Adriatic. They found interrelation between climatic fluctuations over the Northern Hemisphere and small pelagic fish landing data. Years with positive pressure differences, which are years of higher salinity, cause the increase of phytoplankton productivity. Strong correlation of the species landing data to the pressure difference could also be due to the connection of fish to plankton productivity.

We found that catch of bluefin tuna and sardine had the same periods of 3.65, 7.75 and 10.34 years, which coincide with some of the coherent periods for bluefin tuna and NAO.

ACKNOWLEDGMENT

Funded by the Ministry of Education, Science and Technological Development of the Republic of Serbia, within the Project No. TR37009 and No. OI173045.

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SIZE STRUCTURE OF THE TURBOT (*PSETTA MAXIMA MAEOTICA)* LANDINGS FROM THE NORTH BULGARIAN BLACK SEA COAST

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DUŽINSKA I TEŽINSKA STRUKTURA IVERKA (*PSETTA MAXIMA MAEOTICA)* IZLOVLJENOG DUŽ SEVERNE OBALE BUGARSKOG DELA CRNOG MORA

Apstrakt

Iverak (*Psetta maxima maeotica*) je demerzalna vrsta ribe koja se može naći na sprudovima duž obala svih zemalja koje okružuju Crno more. Iverak predstavlja veoma važnu demerzalnu vrstu ribe u Crnom moru, sa velikom potražnjom na tržištu i visokom cenom. Uprkos merama koje su preduzete za zaštitu populacija iverka, uočen je trend opadanja u proračunu relativne biomase ove vrste od 2008. godine na prostoru bugarskog dela Crnog mora. Zbog nepostojanja informacija koje se odnose na validne ribarske statistike i dostupne biološke podatake, ova istraživanja na ulovima iverka duž bugarske obale Crnog mora su imala za cilj sakupljanje bioloških podataka za analizu njegovog ulova. Težinska (W, g) struktura istraživanih iveraka (600 jedinki) iz 20 ulova pokazala je da je prosečna težina ribe iznosila 2.63 kg, sa maksimalno zabeleženom vrednošću od 7.0 kg i minimalnom od 1.25 kg. Najveći procenat u težinskoj strukturi izlovovljenih jedinki imala je težinska grupa od 2.0 do 3.0 kg (44%), a zatim težinska grupa od 1.25 do 2.0 kg (31%). Grupa od 3.0 to 4.0 kg je činila 16%, grupa od 4.0 do 5.0 kg je činila 6%, grupa od 5.0 do 6.0 kg je činila 2% i grupa od 6.0 do 7.0 kg je činila samo 1% od ukupnog izlova. Iverak težine od 1.5 do 2.5 kg činio je 56% od svih izmerenih jedinki. Prosečna totalna dužina (TL, cm) merenih riba je bila 52.8 cm, sa maksimalnom vrednošću od 77.0 cm i minimalnom od 45 cm. U okviru svih dužinskih grupa najbrojnije su bile dužinske grupe od: 45-48 cm sa 24,6%, 48-51 cm sa 23.3% i 51-54 cm sa 20.1% od celokupnog uzorka. Ribe sa totalnom dužinom od 45 cm, koja je minimalna mera dozvoljena za izlov, su činile samo 2.8%, ali su jedinke u okviru dužinske grupe od 45-48 cm obuhvatile jednu četvrtinu uzorka. Generalno, analiza podataka dobijena na osnovu 600 iveraka sa prosečnom težinom od 2.63 kg ili totalnom biomasom od 1 578 kg dovela je do pretpostavke da se značajni deo ulova sastoji od riba sa relativno malom težinom tela. Buduće monitoring studije će podržati ili odbaciti ovu pretpostavku.

Ključne reči: *iverak, Psetta maxima maeotica, Crno more, prijavljeni ulov, dužinska i težinska struktura*
Keywords: *turbot, Psetta maxima maeotica, Black sea, landings, size structure*

INTRODUCTION

Turbot (*Psetta maxima maeotica*) is a demersal species and occurs all over the shelf area of all Black Sea coastal states at depths up to 100 m -140 m, grouped in local shoals. It has a life span of 10-12 years and reaches up to 87 cm in length and up to 15 kg in weight (Karapetkova, Zivkov 2006). The spawning process takes place in spring season between April and June. Species inhabits sandy or silt bottoms, mussel beds or mixed types. Turbot in the Black Sea is represented by several local populations, which migrate and mix in the adjacent zones. The fish is not a highly migratory species but relocates seasonally towards the coast and offshore related to reproduction, feeding and wintering.

Turbot is the one of the most important demersal fish species in the Black Sea with high market demand and prices. Higher turbot catches are registered in spring and autumn periods. Main fishing gear for turbot in Bulgaria are gillnets. According to Art. 35 of the Bulgarian Law on Fisheries and Aquaculture (LFA) the use of bottom trawling and dredging means is prohibited since 1984. The minimum legal mesh size for bottom-set nets used in turbot fisheries is 400 mm (Art. 11 of the Council Commission Regulation 850/98), and the minimum allowable total length at landing is 45 cm (Annex XII of Council Regulation 850/98 and Annex II of the LFA). Not allowed is the catch, retain on board, trans-bordering, landing, first sale and transport of turbot during the reproduction period between April 15 and June 15. Turbot quotas are introduced since 2004 in national waters and after 2008 in community waters, regarding to Council Regulations (EC, 2008 - 2013).

Despite the measures taken to protect turbot populations a decreasing trend in the estimated relative biomass of turbot since 2008 is observed in the Bulgarian Black Sea area. Due to existing gaps of information in terms of accurate fisheries statistics and availability of biological data, the present study on turbot catches at the Bulgarian Black Sea shore aims to collect biological data for analysis of catches and for buildup of a database to follow-up of the structure of catches over the years. The main tasks of the study included measurements of body weight, total length of turbots from landing operations in order to determine the size structure of catches and to analyze the results.

MATERIALS AND METHODS

The data collection was performed in December 2014 in the northern Bulgarian Black Sea aquatory. From ports where landings of turbot are permitted, the Kavarna, Balchik and Varna ports were used for biological data collection. Out of 156 ships authorized for catch of turbots in 2014, 20 vessels (and 20 catches*)* were used for collection of biological data. The total number of fish used for biological data collection was 600. The catches were from the aquatory between latitudes 43^0 12’ N and 43^0 45’ N and longitudes 28^0 19’ E and $29^0$11’

E. The depth of places where fish were caught varied from 60 to 67 m. For the 8 vessels, no data for the places of catch and their depth were available, but they were similar to aforementioned ones. The measurements of fish were made on the board of ship immediately after docking of ships at the port, on fresh ice-cooled subjects. The weight measurement was done with a precision of 1 g, while that of total lengths – with a precision of 0.1 cm. The data processing, graphs and tables were elaborated by means of statistical software.

RESULTS AND DISCUSSION

Number of fish caught from each ship

The number of fish caught from each vessel and their percentage distribution is presented on Fig. 1 and Fig. 2**.** Thirty turbots were caught from each ship on the average, with maximum number of 86 and minimum number of 5 fish. Eight vessels or 40% of all 20 have caught between 10 and 20 fish, 4 ships (20%) between 30 and 40 fish, and 2 ships (10%) between 40 and 50 fish. Catches with less than 5 turbots, 70-80 and 80-90 turbots were each registered in one vessel (Fig 1.).

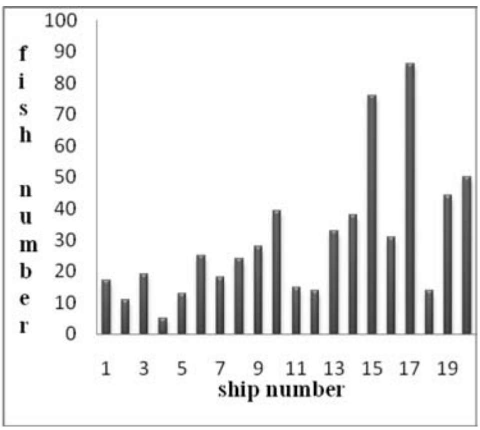


Figure 1. Number of fish caught at each ship

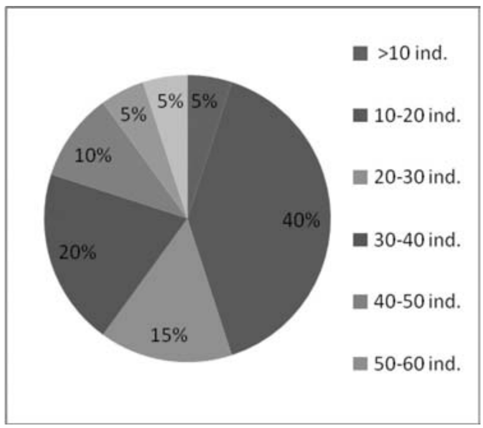


Figure 2. Percentage distribution of catches from each of ship, %

Weight structure of catches

The average, maximum and minimum weights of the landings of turbots are depicted on Fig. 3. The average weight of measured turbots was 2.63 kg, and the maximum-minimum range: 7.0-1.25 kg.

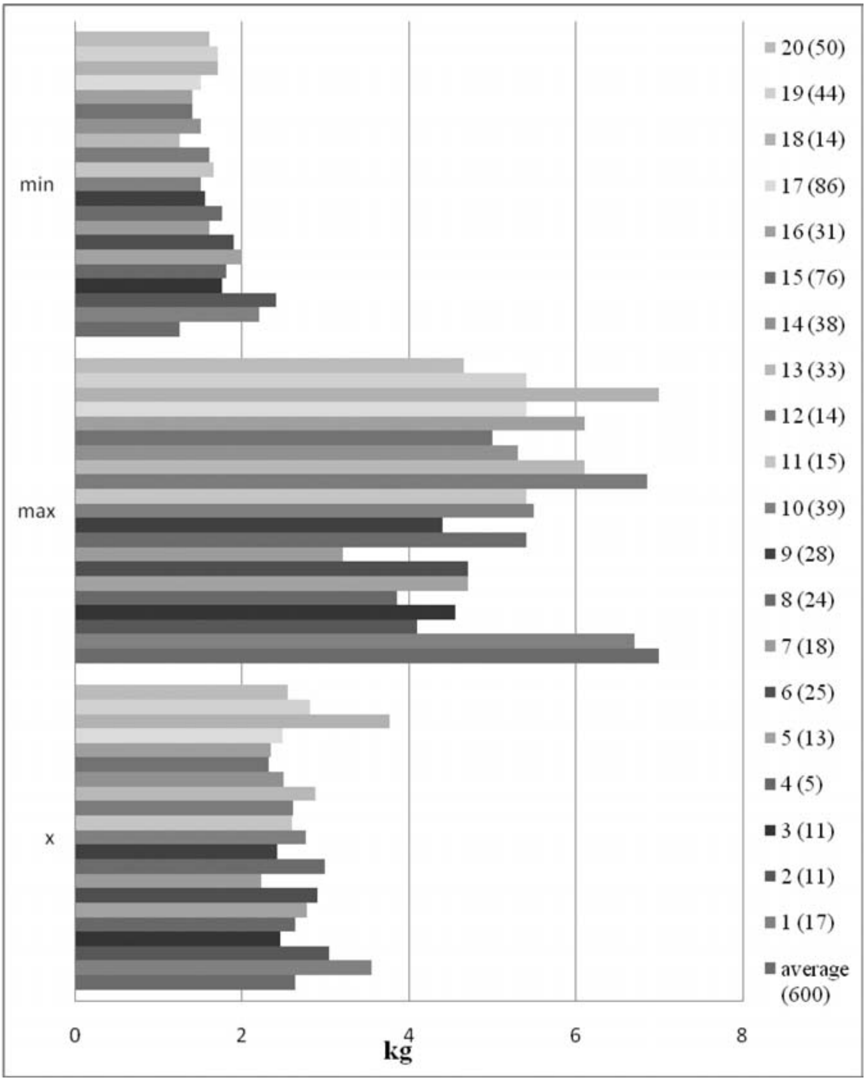


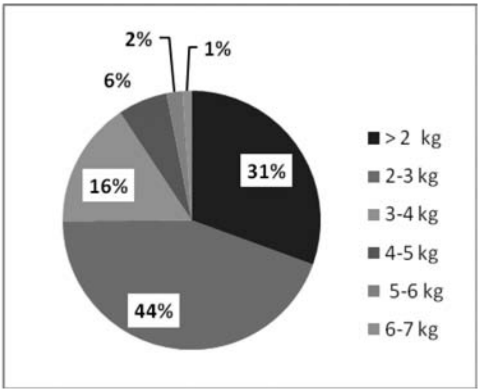
Figure 3. Minimum and maximum weights (W, kg) of turbots from 20 landings.

The number of fish from each ship is given in brackets.

Average weights of turbots from the different catches varied between 3.8 and 2.21 kg, maximum weights – between 7.0 and 3.2 kg, and minimum ones between 2.4 and 1.25 kg.

Figure 4 acquaints with the percentage distribution of the different weight groups for all 600 weighed turbots. The highest share was that of the weight group from 2.0 to 3.0 kg (44%), followed by the weight group from 1.25 to 2.0 kg (31%). The share of the weight group between 3.0 and 4.0 kg , 4.0 to 5.0 kg, 5.0 to 6.0 kg, and 6.0 to 7.0 kg was 16%, 6%, 2% and 1%, respectively.

The graph shows that turbots weighing between 1.25 and 3.0 kg comprised 75% of all weighed fish.



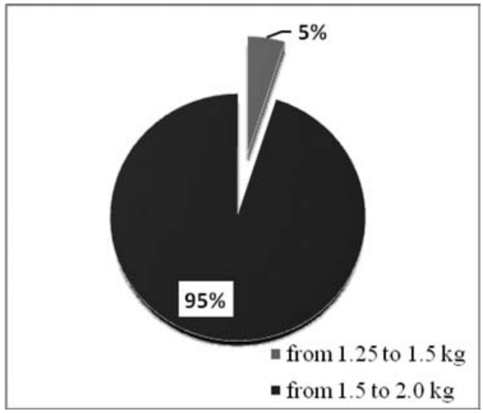


Figure 4. Proportions of the different weight groups of turbots.
Figure 5. Proportions of fish within the weight group from 1.25 to 2.0 kg

When the first weight group (from 1.25 to 2 kg) was divided into two subgroups: from 1.25 to 1.5 kg and from 1.5 to 2.0 kg, it becomes obvious that only 5% of turbots weighed from 1.25 to 1.5 kg, while those weighing between 1.5 and 2.0 kg were 95% (Fig. 5).

The division of the second weight group (2.0 to 3.0 kg) into subgroups of 2.0-2.5 kg and 2.5-3.0 kg demonstrates that the major part of fish (59%) weighed between 2.0 and 2.5 kg. Summing up the results from the analysis of the first two weight groups, it could be seen that turbots with body weight from 1.5 to 2.5 kg were more than one half (56 %) of the entire group of 600 fish.

The average, minimum and maximum total body lengths (TL, cm) of turbots from the landings are presented on Fig. 6. The average total length of measured fish was 52.8 cm, the maximum was 77.0 cm, while the minimum was 45 cm. The average total lengths of fish varied from 60.6 and 51.1 cm among the catches, the maximum was between 77.0 and 56.0 cm, and the minimum between 50.5 and 45.0 cm.

The shares of the different size groups (at 3 cm-intervals) showed that three size groups were the most frequently encountered: 45-48 cm, 48-51 cm and 51-54 cm (Fig. 7).

Turbots with total body length from 45 to 54 cm comprised 68% of the entire sample, whereas those with TL 45-51 cm were almost one half from all measured fish (47%). Fish with total body length of 45 cm, which is the minimum allowance for catch, were only 2.8% of all turbots, but one-quarter of all studied fish had TL within the range 45-48 cm.

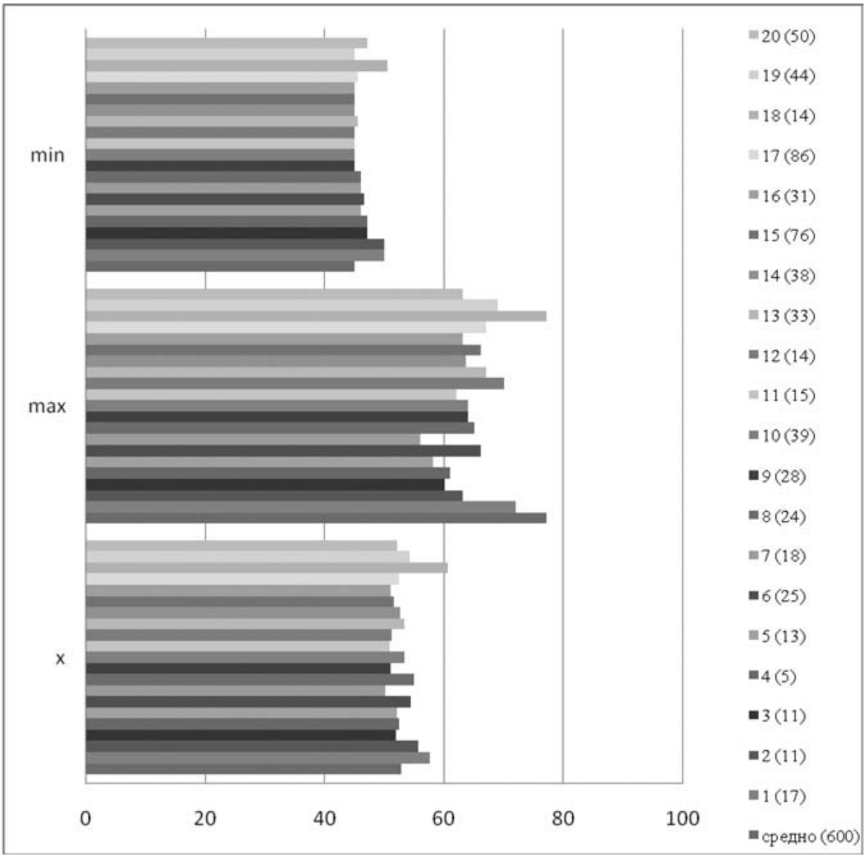


Figure 6. Average, minimum and maximum total body lenghts (TL, cm) of turbots from 20 unloadings. The number of fish from each ship is given in brackets

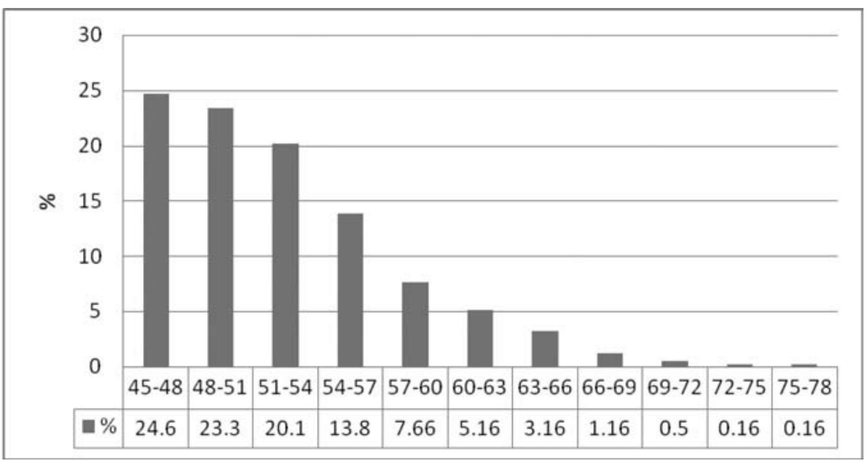


Figure 7. Proportions of the different body size groups (TL, cm) at 3 cm-intervals of turbots from 20 landings, December 2014.

CONCLUSIONS AND RECOMMENDATIONS

In general, the analysis of data obtained from 600 turbots with average weight of 2.63 kg or total biomass of 1 578 kg allowed assuming that a substantial part of catches consisted of fish with relatively low weight. Future monitoring studies would support or reject this suggestion. According to the EC legislation, the mesh size for turbot nets must be at least 400 mm, which, according to professional opinions in the branch should prevent catching specimens weighing less than 2.3-2.5 kg. The results from the present study showed that turbots weighing from 1.25 to 2.0 kg were one-third (31%) of the entire sample whereas those weighing from 1.5 to 2.5 kg more than half of the sample (56%). We suggest that the low weight of turbots with body size around the minimum allowed (45 cm) could be due to the poorer body condition of fish during the study period, as also supported by the results from turbot meat biochemical analysis. We recommend continuation of the biological monitoring on turbot landings at the Bulgarian Black Sea shore in the future in order to obtain a more complete and more objective image of catch structure over the years, which is a parameter for the population structure of the species. Spring catches could be also included in the analysis, as they are considerably bigger than those in the autumn. This would permit to perform a comparative analysis of data from both periods.

ACKNOWLEDGEMENTS

The present study was conducted owing to the financial support from the European Commission under Council Regulation (EC) No.199/2008 establishing a community framework for the collection, management and use of data in the fisheries sector and support for scientific advice regarding the common fisheries policy. The survey was carried out in execution of National Programs of Bulgaria for data collection in 2014.

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INHIBITION OF LIPID OXIDATION IN ANCHOVY OIL BY GRAPEFRUIT ALBEDO EXTRACT

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INHIBICIJA OKSIDACIJE LIPIDA U ULJU INĆUNA POMOĆU ALBEDO EKSTRAKTA IZ GREJPFRUTA

Apstrakt

Ispitan je efekat albedo ekstrakata iz kore grejpfruta na oksidaciju lipida ribe. Ekstrahovano ulje inćuna (*Engrailus engrasicolus*) i albedo ekstrakt iz grejpfruta(*Citrus paradisi*) korišćeni su kao materijal u toku istraživanja.Ekstrakt albedo fragmenata grejpfruta su dodati lipidima ribe u koncentracijama od 0,5, 1,0 i 2,0 mg/g. Lipidi ribe bez dodatog ekstrakta korišćeni su kao kontrolna grupa. Uzorci su čuvani na 25°C i analizirani su u nedeljnim intervalima. Vrednosti za sadržaj fenolna i antioksidativnu aktivnost koje su ustanovljene u albedo fragmentima grejpfruta iznosile su 6,61 ± 0.01g GAE / 100 g i 0.170 ± 0.014 µM troloksa, respektivno. Tretmani sa albedo ekstraktima iz kore grejpfruta dali su uspešne rezultate u suzbijanju oksidacije lipida ribe. Tokom skladištenja, TBA, para-anizidin i peroksidne vrednosti u uzorcima ekstrakta bile su niže od istih vrednosti u kontrolnim uzorcima. Najveće vrednosti UV spektara su uočene u kontrolnim uzorcima na kraju perioda skladištenja. U sprečavanju oksidacije lipida najveća koncentracija ekstrakta (2,0 mg/g) u tretmanu bilaje efikasnija od ostalih koncentracija.Kao zaključak, albedo ekstrakt iz grejpfruta može da se koristi kao prirodni antioksidans, a sporedni proizvodi koji se dobijaju iz kore mogu da imaju ekonomski značaj bez ugrožavanja životne sredine.

Ključne reči: oksidacija lipida, albedo grejpfrut, inćunovo ulje

Abstract

The effect of grapefruit peel albedo extracts on the oxidation of fish lipids was investigated. Extracted anchovy *(Engrailus engrasicolus)* oil and grapefruit *(Citrus paradisi)* albedo extract was used as research material.

The extract of albedo fragments of grapefruit were added into the fish lipid in the concentrations of 0.5, 1.0 and 2.0 mg/g. The fish lipid without extract was regarded as control group. The samples were stored at 25°C and analyses were performed on weekly intervals. The phenolic content and antioxidant activity determined in albedo fragments of grapefruit were 6.61±0.01g GAE/100 g and 0.170±0.014 µM trolox, respectively. Grapefruit peel albedo extract treatments has successful results in suppression of fish lipid oxidation. TBA, para-anisidine, and peroxide values of extract samples were lower than control samples during the storage. The highest UV spectrum values were observed in control samples at the end of the storage period. The highest extract concentration (2.0 mg/g) treatment was more effective than the other concentrations in hinderinglipid oxidation. In conclusion, the extract of grapefruit albedo extracts can be used as a natural antioxidant and the by-products of the peels can be evaluated economically without giving harm to the environment.

Keywords: lipid oxidation, grapefruit albedo, anchovy oil

EFFECTS OF HIGH PRESSURE PROCESSING ON MICROBIOLOGICAL QUALITY OF HERRING (*CLUPEA HARENGUS*)

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UTICAJ VISOKOG PRITISKA PRERADE NA MIKROBIOLOŠKI KVALITET HARINGE (*CLUPEA HARENGUS*)

Apstrakt

Vrste riba obezbeđuju važne komponente za ljudsku ishranu, ali se kvalitet ribe rapidno pogoršava post-mortem, osim ukoliko riba nije podvrgnuta odgovarajućem tretmanu. Uglavnom, kao posledica bakterijskog metabolizma, kvar ribe se ispoljava nastajanjem neprijatnog mirisa i ukusa. Upotreba temperature hlađenja predstavlja koristno sredstvo da se postignu niže stope rasta mikroorganizama, ali za kontrolu mikrobiološkog kvara kod proizvoda od plodova mora taj blagi prostupak nije dovoljan. Sa povećanjem potražnje za minimalno prerađenom hranom, netermička obrada je postala alternativa konvencionalnim metodama. Obrada visokoim pritiskom (HPP, High Pressure Processing) je jedna od novijih tehnologija koja predstavlja hladnu sterilizaciju za očuvanje hrane. Upotreba HPP tehnologije u preradi hrane je od velikog značaja zbog svoje sposobnosti da na niskoj temperaturi inaktivira mikroorganizme koji se prenose hranom i enzime, sa manje promena u teksturi, boji i aromi proizvoda u poređenju sa konvencionalnim tehnologijama. Cilj ovog rada je bio da se procijeni uticaj prerade pod visokim pritiskom (100, 200, 300, 500 i 600 MPa tokom 5 minuta) na mikrobiološki kvalitet haringe upakovane u vakuumu toku skladištenja na 4 ˚C u trajanju od 21 dan. Posle tretmana pritiskom nije bilo značajnih razlika između kontrolne, 100 MPa i 200 MPa pritiskom tretiranih grupa, u odnosu na ukupne psihrofilne bakterija, ukupne mezofilne bakterija i ukupni broj gljivica i plesni. Na početku skladištenja, populacija ukupnih psihrofilnih bakterije i ukupnih mezofilnih bakterija bila je 3.41 Log CFU / g, 3.04 log CFU / g, 3,17 Log CFU / g, 3.02 log cfu / g u kontrolnoj i grupi tretiranoj pod pritiskom od 100 MPa, respektivno. Populacija ukupnih psihrofilnih bakterije i ukupnih mezofilnih bakterija se povećavala tokom perioda skladištenja u kontrolnoj, 100 MPa i 200 MPa pritiskom tretirnim grupama, respektivno. Tokom skladištenja, ukupne *Enterobacteriaceae* su imale najveće vrednosti u kontrolnoj i grupi tretiranoj sa pritiskom od 100 MPa. Za vreme skladištenja, značajno manje populacije bakterija su ustanovljene kod 300 MPa i 500 MPa pritiskom tretiranih grupa, dok rast bakterija uopšte nije ustanovljen u grupi tretiranoj sa pritiskom od 600 MPa.

Ključne reči: obrada pod visokim pritiskom, mikrobiološki kvalitet, haringa

Abstract

Fish species provide important components to human nutrition,but deteriorate rapidly post-mortem unless subjected to an appropriatetreatment.Mostly,spoilage is as a result of the production of off-odours and off-flavours caused by bacterial metabolism. The use of refrigerated temperatures represents a useful mean to achieve lower rates of microbial growth, but it is not a sufficient mild procedure to control the microbial spoilage in seafood product. With the increasing demand for minimally processed foods, nonthermal processing has become an alternative to conventional methods. High pressure processing (HPP) is one of the recent technologies which represent cold sterilisation for food preservation. The use ofHPP in food processing is of great interest because of its ability toinactivate food borne microorganisms and enzymes, at low temperaturewith fewer changes in texture, colour and flavour of the product as compared to conventional technologiesThe objective of this study was to evaluate the influence of high pressure processing (100, 200, 300, 500 and 600 MPa for 5 min) on the microbiological qualityof vacuum-packaged herring during storage at 4^{o}C for 21 days. There were no significant differences between control, 100 MPa and 200 MPa pressure treated groups in terms of total psychrophilic bacteria, total mesophilic bacteria and total yeast and mold after pressure treatment. At the beginning of storage,total psychrophilic bacteria and total mesophilic bacteria populations were 3.41 log cfu/g,3.04 log cfu/g and 3.17 log cfu/g, 3.02 log cfu/g in control and 100 MPa pressure treated groups, respectively. Total psychrophilic bacteria and total mesophilic bacteria populations increased during the storage period in control, 100 MPa and 200 MPa treated groups, respectively. Total Enterobacteriaceae showed the highest values in control and 100 MPa pressure treated groupthroughout the storage. Significantly lower bacteria populations were observed in 300 MPa and500 MPa pressure treated groups, while no growing was determined in 600 MPa treated groupduring storage time.

Keywords: high pressure processing, microbiological quality, herring

EFFECTS OF DIETARY ALLSPICE, *PIMENTA DIOICA* POWDER ON HEMATOLOGICAL AND IMMUNOLOGICAL RESPONSES OF *OREOCHROMIS MOSSAMBICUS* UNDER LOW pH STRESS

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EFEKTI PIMENTA *(PIMENTA DIOICA)* U PRAHU U HRANI NA HEMATOLOŠKE I IMUNOLOŠKE REAKCIJE KOD MOZANBIČKE TILAPIJE *(OREOCHROMIS MOSSAMBICUS)* IZLOŽENE STRESU NISKOG pH

Apstrakt

U spoljnjim sistemima gajenja u akvakulturi, ribe su često izložene fizičkim i hemijskim izvorima stresa kao što je kolebanje nivoa kiseonika u vodi, temperature i pH vrednosti. Brzo kolebanje pH vrednosti često može da bude veći problem kod riba nego pojedinačne pH vrednosti (Roberts and Palmerio, 2008). Tilapija je manje tolerantna na promenu pH vrednosti i može da razvije fiziološke promene prilikom prebacivanje iz vode neutralnog pH u kiselu sredinu (El-Sayed, 2006). Visoka ili niska pH vrednost vode može da dovede do promena u ponašanju, ošteti epitelne ćelije škrga, smanji efikasnost ekskrecije azota i poveća smrtnost Nilske i Mozanbičke tilapije (Yada and Ito, 1997).

U ovom eksperimentu korišćena je zdrava gajena Mozanbička tilapija *O. mossambicus* (prosečne težine ± SD = 16.05±0.08 g). Obrok od semena pimenta (*P. dioica*) dobijen je od Kotanyi, GmbH, (Istanbul, Turska). Ovaj obrok dodavan je hrani za ribe u količini 0. 5, 10, 15 i 20 g/kg. Eksperiment je rađen u triplikatu za svaki od ovih hrana. Petnaest akvarijuma (80 L) nasađeno je sa 270 riba (18 jedinki po akvarijumu). Riba iz eksperimenta hranjena je do vidljivog zasićenja dva puta dnevno u toku 60 dana. Riba nije hranjena 24 sata pre izlaganja stresu. Sve grupe riba bile su izložene stresu. Stres niskog pH postignut je izlaganjem riba vodi pH 5.5 u periodu od 3 dana.

Stresni uslovi značajno su smanjili hematološke i imunološke parametre kod riba koje su hranjenje kontrolnom hranom, osim one koje je bila obogaćena sa 5 –20 g/kg pimenta.

Rezultati ovog istraživanja pokazuju da dodatak pimenta u ishrani od 10 g/kg u trajanju od 60 dana ima pozitivne efekte na poboljšanje nekih hemato-imunoloških parametara kod Mozanbičke tilapije posle izlaganja stresnim uslovima kiselosti. Slične rezultate su objavili i Nayak i Abhilash (2008).

INTRODUCTION

In an outdoor aquaculture system, fish are usually exposed to physical and chemical stressors, such as fluctuations in water oxygen, temperature and pH. Rapid fluctuations in pH are generally more problematic for fish than specific individual pH values (Roberts and Palmerio, 2008). Tilapia are less tolerant to water pH and may develop physiological changes following transfer from neutral water to acidic water (El-Sayed, 2006). Low or high water pH may lead to behavioural changes, damage of gill epithelial cells, reduction in the efficiency of nitrogenous excretion and increased mortality of *Oreochromis niloticus* and *O. mossambicus* (Yada and Ito, 1997).

MATERIAL AND METHODS

Healthy cultured *O. mossambicus* (mean weight ± SD = 16.05±0.08 g) were used in experiment. Allspice (*P. dioica*) seed meal was obtained from Kotanyi, GmbH, (Istanbul, Turkey). It was added to the feed at a rate of 0, 5, 10, 15 and 20 g/kg. The experiment was designed in triplicate for each diet. Fifteen 80–L aquarium were stocked with 270 fish (18 fish/aquarium). The experimental fish were fed to apparent satiation twice a day for 60 days. Fish were not fed for 24 h before exposing them to stress. All group of fish were subjected to stress. An acidic stress was achieved by exposing the sampled fish to acidic water (pH 5.5) for 3 days.

RESULTS

The stressful condition significantly decreased hematological and immunological paramaters in fish that were fed the control diet, except diets that had been supplemented with 5–20 g/kg allspice.

DISCUSSION

The results of the present study demonstrated that supplementation of allspice at 10 g/kg for 60 days, has adequate beneficial effects on improvement of some hemato-immunological parameters of *O. mossambicus* after acidic stress. Similar results were given by Nayak and Abhilash (2008).

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GENETIC VARIABILITY OF AN INVASIVE FRESHWATER FISH, GIBEL CARP *CARASSIUS GIBELIO* (BLOCH, 1782) IN TURKEY REVEALED BY SEQUENCES OF MITOCHONDRIAL CYTOCHROME OXIDASE I GENE

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GENETSKE VARIJACIJE KOD INVAZIVNE SLATKOVODNE VRSTE, BABUŠKE *CARASSIUS GIBELIO* (BLOCH, 1782) U TURSKOJ OTKRIVENO SEKVENCIONIRANJEM GENA MITOHONDRIJALNE CITOHROM OKSIDAZE I

Apstrakt

Invazivne vrste riba veoma lako postaju dominantne vrste u slakim vodama i mogu da negativno utiču na stanište (Paulovits et al., 1998). Jedna od najpoznatijih ne nativnih slatkovodnih vrsta u Turskoj je babuška *Carassius gibelio* (Bloch, 1782). Molekularne analize nam daju značajne informacije za bolje razumevanje činjenica vezanih za rastuću bioinvaziju. Ove molekularne analize izvršene su poređenjem genetskih varijacija vrsta (Doğaç et al., 2015). U ovom istraživanju, naš cilj je da ispitamo genetske varijacije babuške u Turskoj, pomoću mitohondrijalnog gena citohrom oksidaze I (COI).

Uzorci riba izlovljeni su elektroribolovom iz svih krajeva Turske. Tkivo mišića korišćeno je za ekstrakciju DNK. COI amplifikacije izvršene su sa dva para prajmera (Ward et al., 2005). PCR reakcije postavljane su u zapremini od 25 µl, gde je svaka sadržala 2.5 µl 10X Taq Buffer sa KCl (100 mM Tris-HCl, 500 mM KCl, pH 8.8), 2µl MgCl2 (25mM), 0.5 µl dNTPs (10 mM), 0.5 µl svakog prajmera (10 pM/µl), 2 U of Taq polimeraze (5U/µl) i 2 µl DNK (50 ng/µl). Amplifikacije su izvršene u 'termosajkleru'u sledećim uslovima (Keskin and Atar, 2012): preliminarna denaturacija na 95°C u trajanju od 2 minuta, zatim 35 ciklusa koji se sastoje od denaturacije na 95°C u trajanju od 30 sekundi, prvo žarenje i hlađenje na 55°C u trajanju od 30 sekundi, prva ekstenzija na 72°C u trajanju od jednog minuta. Ceo proces završen je krajnjom ekstenzijom na 72°C u trajanju od 10 minuta. Analiza podataka izvršena je sa MEGA 5.0 (Tamura et al., 2011), DNKSP 5.0 (Librado & Rozas, 2009) i Network 4.6 (Bandelt et al., 1999).

Tri haplotipa pronađena su među 220 sekvenci i jedan od tih haplotipa je jedinstven za Tursku. Diverzitet haplotipa bio je 0.27. Diverzitet nukleotida procenjen je kao 0.009.

Mreža haplotipa je pokazala da su haplotipi Turske baubuške blisko povezani sa Japanskom polpulacijom.

Genetska varijacija populacije babuške u Turskoj je bila niska, ali nivoi genetskog struktuiranja sa novim jedinstvenim vrstama su bili visoki. Rezultati ovod istraživanja pokazuju da je babuška izmeštena u Tursku iz svojih nativnih i ne nativnih krajeva.

Ključne reči: genetske varijacije, invazivne vrste, haplotipovi, babuška

INTRODUCTION

Invasive fishes quite become one of the predominant species in freshwaters and may affect the habitat in a negative way (Paulovits et al., 1998). One of the most prominent non-native freshwater fish species in Turkey is gibel carp *Carassius gibelio* (Bloch, 1782). Molecular analyses provide beneficial information for a better comprehension of the facts required for a thriving bioinvasion by comparing genetic variation of a species (Doğaç et al., 2015). In this study, we aim to investigate genetic variation of gibel carp from Turkey, using mitochondrial cytochrome oxidase I (COI) gene.

MATERIAL AND METHODS

Fish samples were collected by electrofishing from all parts of Turkey. Muscle tissues used in DNA extraction. Amplifications of COI were carried out using two primer pairs (Ward et al., 2005). PCR reactions were set up in 25 μl volumes, each containing: 2.5 μl of 10X Taq Buffer with KCl (100 mM Tris-HCl, 500 mM KCl, pH 8.8), 2μl of MgCl2 (25mM), 0.5 μl of dNTPs (10 mM), 0.5 μl of each primer (10 pM/μl), 2 U of Taq polymerase (5U/μl) and 2 μl of DNA (50 ng/μl). Amplifications were conducted in thermal cycler with the following cycling conditions (Keskin and Atar, 2012): preliminary denaturation at 95°C for 2 minutes followed by 35 cycles consisting of denaturation at 95°C for 30 seconds, primer annealing at 55°C for 30 seconds, primer extension at 72°C for 1 minute and concluded by a final extension step at 72°C for 10 minutes. Data analyses were conducted using MEGA 5.0 (Tamura et al., 2011), DnaSP 5.0 (Librado & Rozas, 2009) and Network 4.6 (Bandelt et al., 1999).

RESULTS

Three haplotypes were detected among 220 sequences and one of these haplotypes is unique for Turkey. Haplotype diversity was detected as 0.27. Nucleotide diversity was estimated as 0.009. Haplotype network showed that haplotypes of Turkish gibel carp populations seemed to be closely related with Japanese populations.

DISCUSSION

Genetic variation was found to be low for gibel carp populations in Turkey but also showed high level of genetic structuring with new unique haplotype found. Findings of this study specified that gibel carp was translocated to Turkey from both native and non-native area of the species.

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LENGTH-WEIGHT RELATIONSHIPS OF 18 FISH SPECIES FROM THE PERSIAN GULF

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DUŽINSKO-MASENI ODNOS 18 VRSTA RIBA IZ PERSIJSKOG ZALIVA

Apstrakt

Dužinsko-maseni odnos (LWR) je opisan kod osamnaest vrtsa riba ulovljenih duž obala Hormoz moreuza u Persijskom zalivu, Iran. Izlovljeno je ukupno 2328 jedinki od jeseni 2013. do zime 2014. godine sa strašinom za lov gambora i obalnom klopkom. LWR je izračunat korišćenjem stepene jednačine $W = a \cdot L^b$ Vrednosti parametra *b* su bile bliske vrednosti 3 kod malabarskog crvenog pagara (*Lutjanus malabaricus*), tigrozube kavale (*Otolithes ruber*), srebrnastog silaga (*Sillago sihama*), salaha (*Equulites lineolatus*), glatkozubog konja (*Leiognathus equulus*) i konjića (*Secutor ruconius*), vrednosti parametra *b* značajno veće od 3 su zabeležene kod prugastog konjića (*Aurigequula fasciata*) i indijskog iverka (*Psettodes erumei*), a vrednosti parametra *b* značajno manje od 3 su zabeležene kod vrste pagar žutoperajar (*Acanthopagrus latus*), srpa (*Drepane longimana*), indijskog arbuna (*Lethrinus lentjan*), skakavice (*Liza klunzingeri*), indijskog pagra (*Lutjanus johnii*), plotice (*Parastromateus niger*), kopljastog gruntera (*Pomadasys kaakan*), zmijičnjaka (*Trichiurus lepturus*), šarenog konjića (*Nuchequula gerreoides*) i vrste *Photopectoralis bindus*. Do sada nisu postojale u bazi FishBase LWR kod četiri istraživane vrste u ovom radu, a takođe je zabeležena i nova maksimalna dužina za vrstu pagar žutoperajar. Rezultati dobijeni u ovom radu mogu biti od pomoći biolozima i upravljačima koji se bave ribarstvom u Persijskom zalivu.

Ključne reči: dužinsko-maseni odnos, model rasta, Persijski zaliv
Keywords: Length-weight relationship, growth pattern, Persian Gulf

INTRODUCTION

Length-weight (LW) relationship parameters have basic uses in fish stocks assessment and fisheries management (Froese, 2006). For the Persian Gulf, the numbers of such studies are very limited and mostly related to recent years (Raeisi et al., 2014; Aghajanpour et al., 2015; Daliri et al., 2015). This paper provides the first published reference of length-weight relationships for Yellowfin seabream (*Acanthopagrus latus*), Concertina fish (*Drepane longimana*), Klunzinger‘s mullet (*Liza klunzingeri*) and Ornate ponyfish (*Equulites lineolatus*) and also is the first report for other 14 fish species from Persian Gulf.

MATERIAL AND METHODS

Fish specimens were captured from the northern coast of the Persian Gulf in the Strait of Hormuz. In total, 2328 specimens were collected between autumn 2013 and winter 2014 by shrimp beam trawl and intertidal fishing weirs.

All specimens were identified to the species level according to Fischer and Bianchi (1984),

Carpenter et al. (1997) and rechecked against the FishBase (Froese and Pauly, 2015). Total lengths (TL), were measured to the nearest 0.1 mm with a digital caliper. Weights (W) were measured to the nearest 1 g with an electronic balance. For visual inspection of outliers, log-log plots of length and weight values were performed and only extreme outliers attributed to data error were omitted from analysis (Froese, 2006). The LWR was calculated by applying the power regression equation $W = a \cdot L^b$, where W is the total weight (g), L is the total length (cm), *a*, the intercept and *b* the slope of log-transformed linear regression (Bagenal, 1978).

RESULTS AND DISCUSSION

Sample size, range of total length and weight and the value of parameters *a* and *b* with 95% confidence limit are given in Table 1. In the present study the range of exponent *b* was 2.67-3.36 and within the expected range of 2.5-3.5. Minimum and maximum values of parameter *a* were 0.0031 for Eel-like Largehead hairtail (*Trichiurus lepturus*) fish specimens and 0.0495 for short and deep body Concertina (*Drepane longimana*) fish specimens, respectively (Froese, 2006). The length-weight relationship in fish is affected by a number of factors such as habitat, population, gonad maturity, sex, health, sample size, preservation techniques (Tesch, 1971; Alavi-Yeganeh, et al, 2011), which were not considered in the present study. The new maximum TL record for *Acanthopagrus latus* appears to be 38.6 cm (Table 1). These results will be useful for fishery research, management and conservation in the Persian Gulf.

Table 1 - Length-weight relationships in 12 commercial fish species from the Strait of Hormuz in the Persian Gulf.

Family	Species	Length range (TL, cm)	Weight range (g)	n	Regression parameters				
					a	*a* CI 95%	*b*	*b* CI 95%	r^2
Sparidae	*Acanthopagrus latus*	16.4 - 38.6^c	89 - 1288	74	0.0279	0.0199 - 0.0392	2.883	2.782 - 2.983	0.99
Drepaneidae	*Drepane longimana*	19.8 - 47.3	258 - 2866	91	0.0495	0.0378 - 0.0649	2.895	2.783 - 2.936	0.99
Lethirinidae	*Lethrinus lentjan*	20.3 - 40.9	142 - 982	66	0.0270	0.0164 - 0.0437	2.837	2.696 - 2.978	0.98
Mugilidae	*Liza klunzingeri*	10.8 - 19.9	13 - 74	383	0.0205	0.0149 - 0.0282	2.762	2.645 - 2.880	0.95
Lutjanidae	*Lutjanus johnii*	32.2 - 45.4	479 - 1340	42	0.0390	0.0162 - 0.0944	2.733	2.488 - 2.977	0.96
	Lutjanus malabaricus	23.8 - 73	231 - 6490	82	0.0206	0.0159 - 0.0267	2.931	2.895 - 3.002	0.99
Sciaenidae	*Otolithes ruber*	21 - 51.6	65 - 1433	102	0.0116	0.0094 - 0.0142	2.970	2.912 - 3.027	0.99
Carangidae	*Parastromateus niger*	17.8 - 48	109 - 2213	89	0.0263	0.0205 - 0.0338	2.899	2.828 - 2.970	0.99
Haemulidae	*Pomadasys kaakan*	28.3 - 59.9	322 - 2787	80	0.0207	0.0155 - 0.0277	2.903	2.825 - 2.981	0.99
Psettodidae	*Psettodes erumei*	20.2 - 63.8	80 - 3558	107	0.0038	0.0026 - 0.0054	3.360	3.259 - 3.461	0.99
Sillaginidae	*Sillago sihama*	12.4 - 23.7	14 - 96	95	0.0084	0.0060 - 0.0119	2.949	2.831 - 3.068	0.98
Trichiuridae	*Trichiurus lepturus*	50.5 - 104.5	120 - 937	163	0.0031	0.0016 - 0.0059	2.670	2.520 - 2.821	0.95
Leiognatidae	*Equulites lineolatus*	5.0-11.3	1-10.	139	0.0060	0.004-0.009	3.144	2.943-3.353	0.931
	Aurigequula fasciata	5.8-13.1	3 -37	221	0.0101	0.008-0.012	3.249	3.161-3.336	0.980
	Secutor ruconius	4.3-6.1	1-4	69	0.0231	0.017-0.032	2.833	2.642-3.024	0.964
	Photopectoralis bindus	4.4-10.8	1 -19	239	0.0175	0.016-0.019	2.934	2.889-2.980	0.993
	Leiognathus equulus	9.4-22.1	14-237	22	0.0102	0.007-0.015	3.216	2.912-3.519	0.997
	Nuchequula gerreoides	6.9-11.4	4-18	169	0.0230	0.018-0.029	2.794	2.676-2.912	0.964

n, sample size;; *a*, intercept of log-log relationship; *b*, slope of relationship; CI95%, confidence intervals; r^2, coefficient of determination; c Denotes species with a maximum length greater than previously recorded.

ACKNOWLEDGEMENTS

This study was supported by a research grant from Tarbiat Modares University (Project no. 152/3433).

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THE IMPORTANCE OF BORDER INSPECTION IN CONTROL HISTAMINE POISONING FROM CANNED TUNA

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ZNAČAJ GRANIČNE INSPEKCIJE U KONTROLI TROVANJA HISTAMINOM IZ KONZERVI OD TUNA

Apstrakt

Republika Srbija nema sopstvenu proizvodnju morskih riba i poseduje svega nekoliko objekata za preradu morske ribe. Sva morska riba se uvozi, samim tim su i sve konzerve od komada i komadića tunja na našem tržištu uvezene. Uvoz konzervi od tunja, kao i svih drugih ribljih proizvoda prilikom ulaska u našu zemlju se mora podvrgnuti kontroli granične veterinarske inspekcije. Odmah nakon prispeća pošiljke na granični prelaz lice odgovorno za pošiljku, pored obrasca zajedničkog veterinarskog ulaznog dokumenta za proizvode, podnosi i zahtev za veterinarsko sanitarni pregled. Svaka pošiljka konzervi od tunja u uvozu u Republiku Srbiju mora biti podvrgnuta pregledu dokumentacije, identifikaciji pošiljke i fizičkom, hemijskom, mikrobiološkom i senzorskom pregledu.

Sadržaj histamina u ribama i proizvodima od riba je pre svega kriterijum bezbednosti hrane obzirom da utiče na zdravlje ljudi, ali je u isto vreme i pokazatelj higijene procesa i svežine proizvoda. Cilj ove studije je da se obezbede informacije o prisustvu histamina u uvezenim konzervama od tunja i da se ukaže na značaj granične inspekcije u kontroli histaminske intoksikacije koja nastaje kao posledica konzumiranja konzervi od tunja sa povišenim sadržajem histamina.

Tokom jednogodišnje studije (od januara do decembra 2014. godine) ispitano je 97 proizvodnih partija uvezenih konzervi od tunja. Svih 97 proizvodnih partija je uzorkovano tokom granične inspekcije i ispitano u laboratorijama Naučnog instituta za veterinarstvo „Novi Sad". Ispitivanje sadržaja prisustva histamina u konzervama od tunja je urađeno ELISA metodom sa test kitom HIS-E02 (Immunolab GmbH, Germany).

Rezultati studije pokazuju da je 3,09 % proizvodnih partija uvezenih konzervi od tunja imalo nezadovoljavajuće povećan nivo sadržaja histamina i da nisu bezbedne za ljudsku ishranu. Sve proizvodne partije kod kojih je dokazan povećan nivo histamina, odnosno koje su proglašene za nebezbedne za ljudsku ishranu su vraćene, a uvoz je zabranjen. Granična inspekcija, tj. ispitivanje pri graničnoj inspekcije predstavlja značajnu preventivnu meru u sprečavanju histaminske intoksikacije kao posledice konzumiranja konzervi od tunja sa povišenim sadržajem histamina.

Ključne reči: granična inspekcija, histamin, konzervirana tunjevina
Key words: Border Inspection, histamine, canned tuna

INTRODUCTION

Fish and fishery products are widely consumed in many parts of the world because they have high protein content, low saturated fat and also contain omega fatty acids known to support good health (Swanson et al., 2012).

Tuna is one of Scombroid toxin-forming species in which significant levels of histamine may be produced by decarboxylation of free histidin (Hungerford, 2010). Histamine production in fish is related to the histidine content of the fish, the presence of bacterial histidine decarboxylase and environmental conditions.

Histamine content in fish and fish products is a criterion for food safety due to its effects on human health, but is also an important indicator of hygiene level of the food production process and product freshness. Histamine poisoning is a food-borne chemical intoxication occurring within few minutes to several hours following the ingestion of foods that contain unusually high levels of histamine and possibly other biogenic amines (Taylor, 1986; WHO, 2012; Er et al., 2014). The symptoms of histamine poisoning resemble an allergic reaction and there is potential for misdiagnosis, since symptoms are usually mild, it is likely that the illness is considerably under-reported. All canned tuna in our country is imported. Imports of canned tuna from all other countries must enter the Republic of Serbia via an approved Border Inspection under the authority of an official veterinarian. Import controls are in place to protect human health. Each consigment is subject to a systematic documentary check, identity checks, physical, chemical, microbiological and sensory check.

Some studies revealed that histamine formation could occur during storage of fish products (Veciana-Nogues et al., 1996). Implementation of HACCP concept and prerequisite programs (PRPs) into the fish processing industry is essential to reduce risk of histamine occurrence. The level of histamine in fish products cannot be reduced by cooking, or preservation and freezing since the compound is heat stable (Visciano et al., 2014).

Histamine is generally not uniformly distributed in a fish or in a lot. Because of that, the validity of histamine testing is dependent upon the design of the sampling plan (FDA, 2011). The legislative acts of the Republic of Serbia (Official Gazette RS 72/2010) are harmonized with the Directive of European Union (Commission Regulation 2073/05). The examination of one production lot includes testing of 9 units. The permitted level implies that not more than two out of nine sample units may contain between 100 and 200 mg/kg; however, none of the units may exceed the upper histamine limit of 200 mg/kg.

MATERIAL AND METHODS

During a two-year study (January, 2013 – January, 2015) 97 imported canned tuna production lots were examined in 9 units. All samples which were collected during Border

Inspection were examined in laboratories of Scientific Veterinary Institute „Novi Sad". The presence of histamine in canned tuna was analyzed by enzyme-linked immunosorbent assay method (ELISA), using test kit HIS-E02 (Immunolab GmbH, Germany). Histamine concentrations were determined according to the manufacturer's procedure. Subsamples of 10 g were extracted with 0.1 M hydrochloric acid. After dilution and derivatization, samples or standards containing derivatized histamine and an antibody directed against histamine were placed into the wells of the microtiter plate. A histamine conjugate is bound on the surface of a microtiter plate. Immobilized and free histamine compete for the antibody binding sites. After incubation, washing and removing of unbound material, a peroxidase conjugate directed against the histamine antibody was added into the wells. After the second incubation, the plate was washed again and substrate solution was added and then incubated, which resulted in the development of blue colour. By addition of a stop solution, colour turned yellow. The intensity of yellow colour was measured photometrically at 450 nm (Multiskan FC, Thermo Scientific, China) and it is inversely proportional to the histamine concentration in the sample. Special software, the Rida®Soft Win (Art. No. Z9999, R-Biopharm, Germany) was used for the evaluation of enzyme immunoassay. Laboratory detection limits for histamine determination in canned tuna were 10 mg/kg, recovery of the method was 94.3%. The analytical quality of the ELISA method was assured by the use of reference material (lyophilized tuna muscle, T1134A-1/CM, Progetto, Trieste, Italy), as well as by participation in proficiency testing scheme (canned fish sample, FAPAS 27110).

RESULTS

The results of histamine content in unfit production lots are displayed in Table 1. Out of 97 analyzed production lots, 94 (96.9%) were under the regulatory limit according to the legislative acts of the Republic of Serbia (Official Gazette RS 72/2010).

Table 1. Histamine contents in unfit production lots

No.	Country of origin	Histamine content mg/kg								
		1st unit	2nd unit	3rd unit	4th unit	5th unit	6th unit	7th unit	8th unit	9th unit
1.	Vietnam	124.5	41.38	160.30	10	28.51	52.91	33.61	72.03	162.91
2.	Thailand	75.46	135.21	268.65	274.67	437.32	183.08	158.59	207.37	152.58
3.	Thailand	196.01	35.26	121.04	70.84	40.96	74.85	233.86	178.27	146.95

Our results confirmed that concentration of histamine can vary considerably from unit to unit. which required that examination of one production lot has to include testing of 9 units.

DISCUSSION AND CONCLUSION

The Food and Drug Administration (FDA) established HACCP programme for seafood industry and defined critical control points for analyses of marine fishery products. According to FDA (2010), canned tuna products with histamine level < 10 mg/kg is considered to be of good quality, the level 30 mg/kg indicates significant deterioration, whereas level of 50 mg/kg represents conclusive evidence of deterioration.

Histamine poisoning is one of the most important public health and safety concern and a trade issue. In the United States histamine poisoning is the most prevalent form of seafood-borne disease (Lehane and Olley, 2000). UK, USA and Japan are countries with the highest number of reported incidents. Less frequent incidents have been reported elsewhere in world (Lehane and Olley, 2000). Histamine in fish and fish products was the responsible agent for 17 outbreaks in 2008 in Europe (EFSA, 2011). In 2008, the French Institute for Public Health Surveillance reported an increase in the number of histamine food poisoning outbreaks and cases in France and also during a border control 872.86 ppm levels of histamine in frozen tuna originating from Vietnam was discovered (Guillier et al. 2011). In 2008, in Sweden the findings of exceeding histamine in canned tuna coming from the Philippines were notified. The alert was based on food poisoning (EU, 2008). Between 2005 and 2010 the recorded histamine notifications were 246 of which even 119 notifications were recorded thanks to Border Inspection (EFSA, 2011).

Food safety relevant factors such as global canned fish trade and consumers’ eating habits are changing. Therefore there is a need to periodically revisit the histamine related food safety issues and consider any new related knowledge, and data and trends which may enable further improvements of histamine poisoning risk reduction strategies.

Our analysis revealed 3.09% production lots of canned tuna with significant level of deterioration. All production lots of canned tuna with high content of histamine that were declared unfit for human consumption have been returned and the import was banned. That is the possibility that histamine poisoning was prevented.

ACKNOWLEDGMENTS

This paper is a result of the research within the project TR 31011 “The influence of the quality of the food components for cyprinid fish species on the quality of meat losses and the profitability of production” financed by the Ministry of Science and Technological DevelopmentRepublic of Serbia.

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A PROPOSAL OF METHODS FOR DETERMINING WATER STABILITY OF EXTRUDED FISH FEED

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PREDLOG METODA ZA ODREĐIVANJE STABILNOSTI U VODI EKSTRUDIRANE HRANE ZA RIBE

Apstrakt

Važan parametar fizičkog kvaliteta ekstrudirane hrane za ribe jeste njena stabilnost u vodi, koja se definiše kao svojstvo peleta uronjenih u vodu da zadrže svoj fizički oblik uz minimalno raspadanje i gubitak nutritivnih komponeneti sve do momenta njenog unosa od strane riba. Stabilnost u vodi hrane je važna za sve akvatične vrste, kako za pastrmke i losose koji se hrane sporotonućom hranom, tako i naročito za one koje se sporo hrane, kao što su škampi, gde je potrebno da hrana satima u vodi zadrži oblik i sve potrebne hranljive materije. Mala stabilnost hrane u vodi dovodi do slabog rasta riba, neefikasne konverzije i ekonomskih gubitaka, te stoga hrana za ribe mora posedovati visoku stabilnost u vodi kako bi se ribama obezbedila najveća moguća količina hranljivih materija a tako dovelo i do smanjenje troškova proizvodnje.

U ovom radu određena je stabilnost u vodi tri različite grupe hrane za losose korišćenjem tri metode: statičke metoda, metode mokrog prosejavanja natopljenih peleta i konduktometrijske metode, kao novog pristupa određivanja stabilnosti ekstrudirane hrane za ribe u vodi. Za ispitivanje stabilnosti odabrana su tri uzorka ekstrudirane hrane za losose tako da poseduju različitu stabilnost u vodi. Cilj istraživanja bio je da se testirajem metoda ispita njihov potencijal za određivanje stabilnosti hrane za ribe, kao i da se proveri međusobna korelacija predloženih metoda.

Na osnovu rezultata ispitivanja, za sve tri korišćene metode primećene su statistički značajne ralike ($p<0,05$) između uzoraka ekstrudirane hrane za losose. Rezultati dobijeni korišćenjem predloženih metoda pokazali su da je najmanju stabilnost u vodi imao uzorak hrane A, dok je najveću imao uzorak hrane C. Iako se statički metod pokazao kao pogodan za odreživanje maksimalne vrednosti stabilnosti hrane za ribe u vodi, metod mokrog prosejavanja pokazao se kao bolji izbor jer daje jasnije razlike između pojedinačnih uzoraka. Konduktometrijska metoda na osnovu izračunatog koeficijenta varijacije (c_v=0,02%) imala je najveću tačnost od sve tri predložene metode. Primećena je pozitivna korelacija između statičke i metode mokrog prosejavanja (r=0,999; p=0,02), nešto niža korelacija između konduktometrijske i statičke metode (r=0,999; p=0,03), dok je najniža korelacija prisutna između konduktometrijske i metode mokrog prosejavanja (r=0,997; p=0,05), na granici poverenja od 95%. Prednost konduktometrijske metode u odnosu na ostale ispitivane metode ogleda se u mogućnosti direktnog merenja, brzini i osetljivosti.

Ključne reči: stabilnost u vodi, hrana za ribe, ekstrudiranje, prosejavanje, konduktometrija
Keywords: water stability, fish feed, extrusion, sieving, conductometry

INTRODUCTION

Production of commercial fish feed is mostly done by extrusion process, resulting in products high in energy with high physical quality as well good nutritional value. Production conditions and also physiochemical properties of ingredients, affect physical quality of extruded feed (Oehme et al., 2014).

Water stability is an important physical property of feed for aquatic species. It is defined as the retention of the pellet physical integrity with minimal disintegration and nutrient leaching while it is immersed in the water and until it is consumed by animals (Obaldo et al., 2002). Fish feed should have high water stability to prevent increased cost of feeding and to provide the greatest proportion of available nutrients to the cultured animal (Obaldo et al., 2002). For fish that require slow sinking pellet as a feed, such are salmon and trout, water stability may be important to predict the degradation model of feed in the gastrointestinal tract (Sørensen, 2012). Low water stability of extruded feed resulted in oil and water separation and accumulation of free oil in the stomach of rainbow trout, thus induced risk of oil-belching (Baeverfjord et al., 2006; Aas et al., 2011).

The ideal method for determining water stability of aquatic feed should be quick and simple, and should produce practical, accurate and reproducible results is needed (Obaldo et al., 2002). The method should be also able to clearly discriminate between extruded pellets with different water stability (Baeverfjord et al., 2006).

In this work water stability of three different salmon feed samples were investigated by three different methods developed by ourselves: the static water method, wet sieving method, and conductometric method. The aim was to propose and test these novel methods, find a correlation between them and present the potential of the conductometric method for determining water stability of extruded fish feed.

MATERIALS AND METHODS

Three salmon feeds (feed A, feed B and feed C) with different water stability were produced in pilot plant of Feed to Food Center (FINS, Novi Sad, Serbia). Extrusion parameters were changed during production in order to obtain differences in pellet quality. Feed A was produced to have lowest and feed B the highest water stability. Three methods for determining pellet water stability were defined and tested: static method, wet sieving method and conductometric method. The dry matter (DM) of each feed was determined by drying it at 105°C to constant weight, prior to the water stability tests. All water stability tests were done in duplicate.

Static water method

In the static water method, no pellet or water agitation was involved. Around 25 g of salmon feed pellets were weighed and put in 600 ml glass beaker and 250 ml of distilled water was added. The beaker was covered with aluminum foil and put in the climate chamber (Binder KBF 240, Binder GmbH, Tuttlingen, Germany) where the feed was soaked for 24 hours at 23°C. After soaking, all pellets were transferred on a 2.24 mm sieve and excess of water was gently removed by manually moving the sieve for 20 sec. Pellets were then weighed in a previously dried and weighed glass plate. Soaked pellets were dried in UNB 400 oven (Memmert GmbH, Schwabach, Germany) at 105°C for at least 18 h. After drying, the plate with pellets was again weighed in determining a residual dry matter of the samples. The Water Stability Index (WSI) was calculated as remaining DM weight after soaking divided by initial DM weight of pellets before soaking.

Wet sieving method

The soaking part of this method was the same as for static one and same materials and equipment were used. After soaking pellets were sieved by laboratory sieving device (Retsch AS200 Control, Haan, Germany) and 2.24 mm sieve. The sieving was carried out for 10 min at amplitude of 2, and for the whole sieving duration the pellets were washed directly from the top of the sieve with tap water at maximum flow in order to additionally enhanced disintegration of pellets. Sample drying and results calculations were done in the same manner as it is described above.

Conductometric method

In this method the conductivity of the water solution with immersed pellets was measured during time. In 600 ml glass beaker 25 g of feed pellets were put. After addition of 250 ml, the measuring cell of the Lab960 conductivity meter (SI Analytics, Mainz, Germany) was immediately immersed directly in the middle of the beaker to the point in which the bottom of the cell was at the 100 ml mark. The conductivity measuring device was set to automatically measure and record values of conductivity in the time period of 20 min, during 24 hours. The reference temperature of temperature compensation was preset at 20°C. After 24 h, the conductivity measurement was stopped, and conductivity values and corresponding times were transferred from the measuring device directly to the PC using MultiLabpilot software (WTW, Weilheim, Germany). The obtained conductivity of water after 20 min was chosen as the representative of feed disintegration in distilled water, and it‘s sign was changed in order to be compared to other two proposed methods

Statistical analysis

One-way analysis of variance (ANOVA) by post-hoc Tukey’s HSD test and F-test were used to analyze data at at 95% confidence limit (STATISTICA 12.0, StatSoft Inc., Tulsa, OK, USA). The conductometric method’s validity was confirmed, based on correlations with widely used static and wet sieving methods, obtained by correlation analysis.

RESULTS AND DISCUSSION

Water stability results of salmon feeds obtained by static water and wet sieving method as well as water conductivity after 20 min of pellet soaking with negative sign are presented in table 1.

Table 1. Water stability of tested salmon feeds by all three methods

Sample	Static water method (WSI %)	Wet sieving method (WSI %)	Water conductivity after 20 min (µS/cm)
Feed A	87.53±0.01[a]	42.66±0.03[a]	-624.0±0.13[c*]
Feed B	88.99±0.07[b]	54.25±0.53[b]	-361.0±0.09[b]
Feed C	90.00±0.12[c]	61.19±2.09[c]	-151.4±0.08[a]
Variance	1.54	87.58	56075.32
Coeff. of variation (%)	0.13	3.42	0.02

[a,b,c] *Values with the same letter in a column, written in superscript, are not statistically different at the p<0.05 level, 95% confidence limit, according to Tukey's HSD test*
[*] *Negative value is assigned for a comparing and finding of correlation between conductometric and static and wet sieving method*

Feed A had the lowest while the feed C had highest water stability obtained by all three methods (Table 1). The static water method contributed to the significantly higher values of WSI than the wet sieving method that included shaking of pellets. It was in accordance with the results of Obaldo et. al (2002) where the static water method produced the highest DM retention of two shrimp feeds compared with horizontal and vertical shaking methods. As these authors suggested, that static water method can be used for comparing the maximum pellet water stability. The wet sieving method was proved to be a more fitting method for determining water stability as there were more distinctive significant differences between WSIs of all three feed.

Experimental results of conductivity measurements are presented in Figure 1. Feed C had the lowest value of conductivity after 24h, as well as linear nutrient leaching rate. indicating the highest water stability. The superiority of conductivity measurement compared to other methods can be observed from Table 1, where conductivity data span a much larger range of values (also shown with larger variance) compared to static and wet sieving method, but still, the accuracy of conductivity measurement remains much higher compared to the other fish feed water stability tests (according to the coefficient of variability, which is calculated to be 0.02%).

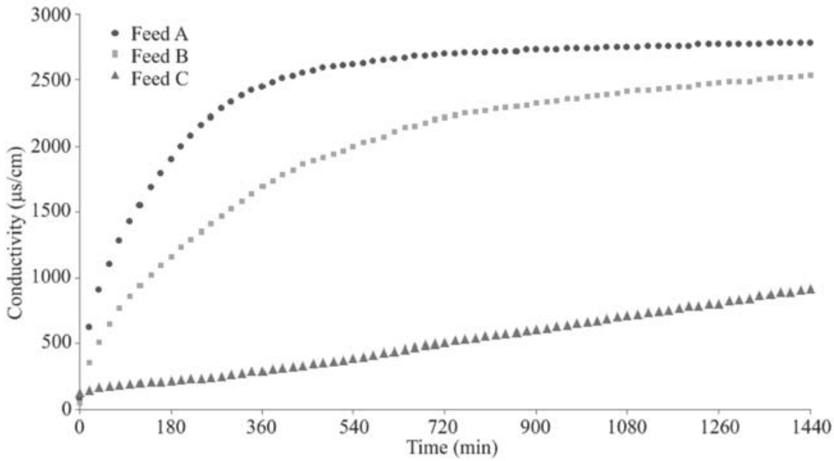

Figure 1. Water conductivity during 24 h soaking of tested salmon feeds

The possibility of direct measurement, high resolution screening of results as well as rapidness and sensitivity are certain advantages of the conductometric approach over other two proposed methods Statistically significant difference ($p<0.05$) has been found in all samples, at significance level (Table 1). This novel assay has been validated through correlation analysis with other two water stability tests employed in this study (Table 2).

Table 2. Correlation coefficients between static water and wet sieving method and conductivity measurement of water

	Wet sieving method	Conductivity of water after 20 min
Static water method	$r=0.999^*$ $p=0.02$	$r=0.999^*$ $p=0.03$
Wet sieving method		$r=0.997^*$ $p=0.05$

* *Significant at 0.05 level*

CONCLUSION

The results of water stability of tested salmon feed obtained by all three proposed methods, significantly differed within the specific method, thus proving that methods can be successfully used for relative comparison water stability between different feeds. The static water method was shown to be suitable for determining maximum water stability of feed, while wet sieving method was proven to be suitable for showing differences in water stability between various samples. Positive correlations between the results of conductometric method and the other two applied methods were obtained. The results indicated that conductometric method is accurate, rapid and simple method which can be successfully used for determining water stability of salmon feed.

ACKNOWLEDGMENTS

These results are part of the research within the projects III46012 and TR31011, financed by the Ministry of Education, Science and Technological Development, Republic of Serbia.

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DETERMINATION OF THE HEAVY METALS' PRESENCE IN THE WATER AND GRAYLING MUSCLE TISSUE (*THYMALLUS THYMALLUS*) OF THE RIVER UNA

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ODREĐIVANJE PRISUSTVA TEŠKIH METALA U VODI I MIŠIĆNOM TKIVU LIPLJENA (*THYMALLUS THYMALLUS)* RIJEKE UNE

Apstrakt

Cilj ovog rada bio je da se prikažu uporedni rezultati hemijskih analiza vode i ribe rijeke Une na različitim lokacijama. Za ispitvanje je uzeta riba lipljen (*Thymallus thymallus*) iz porodice Thymallidae. Lipljen je riba koja preferira hladne vode bogate kisikom, što upravo Una i jeste. Uzorci vode i ribe su uzeti sa tri lokacije. Hemijska ispitivanja su obuhvatila analizu vode rijeke Une i to slijedeće parametre: temperatura, pH, elektrovodljivost, otopljeni kisik, suspendirane materije, $BPK_{5,}$ KPK - Cr, ukupni dušik - N, ukupni fosfor - P, sufati - SO_4, hloridi, nitriti - $NO_{2,}$ nitrati - $NO_{3,}$ fluoridi i teški metali (Cu,Cd i Pb). U fileu lipljena određeni su: proteini, masti, voda, ugljikohidrati i mineralne materije.Rezultati su pokazali dobar kvalitet vode rijeke Une sa različitim koncentracijama na pojedinim lokalitetima kao i na dobar kvalitet mesa ribe.

Sadržaj Pb, Cu i Cd je određen na atomskom apsorpcionom spektrofotometru „Perkin Elmer" AAnalyst - 800, plamenom tehnikom. Količine teških metala (Pb, Cu i Cd) bile su ispod maksimalno dozvoljenih količina (MDK). Sadržaj teških metala u mišićnom tkivu riba u direktnoj vezi je sa zagađenjem rijeka. Sadržaj olova u mišičnom tkivu lipljena izlovljenog u rijeci Uni bio je najveći u uzorcima koji su izlovljeni na drugom lokalitetu i najveći sadržaj bakra identifikovan je na istom lokalitetu. Vrijednosti kadmija u uzorcima mišićnog tkiva ribe na svim lokalitetima iznosio je ispod 0,1 mg/kg.

Sadržaji teških metala u ispitanim uzorcima vode i ribe lipljena rijeke Une, imali su vrijednosti ispod dozvoljenih granica. Takvi rezultati ukazuju da još uvijek nije došlo do značajnijeg zagađenja vodotoka rijeke Une.

Ključne riječi: teški metali, voda, riba, hemijske analize.

Abstract

The aim of this paper is to present comparative results of the chemical analysis of water and fish of the river Una in different locations. Grayling fish (*Thymallus thymallus*) from the family Thymallidae was sampled for analysis. Grayling is the fish that prefers cold water, rich in oxygen, just as it is a case with river Una. Water and fish samples were taken from three locations. Chemical testing included water analysis of the river Una paying attention to the following parameters: temperature, pH, conductivity, dissolved oxygen, suspended matter, BOD_5, COD - Cr, total nitrogen - N, total phosphorus - P, sulfates - SO_4, chlorides, nitrites - NO_2, nitrates - NO_3, fluorides and heavy metals (Cu, Cd and Pb). Protein, fat, water, carbohydrates and minerals were determined in the fillet of grayling. The results showed not only good water quality of the river Una with different concentrations in certain locations, but also good quality of fish meat.

Atomic absorption spectrometer “Perkin Elmer” AAnalyst – 800 was used to determine the content of Pb, Cu and Cd through flame technique. Amounts of heavy metals (Pb, Cu and Cd) were below maximum allowable concentration (MAC). The content of heavy metals in fish muscle tissue is directly related to the pollution of rivers. The lead content in the muscle tissue of grayling being overfished in the river Una was the highest in the samples from site number two and the largest copper content was identified at the same site. Cadmium values in the fish muscle tissue samples were less than 0.1 mg / kg at all sites.

The heavy metal content, in the tested samples of water and grayling fish of the river Una, had values below the acceptable limit. These results indicate that the watercourse of river Una is still not significantly polluted.

Keywords: heavy metals, water, fish, chemical analysis.

OUT OF SEASON QUALITY ASSESMENT AND CRYOPRESERVATION OF EURASIAN PERCH (*PERCA FLUVIATILIS*) SPERM

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PROCENA KVALITETA I METODE KRIOPREZERVACIJE SPERME GRGEČA (*PERCA FLUVIATILIS*) UZORKOVANE VAN SEZONE

Apstrakt

Od svih vrsta koje su introdukovane u evropsku akvakulturu, grgeč (*Perca fluviatilis*) najviše obećava. Mogučnost mrešćenja van sezone je jedan od najbitnijih faktora u veštačkom mrestu bilo koje vrste. Gajenje grgeča (u severnoj i zapadnoj Evropi) se uglavnom obavlja u recirkulacionim sistemima, gde je mogućnost proizvodnje u toku cele godine ključni faktor da bi se zadovoljile potrebe tržišta (Migaud et al. 2002). Krioprezervacija sperme je efikasan način smanjenja troškova koji nastaju držanjem matica i pruža dobar kvalitet gameta tokom cele kalendarske godine. (Cabrita et al. 2010). Matice grgeča (Perca fluviatilis) su izlovljavane u periodu od oktobra do novembra 2014. 13 mužjaka (težina: 39-137 g) su čuvani u vodi čija je temperatura iznosila 6-16°C. Ispuštanje sperme je indukovano hormonima, korišćenjem 500 IU^{-1} kg hCG (humanog horionskog gonadotropina). Sperma je sakupljena 1. i 6. dana nakon ubrizgavanja hormona. Parametri pokretljivosti spermatozoida sveže i odmrznute sperme bez ubrizgavanja hormona (Wo), nakon 1. (1da) i nakon 6. (6da) dana ubrizgavanja hormona su kvantifikovani CASA sistemom. Ukupna zapremina sperme nakon istiskanja je izmerena u svim tretmanima. Sperma grgeča bez i sa injektiranog hormona nakon 1. i 6. dana je prezervirana u skladu sa prethodno definisanim protokolima. Za prezervaciju je korišćen zamrzivač sa automatskim programom hlađenja (od 7.5 ºC do -160 ºC, stopa hlađenja: 56 ºC/min) (Bernáth et al. 2015). Sperma sa najvećom prosečnom zapreminom je istisnuta u 6da grupi riba (1611 ± 1428µl). Prosečna zapremina sperme je bila značajno niža u grupi Wo (58 ± 82µl) u odnosu na grupu 6da. Prosečna za-

premina sperme u grupi 1da (64 ± 49μl) se nije statistički razlikovala od druge dve grupe. Progresivna pokretljivost spermatozoida u sveže istisnutoj spermi je bila slična pokretljivosti nakon hormonalne stimulacije (Wo: 79 ± 10%, 1da: 54 ± 26%, 6da: 75 ± 11%). Ista tendencija je zabeležena u slučaju brzine nepravilnog kretanja (VCL) spermatozoida (Wo: 149 ± 24 μm/s, 1da: 137 ± 23 μm/s, 6da: 145 ± 40 μm/s), kao i pravolinijskog kretanja (STR) spermatozoida (Wo: 76 ± 7%, 1da: 80 ± 1%, 6da: 80 ± 8%) u sveže istisnutoj spermi. Slične vrednosti progresivne pokretljivosti, VCL-a i STR-a su izmerene u krioprezerviranim uzorcima nakon odleđivanja sperme. Ipak, progresivna pokretljivost je značajno redukovana u grupi 6da nakon krioprezervacije (11 ± 7%) u poređenju sa sveže istisnutom spermom u grupama Wo i 6da. Pokretljivost u odleđenoj spermi nije značajno opala u grupama Wo (18 ± 8%) i 1da (14 ± 5%), dok je značajno smanjenje primećeno za parametar VCL u grupi 6da, nakon odleđivanja (70 ± 11 μm/s) u odnosu na sve grupe gde je sperma sveže isceđena. Značajno smanjenje u parametru VCL je primećeno u grupi Wo (88 ± 25 μm/s) nakon krioprezervacije u poređenju sa grupama Wo i 6da kada je sperma sveže istisnuta. Parametar VCL u grupi 1da nakon odleđivanja (101 ± 15 μm/s) se nije promenio u odnosu na sveže isceđene grupe. Vrednosti STR-a su bile jako visoke nakon odleđivanja u svim krioprezerviranim grupama (Wo: 90 ± 5%, 1da: 92 ± 2%, 6da: 88 ± 4%). Značajna razlika je primećena između grupe 1da, posle odleđivanja i sveže istisnute sperme grupe Wo. Ovi rezultati su pokazali da je hormonalna stimulacija uspešno sprovedena kod mužjaka grgeča u cilju indukovanja proizvodnje sperme van sezone parenja.

Abstract

Eurasian perch (*Perca fluviatilis*) is a promising species among those that were recently introduced into European aquaculture. Out-of-season spawning is a remarkable factor in artificial propagation of every species. The production of Eurasian perch is mainly (Northern and Western Europe) maintained in recirculating systems where all year long production is a key factor in the satisfaction of current market demands (Migaud et al. 2002). Cryopreservation of sperm could be an efficient tool to reduce the costs of broodstock management and provide good quality gametes all year round (Cabrita et al. 2010). A broodstock of wild caught Eurasian perch (*Perca fluviatilis*) males was established from October to November 2014. The 13 males (bodyweight: 39-137 g) were kept at the same water temperature in the range of 6-16°C (according to the hatchery temperature). Spermiation was hormonally stimulated using 500 IU^{-1} kg hCG (human chorionic gonadotropin). Sperm was stripped 1 day and 6 days after injection according to the experimental design. Motility parameters of fresh and thawed sperm without injection (Wo), 1 day (1da) and 6 days (6da) after injection were measured using a CASA system. The total volume was estimated in all treated freshly stripped groups. Perch sperm was cryopreserved without injection, 1 day after and 6 days after injection according to our previously developed cryopreservation protocol. A controlled rate freezer with a cooling program (from 7.5 ºC to -160 ºC, cooling rate: 56 ºC/min) was used (Bernáth et al. 2015). The largest volume of sperm was stripped 6 days after injection (1611 ± 1428μl). Average sperm volume was significantly lower in Wo (58 ± 82μl) compared to 6da. Total volume of sperm at 1da did not differ significantly from the other groups (64 ± 49μl). Progressive motility of freshly stripped perch sperm was similar after hormonal stimulation (Wo: 79 ± 10%, 1da: 54 ± 26%, 6da: 75 ± 11%). The same tendency was observed in the case of curvilinear velocity (VCL) of

spermatozoa (Wo: 149 ± 24 μm/s, 1da: 137 ± 23 μm/s, 6da: 145 ± 40 μm/s) and straightness (STR) of sperm movement (Wo: 76 ± 7%, 1da: 80 ± 1%, 6da: 80 ± 8%) in freshly stripped sperm.A similar progressive motility, VCL and STR was measured after thawing among cryopreserved groups. However, progressive motility was significantly reduced after cryopreservation in the group 6da (11 ± 7%) compare to fresh Wo and 6da (see above). Post-thaw motility did not decrease significantly in Wo (18 ± 8%) and 1da (14 ± 5%). A significant reduction was observed after thawing in VCL 6da (70 ± 11 μm/s) compared to all fresh groups. A significantly decreased VCL was recorded in Wo (88 ± 25 μm/s) after cryopreservation compared to fresh Wo and 6da. Post-thaw VCL in 1da (101 ± 15 μm/s) did not change in comparison to freshly stripped groups. STR was quite high after thawing in all cryopreserved groups (Wo: 90 ± 5%, 1da: 92 ± 2%, 6da: 88 ± 4%). A significant difference was observed between thawed 1da and fresh Wo. Hormonal stimulation was succesfully used in the out-of-season induction of spermiation in male Eurasian perch. Eurasian perch sperm can be cryopreserved out-of-season, as well.

The work was supported by the projects EUREKA_HU_12-1-2012-0056, 8526-5/2014/ TUDPOL of the Ministry of Human Resources of Hungary awarded to Szent István University and the GOP-1.1.1- 11.2012-0306.

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EFFECTS OF SUBCHRONIC TONALIDE EXPOSURE ON ZEBRAFISH, *DANIO RERIO*

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EFEKTI SUBHRONIČNOG IZLAGANJA ZEBRICE, *DANIO RERIO* SINTETIČKOM MOŠUSU

Apstrakt

Policiklična jedinjenja sintetičkog mošusa su veoma rasprostranjena i koriste se u velikim količinama kao miris u proizvodima široke potrošnje. Zbog njihove česte upotrebe i otpuštanja mirisa, ova jedinjenja su postala prisutna svuda u životnoj sredini. Toksikološka istraživanja su potvrdila da jedinjenje mošusa predstavlja opasnost za vodene ekosisteme. Cilj ovog istraživanja bio je da se ocene efekti subhroničnog izlaganja sintetičkom mošusu tonalidu na rast riba i odgovore na oksidativni stress kod zebrice (*Danio rerio*). Test rasta kod mlađi zebrice izvršen je prema OECD metodu broj 215. U toku 28 dana, riba stara 30 dana je bila izložena koncentraciji tonalida (50; 500; 5.000 and 50.000 ng/l). Na kraju eksperimenta, sve ribe su žrtvovane, izmerene, određena je njihova specifična prosečna stopa rasta po tanku, a selektivni oksidativni stress markeri su analizirani u homogenatu celog tela (glutation S-transferaze, glutation reduktaze, glutation peroksidaze, katalaze i lipidne peroksidaze). U našem ekperimentu, nismo pronašli značajne razlike između kontrolne i eksperimentalne grupe u specifičnom prirastu, telesnoj težini i dužini. Međutim, primetili smo značajne promene kod većine oksidativnih stres markera naročito kod eksperimentalne grupe koja je bila izložena najvećoj koncentraciji mošusa (tonalida).

U poređenju sa kontrolnom grupom, značajna povećanje je konstatovano u aktivnostima glutation S-transferaze (za koncentracije – 5.000 i 50.000 ng/l) i kataze (za koncentracije – 500, 5.000 i 50.000 ng/l). Sa druge strane, primećeno je značajno smanjenje aktivnosti glutation peroksidaze (za koncentraciju – 500 ng/l) u poređenju sa kontrolnom grupom. Nisu konstatovane promene u aktivnosti glutation reduktaze i nivou lipidne peroksidaze u poređenju sa kontrolnom grupom. Naši rezultati pokazuju da izlaganje zebrice tonalidinu ima značajan uticaj na oksidativne stres markere i enzime za detoksifikaciju. Promene u aktivnostima antioksidantnih enzima se mogu tumačiti kao adaptivni odgovor koji bi zaštitio organizam ribe od toksičnosti prouzrokovane tonalidinom.

Ključne reči: riba; test rasta toksičnosti; policiklična jedinjenja mošusa; oksidativni stres; zagađenje vodene sredine
Keywords: fish; growth toxicity test; polycyclic musk compounds; oxidative stress; contamination of aquatic environment

INTRODUCTION

Polycyclic musk compounds are widely used as fragrances in consumer products in very large quantities. Due to their high use and release, they have become ubiquitous in environment. Toxicological studies confirmed that all musk compounds pose risk for aquatic ecosystems and can be considered toxic for aquatic invertebrates. Tonalide and galaxolide are the two most important compounds in group of polycyclic musk compounds. They probably share over 95% of the market for polycyclic musk compounds (Balk and Ford, 1999ab; Ramirez et al. 2009). The aim of this study was to investigate the effects of subchronic exposure to tonalide on fish growth and oxidative stress responses in zebrafish, *Danio rerio*.

MATERIALS AND METHODS

Juvenile growth tests were performed on *D. rerio* according to OECD method No. 215. For 28 days, fish at an initial age of 30 days were exposed to the environmental tonalide concentrations (50; 500; 5.000 and 50.000 ng/l). The fish were randomly distributed into 20 l glass aquaria, 50 specimens per each. Each test group was performed in duplicate. The experiment was conducted in a flow-through system and the volume of each test solution was replaced twice a day. The fish were fed with dried *Artemia salina* without nutshells to the amount of 8% of their body weight per day. Experimental procedures were in compliance with national legislation (Act No. 246/1992 Coll., on the Protection of Animals Against Cruelty, as amended, and Decree No. 207/2004 Coll., on the Protection, Breeding and Use of Experimental Animals as amended).

At the end of the experiment, the fish were killed, weighed and their tank-average specific growth rates were deterimined. Then, fish were immediately frozen, and stored at –85 °C until spectrophotometric analyses of oxidative stress biomarkers such as activities of glutathione S-transferase (GST), glutathione reductase (GR), glutathione peroxidase (GPx), catalase (CAT) and level of lipid peroxidation using thiobarbituric acid (TBARS). The activity of GST was determined by measuring the conjugation of 1-chloro-2,4-dinitrobenzene with reduced glutathione at 340 nm (Habig et al. 1974) and was expressed as the nmol of the formed product per min per mg of protein. The activity of GR was determined spectrophotometrically by measuring NADPH oxidation at 340 nm (Carlberg et al. 1975). The activity of GPx was calculated from the amount of NADPH oxidation by the reaction with GR at 340 nm (Flohe & Gunzler 1984). The activities of GR and GPx were expressed as the nmol of NADPH consumption per min per mg of protein. Protein concentration was determined by a Bicinchoninic Acid Protein Assay Kit (Sigma-Aldrich, St. Louis, MO, USA) using bovine serum albumin as a standard (Smith et al. 1985). To check lipid peroxidation, malondialdehyde was measured by the TBARS method as described by Lushchak et al. (2005) at 535 nm. The concentration was expressed as nmol per gram wet weight of tissue.

Statistical analysis was performed using Unistat 5.6 software. Indices were tested for normal distribution and after testing of homogeneity of variance across groups, an analysis of variance (one-way ANOVA) was used. Differences between control and each experimental group were assessed with the Dunett test and $p<0.05$ was chosen as the level of significance.

RESULTS AND DISCUSSION

In test groups exposed to sublethal concentrations of tonalid we did not notice any changes in fish behaviour or food intake. We also did not find out any significant differences in specific growth rate, body weight and length among control and experimental groups.

On the other hand, we observed significant changes in most of oxidative stress markers especially in experimental groups exposed to the highest concentrations (Table 1).

Activity of GST significantly ($p<0.001$) increased in experimental groups exposed to tonalide at 5.000 ng/l and 50.000 ng/l compared to the control group. Significant increases were also observed in activity of CAT in experimental groups exposed to tonalide at 500 ng/l ($p<0.05$), 5.000 ng/l ($p<0.001$) and 50.000 ng/l ($p<0.001$) compared to the control group. In case of GPx acitivity, we found out significant decrease ($p<0.05$) but only in experimental group exposed to 500 ng/l. No changes were found in GR activity and level of lipid peroxidation.

Table 1. Results of oxidative stress markes in *D. rerio* after tonalide exposure; data are expressed as mean ± standard error of mean; **p<0.05 ***

Group	**GPx** (nmol/min/mg protein)	**GR** (nmol/min/mg protein)	**GST** (nmol/min/mg protein)	**CAT** µmol/min/mg protein)	**TBARS** (nmol/g)
control	99.96 ± 21.20	15.60 ± 0.65	180.28 ± 5.17	69.42 ± 2.89	12.99 ±0.93
50 ng/l	88.51 ± 19.11	13.69 ± 0.45	197.77 ± 4.48	86.80 ±4.77	15.58 ±1.28
500 ng/l	**44.15 ± 9.72***	14.55 ± 0.53	184.86 ± 4.29	**90.66 ±5.69***	13.62 ± 1.04
5000 ng/l	71.02 ± 16.55	15.53 ± 0.59	**215.62 ± 5.65***	**115.42 ±5.13***	14.47 ±2.74
50000 ng/l	108.64 ± 12.59	14.80 ± 0.71	**238.94 ± 9.39***	**104.55 ±7.85***	17.54 ± 0.72

CONCLUSION

Our results showed that tonalide exposure had profound influence on the oxidative stress markers and detoxifying enzyme of the exposed zebrafish. The changes in antioxidant enzyme activities could be an adaptive response to protect the fish from the tonalide induced toxicity.

ACKNOWLEDGEMENTS

This research was supported by IGA 248/2015/FVHE.

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TROPHIC STATUS ASSESSMENT OF OČAGA RESERVOIR (LAZAREVAC, SRBIJA) BY CARLSON‘S INDEX

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PROCENA TROFIČKOG STATUSA AKUMULACIJE OČAGA (LAZAREVAC, SRBIJA) POSREDSTVOM CARLSON-OVOG INDEKSA

Apstrakt

Trofički status se može definisati kao ukupna težina živog biološkog materijala (biomase) u nekom vodenom telu na određenom lokalitetu i u određeno vreme. Trofički status nekog vodenog tela može se proceniti na osnovu merenja specifičnih parametara i predstavlja biološki odgovor na spoljašnje faktore, kao što su količina nutrijenata, sezonske promene, ispaša, mešanje vode itd. Procena trofičkog statusa jezera ili akumulacije je od velikog značaja. Postoje različiti kriterijumi za procenu trofičkog stanja jezera, kao što su: koncentracija nutrijenata, produktivnost, kvantitativni i kvalitativni sastav flore i faune, dostupnost kiseonika i morfometrija jezera. Međutim, imajući u vidu da je multiparametarski indeks nepraktičan, Carlson je predložio indekse trofičkog stanja (TSIs) koji su znatno jednostavniji za upotrebu od multiparametarskog indeksa. Ovi indeksi kao bazu za klasifikaciju trofičkih stanja koriste algalnu biomasu. U tom smislu koriste se tri parametra za izračunavanje ovih indeksa: koncentracija hlorofila a, ukupni fosfor i providnost izmerena Secchi diskom. Svaka od ove tri promenljive se može koristiti za klasifikaciju statusa vodenog tela, ali je hlorofila a najznačajniji parametar s obzirom da je direktni pokazatelj algalne biomase. Opseg Carlson-ovog trofičnog indeksa obuhvata vrednosti od 0 do 100. Vrednosti ispod 40 odgovaraju oligotrofnim, od 40 do 50 mezotrofnim, od 50 do 70 eutrofnim, a preko 70 hipereutrofnim jezerima i akumulacijama. Glavna prednost ovog indeksa je što odnos između tri parametra može ukazati na određene uslove koji vladaju u datom jezeru ili akumulaciji, a tiču se faktora koji limitiraju algalnu biomasu ili utiču na izmerene parametre. Iako određivanje trofičkog statusa vode nekog vodenog tela ne treba poistovećivati sa samim kvalitetom vode, ono, svakako, predstavlja značajan aspekt istog.

Cilj ovog istraživanja bio je određivanje trofičkog statusa jezera Očaga na osnovu Carlson-ovog trofičnog indeksa i ispitivanje veze između izračunatih indeksa za hlorofil a (TSI(CHL)), ukupni fosfor (TSI(TP)) i providnost merenu Secchi diskom (TSI(SD)).

Veštačko jezero Očaga nalazi se u blizini Lazarevca i isključivo se koristi za rekreaciju. Ispitivanje vode rađeno je jednom nedeljno tokom jula i avgusta, od 2012. do 2014. godine. Sve analize urađene su u Zavodu za javno zdravlje iz Beograda.

Vrednosti dobijenih indeksa (TSIs) varirale su od 41 do 86.25, u ispitanom periodu, a jezero je menjalo status od mezotrofnog, preko eutrofnog do hipereutrofnog, pri čemu su eutrofni uslovi preovladavali. Hipereutrofija je zabeležena tokom avgusta 2013. Godine, kada su bile izmerene i najveće vrednosti sva tri parametra, što je, verovatno, rezultat organskog opterećenja vodenog ekosistema i vremenskih uslova. Prema podacima RHMZ-a, 2013. godina je bila jedna od najtoplijih i najsušnijih godina još od 1951. godine. Nasuprot tome, u avgustu 2014. godine izmerene su najmanje vrednosti svih parametara, što ukazuje na mezotrofne uslove u jezeru te godine. Mezotrofni status jezera je, verovatno, posledica velike količine padavina u prvoj polovini te godine, ali i pražnjenja i ponovnog punjenja jezera nakon majskih poplava.

Ključne reči: jezero Očaga, Carlson-ov trofički indeks
Keywords: Očaga reservoir, Carlson's trophic index

INTRODUCTION

Trophic state can be defined as the total weight of living biological material (biomass) in a waterbody at a specific location and time, and it is understood to be the biological response to forcing factors such as nutrient additions, where the effect of nutrients can be modified by factors such as season, grazing, mixing depth, etc. (Carlson & Simpson 1996).

Carlson (1977) suggested the trophic state indices (TSIs) that use algal biomass as the basis for trophic state classification. TSIs are calculated using only three parameters: log-transformed Secchi disk transparency (SD), concentration of chlorophyll a (CHL) and the total phosphorus (TP). In practice, the index range is from 0 to 100. Generally, the TSIs value below 40 corresponds to oligotrophy, between 40 and 50 – mesotrophy, from 50 to 70 – eutrophy, and above 70 – hypereutrophy of the lake or reservoir (Carlson & Simpson 1996).

When more than one of the three variables are measured, it is possible that different index values will be obtained. Nevertheless, the interrelationships between variables can be used to identify certain conditions in the lake or reservoir that are related to the factors that limit algal biomass or affect the measured variables (Carlson & Simpson 1996).

Trophic state determination is an important aspect of every reservoir survey. On the other hand, trophic state is not the same thing as water quality, but the trophic state certainly is one aspect of the water quality (Carlson & Simpson 1996).

The objective of this study was to determine the trophic status of Očaga reservoir based on TSIs and to examine the relationship between the three calculated trophic state indices: TSI (SD), TSI (CHL) and TSI (TP).

MATERIALS AND METHODS

Sampling site

Očaga artificial lake (44˚23‘24.9”N, 20˚14‘14.88”E) was formed at the old open pit of lignite mining basin „Kolubara” for the purpose of remediation after exploitation. The bottom of the reservoir is concreted, while the coasts were made of stone. The surface area of the reservoir is 0.12 km^2. The reservoir is fed by groundwater as well as precipitation. The last regular cleaning was carried out at the end of 2013, however, after the floods in May 2014 the whole procedure was repeated. Očaga reservoir is primarily used for recreational purposes.

Methods

Sampling was conducted weekly for two summer months during the period of the three years (2012-2014), each time at the same sampling site (middle of the reservoir, from the depth of 0.5 m). The sampling procedure for Chl a and TP was carried out following a standard sampling technique described in SRPS IS0 5667-4:1997 standard. The transparency (m) of the reservoir was measured *in situ* using Secchi disc. In the laboratory, TP (µg/L) concentration was measured using SRPS EN ISO 6878:2008 standard, while Chl a (µg/L) concentration wasdetermined using spectrophotometric method according to standard procedure ISO 10260:1992. All measurements were conducted by the National Institute of Public Health, Belgrade.

The trophic state of the Očaga reservoir was determined by Carlson‘s TSI. Three equations were used for the index calculation: Secchi disk TSI (SD), chlorophyll a TSI (CHL) and total phosphorus TSI (TP), (Carlson & Simpson 1996).

Statistical analyses were done using CANOCO for Windows Version 5.0 (Ter Braak & Šmilauer 2012).

RESULTS AND DISCUSSION

The average values for SD, Chl a and TP are presented in Figure 1. The lowest documented SD value in Očaga reservoir was 0.4 m, in August 2013, while the highest value was in August 2014 (3.1 m). The highest values for TP (207 µg/L) and Chl a (73.04 µg/L) were observed in August 2013, while the lowest values were recorded in August 2014, 6 µg/L for TP and 2.07 µg/L for Chl a. This is in accordance with the obtained SD values during the mentioned periods.

Carlson’s TSI values were calculated using monthly average values for Chl a, TP and SD obtained on the basis of weekly measurements in July and August. The values of TSIs obtained during studied period ranged from 41 to 86.25 (Figure 2).

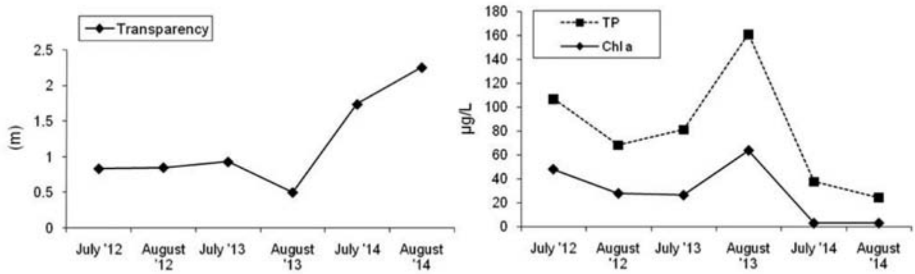

Figure 1. Average values for transparency (left), chlorophyll and total phosphorus (right).

The values of individual indices are more evenly distributed than the measured values of TP, Chl a and SD, but still the highest values of TSI (CHL) and TSI (TP) were observed in August 2013, and the lowest during 2014, and *vice versa* for TSI (SD), (Figure 2).

In general, Carlson's TSI values for all three parameters are high to very high, ranging from mesotrophy to hypereutrophy, but most often correspond to eutrophic model. High values of Carlson's TSI and the high concentration of Chl a and TP in the mentioned periods indicate to a very poor ecological condition. The sudden increase of TP in August 2013 is most probably due to anthropogenic impact and lack of rainfall, as this year was considered the hottest and the most arid since 1951 (RHMZ of Serbia 2014). Lower TSI values recorded during 2014 are probably a consequence of a heavy rainfall that was frequent during the first half of this year (RHMZ of Serbia 2015), as well as of the cleaning of the reservoir.

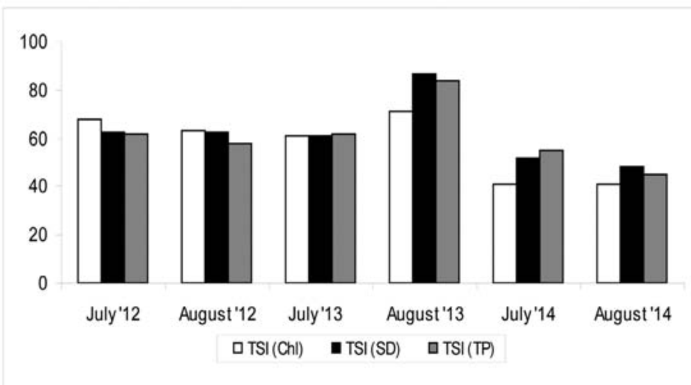

Figure 2. Carlson's TSI values for Chl a, SD and TP during the studied period.

Regarding to monthly average values (Figure 2), all TSI's were close together indicating the same trophic status, except in July 2014, were TSI(CHL) indicated mesotrophic status, while the other two pointed to eutrophy. During the whole study period the eutrophic conditions prevailed in July, while in August ecosystem was characterized as eutrophic in 2012, as hypereutrophic in 2013 and as mesotrophic in 2014.

According to Carlson & Simpson (1996) lake classification regarding trophic condition range, the reservoir in 2012 was eutrophic, in 2013 eu/hypereutrophic, while in 2014 meso/

eutrophic (Figure 3). Interrelationships between TSI’s based on main annual values follow TSI (CHL) = TSI (TP) = TSI (SD) pattern in 2012.

According to Carlson & Simpson (1996) this means that algae dominate light attenuation, where phosphorus is limiting factor. In 2013 and 2014 the ratio is TSI (TP) = TSI (SD) > TSI (CHL), and this pattern shows that non-algal particulates or color dominate light attenuation.

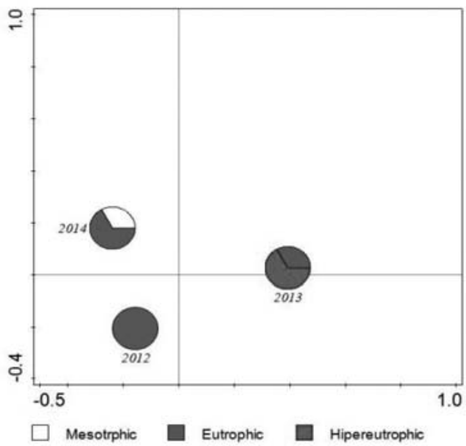

Figure 3. Trophic state based on the main annual values of Očaga reservoir

CONCLUSION

In Očaga reservoir, eutrophic conditions in general prevail, except in August 2013 and 2014. Hypereutrophic state that was observed during August 2013, when high values of all parameters (TP, Chl a and SD) have been measured, probably occurred as a result of organic load on ecosystems and weather conditions. The lowest measured values of all parameters and the state of mesotrophy in August 2014 are probably the consequence of strong rainfall that were frequent during this year and, consequently, emptying of the reservoir. It is worth mentioning that, for all parameters, Carlson’s trophic state indices were uniform during these two months.

ACKNOWLEDGEMENTS

Financial support was provided by the National Institute of Public Health, Belgrade.

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FISH POPULATION FROM KLOKOT AND KRUSNICA RIVERS

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POPULACIJE RIBA RIJEKA KLOKOT I KRUŠNICA

Apstrakt

Vode unsko-sanskog kantona spadaju u slabije istražena područja u Bosni i Hercegovini sa aspekta ihtiofaune, a to se pogotovo odnosi na pritoke Une. Uzevši u obzir činjenicu da je ihtiološko ispitivanje ribljih populacija lijeve pritoke Une - Klokot i desne pritoke Une - Krušnica, važno za ovo područje, provedna su istraživanja tokom dvije sezone, i to jesen - zima 2001.godine i proljeće - ljeto 2002. godine. Istražen je kvalitet i kvantitet izlovljenih ihtiopopulacija ova dva vodena biotopa. U istraživanjima je primjenjeno standardno ribolovno oruđe, elektroagregat i mreže. Dio izlovljenog materijala se direktno ispitivao na terenu, a veći dio je fiksiran i prenesen u laboratorij Biotehničkog fakulteta, Univerziteta u Bihaću. Ispitivanja osnovnih pokazatelja kvalitativnog i kvantitativnog sastava ihtiofaune rijeke Klokot i Krušnice provedena su na tri lokaliteta (izvor, srednji tok i ušće) na njihovom longitudinalnom profilu. U rijeci Klokot ukupno je izlovljeno 287 jedinki deset vrsta riba koje su raspoređene u pet familija, a u rijeci Krušnici 108 jedinki osam vrsta riba također zastupljenih u istih pet familija: *Salmonidae, Thymallidae, Cyprinidae, Esocidae i Cottidae.*

Ključne riječi: populacija, ribe, rijeka
Keywords: population, fish, river

INTRODUCTION

Hydrographic and geological characteristics in the area of basin of river Una are partly examined, which is positive, but, waters of Una-Sana basin belong to less researched areas in terms of ichthyofauna in Bosnia and Herzegovina. These water systems are interesting to explore both ichthyofauna and other characteristics such as quality of ecosystem, and biodiversity of flora and fauna. River Una springs bellow mountain Čemernica and hill Lisina in the village of Donja Suvaja. The length of river flow is 210,35 kilometers. In the upper course, from the river spring to Bihać (69,63 km), river has the features of a real mountain river. From Bihać to Bos. Novi (72,78 km) river has characteristics of hill river, and on the area from Bos. Novi to rivermouth (67,94 km) the river Una is a lowland river. Una flows into Sava River near Jasenovac on 95 meters above sea level. The most important right tributaries of river Una are Unac, Krušnica and Sana. After these three rivers, there are two more tributaries:Mlječanica and Moštanica, while the most important left tributaries of river are Klokot and Žiravac (Spahić, 1991; Alagić i sar., 1994; IBG, 2003). In addition to the above mentioned tributaries, significant amounts of water, especially in periods of intense rainfall, Una receives water from several big border-land sources along the flow, of which the richest with water are sources in the area of Kulen Vakuf. River Klokot springs near Bihać in the foothills of the mountain Plješevice, around 5 kms of the city, and represents the biggest water well for supplying the population of Bihać with drinking water. Length of the river is 6 km, the average width 18-22 m, and the depth of 5-7 m. Along the river course, riverbed is incised into the diluvial and alluvial deposits. River flow direction is from west to east and represents the boundary of the upper and middle course of the river Una. The rivermouth of Klokot into Una is located 1 km downstream of the town Bihać.

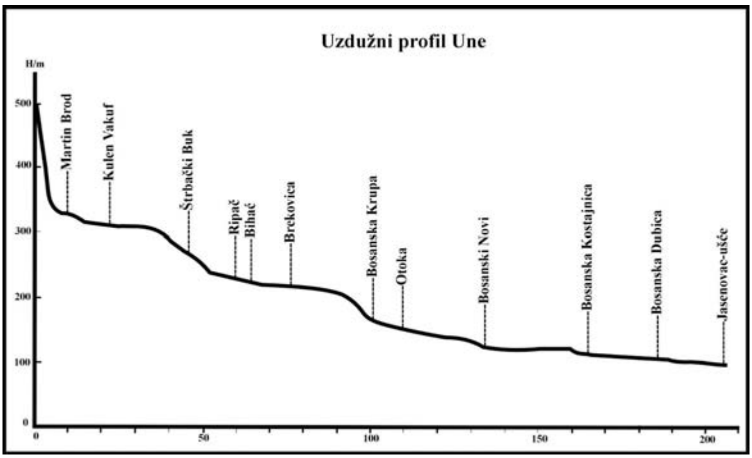

Figure 1. The longitudinal profile of river Una (Sofradžija et al., 2002)

River Krušnica springs around village Gudavac in municipality Bosanska Krupa, at the foot of the mountain of Grmeč. River springs in the cave in which waters of Grmeč and surrounding hills are collected. The length of river is 6,8 km, average width is 20 m, and average depth varies from 5 to 7 m. The width of Krušnica on the rivermouth is 15 m. It springs on 200 m above sea level, and rivermouth of Una is located on 140 m above sea level (DMA, 1994), so the difference in sea level along the course is of 60 m (Ajanović, 1999). Krušnica is right tributary in the middle part of Una's flow and represents oasis of ecology preservation in area of municipality Bosanska Krupa. With its tributary, Una belongs to Black Sea basin, and fish populations which settle this area are spread wider in Europe. There are no endemic species of fish in Una's basin, but it doesn't diminish the importance of study of fish populations of river Una, and especially its smaller tributaries which are interesting from the aspect of applicative fisheries. This fact particularly refers to Klokot, left Krušnica, right tributary of river Una, which attracts great significance recently. There is fish farm on the river Klokot, where trout is reared and grayling is spawned. Taking into account all mentioned above, we consider that it is very important and useful to determine real state of fish populations in this two tributaries. With the realization of this goal we would have complete insight about the composition of fish populations of these two tributaries of river Una, which would create a solid basis for the completion of knowledge about the diversity of ichthyofauna of aquatic ecosystems of Una - Sana Canton. Introduction of fish populations of particular area is significant for fishing, but also from the aspect of protection of. In the spotlight of this work the following aims are set: to determine the qualitative and quantitative composition of ichthyopopulations in three localities (source, flow and the rivermouth) at longitudinal profile of Klokot and Krušnica. 4 families are present in the River Krušnica: *Salmonidae, Thymallus, Cyprinidae* and *Cottidae* (,'UNA I'' 1984). Family of Salmonidae family is represented with 2 species and they are *Salmo trutta fario* and *Hucho hucho. Thymallus thymallus* represents family Thymallidae. From Cyprinidae family 4 species are registered, and they are: *Chondotostoma nasus*, *Leuciscus cephalus*, *Barbus barbus* and *Rutilus pigus virgo.* Only one species from the *Esocidae* family is found: *Esox lucius.* In Klokot River, according to the same literature data as for Krušnica, in period August-October 1984, families Salmonidae, Thymallidae, Cyprinidae i Esocidae are registered.

MATERIALS AND METHODS

A detailed field study of river Klokot, and river Krušnica have been done during two seasons. First field was done in October -November 2001, and second at the end of May, 2002. To determine the qualitative and quantitative composition of ichthyopopulations on longitudinal profile of these rivers, three localities were selected: spring, middle flow and rivermouth. Sampling was done using nets and electrical aggregates. Gillnets, seine net type with mesh diameter of 10-36 mm, are used for sampling. Nets were placed in the evening and taken out in the early morning at middle flow and rivermouth. Sampling at the river spring was carried out using electrical aggregate „Honda" EZ 2.200, volume 2kV and electrical aggregate „ELT 61 II" 300/500 V, while fish net with mesh diameter 3x3 was also used. During the study, a smaller number of individuals of fish were processed on the field, while the largest number of collected material was fixed in 4% formalin and transferred into laboratory of Biotechnical Faculty for further analysis. Fish determination was done according to Vuković i Ivanović (1971). The results of researches were statistically processed according to Petz (1985).

RESULTS AND DISCUSSIONS

Ichthyological studies of rivers Klokot and Krušnica were conducted in three localities of each river during two seasons and resulted in the following number of caught individuals: river Klokot - 287, river Krušnica - 108. In river Klokot in the season autumn-winter 2001, 237 individuals were caught while in the season spring-summer the number of caught individuals was 50. In river Krušnica in the season autumn-winter 30 individuals were caught and in the season spring- summer the number of caught individuals was 78. Based on caught samples, qualitative and quantitative structures of ichthyopopulations of rivers Klokot (Table 1) and Krušnica (Table 2) were made.

Table 1. Qualitatively - quantitative structures of the ichthyofauna of the river Klokot in 2001/2002 - summary view

Family	No.	Fish species	Indivi-duals	%	Total weight(g)	% mass
Salmonidae	1.	Brown trout-*Salmo trutta m. fario*,	36	12,5	9 010,5	49,1
	2.	Rainbow trout-*Oncorhynchus mykiss*	9	3,13	627,2	3,42
Thymallidae	3.	Grayling-*Thymallus thymallus*,	2	0,69	1 286,3	7,0
Esocidae	4.	Pike-*Esox lucius*,	3	1,04	2 121,7	11,5
Cyprinidae	5.	Chub-*Leuciscus cephalus*,	2	0,69	1 239,5	6,76
	6.	Red-eyed fish-*Rutilus rutilus*,	31	10,8	2 451,7	13,3
	7.	Pomfret-*Rutilus pigus virgo*,	3	1,04	1 083	5,91
	8.	Minnow -*Phoxinus phoxinus*,	186	64,8	345	1,88
	9.	Bleak-*Alburnus alburnus*,	2	0,69	47,8	0,26
Cottidae	10.	Lappet-*Cottus gobio*,	13	4,52	102,5	0,55
TOTAL			**287**	**100**	**18 315,2**	100

It is visible from the table above that there is in the ichthyofauna of river Klokot a presence of Ten species from five families were found in River Klokot. One species from the Esocidae is found: *Esox lucius* which is caught in both seasons. This species is found in Klokot River, but not in the Una River. Fish from Cyprinidae family were most numerous, in the river Klokot: *Phoxinus phoxinus*, *Rutilus rutilus*, *Rutilus pigus virgo*, *Leuciscus cephalus* and *Alburnus alburnus*, which was by ichthyological research of Una basin first time registered in Klokot, (Sofradžija i sar., 2002). During spring-summer period of 2002 there no bleak and pomfret fish were present. In the river Klokot in both seasons presence of just one species from the Thymallidae was found *Thymallus thymallus*, typical species widely spread in the waters of the Black Sea basin. The Salmonidae,found in both seasons, is represented by two species: *Salmo trutta m. fario* and *Oncorhynchus mykiss.* Cottidae is represented by one species: *Cottus gobio*, and its presence was found in both seasons too.

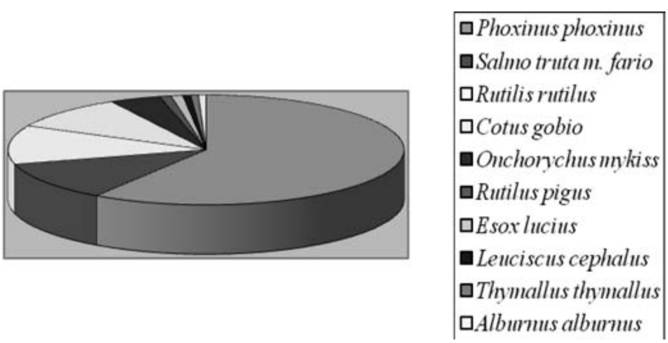

Figure 2. The relative number of fish found in the whole sample of river Klokot, summary view

In river Krušnica Salmonidae is represented by two species: *Salmo trutta m. fario*, registered in both seasons and *Oncorhynchus mykiss* which was catched in autumn-winter season 2001, and it was not registered in 2002, spring-summer season. Esocidae is represented by one species *Esox lucius*, which is found only in the spring- summer season, 2002. Thymallidae is represented by *Thymallus thymallus* whose units were found in both seasons.

According to the number of registered species, the most numerous family is Cyprinidae, represented by three species: *Leuciscus cephalus*, *Phoxinus phoxinus* and *Rutilus pigus virgo* found in both seasons, while the chub was caught only in the autumn-winter 2001. *Cottidae* was represented by *Cottus gobio*, found in both seasons.

Table 2. Qualitative-quantitative composition of the ichthyofauna of the river Krušnica in 2001/2002- summary view

family	No.	Fish species	Number of units	%	Total weight(g)	% mass
Salmonidae	1.	Brown trout -*Salmo trutta morpha fario*,	21	19,4	4 347	36,3
	2.	Rainbow trout- *Oncorhynchus mykiss*,	1	0,92	293,6	2,45
Thymallidae	3.	Grayling -*Thymallus thymallus*	15	13,8	2 072,7	17,3
Esocidae	4.	Pike -*Esox lucius*,	1	0,92	104,2	0,87
Cyprinidae	5.	Chub -*Leuciscus cephalus*,	5	4,62	2 168,3	18,1
	6.	Pomfret-*Rutilus pigus virgo*,	4	3,7	2 551,9	21,3
	7.	Minnow-*Phoxinus phoxinus*	29	26,8	134,7	1,12
Cottidae	8.	Lappet *Cottus gobio*	32	29,6	294,9	2,46
TOTAL			**108**	**100**	**11. 967,3**	**100**

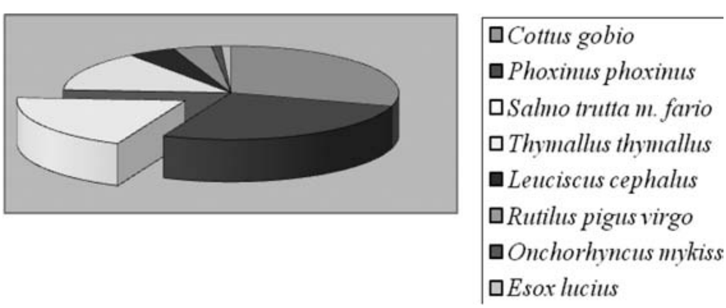

Figure 3. The relative number of fish, found in the whole sample of river Krušnica, summary view

If we omit two types that were not found in Krušnica, bleak and red-eyed fish, but occurred in Klokot, we can conclude that of eight species that occur in both rivers, minnow is definitely the most numerous in Klokot, and lappet in Krušnica. Conducted ichthyological research on rivers Klokot and Krušnica, during two seasons, gives us information about the types of fish that are not registered, although they were found in river Una, according to Sofradžija et al. (2002). In Klokot and Krušnica rivers, *Barbus barbus*, *B. meridionalis petenyi*, *Alburnoides bipunctatus,* from the Cyprinidae family, and *Hucho hucho* from Salmonidaefamily were not found. According to the same research in the tributaries of the Una - Klokot and Krušnica, as well as in the river Una are registered species which occur in all three mentioned biotope: *Salmo trutta m. fario*, *Oncorhynchus mykiss*, *Thymallus thymallus* and *Cottus gobio.* From the previous data, we can conclude that there are no big differences in the qualitative composition of the parent river and its two tributaries. Considering the anthropogenic influence, especially uncontrolled and illegal fishing and the war consequences, as well as industrial and municipal wastewater issue and neglected and solid waste around these rivers, the presence of established number of fish in the whole sample gives satisfying results.

CONCLUSION

According to analysis in this paper and the overall results obtained in ichthyofaunistic study of aquatic ecosystems of river Klokot and Krušnica, which were made in the course of two seasons, autumn - winter 2001 and spring - summer 2002, with the application of a standard fishing tools, it is possible to bring out several important conclusions: It was determined that ten species of fish, which are grouped into five families: Salmonidae, Cyprinidae, Esocidae, Thymallidae and Cottidae inhabit Klokot, and eight species of fish also classified in these five families, inhabit river Krušnica. In the ichthyofauna of rivers Klokot and Krušnica families Esocidae, Thymallidae, Cottidae are presented by one species *Esox lucius, Thymallus thymallus*, *Cottus gobio*. Salmonidae was represented with two species in Klokot and Krušnica, *Salmo trutta m. fario* and *Oncorhynchus mykiss*. Cyprinidae is the most numerous in the both rivers, and it is represented by 5 species in the river Klokot: *Leuciscus cephalus*, *Phoxinus phoxinus*, *Alburnus alburnus*, *Rutilus pigus virgo*, *Rutilus rutilus*. In the river Krušnica Cyprinidae is represented with three species which also appear in Klokot, with the exceptions of fish *Rutilus rutilus*, *Alburnus alburnus*. Considering insufficient exploration of water and river flows in the Una-Sana Canton, it is necessary to make an inventory of ichthyofauna of aquatic ecosystems which are part of Una-Sana Canton waters. In order to take concrete measures of protection, of indigenous fish populations primarily, it is necessary to prevent an unplanned fish stocking, importation of new species and uncontrolled and illegal fishing; this was also recommended within the monitoring and research of aquatic biotopes. In future the research of ichthyofauna of rivers Klokot and Krušnica should be expanded to four seasons; autumn, winter, spring and summer, and on the longitudinal profile of these rivers at least six locations should be chosen for ichthyological studies.

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REINTRODUCTION OF THE BURBOT TO THE HUNGARIAN AQUACULTURE (PRELIMINARY RESULTS)

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PONOVO UVOĐENJE MANIĆA U AKVAKULTURU MAĐARSKE (PRELIMINARNI REZULTATI)

Apstrakt

Manić (*Lota Lota*) je nativna vrsta ribe u Mađarskoj i poznato je da on živi skoro u svim našim rekama. Među pecarošima, ova vrsta je veoma popularna mada je retka kada je reč o ulovu. Dužina većih jedinki varira između 40 i 50 santimetara, retko dostiže dužinu od 60 santimetara, a državni rekord od 3,56 kg dostignut je 2001. godine (Harka and Sallai, 2007). Mađarska industrija za akvakulturu je zainteresovana za gajenje ove vrste već neko vreme, međutim uprkos činjenici da su neki strani istraživački timovi počeli da rade na istraživanju metoda za gajenje manića (Żarski et al., 2010; Trabelsi et al., 2011; Lahnsteiner et al., 2012;), ova vrsta nema detaljno razvijenu tehnologiju za reprodukciiju i uzgoj. Prethodna istraživanja na nivou države (e.g. Keresztessy and Rideg, 2001) i povećane potrebe potrošača i pecaroša podstakli su nas da ponovo započnemo uzgoj i reprodukciju manića.

Nedavno su u Mađarsku dostavljene 2 populacije manića gajene u Poljskoj u RAS sistemu: 08.10.2014 dostavljeno je 1000 larvi prosečne težine 15g, a 15.12.2014 dostavljeno je 100 matica prosečne težine od 210g iz laboratorija Univerziteta Warmia i Mazury. Ribe su i u Mađarskoj uvedene u RAS sistem, na privatnom ribnjaku Zoltána Szabó. Larve su hranjene sa Scretting hranom za pastrmke (proteina: 42%, masti: 14%), na početku sa 140 g/

dnevno, a kasnije i do 13.04.2015 sa 280 g/dnevno. Do tog datuma, prosečna težina jedinki dostigla je 62 g, a ukupno 96 jedinki je uginulo (stopa preživljavanja: 90,4%).

Ista hrana, Scretting, korišćena je i za hranjenje matica za reprodukciju, međutim posle mesec dana, promenili smo je i počeli da koristimo Aquabio (proteina: 54%, masti: 17%). Do 13.04.2015 prosečna težina dostigla je 300 g, a stopa preživljavanja bila je 50% zbog bakterijske infekcije. Temperatura vode bila je konstantna: 14˚C.

Da bi izvršili reprodukciju, stavili smo 40 ženki u tank od 700 l, u kome smo za jako kratak vremenski period snizili temperaturu na 2-2,5 ˚C. Uspeli smo da istisnemo ikru od 13 jedinki u tri različita dana (20.03, 24.03, i 26.03) sa stopom uspeha od PGSI: 11,75 % ±11,75; 24,43 % ±4,40; 12,92% ±3,62).

Osim tehnoloških eksperimenata za gajenje i reprodukciju, izvršili smo preliminarni ekperiment prerade ribe, u kome smo testirali sledeće parametre: težinu creva, jetre, glave, kičme i mesa, karakteristike ribljeg mesa, reakcije u toku pripreme u kuhinji i ukus nakon pripreme.

Rad je podržan projektom 8526-5/2014/TUDPOL Ministarstva Ljudskih Resursa Mađarske.

Abstract

Burbot (*Lota Lota*) is a native species in Hungary and is known to live in most of our rivers. It is a popular but slightly rare catch among anglers. The length of the larger sized individuals varies between 40 and 50 centimeters, rarely reaches 60 cm, the national record is 3,56 kg from 2001 (Harka and Sallai, 2007). The Hungarian aquaculture industry is interested in rearing this species for a while, however in spite of some foreign research teams having started to work on investigating the rearing of burbot (Żarski et al., 2010; Trabelsi et al., 2011; Lahnsteiner et al., 2012;), this species does not have a developed and detailed reproduction and rearing technology. The previous national studies (e.g. Keresztessy and Rideg, 2001) and the increasing consumer and angler needs made us to begin the rearing and reproduction of burbot again.

Two Polish, RAS reared burbot population delivery arrived to Hungary lately: 1000 pcs larvae with 15 g average weight on 08.10.2014 and 100 pcs broodstock with 210 g average weight on 15.12.2014 from the laboratory of the Warmia and Mazury University. The fish were introduced to RAS system here as well, in the fish farm of Zoltán Szabó self-entrepreneur. Larvae were fed with Scretting trout feed (protein: 42%, fat: 14%), at the start in an amount of 140 g/day, later, until 13.04.2015 in an amount of 280 g/day. By this time, the average weight reached 62 g, and altogether 96 individuals died (survival rate: 90,4%)

For feeding the broodstock intended for reproduction, the feed was the same Scretting feed, and after one month, we have changed to Aquabio (protein: 54%, fat: 17%). By 13.04.2015 the average weight reached 300 g, survival rate is 50% due to a bacterial infection. The water temperature was constant 14˚C.

For the purpose of reproduction, we have placed 40 females into a 700 l tank, where we have reduced the water temperature to 2-2,5 ˚C in a very short time. We could strip eggs from 13 individuals at 3 different dates (20.03, 24.03, and 26.03) and success rates (PGSI: 11,75 % ±11,75; 24,43 % ±4,40; 12,92% ±3,62).

In addition to the rearing and reproduction technology experiments, we have conducted a preliminary fish processing experiment as well, where we tested the following parame-

ters: weight of intestines, liver, head, spine, and meat, the characteristics of the fish meat, reactions during kitchen preparation and taste after preparation.

The work was supported by the project number 8526-5/2014/TUDPOL of the Ministry of Human Resources of Hungary.

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EFFECTS OF DIETARY INCLUSION OF ASTAXANTHIN ON GROWTH, MUSCLE PIGMENTATION AND ANTIOXIDANT ACTIVITY OF JUVENILE RAINBOW TROUT (*ONCORHYNCHUS MYKISS*)

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EFEKTI KORIŠĆENJA ASTAKSANTINA U ISHRANI NA RAST, PIGMENTACIJU MIŠIĆA I ANTIOKSIDANTNE AKTIVNOSTI MLAĐI KALIFORNIJSKE PASTRMKE (*ONCORHYNCHUS MYKISS*)

Apstrakt

Cilj ovog istraživanja bio je da testira efekte korišćenja astaksantina u ishrani na rast, pigmentaciju mišića i antioksidantnu aktivnost i biohemijski sastav mlađi kalifornijske pastrmke (*Oncorhynchus mykiss*). Eksperimentalna hrana formulaisana je tako da sadrži 50, 70 i 100 ppm astaksantina (označen kao AS50, AS75 and AS100). Hrana koja nije bila obogaćena astaksantinom smatrana je kontrolnom hranom. U periodu od deset nedelja, ribe su hranjene svakom eksperimentalnom hranom (18.5 grama / ribi) do vidljivog zasićenja, dva puta dnevno. Eksperiment je rađen u triplikatu. AS nivo u hrani ($P > 0.05$) nije uticao na performansu rasta i hemijski sastav mišića ribe. Ukupna koncentracija karotenoida u mišiću ribe koja je hranjena AS50 hranom bila je viša nego kod riba hranjenih kontrolnom hranom, ali nije bila drugačija od riba hranjenih AS75 i AS100 hranom. Koncentracija astaksantina u mišiću riba hranjenih AS50, AS75 i AS100 hranom bila je viša nego kod riba hranjenih kontrolnom hranom. Crvena boja (a*) mišića ribe koja je hranjena AS50, AS75 i AS100 hranom bila je jača nego kod ribe hranjene kontrolnom hranom ($P < 0.05$). Antioksidantna aktivnost DPPH, radikala hidroksila i alkila u plazmi i jetri riba nisu zavisili od nivoa astaksantina osim kod plazme koja je imala antioksidantnu aktivnost alkilnih radikala. Rezultati ove studije nagoveštavaju da se hrana koja sadrži 50 ppm astaksantina može koristiti da bi se poboljšala crvena boja mišične pigmentacije kod mlađi kalifornijske pastrmke.

Abstract

This study was designed to test the effects of dietary astaxanthin on growth, muscle pigmentation, antioxidant activity and biochemical composition of juvenile rainbow trout (*Oncorhynchus mykiss*). Experimental diets were formulated to contain 50, 75 and 100 ppm astaxanthin (designed as AS50, AS75 and AS100). The diet without supplementation of astaxanthin was considered as the control diet. Each experimental diet was fed to three replicate groups of fish (18.5 g/fish) to visual satiation two times a day for 10 weeks. Growth performance and proximate composition of muscle of fish were not affected by dietary AS levels ($P > 0.05$). Total carotenoid concentration in the muscle of fish fed the AS50 diet was higher than that of fish fed the control diet, but no different to that of fish fed the AS75 and AS100 diets. The astaxanthin concentration in the muscle of fish fed AS50, AS75 and AS100 diets were higher than that of control diet. The redness (a*) of the muscle of fish fed AS50, AS75 and AS100 diets were higher than that of fish fed the control diet ($P < 0.05$). DPPH, hydroxyl and alkyl radical scavenging activities in the plasma and liver of fish were not affected by dietary astaxanthin level except for the plasma of alkyl radical scavenging activity. The results of this study suggest that a diet contained 50 ppm astaxanthin could be used for improve red color of muscle pigmentation of juvenile rainbow trout.

PROBIOTICS AND HERBS IN CARP (*CYPRINUS CARPIO* L.) POND AQUACULTURE – IMPACT ON FISH GROWTH, HEALTH AND PRODUCTION EFFICIENCY

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PROBIOTICI I LEKOVITO BILJE U UZGOJU ŠARANA (*CYPRINUS CARPIO* L.) U ZEMLJANIM BAZENIMA - UTICAJ NA PRIRAST RIBE, ZDRAVLJE I PROIZVODNE REZULTATE

Apstrakt

Cilj ovog rada je da prikaže rezultate ishrane šarana tradicionalnom smešom žitarica (tritikale + pšenica) sa dodatkom probiotika i/ili lekovitog bilja.

Kao probiotik korišćen je koncentrat EmFarma, koji je obezbedio „ProBiotics Polska" iz Poljske. Ova smesa sadrži skup sledećih mikroorganizama, bakterija i gljivica: *Bifidobacterium animalis, Bifidobacterium bifidum, Bifidobacterium longum, Lactobacillus acidophilus, Lactobacillus bulgaricus, Lactobacillus casei, Lactobacillus delbrueckii, Lactobacillus plantarum, Lactococcus diacetylactis, Lactococcus lactis, Streptococcus thermophilus, Bacillus subtilis var natto, Saccharomyces cerevisiae and Rhodopseudomonas palustris*. Koncentrat probiotskih mikroorganizama dodat je u mleveni kukuruz u količini od 2 litra preparata na 1 tonu hraniva. Pre dodavanja hranivima probiotski preparat je razređen u vodi. Količina dodate vode bila je oko 10% od težine hrane. Posle mešanja probiotika sa kukuruzom, hranivo je ostavljeno dva sata da nabubri.

Lekovito bilje sastojalo se od praha *Terminalia chebula, Phyllantus emblica, Andrographis paniculata, Tinospora cordifolia* i *Boerhaavia difusa*. Biljni preparat dobijen je od kompanije Farmwet iz Poljske. Mešavina bilja dodata je u količini od 3 kg na tonu kukuruzne prekrupe. Bilje u prahu pomešano je sa prekrupom, zatim navlaženo vodom u količini od oko 10% od doze hrane i ostavljeno dva sata da nabubri.

Ispitivanje je vršeno na šest hranidbenih grupa:
- I - samo prirodna hrana (kontrolna grupa)
- II - smeša sa kukuruznom prekrupom
- III - smeša sa kukuruznom prekrupom obogaćena probioticima
- IV - smeša sa kukuruznom prekrupom obogaćena lekovitim biljem
- V - smeša sa kukuruznom prekrupom obogaćena probioticima i lekovitim biljem
- VI - peletirana hrana Aller Aqua (referentna grupa)

Eksperimentalne smeše korišćene su za ishranu šaranske mlađi (C1), dvogodišnjaka za dalji uzgoj (C2) i konzumnih šarana (C3). Gustine nasada šarana bile su sledeće:
- za C1– 20000 jedinki/ha
- za C2– 5000 jedinki/ha
- za C3–1500 jedinki/ha

Sledeći parametri su mereni i analizirani:
- završna telesna masa (g/jedinka)
- prinos (kg/ha)
- stopa preživljavanja (S)
- stopa konverzije hrane, FCR (kg)
- Fultonov koeficijent (F)
- broj parazita (*Trichodina–Trich., Chilodonella–Chil., Epistylis–Epist., Costia*,)
- nivo lizozima (mg/l)
- nivo gama globulina (g/l)

Ključne reči: šaran, tradicionalan uzgoj u bazenima, održivost, probiotici, bilje

Tabela 1. Rezultati ishrane šaranske mlađi (C1) smešom žitarica sa dodatkom probiotika i/ili lekovitog bilja

Grupa	Težina g/jed.	S (%)	FCR (kg)	F	Prinos kg/ha	*Trich.*	*Chil.*	*Epist.*	*Costia*	Lisozyme	Gama globu-lini
I	29	42	0	1,68	241	18	28	22	17	$2,3^{b}$	$9,6^{b}$
II	66	45	2,6	1,71	594	17	22	19	33	$2,6^{ab}$	$6,9^{a}$
III	66	64	2,4	2,18	845	16	11	9	12	$1,8^{a}$	$7,1^{ab}$
IV	66	50	2,3	1,93	660	5	5	6	10	$2,4^{ab}$	$10,0^{b}$
V	64	63	2,7	1,82	806	8	11	11	11	$3,2^{b}$	$8,7^{ab}$
VI	75	83	0,7	2,01	1245	0	1	0	0	$3,1^{b}$	$10,4^{b}$

U okviru kolona, podaci obeleženi različitim slovima značajno se razlikuju (P<0,05), a obeleženi istim slovima nalaze se u istoj homogenoj grupi.

Tabela 2. Rezultati ishrane dvogodišnjih šarana za dalji uzgoj (C2) smešom žitarica sa dodatkom probiotika i/ili lekovitog bilja

Grupa	Težina g/jed.	S (%)	FCR (kg)	F	Prinos kg/ha	*Trich.*	*Chil.*	*Epist.*	*Costia*	Lisozyme	Gama globulini
I	86	45	0	1,18	196	35	31	42	21	0,82[a]	6,6[a]
II	209	92	2,2	1,56	967	23	39	22	32	0,96[a]	10,6[b]
III	216	82	1,8	1,51	891	12	12	6	11	-	-
IV	214	98	1,7	1,52	1043	12	4	8	0	0,78[a]	16,6[c]
V	315	85	1,2	1,70	1350	3	4	2	3	1,29[b]	15,3[c]
VI	346	98	0,9	2,02	1695	5	0	0	2	1,03[ab]	15,5[c]

U okviru kolona, podaci obeleženi različitim slovima značajno se razlikuju ($P<0,05$), a obeleženi istim slovima nalaze se u istoj homogenoj grupi.

Tabela 3. Rezultati ishrane konzumnih šarana (C3) smešom žitarica sa dodatkom probiotika i/ili lekovitog bilja

Grupa	Težina g/jed.	S (%)	FCR (kg)	F	Prinos kg/ha	*Trich.*	*Chil.*	*Epist.*	*Costia*	Lisozyme	Gama globulini
I	658	67	0	1,32	661	12	18	22	11	1,6[a]	11,8[c]
II	1367	100	4,0	1,93	2051	13	14	22	32	2,3[b]	5,5[a]
III	1386	100	3,5	1,92	2079	10	15	6	21	2,0[ab]	8,2[b]
IV	1325	100	3,8	1,97	1988	0	3	2	2	1,7[ab]	9,4[b]
V	1391	100	3,6	2,02	2087	1	3	4	3	1,9[ab]	9,3[b]
VI	1749	100	2,0	2,10	2624	2	0	1	0	1,8[ab]	9,9[b]

U okviru kolona, podaci obeleženi različitim slovima značajno se razlikuju ($P<0,05$), a obeleženi istim slovima nalaze se u istoj homogenoj grupi.

Korišćenje probiotika i lekovitog bilja imalo je pozitivan efekat na prirast, stopu preživljavanja i ukupni prinos šarana koji su gajeni u zemljanim bazenima i hranjeni tradicionalnom smešom žitarica;

Korišćenje probiotika i/ili lekovitog bilja imalo je pozitivan efekat na uzimanje hrane, a stopa konverzije hrane (FCR) obično je bila smanjena 10% - 15% u grupama u kojima su korišćeni dodaci;

Dodatak probiotika i/ili lekovitog bilja smanjio je broj najčešćih spoljnih parazita kod šarana (*Trichodina, Chilodonella, Epistylis* i *Costia*);

Probiotici i lekovito bilje kod šarana su pokazali pozitivan efekat na otpornost na bolesti. Tradicionalna smeša žitarica obogaćena probioticima i/ili lekovitim biljem stimulativno je delovala na povećanje nivoa gama globulina. Pozitivan efekat bio je posebno uočen kada su oba dodatka korišćena zajedno;

Kod šaranske mlađi (C1) probiotici i lekovito bilje ne treba da se primenjuju zajedno jer je u tom slučaju primećen smanjen prirast i nivo gama globulina.

Abstract

The aim of the paper is to present the results of feeding carp with traditional grain diet (triticale + wheat) supplemented with probiotics and/or herbs.

As probiotics, the EmFarma concentrate, provided by ProBiotics Polska, Poland,was used. This preparation contains consortia of the following microbial bacteria and fungi: *Bifidobacterium animalis, Bifidobacterium bifidum, Bifidobacterium longum, Lactobacillus acidophilus, Lactobacillus bulgaricus, Lactobacillus casei, Lactobacillus delbrueckii, Lactobacillus plantarum, Lactococcus diacetylactis, Lactococcus lactis, Streptococcus thermophilus, Bacillus subtilis var natto, Saccharomyces cerevisiae and Rhodopseudomonas palustris*. The concentrate of probiotic microorganisms was added to the ground corn in an amount of 2 liters of preparation per 1 ton of feed. Prior to adding to the feedstuff, the probiotics were diluted in water. The amount of added water was approximately 10% of feed weight. After mixing probiotics with corn, the feed was left for two hours for swelling.

The composition of herbs consisted of powdered *Terminalia chebula, Phyllantus emblica, Andrographis paniculata, Tinospora cordifolia* and *Boerhaavia difusa*. The herbal preparation was obtained from the Farmwet company, Poland. The blend of herbs was added in the amount of 3 kg per ton of ground corn feed. The powdered herbs were mixed with grinded corn, moistened with water amounting to approximately 10% of feed dose and left for two hours for swelling.

Six feeding groups were examined:

- I - natural food only (control group)
- II - ground mix of corn
- III - ground mix of corn supplemented with probiotics
- IV - ground mix of corn supplemented with herbs
- V - ground mix of corn supplemented with probiotics and herb
- VI - pelleted feed Aller Aqua (referential group)

Experimental diets were used for feeding carp fingerlings (C1), two-year restocking material (C2) and consumable carps (C3). Stocking densities of carp, were as follows:

- for C1– 20000 ind./ha
- for C2– 5000 ind./ha
- for C3–1500 ind./ha

The following parameters were measured and analyzed:

- final body mass (g/ind.)
- yield (kg/ha)
- survival rate (S)
- FCR (kg)
- Fulton's coefficient (F)
- number of parasites (*Trichodina–Trich., Chilodonella–Chil., Epistylis–Epist., Costia*,)
- level of lisozyme (mg/l)
- level of gamma globulins (g/l)

Key words: carp, traditional pond aquaculture, sustainability, probiotics, herbs

RESULTS

Table 1. The results of feeding carp fingerlings (C1) with grain diet supplemented with probiotics and/or herbs

Group	Weight g/ind.	S (%)	FCR (kg)	F	Yield kg/ha	*Trich.*	*Chil.*	*Epist.*	*Costia*	Lisozyme	Gamma globu-lins
I	29	42	0	1.68	241	18	28	22	17	2.3^{b}	9.6^{b}
II	66	45	2,6	1.71	594	17	22	19	33	2.6^{ab}	6.9^{a}
III	66	64	2,4	2.18	845	16	11	9	12	1.8^{a}	7.1^{ab}
IV	66	50	2,3	1.93	660	5	5	6	10	2.4^{ab}	10.0^{b}
V	64	63	2,7	1.82	806	8	11	11	11	3.2^{b}	8.7^{ab}
VI	75	83	0,7	2.01	1245	0	1	0	0	3.1^{b}	10.4^{b}

Within columns, data with different letters differs significantly (P<0,05), with the same letters are in the same homogenous group.

Table 2. The results of feeding two-year carp stocking material (C2) with grain diet supplemented with probiotics and/or herbs

Group	Weight g/ind.	S (%)	FCR (kg)	F	Yield kg/ha	*Trich.*	*Chil.*	*Epist.*	*Costia*	Lisozyme	Gamma globu-lins
I	86	45	0	1.18	196	35	31	42	21	0.82^{a}	6.6^{a}
II	209	92	2.2	1.56	967	23	39	22	32	0.96^{a}	10.6^{b}
III	216	82	1.8	1.51	891	12	12	6	11	-	-
IV	214	98	1.7	1.52	1043	12	4	8	0	0.78^{a}	16.6^{c}
V	315	85	1.2	1.70	1350	3	4	2	3	1.29^{b}	15.3^{c}
VI	346	98	0.9	2.02	1695	5	0	0	2	1.03^{ab}	15.5^{c}

Within columns, data with different letters differs significantly (P<0,05), with the same letters are in the same homogenous group.

Table 3. The results of feeding consumable carps (C3) with grain diet supplemented with probiotics and/or herbs

Group	Weight g/ind.	S (%)	FCR (kg)	F	Yield kg/ha	*Trich.*	*Chil.*	*Epist.*	*Costia*	Lisozyme	Gamma globulins
I	658	67	0	1.32	661	12	18	22	11	1.6^{a}	11.8^{c}
II	1367	100	4.0	1.93	2051	13	14	22	32	2.3^{b}	5.5^{a}
III	1386	100	3.5	1.92	2079	10	15	6	21	2.0^{ab}	8.2^{b}
IV	1325	100	3.8	1.97	1988	0	3	2	2	1.7^{ab}	9.4^{b}
V	1391	100	3.6	2.02	2087	1	3	4	3	1.9^{ab}	9.3^{b}
VI	1749	100	2.0	2.10	2624	2	0	1	0	1.8^{ab}	9.9^{b}

Within columns, data with different letters differs significantly ($P<0.05$), with the same letters are in the same homogenous group.

CONCLUSIONS

- the use of probiotics and herbs has had positive effect on growth, survival rate and total yield of carp reared in earthen ponds and fed traditional grain diet;
- the use of probiotics and/or herbs has had positive impact on food intake, food conversion rate (FCR) was usually reduced in 10% - 15% in groups where supplements were applied;
- the addition of probiotics and/or herbs decreased the number of the most common external parasites of carp (*Trichodina, Chilodonella, Epistylis and Costia*);
- probiotics and herbs presented positive effect on carp disease resistance. Supplementation of traditional grain diet with probiotics and/or herbs stimulated higher gamma globulins level. The positive effect was especially observed when both additives are used together;
- in case of carp fingerlings (C1) probiotics and herbs should not be applied together because when used together reduction of fish growth and gamma globulin level was observed.

OPTIMUM DIETARY PROTEIN AND LIPID LEVELS FOR JUVENILE ROCKFISH (*SEBASTES SCHLEGELI)*

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OPTIMALNE POTREBE JUVENILNIH MORSKIH GRGEČA *SEBASTES SCHLEGELI* U PROTEINIMA I LIPIDIMA

Apstrakt

U ovom istraživanju su analizirane optimalne potrebe juvenilnih morskih grgeča *Sebastes schlegeli* u proteinima i lipidima. 810 juvenilnih riba je izabrano po principu slučajnosti i distribuirano u 27 tankova od po 50 L sa protočnih sistemom. Pripremljeno je 9 eksperimentalnih smeša u vidu 3x3 faktorijalne eksperimentalne postavke: tri nivoa proteina (45, 50 i 55%) x tri nivoa lipida (11, 15 i 19%). Nivo proteina je imao uticaj na prirast riba, dok nivo lipida nije. Prirast riba hranjenih smešom u odnosu 50P-15L (50% proteina i 15% lipida) je bio veći nego prirast riba hranjenih smešama sa 45% proteina, bez obzira na nivo lipida, ali je bio isti kao kod riba hranjenih sa smešama 50P-11L, 50P-19L, 55P-11L, 55P-15L i 55P-19L. Stopa efikasnosti hrane (FER) riba je bila pod uticajem proteina u hrani ali ne i nivoa lipida. Stopa efikasnosti proteina (PER) riba je takođe bila pod uticajem proteina u hrani ali ne i nivoa lipida. Može se zaključiti da je za juvenilne Sebastes schlegeli optimalan nivo proteina i lipida za dobar prirast i iskoristljivost hrane (PER and NRE) 50% i 15% odnosno 45% i 19%, dok je optimalan odnos proteina i energije 27.4 i 23.9 mg protein/kJ.

Ključne reči: morski grgeč, Sebastes schlegeli, optimalan nivo proteina, optimalan nivo lipida, optimalan odnos proteina i energije
Keywords: rockfish, Sebastes schlegeli, optimum protein level, optimum lipid level, optimum protein to energy ratio

INTORDUCTION

Rockfish (*Sebastes schlegeli*, Hilgendorf 1880) is a commercially important marine fish species in Korea and its annual aquaculture production in 2012 reached 17,338 metric tons (MFAFF 2014). Therefore, many feeding trials to determine the dietary requirements (Kim et al., 2001), alternative animal and/or plant protein sources for substitution of fishmeal in the diets (Lee et al., 1996), optimum feeding frequency (Lee et al., 2000), feeding strategy (Oh et al., 2008) and dietary additive to improve immune response (Kim et al., 1999) of rockfish have been performed.

Generally speaking proteins are one of the most expensive components of commercial feed affecting fish performance. In addition, dietary energy level is a critical factor influencing feed consumption and level of lipids in fish. Therefore, the ratio of energy to protein in the diet must be kept in balance. When the dietary energy to protein ratio is low, dietary proteins are utilized as an energy source in fish to satisfy dietary energy requirement. Eventually this would increase fish production cost and deterioration of water quality. On the contrary, when dietary energy to protein ratio is high, feed consumption decreases and it results in reduction of growth due to lack of necessary other nutrients such as essential amino acids and fatty acids for the maximal growth (Lovell, 1989). Therefore, determination of the optimum dietary protein and lipid levels should be made prior to other feeding trials. Limited studies on the optimum dietary protein to energy ratio for growth of juvenile rockfish has been performed, except for the study of Kim et al. (2004a). They determined that the optimum dietary protein to energy ratio fell into 21.5-35.4 mg protein/kJ gross energy for juvenile rockfish, but its range was quite wide and fish performance, such as weight gain (147-187%) and feed efficiency ratio (0.62-0.72) were relatively poor. Thus, the determination of optimum dietary protein and levels for juvenile rockfish needs to be re-evaluated. Therefore, the aim of this study was to determine the effect of different levels of protein and lipid in the diet on growth, body composition and plasma chemistry of juvenile rockfish in order to identify optimum dietary protein-to-energy.

MATERIALS AND METHODS

Eight hundred ten juvenile (initial body weight of 3.2 g) fish were randomly chosen and distributed into 27 of 50 l flow-through tanks (water volume: 40 l) (30 fish per tank). The flow rate of water in each tank was 600-ml/min/tank. The water source was sand-filtered natural seawater and aeration was supplied into each tank. Water temperature monitored daily from 16.0 to 24.9°C (mean ± SD: 20.8 ± 2.61°C) and photoperiod followed natural conditions.

Nine experimental diets were prepared (Table 1) according to a 3 × 3 factorial experimental design: three crude protein levels (45, 50 and 55%) × three crude lipid levels (11, 15 and 19%). Fishmeal, casein and dehulled soybean meal were used as the protein source in the experimental diets. Dextrin, squid liver and soybean oils were used as the carbohydrate and lipid sources, respectively.

The following variables were calculated: feed efficiency ratio (FER) = Weight gain of fish/feed consumed, protein efficiency ratio (PER) = Weight gain of fish/protein consumed, nitrogen retention efficiency (NRE) = Nitrogen gain×100/nitrogen intake.

Two-way ANOVA and Duncan`s multiple range test were used to analyze the significance of the difference among the means of treatments through SAS version 9.3 (SAS Institute, Cary, NC, USA).

RESULTS

Survival of fish ranging from 95.6 to 98.9% was not significantly ($P > 0.05$) affected by either dietary protein or dietary lipid level (Table 2). However, weight gain of fish was significantly ($P < 0.006$ and $P < 0.005$) affected by dietary protein level, but not ($P > 0.05$) by dietary lipid level. Weight gain of fish fed 50P-15L (50% protein and 15% lipid) diet was significantly ($P < 0.05$) higher than that of fish fed the 45% protein diets regardless of lipid level, but not significantly ($P > 0.05$) different from that of fish fed the 50P-11L (50% protein and 11% lipid), 50P-19L (50% protein and 19% lipid), 55P-11L (55% protein and 11% lipid), 55P-15L (55% protein and 15% lipid) and 55P-19L (55% protein and 19% lipid) diets.

Feed consumption (g/fish), feed efficiency ratio (FER), protein efficiency ratio (PER) and nitrogen retention efficiency (NRE) of juvenile rockfish fed experimental diets with various protein and lipid levels are given in Table 3. Feed consumption of fish was significantly ($P < 0.02$) affected by dietary protein level, but not ($P > 0.05$) by dietary lipid level. FER of fish was significantly ($P < 0.0001$) affected by dietary protein level, but not ($P > 0.05$) by dietary lipid level.

FER of fish fed the 45 and 50% protein diets was significantly ($P < 0.05$) higher than that of fish fed with the 55% protein diets at all lipid levels. PER of fish was significantly ($P < 0.0001$) affected by dietary protein level, but not ($P > 0.05$) by dietary lipid level. PER of fish fed the 45% protein diet was significantly ($P < 0.05$) higher than that of fish fed the 50 and 55% protein diets regardless of dietary lipid level. In addition, PER of fish fed the 50% protein diet was significantly ($P < 0.05$) higher than that of fish fed the 55% protein diet regardless of dietary lipid level. NRE of fish fed the 45P-19L diet was significantly ($P < 0.05$) higher than that of fish fed the 45P-11L, 50P-11L, 50P-15L, 50P-19L, 55P-11L, 55P-15L and 55P-19L diets, but not significantly ($P > 0.05$) different from that of fish fed the 45P-15L diet. The lowest NRE was observed in fish fed the 55P-15L diet.

Table 1. Feed consumption (g/fish), feed efficiency ratio (FER), protein efficiency ratio (PER) and nitrogen retention efficiency (NRE) of juvenile rockfish (*Sebastes schlegeli*) fed the experimental diets with the various protein and lipid levels

Experimental diets	Feed consumption	FER[1]	PER[2]	NRE[3]
45P-11L	8.4 ± 0.17	0.97 ± 0.008^{a}	2.1 ± 0.03^{a}	34.6 ± 1.76^{b}
45P-15L	8.5 ± 0.41	0.96 ± 0.001^{a}	2.1 ± 0.01^{a}	36.9 ± 0.38^{ab}
45P-19L	8.7 ± 0.58	0.96 ± 0.006^{a}	2.1 ± 0.04^{a}	37.7 ± 1.51^{a}
50P-11L	8.9 ± 0.33	0.96 ± 0.005^{a}	1.9 ± 0.02^{b}	30.6 ± 0.14^{de}
50P-15L	9.6 ± 0.14	0.97 ± 0.003^{a}	1.9 ± 0.03^{b}	33.9 ± 0.76^{bc}
50P-19L	9.4 ± 0.39	0.96 ± 0.004^{a}	1.9 ± 0.04^{b}	31.3 ± 0.91^{cd}
55P-11L	9.8 ± 0.62	0.94 ± 0.008^{b}	1.6 ± 0.06^{c}	28.3 ± 0.88^{de}

55P-15L	9.4 ± 0.42	0.95 ± 0.003^{b}	1.7 ± 0.02^{c}	28.1 ± 0.43^{e}
55P-19L	9.5 ± 0.27	0.94 ± 0.004^{b}	1.7 ± 0.03^{c}	31.0 ± 0.77^{cde}
Two-way ANOVA				
Protein	$P < 0.02$	$P < 0.0001$	$P < 0.0001$	$P < 0.0001$
Lipid	NS	NS	NS	$P < 0.03$
Interaction	NS	NS	NS	NS

Values (means of triplicate ± SE) in the same column sharing a common superscript are not significantly different ($P > 0.05$).

DISCUSSION

Weight gain of rockfish was affected by dietary protein level, but not by dietary lipid level. The highest weight gain of fish fed the 50P-15L diet indicated that the optimum dietary protein and lipid levels for juvenile rockfish were 50% and 15%, respectively, and ratio of protein to energy was 27.4 mg protein/kJ in this study. Our results agree with the results of Kim et al. (2004a) study determining the optimum dietary protein to energy ratio of 21.5-35.4 mg protein/kJ gross energy. Weight gain of fish in our study ranging from 249.3% to 284.3% was relatively higher than that of fish in the study of Kim et al. (2004a).

FER of rockfish fed 45 and 50% protein diets was higher than that of fish fed the 55% protein diets at all lipid levels. Similarly, FER was reported to decrease when protein levels in the diets were over dietary protein requirement in olive flounder (Lee et al., 2002) and turbot (*Scophthalmus maximus*) (Lee et al., 2003).

PER of fish was significantly affected by dietary protein level, but not by dietary lipid level. PER of fish fed 45% protein diet was higher than that of fish fed 50 and 55% protein diet regardless of dietary lipid level. In addition, PER of fish fed 50% protein diet was higher than that of fish fed 55% protein diet regardless of dietary lipid level. Generally speaking PER tended to decrease as dietary protein level increased (Lee et al., 2002; Kim et al., 2004).

NRE of fish was significantly affected by both dietary protein and lipid levels. Significantly improvement in NRE of fish fed 50% protein diet at 15% lipid level compared to that of fish fed 50% protein diet at 11% lipid level indicated "protein-sparing effect" of lipid, is in line with other studies (Manuel-Vergara et al., 1996; Van der Meer et al., 1997; Helland and Grisdale-Helland, 1998; Lee et al., 2000; Kim et al., 2004). However, further increase in lipid levels from 15% to 19% in 50% and 55% protein diets tended to lower feed efficiency.

CONCLUSION

Optimum protein and lipid levels for growth (SGR) and feed utilization (PER and NRE) for juvenile rockfish were 50 and 15%, and 45 and 19%, respectively, and the optimum dietary protein to energy ratio of 27.4 and 23.9 mg protein/kJ.

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HEMATOLOGICAL CHARACTERISTICS OF *TELESTES METOHIENSIS* (STEINDACHNER, 1901) FROM DIFFERENT HABITATS

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HEMATOLOŠKE KARAKTERISTIKE *TELESTES METOHIENSIS* (STEINDACHNER, 1901) SA RAZLIČITIH STANIŠTA

Apstrakt

Telestes metohiensis (Steindachner, 1901) predstavlja endemičnu vrstu riba područja Istočne Hercegovine, sa specifičnim životnim ciklusom i prilagođenostima da određeni dio godine provodi pod zemljom, a sa pojavom velikih voda izlazi na površinu. Istraživanja fizioloških i ekofizioloških karakteristika ove vrste imaju poseban značaj jer se radi o nedovoljno istraženoj vrsti, pri čemu su podaci ovoga karaktera oskudni i fragmentirani, a radi se o vrsti koja se obuhvaćena Crvenom listom Republike Srpske, dok je dvije najveće baze ovih podataka WCMC i IUCN svrstavaju je u prvu kategoriju ranjivih vrsta. Fiziološka istraživanja endemičnih vrsta riba su od posebnog značaja, jer daju uvid u niz procesa u organizmu, a posredno i u životnoj sredini, što je neophodno pri prilikom planiranja i provođenja mjera zaštite. Hematološki parametri predstavljaju pouzdane indikatore stanja organizma, a posredno i stanja životne sredine. Za utvrđivanje hematološkog statusa koriste se kvantitativni karakteri ćelija crvene i bijele loze, koji pružaju uvid u čitav niz procesa u organizmu, a na osnovu analize različitih komponenti krvi može se suditi o promjenama koje nastaju u određenim sistemima pod uticajem faktora spoljašnje i unutrašnje sredine. Hematološki parametri su obuhvatali: broj eritrocita, koncentraciju hemoglobina, hematokrit, MCV, MCH i MCHC. Praćeni parametri su analizirani kod jedinki gatačke gaovice koje su lovljene u vodotocima: Vrijeka, Opačica i Zalomka. Prve dvije rijeke se nalaze na području Dabarskog polja, dok rijeka Zalomka teče kroz Nevesinjsko polje. Rezultati pokazuju postojanje značajnih razlika u vrijednostima praćenih parametra prilikom komparacije po lokalitetima, pri čemu su jedinke iz rijeke Vrijeke imale veće vrijednosti koncentracije hemoglobina, vrijednost hematokrita, broja eritrocita i srednje vrijednosti količine hemoglobina u eritrocitima (MCH) u odnosu na druga dva vodotoka.

Ključne riječi: Telestes metohiensis, gaovica, hematologoške karakteristike, endemi, ranjive vrste
Key words: Telestes metohiensis, striped prijor, hematological characteristics, endemic species, vulnerable species

INTRODUCTION

Freshwater fish fauna of Bosnia and Herzegovina have a high diversity. Vuković (1977) cites 108 fish species in Bosnia and Herzegovina, while according to recent data, there are 118 fish species (Sofradžija, 2009). Ichthyofauna of B&H is characterized by a large number of endemic species many of which live just in particular localities. Most of the endemic species have restricted distribution areas which is the main reason for their vulnerability (Glamuzina et al., 2010; Dekić et al., 2011). Physiological and morphometric studies of endemic fish species are of particular importance because adequate knowledge is necessary for their protection and preservation (Ivanc, 2012). Endemic fish species are particularly vulnerable and they often have lower ability to adapt to changes in environmental conditions. They are mainly distributed in the former glacial remains and they are closely related to the environment which they inhabit (Glamuzina, 2013). The aim of research was to determine the hematological characteristics of striped prijor from three rivers in the Eastern Herzegovina.

MATERIAL AND METHODS

Telestes metohiensis (Steindachner, 1901) is an endemic fish species characteristic for Eastern Herzegovina. They have a specific life cycle and adjustments that allow them to spend one part of the year in groundwater. With increasing of water level they come out to the surface water. Striped pijor lives in karst habitats such as lakes and watercourses with low flow in lowland areas, but also at higher altitudes. However, during the summer months, it generally retains in the groundwater flows because surface waters dry out. During winter, pijor also enters the groundwater flows where it rests in mud. Before going into the groundwater, specimens form large flocks. Electrofishing was carried out from 6 to 8 September 2013, at localities Opačica, Vrijeka and Zalomka. Electrical aggregates IG 600, power 1.2 KW and ELT62II GI HONDA GCV160, power 3 kW, were used for this purpose. River Opačica is an occasional watercourse of the field Dabarsko polje. At the time of high water Opačica is torrent, and during summer it dries up. It appears in the northwestern part of the field by merging the watercourses Bijeli Potok and Trusina, and it goes underground in the part of the field which is called Lužine Bare. Water from Opačica occurs later at the springs of the watercourses Bregava and Tebišnjica after underground bifurcation (Dekić, 2013). Spring of the river Vrijeka is located at the edge of the field Dabarsko polje. Most of the river dries up during warm summer months, and water is present only in spring (Gnjato, 2004). The length of river is about 2.5 km, and it flows through the field Dabarsko polje along the entire watercourse. Zalomka is a river that flows through the field Nevesinjsko polje, at southeast part of Bosnia and Herzegovina. Spring of this river is near the field Gatačko polje. River flows through the Nevesinjsko polje and plunges into numerous chasms. During the dry season it dries up, while during the wet, rainy period, it is rich in water.

Zalomka flows through the area which represents a tectonic depression which is limited by the mountain ranges (Vuković, 2014).

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, with any anticoagulant, is used for further analyses. Erythrocyte count was performed in the hemocytometer using diluent by Kekić and Ivanc (1982). Hemoglobin concentration (Hb) was determined by hemiglobin cyanide method using Drabkin reagent (Blaxhall and Daisly, 1973). Hematocrit (Hct) was determined by microhematocrit centrifuge. Haematological indices were calculated using values of hematocrit, erythrocyte number and hemoglobin concentration.

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

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

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

RESULTS AND DISCUSSION

Results of the research of haematological parameters are presented in Table 1. The sample from the river Opačica contained 30 individuals, from the river Vrijeka 20 individuals and from the watercourse Zalomka 11 individuals. Comparing the average values of the parameters of erythrocyte lineage among individuals from three different watercourses, it is evident that these groups differ in most of the monitored parameters. Mean corpuscular hemoglobin concentration was significantly higher in specimens from the river Vrijeka in relation to individuals from the river Opačica ($p = 0.002$) and to individuals from the river Zalomka ($p = 0.000$). Significantly higher values of hemoglobin had also individuals from the river Opačica in relation to specimens from the river Zalomka ($p = 0.009$).

Table 1. Parameters of erythrocytes lineage of striped pijor from rivers Opačica, Vrijeka and Zalomka

River	Statistical parameters		Hb g/l	Hct l/l	Number of erythrocytes $x10^{12}/l$	MCV fl	MCH pg	MCHC g/l erit.
Opačica	Mean value		82,35^{bc}	0,410^{b}	1,533^{b}	270,92	54,69^{c}	203,90
	Standard deviation		11,577	0,058	0,182	48,900	11,357	37,059
	Minimim		66,67	0,333	1,250	182,149	36,430	155,555
	Maximim		114,81	0,500	1,960	384,615	90,405	344,444
	95 % Confidence Interval for mean	Lower bound	78,02	0,386	1,463	252,657	50,449	190,064
		Upper bound	86,66	0,430	1,599	289,176	58,926	217,740
	Coefficient of variation %		14,060	14,045	11,934	18,050	20,755	18,175
Vrijeka	Mean value		92,77^{ac}	0,449^{a}	1,651^{a}	276,48	57,14^{c}	211,36
	Standard deviation		11,741	0,055	0,165	33,236	7,885	34,227
	Minimim		66,67	0,330	1,350	214,286	43,150	148,140
	Maximim		103,70	0,560	2,060	340,136	68,680	259,250
	95 % Confidence Interval for mean	Lower bound	87,28	0,420	1,573	259,390	53,465	193,767
		Upper bound	98,27	0,477	1,728	293,567	60,818	228,963
	Coefficient of variation %		12,654	12,327	10,050	12,021	13,799	16,194
Zalomka	Mean value		71,72^{ab}	0,419	1,531	274,21	46,83^{ab}	181,08
	Standard deviation		8,490	0,084	0,078	56,199	5,106	60,540
	Minimim		59,30	0,227	1,390	141,163	37,745	118,518
	Maximim		85,19	0,500	1,610	335,570	53,576	342,222
	95 % Confidence Interval for mean	Lower bound	66,015	0,363	1,479	236,452	43,403	140,411
		Upper bound	77.423	0,430	1,584	311,962	50,263	221,754
	Coefficient of variation %		11,838	20,061	5,110	20,495	10,902	33,432

^{a,b,c} *Different superscript letters indicate statistically significant difference ($p <0.05$) compared to specimens from different localities: a-Opačica, b-Vrijeka and c-Zalomka.*

The average hematocrit value was significantly higher in specimens from the river Vrijeka in relation to individuals from the river Opačica ($p = 0.039$). The same relation was seen between average values of numbers of red blood cells ($p = 0.014$) in specimens from these two localities. The average value of mean corpuscular hemoglobin (MCH) was significantly lower in individuals from the river Zalomka in relation to individuals from the river Opačica ($p = 0.022$) and in relation to individuals from the river Vrijeka ($p = 0.005$). MCHC and MCV values are approximately equal in individuals from all three watercourses. Parameters of erythrocytes lineage of striped pijor show that individuals from the river Vrijeka have highest values of all parameters. On the other hand, comparing the quality of water among these watercourses indicate that they are approximately the same. The river Vrijeka had slightly different values of conductivity, BOD_5 and concentration of total suspended solids. Variations in number of blood elements in fish depend on the species of fish (Langston et al., 2009), environmental conditions (Hickey 1982; Aldrin et al., 1982), nutritional status (Casillas and Smith, 1977), sex (Sidiquie and Nasim, 1979 ; Collazos et al, 1998), size of fish (Garcia, 1992) and seasonal changes (Cech and Wohlschlag, 1981). All these issues should be considered in order to determine hematological values of specific species. When we compare our results with results obtained for striped pijor from the river Pribitul, we can conclude that the average value of hemoglobin concentration in specimens from the river Vrijeka are higher compared to individuals from Pribitul, whose value was 83.54 g/l. While specimens from the river Opačica had an approximate value, individuals from the river Zalomka had slightly lower value of hemoglobin concentration compared to individuals from watercourse Pribitul (Dekić et al., 2012). Hematocrit values in blood of striped pijor from watercourse Pribitul ranged from 0.294 to 0.545 l/l, and the average value of this parameter was 0.411 l/l (Dekić et al., 2012). Compared with our results, only individuals from the river Vrijeka had slightly higher values, while individuals from two other watercourses showed approximate values. The average value of the number of red blood cells in striped pijor from watercourse Pribitul was 1,747 x 10^{12} /l (Dekić et al., 2012), which was slightly higher compared to all three samples we analyzed. However, the number of erythrocytes varies in certain physiological limits and can be changed depending on the physiological state of an organism.

Data on the number of red blood cells in fish are different (Dekić et al., 2012). The number of erythrocytes in striped pijor is relatively high because the majority of our freshwater fish species have lower values of this parameter (Dekić et al., 2012). Mean corpuscular volumes for all three of studied populations are higher than the values of MCV determined in fish from watercourse Pribitul 236,79 fl (Dekić et al., 2012). The average value of MCH in specimens from Pribitul was 48.42 pg (Dekić et al., 2012). Specimens from rivers Vrijeka and Opačica had significantly higher values of this parameter, but individuals from the river Zalomka had slightly lower values of MCH. The average value of MCHC in specimens from Pribitul was 211.32 g/l (Dekić et al., 2012). Specimens from the river Vrijeka had significantly higher values of this parameter, but individuals from rivers Opačica and Zalomka had slightly lower values of MCHC. When we compare results of monitored parameters of erythrocyte lineage of fish in different watercourses with our study, we can see that other authors had similar results. Studies of haematological parameters of carp, grayling (Ivanc et al., 1993; 1994) and trout (Kekić, 1985) showed slightly different values for individuals which live in various environmental conditions. There are significant differences of hematological parameters between balkan barbel from rivers Suturlija and Jakotinska rijeka

(Dekić et al., 2009). Significant differences of the total number of red blood cells, hematocrit values and hematological indices: MCH and MCHC were observed between chub from rivers Krupice and Željeznice (Mitrašinović et al., 2009).

CONCLUSIONS

Observed hematological parameters of striped prijor from three watercourses show significant differences for most parameters. They all have relatively high values of the number of red blood cells and high concentration of hemoglobin.

Comparing parameters of erythrocyte lineage it can be concluded that individuals from the river Vrijeka had higher concentrations of hemoglobin, hematocrit concentration, the number of red blood cells and the mean corpuscular hemoglobin (MCH) in relation to the individuals from other two rivers.

ACKNOWLEDGMENTS

This paper is the result of a scientific research project "Environmental monitoring and biodiversity" that was co-financed by the Ministry of Science and Technology of Repubika Srpska.

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ASSESSMENT OF TOXIC AND TRACE ELEMENTS (As, Cd, Cu, Cr, Hg, Ni, Pb, Sr, Zn) IN ZOOPLANKTON FROM CARP FISH PONDS

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ANALIZA KONCENTRACIJE TOKSIČNIH I ESENCIJALNIH ELEMENTA (As, Cd, Cu, Cr, Hg, Ni, Pb, Sr, Zn) U ZOOPLANKTONU SA ŠARANSKOG RIBNJAKA

Apstrakt

U poslednjih 20 godina zagađivanje slatkovodnih ekosistema toksičnim elementima je u porastu širom sveta. Zagađenjem su pogođeni pre svega izvori za vodosnabdevanje stanovništva i životno okruženje, ali i industrija kao i privreda uopšte. Međutim zbog perzistentnosti i transfera kroz lance ishrane i potencijalnog akumuliranja u ribama i drugim vodenim organizmima koji se koriste u ishrani, toksični elementi predstavljaju stalnu pretnju ljudskom zdravlju. Zagađivanje naših reka teškim metalima nameće pitanje ne samo zdravstvene ispravnosti riba iz reka već i riba iz ribnjaka obzirom da se većina šaranskih ribnjaka napaja vodom iz sistema kanala DTD.

Cilj ove studije je bio da se analizira koncentracija 9 elemenata u zooplanktonu koji predstavlja značajnu prirodnu hranu šarana u poluintenzivnom sistemu gajenja. Istraživanje je obavljeno na 4 ribnjačka objekta, tokom dva ciklusa gajenja šarana, od juna do oktobra, na ribnjaku „Despotovo“. Uzorci zooplanktona za analizu elemenata su uzimani sa tri tačke u svakom jezeru pomoću planktonske mrežice veličine 250 µm jednom mesečno. Na ovaj način su sakupljene samo krupnije veličinske klase zooplanktona (Cladocera i Copepoda), koje šaranska mlađ najviše konzumira. Sa svakog jezera je uziman po još jedan uzorak zooplanktona za kvantitativnu i taksonomsku analizu. Koncentracija elemenata je analizirana induktivno spregnutom plazma masenom (ICP-MS) i optičkom emisionom spektrometrijom (ICP-OES).

Rezultati su obrađeni jednofaktorijalnom analizom varijanse (ANOVA) u statističkom programu PAST 3.06. Značajnost razlika testirana je primenom Tukey's post hoc testa. Podaci su klasifikovani na prolećni, letnji i jesenji aspekt tokom jednog proizvodnog ciklusa.

Cladocera su dominirale u populaciji zooplanktona, osim u junu kada su Copepoda bile zastupljenije. Iako nije bilo značajnih razlika u koncentraciji elemenata između godina, osim za Cu i Sr, uočen je karakterističan sezonski obrazac kretanja koncentracija elemenata tokom celog istraživanja. Prolećni i jesenji aspekti u 2012 su bili veći nego u 2013, dok je letnji aspekt u 2013 bio viši nego u 2012. godini. Izuzetak je bila koncentracija Zn u zooplanktonu gde je situacija bila obrnuta.

Povišene vrednosti većine toksičnih metala u zooplanktonu na ribnjaku Despotovo se mogu objasniti relativno velikim afinitetom egzoskeleta Cladocera za većinu dvovalentih jona. Nakon adsorbcije elementi na površini ljuštura ovih životinja, tokom vremena bivaju absorbovani kroz telesni zid u unutrašnje organe. Neke studije čak navode da površinski akumulirani kontaminanti na plenu mogu biti dostupniji predatorima od onih akumuliranih u tkivima, zbog niskog pH i visokog nivoa jonske kompleksacije koji vladaju na mestu abstorpcije, u digestivnom traktu većine životinja,

Ovi rezultati nameću zaključak da je u budućim istraživanjima kontaminacije vodenih ekosistema i riba poželjno uključiti analize ne samo vode kao izvora toksičnih elemenata, već i odgovarajućih izvora hrane (plena) koji, kako je pokazano, sadrži potencijal ne samo za značajnu akumulaciju elemenata već i njihovu potencijalnu veću biodostupnost konzumentima.

Ključne reči: Cladocera, toksični elementi, egzoskelet, ribnjačka jezera, šaran
Key words: Cladocerans, toxic elements, exoskeleton, fish ponds, common carp

INTRODUCTION

Pollution of freshwater ecosystems with toxic elements is increasing over the past few decades worldwide, and accordingly a lot of attention is raised. The threats are posed primarily on the water supply and environment, but also towards industry and economy in general. However, due to persistence and transfer of toxic elements through food webs, and potential accumulation in fish and other aquatic animals used as food, it presents a constant danger to human health. In recent years, a considerable number of papers dealing with the pollution of fish, water and sediments of the Serbian part of River Danube with toxic elements have been published concluding that the situation needs urgent action and regular monitoring. One of the main issues is the low level of wastewater treatment of municipal and industrial waters, where rivers in Serbia usually serve as collectors of a range of pollutants. One of the concerns that these situations bring is not only the safety of fish from rivers for human consumption but also the level of contamination present at fish farms. The majority of fish farms in Serbia are located in the north of the country and are fed by Danube-Tisza-Danube Canal (DTD) hydro system. The present study is a part of a series of studies investigating element transfer through food webs and sediments at the carp fish farm "Despotovo" during 2012 and 2013. The aim of this research was to analyze the concentration of nine toxic elements (As, Cd, Cr, Hg, Ni, Pb, Sr, Zn and Cu) in zooplankton, a major part of the diet of common carp (*Cyprinus carpio* L.) in semi-intensive fish production.

MATERIAL AND METHODS

The study was carried out at the fish farm Despotovo, located in Vojvodina Province during two production cycles of common carp (in 2012 and 2013), from June to October. For the experiment 4 fish ponds were used. Zooplankton samples were taken monthly from three sites in every pond using a 250 µm mesh plankton net. Samples from each lake were rinsed with distilled water, placed in plastic bottles and frozen prior to analysis of elements. One extra sample of zooplankton per lake was taken and preserved with 4% formaldehyde for taxonomic and quantitative analysis. The content of As, Cd, Cr, Cu, Hg, Ni, Pb, Sr, and Zn in zooplankton samples was determined by inductively coupled plasma mass spectrometry and optical emission spectrometry respectively.

The results of the concentration of toxic elements in zooplankton were tested using analysis of variances (one-way ANOVA). Differences between treatments were tested using Tukey’s post hoc test. The analyses were performed using PAST 3.06. The results were classified into three seasons during one year, spring, summer and autumn aspect.

RESULTS AND DISCUSSION

Overall there are not many field studies that investigated the concentration of toxic elements in freshwater zooplankton, and even less those done in fish ponds. There is one dealing with methyl mercury (Schultz et al., 2012) and another investigating introduction of heavy metals to fish and zooplankton via sediment resuspension (Cheng et al., 2013). Additionally, there are problems in comparing the results from different studies due to different methodologies for element analysis and measures used for expressing element concentration (e.g. µg/L wet weight and µg/g dry weight).

In both years, Cladocerans were dominating (80%) in the Crustacean population, except in June, when the Copepods were more abundant. Except for Cu and Sr, no significant differences were found in the investigated elements between two production cycles. However, there was a pattern that was consistent for most of the elements throughout the study: spring and autumn aspects in 2012 were higher than in 2013, while summer aspect in 2013 was higher than in 2012. Conversely, the level of Zn in zooplankton was higher in spring and summer in 2013 while lower in autumn compared to 2012.

The concentration of elements found in zooplankton of the investigated fish ponds were generally in the range reported for a number of natural uncontaminated lakes (Chen et al., 2000, Farkas et al., 2003). However, mean values of some elements (Cu, Ni, Pb and Zn) in our zooplankton samples (12.1 µg/g dw 3.1 µg/g dw, 2.8 µg/g dw and 75.3 µg/g dw, respectively) were higher than reported by Nguyen et al. (2005) for Lake Balaton during the calm weather period (4.8 µg/g dw, 1.65 µg/g dw, 0.95 µg/g dw and 71 µg/g dw, respectively) and especially higher than those found at Halali reservoir lake (0.022 µg/g dw, 0.009 µg/g dw, 0.048, 0.117 µg/g dw, respectively) (Malik et al., 2013).

Strontium was the most variable heavy metal during the investigation period with the highest values observed especially during spring and summer aspects of subsequent years (115.81 µg/g dw and 142.4 µg/g dw, respectively). The increased level of Sr found in our samples could be due to the high affinity of Cladoceran exoskeleton for this metal. This is also the case with lead that binds to the carapax possibly by the exchange with calcium, cadmium, nickel, zinc, copper and other positively charged contaminants (Robinson et al., 2003). This mechanism seems to be the result of high affinity of chitin, the main constituent of cladoceran carapaces, for heavy metals. However, the adsorbed toxic elements on the surface of animal shells are sooner or later absorbed up to a certain level into the organisms through the integument and distributed to different internal organs (Robinson et al., 2003). Munger et al. (1999) reported that internal accumulation of cadmium in *Ceriodaphnia dubia* is primarily located at the gut diverticula, the main place where nutrients and Ca are uptaken in Cladocerans. Both, external and internal deposition of toxic elements in these organisms raises risk for dietary exposure of consumers/predators (Robinson, 1999). Some studies even suggest that the surface-associated contaminants of prey may be even more bioavailable to predators than the those accumulated in tissues due to the suitable conditions found in the gut of most animals as low pH and high ion complexation (Robinson et al., 2003). Additionally, several recent studies have stressed the relative importance of dietaryborne vs. waterborne exposure route for contamination of predators e. g. fish (Filipović Marijić and Raspor, 2012). This is observed also on lower trophic levels as phytoplankton/zooplankton and zooplankton/invertebrate predators (Taylor et al. 1998).

In conclusion, based on these findings, we propose that future studies on the pollution of aquatic ecosystems and contamination in fish should not only take into account the potential of waterborne routes, but also the significance of element accumulation in prey and additionally their potentially higher bioavailability to consumers.

ACKNOWLEDGEMENTS

This study was supported by the Ministry of Education, Science and Technological Development, through the project Improvement of production capacities of carp (*Cyprinus carpio)* through nutrition and selective breeding programs (TR 31075) and FP7 project AREA (316004).

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MERCURY IN DIFFERENT MARINE FISH SPECIES ON SERBIAN MARKET

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ŽIVA U RAZLIČITIM VRSTAMA MORSKIH RIBA NA SRPSKOM TRŽIŠTU

Apstrakt

Proučavanje žive (Hg) u marinskim sistemima je od velikog interesa, s obzirom da je reč o toksičnom metalu koji ima sposobnost bioakumulacije i biomagnifikacije u lancu ishrane. Vodeni sistemi, a time i ribe, mogu biti kontaminirani živom kao posledicom zagađenja životne sredine prirodnim procesima (vulkanske erupcije, klimatske promene itd.) i antropogenim izvorima zagađenja (industrija, rudarstvo itd.). Živa (Hg) može postojati kao elementarna, neorganska i organska živa. Najtoksičniji oblik žive je metil živa (MetHg) koja čini od 70-100% ukupne Hg u ribi. Naučna ispitivanja ukazuju da velike količine žive u hrani mogu negativno uticati na razvoj fetusa i izazvati oštećenja mozga i jetre.

Cilj ovog rada bio je ispitivanje sadržaja žive u jestivim delovima tri vrste morske ribe na tržištu Srbije: oslić - *Merluccius merluccius* (n=84), skuša - *Scomber scombrus* (n=35) i papalina - *Sprattus sprattus* (n=17)). Prosečna potrošnja ribe u Srbiji iznosi oko 5 kg po glavi stanovnika, što je znatno niže u poređenju sa EU prosekom (21 kg po glavi stanovnika). Svi uzorci uzeti su i analizirani tokom 2014. godine. Analiza sadržaja žive urađena je primenom induktivno-kuplovane plazme sa masenom spektrometrijom (ICP-MS), merenjem izotopa ^{202}Hg.

Najviša srednja vrednost sadržaja žive utvrđena je u uzorcima skuše (0,056 mg/kg), zatim kod oslića (0,034 mg/kg), a najniža kod papaline (0,016 mg/kg). Maksimalno dozvoljene količine Hg u ispitanim ribama, koje su definisane zakonskom regulativom Srbije [MDK (oslić, papalina) = 500 ng/g, MDK (skuša) = 1000 ng/g] nisu prekoračene u svim ispitanim uzorcima. Statističkom evaluacijom dobijenih rezultata ustanovljeno je da postoji statistički zanačajna razlika ($p<0,005$) u sadržaju Hg između oslića i skuše, kao i između skuše i papaline.

Za procenu unosa Hg konzumiranjem ribe korišćeni su podaci iz „GEMS/Food Consumption Cluster Diets database“. Prema ovom izvoru, procenjena prosečna nedeljna potrošnja morske ribe u Srbiji iznosi 106,4 g. Koristeći podatke iz ove studije izračunat je nedeljni unos Hg, baziran na srednjoj vrednosti sadržaja Hg u konzumiranoj ribi i prosečnoj telesnoj težini čoveka od 70 kg.

Na osnovu izračunatih vrednosti za nedeljni unos Hg (oslić: 0,051-0,208 µg/kg telesne težine; skuša: 0,086-0,289 µg/kg telesne težine; papalina: 0,025-0,050 µg/kg telesne težine) može se zaključiti da je unos žive pri konzumaciji oslića, skuše i papaline znatno niži od preporučenih graničnih vrednosti svetske zdravstvene organizacije (1,6 µg MetHg /kg telesne težine).

Ključne reči: živa, morska riba, tržište Srbije
Key words: mercury, marine fish, Serbian market

INTRODUCTION

The investigation of mercury (Hg) behavior in marine systems is of great interest, considering the fact that it is a toxic metal that bioaccumulates and biomagnifies over the entire marine food web (Clayden et al., 2015; Li et al., 2015). Mercury exists as metallic (elemental), inorganic and organic mercury compounds. By both abiotic and biotic processes, inorganic mercury can be transformed into one of the most toxic forms, organic Hg as methylmercury (MetHg), which is predominantly present in fish (Merritt and Amirbahman, 2009; Saei-Dehkordi et al., 2010). Depending on species, MetHg accounts for 70-100% of total Hg in fish (EFSA, 2005). Metal accumulation in fish is of global public health concern, because the consumption of contaminated fish has been associated with several diseases such as neurologically related problems, myocardial infarction and autism (Li et al., 2008). Consumption of freshwater and marine fish as well as seafood is the main environmental source of human mercury exposure (Bille et al., 2015; Vieira et al., 2015). The global increase in fish consumption tallies with trends in food consumption in general. Per capita food consumption has been rising in the last few decades. Annual fish and seafood consumption by county varies from one country to another depending on different factors such as fisheries resources, the economic climate, environmental conditions, dietary habits etc. Average annual fish consumption in Serbia is about 5 kg per capita, which is significantly lower compared to EU average of 21 kg (Baltić et al., 2009).

This study was conducted to determine and compare the content of mercury in the edible portion of three species of marine fish collected from Serbian market and ordinary consumed by the population of Serbia. On the other hand, data from this study were used in order to assess intake of Hg by fish.

MATERIALS AND METHODS

Total concentration of Hg was measured in three fish species collected in Serbian markets during 2014 year. Total 136 samples were analyzed: hake - *Merluccius merluccius* (n=84), mackerel - *Scomber scombrus* (n=35), and sprat - *Sprattus sprattus* (n=17). After collection, samples were labeled and stored in polyethylene bags and frozen at -18 °C prior to analysis. Samples were partially thawed at +4 °C 1 day before analysis. Edible parts were chopped into 2 to 3 cm thick portions and homogenized. Approximately 0.3 g of samples were mineralized by adding 5 ml nitric acid (SIGMA) and 1.5 ml hydrogen peroxide (30%, MERCK). Microwave assisted digestion was performed in Microwave Digestion System (Via Fatebenefratelli, 1/5-24010 Sorisole (BG), Italy). The digested sample solutions were quantitatively transferred into disposable flasks and diluted to 100 ml with deionized water (ELGA).

The analysis was performed by inductively-coupled plasma mass spectrometry (ICP-MS). Measurements were performed using the instrument “iCap Q” (Thermo Scientific, Bremen, Germany), equipped with collision cell and operating in kinetic energy discrimination (KED) mode. The ^{202}Hg isotope was measured. Torch position, ion optics and detector settings were adjusted daily using tuning solution (Thermo Scientific Tune B) in order to optimize measurements and minimize possible interferences. For the qualitative analysis of the samples, five-point calibration curve (including zero) was constructed for the ^{202}Hg isotope in the concentration range of 0.2 – 2.0 mg/L. Additional line of the peristaltic pump was used for on-line introduction of multi-element internal standard (^{45}Sc – 10 ng/mL; ^{71}Ga – 2 ng/mL). Concentrations of each measured sample were corrected for response factors of both higher and lower mass internal standard using interpolation method. The quality of the analytical process was controlled by the analysis of the standard reference material (NIST SRM 1577c, Gaithersburg, USA). Measured concentrations were within the range of the certified values for all isotopes. The limit of quantification was 0,001 mg/kg.

For data analysis a one-way analysis of variance (ANOVA) and Tukey’s test were used for the comparison of the mean content of Hg in different fish species, and they were performed using Minitab 16 software.

RESULTS AND DISCUSSION

Contents of Hg (mg/kg) in three edible parts of fish species as the mean value ± standard deviation (SD) are expressed, and they are graphically presented in Figure 1. Results for ANOVA and Tukey’s test are showed in Figure 1, as well. Statistical analysis of the data showed significant differences ($p<0.05$) between the mercury content in hake and mackerel, as well as in mackerel and sprat. Hg limits defined by Serbian legislation (Službeni glasnik RS, 29/2014) for hake and sprat (500 ng/g fresh weight) as well as for mackerel (1000 ng/g fresh weight) were not exceeded in any of the analyzed samples.

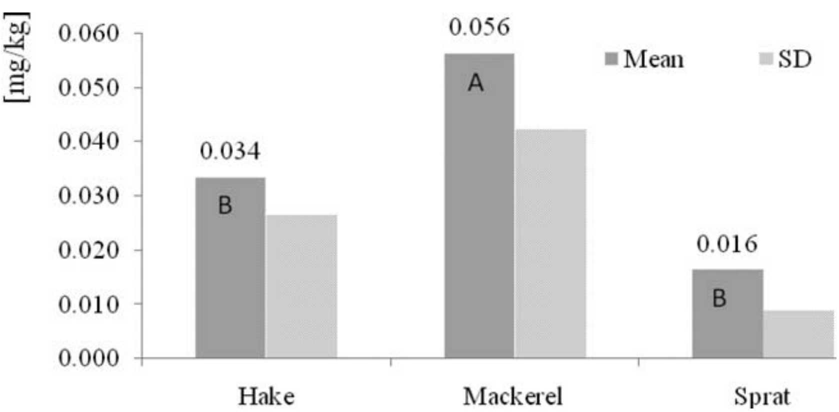

Figure 1. The mean values for Hg content (mg/kg) in three fish species [A, B – values expressed as columns followed by different letters are differ significantly ($p<0.05$)]

According to the GEMS/Food Consumption Cluster Diets database (*FAO/WHO*, 2006) the average weekly consumption of marine fish in Serbia is 106.4 g/week. Using the data of this study, the weekly intakes of Hg via three fish species consumption were calculated (Table 1). Mercury intake expressed as weekly intake (WI) was calculated using the following formula (Janković et al., 2012):

WI = (weekly consumption data x content of compound) / body weight.

Table 1. Weekly intakes (WI) of Hg via fish consumption [µg/kg b.w.]

Fish	Weekly intakes of Hg [µg/kg b.w.]			
	Mean	Maximum	Mean	Maximum
	*50% - 70 kg**		*5% - 51 kg***	
Hake	0.051	0.208	0.070	0.286
Mackerel	0.086	0.289	0.118	0.396
Spart	0.025	0.050	0.034	0.069

*body mass of 70 kg represents 50% of population (Janković et al., 2012).
*body mass of 51 kg represents 5% of population (Janković et al., 2012).

Based on epidemiological studies The Joint FAO/WHO Expert Committee on Food Additives (JECFA) established a provisional tolerable weekly intake (PTWI) of 1.6 µg MetHg/kg b.w. (FAO/WHO, 2003). The estimated weekly intakes of Hg through fish consumption among Serbian population (Table 1) are few times lower than established value.

CONCLUSIONS

Taking into consideration the results of this study, it could be concluded that the current level of total Hg in marine fish available at the Serbian market does not pose a threat to consumers' health.

ACKNOWLEDGEMENTS

This work was supported by grants from the Ministry of Education, Science and Technological Development of the Republic of Serbia (project no. TR 31075).

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BARBEL (*BARBUS BARBUS* LINNAEUS, 1758) ENDOPARASITE FAUNA AND DIET IN THE BELGRADE SECTION OF THE DANUBE RIVER (SERBIA)

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ENDOPARAZITI I ISHRANA MRENE (*BARBUS BARBUS* LINNAEUS, 1758) U BEOGRADSKOM SEKTORU DUNAVA (SRBIJA)

Apstrakt

U radu su prikazani rezultati istraživanja crevnog sadržaja rečne mrene (*Barbus barbus* L. 1758). Tokom dvogodišnjeg perioda studije, 2007-2009, ukupno je prikupljeno 63 jedinki mrena u okviru beogradskog sektora reke Dunav. Cilj je bio da se dobije informacija o inficiranosti mrena crevnim parazitima (endoparaziti, helminti) i utvrdi prisustvo organizama makrozoobentosa u njihovom crevnom sadržaju (Gammaridae, Bivalvia, Gastropoda i Oligochaeta), kao posledica raspoloživosti hrane na području uzorkovanja. Nabrojani organizmi makrozoobentosa imaju veoma značajnu ulogu u ishrani mrena, s obzirom da je ona bentofagna vrsta, i da njena ishrana zavisi od sastava i strukture faune dna. Pojedini akvatični makrobeskičmenjaci (gamarusi, oligohete, školjke, puževi, i neke larve insekata), kojima se hrane mrene, predstavljaju i prelazne domaćine za nekoliko vrsta endoparazita. Pregledom crevnog sadržaja mrena ukupno je nađeno i identifikovano šest vrsta endoparazita (helminta) iz tri filuma (Platyhelminthes, Nematoda i Acanthocephala), sa prevalencom infekcije od 98.41%. Broj endoparazita po jedinki mrene varirao je u rasponu od 6 do 207. Najveća procentualna zastupljenost utvrđena je za parazita iz grupe Acanthocephala – *Pomphorhynchus laevis* (Müller, 1776), sa prevalencom inficiranosti od 7,73%. Takođe, udeo gamarusa u crevnom sadržaju mrena bio je u interval 31.43% do 46.73%, odnosno u zajednici makrozoobentosa od 1.4% do 16.22%. Ova činjenica ukazuje da su gamarusu bili omiljena hrana mreni i da je to doprinelo visokoj abundanci - parazita *Pomphorhynchus laevis* u crevnom sadržaju mrene. Uzimajući u obzir, raznovrsnost i bogatstvo faune parazita na području Srbije, neophodno je nastaviti istraživanja parazita što većeg broja slatkovodnih vrsta riba.

Ključne reči: mrena; endoparaziti; ishrana; makrozoobentosa; Dunav.
Keywords: barbel, endoparasite, diet, makrozoobenthos, Danube

INTRODUCTION

The barbel *Barbus barbus* L. 1758 (Cyprinidae) is a benthopelagic, rheophilic long-lived fish, feeds on benthic organisms, including crustaceans, insect larvae (mayfly and midge larvae), molluscs, crayfish and swan mussels, as well as on juveniles and eggs of other fish species (Vukovic and Ivanovic, 1971). Certain aquatic macroinvertebrates (gammarids, molluscs, oligochaetes and some insect larvae) consumed by barbels represent intermediate hosts for some endoparasites (Adámek and Obrdlík, 1977). Barbel endoparasite fauna composition is directly related to its feeding preferences and the macrozoobenthic fauna composition of its feeding habitat (Hine and Kenedy, 1974; Moravec et al., 1997).

The composition of the barbel helminth community in European surface waters is generally well-studied, especially in the Danube and Elbe river basins (Moravec and Scholz, 1991, 1995; Gelnar et al., 1996). The helminth parasites of the barbel were studied in the open waters of the Danube drainage system of the Balkan Peninsula and Serbia (Roman, 1955; Margaritov, 1966; Kakacheva-Avramova, 1977; Kiskaroly and Tafro, 1988; Djikanovic et al., 2010).

The aim of this study was to analyze the endoparasitic fauna of barbels in the Danube River (Belgrade city area) and to assess the relationship between the composition of the barbel’s endoparasitic fauna, its diet and the macrozoobenthos community in this part of the river.

MATERIALS AND METHODS

A total of 63 barbels were collected at two locations: Zemun (1173 rkm) and Višnjica (1163 rkm), using bentic driftnets (dimension 30-50m x 2m, 32 – 50 mm mesh size), twice a month from November 2007 to November 2009. In laboratory, analysis of their intestines for food items and endoparasites was carried out. Parasites were identified using identification keys (Bykhovskaya-Pavlovskaya et al., 1962; Kakacheva–Avramova, 1983; Bauer, 1987; Moravec, 1994) to the lowest taxonomic level (species). Macrozoobenthic organisms recorded in the fish intestines were determined using the appropriate identification keys (Nilsson, 1997a,b; Glöer & Meier-Brook, 2003). Parasitic prevalence and intensity of infection were estimated as well as Fulton’s body condition factor. Benthic fauna samples were taken during research conducted under the international project “Joint Danube Survey 1 and 2” (Paunovic et al., 2010).

The relationship between the condition factor of particular fish and the number of identified parasites was assessed using basic statistics and correlation matrices of the Pearson correlation, and outliers were omitted from the correlation analysis.

RESULTS AND DISCUSSION

At the Zemun and Višnjica locations, 42 and 21 fish specimens were collected, respectively. A total of six endoparasitic species were found in the intestines of collected barbel specimens, with a 98.41% infection prevalence. The total number of endoparasites per fish individual ranged from 6 to 207. The recorded helminth representatives belonged to 3 phylum - Nematoda (1 species), Acanthocephala (1 species) and Platyhelminthes with two classes – Cestoda (2 species), Trematoda/orderDigenea (2 species). The list of parasitic species, number of infected barbels, prevalence and intensity of infection per endoparasitic species are presented in Table 1.

All except one examined barbel were infected by the acanthocephalan species *Pomphorynchus laevis,* which is in accordance with previous investigations (Laimgruber et al., 2005; Djikanovic et al., 2010). The number of *P. laevis* specimens per individual ranged from 6 to 207. This acanthocephalan is often the dominant parasite species in barbel populations (Hine and Kenedy, 1974; Nedeva et al., 2003; Nachev and Sures, 2009). The prevalence and abundance of *P. laevis* in barbel have been attributed to the dynamics of the abundance of intermediate hosts (amphipods, gammarids) and their importance in the diet of the fish (Hine and Kenedy, 1974; Rumpus and Kennedy, 1974; Moravec and Scholz, 1991; Nachev and Sures, 2009). Moravec et al. (1997) described the barbel as specific in its choice of diet, suggesting that lower parasite diversity but higher abundance of one parasitic species can be a consequence of such food preferences. The presence of only one parasite species per individual could be explained by a high *P. laevis* intensity of infection, which results from the barbel’s preferred diet that consists of amphipods, even though they represent a very small part of the bottom fauna.

Table 1. The barbel`s helminth fauna (*Barbus barbus* L.) in Belgrade region of Danube River 2007-2009.

SITES	ZEMUN		VISNJICA	
PARASITIC SPECIES	**Cestoda** *Caryophyllaeus brachycollis* (Janiszewska, 1951) **Trematoda** *Allocreadium isoporum* (Looss, 1894) *Allocreadium dogieli* (Koval, 1950) **Nematoda** *Rhabdochona hellichi* (Šrámek, 1901) **Acanthocephala** *Pomphorhynchus laevis* (Müller, 1776)		**Cestoda** *Caryophyllaeus brachycollis* (Janiszewska, 1951) *Caryophyllaeus laticeps* (Pallas, 1781) **Trematoda** *Allocreadium isoporum* (Looss, 1894) **Nematoda** *Rhabdochona hellichi* (Šrámek, 1901) **Acanthocephala** *Pomphorhynchus laevis* (Müller, 1776)	
NUMBER OF EXAMINED BARBELS	42		21	
NUMBER OF INFECTED BARBELS	42		**20**	
PREVALENCE (%)	**100**		**95.24**	
NUMBER OF INFECTED BARBELS	*C. brachycollis* *A. isoporum* *A. dogieli* *R. hellichi* *P. laevis*	**3** **3** **1** **3** **42**	*C. brachycollis* *C. laticeps* *A. isoporum* *R. hellichi* *P. laevis*	**1** **2** **2** **3** **20**
INTENSITY OF INFECTION	*C. brachycollis* *A. isoporum* *A. dogieli* *R. hellichi* *P. laevis*	**5-9** **2-8** **11** **3-7** **6-207**	*C. brachycollis* *C. laticeps* *A. isoporum* *R. hellichi* *P. laevis*	**3** **9-11** **5-8** **1-7** **9-124**

The results of our study correspond well with the data of Laimgruber et al. (2005), Moravec et al. (1997) and Nachev and Sures (2009) who found the parasite *Rabdochona hellichi* (Nematoda) in barbel intestines. They also published finding *Caryophyllaeus barchycollis* and *Allocreadium isoporum*. The parasite list of *B. barbus* published by Margaritov (1966) and Kakacheva-Avramova (1977) for the Bulgarian section of the Danube River contained three cestode species (*Caryophyllaeus brachycollis, C. laticeps, C. fennica*), which supports our findings.

The relationship between the macrozoobenthos community composition and structure and fish intestine contents is presented in Table 2. The dominant invertebrate hosts in the diet of the barbel were Gammaridae represented in fish gut contents with 31.4% and 46.7%, in the two studied locations respectively. Our results indicate that the fish gut content resulted from selective feeding behaviour, since Gammaridae were represented by a higher ratio in the fish's diet than in the benthic fauna composition (1.4% to 16.22%). A major characteristic of the principal invertebrate fauna of the Danube River in Serbia is the high abundance of gammarids (*Dikerogammarus villosus* being the most frequent), except in the Belgrade are, where gammarids represent a small part of bottom fauna (Paunovic et al., 2010). Representatives of Oligochaeta, Molluscs and Ephemeroptera, identified in barbel gut contents, are intermediate hosts for recorded endoparasitic species (*Caryophyllaeus brachycollis*, *C. laticeps, Allocreadium isoporum, A. dogieli)* (Kakacheva-Avramova, 1983; Bauer, 1987; Moravec et al., 1997). According to Moravec and Scholz (1995) trichopteran larvae (*Hydropsyche*) serve as intermediate hosts for the transmission of Nematoda *Rhabdochona hellichi* (Moravec, 1995; Moravec et al., 1997; Laimgruber et al., 2005). The recorded Trematoda parasites have complex life cycle, which includes two intermediate hosts; the first is a mollusc (*Sphaerium* sp, *Bythinia tentaculata*), and the second is some type of insect larva (i.e. Ephemeroptera) (Bauer, 1987).

Table 2. Bottom fauna and intestine content of examined barbels in Belgrade section of the Danube River, Zemun and Visnjica locality

Group of bottom fauna/ locality	ZEMUN		VISNJICA	
	Bottom fauna (%)	Intestine content (%)	Bottom fauna (%)	Intestine content (%)
Gammaridae	1.4	31.43	16.22	46.73
Gastropoda	11.46	6.08	8.7	4.20
Bivalvia	27.54	2.08	4.18	5.40
Oligochaeta	60.8	6.45	71.45	3.60
Others (Insects, Crustacea)		53.96		40.07

There was a negative correlation between the Fulton condition factor (CF) and the number of parasites per barbel specimen (r =0.3568; **Fig 1**).

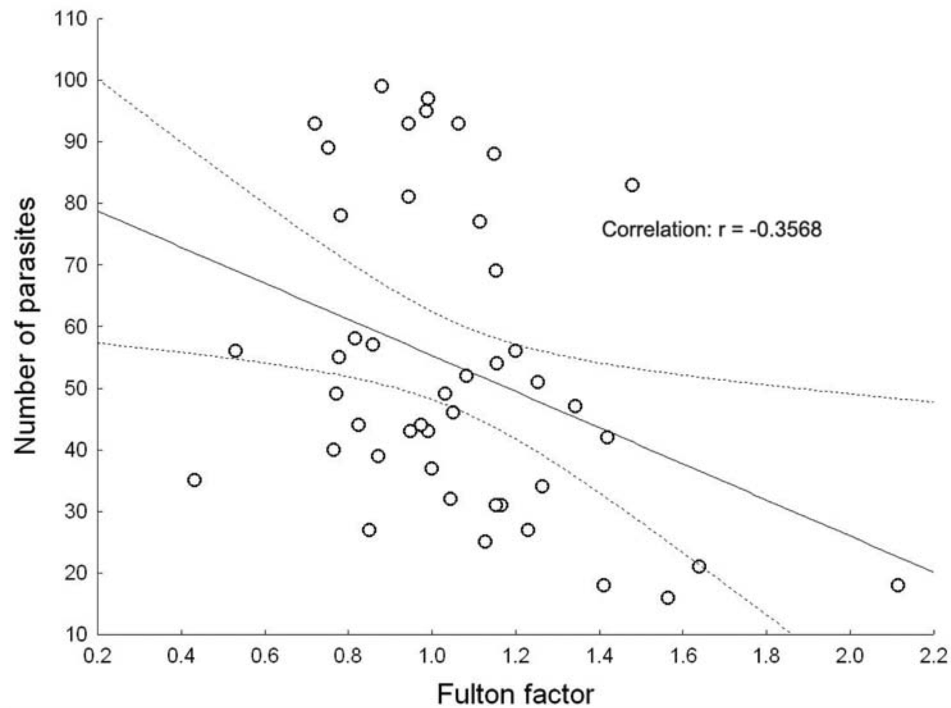

Figure 1. Correlation between Fulton condition factor and number of parasites

CONCLUSION

The feeding habits of the barbel and its diet are influenced by the available local invertebrate fauna, which is, in turn, determined by water quality and habitat composition.

ACKNOWLEDGEMENTS

This work is supported by Ministry of Education, Science and Technological Development Republic of Serbia, Project No. 173045.

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INVESTIGATION OF THE ABILITY OF FLOATING REEFS TO COLLECT YELLOWTAIL (*SERIOLA DUMERILI*) FRY FROM THE NATURE

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ISPITIVANJE MOGUĆNOSTI PLUTAJUĆIH GREBENA ZA SAKUPLJANJE MLAĐI GOFA (*SERIOLA DUMERILI*) IZ PRIRODE

Apstrakt

Cilj ovog istraživanja bio je da se mlađ gofa (*Seriola dumerili*) izlovi uz pomoć plutajućih grebena iz prirode. U svrhe ovog istraživanja, napravljena su četiri plutajuća grebena sa palminim listovima na površini i u vodi. Ribe koje su težile manje od 5g nisu bile izlovljene pod plutajućim i statičnim grebenima. Međutim, ustanovljeno je da se mlađ sakupljala ispod materijala kao što je plastika, drvo, morska trava. Morske struje su nosile ovu mlađ i mlađ se ponašala kao plankton. Mlađ od 5-200g se sakupljala ispod grebena u vodi. 1000 jedinki mlađi pronađeno je ispod grebena blizu mesta Sıcanadası u zalivu Antalija, 500 jedinki mlađi ispod drugog grebena koji je lociran nešto južnije, 350 jedinki još južnije i 200 jedinki je pronađeno ispod grebena koji je lociran još južnije od prethodnog. Jednike su prebrojane uz pomoć tehnologije koja omogućava snimanje is fotografisanje. Mlađ gofa koja je težila između 250 i 300 g je takođe prebrojana.

Ključne reči: Plutajući greben; gof, Seriola dumerili; mlađ

Abstract

The aim of this study was to catch of yellowtaıl (greater amberjack) (*Seriola dumerili*) fry using floating reefs from the nature. For this purpose, four floating reefs with palm leaves were created on the surface and in the mid-water. Under the reef in floating and fixed position, less than 5g of the fish were not caught. However, it was determined that under the materials such as plastic, wood, seaweed etc. fry accumulated. Moreover it was determined that these fry were moved by currents and showed planktonic behaviour. Fry of 5-200g accumulated under the reef in the mid-water. One thousand fry under the reef located near Sıcanadası in the Gulf of Antalya, 500 fry under the second reef located in south of first reef, 350 fry under the third reef located in south of second reef, 200 fry under the fourth reef located in south of third reef were counted using video and photograph techniques. Yellowtail fry of 250-300g were counted.

Keywords: Floating reef; yellowtail, Seriola dumerili; fry

EFFECTS OF TUMBLING PROCESS ON PHYSICOCHEMICAL PROPERTIES OF OCTOPUS (*OCTOPUS VULGARIS*)

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EFEKTI TUMBANJA NA FIZIČKE I HEMIJSKE ODLIKE HOBOTNICE (*OCTOPUS VULGARIS*)

Apstrakt

Hobotnica (*Octopus vulgaris*) je glavonožac koji se konzumira pretežno na Mediteranu, u Južnoj Africi i Istočnim zemljama sveta. Najčešće se plasira na tržište sveža, zaleđena ili suva usoljena. U načelu, sveži glavonošci, a naročito hobotnica, imaju veoma čvrstu i žilavu strukturu. Ova čvrstina potiče iz strukture i sastava kolagena, i njegovog nakupljanja u mišićima glavonožaca. Kod hobotnice, kolagen se najčešće opušta u vrućoj, najčešće ključaloj vodi. Tradicionalan način ublažavanja čvrstine kod hobotnice bio je 'mlaćenje' sveže ulovljene hobotnice o stene pored mora. Industrija je usvojila ovu proceduru, tako da se mehanično 'mlaćenje' primenjuje u specijalizovanoj opremi za tumbanje, gde se hobotnica tumba u vodi. Proces tumbanja se često koristi da se poremeti struktura mišića, razgrade spoljne površine komada mesa i otpuste protein iz miofibrila. Tumbanje poboljšava nežniju strukturu hobotnice dok NaCl (u niskim koncentracijama) pomaže izlučivanje proteina i poboljšava kapacitet mišića za zadržavanjem vode i na taj način povećava nežnost krajnjeg proizvoda. Proces tumbanja ispitivan je za omekšavanje drugih vrsta mesa. Međutim, postoji ograničen broj studija koje se bave upotrebom tumbanja na hobotnice.

Osnovni cilj ovog istraživanja jeste da ispita efekte koje process tumbanja ima na fizičkohemijske odlike mišića hobotnice. Hobotnica (*Octopus vulgaris*) sa glavne pijace ribe u Antaliji. Odmah pošto je dospela na kopno, hobotnica je kupljena i stavljena u hladne kese za skladištenje sa ledom. Proces tumbanja izvršen je korišćenjem mašine za tumbanje koja je posebno dizajnirana za ovu studiju. Proces tumbanja izvršen je u kontinuitetu, sa 3 različite dužine trajanja procesa (2, 4, 6 sati). Posle tumbanja, uzorci su podvrgnuti analizi. Rezultati su pokazali da je tumbanje imalo pozitivan efekat na fizičkohemijske odlike hobotnice. Dužina trajanja tumbanja uticala je na sve parametre. Sa povećanjem trajanje tumbanja, povećavala se i nežnost mišića hobotnice. Ukupna količina rastvorljivog proteina, ukupna količina amino kiselina i pH vrednosti su se povećale sa povećanjem trajanja tumbanja. Kapacitet za zadržavanjem vode se smanjio a gubitak pri kuvanju povećao. Prema merenjima teksture instrumentima, čvrstina i vrednosti poprečne sile su se smanjile posle procesa tumbanja. Zaključeno je da je process tumbanja efikasan metod za opuštanje mišića hobotnice.

Ključne reči: Hobotnica, omekšavanje, tumbanje, tekstura

Abstract

Common octopus (*Octopus vulgaris*) is a cephalopod eaten mainly in Mediterranean, South American and Oriental countries and is typically marketed fresh, frozen and dried salted. Cephalopods in general and octopus in particular have a very firm and tough texture, especially when fresh. This toughness is associated with the collagen structure, content and aggregation in the cephalopod muscles. Collagen in octopus is tenderized mainly by heating, usually in boiling water. The traditional way to overcome octopus toughness has been the repeated "beating" of the freshly caught octopus on the rocks by the sea. This procedure has been adopted by the industry the mechanical "beating" of octopus is performed in specialized tumbling equipment, where octopus is tumbled in water. Tumbling processes are commonly used to disrupt the muscle structure, disintegrate external surfaces of meat pieces and to release myofibrillar proteins. In general, tumbling improves tenderness while NaCl (in low concentrations) helps the extraction of proteins and improves the water-holding capacity of the muscle increasing the tenderness of the final product. Tumbling process has been studied for other meats for tenderization. However there is in limited amount of study on use of tumbling on octopus.

The main objective of this study is to investigate the effects of tumbling process on physicochemical properties of octopus muscle. Octopus (*Octopus vulgaris*) was obtained from the main fish market of Antalya. They were purchased just after landing and placed in cold storage bag with ice. Tumbling process was carried out using a tumbler specially designed for this study. Tumbling process was performed continuously in 3 different tumbling times (2, 4, 6 h). After tumbling, the samples were analysed. According to results tumbling was effective on physicochemical properties of octopus. Tumbling time affected all parameters. With increase in tumbling time increased in tenderness of octopus muscle. Total soluble protein, total free amino acid and pH values increased with increase of tumbling time. Water holding capacity decreased and cooking loss increased. According to instrumental texture measurements, hardness and shear force values decreased after tumbling process. As a result, tumbling was found as effective method to tenderize of octopus muscle.

Keywords: Octopus; tenderization; tumbling; texture

DISTRIBUTION OF SPECIES OF THE GENUS *PSEUDOMONAS* IN ARTIFICIAL PONDS OF ARMAVIR REGION IN ARMENIA

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DISTRIBUCIJA VRSTA RODA *PSEUDOMONAS* U VEŠTAČKIM JEZERIMA REGIONA ARMAVIR U JERMENIJI

Apstrakt

Obavljena je identifikacija i izolacija bakterija roda *Pseudomonas* iz uzoraka podzemne vode koja se koristi za gajenje pastrmke.

Urađena je komparativna analiza vrsta *Pseudomonas* u uzorcima dovodne i odvodne vode. Analizirano je 80 uzoraka od kojih je 50 uzorkovano iz 5 pastrmskih jezera regiona Armavir.

Ispitivanje je obavljeno korišćenjem metode membranske filtracije upotrebom visoko selektivnih medija na rod *Pseudomonas.* Korišćeni su kitovi za brzu biohemijsku identifikaciju vrsta pomenutog roda.

Ispitivan je odnos između kvantitativnog i kvalitativnog sastava vrsta *Pseudomonas* i fizičko hemijskih osobina vode. Vrste *P. fluorescens, P. aeruginosa* i *P. diminuta* su imale visoku frekvenciju pojavljivanja u arteškoj void. *P. fluorescens* i *P. aeruginosa* su izolovane iz uzoraka vode uzetih iz jezera koj su sadržala ribu bez znakova bolesti. Vrste *P. anguilliseptica, P. putida, P. diminuta* poznate kao izazivači pseudomonada kalifornijske pastrmke su identifikovane iz vode gde se nalazila bolesna riba sa ulceracijama u blizini repnog peraja i na leđnoj strain. Zapaženo je i istovremeno prisustvo *P. anguilliseptica* i *P. alcaligenes.*

Abstract

Identification and isolation of bacteria from genus *Pseudomonas* has been carried out form ground water samples used for rainbow trout aquaculture.

The comparative analysis of *Pseudomonas* species in inlet and outlet water samples has been done. 80 water samples were analyzed, 50 from which have been taken from five rainbow trout ponds from Armavir region.

Examination of water has been performed by membrane filtration method, using high selective media particularly for genus *Pseudomonas.* The rapid biochemical identification kits were used to identify the species of the mentioned genus.

The relationship between quantitative and qualitative composition of *Pseudomonas* species and physical-chemical properties of water have been studied. *P. fluorescens, P. aeruginosa* and *P. diminuta* had high frequency of occurrence in artesian water. *P. fluorescens* and *P. aeruginosa* have been isolated from water samples taken from ponds contaning fish without symptoms of illness. The following species *P. anguilliseptica, P. putida, P. diminuta* known as a causative agents of pseudomonades of rainbow trout were identified in water where ill fish with ulcers near caudal fins and on back side were present. Co-occurrence of *P. anguilliseptica* and *P. alcaligenes* has been noticed.

EFFECTS OF CITRUS ESSENTIAL OIL SUPPLEMENTATION ON THE GROWTH AND SERUM BIOCHEMICAL RESPONSES OF *OREOCHROMIS MOSSAMBICUS*

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EFEKTI ETERIČNOG ULJA CITRUSA KAO DODATKA U ISHRANI NA PRIRAST I BIOHEMIJSKE REAKCIJE SERUMA KOD MOZANBIČKE TILAPIJE *OREOCHROMIS MOSSAMBICUS*

Apstrakt

Akvakultura se razvila veoma brzo u proteklih 30 godina. Cilj akvakulture je da vodi računa o zdravlju riba i da poboljša njihove performanse. Antibiotici se često koriste protiv bolesti riba, za jačanje imunog sistema i u svrhe povećanja prirasta. Međutim, upotreba antibiotika je ograničena u mnogim zemljama zbog otpornosti koje stvaraju kod bakterija i zbog ostataka koje mogu da budu koncentrovani u vodenim organizmima i otpušteni u vodenu sredinu (Citarasu, 2010). Zbog toga istraživači pokušavaju da nađu alternativna rešenja, umesto upotrebe antibiotika u akvakulturi. Cilj ovog rada je da oceni efekte limunovog eteričnog ulja na prirast, hematološke i imunološke odgovore kod *O. mossambicus*.

U eksperimentu koji je trajao 90 dana, zdrava Mozanbička tilapija (prosečne težine ± SD = 0.91±0.03 g) nasađena je u 12 akvarijuma (40L), svaki akvarijum sa po 25 riba. Hidro destilacijom dobijeno je limunovo eterično ulje iz sveže kore limuna. U ove svrhe korišćen je Clevenger system sa 150 g biljne suve materije i 1500 mL vode. Eterično ulje dodato je hrani za ribe sa 0, 1, 3 i 5 g/kg.

Hrana sa dodatakom limunovog eteričnog ulja od 1 g/kg uticala je na prirast i neke biohemijske parametre seruma.

Rezultati ovog istraživanja pokazuju da dodatak limunovog eteričnog ulja od 1 g/kg u ishrani ribe u toku od 90 dana ima pozitivan efekat, utiče na poboljšani prirast i neke biohemijske parametre u krvi *O. mossambicus*. Slični rezulatati dobijeni su korišćenjem eteričnog ulja origana i nekih biljnih imunostimulansa (Gültepe et al., 2014).

INTRODUCTION

Aquaculture sector has showed a rapid growth in the last 30 years. The aim of aquaculture is to maintain fish health as well as to improve fish performance. Antibiotics are commonly used to control fish disease, enhance the immune systems and as an increase of growth performance. However, the usage of antibiotics has been restricted in many countries due to the resistance inducted to bacteria and the residues that they could release in aquatic organisms and food (Citarasu, 2010). Therefore, researchers are focusing on alternatives to the use of antibiotics in aquaculture. Thus, the aim of the present study is to assess the effects of citrus essential oil on growth performance, hematological and immunological responses in *O. mossambicus*.

MATERIAL AND METHODS

In a 90-day feeding trial, healthy cultured *O. mossambicus* (mean weight ± SD = 0.91±0.03 g) were stocked 25 fish per aquarium (40–L) on 12 aquariums. Citrus essential oil was obtained from fresh peel by hydro-distillation, using a Clevenger system with 150 g dry plant material and 1500 mL water. The essential oil was added to the feed at a rate of 0, 1, 3 and 5 g/kg.

RESULTS

Supplementation of citrus essential oil at level of 1 g/kg diet influenced the growth performance and some serum biochemical parameters.

DISCUSSION

In conclusion, the results of the present study demonstrated that, feeding with diet supplemented with citrus essential oil at 1 g/kg for 90 days has adequate beneficial effects on improvement of growth performance and some biochemical parameters of *O. mossambicus* blood. Similar results were obtained by the use of oregano essential oil and some herbal immunostimulants in fish diets (Gültepe et al., 2014).

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CHEMICAL AND FATTY ACID COMPOSITION OF TURBOT (*PSETTA MAXIMA MAEOTICA*) MEAT FROM THE BULGARIAN BLACK SEA SHORES

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HEMIJSKI I MASNO KISELINSKI SASTAV MESA IVERKA (*PSETTA MAXIMA MAEOTICA*) IZ BUGARSKOG DELA CRNOG MORA

Apstrakt

Cilja rada je bio da se analizira hemijski i masno kiselinski sastav mesa ivera (Psetta maxima maeotica) ulovljenog u Bugarskom delu Crnog mora. Iver je lovljen kočarenjem u periodu od 12 do 30 novembra 2014 sa 5 proizvoljno određenih zona od ukupno 38 zona. Ovo istraživanje je pokazalo da postoje izražene razlike u hemijskom sastavu i energetskoj vrednosti mesa ivera uhvaćenog sa različitih lokaliteta u severnom i južnom sektoru Crnog mora. Konstatovana su veća variranje u proteinskom sastavu od 16.87 % (južni sektor) do 19.32 % (severni sektor) u odnosu na variranje nivoa masti u mesu (1.29 - 2.08 %). *Na osnovu nivoa masti, ribe sa istraživanih područja bi mogle da se klasifikuju kao posna (niskomasna) grupa riba*.

Na osnovu prisustva specifičnih masnih kiselina u mesu ivera iz zona C17-E9 i E11-E13 i dubine kočarenja od 36-53 m, ove ribe se mogu svrstati u palmitinski tip, dok se ribe ulovljene iz zona L1-7/M2/N1-2 i dubine od 52-64 m mogu svrstati u oleinsko-palmitinski tip. Biološki važan odnos n-3 i n-6 masnih kiselina je varirao u opsegu od 0.06 do 0.13 što je zadovoljavajući nivo propisan od strane WHO (Svetske zdravstvene organizacije) koji smanjuje rizik po ljudsko zdravlje (<5.0). Odnos PUFA/SFA u lipidima ivera je varirao od 0.36 do 0.54, sa boljim balansom masnih kiselina u mesu riba iz severnog sektora Crnog mora (0.54).

Ključne reči: iver, masne kiseline, odnos masnih kiselina, lipidne grupe
Keywords: turbot, fatty acids, fatty acid ratios, lipids groups

INTRODUCTION

The turbot (*Psetta maxima maeotica*) is one of commercial fish species in the Black Sea. The Black Sea turbot is a demersial fish, spawning in the spring, during autumn it approaches the shores at a depth of 10-30 m for fattening, whereas winter gatherings are at a depth of 70-90 m. It prefers sandy mussel beds. The adult turbot is a predator, and feeds mainly on demersial fish, gobies, whitings etc., crustaceans and molluscs. Turbot meat is white, tender and with excellent flavour, that is why it is a valuable and much sought fish. Deep-sea fish including Black Sea turbots are not only a dietary source of biologically valuable proteins, but also a food with healthy components (Suseno et al., 2010), as vitamins, essential fatty acids, macro- and trace minerals (Özyrut et al., 2009). In natural basins, the nutritional value of fish is mainly modulated by the available natural food. According to Steffens (2006), the lipid content of naturally inhabiting species could not be altered and is highly dependent on the species and amount of available food.

During recent years, studies on the seasonal patterns of total fat content and fatty acids of Black Sea turbots have been carried out (Merdzhanova, 2014). Systemic observations on the age, season, environmental conditions etc. that are acknowledged to influence the meat biochemical composition, are scarce on a national scale.

The investigation of changes in the plastic metabolism, quantity and quality of fatty acids and tocopherols with regard to the autumn catch places at the Bulgarian Black Sea shores allows for objective information and evaluation of the nutritional value of Black Sea turbots, hence contribution to the study of the species' biology.

Therefore, the aim of the present study was to analyse the chemical and energy content of meat, as well as fatty acid composition of meat lipids in turbots (*Psetta maxima maeotica*) caught at the Bulgarian Black Sea shore.

MATERIAL AND METHODS

In this study, turbots (*Psetta maxima maeotica*) caught at the Black Sea by trawling of 5 areas, randomly selected from the total number of 38 areas between 12 and 30 November 2014 were used (Table 1). The standard methodology for stratified sampling from the seasonal aquatory of fish, the swept area method, was applied (Sparre and Venema, 1998; Sabatella and Franquesa, 2004). Trawlings were performed by means of bottom trawl, 10×10 cm mesh size, holding the net for 60 min at towing speed of 1.2 knots. Turbots were divided into 5 groups according to catch area and depth. Fish with size average for the group were selected, ranging from 800 to 1600 g from strata 2 and 3.

Table 1. Place and date of turbot catch

Black Sea	Southern sector		Northern sector		
Date	19-28.11.2014	17.11.2014	12.11.2014	12.11.2014	12.11.2014
Place of catch	C17-E9	E11-E13	L1-7	M-2	N1-2
Depth, m	36-41	53	52	61	64

For meat chemical analysis, fillet samples were obtained from the same body part. The following parameters were determined: water content % (drying at 105 ^oC, 24 h), protein content % (Kjeldahl method, semi-automated DK 6 digester unit and UDK 132 distillation system, Velp Scientifica;), fat content % (by the method of Smidt-Boudzynski Ratzlaff); mineral content % (by burning in a muffle furnace at 550 ^oC). The energy content of turbot meat was calculated on the basis of the chemical composition using the following coefficients: 17 $kJ.g^{-1}$ for proteins and carbohydrates and 37.0 $kJ.g^{-1}$ for fat (Ordinance 23/2001, Ministry of Health).

The fatty acid composition of meat triglycerides was analysed by gas chromatography using a HP 5890 II gas chromatograph with flame ionisation detector, 60 m DB-23 capillary column, column temperature – 130 ^oC (1 min), 6.5 ^oC/min increments up to 170 ^oC, 3.0 ^oC/min increments up to 215 ^oC (12 min); 40.0 ^oC/min up to 230 ^oC (1 min); detector temperature – 280 ^oC; injector temperature – 270 ^oC, carrier gas – hydrogen (H_2), split 1:50 and software Data Apex Clarity™ 2.4.1.93/2005. The individual fatty acid content was identified with standards and through retention times. Fatty acid groups, fatty acid ratios, as well as group ratios were calculated.

RESULTS AND DISCUSSION

Chemical composition and energy content of turbot meat

Water, protein, fat and mineral content of meat of studied groups of turbot is presented in Table 2. Water content varied within 78.23 % - 79.26% (northern sector) to 80.03 - 80.37 % (southern sector). The difference between values was < 3%. Data demonstrated that water was the main chemical constituent of studied turbot groups. This parameter is important for organoleptic properties of meat.

Table 2. Chemical composition (% of wet matter) and energy value ($kJ.100\ g^{-1}$) of meat

Place of catch	C17-E9	E11-E13	M2	N1-2	L1-7
Water	80.37	80.03	78.30	79.26	78.56
Proteins	19.87	16.99	19.32	18.56	18.88
Fats	1.76	2.08	1.52	1.29	1.69
Ash	1.00	0.90	0.86	0.89	0.84
Energy (total), $kJ.100\ g^{-1}$	351.91	365.79	384.68	363.25	383.49

Absolute values of protein content were from 16.87 % (C17-E9) to 19.32 % (M2). The studied groups of fish were distinguished with high relative proportion of protein on the dry matter basis from 85.08 - 85.94% (southern Black Sea sector) to 88.2-89.49 % (northern Black Sea sector). The high protein content of turbot meat predetermines its good nutritional quality.

Turbot meat fat values varied from 1.29 % to 2.08 % depending on sea sector, whereas the relative fat content on DM basis was 6.22 - 10.42 %. Detected values were lower than total fat content of turbot from the autumn catch (October-November) reported by Merdzhanova (2014). On the basis of the classification of Kyosev and Dragoev (2009), the turbot groups from studied fields were referred to the lean fish group (with meat fat content < 2%). The low fat content indicated that by the end of November, meat of turbots still gathered energy nutrients in relation to the fattening habitat and local trophic level. Mineral content of turbot meat varied from 0.84 to 1.00 %. Energy value of meat in studied fields ranged between 351.91 kJ.100 g^{-1} and 384.68 kJ.100 g^{-1}.

Quantity and quality of fatty acids in turbot meat lipids

The main groups of fatty acids in turbot meat lipids are shown in Table 3.

Turbot meat lipids contained all three groups of fatty acids – saturated (SFA), monounsaturated (MUFA) and polyunsaturated (PUFA) distributed as followed: MUFA > SFA > PUFA.

SFA content varied within a narrow range: 38.1-41.9 %. The values were comparable to those established by Merdzhanova (2014) in her study on the fatty acid composition of Black Sea turbots caught during the autumn. The saturated fatty acids in studied samples were mainly represented by the palmitic (C16:0) and myristic (C14:0) acids, followed by stearic acid (C18:0), in the following order: C16:0 > C14:0 > C18:0. The group of fish from the fields L1-7/M2/N1-2 had lower SFA level in their meat (by 7.3 %) but higher MUFA concentration (by 5.8%) as compared to all other groups. Saturated fatty acids with odd number of C atoms – C15:0 and C17:0 which had no nutritional value, were under 1%.

Unsaturated fatty acids in the meat of the three studied groups of turbot were approximately 60% of the total FA content of meat lipids. This was in line with the results of Merdzhanova (2014) from the autumn catch of Black Sea turbot, as well as those of Guner et al. (1998) in other sea fish species as anchovies and whitings.

Within the MUFA group, predominating fatty acids were the oleic acid C18:1 ω-9 (for fish caught in fields L1-7/M2/N1-2), vaccenic acid C18:1 ω-7 (for fish caught in C17-E9 and E11-E13 fields), and palmitoleic acid (C16:1). The results showed difference with regard to the oleic acid content. Its average concentration in the meat of fish from the northern sector was by 73% higher than that from the southern sector (15.2-15.4%). The values of the palmitoleic acid varied within a narrowed range (6.9 - 8.9 %).

The polyunsaturated fatty acid group was presented with levels from 13.1 to 20.4 %, with higher contents in fish caught from the northern sector. Omega-6 fatty acids in turbot meat lipids from studied strata was determined at a higher extent by linoleic acid C18:2 ω-6 proportion (0.6-1.7 %) and a lesser extent, by linolenic acid (C18:3 ω-6) content.

Differences were observed with respect to the PUFA from the omega-3 group. Again, they were more in turbots from fields L1-7/M2/N1-2 (18.1 %) than in the other groups (13.9 % and 12.0 %). From the omega-3 fatty acids group, docosahexaenoic acid C22:6 ω-3 was the predominant one, with comparable values for C17-E9 and E11-E13– 10.1-11.9 %, and by 28.6 % higher for fish from fields L1-7/M2/N1-2 (15.3 %). At the same time, docosahexaenoic acid C22:6 ω-3 values were substantially higher than those of eicosapentaenoic C20:5 ω-3 acid – 1.9-4.2%. This is in agreement with the studies of other authors (Seibel and Drazen, 2007; Iverson, 2009), stating that in predatory fish, DHA level exceeded that of EPA while in fish feeding on plankton, the opposite relationship was seen: EPA > DHA. The most probable reason for this is that turbots are predatory fish from a higher trophic level of the food chain. Steffens and Wirt (2005) and Ackman (1994) affirm that long-chain fatty acids C20 and C22 are at a higher level in sea plankton. This explains the higher levels of C20:1 (3.3-4.7%) and C22:6 ω-3. According to Ugoala et al., (2008) and Abbas et al., (2009) sea fish species contained more docosahexaenoic 22:6 from the ω-3 group, while freshwater fish – more C18:2 and C20:4 from the ω-6 group of fatty acids.

Table 3. Individual fatty acid composition of turbot

FA*, %	Place of catch		
	C17 – E9	**E11 – E13**	**N1-2/M2/L1-7**
C 12:0	0.1	0.1	-
C 14:0	3.9	5.2	5.8
C 15:0	0.5	0.6	0.6
C 16:0	31.5	30.4	24.8
C 16:1	6.9	8.4	8.9
C 17:0	0.2	0.2	0.1
C 18:0	3.0	2.9	2.5
C 18:1 (ω-9)	15.4	15.2	26.7
C 18:1 (ω-7)	17.8	16.7	1.8
C 18:2 (ω-6)	0.6	0.9	1.7
C 18:3 (ω-6)	0.2	0.2	0.5
C 18:3 (ω-3)	-	-	0.4
C 20:1	4.1	4.7	3.3
C 20:2 (ω-6)	-	-	0.1
C 20:5 (ω-3)	2.0	1.9	2.4
C 22:0	1.9	2.5	4.2
C 22:1	-	-	0.8
C 22:6 (ω-3)	11.9	10.1	15.3
C 24:0	-	-	0.1
SFA	**41.1**	**41.9**	**38.1**
UFA	**58.9**	**58.1**	**61.9**
MUFA	**44.2**	**45.0**	**41.5**
PUFA	**14.7**	**13.1**	**20.4**
∑ω-6	**0.8**	**1.1**	**2.3**
∑ω-3	**13.9**	**12.0**	**18.1**
ω-6/ω-3	**0.06**	**0.09**	**0.13**

The higher levels of specific MUFA and PUFA in the meat lipids of fish groups from the northern Black Sea sector (L1-7/M2/N1-2) were most probably due to the species diversity and trophic level in the different studied fields and depths. This reflected on the biologically important omega-6/omega-3 ratio in fish, which ranged within 0.06-0.13. The fish from L1-7/M2/N1-2 had higher ratios than the other studied groups, with 1.4 to 2.1-fold differences.

The PUFA/SFA ratio calculated on the basis of fatty acid groups values (Table 3) varied from 0.38 to 0.54. According to the British Department of Health (1994) the minimum recommended value for this ratio is 0.45. Other researchers and sources (Kang and Leaf, 2000; FAO/WHO, 2008; EFSA, 2009) have set optimum value of 1.0 ± 0.2, therefore the recommended range of PUFA/SFA is from ≥ 0.45 to 1.0. According to our data, the fish caught in the northern Black Sea sector are within the recommended range (0.54). According to Simopolous (2013) values < 1.0 indicate a balanced distribution of fatty acid groups, as shown for other fish species from this sector.

CONCLUSIONS

The study provides evidence for specific differences in meat chemical composition and energy value of turbots caught from different localities in the northern and southern Black Sea sectors. A higher variation in protein content from 16.87 % (southern sector) to 19.32 % (northern sector) was observed as compared to meat fat contents range (1.29 - 2.08 %). With relation to fat content, fish from studied fields and sectors could be classified as the lean fish group.

It was demonstrated that according to the presence of the specific fatty acids, turbot meat lipids from fields C17-E9 and E11-E13 and trawling depth 36-53 m were of the palmitic type, whereas those from fish caught in L1-7/M2/N1-2, depth 52-64 m: of the oleic-palmitic type.

From the omega-3 fatty acid group, docosahexaenoic (C22:6 ω-3) acid in turbot meat lipids was at a higher amount than the eicosapentaenoic acid (C20:5 ω-3).

The biologically important ω-6:ω-3 ratio varied from 0.06 to 0.13, falling within the WHO range related to low risk for human health (<5.0).

The PUFA/SFA ratio in turbot lipids varied from 0.36 to 0.54, with a more balanced distribution for fatty acid groups in the meat of fish from the northern Black Sea sector (0.54).

According to the present study, Black Sea turbots caught in November 2014 from the northern sector (cape Galata, Kamchia and Shabla) at a depth of 52-64 m had higher absolute (19.32 %) and relative (89.49 %) meat protein contents, more balanced distribution of fatty acids (PUFA/SFA-0.54), higher unsaturated fatty acid content (61.9 %), including PUFA (20.4 %) and ω-3 (18.1 %) and more favorable ω-6:ω-3 ratio than respective values obtained from fish caught in the southern sector.

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LEACHING OF DIETARY MALTOSE, ECONOMIC EFFICIENCY TO NILE TILAPIA (*OREOCHROMIS NILOTICUS*) FINGERLINGS

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EKONOMSKA EFIKASNOST DODAVANJA MALTOZE U ISHRANI MLAĐI NILSKE TILAPIJE (*OREOCHROMIS NILOTICUS*)

Apstrakt

Hrana koja se proizvodi za akvakulturu zahteva dobru stabilnost, kako bi se izbeglo njeno raspadanje u toku primene, naročito ugljenih hidrata. Pet eksperimentalnih hrana je formulisano tako da sadrže različite količine maltoze (20, 25, 30 i 35%). Postojanost maltoze u toku 15, 30, i 60 minuta ustanovljeno je u laboratorijskim uslovima. Eksperimentalni rezultati su pokazali da se natapljenost maltozom povećava sa povećanjem vremena potapanja (30 min) i bilo je 100% za hranu 20, sa 25% maltoze i 95% za hranu 30, sa 35% maltoze. Porast je bio u korelaciji sa podacima koji su dobijeni za natapanje dijetetske maltoze, a ustanovljene su značajne razlike (p <0,05) u srednjim vrednostima finalne mase kao i u prirastu srednjih vrednosti masa između grupa. Riba koja je hranjena hranom koja je sadržala 35% maltoze ostvarila je najbolji prirast, ekonomsku efikasnost i relativne ekonomske efikasnosti u poređenju sa drugim ispitanim hranama.

Ključne reči: natapanje maltozom, tilapija, rast, ekonomska efikasnost

Abstract

Diets developed for aquaculture require good stability to avoid dissolution from exposure, especially carbohydrates. Five experimental diets were formulated to contain different maltose levels (20, 25, 30 and 35%). Maltose retention at 15, 30, and 60 minutes was investigated in the laboratory. The result of the experiments showed that maltose leaching increased by increasing time of immersion (30 min),and it was 100% for feed 20, containing25% maltose, and 95% for feed 30,containing 35% maltose. The growth was correlated to the data produced from the leaching of dietary maltose, and significant differences (P <

0.05) in mean final weight and mean weight gain between groups have been recorded. The fish fed diet which contained 35% maltose achieved the best growth, economic efficiency and relative economic efficiency comparing with other tested diets.

Keywords: leaching maltose, Tilapia, growth, economic efficiency

SOME BIOLOGICAL PARAMETERS OF BLACK-BELLIED ANGLER FISH (*LOPHIUS BUDEGASSA* SPINOLA, 1807) IN MONTENEGRIN WATERS (SOUTH-EAST ADRIATIC)

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NEKI BIOLOŠKI PARAMETRI GRDOBE (*LOPHIUS BUDEGASSA* SPINOLA, 1807) U VODAMA CRNE GORE (JUGOISTOČNI JADRAN)

Apstrakt

U ovom istraživanju obrađene su 264 jedinke grdobe (LophiusbudegassaSpinola, 1807), od kojih su 128 (ili 48,5%) bili mužjaci, 114 (43,2%) ženke, dok kod 22 jedinke (8.3%) nije bilo moguće odrediti spol. Odnos ženki i mužjaka bio je 1:1,12. Omjer spolova (SR) procijenjen je na 47,1. Testiranje uzorka χ^2-testom pokazalo je da ne postoji statistički značajna razlika između broja mužjaka i ženki u uzorku ($\chi^2 = 0.8099$, $p > 0.05$).

Totalna dužina (TL) jedinki u uzorku kretala se od 15,6 do 67,0 cm, sa prosječnom vrijednošću od 29,2 ± 8,1 cm (prosječna dužina ± standardna devijacija). Shapiro-Wilkesov test normalnosti pokazao je da distribucija dužinskih frekvencija ukupnog uzorka nije pratila normalnu distribuciju ($W = 0,9003$; $p < 0,05$), kao ni distribucija mužjaka ($W = 0,9563$; $p < 0,05$) ni ženki ($W = 0,8959$; $p < 0,05$). Raspon dužina kod ženki varirao je 18,8 do 67,0 cm TL, sa prosjekom od 31,9 ± 9,6 cm TL. Prosječna dužina mužjaka bila je 82,3 ± 5,7 cm TL, dok su minimalna i maksimalna dužina bile 16,6 cm i 48,4 cm TL.

Prosječna masa jedinki u uzorku bila je 444,15 ± 427,24 g, sa rasponom od 57,14 g do 3000,00 g. Masa ženki kretala se od 114,50 g do 3000,00 g, sa srednjom vrijednošću od 584,10 ± 557,15 g. Najmanja izmjerena masa mužjaka bila je 66,70 g, a najveća 1791,96 g, sa prosjekom od 372,40 ± 249,28 g.

Analiza dužinsko-masenog odnosa pokazala je vrijednost parametra b manju od idealne izometrijske vrijednosti 3, što znači brži rast jedinki u dužinu od povećanja mase, odnosno da jedinka sa rastom dobiva izduženiji oblik tijela. Vrijednosti parametra b po spolovima su također bile manje od 3. Studentov t-test pokazao je da se parametar b dužinsko-masenog odnosa kod ukupnog uzorka i ženki statistički značajno razlikovao od vrijednosti 3, dok kod mužjaka to nije bio slučaj.

Dužina prve spolne zrelosti ($L_{m50\%}$) ukupnog uzorka procijenjena je na 26,37 cm TL, odnosno 30,50 cm TL kod ženki i 26,26 cm TL kod mužjaka.Procijenjene su i dužine pri kojima 25% ($L_{m25\%}$), odnosno 75% ($L_{m75\%}$) populacije dosegne spolnu zrelost, kao i parametri α i β krivulje zrelosti.

Ključne riječi: grdoba, Lophius budegassa, dužinsko-maseniodnos, spolnazrelost, Jadransko more
Keywords: Black-bellied angler fish, Lophius budegassa, length-weight relationship, sexual maturity, Adriatic Sea

INTRODUCTION

Black-bellied angler fish, *Lophiusbudegassa* (Spinola, 1807) (Figure 1) is a demersal-dwelling species of bony fishes (Teleostei) that is distributed in the entire Mediterranean and the eastern Atlantic, from Ireland, Great Britainand the North Sea to the western coasts of West Africa (Sierra Leone), but also in Namibia (Jardas, 1996; Bianchi *et al*., 1999; Vrgoč*et al*., 2004). It is widespread in the entire Adriatic Sea (Jardas, 1987; Vrgoč*et al*., 2004). It is one of the two species of angler fish found in the Adriatic (the other being *L. piscatorius*), but is the more abundant one (Vrgoč*et al*., 2004).

Figure 1. Black-bellied angler fish (*L. budegassa*) (photo by A. Joksimović)

L. budegassa lives on soft bottoms, but shows no preference for any specific type of sediment. (Jardas, 1996; Vrgoč*et al*., 2004). It is found at depths between 13 and 404 m (Jardas, 1987; Vrgoč*et al*., 2004), but the majority of fished individuals come from depths between 90 and 170 m (Jardas, 1987; 1996). Depth, and not the type of sediment, seems to be decisive for the distribution of species (Jardas, 1987; Vrgoč*et al*., 2004).

In Montenegrin waters, Merker&Ninčić (1973) reported*L. budegassa* in the open waters of the South Adriatic at depth strata from 25 to 500 m.

MATERIALS AND METHODS

The *L. budegassa*samples were taken in period from 2007 to 2014, obtained upon landing from trawlersoperating in Montenegrin territorial waters (ports of Herceg Novi, Budva and Bar), and then analysed in laboratory. The length was measured to the nearest mm, and the total body weight (using a precise electronic balance) to the nearest 0.01 g.

Shapiro-Wilkes test was used to test whether the length frequency distribution of *L. budegassa* followed the normal (bell-curve) distribution.

Sex ratio (SR) was given as a proportion of females in the sample, according to the formula: $SR = \frac{N_f}{N_f+N_m} \cdot 100$, where $N_f$is the number of females and $N_m$number of males in the total sample.

The χ^2-square (chi-square) test was used to test whether there was a statistically significant difference between the number of females and males.

Length-weight relationship was determined for the entire sample, according to the formula $W = a \cdot L^b$. Parameters *a*and*b* were estimated using ordinary least–square regression after transforming the data in natural logarithms ($\ln W = \ln a + b \cdot \ln TL$) (Huxley, 1924; Jensen, 1976).

Modified Student's *t*-test was used to test whether there was a statistically significant difference between the value of slope (parameter *b*) of the length-weight relationship and the ideal, isometric value of 3.

Maturity stages were determined according to the MEDITS Instruction manual V.6 (2012).Maturing and mature individuals were used to determine the length of first sexual maturity (MEDITS stages 2B, 2C, 3, 4A and 4B). Logistic model was used to estimate the length of first sexual maturity ($L_{m50\%}$):$P = \frac{1}{1+e^{(\alpha-\beta \cdot TL)}}$, where *P* is the estimated ratio of sexually mature individuals of a given total length, *α* and *β* are constants, and TL is the total length of the fish.

Based on parameters *α* and *β*, it is possible to estimate lengths at which 25% ($L_{m25\%}$), 50% ($L_{m50\%}$) and 75% ($L_{m75\%}$) of the population reaches sexual maturity:$L_{m50\%} = \frac{\alpha}{\beta}$, $L_{m25\%} = \frac{\alpha - \ln 3}{\beta}$, and $L_{m75\%} = \frac{\alpha + \ln 3}{\beta}$.

RESULTS AND DISCUSSION

There were 264 individuals in the total sample of *L. budegassa*, of which 128 (or 48.5%) were male, 114 (43.2%) female and 22 (8.3%) were individuals of undetermined sex. Female-to-male ratio was 1:1.12, while the sex ratio (SR) was estimated to 47.1.The χ^2-test performed on the sample showed that there was no statistically significant difference between the number of males and females ($\chi^2 = 0.8099$, $p > 0.05$).

Total lengths of individuals are presented in Table 1.Shapiro-Wilkes normality test showed that the length frequency distribution of the total sample did not follow the normal (Gaussian) distribution (W = 0.9003, $p < 0.05$) (Figure 2), and neither did females (W = 0.8959, $p < 0.05$) nor males (W = 0.9563, $p < 0.05$).

Maximum length reported for the species was 100 cm (Caruso, 1986), and Ungaro*et al.* (2002) report lengths of up to 95 cm during the MEDITS survey. Jardas (1996) gives maximum size for *L. budegassa*at 70 cm, with lengths from 20 to 40 cm being the most common. This is in line with present findings, and it could be that this species in the Adriatic doesn’t reach maximum lengths reported in the Atlantic and Mediterranean.

Table 1. Total length (TL) and weight (W) of analysed specimens of *L. budegassa*

Sex	TL (min – max, cm)	TL (mean ± SD)	W (min – max, g)	W (mean ± SD)
Total sample	15.6 – 67.0	29.2 ± 8.2	57.14 – 3000.00	444.15 ± 427.24
Female	18.8 – 67.0	32.0 ± 9.6	114.50 – 3000.00	584.10 ± 557.15
Male	16.6 – 48.4	28.3 ± 5.8	66.47 – 1791.96	372.40 ± 249.28

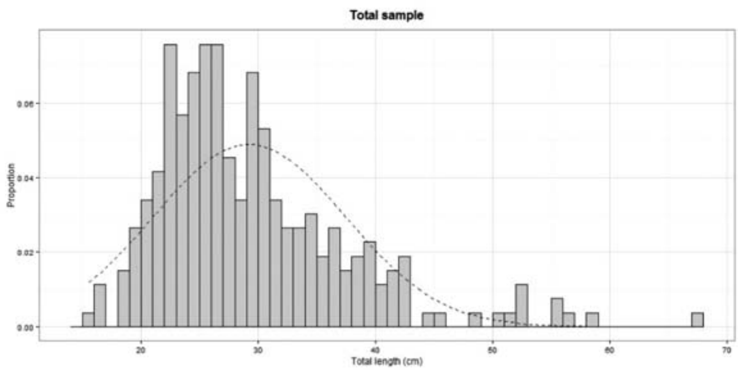

Figure 2. Length frequency distribution of *L. budegassa* in Montenegrin waters (gray bars – actual length frequency categories, dashed line – theoretical normal distribution of the sample)

The predominance of smaller individuals appears to be a common occurrence in *L. budegassa* trawl fisheries, and is reported by other authors (Jardas, 1987; Ungaro*et al.*, 2002; Vrgoč*et al.*, 2004; Piccinetti*et al.*, 2012; Ikica*et al.*, 2013).

The average body weights ofthe total sample, and separately for females and males are presented in Table 1.

The length-weight relationship parameters for the total sample showthat the parameter *b*(slope) has a value lower than the ideal, isometric value of 3, implying a faster growth in length compared to the gain in weight, or, alternatively, the body assuming a more elongated shape with growth. Likewise, values of the *b*coefficient for both sexes show values of *b* lower than 3 (Figure 3).

For females and the total sample, Student’s *t*-test showed a statistically significant difference between the estimated value of coefficient *b* and the isometric value of 3, $t = 0.2161$ ($p = 0.05$) and $t = 0.2426$ ($p = 0.05$), respectively. The*b* value of males was not statistically different from 3, $t = 0.1248$ ($p = 0.05$).

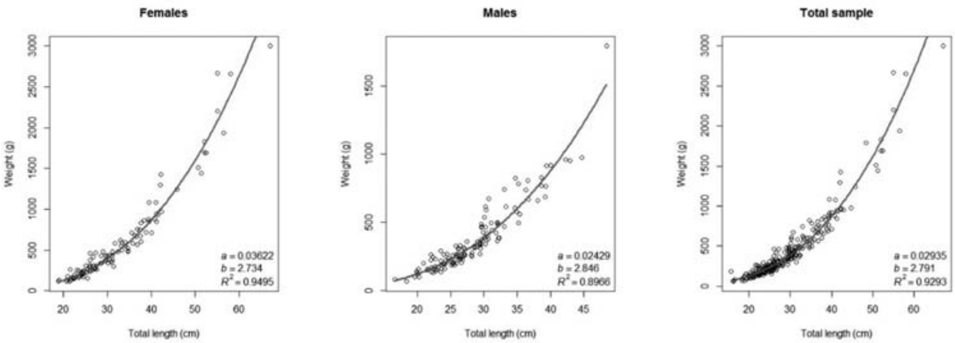

Figure 3. Length-weight relationship of females, males and total sample of *L.budegassa* in Montenegrin waters

Jardas (1987) gives $b = 2.089$ for juvenile *L. budegassa* in the Adriatic, and $b = 3.024$ for adult individuals (sexes combined), and states that juveniles were predominant in the sample. In this study, parameter b of the juvenile*L. budegassa* (TL < 27 cm), also dominant in the sample, was estimated to 2.697, which is agrees with Jardas’s findings. Previous study by Ikica *et al.* (2013) also listed $b < 3$ for the area, which is different from data reported by other authors for the Adriatic (Jardas, 1987; Dulčić&Glamuzina, 2006), who report values of b parameter above 3. However, several authors (Stergiou&Moutopoulos, 2001; Torres *et al*., 2012; STECF, 2013; Stergiou*etal*., 2014) consistently report values of b lower than 3 in Greek and Spanish waters.

Length at first maturity ($L_{m50\%}$) for the total sample was estimated at 26.37 cm TL, for females at 30.50 cm TL and 26.26 cm TL for males (Figure 4, Table 2). Lengths at which 25% ($L_{m25\%}$)and 75% ($L_{m75\%}$)of the population reach maturity, as well as the maturity range ($L_{m75\%} - L_{m25\%}$; MR) are given in Table 2.

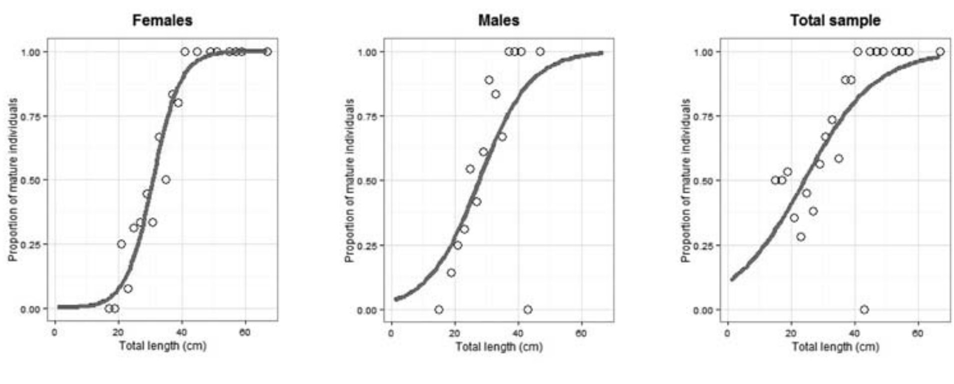

Figure 4. Maturity ogives and length at first maturity for *L. budegassa*females, males, and total sample (circles – observed values, red line – predicted values)

Table 2. Estimation of maturity ogive parameters (α, β) and lengths at which 25% ($L_{m25\%}$), 50% ($L_{m50\%}$) and 75% ($L_{m75\%}$) of population reach sexual maturity and maturity range (MR) for males, females and total sample of *L. budegassa*

Sex	α	β	$L_{m25\%}$	$L_{m50\%}$	$L_{m75\%}$	MR
Males	4.8054	0.1830	20.25	26.26	32.26	12.01
Females	6.0783	0.1993	24.99	30.50	36.01	11.02
Total sample	3.0516	0.1157	16.87	26.37	35.86	18.99

Previous data for the Adriatic give the estimated length of first maturity (with no mention of sex) at 33-34 cm (Jardas, 1987; Jardas 1996; Vrgoč*et al.*, 2004), and 25.72 cm for Montenegrin waters (total sample; Ikica *et al*, 2013). Estimations of $L_{m50\%}$ for Atlantic Iberian Coast were significantly higher at 44.7 cm for both sexes and 53.6 cm for females (Duarte *et al*., 2001), and higher still for the Mediterranean (66.2 cm, females only; Ungaro*et al*., 2002).

Various differences between the data from Montenegrin waters compared to other areas of the Adriatic and, especially, the Mediterranean and/or Atlantic waters can be explained by the fact that the Adriatic Sea is a part of the Central Mediterranean (Jardas*et al*., 2008; Piccinetti*et al*., 2012) with inflow of water masses from the Eastern Mediterranean (Zore-Armanda, 1963, 1968), and according to the MEDITS data, the smallest individuals (TL < 30 cm) of *L. budegassa* are more represented in the catchesin the Central and Eastern Mediterranean (Ungaro*et al*, 2002). This fact would then influence any analysis based on length frequencies. Additionally, trawling in Montenegro is limited mostly to the shelf area, which makes for about 43%(or about 3500 km^2) of Montenegrin territorial waters and epicontinental belt combined, and majority of the hauls are performed at the same locations over and over again, likely influencing the length frequency distribution of catches, favouring the smaller individuals.

CONCLUSIONS

The results in this study could indicate that the population of *L. budegassa* in the Adriatic Sea shows different patterns of growth and sexual maturity than the populations studied in the Mediterranean and the eastern Atlantic Ocean. However, even though the specimens used in the present study were obtained over a number of years, the total sample was still relatively small and heavily biased towards individuals of smaller lengths, a fact that could influence the final results of the analyses. Therefore, a deeper study on a more representative sample would be advisable in the area, in order obtain a better and more detailed of the various biological parameters.

ACKNOWLEDGMENTS

The research presented in this paper was made possible through the cooperation with the FAO AdriaMed project – Scientific Cooperation to Support Responsible Fisheries in the Adriatic Sea and the Ministry of Agriculture and Rural Affairs of Montenegro. The authors would like to thank anonymous reviewers, whose helpful comments and corrections significantly helped raise the quality of this paper.

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WATER QUALITY IN CARP FARMING SYSTEMS WITH DIFFERENT LEVEL OF INTENSIFICATION

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KVALITET VODE U SISTEMIMA GAJENJA ŠARANA SA RAZLIČITIM STEPENOM INTENZIFIKACIJE

Apstrak

Cilj ovog istraživanja je bila procena kvaliteta vode na osnovu fizičko hemijskih parametara u sistemima gajenja šarana u kojima je prisutan različit stepen intenzifikacije. Eksperimenti su rađeni u različitim sistemima za proizvodnju konzumnog šarana *(Cyprinus carpio L.)*. Sistemi uzgoja se razlikuju po geografskoj lokaciji, tipu sistema za gajenja (zemljani bazeni, akumulaciona jezera, kavezi), stepenu intenzifikacije (polu intenzivno, intenzivno i veoma intenzivno gajenje). Određen je kvalitet vode u 9 proizvodnih sistema za konzumnog šarana u kojima se razlikuju: stepen intenzifikacije, tip ribnjaka i izvor vodosnabdevanja. U odnosu na 9 primarnih fizičko hemijskih parametara sva voda je bila usaglašena sa tehnološkim normama za gajenje šarana. Na kvalitet vode u ispitivanim sistemima najviše je uticala temperatura, te sadržaj mineralnih i biogenih materija u vodi za vodosnabdevanje.

Ključne reči: kvalitet vode, šaran, proizvodni sistemi, fizičko hemijski parametri
Keywords: quality of water, carp, production systems, physico-chemical parameters

INTRODUCTION

The share of warmwater fish culture in freshwater fish production in Bulgaria is substantial. This is related to the favourable climatic conditions in the country water basins and the traditions in this type of fish farming. One of the main freshwater fish species produced in Bulgaria is the carp (*Cyprinus carpio* L.). The annual carp production for human consumption in the period 2003-2012 was between 1091 and 1288.4 tonnes. Carp is farmed either independently (monoculture) or together with other fish species (polyculture) in various production systems (earthen ponds, dam lakes, net cages). Regardless of the used production system, methods and technologies, the quality of water in aquaculture facilities is dependent on a number of abiotic (physical and chemical) and biotic factors. Physico-chemical parameters of water are of primary importance in the evaluation of its quality for aquaculture purposes, as water temperature, dissolved oxygen, content of biogenic elements, organic matter load etc. are essential for optimisation of the environment in aquaculture farms. All this creates preconditions for production of high-value fish. Its flavor, nutritional qualities become more and more important for consumer preferences in the purchase of fish and retail trade (Pieniak et al., 2007; Bauer and Schlott, 2009). That is why the monitoring of water quality at fish farms is a mandatory element of the good production practices in aquaculture (Hadjinikolova, 2013). The purpose of the present study was to evaluate the quality of water in carp production systems with different level of intensification on the basis of physico-chemical indicator parameters.

MATERIAL AND METHODS

The experiments were carried out in carp (*Cyprinus carpio* L.) production systems for human consumption, differing by their geographical location, types of farming system (earthen ponds, dam lakes, net cages), level of intensification (semi-intensive, intensive, super-intensive). Semi-intensive systems (SemiIS) were as followed: The Tri Voditsi/10 Experimental base (SemiIS-1), and Tsarimir 1 Dam Lake (SemiIS-2), free aquatory of Bistritsa Dam Lake (SemiIS-3), Budak dere dam lake (SemiIS-4). The intensive systems (IS) were the Tundzha 73/ 4 fish farm (IS-1), the Tundzha 73/ 5 fish farm (IS-2), and Tsarimir 2 Dam Lake (IS-3). Super-intensive systems consisted of net cages of Bistritsa Dam Lake (SuperIS-1) and net cages in Kardzhali Dam Lake (SuperIS-2). The 40 Izvora Dam Lake intended for amateur fishing only and no organised farming activities was used as control aquatic ecosystem (CAES). Carps were fed diets of different composition and structure: grain feeds, high-protein meals, specialised pelleted and extruded feeds (Table 1).

Table 1.Characteristics of the studied production systems

Production systems	**Semi-intensive**				**Intensive**			**Super-intensive**		**Control aquatic ecosystem**
	Semi IS-1*	**Semi IS-2**	**SemiIS-3**	**Semi IS-4**	**IS-1**	**IS-2**	**IS-3**	**Super-IS-1**	**Super-IS-2**	**CAES**
Typeof feed	grain / meal	grain / meal+ CEF**	grain / grain screenin-gs + CEF	grain	CPF	CEF	CEF	CEF	CEF	for an-gling
Ratioof feed,%	50:50	73:16:11	70:30	100	100	100	100	100	100	-
area										
dka	45	500	204	250	750	200	40	-	-	489
m^3	-	-	-	-	-	-	-	80	156	-

(SemiIS-1)- *TriVoditsi/ 10 Experimentalbase;* ***(SemiIS-2)*** *-Tsarimir 1 damlake;* ***(SemiIS-3)*** *- thefreeaquatoryoftheBistritsadamlake;****(SemiIS-4)*** *–Budakdere dam lake;* ***(IS-1)*** *-Tundzha 73/ 4 fishfarm;* ***(IS-2)*** *- Tundzha 73/ 5 fishfarm;* ***(IS-3)*** *-Tsarimir 2 damlake;* ***(SuperIS-1)*** *- netcagesinBistritsadamlake;* ***(SuperIS-2)*** *- netcagesintheKardzhalidamlake;* ***(CAES)*** *- the 40 Izvoradamlake*
****CEF**- *extruded feed;* ***CPF***- *pelleted feed*

The systems also differed with respect to the stocking structure, which consisted mainly of one-year-old carps (K_1) from 70 to 200 ind.dka^{-1} in semi-intensive and intensive systems, two-year-old carps (K_2) – 140 ind.dka$^{-1,}$ one-year-old bighead carp (T_1) from 8 to 30 ind.dka^{-1}, two-year-old bighead carp (T_2) ‒ 30 ind.dka^{-1}, two-year-old grass carp (A_2) from 3 to 10 ind. dka^{-1}. In intensive systems (IS-1 and IS-2), European catfish and pikes were periodically added. Super intensive systems were stocked with one-year-old carp (K_1) at 14-18 ind.m^{-1}. The control ecosystem (CAES) were periodically (every2-3 year) stocked with carp, grass carp and bighead carp, and the ichtyofauna included also common ruds (*Scardinius erythrophthalmus*), Danube bleaks (*Alburnus alburnus*), carassius (*Carassius carassius*) etc.

The different farming systems were supplied with water by various sources: karst springs (SemiIS-3, SuperIS-1), drilling water wells (SemiIS-1), dam lakes and irrigation facilities (SemiIS-2, IS-1, IS-2, IS-3, SuperIS-2), rivers (SemiIS-4), underground springs and rivers (CAES).

During the experimental period (2012‒2014) between May and October, physicochemical parameters of waters in surveyed carp farming ponds were analysed monthly. The analysis of water included instrumental and analytical measurements of: water temperature (T, ^{0}C) – by means of microprocessor oximeter type WTW 315/SET (BSS 17.1.4.01-77); electric conductivity – by means of microprocessor conductivity meter type WTW 315/SET (µS.cm^{-1}); water pH – with pH-meter type WTW 315/SET (BSS 3424-81, ISO 10523, 1994); dissolved oxygen content mg.l^{-1} – by means of microprocessor oximeter type WTW

315/SET (BSS EN 25814-2002); chemical oxygen demand (permanganate), mg $O.l^{-1}$ – by a standard analytical method (BSS EN ISO 8467, 2001); ammonia nitrogen N-NH4, $mg.l^{-1}$ – spectrophotometrically by the method of Nessler (BSS 3587-79, ISO 5664); nitrate nitrogen N-NO3, $mg.l^{-1}$ – spectrophotometrically (ISO 7890-3, 1998); orthophosphate content $P\text{-}PO_4$, $mg.l^{-1}$– spectrophotometrically (BSS EN ISO 6878-1:2004). Water temperature and dissolved oxygen were determined in-situ.

RESULTS AND DISCUSSION

The environmental conditions of carp farming systems with the respective physicochemical parameters of water are summarised in Table 2.

Water temperature. The average seasonal water temperature in SemiIS varied from 19.3 to 24.6^{0}C, in IS from 23.2 to 25.3^{0}C, in Super IS within 20.8-22.6^{0}C, and in CAES it was 25.7^{0}C. Maximum registered values were in July and august: between 24.2 and 29.7^{0}C, with exception of SemiIS-3 (21.3^{0}C). Lower water temperature values were measured in May-October, which was related to climatic characteristics and the seasonal character of the parameter. The lower mean seasonal and maximum water temperature values for production systems located in the aquatory of the Bistritsa dam lake were attributed to the fact that it is supplied with water by karst springs, whose average temperature for the season was 13^{0}C. Despite that, the water temperature was within the technological allowances for carp farming in SemiIS-3 and SuperIS-1. For other production systems, it was within the optimum range (Hadjinikolova, 2013, Privezentsev, 2000).

Electric conductivity. The average seasonal values of this ecological parameter varied between 327 and 705 $\mu S.cm^{-1}$. Data show that both minimum and maximum registered values for all production systems, regardless of intensification level and farming system type, were within the allowances for freshwater in ponds from the lake type, such as dam lakes (650 - 750 $\mu S.cm^{-1}$, Ordinance H-4/14.09.2012 of the Ministry of Environment and Water). The only exception was SemiIS-4, located in the aquatory of Budak dere dam lake, where water electric conductivity varied from 1301 $\mu S.cm^{-1}$ to 1385 $\mu S.cm^{-1}$, and average values for the season in the different stations were within 1328.67–1357.33 $\mu S.cm^{-1}$. All these values exceeded the allowances for freshwater lake-type ponds and indicated the presence of external contamination of dam waters from anthropogenic contamination of rivers supplying the dam.

Water pH. The average seasonal values of water pH of studied production systems varied from 7.57 to 8.40; i.e. were compliant with optimum technological values for carp farming. The same was true for minimum pH values. In some of water samples collected in July and August, water pH was within the range 8.68-9.10, which did not influence the overall water quality with respect to this parameter.

Dissolved oxygen content of water. The average for the season dissolved oxygen content of water was from 6.69 $mg.l^{-1}$ to 10.4 $mg.l^{-1}$, and maximum absolute values were from 8.8 $mg.l^{-1}$ to 13.7 $mg.l^{-1}$. All measurements were optimum for farmed fish species. An exception was demonstrated for station 1/pond 5 of Tundzha 73 fish farm in August, with in situ dissolved oxygen concentration of 3.4 $mg.l^{-1}$. Data indicate that with respect to water dissolved oxygen content, the conditions in studied production system were optimum which benefited the normal feeding and feed conversion ensuring good growth performance of fish and resistance to adverse environmental conditions (Hadjinikolova, 2013).

Table 2. Values (minimum, maximum and average) of primary physico-chemical parameters of water

Production systems / Parameter		Semi-intensive				Intensive			Super-intensive		Control Aquatic ecosys tem
		Semi IS-1*	Semi IS-2	Semi IS-3	Semi IS-4	IS-1	IS-2	IS-3	Super IS-1	Super IS-2	CAES
T,^{0}C	min	13.7	21.9	13.0	16.4	17.8	18.3	23.7	17.1	21.0	23.1
	max	27.8	27.1	21.3	29.7	26.8	27.6	26.4	25.0	24.2	28.5
	x	23.7	24.6	19.3	24.6	23.2	23.3	25.3	20.8	22.6	25.7
Electrical conductivit, µS.cm^{-1}	min	-	486	498	1301	679	630	458	480	324	469
	max	-	722	672	1385	733	719	647	673	330	586
	x	616	647	663	1343	705	690	552	666	327	526
pH, units	min	7.87	7.87	7.77	8.35	7.59	7.53	7.90	7.82	7.41	8.21
	max	8.65	8.53	8.15	8.45	8.68	8.69	9.10	8.37	7.75	8.47
	x	8.20	8.13	8.01	8.40	8.24	8.08	8.35	8.07	7.57	8.35
O_2, mg.l^{-1}	min	4.4	5.3	9.9	9.33	5.6	3.4	6.4	9.0	8.3	8.2
	max	11.4	8.8	10.6	13.00	10.3	10.8	10.0	13.7	11.3	12.6
	x	8.2	6.7	10.1	9.98	7.9	6.9	7.68	10.2	9.5	10.4
Oxidability by KMnO4, mg.l^{-1}	min	2.22	5.33	1.28	11.31	5.82	6.40	5.72	0.35	0.35	2.00
	max	11.47	7.20	4.29	20.18	13.2	11.25	9.90	6.97	1.91	3.17
	x	5.46	6.17	2.50	11.80	8.88	8.50	7.16	3.26	1.27	2.57
N-NH4, mg.l^{-1}	min	0.07	0.01	0.01	0.16	0.03	0.01	0.01	0.03	0.01	0.01
	max	0.94	0.06	0.07	0.66	0.32	0.10	0.07	0.17	0.04	0.05
	x	0.47	0.04	0.04	0.33	0.08	0.05	0.05	0.09	0.03	0.02
N-NO3, mg.l^{-1}	min	0.02	0.06	1.32	0.02	0.20	0.20	0.36	1.44	1.02	0.34
	max	1.20	1.20	5.10	2.21	1.44	1.06	0.88	4.50	2.71	0.98
	x	0.76	0.71	3.13	1.20	0.75	0.53	0.65	3.00	1.74	0.67
Total nitrogen (Nt), mg.l^{-1}	min	0.09	0.09	1.33	0.18	0.23	0.21	0.37	1.02	1.03	0.35
	max	2.14	1.26	5.17	2.87	1.76	1.16	0.95	2.71	2.75	1.03
	x	1.23	0.75	3.17	1.53	0.82	0.58	0.70	1.47	1.77	0.69
P-PO_4, mg.l^{-1}	min	0.14	0.17	0.40	0.44	0.23	0.13	0.16	0.16	0.14	0.14
	max	0.93	0.94	0.55	0.61	0.59	0.59	0.62	0.78	0.81	0.75
	x	0.41	0.36	0.48	0.44	0.37	0.37	0.35	0.50	0.42	0.33

Chemical oxygen demand (permanganate). The average seasonal values of the permanganate oxidation ranged between 1.27 mg.l^{-1} and 11.80 mg.l^{-1}. The minimum detected values were from 0.35 mg.l^{-1} to 11.31mg.l^{-1}, and the maximum ones – from 1.91 mg.l^{-1} to 20.18 mg.l^{-1} (SemiIS-4, July). The data suggest that oxidation was compliant to technological norms for warmwater fish species. Permanganate oxidation levels of 15.0 to 30.0 mg.l^{-1} for summer months are typical for carp farming ponds (Grozev et al., 1999, Hadjinikolova, 2013). This allowed concluding that studied water ponds were not loaded with organic waste.

Ammonia and nitrate nitrogen. Average seasonal values of ammonia nitrogen varied within a very large range – from 0.02 to 0.47 mg.l^{-1}, with higher levels in SemiIS-1 and SemiIS-4.

The average amount of nitrate nitrogen were between 0.53 mg.l^{-1} – 1.34 mg.l^{-1} (station 2 and station 1) and peak values – from 1.69 mg.l^{-1} to 3.13 mg.l^{-1}. A higher level of nitrate nitrogen was registered for production systems in the Bistritsa dam lake aquatory resulting from higher background values in karst waters supplying the water basin (10.5 mg.l^{-1}).

Total nitrogen. The average seasonal values of total water nitrogen in the major part of studied systems were under the optimum value of the parameter (2.0 mg.l^{-1}) varying between 0.58 mg.l^{-1} and 1.77 mg.l^{-1}. The only exception was SemiIS-3 whose total nitrogen water level of 3.17 mg.l^{-1} was associated with higher nitrate nitrogen concentration of karst water supplying the dam lake. In general, the dynamics of total nitrogen content of water follows that of nitrate nitrogen content, as their relative proportion in total nitrogen (TN) is limiting. This tendency was established for all studied aquatic ecosystems.

Phosphorus (phosphate P). The average seasonal values of soluble inorganic phosphorus (phosphate P) for the different studied production systems were between 0.33 mg.l^{-1} and 0.50 mg.l^{-1}. The registered maximum values (0.55 mg.l^{-1} – 0.94 mg.l^{-1}) were optimum for carp farming ponds.

To sum up, the results from physico-chemical analysis of water indicate that factors with limiting significance for carp farming (temperature, dissolved oxygen content, water pH, chemical oxygen demand (permanganate), phosphates) were maintained within the technological reference ranges (Privezentsev, 2000; Hadjinikolova, 2013). A temperature lower than 19-20^{0}C, which is assumed to be the low optimum limit of this parameters for carps (Backiel, 1964; Mazurkiewcz, 2009; Szumiec and Szumiec, 1985) was observed in the Bistritsa dam lake production system due to the fact that the dam lake is supplied with water from several karst springs with flow rate of 70 l.sec^{-1}, and average seasonal temperature of 13^{0}C, located in close vicinity to the tail of the reservoir. For the other studied systems, a similar temperature was measured by the end of the vegetation period, in October. According to several authors (Adelman, 1975; Wolny, 1974; Karpinski, 1994; Wojda, 2006) the effective temperature for carp farming, accounts for maximum weight increase, is from 23-28^{0}C to 32^{0}C. Data indicate that during the active vegetation period (June-September), water temperature of studied production systems remained within the optimum limits for carps (22.0 – 28.0^{0}C).

The electric conductivity of water in Budak dere dam was over the allowances for freshwater in lake-type basins, pointing out at external pollution of the pond.

CONCLUSIONS

The quality of water in nine systems producing carp for consumption with different level of intensification, type of used ponds and supply water sources was determined.

It was found out that with regard to the analysed 9 primary physico-chemical parameters, the water was compliant with technological norms for carp farming.

It was found out that the quality of water in studied production systems was influenced at a greater extent by the temperature and background mineral, solids and biogenic substances content of supply water.

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METALS CONCENTRATIONS IN WATER AND SEDIMENT FROM THE DANUBE RIVER

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KONCENTRACIJA METALA U VODI I SEDIMENTU REKE DUNAV

Apstrakt

Voda je osnov opstanka živog sveta koji je u njoj i nastao. Dve trećine zemljine površine je pod vodom. Bez obzira na značaj vode na opstanak živog sveta ljudska vrsta prema vodi nema odgovarajući odnos. O tome govore podaci o zagađenju okeana, mora, vodotokova (reke, potoci) i jezera. Antropogeno zagađenje vode, dakle, poreklom od čoveka, javlja se kao posledica direktnog ili indirektnog ispuštanja zagađivača u vodu, bez adekvatnog postupka sa štetnim i opasnim jedinjenjima. Najčešći zagađivači vode posledica su otpadnih voda i voda koje dolaze sa površina zemlje (naročito obradivih površina). Zagađenost vode je blisko povezana za povećanim potrebama stanovništva za vodenim resursima. Povećane potrebe rastu sa porastom stanovništva, razvoja privrede i tehnologije. Veća potrošnja vode znači i veću količinu otpadnih voda. Dospevanjem u reke otpadne vode menjaju fizička (boja, miris, ukus, providnost), hemijska (sastav) i biološka svojstva (živi svet reka). Naša zemlja nema odgovarajuće propise za procenu kvaliteta sedimenta pa se, zbog toga, koriste standardi kvaliteta prema kanadskom zakonodavstvu, preporuke ICPDR-a (International Commission for the Protection of the Danube River), a pojedini parametri se procenjuju korišćenjem holandske metodologije. Prema kanadskom zakonodavstvu definisane su dve vrednosti: niža vrednost ISQGs (Interim Sediment Quality Guideline) predstavlja privremene preporuke koje su dobijene teorijskim putem i iznad kojih je moguć uticaj na akvatične organizme, dok je druga, viša, vrednost PEL (Probable Effect Level), vrednost iznad koje je uticaj na akvatične organizme verovatan. Poređenjem sa kanadskim preporukama, sadržaj žive je prekoračen na skoro svim ispitanim lokalitetima, prema ISQG vrednosti. Viša PEL vrednost ukazuje na verovatno prisutne negativne toksične efekte na akvatične organizme i sedimente u dva ispitivana profila u Dunavu (profil A i profil

B). Pojava žive u sedimentu je posledica ispuštanja industrijskih otpadnih voda (naročito iz pogona za proizvodnju hlora), upotrebe živinih pesticida za zaštitu semena, upotrebe živinih jedinjenja u upaljačima eksplozivnih sredstava (posledice ratnog konflikta 1999. godine) i upotreba žive u mernim uređajima (u toku razaranja industrijskih postrojenja za vreme konflikta 1999. godine uništen je deo ove merne opreme). Arsen je poznat po negativnim ekotoksičnim efektima, a negov sadržaj u sedimentu reke Dunav nije bio iznad ICPDR vrednosti. Sadržaj drugih teških metala, cinka, bakra, kadmijuma i olova (profil B) je bio u ispod vrednosti propisane ICPDR. Kako se sam proces zagađivanja vode teško može sprečiti, zaštita voda, prvenstveno, je usmerena na smanjenje uticaja, a u najboljem slučaju, potpuno uklanjanjanje uticaja teških metala. To se postiže kroz opšte ekološke (radne akcije, ekološki aktivizam) kao i specifične mere (obrazovanje, sredstva javnog informisanja). Najvažnije je utvrđivanje kvaliteta vode i mogućnosti vodotoka da primi otpadne vode, registovanje svih mogućih zagađivača (njihova lokacije i stepen zagađivanja), obavljanje stalnih kontrola otpadnih voda u blizini vodotokova, prečišćavanje otpadnih voda i izmeštanje industrije na mesta na kojima će zagađivanje biti minimalno.

Ključne reči: sediment, voda, Dunav, teški metali
Keywords: sediment, water, Danube, heavy metals

INTRODUCTION

Chemical elements belong to the most common environmental pollutants, and they are equally undesirable in the air, water and soil (Mendil et al., 2010). Nowaday, environmental pollutants of aquatic ecosystems, especially heavy metals are the major problem. In aquatic systems, they are deposited into the sediments (Monroy et al., 2014). Metal pollutants can accumulate in aquatic organisms from water, sediments or through the food chain. Some metals like Cu, Zn or Fe are important for many biochemical processes in living organisms. Also, they are essential elements for aquatic plants and animals. Presence of pollutants in the Danube is the topic of many previous researches and international treaties and agreements, such is the Danube Convention issued by the International Commission for the Protection of the Danube River (Milenković et al., 2005; Pajević et al., 2008). Serbia is the full member of this association since August 2003, which stipulates monitoring of water quality as well as ecosystem. The aims of the study was to measure concentrations of heavy metals in samples of sediment and water.

MATERIAL AND METHOD

The samples were taken from the river Danube in Belgrade region, near Vinča - Profile A (N 44º 40’, E 20º 43’) and Belegiš - Profile B (N 45º 01’, E 20º 20’). Sediments samples were collected during 2013. The samples of sediments were first dried at 110 ºC for 24 h and then were mechanically homogenized to a powder. Amounts of 0.5 g of each sample of dry sediments were wet digestion with nitric acid and hydrogen peroxide in a microwave closed system on temperature program of 180 – 240 ºC for 35 min. All reagents used in the analysis were of reagent grade. Double-deionized water (18.2 MΩcm^{-1} resistivity at 25ºC) obtained using a Milli-Q system (Millipore, Bedford, USA) was used for all dilutions. Chemicals used for microwave digestion (nitric acid, HNO_3, 65%) and hydrogen peroxide (H_2O_2, 30%) were of high pure quality (Merck, Germany). The element standard solutions (Merck, Germany) that were used for the calibrations were prepared by diluting the stock solutions of 1000 mg/L concentration. Sample digestion was carried out using a microwave closed system Berghof MWS-2 (Berghof Products+ Instruments GmbH, Eningen, Germany). After digestion, concentrations of heavy metals were analyzed by absorption spectroscopy using the GBC 932 plus atomic absorption spectrometer (GBC Scientific Equipment, USA). Detection of Cu, Fe, Zn and Ni were determinate in air-acetylene flame, while analysis of Pb and Cd were conducted by graphite furnace (GBC SensAA spectrometer with Hyper-Pulse background corrector) with an auto sampler. Mercury and arsenic were analyzed by the cold vapor technique with a flow injection system. The operating parameters for the working elements were set as recommended by the manufacturers, and are that were given in Table 1.

Water samples (n=74) were collected at the depth of 20-30 cm under the water surface with a 5 l Friedinger bottle (SCHOTT DURAN®, Elmsford, North America), and mixed. A 500 ml subsamples were bottled in the pre-cleaned plastic flasks. The sampled material was stored in darkness at 4 °C, and before analyses all water samples were filtered through Whatman filters (Sigma-Aldrich Co, United Kingdom) to remove suspended particulate matter.

Table 1. Instrumental analytical conditions for measurement heavy metals recommended by FAAS

Element	Acetylene (L/min)	Air (L/min)	Wavelength (nm)	Slit width (nm)
Fe	2.0	17.0	248.3	0.2
Cu	2.0	17.0	324.7	0.5
Zn	2.0	17.0	213.9	0.5
As	2.0	17.0	193.7	1.0

Instrumental conditions	Pb	Cd
Wavelength (nm)	217.0	228.8
Slit width (nm)	1.0	0.5
Argon flow (mL/min)	250	250
Sample volume (μL)	20	20
Heating program temperature (°C)		
Drying 1	80 (10,10)[a]	80 (10, 10)
Drying 2	120 (20, 5)	120 (20, 5)
Pyrolysis	400 (5, 15)	300 (5, 15)
AZ (auto zero)	400 (0.5, 1)	300 (0.5, 1)
Atomization	1600 (1, 2)	1700 (0.9, 2)
Cleaning	2100 (1, 1)	2100 (1, 1)

Conditions for GFAAS [a] - ramp time (s), hold time (s)

The analyses were performed by included the assessment of concentrations of the following elements: As, Al, Zn, Fe, Cu, As, Sd, Hd, Pb. The concentrations of heavy metals were compared with the probable effect levels (PELs). According to the Canadian Sediment Quality Guidelines for the Protection of Aquatic Life, PELs for Zn, Cu, As, Sd, Hg and Pb are 315 mg kg^{-1}, 197 mg kg^{-1}, 17.0 mg kg^{-1}, 3.5 mg kg^{-1}, 0.486 mg $kg^{-1,}$ 91.3 mg kg^{-1} dw, respectively.

Statistical analysis of the results were elaborated using software GraphPad Prism version 5.00 for Windows, GraphPad Software, San Diego California USA, www.graphpad.com.

RESULTS AND DISCUSSION

Presence of chemical elements in water depends on many environmental factors, like the influence of industry and other forms of pollutions (Adhikari et al.,2009). The obtained results may be explained by the fact that several rivers flow into the Dunabe near the sampling site, but also large cities lying on this river, as well as the industry, can be serious polluters of the environment (Subotić et al., 2013). Concentration of the metals in samples of sediments and water from Danube river are presented in table 2.

Table 2. Heavy metal concentration in water samples (mg L) and sediment (mg kg^{-1}) from river Danube, expressed as means±deviation (range)

Metals	Profile A		Profile B	
	Water (mg/L)	Sediment (mg kg^{-1})	Water (mg/L)	Sediment (mg kg^{-1})
Zn	0.032±0.002	270.40±17.98	0.063±0.007	139.4 ± 8.71
Fe	0.33±0.02	17530.00±971.7	0.41±0.01	16104 ± 1068.0
Cu	0.004±0.001	50.93±3.34	0.004±0.001	35.95 ± 1.40
As	0.004±0.001	13.89±1.05	0.006±0.001	8.90 ± 0.25
Cd	ND	1.69±0.13	ND	0.61 ± 0.11
Hg	ND	0.80±0.09	ND	0.69 ± 0.08
Pb	ND	64.92±2.39	ND	32.58 ± 2.61

Legend: *ND- not detected

It was found that the environmentally mobile elements were arranged in the order Fe> Zn> Pb>Cu>As> Cd> Hg in samples collected in the examined profile A. In profile B, elements were arranged Fe> Zn> Cu>Pb>As> Hg> Cd. Vasiljevic and Tomasevic (1985), 30 years ago, were conducted a survey on the heavy metal concentrations in the Danube sediments in Serbia. They selected six sites, two of which corresponded to those investigated in this study (Veliko Gradiste and Tekija), thus allowing a direct comparison between the sites over the intervened time period. Their results also showed higher concentrations at those sites, where higher sedimentation occurs due to slowing down of the river.

Zinc concentration in profile A and profile B did not exceed PeLs prescribed by Canadian Sediment Quality Guidelines for the Protection of Aquatic Life. Comparing with the profile examined in 2001 (Škrbić et al., 2004), zinc concentration increased during the same period. Copper concentrations were below the PeLs in profile A and profile B, but it was higher in profile A. Crnković et al. (2008) found that copper concentration was below the

PELs. In 2005, the obtained results for copper concentration were in agreement with the data presented in the study of copper content in Sava sediment around Belgrade (Ščančar et al., 2007). Arsenic concentrations were below PELs prescribed by Canadian Sediment Quality Guidelines for the Protection of Aquatic Life. In 2001 (Škrbić et al. 2004) arsenic concentration increased in sediment from Novi Sad. In the study of Crnković et al. (2008) it was found the arsenic concentration always below PELs value. Cadmium concentration has been below the PELs value. In 2001, on Novi Sad spot, Šrkbić et al., 2004 noted that the examined profile in their work contained higher amount of cadmium. According to the Canadian Sediment Quality Guidelines for the Protection of Aquatic Life the concentration of mercury in profile A (0.80±0.09 mg kg-1) and profile B (0.69 ± 0.08 mg kg-1) exceed PELs, which are 0.486 mg kg^{-1}. The obtained results are not in argeement with the previous studies of mercury content in sediment of Danubian tributaries around Belgrade (International Commission, 2000). In the study of Crnković et al. (2008) concentration of mercury was above the PELs.

CONCLUSIONS

This study provides information about heavy metals content in water and sediment from the Danube river. Sediment samples contained mercury above the PELs prescribed by the Canadian Sediment Quality Guidelines for the Protection of Aquatic Life.

ACKNOWLEDGEMENTS

The research was carried out within the project TR 31011, funded by the Ministry of Education, Science and Technological Development of the Republic of Serbia.

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METAL CONCENTRATION IN MUSCLE TISSUE OF FISH FROM DIFFERENT FISH PONDS

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KONCENTRACIJA METALA U MIŠIĆNOM TKIVU RIBA IZ RAZLIČITIH RIBNJAKA

Apstrakt

Riba predstavlja važan deo ljudske ishrane, a takođe, riba je dobar pokazatelj kontaminacije životne sredine od strane jednog broja supstanci, uključujući i tragove metala u slatkovodnim sistemima, posebno zbog toga što se ribe, kao tercijelni potrošači, nalaze na vrhu lanca ishrane u vodenom ekosistemu (Noël i sar., 2013). Mnogo pažnje posvećeno je elementima kao što su olovo (Pb), kadmijum (Cd), Živa (Hg) i Arsen (As) i efekti izloženosti ovim elementima su sveobuhvatno istraženi (Castro-González and Méndez-Armenta, 2008; Has-Schön et al., 2008). Zbog svoje toksičnosti, otpornosti i bioakumulacije u vodi i sedimentima, kada se nalaze u visokim koncentracijama, ovi elementi predstavljaju opasnost za sve žive organizme. U pogledu bezbednosti javnog zdravlja, kod riba, prate se koncentracije olova, kadmijuma i žive, gde postoje jasno definisani maksimalni nivoi teških metala u namirnicama koji su određeni od strane Evropske Komisije, br. 1881/2006 (EC, 2006), izmenjena i dopunjena EC 629/2008 (EC, 2008). Za arsen, maksimalni nivo još nije uspostavljen na evropskom nivou, ali se očekuje da će granične vrednosti za arsen biti postavljene u bliskoj budućnosti, kao i metode za određivanje arsena (Noël et al., 2013). Za utvrđivanje koncentracije teških metala u tkivu riba prikupljeno je deset uzoraka dve različite vrste riba - šarana (*Cyprinus carpio*) i evropske ili severne štuke (*Esox lucius*). Uzorci su ulovljeni od strane profesionalnih ribara tokom rane jeseni 2013. godine iz četiri različita ribnjaka na području Beograda. Dobijeni rezultati su izraženi kao srednja vrednost ± standardna devijacija. Statistička analiza je urađena je korišćenjem Studentovog t-testa i analizom varijanse (ANOVA) sa višestrukim poređenjem Turkey test za utvrđivanje značajnih razlika između srednjih vrednosti. Primenjen je nivo značajnosti od 0.01 i 0.05. U različitim ribnjacima, koncentracija metala u mišićnom tkivu šarana je varirala u zavisnosti od vrste metala. Između sva četiri poređena ribnjaka nije utvrđena statistički značajna razlika jedino u koncentraciji kadmijuma u mišićnom tkivu. U ostalim slučajevima poređenja utrvđene su statistički značajne razlike ($p<0.01$). Koncentracija metala u mišićnom tkivu štuke razlikovala se između ribnjaka, zavisno od vrste metala. U svim slučajevima poređenja utvrđene su statistički značajne razlike ($p<0.01$). Koncentracija olova u mišićnom tkivu štuke u svim poređenim ribnjacima bila je statistički značajno veća ($p<0,01$) u odnosu na koncentraciju olova u mišićnom tkivu šarana. Koncentracija kadmijuma u mišićnom tkivu štuke bila je statistički značajno veća ($p<0,01$; $p<0,05$) od koncentracije kadmijuma u mišićnom tkivu šaran u svim poređenim ribnjacima. Za razliku od koncentracije olova i kadmijuma, koncentracija žive u mišićnom tkivu štuke u poređenim ribnjacima bila je statistički značajno niža ($p<0,01$) u odnosu na koncentracije žive u mišićnom tkivu šarana. Koncentracija arsena bila je statistički značajno veća ($p<0,01$) u mišićnom tkivu šarana od koncentracije arsena u mišićnom tkivu štuke. Rezultati koncentracija ispitanih elemenata u mišićnom tkivu riba pokazuju varijacije u opsegu koji se čini tipičan za ribnjake u Srbiji, ali su ove koncentracije niže od onih u rekama sa značajnim antropogenim uticajem.

Ključne reči:koncentracija metala, ribnjak, šaran, štuka
Keywords: metal concentration, fish pond, Common carp, Northern pike

INTRODUCTION

Fish is an important part of the human diet, but also a good indicator of environmental contamination by a number of substances, including trace metals in freshwater systems, notably because fish are at the top of the food chain in the water ecosystem (Noël et al., 2013). Much attention has been paid to hazardous elements such as lead (Pb), cadmium (Cd), mercury (Hg), and arsenic (As) and the effects of exposure to these elements have been comprehensively studied (Castro-González and Méndez-Armenta, 2008; Has-Schön et al., 2008). Indeed, due to their toxicity, persistence and bioaccumulation in water and sediment, when occurring in high concentrations, these elements become severe poisonous for all living organisms. In terms of public health food safety, Pb, Cd and Hg, are monitored in fish with maximum levels of heavy metals in foodstuffs fixed by Commission Regulation (EC) No. 1881/ 2006 (EC, 2006) amended by EC 629/2008 (EC, 2008). For As, no maximum level has yet been established at European level, but discussions are ongoing on this topic and it is anticipated that limits will be set for arsenic in the near future, as the methodology for the determination of arsenic and its speciation improves (Noël et al., 2013). Although many studies have examined the relationship between metal exposure, accumulation and toxicity under laboratory conditions, prediction of toxic effects based on environmental or tissue concentrations remains difficult under natural exposure conditions (Visnjic-Jeftic et al., 2010; Skorić et al., 2012). Data from the literature indicate that the contents of these elements in fish varied as a function of the different localities, but depended on species and feeding behaviour and also, by various biotic and abiotic factors (Has-Schön et al., 2006; Noël et al., 2013). This study was undertaken to determine the contamination data of Pb, Cd, Hg and As in the muscle of two fish species (common carp-*Cyprinus carpio* and Northern pike- *Esox lucius*) from four different fish ponds and to compare the level of contamination and the differences in the concentration of these elements between these two fish species and between compared fish ponds.

MATERIALS AND METHODS

To determine heavy metals in fish tissue ten samples of two different fish species Common carp (*Cyprinus carpio*) and Northern pike (*Esox lucius*) were collected. The samples were caught by professional fishermen's during early autumn of 2013 from four different fishponds in Belgrade area. All individuals were identified to species level and a random sub-sample of 10 individuals per species at each location was used for metal analysis. Fish were dissected, and a sample of muscle (ca. 200 g) below the dorsal were stored for metal analyses in polypropylene vials previously pre-cleaned with nitric acid (10%) and rinsed three times in water. Muscle was selected to determine the risk posed by metal pollution to humans (Miller et al., 1992). The total weight (g) of fishes was measured and transported in refrigerator at 5 ºC daily to the laboratory. The samples were dissected to obtain muscle samples, then were mixed homogeneously and immediately frozen and stored at -20 ºC. The analysis was performed by included the assessment of concentrations of the following elements: As, Al, Zn, Fe, Cu, As, Sd, Hd, Pb. The concentrations in fish meat (i.e muscle samples) were also recalculated to the wet tissue weight (ww) and compared with the maximum allowed concentrations (MAC) in fish meat for the utilization in human diet. As established by the Europea Union (EU) and the national legislation. According to the EU legislation (European Commission Regulation, 2006), MAC for Cd, Hg and Pb are 0.05, 0.05 and 0.30 µg g-1 ww, respectively. The national legislation prescribed MAC for As, Cd, Hg, Pb, Cu, Fe and Zn in fish meat at 2.0, 0.1, 0.5, 1.0, 30.00, 30.00 and 100.00 µg g-1 ww. respectively (Official Gazette of RS 2011). All samples were collected and analyzed in duplicate and the results are expressed as the means ± standard deviation. Statistical analysis of the results was elaborated using software GraphPad Prism version 5.00 for Windows, GraphPad Software, San Diego California USA, www.graphpad.com. The statistical analysis was performed using Student's t-test and analysis of variance (ANOVA) with the multiple comparison Turkey's test to determine the significant differences between means. Significant level of 0.01 and 0.05 was applied.

RESULTS

Concentrations of different metals in carp muscle tissue from four different fish ponds are shown in Table 1. The lead concentration in carp muscle tissue from fishpond C was significantly higher ($p<0.01$) than the lead concentration in carp muscle tissue from other fish ponds. In various fishponds, metal concentration was varied depending on the type of metal. Thus, mercury concentration in carp muscle tissue from fishpond C was significantly higher ($p<0.01$) than mercury concentration muscle tissue of carp from other fish ponds. Also, mercury concentration in carp muscle tissue from fishpond A was significantly higher ($p<0.01$) than the mercury concentration in muscle tissue of carp from fishpond D. Completely different results were obtained for arsenic concentration in carp fishponds. In carp fish pond D arsenic concentration in muscle tissue of fish was significantly lower ($p<0.01$) than the concentration of the same metal in muscle tissue of carp from other fish ponds. Also, the arsenic concentration in carp muscle tissue from fishpond C was significantly higher ($p<0.01$) than the arsenic concentration in carp muscle tissue from fishpond B.

Table 1. Metal concentration in carp muscle tissue

Fishpond	Metal Concentration (μg/g)			
	Lead $\bar{X}$±Sd	Cadmium $\bar{X}$±Sd	Mercury $\bar{X}$±Sd	Arsenic $\bar{X}$±Sd
A	$0.0189^{A}\pm0.0031$	0.067 ± 0.0038	$0.401^{A,B}\pm0.0105$	$0.353^{A}\pm0.0256$
B	$0.0186^{B}\pm0.0039$	0.061 ± 0.0038	$0.393^{C}\pm0.0111$	$0.343^{B,C}\pm0.0267$
C	$0.0301^{A,B,C}\pm0.0043$	0.065 ± 0.0043	$0.485^{A,C,D}\pm0.0082$	$0.378^{B,D}\pm0.0085$
D	$0.0204^{C}\pm0.0017$	0.058 ± 0.0145	$0.387^{B,D}\pm0.0063$	$0.252^{A,C,D}\pm0.0040$

Legend: same letters ^{A,B,C,D} - p<0.01;

Metal concentration in pike muscle tissue differed from the fishpond, depending on the type of metal. The lead concentration in pike muscle tissue from fishpond D was significantly lower (p<0.01) than the lead concentration in pike muscle tissue from other fish ponds. Also, the lead concentration in pike muscle tissue from fishpond A was significantly higher (p<0.01) than the lead concentration in pike muscle tissue from fish pond B. The cadmium concentration of muscle tissue in pike was the highest in fish pond C, and it was significantly higher (p<0.01) than the cadmium concentration in pike muscle tissue from other fish ponds. The muscle tissue of pike from fishpond A has significantly higher (p<0.01) cadmium concentration than the muscle tissue of pike from fishpond D. A similar situation was observed with a concentration of mercury in muscle tissue of pike, which was significantly higher (p<0.01) in the fish pond C compared to other fish ponds. The mercury concentration in pike muscle tissue from fishpond D was significantly lower (p<0.01) than the mercury concentration in pike muscle tissue from fishpond A and B. The arsenic concentration in pike muscle tissue was also significantly higher (p<0.01) in fish pond C than the arsenic concentration in pike muscle tissue from other fish ponds (Table 2).

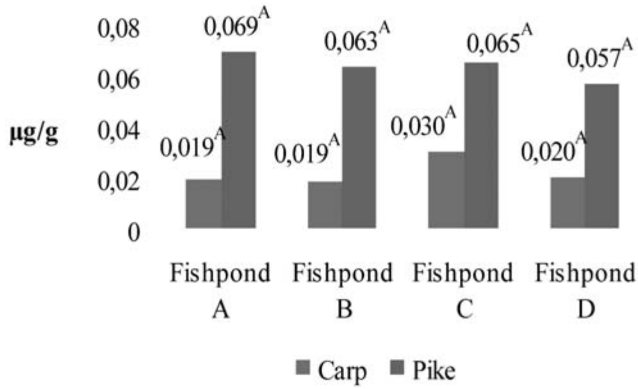
Table 2. Metal concentration in pike muscle tissue

Fishpond	Metal Concentration (μg/g)			
	Lead $\bar{X}$±Sd	Cadmium $\bar{X}$±Sd	Mercury $\bar{X}$±Sd	Arsenic $\bar{X}$±Sd
A	$0.069^{A,B}\pm0.0031$	$0.071^{A,B}\pm0.0031$	$0.294^{A,B}\pm0.0058$	$0.224^{A,B,C}\pm0.0096$
B	$0.063^{A,C}\pm0.0039$	$0.067^{C}\pm0.0030$	$0.289^{C,D}\pm0.0067$	$0.206^{A,D,E}\pm0.0087$
C	$0.065^{D}\pm0.0014$	$0.079^{A,C,D}\pm0.0046$	$0.313^{A,C,E}\pm0.0064$	$0,249^{B,D,F}\pm0.0068$
D	$0.057^{B,C,D}\pm0.0006$	$0.063^{B,D}\pm0.0045$	$0.217^{B,D,E}\pm0.0048$	$0.159^{C,E,F}\pm0.0074$

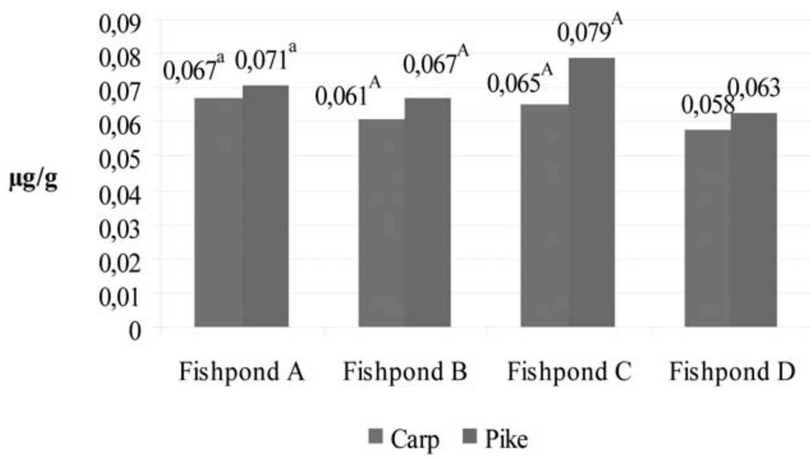
Legend: same letters ^{A,B,C,D,E,F} - p<0.01;

DISCUSSION

The lead concentration in muscle tissue of pike from all observed fish ponds was significantly higher (p<0.01) than the lead concentration in carp muscle tissue (Fig. 1). The cadmium concentration in pike muscle tissue was significantly higher (p<0.01; p<0.05) than the cadmium concentration in the carp muscle tissue of all compared fish carps (Fig. 2).

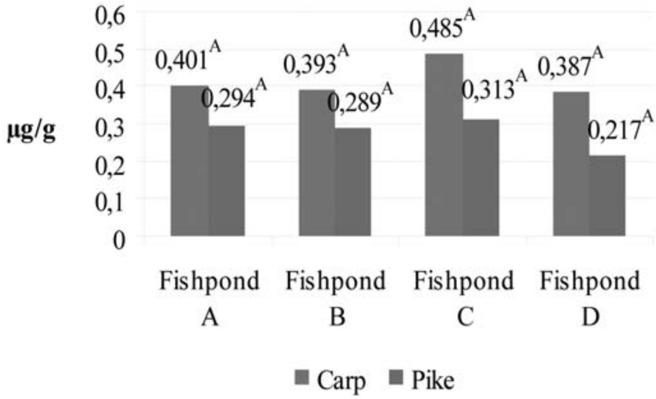


Legend: same letter ^{A} - $p<0.01$;
Figure 1. Lead concentration in carp and pike muscle tissue



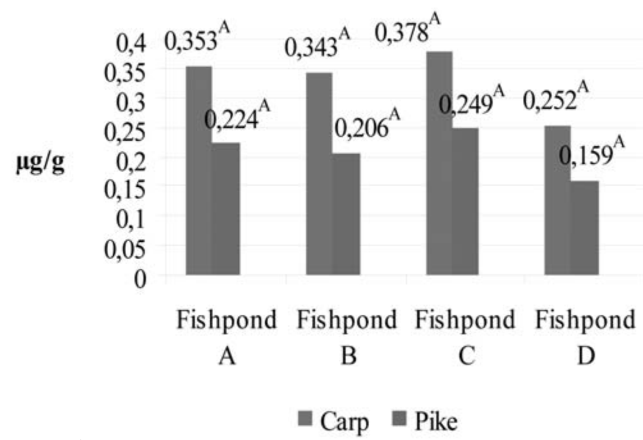
Legend: same letter ^{A} - $p<0.01$; ^{a} – $p<0.05$;
Figure 2. Cadmium concentration in carp and pike muscle tissue

As opposed to concentration of lead and cadmium, the mercury concentration in muscle tissue of pike from all observed fish ponds was significantly lower ($p<0.01$) than the mercury concentration in carp muscle tissue (Fig. 3). The arsenic concentration was significantly higher ($p<0.01$) in carp muscle tissue than the arsenic concentration in pike muscle tissue (Fig. 4).



Legend: same letter A - p<0.01;

Figure 3. Mercury concentration in carp and pike muscle tissue



Legend: same letter A - p<0.01;

Figure 4. Arsenic concentration in carp and pike muscle tissue

The results for the four elements in fish muscle show variation in a range that seems to be typical for the Serbian fish ponds, but are lower than those in rivers with considerable anthropogenic impact.

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HUMAN EXPOSURE TO 17 ELEMENTS THROUGH MUSSELS CONSUMPTION

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IZLOŽENOST LJUDI DELOVANJU 17 ELEMENATA PUTEM KONZUMIRANJA DAGNJI

Apstrakt

Elementi dospevaju u životnu sredinu iz prirodnih izvora i posredstvom ljudske aktivnosti. Toksični elementi, odnosno njihova sve veća zastupljenost u vazduhu, vodi, sedimentu i zemljištu, postali su globalni problem. Pojedini elementi potencijalno su opasni po biljke, životinje i ljude, posebno zbog svoje toksičnosti, sposobnosti da se bioakumuliraju kao i zbog njihove bionerazgradive prirode. Kao glavni uticaji mogu se navesti kontaminacija ekosistema i zdravstveni problemi koje mogu izazvati kod ljudske populacije. Ovi problemi su uzrok povećane zabrinutosti javnosti širom sveta.

S obzirom na rastuću populaciju ljudi na planeti i sve veći broj stanovnika koji žive na obalama mora, morski organizmi, kao jeftin i veoma lako dostupan oblik hrane, počinje sve više da se koriste u ishrani ljudi. Jedan od komercijalno veoma važnih proizvoda u svetu danas je morska dagnja *Mytilus galloprovincialis*. Ručno sakupljanje i uzgajanje ove vrste školjki mnogih zemalja datira iz ranog perioda. Vremenom se sa divljih žetvi prešlo na različite tehnike uzgajanja, što je doprinelo, pre svega, većoj produktivnosti morske dagnje. Najveći svetski proizvođač školjki *M. galloprovincialis* je Kina, dok je u Evropi najveći proizvođač Španija.

Crna Gora je Mediteranska zemlja koja se nalazi u jugoistočnoj Evropi, na obalama Jadranskog mora. Dagnja *M. galloprovincialis* je nativna vrsta u ovoj oblasti i postoje naznake da je gajena na ovom području još pre sto godina. Komercijalni uzgaoj i proizvodnja ove dagnje datira od pre 30-tak godina, a danas se ona uspešno uzgaja na više manjih farmi, prvenstveno stacioniranih unutar Bokokotorskog zaliva. Zbog povoljnih uslova u Bokokotorskom zalivu za akvakulturu *M. galloprovincialis* smatra se da njihova proizvodnja ima veliki potencijal za budući razvoj ovog područja.

Dagnje *Mytilus galloprovincialis* predstavljaju jeftinu visoko proteinsku hranu sa niskim sadržajem masti i kalorija, što ih čini potencijalno zdravijom hranom od drugih komercijalno dostupnih proizvoda. Dagnje su, takođe, odličan izvor omega-3 masnih kiselina, vitamina B12, B1 (tiamin), B2 (riboflavin), B3 (niacin) i B9 (folna kiselina), vitamina A i C, selena, gvožđa, cinka, joda, kalcijuma, natrijuma, bakra, magnezijuma, mangana, kalijuma, itd. i izuzetno su korisne za ljudsko zdravlje. Samo 100 grama dagnji obezbeđuje kod odraslih osoba oko 18,5%, 39-67%, 68% i 1260% dnevnih potreba za folnom kiselinom, gvožđem, selenom i vitaminom B12, respektivno. Međutim, dagnje mogu biti kontaminirane različitim zagađujućim materijama koje mogu kod čoveka izazvati trovanje i različite bolesti. Istraživanja mineralnog sastava pojedinih morskih organizama izuzetno je važno i neophodno, jer su pojedine vrste, uključujući i dagnje, postale svetski delikates kod ljubitelja plodova mora. Stoga je poznavanje elementarnog sastava dagnji od suštinskog značaja za procenu dostupnosti hranjivih materija, ali i sa apekta procene i smanjenja potencijalno negativnih efekata po zdravlje ljudi koje može izazvati prekomerna konzumacija kontaminiranih dagnji.

Cilj ovog rada bio je da se utvrdi uticaj 17 ispitanih elemenata (Fe, Zn, Mn, Sr, I, As, Cr, Ba, Cu, Ni, Sb, Sn, Cd, V, Co, Zr i MeHg) na zdravlje potrošača dagnji *M. galloprovincialis* iz Bokokotorskog zaliva, Crna Gora. Procenjeni rizik od ispitanih elemenata sugeriše da nedeljna potrošanja od 100 grama dagnji tokom ljudskog života neće izazvati negativne posledice po ljudsko zdravlje.

Ključne reči: Mytilus galloprovincialis, Ispravnost hrane, Procena rizika po zdravlje ljudi, Bokokotrski zaliv, ED-XRF.
Keywords: Mytilus galloprovincialis, Food safety, Health risk assessment, Boka Kotorska Bay, ED-XRF.

INTRODUCTION

Many minerals are constituents of the body composition of organisms. They play many fundamental roles, and many of them are essential to the normal vital functions of a live organisms. Generally, the elements can be classified into major, present in higher concentrations, and micro, nonessential, and toxic elements, present in lower amounts (Soetan et al., 2010; Stanković et al., 2012). Risk assessment of essential elements has to take into account the two ends of the toxicity spectrum: that associated with intakes that are to high (toxicity), and that associated with intakes that are to low (nutritional deficiencies). Nonessential and toxic elements can also be found in some marine species, and they are available in waters from natural sources, such as rocks, and as a result of different human activities (Jović et al., 2011; Maulvault et al., 2013). These elements tend to accumulate in species from higher trophic levels, like fish and shellfish (Stanković et al., 2012).

Almost all elements considered as essentials and nonessentials, i.e. those provided through the diet and required to maintain normal physiological functions, or the toxic elements, or those without a significant biological role can be found in seafood and mussels (Stanković et al., 2012; Maulvault et al., 2013). Permanent consumption of contaminated seafood can cause poisoning and promote diseases. Therefore, it is very important to know the elemental composition and the content of elements in the food in order to minimize the potential adverse health effects. Hence, the aim of this research was to determine the impact on consumer health and the possible alert regarding adverse health effects that may be caused by the examined elements (Fe, Zn, Mn, Sr, I, As, Cr, Ba, Cu, Ni, Sb, Sn, Cd, V, Co, Zr and MeHg) ingested through consumption of *Mytilus galloprovincialis*mussel .

MATERIALS AND METHODS

Mussel samples were collected at seven stations in Boka Kotorska Bay, Montenegro, on the Southeastern part of Adriatic coast. From five locations the colected mussel samples were wild (Tivat Arsenal, Opatovo, Sv Stasija, Perast, Herceg Novi) and from two spots the mussels were cultivated (Krašići and Kukuljina), Fig. 1.

Figure 1. Sampling stations in Boka Kotorska Bay, Montenegro: 1. Krašići, 2. Kukuljina, 3. Tivat, 4. Opatovo, 5. Sv Stasija, 6. Perast and 7. Herceg Novi

At each sampling site around 2 kg of mussels were collected, then placed in nylon bags containing seawater and transported to the laboratory. The largest 25–30 individuals of the approximately same size were washed and cleaned out, raw opened, and the flesh was scraped out of the shells, which was then freeze-dried at -40 °C for 48 h, weighed, homogenized and ground to a fine powder. The powdered sample was pressed with a hydraulic press by applying a pressure of 7 t for 20 s. No binder material was applied. The resulting pellets had a diameter of 32 mm and a uniform mass of 400 ± 3 mg. The samples prepared in this manner, in the form of pressed pellets, were dedicated to the energy dispersive X-ray fluorescence analysis (ED-XRF).

The measurements were performed by using a MiniPal 4 ED-XRF spectrometer (PANalytical, Almelo, Netherlands). The accuracy of the applied method and of the calibration curves obtained was checked by the measurement of a standard reference material SRM 2976 (Mussel homogenate, NIST).

RESULTS AND DISCUSSION

Health risk assessment to mussels consumers' was performed based on the target hazard quotient (THQ) developed by the United States Environmental Protection Agency (US EPA, 1989). THQ is well recognized and used parameter by a scientific community for the risk assessment of heavy metals in contaminated foods (Jović and Stanković, 2014), and is expressed by the following equation:

$$THQ = \frac{EF * ED * MS * C}{RfD_0 * BW * AT} * 10^{-3}$$

EF: exposure frequency (365 days/year); ED: exposure duration (70 years); MS: food meal size (14.3 g/day, i.e. one meal of 100g mussels per week); C: element content in mussels (mg/kg wet weight); RfD_0: oral reference dose (µg/g/day) provided by the EPAs' Integrated Risk Information System online database (IRIS, 2014); BW: body weight (adults 60 kg); AT: averaging time.

Calculated THQ values of individual elements through mussels' consumption from sampling locations in Boka Kotorska Bay are shown in Table 1.

Table 1. Target hazard quotient values of elements due to consumption of the mussels from Boka Kotorska Bay

	Target hazard quotient (THQ)						
	Krašići	Kukuljina	Tivat	Opatovo	Sv.Stasija	Perast	H. Novi
Fe	0.020	0.061	0.033	0.012	0.008	0.008	0.011
Zn	0.012	0.018	0.033	0.019	0.011	0.013	0.022
Mn	0.024	0.026	0.029	0.026	0.024	0.024	0.029
Sr	0.004	0.003	0.006	0.002	0.003	0.002	0.003
I	0.177	0.161	0.111	0.177	0.144	0.111	0.186
As	0.468	0.329	0.329	0.329	0.357	0.344	0.371
Cr	0.344	0.384	0.344	0.357	0.357	0.344	0.357
Ba	0.003	0.003	0.003	0.002	0.002	0.002	0.002
Cu	0.009	0.009	0.013	0.009	0.006	0.008	0.008
Ni	0.005	0.012	0.009	0.006	0.004	0.005	0.006
Sb	0.227	0.237	0.175	0.216	0.247	0.247	0.227
Sn	0.0001	0.0001	0.0002	0.0001	0.0001	0.0003	0.0001
Cd	0.058	0.041	0.050	0.066	0.041	0.037	0.045
V	0.005	0.005	0.007	0.004	0.001	0.019	0.003
Co	0.071	0.118	0.111	0.104	0.071	0.036	0.124
Zr	0.566	0.875	0.205	0.104	0.104	0.104	0.104
MeHg	0.003	0.014	0.014	0.016	0.037	0.016	0.003

THQ values for all elements are lower than 1, what signifies that the level of exposure is lower than the reference dose, which assumes that a daily exposure at this level (one meal of 100g mussels per week) is not likely to cause any negative health effect during a lifetime in a human population. From Table 1 it can be seen that THQ values for Zr in the mussels from the sites Krašići and Kukuljina are higher than the THQ values for all of the rest elements. Both geogenic and anthropogenic sources for Zr exist, but the first generally being more abundant (Abollino et al., 2002). Increased THQ value for Zr primarily can be attributed to its native origins and its incorporation in sediment particles, and as such are taken by mussels through water filtration. In the case of wild mussels from the sites Tivat, Opatovo, Sv Stasija, Perast and H. Novi the limiting factors based on the obtained THQ values are Cr and iAs. THQ values for Zr, Cr and iAs at assumed level of mussels were still below 1 indicated that no health risk was present.

CONCLUSION

The risk assessment of investigated elements through mussels' consumption suggests that a weekly exposure to 100g mussels during a human lifetime is not likely to cause negative health effects. Calculations based on Fe, Zn, Mn, Sr, I, Ba, Cu, Ni, Sb, Sn, Cd, V, Co and MeHg concentrations present in the mussels *M. galloprovincialis* suggest that a large amount of mussels would have to be consumed to exceed the permissible values for target hazard quotient. In the case of cultivated mussels from Krašići and Kukuljina the limiting factor is Zr, while in the case of wild mussels (rest of the locations) the limiting factors are Cr and iAs. On the basis of the medical literature data in relation to the Zr concentrations in mussels from the Bay their consumption in quantities higher than obtained does not necessarily has implications to human health during a human lifetime.

ACKNOWLEDGEMENTS

The authors acknowledge funding from the Ministry of Education, Science and Technological Development of the Republic of Serbia, Contract No. III43009.

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THE EFFECT OF TRANSGLUTAMINASE ADDITION ON THE QUALITY ALTERATIONS OF RAINBOW TROUT MINCE

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EFEKTI DODATKA TRANSGLUTAMINAZE NA PROMENE KVALITETA MLEVENOG MESA DUŽIČASTE PASTRMKE

Apstrakt

Efekat transglutaminaze na kvalitet ribljeg mlevenog mesa ispitan je u toku skladištenja u frižiderima. U ove svrhe, enzim mikrobijalna transglutaminaza (MTGase) u razmeri od 0.2%, 0.5% i 1.0% je dodavana u mleveno meso pastrmke (*Oncorhynchus mykiss)*.

pH vrednost mlevenog mesa pastrmke je opala na kraju skladištenja na hladnom, a najniži skor (6.00±0.01) je određen kod uzoraka koji su tretirani sa 5% enzima. U zavisnosti od vrednosti TVB-N (ukupni isparljivi azot), riblje mleveno meso je održalo svoj kvalitet tako da je moglo da se konzumira osmog dana nakon skladištenja na hladnom. Međutim, TVB-N vrednost kontrolnih uzoraka se povećala mnogo brže u odnosu na druge tretmane. TMA-N (trimetilamin azot) vrednosti su se povećale tokom skladištenja, a najviši skorovi su zabeleženi kod kontrolnih uzoraka na kraju peroda skladištenja. Dodatak MTGaze (mikrobialna transglutaminaza) nije uticala na ukupan sastav slobodnih amino kiselina među tretiranim grupama. Zabeleženo je da je progres ukupnih slobodnih amino kiselina potisnut dodatkom MTG-a. Povećanje koncentracije enzima je pozitivno uticalo na sprečavanje rasta ukupne količine psikrofilnih i koliformnih bakterija i najbolji rezultati dobijeni su dodavanjem MTG-a koncentraciji od 1.0%. Povećanje koncentracije enzima je pozitivno uticalo na senzorna svojstva ribljeg mlevenog mesa.

Ključne reči: transglutaminaze, dužičasta pastrmka, rok trajanja, hladno skladištenje.

Abstract

The effect of transglutaminase on the quality of fish mince was determined during refrigerated storage. For this purpose, the proportions of 0.2%, 0.5% and 1.0% microbial transglutaminase enzyme (MTGase) was added into trout (*Oncorhynchus mykiss)* mince meat. The pH value of trout mince decreased at the end of the cold storage and the lowest scores (6.00±0.01) were determined with the samples treated with 0.5% enzyme. The fish mince kept its consumable “good” quality on the 8^{th} day of cold storage depending on total volatile basic nitrogen (TVB-N) values. However, TVB-N values of control samples increased rapidly comparing to the other treatments. The trimethylamine nitrogen (TMA-N) values increased during storage and the highest scores were recorded in control samples at the end of the storage. The addition of MTGase caused no difference on the total free amino acid content among the treatment groups. It was recorded that the progress in total free amino acids was suppressed with the addition of MTGase. The increase in enzyme concentration met successful results in hindering the growth of total psychrophilic bacteria and coliform bacteria and the best results were obtained with the addition of MTGase in the concentration of 1.0%. The increase in the enzyme concentration affected the sensory scores of fish mince positively.

Keywords: Transglutaminase, rainbow trout, shelf life, cold storage

PRESENCE OF ORGANOCHLORINE PESTICIDES IN HAKE FILLETS

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ZASTUPLJENOST OGANOHLORNIH PESTICIDA U FILETIMA OSLIĆA

Apstrakt

Organohlorni pesticidi (OCP) su grupa jedinjenja široko zastupljena u prirodi a samim tim i u hrani koju konzumiramo. Da bismo za proizvod rekli da je bezbedan za upotrebu neophodno je da sadržaj OCP-a u njemu bude manji od maksimalno dozvoljenih vrednosti koje su propisane važećim Pravilnikom. Identifikacija ove grupe jedinjenja se vrši gasnom hromatografijom sa masenim detektorom. Priprema uzoraka za analizu sa brzom QuECHERS metodom obezbeđuje da za kratko vreme, pouzdano ekstrahujemo analite od interesa. U našoj studiji ispitivali smo sadžaj 19 organohlornih pesticida u filetima oslića, ribljem proizvodu popularnom u našoj zemlji. Ispitivanja su pokazala da se koncentracija OCP-ova u ovim proizvodima nalazi znatno ispod maksimalno dozvoljenih koncentracija. Organohlorni pesticidi se akumuliraju u hrani i okruženju što izaziva zabrinutost zbog potencijalnih rizika po ljudsko zdravlje kao i zbog narušavanja ekološke ravnoteže (Kartalovic et al, 2015). U predhodnim decenijama organohlorni pesticidi su se koristili širom sveta kako bi unapredili poljoprivrednu proizvodnju. Globalna upotreba ove grupe jedinjenja je od 1950. godine dovela je do povećanja njihove potrošnje i do pedeset puta, paralelno sa rastom stanovništva (Nath, 2013). U našoj studiji ispitivali smo slučajno izabrane uzorke iz marketa. Uzorci su do ispitivanja čuvani u orginalnom pakovanju u skladu sa preporukom proizvođača. Na osnovu ispitivanja 18 uzoraka zaključeno je da je sadržaj organohlornih pesticida znatno niži od maksimalno dozvoljenih koncentracija u ribi, koje su propisane važećim Pravilnikom Republike Srbije. Opseg koncentracija OCP-a se kretao od 0.0065-0.0097 mg/kg.

Generalno, svi pesticidi su toksične supstance dizajnirane da ubiju štetočine sa svojim otrovnim ili štetnim efektima. Direktno ili indirektno pesticidi mogu ući u ljudsko telo kroz lanac ishrane i konačno, mogu prouzrokovati različite efekte na ljudsko zdravlje, poput alergijskih reakcija uključujući i sterilitet i kancer. Zagađivanje hrane sa ovim supstanca-ma se smatra jednim od najopasnijih aspekata u poslednjih nekoliko godina. Riba i riblji proizvodi mogu biti kontaminirani sa hloriovanim ostacima kroz različite izvore. Ovi kvaliteti ih čine najopasnijom grupom hemikalija kojima prirodni sistemi mogu biti izloženi i funkcionišu kao hemijski indikatori antropogenog pritiska i zagađenja (Baiarri i dr., 2001; Storelli et al.,2004). Zbog činjenice da su OCP ipak prisutni u ovim proizodima neophodno je iste izloziti stalnom monitoringu s ciljem obezbeđivanja plasmana samo bezbednih proizvoda na naše trzište.

Ključne reci: fileti oslica, organohlorni pesticidi, gasna hromatografija
Keywords: hake fillets, organochlorine pesticides, gas mass chromatography

INTRODUCTION

Due to accumulation of residues in food and in the environment, the concern was raised regarding potential risks to human health and also because of potential harm to the ecological balance (Kartalović et al, 2015). In previous decades, organochlorine pesticides have been used around the world with the aim to improve the agricultural production. However, global usage of these chemicals since 1950 increased their use 50 times, in parallel with the growth of the human population (Nath, 2013). The main sources of OCPs are food and soil contaminated by them (Snelson, 1979, Valiszevski 1997). Organochlorine pesticides are chemical substances and they contain a lot of combined chlorine and carbon atoms. They can be classified into three general groups: dichlorodipheniletans (DDT, DDD, dicofol, etc.), chlorinated ciclodines (Aldrin, dieldrin, heptachlor, etc.), hekachlorociclohekanes (lindane). These compounds differ considerably between and within the group in terms of the toxic dose, the skin absorption, fat storage, metabolism and elimination. Signs and symptoms of toxicity in humans however, are very similar except for DDT (Abdel-Wahab, 2004). OCP were widely used throughout the world, until the restrictions on their usage were imposed in the late seventies, both in Europe and in the United States, initially only for DDT (Fontcuberta, 2008). Generally, all pesticides are toxic substances designed to kill pests with its poisonous or harmful effects. Directly or indirectly pesticides can enter human body through the food chain and eventually, cause a variety of effects on human health, such as allergic reactions to deadly effects, even including sterility and cancer. Contamination of food with these substances is considered as one of the most dangerous aspects in recent years. Fish and fish products may be contaminated with chlorinated remnants through various sources. Organochlorine pesticides persist and tend to bioaccumulate in the environment. These qualities make them the most dangerous group of chemicals to which natural systems can be exposed and function as chemical indicators of anthropogenic pressure and pollution (Bayarri et al., 2001; Storelli et al., 2004). Analysis of organochlorine compounds are carried out because of their potential health hazardous effect on humans. In this study, hake fillets were monitored using GCMS, for 19 organochlorine pesticides to determine the degree of contamination with them.

MATERIAL AND METHODS

Samples were collected from local markets and shops in region of Vojvodina during the period from 1 January to 1 May 2015. In this study we have collected 20 samples of hake fillets in original packing. All samples were stored in room temperature before analysis in accordance with declaration. All chemicals and reagents used were of analytical grade with high purity. The OCP stock standard solution (1000 μg/ml of 20 organochorine congeners) was diluted in n-hexaneto yield spiking solutions 0.005 to 0.5 μg/ml. The spiking solution was used to prepare the calibration curves in the matrix blank extract by appropriate dilutions. First of all, portion of the samples were homogenized. For extraction we took 3g of homogenized sample portion and added 3ml of water and 6ml of ACN. then we added 3g $MgSO4$ and 1g sodium acetate in the tube of 50ml and mixed it well. The tube was then centrifuged on 4000 rpm for the period 10 min. After that, we took 1 ml of aliquot and transferred it in tube of 5 ml which contained 150 mg $MgSO4$, 50 mg PSA, 50 mg C18, and vortexed it for 1 minute. The dSPE tube was then centrifuged on 4000 rpm for period of 5 min. Finally, the liquid from the tube, was traversed to a GC vial and analyzed by SIM GCMS. The identification of OCP was based on comparison of the retention times of the peaks and target ions, with those obtained from standard mixture of OCP (standards supplied by instrument manufacturer). Quantification was based on external calibrations curves prepared from the standard solution of each of the pesticides congener. The coefficients of determination (r2) for the OCP standard calibration plots were in the range of 0.99675 to 0.99982. The gas - mass chromatography was Agilent 7890B/5977A MSD, with fused silica column [30m*0.25μmfilmof HP-5M (thickness)]; injection temperature was set at 280 °C using splitless mode and volume injected was 4 μL.

RESULTS AND DISCUSSION

Humans take up POPs through skin absorption, respiration and ingestion of contaminated food. Skin absorption and respiration are not the main route. Some researchers have confirmed that more than 90% of contaminants come from food (Furst, Furst, & Groebel, 1990). In our study we shows that hake filet have OCP in concentration less than MDK. OCP range of 20 investigated fillets was in range of 0.0065-0.0097 mg/kg.

Among all foods, fish is one of the main sources of contaminants although fish products account only for about 10% of diet (Alcock, Behnisch, Jones, & Hagenmaier, 1998; Harrisonet al., 1998) or less. POPs in fish from some areas were detected to assess the risk for human health (Binelli &Provini, 2004; Jiang et al., 2005; Yang, Matsuda, Kawano, & Wakimoto, 2006). With the banning of massive usage and production of the compounds, the residual levels in foodstuffs have decreased significantly (Xuemei Li, 2008). Toxic effects of pesticides vary in different organs of the fish. Liver, gill, kidney are tissues that can accumulate high level of pollutants as well as other factors, such as salinity temperature, hardness, etc (Mukesh Kumar Napit, 2013). The earliest study was done by Lincer et al., (1981) on the lake Naivasha he reported undetectable to very low levels of DDE in fish from Lake Naivasha in a study conducted to investigate organochlorine pesticide residue levels in Kenya’s rift valley lakes. A predatory fish from Lake Baringo showed the highest level 2.13mg/kg of DDE in the study. Forty fish samples were analyzed from Lake Naivasha and no individual sample had detectable levels of pesticide residues. A composite fillet sample of 10 fish had α -HCH at a concentration of 0.014mg/kg. The single value compares very well with the concentrations in the present study where the mean concentration of α -HCH was 0.118mg/kg. Other organochlorines pesticides residues studies mostly in other countries have reported higher concentrations.

CONCLUSION

According to the presented investigation of hake fillets from Serbian retail it can be concluded: the present GCMS analytical method was developed for the simultaneous determination of 18 pesticides of hake fillets. The concentrations detected were lower than recommended values in some countries but since there is a possible health risk, it is important to monitor the presence and concentrations of OCP in these products. It could be concluded that OCP pesticide residues were detected in hake fillets, as they were persistent in nature due to their slow decomposition rate, long half - life and high stability in the environment. Chemical analysis showed that the product complies with the Regulations of Republic Serbia.

ACKNOWLEDGEMENTS

The paper is part of the project TR 31011, funded by the Ministry of Science and Technology of the Republic of Serbia.

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MATRIX INFLUENCE IN DETERMINING ANTIPARASITIC BY HPLC

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UTICAJ MATRIKSA NA ODREĐIVANJE ANTIPARAZITIKA HPLC METODOM

Apstrakt

Tečna hromatografija visokih performansi (HPLC, tečna hromatografija pod visokim pritiskom) je oblik kolonske *hromatografije* koji se često koristi u *analitičkoj hemiji*. HPLC se koristi za razdvajanje komponenti iz smese na osnovu hemijskih interakcija između supstance koja se analizira i stacionarne faze u koloni. Princip rada HPLC-a je forsiranje prolaska analizirane supstance (ili *smeše*) kroz kolonu (cev napunjenu materijalom sitnih čestica, a time i velike površine) pumpanjem tečnosti (mobilna faza) pod visokim pritiskom kroz kolonu. Unosi se mala zapremina uzorka u tok mobilne faze i na osnovu specifičnih hemijskih i fizičkih interakcija, dolazi do različitog zadržavanja komponenata smeše. Vreme zadržavanja zavisi od prirode supstance koja se analizira, stacionarne faze i sastava mobilne faze. Vreme za koje se supstanca eluira (dođe do kraja kolone) naziva se retenciono vreme i one je karakteristično za određenu supstancu. Korištenje visokog pritiska povećava linearnu brzinu i daje komponentama manje vremena za zadržavanje, što poboljšava rezoluciju hromatograma. Koriste se uobičajeni *rastvarači*, čisti ili u bilo kojoj kombinaciji (npr. voda, *metanol*, organski rastvarači, itd). Voda može sadržavati i neki *pufer*, kako bi se poboljšalo razdvajanje. Moguće je koristiti i gradijentno eluiranje, što podrazumeva promenu sastava mobilne faze u toku eluiranja. Uređaj za HPLC se sastoji od sledećih komponenata: rezervoar mobilne faze, pumpe i injektora. Detektor ima važnu ulogu da određivanja komponenti koje izlaze iz kolone nakon eluiranja. Detektor generiše električni signal koji je proporcionalan intenzitetu neke osobine mobilne faze ili supstance koja se eluira. Tipovi detektora u HPLC-u su: UV-VIS detektor, fluorescentni detektor, elektrohemijski detektor, detektor indeksa loma i maseni spektrometar (MS). Uticaj matrixa uzorka koji se analizra ima veliki uticaj na izlazne analiticke rezultate. Kako bi se eliminisao uticaj matrixa pozeljno je uraditi kalibraciju kroz matrix, na blank uzorku koji ne sadrzi analite od interesa (Olives, 2006). U našoj studiji smo ukazali na značaj kalibracije kroz matrix, kako bismo neutralisali negov nepovoljan uticaj na rezultat. S tim ciljem je urađena i analiza uzoraka mesa ribe koja je spajkovana sa razlicitim koncentracijama parazitika. Uzorci su ekstrahovani sa acetonitrilom u prisustvu soli, anhidrovanog magnezijum sulfata i natrijum acetate. Kvantifikacija prečišenog ekstrakta je urađena na Thermo HPLC-u, sa UV detektorom. Na osnovu sprovedenog ispitivanja utvrđeno je da procenat recovery kod kalibracije kroz matrix se krece u opsegu od 75-95 % a u slučaju kalibracije sa standardima kreće se u opsegu od 40-55%. Zbog značaja praćenja parazitika u mesu ribe neophodno je optimizovati uslove analize s ciljem dobijanja pouzdanih rezultata i pračenja kvaliteta proizvoda.

Ključne reči: HPLC, parazitici, kalibracija, matriks
Keywords: HPLC, antiparasitics, calibration, matrix

INTRODUCTION

High performance liquid chromatography is basically a highly improved form of column chromatography. Instead of a solvent being allowed to drip through a column under gravity, it is forced through under high pressures of up to 400 atmospheres. That makes it much faster. It also allows the use of much smaller particle size for the column packing material which gives a much greater surface area for interactions between the stationary phase and the molecules flowing past it. This allows a much better separation of the components of the mixture. The other major improvement over column chromatography concerns the detection methods which can be used. These methods are highly automated and extremely sensitive. **Normal phase HPLC** -This is essentially just the same as in thin layer chromatography or column chromatography. Although it is described as „normal”, it isn‘t the most commonly used form of HPLC. The column is filled with tiny silica particles, and the solvent is non-polar - hexane, for example. A typical column has an internal diameter of 4.6 mm (and may be less than that), and a length of 150 to 250 mm. Polar compounds in the mixture being passed through the column will stick longer to the polar silica than non-polar compounds will. The non-polar ones will therefore pass more quickly through the column. **Reversed phase HPLC** - In this case, the column size is the same, but the silica is modified to make it non-polar by attaching long hydrocarbon chains to its surface - typically with either 8 or 18 carbon atoms in them. A polar solvent is used - for example, a mixture of water and an alcohol such as methanol. In this case, there will be a strong attraction between the polar solvent and polar molecules in the mixture being passed through the column. There won‘t be as much attraction between the hydrocarbon chains attached to the silica (the stationary phase) and the polar molecules in the solution. Polar molecules in the mixture will therefore spend most of their time moving with the solvent. Non-polar compounds in the mixture will tend to form attractions with the hydrocarbon groups because of van der Waals dispersion forces. They will also be less soluble in the solvent because of the need to break hydrogen bonds as they squeeze in between the water or methanol molecules, for example. They therefore spend less time in solution in the solvent and this will slow them down on their way through the column. That means that now it is the polar molecules that will travel through the column more quickly. Reversed phase HPLC is the most commonly used form of HPLC. There are several ways of detecting when a substance has passed through the column. A common method which is easy to explain uses ultra-violet absorption. Many organic compounds absorb UV light of various wavelengths. If there is a beam of UV light shining through the stream of liquid coming out of the column, and a UV detector on the opposite side of the stream, a direct reading of how much of the light is absorbed can be obtained. The amount of light absorbed will depend on the amount of a particular compound that is passing through the beam at the time different compounds absorb most strongly in different parts of the UV spectrum. Methanol, for example, absorbs at wavelengths below 205 nm, and water below 190 nm. If methanol-water mixture is used as the solvent, therefore a wavelength greater than 205 nm to avoid false readings from the solvent should be used. The sample matrix can have a profound impact on the analytical results for an HPLC method. As a result of this, a matrix-based calibration curve is recommended almost universally. There are several goals of sample preparation. One is to remove materials that might interfere chromatographically with the analyte, another is to get acceptable recovery of the analyte and a third is to remove "column killers" – those matrix component that shorten column lifetime. A common way to test recovery is to spike known amounts of reference standard into a blank matrix, or placebo, mix it well, then perform sample extraction. The recovered amount then can be compared to the amount spiked into the matrix to determine recovery. This is done by comparing the signal of the extracted sample to the signal obtained from pure reference standard. Although it is ideal to get 100% recovery of the analyte, this seldom occurs. In many cases, recovery of 80-95% can be obtained, but in others, recovery may be below 50%. Although the method usually will perform more reliably and reproducibly the higher the recovery, this is not necessarily the case or a requirement for a successful method 86% of the sample that one spikes into a blank matrix, it is expected that one will also recover 86% of the analyte from a real sample. Then the extracted samples can be directly related to the true amount of analyte present. The simplest way to do this is to use a matrix-based calibration curve. This is done by spiking different concentrations of reference standard into blank matrix, extracting the standard back out, and using the resulting solutions to construct the calibration curve. This will correct for the low recovery from the sample matrix of both real samples and the calibrators so that the reported values should be close to the real values. Because the matrix can have such a profound influence on the final results, it is recommended to match the matrix for the calibration curve to the sample. Sometimes one can get away without using a matrix-based calibration curve, but this should be done only when it is shown that there is no difference in the analytical results with and without the matrix present.

MATERIAL AND METHODS

In the present study used the flesh of fresh fish that is spiked with a known concentration of antiparasitic. All standards are satisfactorily high HPLC purity. Niclosamide (2',5- dichloro-4-nitrosalicylanilide) were obtained from Sigma (St. Louis, MO, USA; USP or BP grade; purity above 98%). HPLC methanol and acetonitrile were used. The HPLC system was Thermo. The flow-rate employed was 1 ml min -t. For determination was used UV detector at 254 nm.

Standard solutions of the antiparasitics niclosamide 0.0023g were dissolved in acetonitrile to obtain concentration of 100 mg/mL. A volume of 1 mL of solution was diluted to 10 mL with acetonitrile to get concentration of 10 mg/mL.

RESULTS AND DISCUSSION

We analyzed 15 samples spiked fish meat with different concentration of niclosamide and we found that recovery goes from 75 to 94% when we calibrate through matrix, and when not about 45%. In the study of HPLC determination of niclosamide Schreier et al. (1996) found that recovery of NIC from rainbow trout (n = 7) fortified at 0.04 mu g/g was 77 +/- 6.5% and from channel catfish (n = 7) fortified at 0.02 mu g/g was 113 +/- 11%. NIC detection limit was 0.0107 mu g/g for rainbow trout and 0.0063 mu g/g for catfish. Percent recovery of incurred radioactive residues by this method from catfish exposed to [C-14] NIC was 89.3 +/- 4.1%. Percent recoveries of NIC from fortified storage stability tissue samples for rainbow trout (n = 3) analyzed at 5 and 7.5 month periods were 78 +/- 5.1 and 68 +/- 2.4%, respectively. Percent recoveries of NIC from fortified storage stability tissue samples for channel catfish (n = 3) analyzed at 5 and 7.5 month periods were 88 +/- 13 and 76 +/- 21%, respectively .The lowest recovery after ten replicate injections of known samples was 100.2% and the highest 104.2%, with an overall mean deviation from 100% of 1.1% (Elsa, 1997). The antiparasitic are drugs that have been used for about 50 years in crop protection, human and animal health. The treatment of antiparasitic diseases is a topic of great importance in these days for the pharmaceutical industry, because it becomes increasingly necessary to improve the pharmaceutical formulations due to the mix presentation of these diseases in many animals (César Soto, 2013).

CONCLUSION

The use of the HPLC method allows a selective and quantitatively accurate analysis of several anthelmintics in veterinary dosage forms. The chromatographic method is sufficiently specific, precise and sensitive for the purpose of analytical characterisation of niclosamide. Preliminary results not given here also showed that the method was applicable for the analysis of other anthelmintic.

ACKNOWLEDGEMENTS

The paper is part of the project TR 31011, funded by the Ministry of Science and Technology of the Republic of Serbia.

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VITRIFICATION OF THE SPERM OF EURASIAN PERCH *(PERCA FLUVIATILIS)*

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VITRIFIKACIJA MLEČA GRGEČA (*PERCA FLUVIATILIS*)

Apstrakt

Vitrifikacija je proces dovođenja vode ili rastvora u čvrsto stanje, odnosno u amorfno ili staklasto stanje koje može da se dostigne veoma brzom hlađenjem (10^6-10^{10} °C/s). Nedavno je objavljeno nekoliko istraživanja o vitrifikaciji mleča različitih vrsta riba, međutim nema dostupnih informacija o vitrifikaciji mleča grgeča *(Perca fluviatilis).*

Mužjaci grgeča su uzorkovani 6 dana posle hormonske injekcije (250 IU kg^{-1} hCG). Evaluirana je pokretljivost spermatozoida pomoću sistema kompjuterske analize sperme CASA. Za process vitrifikacije mleč je razblažen modifikovanim Tanaka ekstenderom na finalni odnos 1:5 (sa krioprotektantima). Posle preliminarnih testova sa kombinacijom metanola i propilen glikola (PG) u različitim koncentracijama, odlučeno je da se koristi 15% metanola i 15 % PG (ukupno 30% krioprotektanata). Suspenzija mleča je ubačena direktno u tečni azot bez prethodnog hlađenja u njegovoj pari. Za sve eksperimente vitrifikacije za hlađenje su korišćene cevčice Cryotop (Kitazato-Dibimed, za 2 µl rastvora).

Za fertilizacioni test su prikupljena jaja ženki grgeča. Vitrifikovane Cryotop cevčice otopljene su direktno u 10 µl rastvora za aktivaciju (50 mm NaCl) u petri šoljama koje su sadržale jaja. Svež mleč služio je za kontrolu. Oplođena jaja su inkubirana u plivajućem sistemu. Izvedena su 3 ogleda da bi se utvrdio odgovarajući broj cevčica Cryotop za svaku seriju jaja: 1, 6 i 18 cevčica Cryotop je isprobano za svaku seriju jajnih ćelija. U 2 µl rastvora mleča jedne Cryotop cevčice bilo je oko 0,33 µl mleča. Na osnovu stepena oplođenja u tri ogleda može se zaključiti da povećanje broja Cryotop cevčica pojačava stepen oplođenja. Dalja sitraživanja su neophodna da bi se razvio metod vitrifikacije sa većim preživljavanjem larvi posle oplođenja vitrifikovanim mlečom. Takođe je potrebno ispitati stepen izvaljenih embriona iz ogleda sa vitrifikovanim mlečom, kao i potencijalni uticaj vitrifikacije na larve, pre svega na deformitete i morfološke promene.

Ključne reči: vitrifikacija spermatozoida, veoma brzo hlađenje, krioprezervacija mleča, Perca fluviatilis, Cryotop
Keywords: spermatozoa vitrification, ultra-rapid cooling, fish sperm cryopreservation, Perca fluviatilis, Cryotop

INTRODUCTION

For preservation of cells and tissues without formation of ice crystals, two main methods have been developed: slow programmable freezing and vitrification. Programmable freezing requires expensive instrumentation and laboratory conditions during the cooling process. Accordingly, ultra-fast cooling or vitrification, which does not require special equipment, has attracted increasing interest in recent years. Vitrification is the solidification of a liquid into an amorphous or glassy state which can be attained at very fast cooling rates (10^6-10^{10} °C/s). The success of the ultra-rapid cooling relies principally on achieving ultra-fast cooling and thawing rate and determining an appropriate concentration of the cryoprotective agent combined with suitable cooling media, preventing ice formation during the process. Although high concentrations of cryoprotectants lower the ice formation temperature, they could be toxic to cells. Consequently, cryoprotectant concentration has to be reduced and the cooling rate has to be enhanced. For this reason, the material and capacity of the cooling device is highly important for achieving fast heat transfer and to avoid creation of ice crystals (Tsai et al, 2015). Recently, several studies have been published on the vitrification of fish sperm (Cuevas-Uribe et al., 2011/a, b, Figueroa et al., 2013, 2015). These studies report results on different fish species, such as Channel catfish (*Ictalurus punctatus)*, Green Swordtail *(Xiphophorus hellerii)*, Rainbow trout *(Onchorynchus mykiss)*, Atlantic salmon *(Salmo salar)*, but no information is available on the vitrification of Eurasian perch *(Perca fluviatilis)* sperm.

MATERIALS AND METHODS

A broodstock of wild caught Eurasian perch males and females was maintained at the hatchery of the Department of Aquaculture, Szent Istvan University, Gödöllő, Hungary. We have collected sperm from Eurasian perch males. After drying the genital area with paper (to avoid contamination of samples with water or mucus), sperm was collected into 1,5 ml Eppendorf tubes by applying gentle abdominal pressure to anaesthetised (with MS-222) males (Fig. 1.). The fish were sampled 6 days after hormonal injection (250 IU kg^{-1} of hCG (human chorionic gonadotropin, Ferring, Saint Prex, Switzerland)). We have kept the sperm on ice during the preparation method. Progressive motility of fresh sperm was evaluated with computer assisted sperm analysis (CASA, Sperm VisionTM v. 3.7.4., Minitube of America, Venture Court Verona, USA). Modified Lahnsteiner’s activating solution (75 mM NaCl, 2 mM KCl, 1 mM $MgSO_4$•$7H_2O$, 1 mM $CaCl_2$• $2H_2O$, 20 mM Tris, pH 8 (Lahnsteiner et al, 2011)) in a mixture of 0.01 g/mL BSA (Bovine Serum Albumin) was used for CASA analysis.

For the vitrification, sperm was diluted with modified Tanaka extender (137 mM NaCl and 76.2 mM $NaHCO_3$ (Szabó et al, 2005)) to the final ratio of 1:5 (including cryoprotectants). Methanol (MeOH) and propylene glycol (PG) were used in three combinations: 10% MeOH

– 10% PG (20% total concentration), 15% MeOH – 15% PG (30%) and 20% MeOH – 20% PG (40%). Chemicals were purchased from Reanal (Budapest, Hungary) and Sigma-Aldrich (Budapest, Hungary). The sperm suspension was plunged directly into liquid nitrogen without pre-cooling in its vapour. For all vitrification experiments Cryotops (Fig. 2.) were used as cooling device (Kitazato-Dibimed, for 2 µl of solution). Motility of vitrified samples was determined using CASA following thawing of Cryotops directly into the activating solution under the microscope.

We have collected eggs from Eurasian perch females (Fig. 3.). After the use of single hormonal injection (hCG, 500 IU/g fish) the females were checked daily by ovarian biopsy, enable to predict the accurate time of the ovulation. One day before the planned ovulation the genital pore were sutured to avoid spontanous spawning into the tank. After removing the suture and drying the genital area the eggs were stripped into a dry dish.

For fertilization tests approximately 100 eggs were used for each sample. Sperm was vitrified in the presence of cryoprotectants at a final concentration of 30%. We thawed the vitrified Cryotops directly into 10 ml of activating solution (50 mm NaCl) in a petri dish containing the eggs. Fresh sperm was used for a control fertilisation test. Fertilized eggs were incubated in a floating system (styrofoam boards with filters on a tank, Fig.4.).

Three trials were realized to find the most appropriate number of cryotops per egg batch. During the experiments we have used 1, 6 and 18 cryotops for the fertilisation of one portion of eggs. The 2 µl diluted sperm on one cryotop contained approximately 0,33 µl sperm. Ferilization ratios were counted with a steromicroscope (Fig. 5.).

To analyze the results, statistical software GraphPad Prism 5.0 for Windows (GraphPad Software, La Jolla, California, USA) was used.


Figure 1. *Sperm stripping*

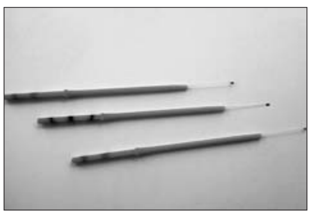
Figure 2. *Cryotops*


Figure 3. *Stripping the eggs*

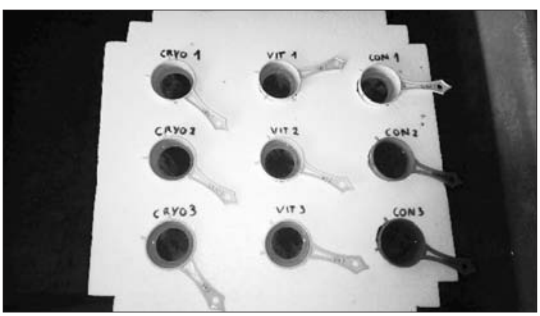

Figure 4. *Floating incubation*

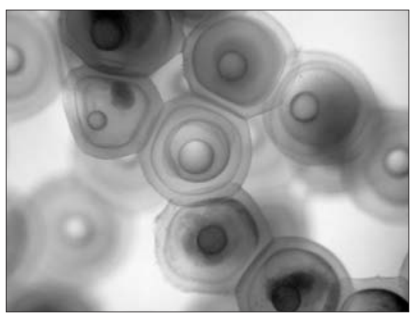
Figure 5. *Developing embryos*

RESULTS AND DISCUSSION

According to the motility data, the use of 20% cryoprotectant (10% methanol and 10% PG), the measured average progressive motility after thawing was 10,31% (±1,92). In the case of 30% cryoprotectant (15% methanol and 15% PG) this value was 13,95% (±1,67), and with using 40% total cryoprotective agent we reached 7,07% (±4,05). Several studies suggest that fish spermatozoa can tolerate high cryprotectant concentrations when the proportion of the chemicals is appropriate (Cuevas-Uribe et al., 2011a, b). During previous experiments with other fish species we have also found that the most appropriate cryoptotectant concentration is around 30% of the total solution, because with lower alcohol content the creation of harmful ice crystals is not fully inhibited, and higher concentrations of cryoprotectants are toxic for spermatozoa.

Thus we can conclude that best vitrification method was carried out with using the following protocol: 1:5 dilution ratio, Tanaka extender, 30% cryoprotectant (15% methanol + 15% propylene-glycol), cooling device: Cryotop, 2 µl droplets (Fig. 6.).

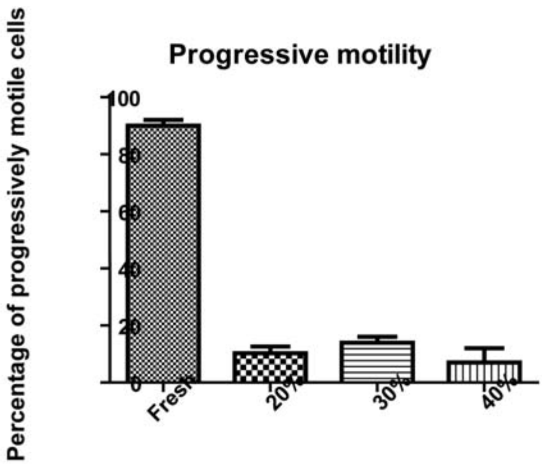

Figure 6. Progressive motility measured in fresh sperm, and sperm vitrified with 20%, 30% and 40% cryoprotectant. Columns represent the datas from 3 experiments, vitrified 3 samples in 4 replicates.

According to the fertilisation rates of the three trials, we can conclude that increasing the number of used Cryotops enhances the fertilisation ratio: fertilising with one Cryotop resulted 1,44% (±1,58) fertilisation, 6 Cryotops per egg batch resulted 4,9% ±4,77), and 18 Cryotops resulted 1,27% (±1,51). In the case of the third experiment (with 18 Cryotops), egg quality was moderate (under 70% fertilisation in the control group), thus further studies are needed to achieve more accurate results.

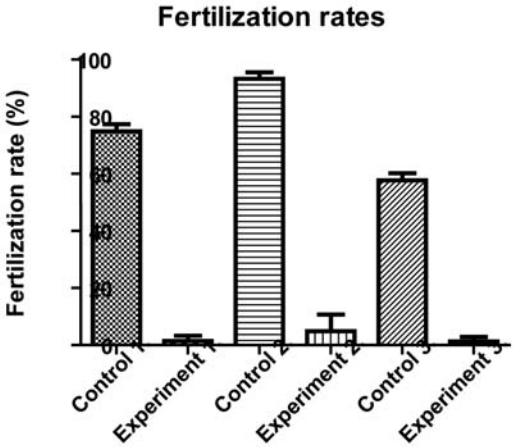

Figure 7. Fertilizaton rates measured in controlls, and vitrified sperm with 1/6/18 cryotops per egg batch. Columns represent the datas from 3 experiments.

CONCLUSION

Successful vitrification of Eurasian perch sperm was conducted for the first time. Motile spermatozoa were recovered following vitrification of sperm and fertilization of eggs with vitrified sperm resulted in developing embryos in this species. Thus, vitrification of sperm is feasible in the Eurasian perch, although futher studies are needed to improve this technique.

ACKNOWLEDGEMENTS

The work was funded by the OTKA grant number 109847. The work was supported by the project number 8526-5/2014/TUDPOL of the Ministry of Human Resources of Hungary and the project EUREKA_HU_12-1-2012-0056 (PERCAHATCH)

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THE RELATION BETWEEN TANK COLOR AND EUROPEAN SEABASS (*DICENTRARCHUS LABRAX*) JUVENILES GROWTH PERFORMANCE

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ODNOS IZMEĐU BOJE BAZENA I PRIRASTA MLAĐI EVROPSKOG BRANCINA (*DICENTRARCHUS LABRAX*)

Apstrakt

Dizajn sistema za gajenje riba je veoma bitan za održivu i visoko profitabilnu proizvodnju u akvakulturi. Različitim vrstama riba potrebani su drugačije dizajnirani sistemi i veštačke sredine. Sistemi u zatvorenom prostoru su korisni za mrestilišta a tankovi su veštačka staništa za vrste gajene u tim sistemima. Prethodna istraživanja pokazuju da boja zida bazena utiče na nivo stresa kod riba (Rotlant et al., 2003) i parametre koji utiču na rast, a dobrobit riba može da bude ugrožena u stresnim uslovima (De Silva and Anderson 1994). Cilj ovog istraživanja je da ispita efekte koje različite boje zidova tankova imaju na prirast mlađi Evropskog brancina (*Dicentrarchus labrax*).

480 jedinki mlađi nasumice su raspoređene u 12 identičnih plastičnih tankova (40 jedinki po tanku). Zapremina svakog bazena iznosila je 40 litara. U triplikatu su korišćene četiri različite boje bazena (crvena, zelena, plava i svetlo žuta). Riba je hranjena komercijalnom hranomn za brancina 2 puta dnevno u period od 60 dana.

Najveći prirast dostigla je riba gajena u crvenim bazenima, dok je riba gajena u žutim bazenima imala najmanji prirast.

Prethodna istraživanja su pokazala da boja zida bazena utiče na prirast ribe u uslovima gajenja i da je različitim vrstama riba potrebna drugačija boja bazena da bi postigle najbolji prirast (Duray et al., 1996; Rotland et al., 2003; Imanpoor and Abdollahi, 2011). Rezultati pokazuju da boja bazena utiče na prirast riba u uslovima gajenja.

INTRODUCTION

System design is very important for sustainable and high profit aquaculture production. Different species need various system design and artificial area. Indoor aquaculture systems are useful for hatcheries and tanks are artificial habitats for culture species in these systems. Previous studies reported that, tanks wall color can affected stress level of fishes (Rotlant et al., 2003) and growth-related parameters and welfare of the fish may be negatively affected under stressful conditions (De Silva and Anderson 1994). In this study it was intended to research the effects of different tank colors on growth performance of Seabass (*Dicentrarchus labrax*) juveniles.

MATERIAL AND METHODS

A total of 480 juveniles were randomly distributed in 12 identical plastic tanks (40 fish per tank) with a water volume of 40 L. Four different tank colors (red, green, blue and light yellow) with triplicate treatments were used. Fish were fed on commercial seabass diet 2 times a day for 60 days.

RESULTS

The best growth performance was obtained in red color, while the lowest growth of fish was recorded in the yellow colored tanks.

DISCUSSION

Previous studies showed that fish growth performance under culture conditions were affected by tank wall color and different species need different tank color for best growth performance (Duray et al., 1996; Rotland et al., 2003; Imanpoor and Abdollahi, 2011). According to results, tank color affects growth performance in fish under culture conditions.

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EFFECT OF DIETARY INCLUSION OF THE VARIOUS SOURCES OF ADDITIVES ON GROWTH, BODY COMPOSITION AND CHALLENGE TEST OF JUVENILE ROCKFISH (*SEBASTES SCHLEGELI)*

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EFEKAT UKLJUČIVANJA U HRANLJIVE SMEŠE ADITIVA RAZLIČITOG POREKLA NA RAST, TELESNI SASTAV I OTPORNOST MLAĐI CRNOG MORSKOG GRGEČA (*SEBASTES SCHLEGELI*)

Apstrakt

U radu je tvrđen efekat uključivanja u hranljive smeše aditiva različitog porekla [đumbir (CG), fermentisana sojina pasta (CJ), borovnica (BB), japanska jabuka (PM), paradajz (TT), brokoli (BC) i jakon (YC)] na rast, telesni sastav i otpornost mlađi crnog morskog grgeča. Hiljadu šesto osamdeset riba je nasumično raspoređeno u 24 protočna tanka zapremine 200 L. Pripremljeno je osam oglednih smeša za ishranu: kontrolna bez dodataka (Con) i smeše sa dodatkom GG, CJ, BB, PM, TT, BC i YC. Svaka od smeša korišćena je u tri tanka, a ribe su 7 nedelja ručno hranjene do sitosti. Posle isteka ovog perioda, dvadeset riba iz svakog tanka inficirano je sa *Streptococcus parauberis* i praćeno narednih 10 dana. Prirast i specifična stopa rasta (SGR) bili su veći kod riba koje su u hrani dobijale jakon (YC) nego kod onih koje su hranjene drugim smešama. Kumulativni mortalitet do 5. dana posle infekcije bio je niži kod riba koje su hranjene smešama sa dodatkom GG, BB i YC nego kod ostalih jedinki. U zaključku, smeša sa dodatkom YC pokazala se kao najbolja u smislu poboljšanja prirasta i SGR kod crnog morskog grgeča. Osim toga, dodatak GG, BB i YC u smeše bio je najefikasniji u smanjivanju mortaliteta crnog morskog grgeča usled infekcije sa *S. parauberis*

Ključne reči: crni morski grgeč (Sebastes schlegeli), aditivi, prirast, specifična stopa rasta, kumulativni mortalitet

Keywords: rockfish (Sebastes schlegeli), additives, weight gain, specific growth rate, cumulative mortality

INTRODUCTION

Annual aquaculture production of rockfish, *Sebastes schlegeli* has been highly ranked (MFAFF, 2014). Many feeding trials to determine dietary nutrient requirements (Lee et al., 2002), digestibility of various feed ingredients (Lee, 2002), alternative animal and/or plant protein sources for fishmeal in the diet (Lee et al., 1996), optimum feeding rate (Mizanur et al., 2014), optimum feeding frequency (Lee et al., 2000) and dietary additives to improve lysozyme activity and stress recovery (Hwang et al., 2013) for rockfish have been reported.

However, since high mortality of fish commonly occurs at fish farm every year due to outbreak of disease throughout year-round culture and dietary administration of the certain synthetic chemicals such as antioxidant to fish which can be used for human consumption is prohibited in some countries (Tang et al., 2001), development of the natural resource of new dietary additive to improve growth performance and/or immune response and to lower mortality of fish at the event of disease occurrence keeps being highly needed.

Ginger (GG), *Zingiber officinale* Roscoe, containing the gingerols and shogaols, was known to have an antioxidant activity and used for treatments of the several diseases (Ali et al., 2008) and its dietary administration improved the antioxidant effect on animals (Kota et al., 2008). Cheongkukjang (CJ) containing isoflavone and anthocyanin, traditional fermented soyfood, had the antioxidant and free radical scavenging activities (Kim et al., 2009). Blueberry (BB), *Vaccinium ashei*, containing anthocyanin had an antioxidant activity and showed the antioxidant effect on animals (Papandreou et al., 2009). Persimmon (PM), *Diospyros kaki* L., containing polyphenols was known to have an antioxidant activity and its dietary supplementation improved the antioxidant effects on animals (Kim et al., 2003). Tomato (TT), *Solanum lycopersicum*, containing lycopene was reported to have an antioxidant activity and its dietary inclusion improved the antioxidant effects on animals (Moreira et al., 2005). Broccoli (BC), *Brassica oleracea*, containing gluconsinolate showed an antioxidant activity and its dietary administration improved the antioxidant effects on animals (Muller et al., 2012). Yacon (YC), *Polymnia sonchifolia*, containing polyphenols also had an antioxidant activity and its dietary supplementation increased the antioxidant effect on animals (Kim, 2013).

Therefore, dietary inclusion of the various sources of additives on growth, body composition and challenge test of juvenile rockfish was determined in this study.

MATERIALS AND METHODS

One thousand six hundred and eighty fish averaging 3.0 g were randomly distributed into 24 of 200 L flow-through tanks. The water source was the sand-filtered natural seawater, while constant aeration was supplied to the each tank. Water flow rate into the tanks was 4.6 L/min. Water temperature was ranged from 15.8 to 23.1° C (mean ± SD: 20.5 ± 2.64° C).

Eight experimental diets were prepared; the control diet (Con) with no additive, GG, CJ, BB, PM, TT, BC and YC. One percent of each additive was included in the each experimental diet at the expense of wheat flour except for the Con diet. The Con diet was prepared to satisfy dietary nutrient requirements for rockfish (Kim et al., 2001; Kim et al., 2004).

Twenty externally normal fish shown to be free from bacterial infection were selected from each tank and stocked into 24 static 200 L tanks at the end of the 7-week feeding trial. Fish was used for the *Streptococcus parauberis* challenge and water was not exchanged throughout the challenge test. The bacteria used for the challenge were reference pathogenic strainof gram positive-*S. parauberis* (KCTC11980BP) isolated from rockfish.

SAS version 9.3 (SAS Institute, Cary, NC, USA) was used to conduct a one-way ANOVA. Tukey’s honestly significant difference (HSD) test was used to determine the significance (P< 0.05) of the differences among the means responses to dietary treatments.

RESULTS AND DISCUSSION

Survival of the juvenile rockfish ranging above 98% was not significantly (P > 0.05) affected by the various sources of dietary additives. However, weight gain (g/fish) and SGR (%/day) of fish fed the YC diet was significantly (P < 0.05) higher than that of fish fed the all other diets, followed by the GG, BB and Con diets. Weight gain and SGR of fish fed the CJ, PM and BC diets was significantly (P< 0.05) lower than those of fish fed the Con diet. The poorest weight gain and SGR was observed in fish fed the BC diet. Similarly, the oral administration of CJ containing polyphenols containing isoflavone and anthocyanin into the diets lowered weight gain of rats fed the high cholesterol diet (Kim et al. 2009). However, weight gain of rats fed the high cholesterol diet was not affected regardless of oral administration PM leaf extract (Kim et al., 2003). Dietary inclusion of BC extract did not affect either weight gain or feed consumption of broilers compared to the control diet without additive (Mueller et al., 2012).

Feed conversion ratio (FCR) and protein efficiency ratio (PER) of fish fed the TT and YC diets were significantly (P< 0.05) higher than those of fish fed the all other diets. FCR and PER of fish fed the Con and GG diets were significantly (P< 0.05) higher than those of fish fed the CJ, BB, PM and BC diets. The poorest FCR and PER were observed in fish fed the BC diet. Less feed consumption, but comparable weight gain of fish fed the TT diet led to the improved FCR and PER compared to those of fish fed the Con diet in this study. The poorest PR was obtained in fish fed the BC diet. Improved weight gain, FCR and PER of rockfish fed the YC diet could be resulted from the increased feed consumption compared to those of fish fed the Con diet in this study. Weight gain of rats fed the high fat-high cholesterol diet supplemented with 10% YC powder was lower than that of rats fed the high fat-high cholesterol diet without YC powder although food intake in the former was slightly, but not significantly, increased (Kim et al., 2010). Weight gain, feed intake and feed conversion of broiler chicken not affected by dietary inclusion of YC when 0.5, 1 and 2% YC byproduct was included and fed for 5 weeks (Kim, 2013).

A cumulative mortality of rockfish fed the Con diet was significantly (P< 0.05) higher than that of fish fed the all other diets since 36-hr after infection throughout the 10-day observation (Figure 1). This indicated that all dietary additives used in this study were effective to lower mortality of rockfish at occurrence of *S. parauberis*. The cumulative morality of fish fed the GG and BB diets was significantly (P< 0.05) lower than that of fish fed the BC diet in 60 hr after infection, but not significantly (P> 0.05) different from that of fish fed the CJ, PM, TT and YC diets.

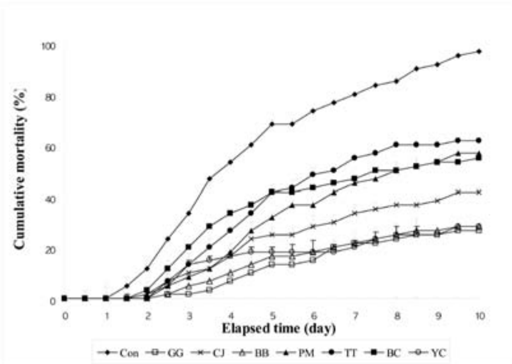

Figure 1. Cumulative mortality (%) of juvenile rockfish, *Sebastes schlegeli*, fed the experimental diets containing the various sources of additives for 7 weeks and then infected by *Strepcococcus parauberis*(means of triplicates ± SE).

The cumulative mortality of fish fed the GG, BB and YC diets was significantly (P< 0.05) lower than that of fish fed the all other diets in 5-day after infection, but no significant difference was found among fish fed the GG, BB and YC diets throughout 10-day observation. At 10-day, the lowest cumulative mortality was observed in fish fed the GG diet, followed by the BB, YC, CJ, BC, PM, TT and Con diets in order. This indicated that GG, BB and YC would be the good dietary additive to lower mortality of rockfish at occurrence of *S. parauberis*. An oral administration of aloe at 0.5% lowered cumulative mortality of rockfish infected by *Vibrio alginlyticus*.

CONCLUSION

YC was the best dietary additive to improve weight gain and SGR of rockfish. In addition, YC and TT was the most effective to obtain improved FCR and PER of fish. However, dietary inclusion of GG, BB and YC were the most effective to lower mortality of rockfish at infection of *S. parauberis*.

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GUESTS FISH SPECIES OF THE GULF OF ANTALYA

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INTRODUKOVANE VRSTE RIBA U ANTALIJSKOM ZALIVU

Apstrakt

Uvođenje novih, ne nativnih vrsta riba u ekosistem utiče na njegov biodiverzitet i strukturu. U Mediteranu, sa bogatim biodiverzitetom, naročito od otvaranja Sueckog kanala 1869. godine, ima veoma mnogo ne nativnih vrsta riba. Postoje izveštaji o 149 novih vrsta riba u Mediteranu, od toga 49 vrsta u vodi blizu obale Turske i 18 egzotičnih vrsta blizu Antalijskog zaliva.

Ovo istraživanje sprovedeno je u decembru 2011. godine i daje nam informacije o statusu ne nativnih vrsta riba putem kočarenja u Antalijskom zalivu. Uzorkovanje je izvršeno na tri dubine (20-50 m, 51-100 m i 101-200 m) korišćenjem mreže za dno. Sve operacije izvršene su u zonama za komercijalno ribarstvo sa geografskim koordinatama; 36°50'730''N - 30°45'480''E, 36°45'928''N - 31°23'092''E.

Ukupno je izlovljeno 10 038 jedinki, pripadale su 109 različitih vrsta riba. 26 % ulova predstavljalo je introdukovane, ne nativne vrste riba. Najveća abundanca novih vrsta riba zabeležena je na dubini od 20 - 50 m i činila je 71% ukupnog ulova. Na dubini od 51 - 100 m bilo je 36%, a na dubinu od 101 - 200 m, 2% ulova predstavljalo je ne nativne vrste.

Od ukupno 17 ne nativnih vrsta riba, *Equulites klunzingeri* je imala najveću abundancu, dok su *Upeneus moluccensis*, *Saurida undosquamis*, *Nemipterus randalli, Etrumeus teres, Sphyraena chrysotaenia* komercijalne vrste riba.

Broj introdukovanih vrsta riba se povećava u Antalijskom zalivu. S obzirom na ovakvu situaciju u ribarstvu, prisustvo komercijalnih vrsta kao što su *Upeneus mollucensis* i *Saurida undosquamis* ima pozitivan efekat koji je kratkotrajan. Međutim, nove vrste riba, kao što je « naduvana riba », opasne su za ribare. Da bi se smanjio negativan efekat migracija na životni sredinu i biodiverzitet, potrebno je ispitati ponašanje novih vrsta pri gajenju i hranjenju kao i njihove interakcije sa nativnim vrstama.

Ključne reči: ne nativne vrste riba, Antalijski zaliv, kočarsko ribarenje

Abstract

Biodiversity and structure of ecosystems are affected with the introduction of alien species. The Mediterranean, which has a very rich biodiversity, especially with the opening of the Suez Canal in 1869, contains many of alien species. 149 new fish species were reported from Mediterranean, 49 of them were recorded off the coast of Turkey and 18 exotic fish species were reported from the Gulf of Antalya.

This study was conducted in December 2011 and provided information about the status of alien fish species in demersal trawl fisheries in the Gulf of Antalya. Sampling was carried out at three depths (20-50 m, 51-100 m and 101-200 m) with bottom trawler. All trawling operations were performed in the commercial fishing zone with geographical coordinates; 36°50'730''N - 30°45'480''E, 36°45'928''N - 31°23'092''E.

Totally 10 038 individuals belonging to 109 species were caught in the study and 26 % of which were alien species. The highest abundance of the alien fish species was recorded at the depth of 20-50 m with 71% percentage of total catch. These values for depth of 51-100 m and 101-200 m were determined as 36% and 2%, respectively.

Among the total of 17 alien fish species, *Equulites klunzingeri* has the highest abundance and *Upeneus moluccensis*, *Saurida undosquamis*, *Nemipterus randalli, Etrumeus teres, Sphyraena chrysotaenia* are commercial species.

The number of alien fish species is increasing in the Gulf of Antalya. Considering this situation in terms of fisheries; the participation of commercial species such as *Upeneus mollucensis* and *Saurida undosquamis* providing positive contributions is temporary. However, non-commercial species such as puffer fish are harmful for fishermen. To minimize the negative effects of migration on the environment and biodiversity, breeding and feeding behavior of alien species, and interactions with native species should be investigated especially.

Keywords: Alien fish species, Gulf of Antalya, trawl fisheries

WATER QUALITY ASSESSMENT OF VRUTCI RESEROVIR TRIBUTARIES BASED ON DIATOM INDICES

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ANALIZA KVALITETA VODE PRITOKA VRUTCI AKUMULACIJE POMOĆU DIATOMNIH INDEKSA

Apstrakt

Silikatne alge su važna grupa akvatičnih organizama osetljivih na promene u svojoj životnoj sredini. Međutim, njihova praktična upotreba kao bioindikatora je relativno nova u Srbiji. U ovom istraživanju sakupljeni su epilitski uzorci iz 14 pritoka akumulacije Vrutci u toku septembra i oktobra 2014. godine. Identifikovano je ukupno 84 taksona silikatnih algi. Najveće populacije u okviru epilitske zajednice silikatnih algi u većini pritoka grade Cocconeis placentula var. lineata i *Achnanthidium minutissimum* var. *minutissimum*. Rezultati izračunavanja dijatomnih indeksa, uz pomoć softverskog paketa OMNIDIA, ukazuju da je ekološki status vode većine ispitivanih pritoka odličan do dobar. Samo rezultati dijatomnih indeksa za pritoke Simića potok, Konjski potok, Bioštanska Banja i neimenovanu pritoku 13 pokazuju prisustvo umerene antropogene eutrofikacije.

Ključne reči: biomonitoring, silikatne alge, dijatomni indeksi, akumulacija Vrutci

Keywords: biomonitoring, diatoms, diatom indices, Vrutci reservoir

INTRODUCTION

Algae are essential components of primary production in aquatic ecosystems. Diatoms are so ecologically important that they are used as bioindicators in environmental monitoring of waters. They form a large part of the benthos communities and therefore they could become an important part of water quality monitoring (Ács et al. 2004). Diatoms have been used in a number of countries as bioindicators of river pollution (Kelly and Whitton 1995, Ziller and Montesanto 2004, Ács et al. 2006, Blanco et al. 2004, Gosselain et al. 2005, Solak et al. 2012). However, in Serbia it is still a new topic (Andrejić 2012, Krizmanić et al. 2013, Vidaković 2013, Jakovljević et al. 2014).

Catchment area of Vrutci reserovir represents a part of Starovlaško-Raških mountains, which are part of the Dinarides. Altitude in the area is mainly within the interval from 570 to 1250 meters above sea level (KRO" Bioktoš" 1986). Average altitude of the catchment area of reservoir „Vrutci" of 915 m.a.s.l. (area of 127 km^2) indicates an area of particularly mountainous and hilly terrain (mountain massifs of Tara, Zlatibor and Mokra Gora). Geologically, catchment of reservoir „Vrutci" is predominantly built from limestone. The dominant tributary of reservoir is the Đetinja River, at which a dam was built. The catchment area of reservoir is abundant in streams which have a torrential character.

MATERIAL AND METHODS

The material used in the present study was collected in autumn 2014, from 15 tributaries of the Vrutci reservoir. Epilithic samples were scraped from the surface of gravel and boulders by using scalpel blade and brush. Samples were fixed immediately with formaldechyde to a final concentration of 4%. In the laboratory samples were prepared using the standard method with cold acid (Krammer and Lange-Bertalot 1986). After this process the material was airdried on coverglasses and mounted in Naphrax®. Light microscope observations and micrographs were made using a Zeiss AxioImager.M1 microscope with DIC optics and AxioVision4.8 software. The relative abundance of taxa within the diatom community in the samples was determined by the valve percentage representation of each taxon relative to 400 numbered valve at every permanent slide. The biological assessment of water quality was performed using 17 diatom indices calculating by OMNIDIA 5.3 software (Lecointe, Coste & Prygiel 1993). The ranges of diatom indices were used together with the water quality classes and ecological status according to the regulation of the Ministry of Agriculture and Environmen (Sl. Glasnik 74/2011).

RESULTS AND DISCUSSION

In this study, a total of 84 diatom taxa were identified. The most numerous were taxa of genus: *Navicula*, *Gomphonema* and *Nitzschia*. Seven taxa were defined as dominant in all tributaries, those whose percentage participation at a given site was 10% or more. The biggest populations were consisting of *Cocconeis placentula* var. *lineata* (Ehr.) van Heurck and *Achnanthidium minutissimum* var. *minutissimum* (Kütz.) Czarnecki, which dominated in almost all tributaries. These two taxa dominated in all tributaries alternately or in combination with some of the other dominant taxa (Tab. 1). These are some of the most common diatoms, with a wide ecological range. They prefer waters from oligo- to eutrophic, and develop numerously in mountain streams with no anthropogenic impact (van Damm et al. 1994, Krammer and Lange-Bertalot 2004, Hofman et al. 2013).

Dominant and frequent species identified in the epilithic communities of the Vrutci reservoir tributaries, such as: *Cocconeis placentula var. pseudolineata* (Geitler) Lange-Bertalot, *Amphora pediculus* (Kütz.) Grunow ex A.Schmidt, *Planothidium frequentissimum* (Lange-Bertalot) Lange-Bertalot, *Planothidium lanceolatum* (Brébisson ex Kützing)

Lange-Bertalot, *Achnanthidium pyrenaicum* (Hust.) Kobayasi, were also found in other aquatic ecosystems with a good water quality, e.g. Baryczka stream (Noga et al. 2013).

Rivers and streams with water characterized by a low level of pollution are also characterized by higher species diversity (Kwandrans et al. 1998; Rakowska and Szczepocka 2011), which is the case with tributaries of the Vrutci reservoir. Further, a substantial part of the diatom species identified in these tributaries are species that prefer water with low level of pollution, β-mesosaprobic zones, according to indicator values after the OMNIDIA 5.3 database.

Table 1. Dominance in diatom communities in tributaries of the Vrutci reservoir in September and October 2014

Tributaries	2	4	5		6		7		8		9		10		11		12	13		14		15	
Taxon	oct	oct	sep	oct	sep	oct	sep	oct	sep	oct	sep	oct	sep	oct	sep	oct	sep	sep	oct	sep	oct	sep	oct
Achnanthidium minutissimum	<10	<10	21-35	10-20	<10	<10	21-35	10-20	10-20	0	10-20	10-20	>35	0	<10	0	10-20	<10	<10	<10	>35	<10	>35
Achnanthidium pyrenaicum	>35	<10	<10	0	<10	0	0	0	<10	0	0	<10	21-35	0	10-20	>35	0	0	10-20	<10	<10	<10	0
Amphora pediculus	<10	<10	<10	10-20	10-20	10-20	<10	<10	<10	0	<10	10-20	0	0	0	<10	<10	<10	<10	21-35	<10	<10	<10
Cocconeis placentula var. *lineata*	<10	>35	10-20	>35	>35	0	10-20	<10	<10	<10	21-35	<10	0	<10	21-35	<10	21-35	>35	21-35	21-35	<10	21-35	<10
Cocconeis pseudolineata	21-35	10-20	0	<10	<10	21-35	21-35	<10	0	0	<10	0	0	0	10-20	0	10-20	<10	0	<10	<10	0	0
Cyclotella ocellata	0	0	0	0	0	0	0	<10	0	21-35	0	<10	0	0	0	0	0	0	<10	0	0	0	0
Cymbella excisa	0	0	0	0	0	0	0	0	0	0	0	0	10-20	0	0	<10	0	0	<10	0	<10	0	0
Diadesmis perpusilla	0	0	<10	0	0	0	0	<10	21-35	0	0	0	0	0	0	0	0	<10	0	0	0	0	0
Fragilaria crotonensis	0	0	0	0	0	10-20	0	>35	0	>35	0	>35	0	0	0	0	0	0	<10	0	0	0	0
Planothidium frequentissimum	<10	<10	10-20	21-35	<10	<10	<10	<10	<10	0	<10	<10	0	<10	21-35	0	<10	<10	<10	0	0	<10	<10
Planothidium lanceolatum	<10	<10	<10	<10	10-20	21-35	10-20	<10	<10	0	21-35	0	0	0	10-20	21-35	0	10-20	10-20	<10	<10	>35	10-20

[%] occurrence:

0	<10	10-20	21-35	>35

Diatom indices

In order to determine the water quality of Vrutci reservoir tributaries 17 diatom indices were counted with OMNIDIA software. IPS and CEE were taken into consideration as legally obliged indices in the assessment of ecological status of small rivers and their water quality in Serbia.

The results of the diatom indices analysis show an excellent ecological status of the tributaries Krnda 2 and Cvetića bay stream in the investigated period, with the absence of organic pollution and anthropogenic eutrophication.

Ecological status of tributaries Simića stream and Konjski stream, based on the analysis of phytobenthos community, could be classified as moderate to good, with moderate anthropogenic eutrophication.

The tributaries Jasik, Jovac, Bioštanska Banja and nameless tributary marked with 10 in the investigated period showed good to moderate ecological status accompanied by the absence of organic pollution and anthropogenic eutrophication. These tributaries in October changed their composition of phytobenthos community, which was manifested by the dominance of typical planktonic algae from the Vrutci reservoir *Fragilaria crotonensis* and

Cyclotella ocellata. This was due to changes of the water level in the reservoir, when the water from the reservoir spilled over into these tributaries.

Ecological status of the tributary Ročnjak and two unnamed tributaries marked with 12 and 13 in the investigated period, can be classified as excellent to good, along with moderate anthropogenic eutrophication in September.

The tributaries Đetinja and nameless tributary marked with 15 in the investigated period showed good to moderate ecological status accompanied by the absence of organic pollution and anthropogenic eutrophication.

CONCLUSIONS

Collected samples of benthic communities consisted of 84 diatom taxa. The most dominant in all tributaries were *Cocconeis placentula* var. *lineata* and *Achnanthidium minutissimum* var. *minutissimum*.

Based on the obtained values of diatom indices it can be concluded that all tributaries of the reservoir Vrutci had no major variations of water quality during the observed period. The water had mostly excellent to good ecological status. Moderate anthropogenic eutrophication can be noticed based on the results from Simića stream, Konjski stream, Bioštanska Banja and unnamed tributary 13.

ACKNOWLEDGMENT

This study was supported by the Ministry of Education, Science and Technological Development of Republic of Serbia (project numbers TR37009 and ON 176020).

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RECENT ADVANCES IN WATER QUALITY MONITORING IN AQUACULTURE

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SAVREMENI PRISTUPI U MONITORINGU KVALITETA VODA U AKVAKULTURI

Apstrakt

Merenje fizičkih, hemijskih, bioloških parametara je važno za praćenje stanja kvaliteta voda, a samim tim i veoma važno i u akvakulturi. Visokofrekventna merenja kvaliteta voda se poslednjih godina uspešno obavljaju i u Srbiji upotrebom multiparametarske sonde, jednostavne za rukovanje a složene po pitanju parametara koje može meriti u istom trenutku. Potreba za kontrolom kvaliteta vode raste sa povećanjem produkcije ribnjaka. Od ekstenzivnog gajenja, poluintezivnog, preko intenzivnog i superintenzivnog gajenja ribe, proces kontrole kvaliteta vode se usložnjava, dakle od povremenog kontrolisanja kvaliteta (mesečno, kod ekstenzivne proizvodnje), preko dnevne, kontrole na sat, i konačno do kontinuiranog praćenja kontrole kvaliteta (super-intenzivno). Praćenje kvaliteta senzorima i sondom je moguce u svim navedenim tipovima ribnjaka, ali je svakako primena takve metode najpotrebnija u superintenzivnoj ribnjačkoj proizvodnji.

Ključne reči: kvalitet vode, visoko frekventana merenja, monitoring, fish, akvakultura
Keywords: water quality, high frequent measurements, monitoring, fish, aquaculutre

INTRODUCTION

Water can dissolve more substances than any other liquid. Over 50 percent of the known chemical elements are found in natural waters (http://www.extension.org/). Successful aquaculture depends on healthy fish and proper water quality management. Fish diseases usually occur after stress from impaired water quality. Most important parameters to be monitored regarding water quality in every fish pond are: temperature, dissolved oxygen, nitrogenous compounds, pH, alkalinity, hardness, carbon dioxide, salinity, iron, chlorine, hydrogen sulfide, water clarity. If the productivity of pond is maintained at high densities, the temperature, dissolved oxygen, ammonia, nitrite, and pH should be monitored daily or more frequently, while clarity of the water, alkalinity, hardness can be measured less frequently, perhaps one or two times per week. Other parameters like salinity, iron, and chlorine should be determined when a water source is planned to be examined, then corrective measures may be done directly during the design (Buttner et al., 1995).

Intensive monitoring of the water quality using multiparameter probes and temperature loggers in Serbia is in its infancy. Some measurements have been conducted at reservoir lakes: Ćelije, Gruža and Vrutci and on Lake Sava, small artificial urban lake in Belgrade. Continuous measurements of water temperature were established on Lake Sava in Belgrade by the aid of high frequency temperature loggers.

MATERIAL AND METHODS

We have started to collect water temperature data with temperature HOBO sensors in real-time, off line manner while other parameters were collected once per month with the multiparameter probe YSI 6600 V2-2. Most of our surveys were conducted on Lake Sava. This lake is an immensely popular recreational zone, which has the status of a special fishing water body ("catch-and-release fishing"). This lake can be observed as an extensive pond, inhabited by 20 introduced fish species (Hegediš et al., 2008). The community is dominated by cyprinid fish species with significant presence of invasive, allochthonous fish species.

Data from HOBO temperature loggers HOBO UA-001-64 are collected with 15 minutes frequency. Ten HOBO temperature loggers hang on a string at ten different depths (in meters): 0; 0.5; 1; 1.5; 2.5; 3.5; 4.5; 5.5; 6.5 and 7.5.

Multiparameter water quality probe YSI 6600 V2-2 collect water quality data by using indicators that can be measured at the same time: water temperature, pH value, conductivity, TDS, DO (luminescence time based optical sensor), DO saturation, Chlorophyll *a*.

RESULTS AND DISCUSSION

After oxygen, water temperature is the most important factor affecting the welfare of fish. Selection of species should be based in part on the temperature of the water. The temperature of the water affects the activity, behavior, feeding, growth, and reproduction of all fishes. Metabolic rates in fish depend on the temperature. Fish are generally categorized into warmwater, coolwater, and coldwater species based on optimal growth temperatures. Temperature also determines the amount of dissolved gases (oxygen, carbon dioxide, nitrogen, etc.) in the water. Figure 1 presents records of water temperature in 15 minutes intervals on 5 different depths measured at Lake Sava from May 23rd to June 2nd. Presence of water column stratification is obvious and sensors are capable of measuring both, daily and diurnal changes in water temperature. In this part of the season fish settle in a certain part of water column in accordance with their preference to water temperature.

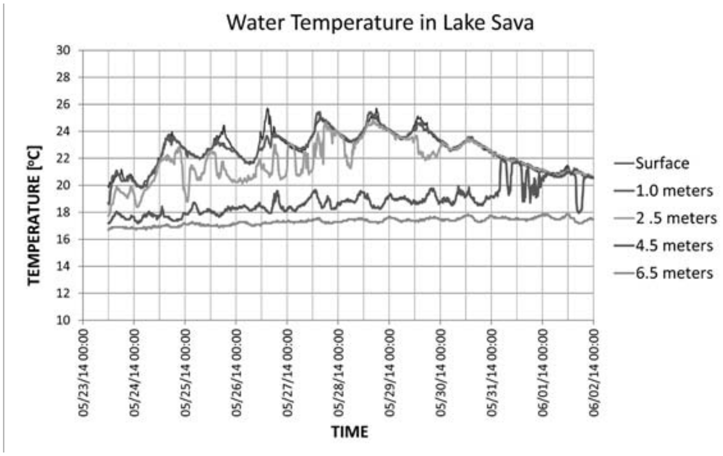

Figure 1. Temperature records at Lake Sava, five depths from May 23rd to June 2nd

Dissolved oxygen (DO) is the most important chemical parameter in aquaculture (Simões et al., 2008). Low-dissolved oxygen levels are responsible for more fish kills, than all other problems combined. Smaller size fish consume more oxygen because of their higher metabolic rate. Dissolved oxygen requirements in parts per million (ppm) for fish are: 5-10 safe, 3-5 caution, 0-3 lethal. Figure 2 shows DO vertical profiles in period of summer thermal stratification. DO concentrations are quite uniform over the depth with the exception of hypoxic conditions near the of bottom sediments observed in August 2014. This could affect benthic fish species.

Other important parameters to be monitored regarding water quality in every fish pond are: nitrogenous compounds, pH, alkalinity, hardness, carbon dioxide, salinity, iron, chlorine, hydrogen sulfide, water clarity. All of these parameters could be measured directly or indirectly in real time by the use of water quality sensors.

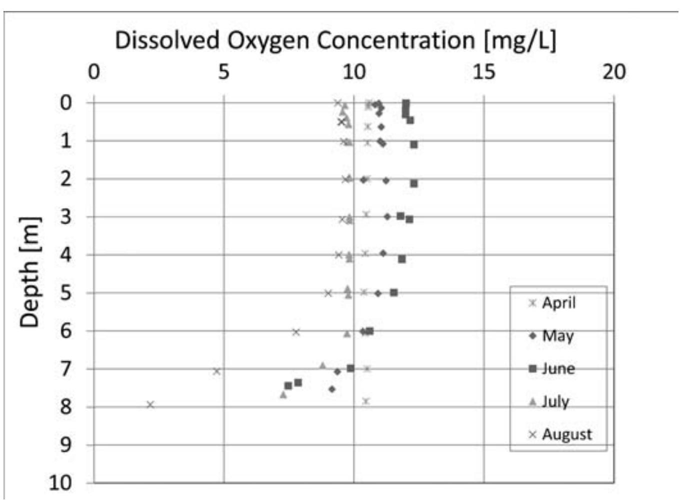

Figure 2. Vertical profiles of DO measured in spring and summer of 2014 at Sava Lake.

Two forms of ammonia occur in aquaculture systems, ionized and un-ionized. The un-ionized form of ammonia (NH3) is extremely toxic, while the ionized form (NH4+) is not. Both forms are grouped together as „total ammonia.” Un-ionized ammonia levels rise as temperature and pH increase.

The quantity of hydrogen ions (H+) in water will determine if it is acidic or basic. The scale for measuring the degree of acidity is called the pH scale, which ranges from 1 to 14. pH levels may fluctuate in ponds from approximately 4-5 to over 10 during the day. In recirculating systems constant fish respiration can raise carbon dioxide levels high enough to interfere with oxygen intake by fish, in addition to lowering the pH of the water. The acceptable range for fish culture is normally between pH 6.5-9.0.

Conductivity is commonly used in aquaculture systems for monitoring of amount of salts, nutrients or impurities in the water. Generally it is used to measure concentration of ionized chemicals in water (Zhang et al., 2013). It can be used as indicator of primary production (chemical richness) and thus fish production (Bhatnagar and Devi, 2013).

There is an interacting effect of oxygen saturation and ammonia level on fish growth (Foss et al., 2003). Increased toxicity of ammonia was observed with decreasing oxygen level. Under reduced oxygen level most of the mortality occurs within a few hours, apparently as a consequence of an additive toxic effect. Increasing oxygen level by aeration is sufficient to reduce acute ammonia toxicity under certain situations (Wajsbrot et al., 1991).

CONCLUSIONS

Appropriate water quality is essential for good results in aquaculture. Poor water quality reduces growth and affects health of fish. Therefore, reliable information about water quality in fish ponds is crucial for proper management in aquaculture. Modern systems for water quality monitoring allow measurement of many water quality indicators in real time. Those systems allow measuring of both seasonal and diurnal water quality dynamics. However, high market prices of these systems are still an obstacle for their mass usage in fish farming.

Any kind of changes in pond environment can be stressful to the fish, so parameters of the water are essential for obtaining maximum yield in a fish pond. Good water quality is characterized by adequate oxygen, proper temperature, transparency, limited levels of metabolites and other environmental factors affecting fish culture (Bhatnagar and Devi, 2013). Even with the new approach regarding conditions of water in ponds, fish mortality is still a present problem, which seeks better monitoring measures applied continuously. As a part of monitoring measures in fish ponds, collected data from water quality analyses should be recorded and stored for future reference. Problems in aquaculture can be avoided by responsible monitoring system.

ACKNOWLEDGMENT

Funded by the Ministry of Education, Science and Technological Development of the Republic of Serbia, within the Project No. TR37009 and No. OI173045.

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THE POSSIBILITY OF CONSERVATION AND SUSTAINABLE USE OF NOBLE CRAYFISH *ASTACUS ASTACUS* IN SERBIA

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MOGUĆNOST KONZERVACIJE I ODRŽIVOG KORIŠĆENJA PLEMENITOG RAKA *ASTACUS ASTACUS* U SRBIJI

Apstrakt

Dekapodni rakovi su ključna komponenta biodiverziteta reka, jezera i močvara u kojima žive. Imaju važnu ekološku ulogu u pravilnom funkcionisanju slatkovodnih ekosistema. U svetu, trećini do polovini populacija slatkovodnih rakova preti smanjenje brojnosti, čak i iščezavanje. Isto se dešava i sa autohtonim populacijama plemenitog raka (*Astacus astacus*) širom Evrope, a posledica je klimatskih promena, zagađenja, degradacije staništa i račije kuge.

Ni populacije ove vrste u slatkovodnim ekosistemima Srbije nisu izostavljene. Situacija je prilično ozbiljna kada se ima u vidu da im je brojnost opala: više od 65% u poslednjih 50 godina. Inače, plemeniti rak je jedna od tri vrste autohtonih, dekapodnih rakova koji naseljavaju slatkovodne ekosisteme Srbije. Nekada brojne populacije, sada su ograničene na fragmentisana staništa. Za razliku od drugih evropskih zemalja, u Srbiji ne postoji kultura konzumiranja ove vrste u ishrani, ne uzgaja se u akvakulturi i shodno tome izostaje ekonomski značaj. Jedino je ribolovci koriste kao mamac za lov riba, pa je tom prilikom nekontrolisano prenose iz jednog vodenog ekosistema u drugi.

Poznato je da rakovi imaju ekološku, ekonomsku i društvenu vrednost, pa je razumljivo povećano interesovanje širom sveta za njih i stanje njihovih populacija poslednjih godina. Shodno tome, veliki broj naučnih radova je objavljen u vezi strategije očuvanja, menadžmenta/upravljanja i održivog korišćenja populacija plemenitog raka. Mogućnost za eksploataciju i ekonomska vrednost, kao i rekreativna korist možda će uticati da se lokalno stanovništvo više pozabavi zaštitom ove vrste.

Cilj ovog rada je da ukaže na potrebu za održivo korišćenje i očuvanje vrste plemenitog raka.

Ključne reči: Astacus astacus, konzervacija, menadžment, Srbija
Keywards: Astacus astacus, conservation, management, Serbia

INTRODUCTION

Crayfish are key components of biodiversity in rivers, lakes and wetlands; with crucial ecological role for the appropriate functioning of freshwater ecosystem they inhabit (Gherardi, 2011). Worldwide one third to one half of the freshwater crayfish species are threatened with population decline or extinction (Schrimpf, 2013). Native population of noble crayfish, *Astacus astacus* (Linnaeus), throughout Europe have been greatly reduced or become extinct due to climate changes, pollution, habitat degradation and crayfish plague (infection by the fungus *Aphanomyces astaci*) (Fevolden & Hessen, 1989). The populations in freshwater ecosystems in Serbia have not been omitted as well. The situation is quite serious when one takes into account that the population of the noble crayfish declined, according to Simić *et al.* (2008), by more than 65% in the past 50 years.

It is known that crayfish have ecological, economical, social and cultural values, therefore the increasing awareness in recent years is understandable. In relation to that a lot of scientific papers were published concerning conservation strategies, management and sustainable use of this species (Souty-Grosset *et al.*, 2003; Schrimpf *et al.*, 2011; Kozák *et al.*, 2011; Gherardi, 2011; Bohman & Edsman, 2011; Zimmerman, 2012). We can use Europeans’ experience in this field, adapt them to our conditions and apply to our own species. The possibility for exploitation along with economical value as well as recreational benefits may make local inhabitants more eager to protect the species (Taugbøl, 2004; Jones *et al.*, 2006).

Serbia is at the beginning of this process. Simić *et al.* (2015) developed a conservation strategy for crayfish from the Astacidae family whose populations inhabiting the aquatic ecosystems of the Central Balkans. We also started researches in genetic of this species.

The aim of this article is to is to point out the probable ways of further research and indicate the need for sustainable use and conservation of this species.

DISCUSSION

Freshwater crayfish species are among the largest and longest-living of freshwater or land invertebrates. Their keystone impacts on community structure play a role in maintaining biodiversity of lentic freshwater systems (Reynolds & Souty-Grosset, 2003). They are used as indicator species for good water quality (Richardson, 2012).

Five crayfish species are present in freshwater ecosystems of Serbia. The noble crayfish (*Astacus astacus* (Linnaeus)) is one of the three indigenous species, along with two non-indigenous species. It is listed as ”vulnerable” in the IUCN Red List of Threatened Species and “endangered” on the Serbian Red List (Official Gazette of the Republic of Serbia No. 5/2010 i 47/2011).

Habitat alteration, river engineering, water pollution, (eutrophication and acidification, in particular), along with non-indigenous species (that are carriers of many pathogens and diseases) seem to be the most important factors causing decline of populations and the reason why the populations became restricted to fragmented habitats. Additionally, research of Simić *et al.* (2008) indicates that *Astacus leptodactylus* behaves in an expansive way and substitutes *Astacus astacus* in some habitats.

In our country there is no culture of consumption in the diet of this species, only the local people used it occasionally as a food, however, anglers use it as a bait for catching fish and that is the reason of uncontrolled translocations from one to another aquatic ecosystem (pers. communication with anglers).

It is not cultivated in aquaculture, so there is no economic importance in Serbia. In Europe is quite different situation. Noble crayfish are mainly used in culture in the Nordic and Baltic countries and also in Germany, France, Switzerland and Austria. This species is commercially important, for ages recognized as a delicacy in gastronomy, with high demand on markets (Swahn, 2004; Gherardi, 2011). Since reaching high prices in the markets, there is a clear economic value. For instance, it is estimated that in Sweden the annual catch of crayfish in lakes and rivers is approximately 1500 tonnes and the value of the annual catch is 30 – 40 million Euros (wholesale price) (Bohman & Edsman, 2011). Perhaps this could be the initial motive to start with crayfish production in aquaculture, in our country, for future restocking and commercial exploitation. We should definitely have in mind that the European market is large and has huge demands for the noble crayfish.

In Europe, reintroductions are common conservation actions for noble crayfish (Šmietana *et al.*, 2004; Sint & Füreder, 2004; Taugbøl, 2004; Taugbøl & Peay, 2004; Jussila *et al.*, 2008; Erkamo *et al.*, 2010; Kozák *et al.*, 2011). In the past, conservation programmes for a given species consisted of restoring the habitat and translocating individuals without the knowledge of their taxonomic status, but today, conservation genetics aims to maintain the genetic specificity of populations (genetic integrity principle) and the genetic diversity within and between populations (biodiversity principle), these basic principles being considered both at the level of protection measures and management measures (Souty-Grosset et al., 2003). The guidelines for the protection of threatened species recommend the identification of evolutionary significant units (ESU, Ryder, 1986). In restocking programs, it is recommended to use donor populations from the same ESU whenever possible, to conserve the local specificity and maintain the maximum within-species diversity (Schrimf, 2013). Therefore we started genetic studies and analysis. According to them we will be able to localize ECU.

Sustainable use is deemed to be a valuable tool to promote conservation of biological diversity, since in many instances it provides incentives for conservation and restoration because of the social, cultural and economic benefits that people derive from that use (Gherardi, 2011).

CONCLUSION

Aquatic environments are strongly affected by changes in the terrestrial environment, whether from natural or anthropogenic causes. The noble crayfish is the largest macroinvertebrate in Serbian aquatic ecosystems. It plays an important role in the food chain and has many effects on the community of aquatic organisms.

It is necessary to inform local population about crayfish (especially on the noble crayfish), to point out their importance for the proper functioning of freshwater communities, and the need of their conservation, in order to preserve the overall biodiversity of freshwater ecosystems, but also due to the possibility of using this species in aquaculture and the possible economic benefits.

An important goal is to strengthen research resources and establishment of collaboration with colleagues and institutions in Europe and the world, through joint research and practice.

Encouraging relevant institutions to introduce stimulating measures and promote freshwater crayfish farming (astaciculture) also required.

It is our responsibility to conserve this species for future generation.

ACKNOWLEDGEMENTS

This investigation was supported by the Ministry of Science and Technological Development of the Republic of Serbia as part of project number 31011.

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HOW FLOODING MODIFIES GENOTOXIC RESPONSE IN FRESHWATER FISH?

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KAKO POPLAVE MODIFIKUJU GENOTOKSIČNI ODGOVOR SLATKOVODNIH RIBA?

Apstrakt

U ovom radu ispitivan je potencijal dve vrste roda *Abramis* (*Abramis bjoerkna* i A*bramis sapa*) kao bioindikatora genotoksičnog potencijala vode reke Save primenom komet testa. Izabrani lokalitet pripada oblasti intezivne poljoprivredne aktivnosti, izložen je otpadnim vodama grada Obrenovca i nalazi se u blizini najveće termoelektrane u Srbiji, “Nikola Tesla”. Uzorkovanje je vršeno od januara do avgusta 2014. godine, obuhvatajući mesec maj kada je došlo do intezivnih poplava u slivu reke Save. Dobijeni rezultati ukazuju da se ispitivane vrste mogu koristiti za procenu genotoksičnog potencijala.

Ključne reči: poplave, reka Sava, genotoksikologija, komet test, Abramis sp.
Key words: flooding, Sava River, genotoxicology, Comet assay, Abramis sp.

INTRODUCTION

Chemical compounds from various sources significantly contribute to the contamination of both, the water column and sediments in rivers (Vargas et al., 2001). Many of these substances exhibit genotoxic potential causing formation of lesions in DNA molecule (Theodorakis, 2001). Due to their possible toxicity, genotoxicity and bioaccumulation potential, heavy metals are receiving significant attention (Višnjić-Jeftić et al., 2010; Sunjog et al., 2012). Chronic exposure of fish to sublethal trace metal levels causes among others disturbed ion regulation, reduced swimming speed and reduced growth (Bervoets and Blust, 2003). Species of the genus *Abramis (A. bjoerkna* and *A. sapa)* are widely distributed in many European and Asian freshwater ecosystems (fishbase.org). These cyprinid fishes are closely associated with superficial sediment due to their benthic way of life (Breukelaar et al., 1994). Flood events account for 40% of all natural disasters which are affecting many countries worldwide. Since the sediment plays an important role as a reservoir of many pollutants (Yang et al., 2008) it is considered that remobilization of sediments under the impact of flooding introduce pollutants into the water column (Ockenfeld et al., 2005) increasing their availability to aquatic organisms. Knowing that various pollutants may exhibit genotoxic potential, Comet assay or single cell gel electrophoresis (SCGE), a rapid and sensitive method for measuring the level of DNA damage in individual cells, has found a wide application in genotoxicity studies (Tice et al., 2000). The aim of this study was to investigate application of comet assay for the assessment of DNA damage in blood cells of two *Abramis* species (*A. bjoerkna* and *A. sapa*) and their potential use as bioindicator organisms. Considering that the flooding occurred during our sampling period (from January to August 2014) a parallel examination of the flooding potential has been made to modify effects of pollutants already present in the environment on the freshwater fish.

MATERIAL AND METHODS

Sampling was performed on a monthly basis, from January to August 2014, with exception of April, due to unfavorable environmental conditions, on the site Duboko, approximately 23 km upstream of the Belgrade city center. This site was chosen due to the exposure to various pollutant pressures, intensive agricultural activity and untreated waste waters from the town of Obrenovac. Moreover, the largest Thermal Power plant in Serbia „Nikola Tesla“ and associated ash fields are situated a few kilometers upstream from the sampling site. Physical and chemical parameters were measured on site. Present levels of metals micro constituents (Zn, Cu, Cr, Cd, Ni, As), total and dissolved, were obtained from the Agency for Environmental Protection (Serbia). For the analyses of microbiological water quality indicators of fecal pollution were monitored. Total coliforms (TC) and *E. coli* (EC) were determined by Most Probable Number (MPN) using Colilert 18 (IDEXX, Ludwigsburg, Germany). *Enteroocci* (EF) were determined by the MPN using MUD/SF microtiter plates (BIORAD, Vienna, Austria) according to ISO 7899-1:1998. For the isolation of presumptive *Clostridium perfringens* (CP) membrane filtration method and incubation on the tryptose-sulfite-cycloserine (TSC) media were applied, according to ISO 14189:2013. The study was carried out on the blood samples from the genus *Abramis* species *(A. bjoerkna*/*A. sapa*), total of 32 specimens ranging in length from 15-22.5 cm, and in weight 31-139 g. To obtain the baseline level of DNA damage we have implemented acclimation strategy, successfully applied in our previous studies (Kolarević et al., 2013; Vuković-Gačić et al., 2014). Cells viability was assessed with differential Acridin orange/Ethidium bromide staining. Under fluorescence viable cells appear uniformly stained in green, whereas cells with orange/red nuclei are considered dead (Squier and Cohen, 2001). The alkaline comet assay was performed according to the method of Singh et al. (1988) with slight modifications (Aborgiba et al., 2015). For statistical analysis data were analyzed using Statistica 6.0 Software (StatSoft, Inc.). Kolmogorov-Smirnov test for normality of distribution was used for the OTM values. Non-parametric Man-Whitney U test was applied with significance level $p < 0.05$ for differences between samples and corresponding negative control (acclimation). Correlations between the level of DNA damage and concentrations of heavy metals in water were investigated using Spearman correlation test ($p < 0.05$).

RESULTS

The highest water level was recorded in May, when flooding occurred in the Sava River Basin (Fig. 1-a). As soon as the flooding occurred, the majority of inhabitants from the town of Obrenovac were evacuated. During the most critical months of flooding (May and June) the highest concentrations of heavy metals in water (Mn, Cd, Pb, Co, As and B) were recorded. High positive correlation was shown between the water level and concentrations of Ni ($r = 0.81$), Cd ($r = 0.71$), Co ($r = 0.65$), Mn ($r = 0.63$) and Pb ($r = 0.61$). In the month with the highest water level (May) the highest concentrations of TC were observed, while during this and following month there has been a drastic decline in the concentrations of fecal pollution indicator bacteria - EC, EF and CP (Fig. 1-c). Additionally, significant negative correlation was observed between the water level and EC concentrations ($r = -0.86$). Viability of blood cells ranged from 87-100%. Compared to the baseline level of DNA damage (OTM = 2.2 ± 0.2), obtained after 10 days of acclimation, the highest level of DNA damage was recorded in June, while March was the month with the lowest level of DNA damage (Fig. 1-b). Significant positive correlation was observed between the DNA damage level and concentration of dissolved arsenic ($r = 0.76$) and high for total arsenic concentrations ($r = 0.71$). DNA damage level was in negative correlation with Zn levels ($r = -0.79$), with the level of $NH4^{+}$ ($r = -0.77$), EF ($r = -0.79$) and CP ($r = -0.79$).

DISCUSSION

In this study we have assessed the level of DNA damage in blood cells of two *Abramis* species (*A. bjoerkna* and *A. sapa*) and their potential use in genotoxicity monitoring of the Sava River. Extremely unfavorable hydrological conditions led to the occurrence of flooding in May 2014. This caused the evacuation of the entire town of Obrenovac thus excluding the impact of domestic waste water on the studied site. Due to this sudden decrease in concentrations of main fecal indicator bacteria was observed. This is evidenced with the observed negative correlation between fecal indicator bacteria concentrations and the water level.

On the other hand the impact of the Thermal Power Plant “Nikola Tesla“ and the ash fields increased. It is well established that flooding leads to sediment movement and perturbation, causing the release of many hazardous substances already present in the environment, such are heavy metals and metalloids (Wölz et al., 2009). When the flood wave reached its peak, during May and June, the highest concentrations of Cd, Co, Mn, Pb, As and B were recorded. The study of Kostić et al. (2012) is dealing with the presence of majority of these elements in the fly ash disposal field of power plant “Nikola Tesla”. Thus, it could be assumed that they were displaced when flooding occurred which elevated their concentrations in water. This is supported with the observed positive correlation between concentrations of Ni, Cd, Co, Mn, Pb and water level. The level of DNA damage in blood cells significantly correlated with dissolved arsenic in water, and highly correlated with total arsenic level. The study of Ramírez and García (2005) reported that arsenic induced increase in micronucleus frequencies in gill cells of zebra fish (*Danio rerio*). Significant negative correlation between DNA damage level and zinc levels in water is not surprising, since according to Galaris and Evangelou (2002) zinc appears to prevent and reduce oxidative stress. Negative correlation between the concentrations of fecal indicator bacteria EF, CP and $NH4^+$ concentration in water points out that observed genotoxic effects in fish should not be linked to wastewaters of the town Obrenovac.

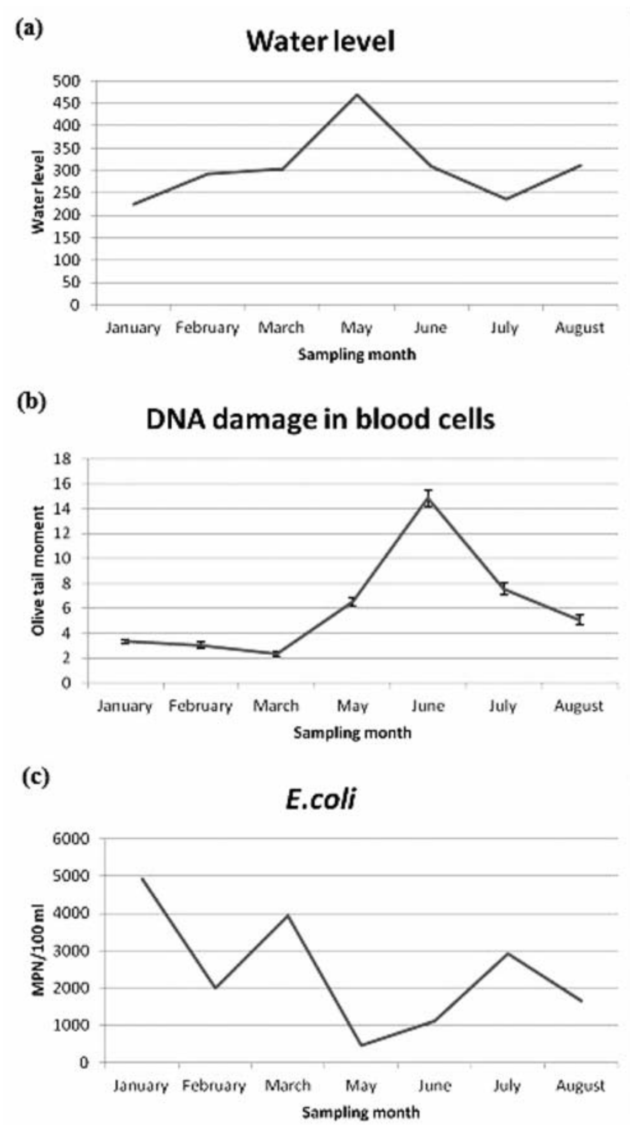

Figure 1. Comparative view in variation pattern of water level (a), DNA damage in blood cells (b) and *E.coli* concentration (c), during sampling period.

CONCLUSIONS

Obtained results show that examined species of the genus *Abramis* may be useful organisms for the assessment of genotoxic potential by the comet assay. Additionally, we showed the potential of flooding to modify water quality by remobilisation of pollutants already present in the environment which is reflected on the DNA damage in studied organisms.

ACKNOWLEDGEMENTS

This study is funded by the Ministry of Education, Science and Technological Development of the Republic of Serbia, Project No. OI173045. The authors would like to acknowledge the Serbian Environmental Protection Agency for provided data.

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DISTRIBUTION OF PHYTOPLANKTON AT FISH POND PREVLAKA (BARDAČA)

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DISTRIBUCIJA FITOPLANKTONA U RIBNJAČKOM JEZERU PREVLAKA (BARDAČA)

Apstrakt

Područje Bardače je smješteno na krajnjem sjeveroistoku Lijevča polja, oko 50 km sjeverno od Banjaluke. Od 2007. godine je uvršteno u svjetsku listu zaštićenih močvarnih područja (tzv. „Ramsarsko područje“). Područje ribnjaka se sastoji od 11 bazena od kojih je bazen Prevlaka sa površinom od oko 160 hektara drugi po veličini. Šaran čini 95% ukupnog nasada ribe, dok su sa po 2% zastupljeni sivi tolstolobik i amur, a 1% nasada čine som i smuđ. Ribnjaci generalno trpe znatno veći antropogeni uticaj nego bilo koji drugi tip kopnenih voda. U šaranskim ribnjacima se odvijaju posebno komplikovani fizičko-hemijski i biološki procesi budući da je neophodno osigurati optimalnu sredinu za život riba i pritom racionalno iskoristiti biološko kruženje materije u vodi. Biološka produktivnost ribnjaka je uslovljena prije svega prisustvom biogenih elemenata u vodi, dinamikom njihove potrošnje i dodavanja u obliku stajskih ili mineralnih đubriva. Najjeftiniji način proizvodnje ribljeg mesa je iz prirodne hrane čiji razvoj se pospješuje đubrenjem. Jedan od efekata đubrenja jeste i povećavanje razvoja primarnih producenata, tj. fitoplanktona i makrofita. Planktonske alge najčešće pripadaju razdjelima Euglenophyta, Pyrrophyta i Chrysophyta, dok se predstavnici Cyanobacteria, Chlorophyta i Bacillariophyta mogu podjednako naći i u planktonu i u bentosu. Svaka vrsta pritom ima svoje posebno kretanje u toku godine, poseban životni ciklus, tempo razmnožavanja i specifične reakcije na spoljašnje uticaje. Unošenje nutrijenata u ribnjake dovodi do narušavanja homeostaze ekosistema. Prirodna sukcesija biocenoza u ribnjacima se narušava krajem uzgojne sezone kada se vrši ispuštanje vode. Tokom 2011. godine je praćen kvantitativni i kvalitativni sastav fitoplanktona u vodi bazena Prevlaka. Analize su pokazale da se bazen Prevlaka odlikuje znatnim diverzitetom ove grupe organizama, ali i visokom organskom produkcijom. Tokom 2011. godine u vodi je konstatovano prisustvo 85 taksona iz 7 razdjela fitoplanktona, uključujući i cijanobakterije. Kvalitativno je bilo najviše predstavnika razdjela Chlorophyta koje su bile zastupljene sa 31 taksonom. Sledeće po zastupljenosti su bile Bacillariophyta-e, Euglenophyta-e i Cyanobacteria-e. Krečenje vode, koje se provodi od aprila do jula, dovelo je do pomjeranja ili potpunog izostanka proljetnog maksimuma razvoja fitoplanktona. Znatno višu brojnost fitoplankton je dostigao u drugom, tzv. ljetno/jesenjem cvjetanju algi. Zbog konstantno visokog sadržaja organske materije u vodi izostala je faza čiste vode. I pored provedenih agrotehničkih mjera u vodi su bile brojne nepoželjne vrste cijanobakterija, dok su poželjne zelene alge u ljetnom periodu bile zastupljene u znatno manjem broju. U cilju poboljšanja ribnjačke proizvodnje trebalo bi vršiti kontinuiran monitoring fitoplanktona i u skladu sa njegovim razvojem provoditi odgovarajuće agrotehničke mjere.

Ključne riječi: Bardača, fitoplankton, ribnjak
Keywords: Bardaca, phytoplankton, fish pond

INTRODUCTION

Area of Bardača is located in the northeast part of the field Lijevče polje (Republic of Srpska, BiH), about 50 km north of Banja Luka. Since 2007 Bardača has been in the world‘s list of protected wetland areas (known as „Ramsar Site”). It is a fish farm consisting of 11 ponds and among them pond Prevlaka, with a surface area of about 160 hectares, as the second-largest (Gašić i Dujaković, 2009). Carp comprise up to 95% of the total of fish composition, while 2% consists of bighead carp and grass carp, and 1% of catfish and perch. Fish ponds are generally exposed to significantly higher anthropogenic impacts than any other type of inland waters (Bojčić, 1982). In carp fishponds, particularly complex physical, chemical and biological processes occur since it is necessary to provide the optimal environmental conditions and rational usage of cycling of matter in the water. Biological productivity of ponds is primarily conditioned by the presence of biogenic elements in water, whose concentrations depend on their introduction from manure or mineral fertilizers, and on their consumption by primary producers. The cheapest way of producing fish meat is from natural food whose growth can be increased by fertilization. One of the effects of fertilization is increased growth of primary producers, i. e. phytoplankton and macrophytes. The change of qualitative and quantitative composition of phytoplankton during the year is called seasonal variation of phytoplankton. Planktonic algae usually belong to phylums: *Euglenophyta*, *Pyrrophyta* and *Chrysophyta*, while representatives from phylums *Cyanobacteria*, *Chlorophyta* and *Bacillariophyta* can be found in plankton and benthos (Kvet *et al.,* 2002). Most planktonic algae are called holoplanktonic species. During the largest part of the annual cycle they are present in the water column. On the other hand, meroplanktonic algae are present in the water column only in a certain part of the year, while during the largest part of the annual cycle they are dormant in the sediment (Sigee, 2004). Each species has its particular dynamics during the year, a specific life cycle, reproduction processes and specific reactions to external influences. External introduction of nutrients in the pond leads to the disruption of the ecosystem’s homeostasis. Natural succession of biocenoses in ponds is interrupted at the end of the growing season when water is drained out of the fish ponds due to the fish harvest.

MATERIAL AND METHODS

Samples for analysis of phytoplankton of pond Prevlaka were collected once a month from January to October 2011. In November and December the pool was drained due to fish harvest. Samples were collected from a boat, at least 10 meters from the lakeshore. Depending on the time of year, 5 to 20 liters of water was filtered through the plankton net with pore diameter of 20 microns. Filtered samples were then fixed with acidic Lugol‘s iodine solution. Identification of algae was performed using the following keys: Blaženčić and Cvijan (1996), Hindak (1978, 2005 and 2008), John et al (2005), Krammer & Lange-Bertalot, (1988a and 1988b), Lazar (1960) and www.algaebase.org. For analysis was used Leica DM 1000 microscope with the corresponding camera Leica DSF245.

RESULTS AND DISCUSSION

Presence of 85 different taxa of phytoplankton within 7 phylums, including cyanobacteria, was identified in the pond Prevlaka during 2011. Qualitatively, the largest number of taxa was from phylum *Chlorophyta*, which were represented by 20 genera and 31 species. The next most numerous phylums were *Bacillariophyta* with 23 taxa, *Euglenophyta* with 15 and *Cyanobacteria* with 13 taxa. Phylums *Chrysophyta, Pyrrophyta* and *Xanthophyta* were present with one species. In the sample from March only 19 taxa were identified, while in the sample collected in September highest number of algae, both, in qualitative and quantitative terms were found. There were 15.62 x 10^6 individuals per liter within 42 different taxa. In January and February 2011 siliceous algae were dominant. Quantitatively, the most numerous representatives in the sample from January were from genera *Melosira* and *Synedra*, and in February were algae of the genus *Navicula*. After extremely cold weather in February, there has been an intensive growth of golden algae *Dinobryon divergens* which accounted for 50.6% of total phytoplankton in sample from March (Chart 1). With the further increase of water temperature and increase of the duration of days, green algae developed in large numbers. Among them, genus *Pediastrum* was the dominant representative. These algae were prevailing until June, when the most abundant become cyanobacteria *Merismopedia tenuissima*.

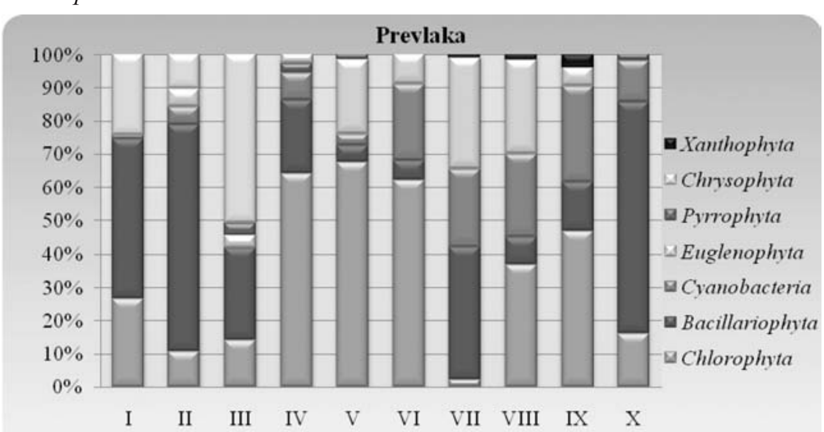

Chart 1. Quantitative distribution of phytoplankton in fish pond Prevlaka

The other species of cyanobacteria developed in great numbers in July, especially representatives of the genus *Microcystis*, as well as *Euglenophyta* from genus *Phacus*. *Euglenophyta* were qualitatively dominant, while in the entire sample only one green algae *Selenastrum bibraianum*, was identified. The absence of the green algae from genera *Pediastrum* and *Scenedesmus* was unusual. This may be caused by the introduction of some of agrotehnical measures and due to predation by zooplankton which was highly abundant in this period. The most abundant algae in August were *Microcystis aeruginosa* and *Pandorina morum*. In the sample from September there were large numbers of *Microcystis* and *Oscillatoria*, and different species from genus *Pediastrum* that developed again. In the sample from October, there were still large numbers of cyanobacteria of the genera *Microcystis* and *Oscillatoria*. Beside them, with the disturbance of mud due to water drainage, there were large numbers of diatoms, especially of species *Nitzschia acicularis*.

During the summer in the fish pond Prevlaka cyanobacteria *Cylindrospermopsis raciborskii* was present. This algae has never been isolated before at the area of Bardača. It is invasive species that produces toxins cilindrospermopsin and saxitoxin. Saxitoxin belongs to neurotoxins and it is dangerous for humans, while cilindrospermopsin mainly causes skin irritation, but can also seriously damage the liver (Simeunović, 2009). Stević found this species in the Danube in the area of Kopački rit in 2011. It is possible that birds brought this alga at the area of Bardača.

Bojčić et al (1982) reported that in fishponds during the summer period green algae and cyanobacteria generally dominate. However, at Bardača, the presence of diatoms cannot be ignored, since they were present in the water column in substantial numbers throughout the year. Also, Sigee (2004) reported that the genus *Melosira* is present in the plankton during colder periods of the year, while in summer it moves to the bottom, while the genus *Microcystis* goes opposite, i.e. in summer it is present in the plankton, and in winter it sinks to the bottom. Analysis of phytoplankton in 2011 showed that *Melosira* was present in significant numbers in all samples throughout the year, while *Microcystis* could be found in low numbers in samples collected in January. In water of Kopački Rit dominant species during the winter was *Chrysophyta Synura uvella* (Stević, 2011). At the fish pond Prevlaka this alga was not very abundant. In summer in Kopački Rit cyanobacteria dominated, and in autumn the dominant was *Pyrrophyta* from genus *Peridinium*. In the fish pond Zobnatica (Serbia) *Pyrrophyta Peridiniopsis cunningtonii* also dominated in autumn (Matavulj i sar., 2007). This algae was present in pond Prevlaka throughout the year, but has never prevailed in the total phytoplankton. In the fish pond Zobnatica very abundant were green algae from genera *Pediastrum* and *Scenedesmus, Euglenophyta* from genera *Euglena* and *Phacus*, and cyanobacteria from genera *Anabaena* and *Microcystis* were, like at Bardača. Dominant siliceous algae at Zobnatica were: *Amphora*, *Cymbella, Fragilaria*, *Navicula* and *Cyclotella*, while in the fish pond Prevlaka dominating genera were: *Melosira*, *Cyclotella, Navicula, Nitzschia, Synedra* and *Gyrosigma*. Genera *Amphora*, *Cymbella* and *Fragilaria* were present only sporadically or in low numbers.

CONCLUSIONS

According to the analysis of qualitative and quantitative distribution of phytoplankton it can be concluded that the fish pond Prevlaka is characterized by considerable diversity of phytoplankton, but also with high organic production. During 2011 presence of 85 different taxa of phytoplankton within 7 phylums, including cyanobacteria, was determined. Qualitatively, the highest number of taxa was from phylum *Chlorophyta*, which were represented by 31 taxa. The next most numerous phylums were *Bacillariophyta*, *Euglenophyta* and *Cyanobacteria*. Draining of fishponds for harvest and application of agrotechnical measures disturb normal seasonal dynamics of phytoplankton. Introduction of lime into the water, which is carried out between April and July, leads to a shift or complete absence of the spring phytoplankton maximum development. Significantly higher abundance of phytoplankton is reaches in the second, summer/autumn algal bloom. Due to the constant high level of organic matter in the water caused by fertilization, clear water phase was absent. Despite implemented agrotechnical measures numerous undesirable species of cyanobacteria were present, while the desirable green algae in the summer period were represented in lownumber. Agrotechnical measures significantly affected the phytoplankton at fish pond Prevlaka, providing differences in their distribution compared to fish ponds in the region. Continuous monitoring of phytoplankton should be carried out in order to improve the fish production, and in accordance with results appropriate agrotechnical measures should be implemented.

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VEGETABLE OILS IN NUTRITION OF CYPRINID FISH SPECIES

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ULJA BILJNOG POREKLA U ISHRANI CIPRINIDNIH VRSTA RIBA

Apstrakt

Meso ciprinidnih vrsta riba predstavlja značajan izvor proteina, masti, vitamina i minerala u ishrani ljudi. Masti ovih ribljih vrsta su bogate nezasićenim masnim kiselinama (USFA), kako mononezasićenom (MUFA), tako i polinezasićenim (PUFA) masnim kiselinama. Navedene grupe masnih kiselina imaju mnogobrojne povoljne efekte na održavanje fizioloških procesa u organizmu, pa samim tim i na očuvanje zdravlja ljudi. Hemijski sastav mesa ciprinidnih vrsta riba, kao i masnokisleinski sastav istog su pod uticajem brojnih faktora, među kojima je ishrana jedan od najznačajnijih. Glavni izvor masti u hrani za ribe je dugo bilo riblje ulje, ali je zbog drastičnog povećanja proizvodnje ribe iz akvakulture, njegova dalja upotreba u istoj meri postala neodrživa. U zavisnosti od vrste ribe, kao izvor masti se sve više koriste ulja biljnog porekla, kao delimična ili potpuna zamena za riblje ulje u kompletnoj hrani za ribe. Ovaj alternativni izvor masti može imati i negativnih efekata na ribe iz akvakulture, pre svega jer su ulja biljnog porekla siromašna ili su potpuno bez n-3 visoko nezasićenih masnih kiselina (n-3 HUFA), dok je riblje ulje poznato kao jako dobar izvor pomenutih masnih kiselina. Ovaj problem je slabije izražen kod ciprinidnih vrsta riba, koje su omnivori ili herbivori i prema rezultatima dosadašnjih istraživanja imaju veće potrebe za n-6 nego za n-3 masnim kiselinama za optimalan rast i održavanje bitnih fizioloških funkcija. Biljna ulja koje se najčešće koriste u ishrani riba su sojino, laneno, ulje uljane repice, suncokretovo, palmino i druga ulja. Upotrebljavaju se u komercijalnim smešama za ishranu ciprinidnih vrsta riba, bez negativnog uticaja na proizvodne parametre, ali mogu nepovoljno uticati na masnokiselinski sastav mesa ovako hranjenih riba. Međutim, najveći broj istraživanja o zameni ribljeg ulja uljima biljnog porekla je izveden na salmonidnim vrstama riba, pa su poželjna dalja ispitivanja o uticaju različitih vrsta ulja biljnog porekla na hemijski i masnokiselinski sastav mesa, kao i na zdravstveno stanje ciprinida. Meso cipirinidnih vrsta je uglavnom pogodnog hemijskog i masnokiselinskog sastava i predstavlja važnu komponentu u zdravoj ishrani ljudi. Sve navedeno ukazuje na neophodnost istraživanja o daljem unapređenju kvaliteta mesa ciprinidnih vrsta riba upotrebom kompletnih smeša, kako bi se zadovoljile nutritivne potrebe ovih vrsta, povećala proizvodnja po jedinici površine, dobila riba kao finalni proizvod što boljeg kvaliteta i postigla dugoročna ekonomska održivost i zadovoljile potrebe potrošača. Pored toga, potrebno je kontinuirano vršiti promociju ribe gajene na ribnjacima Srbije kao nacionalog zdravog proizvoda poželjnog u svakodnevnoj ishrani ljudi.

Ključne reči: ciprinidne vrste riba, biljna ulja, lipidi, sastav masnih kiselina, ishrana
Keywords: cyprinid fish species, vegetable oils, lipids, fatty acid composition, nutrition

INTRODUCTION

Alongside with intensifying the production of cyprinid fish species, increasing attention is being paid to industrially produced fish feed. Large part of our research was focused on the changes in the chemical composition of the cyprinid fish meat and fatty acid composition of their muscles, which were the result of feeding with oils and other feedstuffs of vegetable origin, which proved to be very good in the nutrition of freshwater fish species. In the following text the possibilities of the use of oils of vegetable origin (VO) in the nutrition of the common carp and other cyprinid fish species will be highlighted. Moreover, the advantages and disadvantages of the implementation of these sources of fat into the feed for cyprinid fish species will be discussed.

CURRENT TRENDS IN FISH OIL USE FOR AQUACULTURE

Traditionally, raw fish has been used in fish feed production as a source of proteins and fat, but it is estimated that the demand for this raw material will exceed the possibilities of production in the next decade (Tacon and Metian, 2008). Despite increase in total world consumption of fish oil (FO) in aquaculture, the average share of FO in feed for particular species steadily decline (Tacon and Metian, 2008). The great number of previous studies, which more than two decades is dealing with this problem, certainly contributed to the above mentioned fact, but there is still a need to reduce consumption of this ingredient in order to preserve natural resources and to ensure economically sustainability of fish production.

REQUIREMENTS OF CYPRINIDS FOR ESSENTIAL FATTY ACIDS

Relatively low requirements for n-3 fatty acids in cyprinid fish species are related to low requirements in fats, which was proved in the grass carp (Du et al., 2008), tench (Ljubojević et al., 2014) and in other cyprinid fish species (Ćirković et al., 2012; Ljubojević et al., 2013a,b,c). Today, the requirements for only two fatty acids are determined: linoleic acid (LA, C18:2, n-6) and alpha linoleic acid (ALA, C18:3 n-3), which should make 1% of fats in the carp feed (Takeuchi et al., 2002). The symptoms of essential fatty acid deficiency rarely develop in the common carp and include slower growth, high mortality and depigmentation of the skin (Takeuchi et al., 2002). Since the cyprinids need relatively little fatty acids of both the n-3 and n-6 series, it is supposed that they can be obtained from fatty acids of vegetable origin which contain 18 carbon atoms (Takeuchi et al., 2002).

CHALLENGES AND RESTRICTIONS CONCERNING THE USE OF VEGETABLE OILS

Every change in the fish feed which means the use of alternative components of vegetable origin must ensure normal growth. Thus, when choosing VO which is to partially or completely replace FO in fish feed, it is inevitable to consider its fatty acid composition. Thus, VO which is considered to be adequate source of fats in the feed should contain high levels of SFA and MUFA, which could be used as energy source, and low levels of LA because it is poorly oxidised and is difficult to be removed from the tissues with the use of the finisher. Moreover, the use of VO is challenging because the fact that they do not contain n-3 HUFA which are present in considerable amounts in FO. The content of n-3 HUFA in FO is about 20-30% of total fats, whilst VO contain only moderate levels of C18 PUFA, 18:3n-3. Besides, FO contain low percentage of n-6 PUFA, whilst VO are rich in C18 PUFA (Ljubojević et al., 2015).

INFLUENCE OF VEGETABLE OILS IN CYPRINIDS FISH FEED ON GROWTH PARAMETERS

Several studies have shown that total replacement of fish oil with vegetable oils in diets of cyprinid fish species, has no negative effects on fish growth and growth parameters (Turchini et al., 2007; Du et al., 2008; Zakęś et al., 2010; Ljubojević et al., 2014; 2015). Experiments conducted to date indicate that, when nutritional needs for essential fatty acids for omnivore species such as common carp, but also herbivores, such as grass carp are fulfill, the use vegetable oils in feed for these species does not affect growth performance or the feed efficacy. In the majority of research the type of oil used as the source of fats did not influence the feed intake, which means that the lipid fraction of the feed has subtle influence on its palatability.

INFLUENCE OF VEGETABLE OILS IN CYPRINIDS FISH FEED ON PROXIMATE AND FATTY ACID COMPOSITION

According to results obtained by Turchini et al. (2007) and Ljubojević et al. (2014; 2015) total replacement of FO with VO in diets of cyprinid fish species, has no negative effects on fish proximate composition. Higher contents of LA and ALA in the fillets of cyprinid fish species fed feeds with VO were noted by Turchini et al. (2007). Ljubojević et al. (2014) reported that the intermediate gamma-linolenic (18:3n-6), eicosadienoic (20:2n-6), dihomo-γ-linolenic (20:3n-6) and eiocosatrienoic (20:3n-3) acids were detected in tench fillets in all groups. Since VO are deprived of these fatty acids which are part of the biosynthetic pathways of n-6 and n-3 HUFA, this result features adaptive attempts to moderate HUFA deficiencies. A similar phenomenon was observed in common carp (Ljubojević et al., 2015).

The implementation of VO in the fish feed results in decrease of EPA, DHA and in the n-3/n-6 ratio, in comparison to the feed which contains FO, and has direct consequences on the nutritive quality of the final product. This is important from the consumers’ viewpoint since the high contents of EPA, DHA and the n-3/n-6 ratio are connected with numerous favourable effects on human health and their decrease is considered to be unwanted. This implies that vegetable oils can be successfully used in cyprinid fish species as a source of fat in their feed. Given the results, the high price of FO and its tendency to oxidate and with consequential storage difficulties, it can be concluded that all of the assessed vegetable oils showed satisfactory effects and that the use of each is justifiable. When deciding on their use in cyprinids feed the choice should be based on their cost and availability on the market.

CONCLUSION

VO can serve as a useful source of lipids for cyprinid fish species and represent a suitable source of energy and essential fatty acids. The production performance of VO fed cyprinids is very often unchanged. The usage of VO in cyprinid fish species nutrition is still limited due to possible disorders in fatty acid flesh composition. Using VO as partial or total replacement of animal origin fat sources can further improve productivity and nutritive value of cyprinid fish species. Generaly, cyprinid fish muscles have a favourable fatty acid composition and should be regarded as healthy products in human nutrition.

ACKNOWLEDGMENTS

This work was supported by grants from the Ministry of Education, Science, and Technological Development of the Republic of Serbia (project no. TR31011).

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INTENSIVE REARING PERFORMANCE OF THREE PIKEPERCH (*SANDER LUCIOPERCA)* FINGERLING POPULATIONS FROM HUNGARY

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PERFORMANSE TRI POPULACIJE MLAĐI SMUĐA (*SANDER LUCIOPERCA*) IZ MAĐARSKE U INTENZIVNOM UZGOJU

Apstrakt

Zahvaljujući blagom ukusu i niskom sadržaju masti uz poželjan masno-kiselinski sastav smuđ (*Sander lucioperca*) je cenjen među potrošačima i uz pad izlova i povećanje potražnje dobija sve veću pažnju među uzgajivačima. S obzirom da su osnovni izvori matica smuđa iz ekstenzivnog uzgoja ili divljeg porekla, informacija o podobnosti mlađi pomenutog porekla sa svrhom intenzivnog uzgoja je od izuzetnog značaja. S tom svrhom, naša namera bila je da ovom studijom ocenimo performanse dve grupe mlađi poreklom od matica iz ekstenzivnog uzgoja i jedne grupe mlađi poreklom od divljih matica iz najveće vodene mase u državi, reke Dunav, kao i da ocenimo stepen zadržavanja NMT Alpha vidljivih oznaka u obraz riba. Iako bez statistički značajne razlike, mlađ poreklom iz ekstenzivnog uzgoja ispoljila je brži rast. Ovakav rezultat nagoveštava obimnu selekciju među maticama iz jezerskog uzgoja, posebno uzimajući u obzir stres rukovanja i suboptimalne uslove kvaliteta vode što sezonski karakteriše jezerski uzgoj. Kakogod, treba nagovestiti da je u svakoj grupi zabeležena određena količina iznurenih individua. Konačno, primanje NMT Alpha vidljivih oznaka je bilo visoko u sve tri grupe i bez statistički značajnih razlika.

Ključne reč: smuđ, poreklo, matice, NMT vidljive oznake.

Keywords: pikeperch, origin, broodstock,NMT Alpha visible tags

INTRODUCTION

Pikeperch, *Sander lucioperca*, has a wide range of distribution (both natural and artificially stocked) and is present throughout Europe, in Northern Africa and territory of former Soviet Union (Schlumberger and Proteau, 1996; Fuller, 2011). Due to its mild taste and low fat content with favourable fatty acid composition (Kowalska et al., 2011) it is respected among consumers and with decline of capture and increased demand it gets more attention among farmers. Production of this species in Hungary is mainly based on the extensive pond production but still it has considerable share in the total European production (Dill and Teletchea, 2008). This type of production is characterized by relatively low and variable yield, therefore increased interest is pointed towards intensive rearing technologies. Taking into account that main sources of breeders are pond reared and wild fish, it is of significant importance to get the information which breeders would be more suitable for fingerling production with purpose of intensive rearing. Therefore, in this study the intent was to evaluate intensive rearing performance of two groups of fingerlings originating from pond reared breeders and one group originating from the largest water body in the country, river Danube.

MATERIALS AND METHODS

Three batches of pikeperch fingerlings prepared for intensive on-grow were obtained from two producers from Hungary. Each batch consisted of 200 individuals of average mass of 10 g. Two batches obtained from H&H Carpio Ltd, Ócsárd, Hungary, were originating from pond reared breeders (Attalai Halászati és Értékesítő Kft, Hungary and ÖKO2000 Kft., Hungary). One batch obtained from SZAT Akvárium Bt., Vonyarcvashegy, Hungary, was originating from wild Danube breeders. With this respect, three populations were named Akaszto, Attala and Duna.

Upon arrival to the Research Institute for Fisheries and Aquaculture NARIC HAKI, Szarvas, Hungary, fish were stocked in the separated tanks with volume of 275 L in the recycling system equipped with trickling bio-filter and sedimentation tank where the two month quarantine was carried out. After the quarantine period, all fish were tagged in the right cheek with NMT Alpha visible tags (NMT INC Northwest Marine Technology, WA, USA) and individual weight was recorded. One week after fish were transported to the experimental recirculation system consisted of five 275 L tanks, mechanical screen filter, moving bead bioreactor and UV disinfection. After 10 days of acclimatization, 60 fish with similar masses from each group were distributed in three tanks in equal proportion 20:20:20 = Akaszto: Attala : Duna. Therefore, each tank was stocked with 60 individuals making a triplication of each origin.

At the beginning of the trial individual total length (TL ± 0.1 cm) and body weight (BW ± 0.05 g) was recorded. A week after, all the fish were retagged in left cheek and the individual BW was recorded. This was done in order to prevent the loss of the information. Further on, individual BW measurement was done on a two weeks period. At the end of the trial both BW and TL were recorded for each fish. Prior to each manipulation fish were anaesthetized in the clove oil solution (0.1 mL L^{-1}, 1:1, clove oil: 70% ethanol).

Assessed parameters: BW_i – initial body weight (g); CV BW_i – coefficient of variation for BW_i (%); TL_i - initial total length (cm); CV TL_i - coefficient of variation for TL_i (%); B_i

– initial stocked biomass (g); BW_f– final body weight (g); CV BW_f - coefficient of variation for BW_f (%); TL_f – final total length (cm); CV TL_f– coefficient of variation for TL_f (cm); B_f– final biomass (g); FBG – fish biomass gain per cubic meter of rearing volume (kg m^{-3}) calculated as $(B_f - B_i)\ V^{-1}$ (V- volume of rearing tank); DGR BW – daily growth rate for BW (g day^{-1}) calculated as $(BW_i - BW_f)\ \Delta T^{-1}$; DGR TL - daily growth rate for TL (mm day^{-1}) calculated as $(TL_i - TL_f)\ \Delta T^{-1}$; SGR – specific growth rate (% day^{-1}) calculated as 100 $(LnW_f - LnW_i)\ \Delta T^{-1}$; ΔT – rearing period in days; Starved fish – percentage of fish with negative SGR for minimum three consecutive weeks (%); Survival – survival during the trial (%); Tag retention – percent of retained tags (%).

During the trial fish were fed with SteCo PRE GROWER-14 2.0 mm (protein 50%, fat 14%, crude fibre 0.8%, ash 8.6%, total phosphorus 1.4%, Coppens International, The Netherlands) in first 10 weeks and SteCo SUPREME-10 3.0 mm (protein 49%, fat 10%, crude fibre 0.8%, ash 8.3%, total phosphorus 1.3%, Coppens International, The Netherlands) further on. Feed was distributed with mechanical FIAP belt feeder (4305 FIAP belt feeder; Aquacultur Fishtechnik, Germany) two feedings per day in excess. Each feeding lasted 6 hours. This has been done in order to maximize feed availability and eliminate the feed as the limiting factor for growth. Due to excessive feeding, unconsumed feed was siphoned twice per day, while tank walls were cleaned once per day (at 8 AM). Salinity was maintained between 1.5 and 3 g L^{-1} during the trial measured on the water conductivity basis. This was done in order to prevent parasitic infection (Németh et al., 2013) and to enhance the recovery of acute stress (Barton and Zitzow, 1995). Basic water quality parameters (nitrogen compounds and pH) were assessed twice per week. The ammonium nitrogen $N\text{-}NH_4^+$, nitrite nitrogen $N\text{-}NO_2$, and nitrate nitrogen $N\text{-}NO_3$ were kept under 0.3, 0.45 and 33 mg L^{-1}, respectively. Oxygen content, temperature and conductivity were measured at the outflow 5 times per week after the first feeding. Oxygen was kept above 67% satiation. Temperature and pH were kept on 21.6 ± 0.9 °C and 8.0 ± 0.1, respectively (mean ± standard deviation (SD)), while the conductivity ranged from 4.7 to 7.1 mS cm^{-1}.

Statistical analysis was based on one-way Analysis of Variance (ANOVA). All the percentage data were arcsine transformed prior to statistical analysis. Significant differences between treatments were estimated using a post-hoc Duncan's Multiple Range Test with a significance level at $P \le 0.05$. Analyses were performed using SPSS 22.0 software (IBM, New York, NY, USA).

RESULTS AND DISCUSSION

Growth parameters are presented in Table 1. Higher growth was noticed in stocks originating from pond reared breeders but without statistically significant differences. Recorded SGR was below the previously reported growth for pikeperch of this size (Zakes et al., 2006; Schram et al., 2014). This can be explained by excessive handling due to tagging and recording of individual weight during the trial. Based on the growth curve (Figure 1), we can observe higher growth trend among the fish originating from pond reared breeders. This fact, together with higher achieved SGR might be the outcome of extensive selection process among the pond reared fish, especially with regard to handling stress and suboptimal water quality parameters which are seasonally characterizing pond on-grow.

Table 1. Performance of the three pikeperch populations based on the assessed parameters

Parameter	Akaszto	Atala	Danube
BWi (g)	22.4±0.1^{a}	22.3±0.2$^{a.b}$	22.7±0.2^{b}
CV BWi (%)	12.5±0.0^{a}	12.3±0.0^{a}	10.6±0.0^{b}
TL_i (cm)	14.5±0.1	14.3±0.1	14.6±0.3
CV TL_i (%)	4.1±0.5	4.1±0.4	3.5±0.2
B_i (g)	447.6±1.5	446.6±3.8	454.0±4.7
BW_f (g)	56.8±8.6	53.8±10.4	47.1±5.6
CV BW_f (%)	39.5±10.3	36.2±5.5	37.1±2.6
TL_f (cm)	19.6±0.8	19.1±1.0	18.5±0.6
CV TL_f (%)	11.5±2.5	10.0±0.3	10.0±0.3
B_f (g)	1136.5±171.1	1076.4±207.4	957.3±118.5
FBG (kg m^{-3})	2.8±0.7	2.5±0.8	2.0±0.5
DGR BW (g day^{-1})	0.33±0.08	0.30±0.1	0.23±0.05
DGR TL (mm day^{-1})	4.8±0.7	4.6±0.9	3.7±0.3
SGR (% day^{-1})	0.88±0.15	0.83±0.18	0.69±0.11
Starved fish (%)	16.7±15.3	16.7±16.1	21.7±12.6
Survival (%)	100±0	100±0	100±0
Tag retention (%)	94.2±1.4	92.5±4.3	88.3±7.6

Values (mean±SD) in the same row with different superscript (a, b) are significantly different ($P \leq 0.05$).

There were no observed mortalities during the trial period. However, among each batch emaciation by the fingerlings was noticed. Negative SGR for at least six consecutive weeks starting at the various times during trial was seen. Similar phenomenon has been previously reported by Schram et al. (2014), where the fish emaciation was not found to be the function of the tested factors. In our trial, observed ratio of starved fish was 16.7 ± 15.3, 16.7 ± 16.1, 21.7 ± 12.6 % (mean ± SD) for Akaszto, Attala and Duna respectively, without significant differences between the groups. Nevertheless, it is worth mentioning that as well as for growth parameters, more favourable results were noticed among pond origin fish. This is in agreement with previous indication of an early domestication process among pond reared breeders.

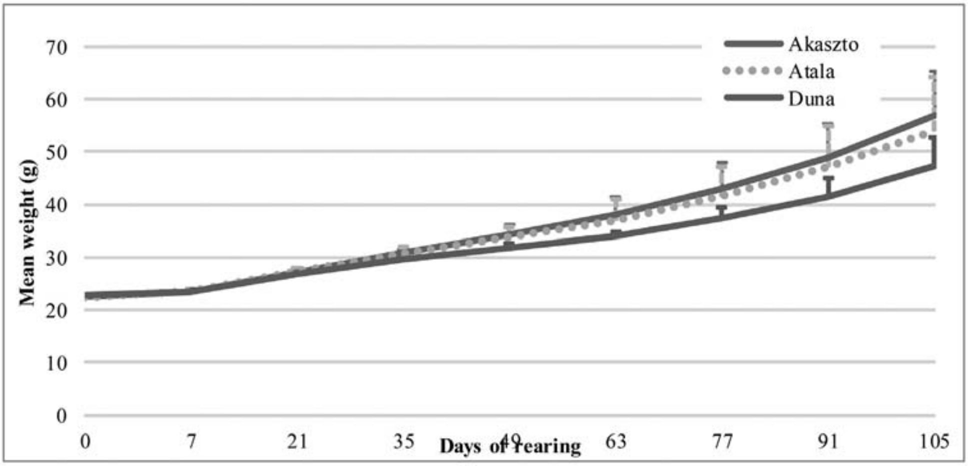

Figure 1. Mean weight + SD of fish throughout the 105 days of trail

Tagging the pikeperch fingerlings with NMT Alpha visible tags seems to yield rather favourable outcome in this size class of fish. Previous reports on tagging juvenile pikeperch considered higher size classes of fish (Hopko et al., 2010; Zakes and Hopko, 2013). These studies reported high rates of tag retention without significant impact of tagging on the growth of the fish. By our observations, tagging lower size classes with these visible implants presents rather fast and non-harmful procedure. Tag retention was 94.2 ± 1.4, 92.5 ± 4.3 and 88.3 ± 7.6 % (mean ± SD) for Akaszto, Attala and Duna respectively, without significant differences between the groups. Once more, we must point to the lower observed tag retention among the fish with wild origin which is indicating more aggressive and stressful behaviour of these fish.

CONCLUSIONS

Considering the small sample size used in this study, we may characterize this study as the preliminary. However, the indications of the more favourable results of intensive rearing with fish originating from pond reared breeders presents basis in which direction further studies could address. Including fingerlings originating from intensively reared breeders should generate significant conclusions. Considering the wide area of distribution of this species, more comprehensive program should yield the relevant answers in terms of suitability of traits of different populations for the circumstances of intensive on-grow. Finally, tagging the fish with NMT alpha implants may be suitable method for lower size classes in further similar studies.

ACKNOWLEDGMENTS

This study was financed by the Hungarian Ministry of Agriculture though project “Improvement of artificial propagation of economically important percid fish species (pikeperch, perch) with special regards to geographical distribution”. Special gratitude goes to fish farm H&H Carpio Ltd, Ócsárd, Hungary for their guidance through the quarantine treatments and provision of stocking material and feed. Finally, acknowledgements are addressed to EU FP-7 project AQUAREDPOT (reference number: 316266) for supporting the researchers involved.

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Cu AND Zu BIOACUMULATION IN CERTAIN ACQUATIC MACROPHITES IN THE AREA OF FISH POND BARDAČA

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BIOAKUMULACIJA Cu I Zu U NEKIM AKVATIČNIM MAKROFITAMA NA PODRUČJU RIBNJAKA BARDAČA

Apstrakt

Rad se bavi analizom sadržaja Cu i Zn u vodi, sedimentu i tkivu Phragmites communis Trin., Salvinia natans L. All. i Utricularia vulgaris L. na području ribnjaka Bardača (Necik i Sinjak-aktivni ribnjački bazeni). Na osnovu izmjerenih vrijednost Cu u vodi, a prema Uredbi o klasifikaciji voda i kategorizaciji vodotoka, istraživani lokaliteti pripadaju vodama II kategorije, a na osnovu sadržaja Zn vodama III/IV kategorije. Sadržaj bakra i cinka u sedimentu nije prelazio vrijednosti maksimalno dozvoljene koncentracije prema Službenom glasniku RS (23/1994). Dobijeni rezultati ukazuju da sadržaj Cu i Zn u biljnom tkivu znatno varira u odnosu na vrstu, lokalitet i period uzimanja uzoraka. U tkivu Salvinia natans tokom istraživanog perioda najniža koncentracija Zn izmjerena je tokom septembra na oba lokaliteta (32 mg/kg), dok je najveća koncentracija prelazila maksimalno dozvoljene vrijednosti (163,55 mg/kg) što ukazuje na izvjestan stepen zagađenosti istraživanog lokaliteta. Koncentracija Zn u tkivu Utricularia vulgaris na oba lokaliteta bila je u opsegu 39,14-55,20 mg/kg, pri čemu su dobijene vrijednosti za 35 % bile niže u odnosu na Salvinia natans na lokalitetu Necik, dok su na lokalitetu Sinjak koncentracije Zn kod obje vrste bile slične (44,55 mg/kg). Najznačajnija razlika u akumulaciji Zn utvrđena je za Phragmites communis kod koje je dobijen 3-6 puta niži sadržaj Zn u odnosu na Salvinia natans i Utricularia vulgaris. Sadržaj Cu u tkivu Utricularia vulgaris tokom perioda istraživanja je bio u opsegu 1,7-10,18 mg/kg, dok su u tkivu Salvinia natans izmjerene neznatno niže vrijednosti u odnosu na Utriculara vulgaris. Koncentracija Cu u tkivu Phragmites communis tokom istraživanog perioda na oba lokaliteta bila je ispod detekcionog limita (<0,023 mg/kg). Kao najbolji biokumulator Zn pokazala se vrsta Salvinia natans dok je Utricularia vulgaris bolje akumulirala bakar, što ukazuje na postojanje različitog trenda u pogledu akumulacije određenih teških metala tokom sezone u različitim organima i tkivima. U ovom radu je

naglašena uloga različitih tipova vodenih makrofita u akumulaciji teških metala tokom sezone i njihov potencijal primjene u tehnici fitoremedijacije.

Ključne riječi: Cu, Zn, akvatične makrofite, bioakumulacija

Abstract

The paper deals with Cu and Zn content analysis in water, sediment and tissue of Phragmites communis Trin., Salvinia natans L. and Utricularia vulgaris L. in the area of Bardača fish pond (Necik and Sinjak- active fish ponds). Based on the measured value of Cu in water, and according to the Regulation on Classification of Water and Water Streams, the researched localities belong to category II waters, and based on the Zn content they belong to category III/ IV waters. The content of copper and zinc in sediments did not exceed the maximum permitted concentration values according to the RS Official Gazette (23/1994). The obtained results indicate that Cu and Zn content in plant tissue significantly varied in relation to species, locality and sampling period. In the tissue of Salvinia natans during the researched period, the lowest Zn concentration was measured in September on both localities (32 mg/kg), while the biggest concentration exceeded the maximum permitted values (163,55 mg/kg) which indicates a certain degree of contamination of researched localities. Zn concentration in the tissue of Utricularia vulgaris on both localities ranged from 39,14 to 55,20 mg/kg, whereby the obtained values were by 35 % lower in relation to Salvinia natans on Necik locality, while on Sinjak locality the Zn concentration at both species was similar (44,55 mg/kg). The most significant difference in Zn accumulation was determined for Phragmites communis where 3-6 times lower Zn content was obtained in relation to Salvinia natans and Utricularia vulgaris. The Cu content in tissue of Utricularia vulgaris during the research period was in the range from 1,7-10,18 mg/kg, while in the tissue of Salvinia natans insignificantly lower values were measured as compared to Utriculara vulgaris. The Cu concentration in the tissue of Phragmites communis during the researched period on both localities was below the detection limit (<0,023 mg/kg). Species Salvinia natans has shown to be the best Zn bioaccumulator, while Utricularia vulgaris accumulated copper the best, which indicates the existence of different trend in terms of accumulation of certain heavy metals during the season in different organs and tissues. The role of different types of aquatic macrophytes in heavy metal accumulation during the season was emphasized here and their potential of application in phytoremediation technique.

Keywords: Cu, Zn, aquatic macrophytes, bioaccumulation

TOWARDS IMPROVING GROWTH ESTIMATION IN FISH: MULTI-MODEL INFERENCE AND ITS APPLICATION IN FRESHWATER FISH

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U SUSRET POBOLJŠANJU PROCENE RASTA RIBE: MULTI-MODELNA ANALIZA I NJENA PRIMENA KOD SLATKOVODNIH RIBA

Apstrakt

Cilj ovog istraživanja je bio da se uporedi multi-modelna analiza (MMI) u modelovanju rasta riba sa tradicionalnom metodom odabira najbolje odgovarajućeg modela kod slatkovodne ribe *Carassius gibelio* (Bloch 1782). Korištena su četiri modela (Von Bertalanffy, Gompertz, Robertson i power funkcije) za modelovanje rasta jedinki vrste *Carassius gibelio* iz reke Stari Begej. Iako je Power funkcija bila najbolje odgovarajuća, u svim slučajevima je više od jednog modela bilo značajno podržano te je stoga primenjeno usrednjavanje modela kako bi se dobili srednji model i tempo rasta. Kada je više od jednog modela podržano, preporučeno je da se koristi MMI koja omogućuje preciznije određivanje parametara rasta.

Ključne reči: srebrni karaš, usrednjavanje modela, multi-modelna analiza

Keywords: Prussian carp, model averaging, multi-model inference

INTRODUCTION

Information regarding fish age and growth is of great importance for stock assessment, fisheries management and conservation strategies (Liu et al., 2009; Wells et al., 2013). Therefore, a mathematical expression for relating fish size to its age is needed (Katsanevakis, 2006; Katsanevakis and Maravelias, 2008). Most of the studies dealing with fish growth are based on fitting one growth model (most commonly the von Bertalanffy growth function, hereafter VBGF) or several *a priori* determined models (such as Gompertz, Robertson and others) to the length-at-age data. In this case, one model is selected as the best fitting based on the principle of parsimony according to Akaike's Information Criterion (AIC) (Katsenevakis, 2006). However, this method leaves a high degree of uncertainty, leads to bias in estimates and overestimation of precision. In order to compensate for these deficiencies, a new approach was recently proposed, one that will have a significant advantage over the traditional picking of the 'best fitting model'. By model averaging, multi model inference (MMI) provides a more stable inference, reduces model selection bias and acquires higher precision when compared to the selection of 'best fitting model' (Burnham and Anderson, 2001; Katsanevakis, 2006).

Our aim was to implement multi-model analysis in evaluating growth of Prussian carp *Carassius gibelio*, use model-averaging to derive average growth parameters and growth rates and compare this method to the traditional one.

MATERIAL AND METHODS

Sampling was conducted along the Stari Begej River (45°15'17.60"N, 20°23'54.06"E) during July to October 2007 and July to October 2008 with gill nets of various mesh sizes and standard electrofishing device. Every individual was measured for total and standard length (±1 mm) and weighted for body weight (±1 g). Sex was determined by macroscopic observation of the gonads. Scales from the left side, above the lateral line in front of the dorsal fin, of every individual were removed for age determination.

Four models were fit to length-at-age data for both sexes independently: the asymptotic (1) von Bertalanffy growth function (VBGF): $L_t = L_\infty(1 - exp^{-k(t-t_0)})$, (2) Gompertz growth function (GGF): $L_t = L_\infty exp^{-exp^{-k(t-t_0)}}$, and (3) Robertson growth function (RGF): $L_t = L_\infty / (1 + exp^{-k(t-t_0)})$, where L_t is the total length, L_∞ is the asymptotic length, k is the growth coefficient, t is the age and t_0 is the theoretical age when length equals zero (L_t = 0) in all three models; and the non-asymptotic (4) power function (PF): $L_t = a_0 + a_1 t^b$. Models were fit using least squares non-linear regression in STATISTICA v12 software (Statsoft Inc., Tulsa, OK, USA). The best fitting models were compared between sexes by using F-statistic (Chen et al., 1992).

Selection of the best fitting model among the candidate models was done by using the Akaike's Information Criterion corrected for small sample sizes (AIC_c) (Katsanevakis, 2006): $AIC_c = N \log \frac{RSS}{N} + 2k + \frac{2k(k+1)}{(N-k-1)}$ where N is the sample size, RSS is the residual sum of squares, k is the number of estimated parameters. The model with the smallest AIC_c ($AIC_{c,min}$) was considered to be the best fitting model among the candidate models. Furthermore, differences from the $AIC_{c,min}$ were calculated as $\Delta_i = AIC_{c,i} - AIC_{c,min}$ where i indexes the growth models. Models with $\Delta_i > 10$ were considered to have no support, models with $2 < \Delta_i < 10$ were considered to have little support, and models with $\Delta_i < 2$ were considered to have significant support. In addition, Akaike's weight (w_i) was used to assess the best fitting model and was calculated as: $w_i = \frac{exp^{(-0.5 \times \Delta i)}}{\sum_{i=1}^{8} exp^{(-0.5 \times \Delta i)}}$. The model with the highest Akaike's weight was considered to be the best fitting among the candidate models. In many cases more than one model was significantly supported ($\Delta_i < 2$) and Akaike's weight displayed no 'clear winner' ($w_i < 0.9$). Therefore, we included multi-model inference (MMI) by model-averaging in the study and calculated average model and average growth rate as described in Katsanevakis and Maravelias (2008).

RESULTS

Among the 515 individuals sampled, 240 individuals were females (W: 7-128 g; TL: 8.5-19 cm), 178 were males (W: 13-144 g; TL: 9.5-20.5 cm) and 97 were juveniles (W: 2-35 g; TL: 5.5-12.5 cm). Female: male sex ratio differed significantly from the expected value of 1:1 (χ^2=9.196, d.f.=1, p<0.01). ANOVA showed no significant differences in weight and total length between sexes ($p > 0.05$). Age of juvenile individuals ranged from 0^+ to 2^+, while age of females and males ranged from 1^+ to 4^+.

Considering that length-at-age data did not significantly differ between sexes, length-at-age data for the juvenile individuals was added to both sexes for growth modeling. Based on AIC_c, PF was the best fitting model in describing growth for both sexes (Table 1, Fig. 1A). However, it was not the only model significantly supported by the data since all chosen models had Δ_i values below 2 and Akaike's weights above 0.1. Since the F statistic displayed no significant difference between sexes in the best fitting growth model ($F = 0.14$, $p > 0.05$), data for both sexes were pooled and growth models were recalculated. PF was the best fitting model for the combined sexes, followed by VBGF and GGF which were also significantly supported, and RGF which had little support (Table 1).

Table 1. Estimated growth models for *Carassius gibelio* from the Begej River per sex using the asymptotic von Bertalanffy (VBGF), Gompertz (GGF) and Robertson (RGF) growth functions and the non-asymptotic power function (PF). Parameters estimated: Akaike's Information Criterion (AIC_c), differences from $AIC_{c,min}$ (Δ_i) and Akaike's weights (w_i) for each model. The best fitting models are bolded.

Sex	Model	AIC_c	Δ_i	w_i
Females	VBGF	111.44	0.45	0.28
	GGF	111.97	0.98	0.21
	RGF	112.48	1.50	0.16
	PF	**110.99**	**0.00**	**0.35**
Males	VBGF	81.13	0.73	0.27
	GGF	81.81	1.42	0.19
	RGF	82.48	2.08	0.14
	PF	**80.39**	**0.00**	**0.39**
Combined	VBGF	161.66	0.85	0.28
	GGF	162.53	1.73	0.18
	RGF	163.41	2.61	0.12
	PF	**160.80**	**0.00**	**0.43**

Since more than one model was significantly supported, multi-model inference by model averaging was used to develop an average growth rate (Fig. 1B).

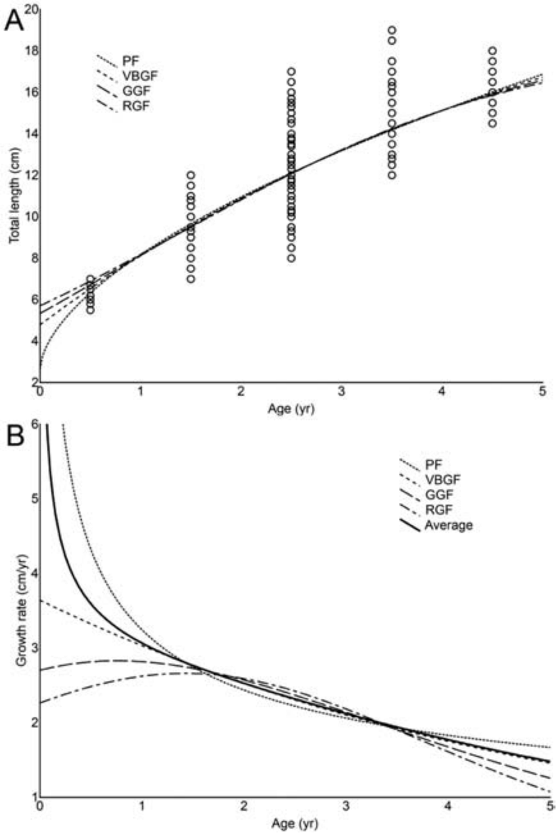

Figure 1 (A, B). Estimated growth functions (A) and growth rates (B) for *Carassius gibelio* sampled from the Stari Begej River. VBGF – Von Bertalanffy growth function; GGF – Gompertz growth function; RGF – Robertson growth function; PF – Power function. Lines indicate growth functions while circles indicate observed length-at-age data.

DISCUSSION

Growth assessment in fish is essential for stock assessment and management strategies (Liu et al., 2009; Wells et al., 2013). The most commonly applied growth model in fisheries science is the VBGF (Katsanevakis, 2006; Katsanevakis and Maravelias, 2008). However, several studies have reported that this growth model is not always the best fitting one. This was observed for *Thunnus albacores*, *Carcharhinus plumbeus* (Katsanevakis, 2006), *Bathyraja interrupta* (Ainsley et al., 2014), *Galeus sauteri* (Liu et al., 2011) and other. Therefore, the usage of different competing growth models in fitting length-at-age data can offer an advantage in providing a more realistic growth assessment. Furthermore, using methods as *AIC* which is indicative of the robustness of the fit can reduce error of growth fits and point out the ‘best fitting’ model.

In the present study we have successfully implemented multi-model analysis in growth assessment of Prussian carp. To the authors' knowledge, this is one of the first studies to implement multi-model analysis in growth estimation of this species, and in freshwater species in general. According to AIC_C, PF was the best fitting model in all cases. Since this model was not the only one significantly supported, picking only this model as the best fitting would leave a great deal of uncertainty and overestimate precision. Therefore, an average growth model which would account for the left uncertainty and would provide a more stable inference was developed. When looking at the average growth rate (Fig. 1B), the average model follows the pattern of the power model during the first year, but then changes and completely follows the pattern of the VBGF. When taking biometric data into account, young of the year individuals have length span from 5 cm to 7 cm, and after gain on average 2 – 3 cm per year afterwards. Therefore we believe that the average model did provide a more stable and precise inference than any of the chosen models.

CONCLUSION

As discussed above, model averaging has significant advantages over the picking of the 'best fitting' model. Since this method was most commonly applied in marine species, we demonstrate that model averaging should be used in freshwater species as well. Since many of these species are threatened or endangered because of overfishing and other anthropogenic activities, precise growth estimates may provide a more suitable and successful management strategies for endangered species, but may also provide a better insight into life-history characteristics of invasive species and enable creating better management strategies for them as well.

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ULTRASOUND DIAGNOSTIC OF STURGEONS AND EXPLOITATION OF REPRODUCTIVE FEMALES AT STURGEON HATCHERY OF AZERBAIJAN

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ULTRAZVUČNA DIJAGNOSTIKA JESETARSKIH VRSTA I KORIŠĆENJE REPRODUKTIVNIH ŽENKI NA MRESTILIŠTU ZA JESETARSKE VRSTE U AZERBEJDŽANU

Apstrakt

Formiranje matičnog stada jesetarskih vrsta za obnavljanje populacija započeto je još 2004. godine u Azerbejdžanu, gde je ostvaren uzgoj riba od stadijuma larvi do adultnih jedinki. Reproduktivne ženke perijske (Kura) jesetre (*Acipenser persicus* Borodin) koje su uzgajane “od ikre” u uzgojnim sistemima za ribe u Azerbejdžanu su 2013. godine po prvi put korišćene za veštački mrest u uslovima mrestilišta.

Cilj ovih studija je bila komparativna evaulacija kvaliteta reproduktivnih ženki koje su uzgajane u uslovima mrestilišta za jesetarske vrste riba i dobijanje njihovog potomstva. Istraživanja su takođe trebala da razviju naučno potkrepljenu dokumentaciju za eksploataciju matičnog stada jesetarskih vrsta, koja bi se bazirala na mrestilišima za jesetarske vrste u Azerbejdžanu.

Studija formiranja matičnog stada jesetarskih vrsta “od ikre” u uslovima Khylly ribnjaka za jesetarske vrste u Azerbejdžanu je bila eksperimentalne prirode. Rad na proizvodnji potomstva/mlađi od reproduktivno zrelih ženki je obavljen 2013. godine. Da bi se stimulisalo sazrevanje dve selektivno odabrane ženke persijske (Kura) jesetre iz generacije 2004. uzgajane u mrestilištu, data im je intramuskularno injekcija sa suspenzijom aceton-vodenog ekstrakta hipofize jesetri u odnosu 2.5 mg na 1 kg težine tela. Da bi se izbegao inbriding putem inseminacije ikre, uzeta su dva mužjaka persijske (Kura) jesetre iz generacije 2005. uzgojene u mrestilištu. Ultrazvučna dijagnostika pola i stepena polne zrelosti, hormonalna stimulacija matičnih riba, fertilizacija i sprečavanje adhezija ikre (odlepljivanje), kao i inkubacija u “Osetr” inkubacionom sistemu je sprovedena u saglasnosti sa postojećim metodama (Dettlaff et al., 1981; Chebanov and Galich, 2010; Mamedov et al., 2009).

Analiza uzgoja riba i bioloških indikatora juvenilnih riba koje su čuvane u mrestilištu je pokazala da je razvoj produkovane mlađi korišćenjem reproduktivno zrelih ženki iz matičnog stada bio u saglasnosti sa normama. Jedan deo proizvedene mlađi je zadržan u mrestilištu sa ciljem daljeg istraživanja njihovih morfoloških, bioloških i fizioloških karakteristika.

Time su u praksi uzgoja riba u Azerbejdžanu po prvi put korišćene za veštački mrest ženke persijske (Kura) jesetre iz matičnog stada koje je uzgojeno "od ikre" u mrestilištu. Ovi podaci ukazuju na mogućnost dostizanja reproduktivnog stanja različitih jesetarskih vrsta u uslovima mrestilišta. Stvaranje reproduktivnog matičnog stada jesetarskih vrsta u mrestilištima će omogućiti očuvanje genetskog pula jesetarskih vrsta i proširiti mogućnosti za njihov veštački mrest u cilju poriblajvanja otvorenih prirodnih vodenih ekosistema.

Ključne reči: *matično stado, ultrazvuk, reproduktivne ženke, persijska (Kura) jesetra, „od ikre", mrestilište za jesetarske vrste*
Keywords: *Broodstock, ultrasound, reproductive females, Persian (Kura) sturgeon, „from eggs", sturgeon hatchery.*

INTRODUCTION

Formation of recovery-maternal stock of sturgeon fish «from roe» assumes long growing of fish industrially. Meanwhile it is known that quality of a breeding material in many respects depends on conditions of their maintenance (Mamedov and Salmanov, 2009; Mamedov, 2011, 2012). Considering that the morphological analysis of blood is one of delicate and objective methods of an estimation of a physiological condition of an organism (Ivanova, 1983), we have previously studied the fish breeding and biological and hematological parameters of individuals of different ages Persian (Kura) sturgeon in the course of their long-term breeding in captivity.

Definition of the sex and maturity stage at sturgeon grown in aquaculture conditions at early ages and in the reproductive runs of various age groups by using the non-invasive diagnostic methods (ultrasound) and using them depending on development stages for fish growing purposes are one of the urgent matters.

The aim of the present study is a comparative evaluation of the quality of reproductive females holding under the conditions of sturgeon hatcheries and obtaining their off spring. This research is also needed to develop scientifically substantiated documents on exploitation of sturgeon broodstocks on the basis of sturgeon hatcheries of Azerbaijan.

MATERIALS AND METHODS

The study was conducted in April 2012 at Khylly Sturgeon Fish Farm of Azerbaijan within the framework of the regional training project "The formation of sturgeon-getter, using them and ultrasound definition of their sex" of the UN Food and Agriculture Organization (FAO). Ultrasound examination was carried out in repair-getter Persian (Kura) sturgeon (*Acipenser persicus*) formed since 2004, in the Russian sturgeons (*A. gueldenstaedtii*) formed in 2005, in the Russian and Siberian sturgeon hybrids (*A. gueldenstaedtii x A. baerii*), sterlet (*A. ruthenus*) and beluga (*Huso huso*) fishes, as well as on part of stellate sturgeon (*A. stella-*

tus) formed in 2006, and their sex and maturity stages were determined. The total number of repair-getter sturgeon fishes examined was 70 pcs. The „Mindray DP-6600” device was used at ultrasound examination. Methods of research works and required recommendations have been described in details in the scientific literature (Chebanov and Galich, 2010).

Works on the production of off spring/fry from reproductive females carried out in 2013. To stimulate maturation of selected two elite females of the Persian (Kura) sturgeon of the generation 2004 from hatchery – reared broodstock intramuscularly was injected by the suspension of acetone-water extract of sturgeon pituitaries based on the rate of 2.5 mg per 1 kg of body weight. To avoid inbreeding with the view of insemination eggs two males of Persian (Kura) sturgeon of the generation 2005 from hatchery were used. Ultrasound diagnosis of sex and maturity stages, hormonal stimulation of breeders, fertilization and de-adhesion of eggs (unsticking), as well as its incubation in the “Osetr” incubation system were conducted according to the existing method (Dettlaff et al., 1981; Mamedov et al., 2009).

The entire biotechnical process starting from the maturation of females until the release of juveniles into a natural water area has been traced. Research on the reproductive females included a comparative study of the size-weight characteristics, gamete-somatic index, and fecundity in both absolute and relative values as well as a response to hormonal simulation/treatment. The quality of offspring was evaluated by fish cultural and biological indicators of eggs, larvae and fries. The Persian (Kura) sturgeon natural population’s off spring was served as control.

RESULTS AND DISCUSSION

The global practice of fresh-water aquaculture proves a possibility of the maintenance and growing of sturgeon fishes in artificial conditions (Smolyanov, 1987; Popova et al., 2007; Chebanov and Galich, 2010; Cotenev et al., 2001). The principle of a bundling of maternal stock of sturgeon fish from spawn up to spawn is based on selection of elite posterity with the subsequent growing within 8-10 years up to mature sires. On the basis of posterity of artificial generation the recovery-maternal stock of sturgeon fish is formed on Khylly Sturgeon Hatchery since 2004 (Mamedov and Salmanov, 2009; Mamedov et al., 2009). At the moment ten-year old (400 pieces), nine-year old (250 pieces), eight-year old (200 pieces), seven-year old- (1200 pieces), six-year old- (500 pieces), five-year old – (500 pieces) Persian sturgeons and different-years old Persian sturgeon stellate sturgeon and Kura-river ship (*A.nudiventris*) (more than 1000 pieces) of industrial manufacture are grown as an experiment in conditions of the Khylly Sturgeon Hatchery. Additionally, some ten of specimen of Siberian sturgeon (*A.baerii*), sterlets beluga besters (*H.huso x A.ruthenus*) and Russian sturgeons are grown as an experiment in the Khylly Sturgeon Hatchery. The general quantity of recovery–maternal stock of different kinds of sturgeon fish on Khylly Sturgeon Hatchery constitutes about 5000 specimens.

It is necessary to note that ecological conditions at growing of young fish and adult forms of sturgeon fish in pools radically differ from conditions in which their various stages of ontogenesis passed during many millions years of evolution. For this reason the long maintenance of young fishes and adult forms of sturgeon in the closed space, that is in ecologically impoverished environment, can lead to simplification of their behavioral reactions, to decrease in impellent activity, formation of the certain stereotype of food behavior, so, to serious changes in functioning of various physiological systems.

In 2012 based on these studies on the definition of sex and stage of maturity of the sturgeon broodstock by ultrasonic diagnosis we were able to identify several females of Persian (Kura) sturgeon from the broodstock of the generation 2004, the state of maturity of the gametes were at III-IV and at IV unfinished stages of development (Table 1). These females were replaced in a separate tank for the purpose of formation of reproductive females at the hatchery.

So, creation of sex in sturgeon having no signs of external dimorphism at early ages and the reproductive run by using the non-invasive diagnostic methods of the maturity stage and implementation of these works according to required rules may be used in sturgeon fish farms of our republic and for commodity fish products.

In the 2013 year during the hatchery season previewing reproductive females by diagnostic ultrasound was done after intramuscular injections of aqueous suspension acetone-dried sturgeon pituitary. The duration of maturation of reproductive females after hormonal injection was 30-32 hours.

The average fecundity of two females was 131,150 of eggs. The number of eggs in 1 g sample amounted to 52 and 53 pieces, respectively. The incubation of sturgeon eggs took place in the individual “Osetr” incubation unit at a water temperature of 18,0-18,5^{0}C. The incubation period was 4,5 days. The percentage of eggs development determined at the stage of the small yolk tube (17th stage) was 90.4 and 88.5%, respectively.

The duration of the early ontogenesis of the experimental and control larvae was similar and was made up 8 days at the water temperature from 18.5^{0}C to 19.5^{0}C. The onset of transition of larvae to active exogenous feeding was determined by their behavior, the release of melanin and the flow rate of the yolk mass, which by this time accounted for both versions 91.8%.

Table 1. Results of ultrasound diagnosis of sex and maturity stages of gonads of broodstock sturgeon in Khylly Sturgeon Hatchery of Azerbaijan (2012)

Species of sturgeons	Years of hatching	Sex	Stage of maturity						The total number of sturgeon fishes	
			I	II	II-III	III	III-IV	IV		
Acipenser persicus	2004	♂		16	3	5	3	1	28	47
		♀		10	4	3	1	1	19	
Acipenser gueldenstaedtii	2005	♂					1		1	3
		♀					1	1	2	
Acipenser gueldenstaedtii x A. baerii	2005	♂				1	2		3	7
		♀				2	2		4	
Acipenser stellatus	2006	♂		1	1	2	2		6	10
		♀		1	1	2			4	
Acipenser ruthenus	2005	♂		1	1				2	2
		♀								
Huso huso	2005	♂		1					1	1
		♀								

During the transition to active feeding the larvae length increased up to 19.5 mm (experiment) and 20.0 mm (control), the weight of larvae increased respectively to 38.0 mg and 42 mg. The average daily growth rate on length of larvae in the experimental and control conditions amounted to 6.7 and 7.0% by weight 9.6% and 12.0%, respectively. The survival rate of larvae switched to active feeding averaged 86.5% (experiment) and 92.5% (control) with the standard index of 90%. The obtained offspring subsequently reared in plastic basins and after reaching the standard sample of 1.0 g were released into the Kura River's estuary.

The results of growing out of juveniles were assessed by using the method of total enumeration. The survival rate of stocking larvae to fingerlings was in average 67.4%. On separate basins this index varied from 64.5% to 73.6%. For the offspring obtained from fish of the natural complex, it is equal to 72.4% (standard - 70% - the temporary biotechnology standards for hatcheries of Azerbaijan 2007).

The analysis of fish breeding and biological indicators of hatchery-reared juveniles showed that the development of produced fry using reproductive females from broodstock was in compliance with the norm. Some of hatchery-reared fingerlings were kept at the hatchery with the view of further studying their morphological and biological and physiological features.

Thus, in fish cultural practices of Azerbaijan for the first time hatchery-reared broodstock females of the Persian (Kura) of sturgeon raised «from eggs» at the hatchery were used for artificial reproduction. These data indicate the prospects of the formation of the reproductive stage of various species of sturgeon at hatcheries. The creation of reproductive broodstock at sturgeon hatcheries will allow conservation of the sturgeon gene pool and expanding opportunities of their artificial reproduction to be released into natural water bodies.

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INTESTINAL HISTOLOGICAL RESPONSE AFTER A MEAL IN RAINBOW TROUT (*ONCORHYNCHUS MYKISS*)

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HISTOLOGIJA CREVA PASTRMKE (*ONCORHYNCHUS MYKISS*) PRE I POSLE OBROKA

Apstrakt

Peharaste ćelije se nalaze u crevu riba, one sintetišu neutralne i sulfatne mucine i izlučuju sluz, podmazujući nesvareni materijal koji napreduje prema rektumu i štiteći sluzokožu digestivnog trakta. Promene u peharastim ćelijama creva riba posle obroka mogu pokazati odgovor na pojedinačni obrok obzirom na njihovu ulogu u procesu varenja. Cilj ovog istraživanja bio je procena promena histologije creva, pre hranjenja i 6h i 12 h posle obroka. Izgleda da 6h posle obroka kada je vrhunac sinteze proteina kod pastrmke, proces varenja je u toku, a peharaste ćelije izlučuju sluz pa se njihova veličina smanjuje. Slične dimenzije ovih ćelija pre obroka i 12 h posle ishrane potvrđuju da pastrmku treba hraniti 2 puta dnevno, a da drugo hranjenje treba obaviti 6 sati posle prvog. Ovo istraživanje daje više uvida u upravljanje ishranom u procesu uzgoja pastrmki.

Kjlučne reči: *peharaste ćelije, histologija creva, pastrmka*
Keywords: goblet cells, intestine histology, trout

INTRODUCTION

Goblet cells are common components of the post-gastric mucosa in fish and they are the dominant mocous cells in the intestine (Buke, 1971; Groman, 1982). Their nucleus can be found to the bottom part of the cell while mucus fills the upper part and is discharged through an apical pore. Goblet cells can synthesize neutral and sulphate mucins, and sialomucins containing sialic acid (Khojasteh, 2012). The mucus secreted by goblet cells lubricates undigested materials for onward progression into the rectum and protects the mucosa of the digestive tract. Intestinal mucins may have a possible role in osmoregulation (Khojasteh, 2012). Post-prandial changes in the goblet cells in fish intestine may be a response to a single meal since they have a potential role in the digestion process. Measurement of protein synthesis rates in fish can be used as a tool to compare diets and explain some nutritional effects. In fish, generally protein synthesis rates are higher between 4 and 12 hours after a meal. The aim of this study was to assess the changes of intestine histology following a single meal, before feeding and at 6 hours (6h) and 12 hours (12h) after feeding.

MATERIALS AND METHODS

120 juvenile rainbow trout individuals mixed sex (*Oncorhynchus mykiss*), weighing approximately 44.98±1.08 g, were stocked in three 250 l freshwater tanks. Fish were fed *ad libitum* by hand two times daily at 09:00 and 15:00 for 5 weeks a commercial diet. At the end of the experiment, fish were fasted for 24h. Three fish, one fish from each tank were removed, sacrificed by anaesthesia (diluted 1:1 phenoxyethanol in ethanol) and a blow to the head and used as the prefeeding group for measurements of the intestine histology. The remaining trout were fed normally and a group of 6 trout was selected at random, sacrificed and removed at 6h and 12h after feeding, respectively. For light microscopy, rainbow trout midgut samples were first fixed in 10% buffered formalin for 24 h at 4ºC and then immediately dehydrated in graded series of ethanol, immersed in xylol, and embedded in paraffin wax. Sections of 5-7 μm were mounted. After they had been deparaffinized, the sections were rehydrated, stained with Alcian blue, and mounted with Cristal/Mount. Digital images of random cross sections of the midgut were selected in order to measure the mean number of the goblet cells per μm of intestinal fold and the size of the goblet cells. All measurements were made by the ZEN microscope software of ZEISS.

RESULTS AND DISCUSSION

The mean number of the goblet cells per μm of intestinal fold before feeding and at 6h and 12h after feeding was similar (0.07 ± 0.004, 0.06 ± 0.004 and 0.06 ± 0.003, respectively) ($p > 0.05$, Table 1). Regarding the size of the goblet cells the results were 174.69 ± 15.97 μm^2 before feeding, and 108.82 ± 6.97 μm^2 and 159.95 ± 9.99 μm^2 at 6 h and 12 h after feeding respectively ($p < 0.05$, table 2). Smaller goblet cells appeared at 6 h after feeding while the intestine 12 h after feeding and before feeding had similar size of goblet cells ($p > 0.05$).

Table 1. Goblet cells mean number per μm of intestinal fold before feeding, 6 hours and 12 hours after feeding.

Time	Mean number of goblet cells
Before feeding	$0.07^a \pm 0.004$ (12)
6 hours after feeding	$0.06^a \pm 0.004$ (17)
12 hours after feeding	$0.06^a \pm 0.003$ (10)

Data are presented as means ± S.E. The number of intestinal folds is given within parenthesis. Means in a column followed by the same superscript are not significantly different ($p > 0.05$).

Table 2. Goblet cells size (μm^2) before feeding, 6 hours and 12 hours after feeding.

Time	Mean size (μm^2) of goblet cells
Before feeding	$174.69^a \pm 15.97$ (50)
6 hours after feeding	$108.82^b \pm 6.97$ (50)
12 hours after feeding	$159.95^a \pm 9.99$ (50)

Data are presented as means ± S.E. The total numbers of goblet cells are given within parenthesis. Means in a column followed by the same superscript are not significantly different ($p > 0.05$).

Goblet cells are very important for the nutrition of the fish and its health. According to van den Ingh et al. (1991) an increased amount of goblet cells in the epithelium of the intestine could be a sign of enteritis. Such damage is usually related to distal parts of the gastrointestinal tract and characterized also by goblet cell hypertrophy and hyperplasia. Bozic *et al.,* (2001) observed that starvation induced an increase in the number of intestinal goblet cells in carp. The mucus secreted by goblet cells lubricates undigested materials for onward progression into the rectum. Our result shows that the size of the goblet cells is decreased 6 h after feeding a single meal and gained their original size 12 h after feeding. It seems that at 6 h after feeding which is the peak of the protein synthesis rates in trout, the digestion process is on progress and goblet cells secrete their mucous by decreasing their size. 12 h after feeding the protein synthesis rates are decreasing and the digestion process is towards the end, thus the production of mucous by the goblet cells is not needed. The similar size of the goblet cells before feeding and at 12 h after feeding confirms that rainbow trout should be fed twice per day and the second feeding should take place 6 h after the first feeding. This study gives more insight into the feeding management of trout aquaculture.

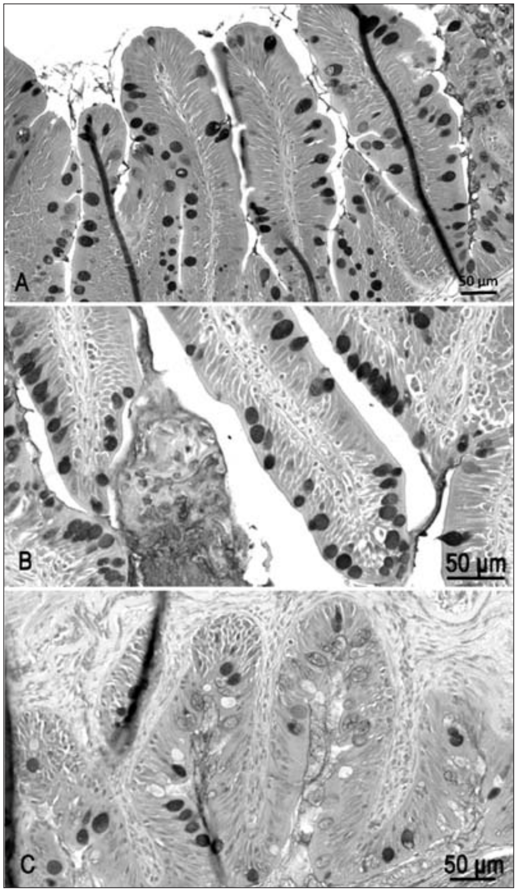

Figure 1. Rainbow trout intestine A: Before feeding. Goblet cells are blue. B: 6 h after feeding. The goblet cells are smaller in size than the ones before feeding. C: 12 h after feeding. The goblet cells have similar size with the ones before feeding.

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BLEAK (*ALBURNUS ALBURNUS*) AS POTENTIAL BIOINDICATOR OF HEAVY METAL POLLUTION

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UKLIJA (*ALBURNUS ALBURNUS*) KAO POTENCIJALNI BIOINDIKATOR ZAGAĐENJA TEŠKIM METALIMA

Apstrakt

Analize vode i/ili sedimenta mogu biti neefikasne u identifikovanju metala u fluvijalnim ekosistemima usled nerazdvojive varijabilnosti rečnog toka i koncentracije zagađivača. Monitoring zagađenja ribljih tkiva ima važnu ulogu ranog alarma koji ukazuje na probleme kvaliteta vode i sedimenta, a takođe omogućava detekciju toksičnih materija u ribama koje dalje mogu da imaju negativan efekat na konzumente.

Reka Sava je tipična nizijska reka i najveća desna pritoka Dunava koja protiče kroz tri zemlje: Sloveniju, Hrvatsku i Srbiju. Do 1990-ih je bila izložena zagađenju iz metalurgije, hemijske, kožne, tekstilne, prehrambene i industrije celuloze i papira, ali i usled poljoprivrednih aktivnosti. Takođe je i glavni recipijent otpadnih voda mnogih gradova i zagađenih pritoka.

Ciljevi ove studije su da se utvrde koncentracije Al, As, Cd, Co, Cr, Cu, Fe, Hg, Mn, Ni, Pb, Se, Sn i Zn u kompostu uklije (*Alburnus alburnus*) kao potencijalne bioindikatorske vrste zagađenja teškim metalima reke Save, kao i da se izračuna Indeks zagađenja metalima (MPI-*Metal Pollution Index*) kako bi se uporedio status zagađenja različitih lokaliteta reke Save.

Terensko istraživanje je sprovedeno tokom septembra 2014. godine. Uklije su sakupljene iz reke Save na sledećim lokalitetima: Čatež u Sloveniji, Zagreb i Slavonski Brod u Hrvatskoj, Jarak i Umka u Srbiji. Koncentracije Al, As, Cd, Co, Cr, Cu, Fe, Hg, Mn, Ni, Pb, Se, Sn, i Zn su merene u kompozitnom uzorku uklije uz pomoć Thermo Scientific iCAP 6500 Duo ICP-OES (Thermo Fisher Scientific, Cambridge, United Kingdom).

Prema dobijenim rezultatima, izdvaja se Zagreb sa najvećim brojem najviših koncentracija elemenata (Cd, Co, Cr, Fe, Pb, i Se). Takođe, najviši MPI je izračunat za Zagreb (0.23) u odnosu na Umku sa najnižim MPI (0.14). Na osnovu dobijenih rezultata, možemo označiti

gornji tok reke Save kao znatno zagađeniji (posebno deo kod Zagreba u Hrvatskoj) od donjeg toka i ušća u Dunav kod Beograda (Umka).

Ključne reči: reka Sava. uklija, bioindikator, indeks zagađenja metalima
Keywords: Sava River, bleak, bioindicator, metal pollution index

INTRODUCTION

Among the various contaminants, heavy metals in rivers has become a matter of great concern, due to environmental persistence, biogeochemical recycling and ecological risks, the threat it poses to public water supplies, and also because of the hazard to human consumption of fishery resources (Terra et al., 2008).

Water and/or sediment analysis may be inefficient at identifying metal inputs to fluvial systems of the inherent variability of flow and contaminant concentrations (Ricart et al., 2010). Fish tissue contamination monitoring has an important role as an early warning indicator regarding problems related to water and sediment quality, and it also enables detection of toxic chemicals in fish, which can produce adverse effects on consumers. Thus, such monitoring allows taking appropriate and timely measures to protect public health and the environment (Lenhardt et al., 2012).

The Sava River as typical lowland and the largest right side tributary of the Danube River is located on the southern fringe of Central Europe. It flows through three countries: Slovenia, Croatia and Serbia. Until the 1990s it was affected by heavy pollution from the metallurgical, chemical, leather, textile, food, cellulose and paper industries, as well as from agricultural activities. Also Sava is the main recipient of waste water from many cities and it is impacted by the polluted water of the tributaries.

The aims of this study were to determine the concentrations of Al, As, Cd, Co, Cr, Cu, Fe, Hg, Mn, Ni, Pb, Se, Sn and Zn in the whole body composite sample of bleak (*Alburnus alburnus*) from Sava River as potential fish bioindicator of heavy metal pollution and to calculate Metal Pollution Index (MPI) in order to compare the pollution status of different locations and to establish locations with the highest or the lowest level of pollution in Sava River.

MATERIALS AND METHODS

The field work was conducted during the September of 2014. Bleak specimens were collected on different locations from Sava River: Čatež (Slovenia), Zagreb and Slavonski Brod (Croatia), Jarak and Umka (Serbia). In the field, all samples were washed with distilled water and in plastic bags transferred to the laboratory. In the laboratory, all samples are measured, length (to the nearest cm), and weigh (to the nearest g), grinded in a Laboratory homogenizer Sterilmixer (International P.B.I. S.p.A.) and whole body composite (wbc) sample stored at -20°C prior to analysis. In the laboratory, fish samples (~1.5 g) were dried in a lyophilizer (Christ Alpha 2-4 LD, Harz, Germany), and then 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 220°C 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. Concentrations of Al, As, Cd, Co, Cr, Cu, Fe, Hg, Mn, Ni, Pb, Se, Sn and Zn were measured in wbc of bleak using a Thermo Scientific iCAP 6500 Duo ICP-OES 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. Standards for the instrument calibration were prepared on the basis of multi-element (SS-Low Level Elements ICV Stock, 10 mg/L) and mono-element (Hg Calibration Stock, 10 mg/L Hg; Sn LSN-100, 10 mg/L Sn) certified reference solution ICP Standard (VHG Labs, Inc-Part of LGC Standards, Manchester, NH 03103 USA). The detection limits for Al, As, Cd, Co, Cr, Cu, Fe, Hg, Mn, Ni, Pb and Se were: 0.1, 0.00022, 0.00047, 0.00005, 0.003, 0.0028, 0.0018, 0.00027, 0.0025, 0.007, 0.0093, 0.0001, 0.00027, 0.109 $mgkg^{-1}$, respectively.

The analytical process quality control, performed by the use of fish protein certified reference material for trace metals DORM 4 (NRCC, Canada), indicated that the resulting concentrations were within 85.8-116.25%. There were no certified values for Al, Mn, and Co. Concentrations of all metals were expressed as mg kg^{-1} wet weight basis (ww).

In order to assess significant differences between the levels of elements (Al, As, Cd, Co, Cr, Cu, Fe, Hg, Mn, Ni, Pb, Se, Sn and Zn) at five different locations, non-parametric Kruskal-Wallis test was applied. Post hoc inter-group comparisons of element levels (between pairs of locations) were performed by the non-parametric Mann-Whitney test for two independent samples. All statistical analysis of data was carried out using SPSS 16.0 statistical package programs for Windows (SPSS Inc., Chicago, IL, USA).

The metal pollution index (MPI) was calculated to compare the total metal content in the different sampling sites using the following equation (Usero et al., 1997):

$$MPI = (Cf_1 \text{ x } Cf_{2\ldots} Cf_n)^{1/n} \text{ where } Cf_n = \text{concentration of the metal } n \text{ in the sample.}$$

RESULTS AND DISCUSSION

The main characteristics (number of individuals, total body length, and weight) and average element concentrations ($mgkg^{-1}$) determined on the wet weight basis in the wbc of bleak are summarized in Table 1.

Muscles are often a major tissue of interest for routine environmental monitoring. However, since they are not always the best indicators of element contamination present in

fish, the analysis of other tissues is recommended as well (Has-Schön et al., 2006). Gills are the primary site of metal uptake from water (Dogru et al., 2011), especially if metals are bound to particulate matters (Klavins et al., 2009), while the liver as metabolically active tissue is the accumulation place of metals (Yilmaz et al., 2007). The accumulation in muscle tissue is, except Hg, usually lower or the lowest (Jarić et al., 2011; Poleksić et al., 2010; Višnjić-Jeftić et al., 2010).

Table 1. The main characteristics of bleak and average element concentrations (mean ± SD) determined on the wet weight basis in the wbc of bleak from Sava River

Ecosystem/ Metal	Čatež (n* = 10)	Zagreb (n = 10)	Slavonski Brod (n = 10)	Jarak (n = 10)	Umka (n = 10)
total body length (cm)	12.33 ± 0.65	11.68 ± 0.96	10.04 ± 1.55	12.01 ± 1.31	11.21 ± 1.64
weight (g)	12.6 ± 2.27	10.16 ± 3.2	6.8 ± 3.03	12 ± 4.92	9.4 ± 5.38
Al	3.31 ± 0.18[a]	2.92 ± 2.42	2.08 ± 0.88	1.55 ± 0.43	1.79 ± 0.93
As	0.15 ± 0.06	0.12 ± 0.025	0.13 ± 0.09	0.12 ± 0.04	0.1 ± 0.086
Cd	0.008 ± 0.002	0.1 ± 0.002[a]	0.016 ± 0.008	0.008 ± 0.002	0.013 ± 0.008
Co	0.0007 ± 0.0006	0.001 ± 0.0007	0.0008 ± 0.0004	0.0005 ± 0.0004	0.0006 ± 0.0004
Cr	0.17 ±0.022	0.18 ± 0.045	0.154 ± 0.024	0.18 ± 0.03	0.18 ± 0.02
Cu	0.48 ± 0.05	0.67 ± 0.12[a]	0.68 ± 0.34[a]	0.43 ± 0.12	0.44 ± 0.14
Fe	9.74 ± 3.2	14.5 ± 8.27	11.44 ± 6.67	9.21 ± 2.71	8.76 ± 3.44
Hg	0.02 ± 0.004[a]	0.013 ± 0.003	0.011 ± 0.003	0.013 ± 0.004	0.011 ± 0.004
Mn	1.37 ± 0.48	1.55 ± 0.71	1.2 ± 0.47	1.6 ± 0.63	1.73 ± 0.72
Ni	0.02 ± 0.004	0.032 ± 0.033	0.034 ± 0.01	0.047 ± 0.064	0.025 ± 0.015
Pb	0.11 ± 0.02	0.17 ± 0.03	0.1 ± 0.01	0.12 ± 0.02	0.11 ± 0.26
Se	0.24 ±0.06	0.34 ± 0.066[a]	0.11 ± 0.025	0.08 ± 0.033	0.085 ± 0.04
Sn	0.016 ± 0.0007	0.016 ± 0.0008	0.015 ± 0.002	0.017 ± 0.002	0.016 ± 0.002
Zn	24.74 ± 7.2	24.47 ± 6.6	23.48 ± 4.82	23.1 ± 4.07	23.09 ± 6.65

[*] the number of sampled bleak per location
[a] the value with this letter is significantly different ($p < 0.05$)

Although literature data indicate that different tissues of the fishes showed significant difference for heavy metal accumulation, we used wbc to assume water pollution with heavy metals. The Kruskal-Wallis test revealed significant differences between locations in regard to Al, Cd, Cu, Hg, and Se concentration ($p < 0.05$). The post hoc Mann-Whitney test showed that concentrations of Cd, Cu, and Se were significantly higher ($p < 0.05$) in Sava River near Zagreb in Croatia, while concentrations of Al and Fe were significantly ($p < 0.05$) higher in Sava River near Čatež in Slovenia (Table 1).

MPI has been calculated to enable presentation of all results from the element concentrations (Al, As, Cd, Co, Cr, Cu, Fe, Hg, Mn, Ni, Pb, Se, Sn and Zn) as one value, with both application and understanding of demanding statistical analysis. The highest MPI was calculated for Zagreb; followed by Čatež, while the lowest was calculated for Umka (Figure 1). It follows that Zagreb site is the most polluted area, while Umka site is the least polluted compared to other areas. According to MPI values, it can be seen that the highest MPI values were recorded for the Zagreb location. To conclude, on the basis of these results, we can mark upper stream of Sava River as more polluted than lower reaches of Sava River.

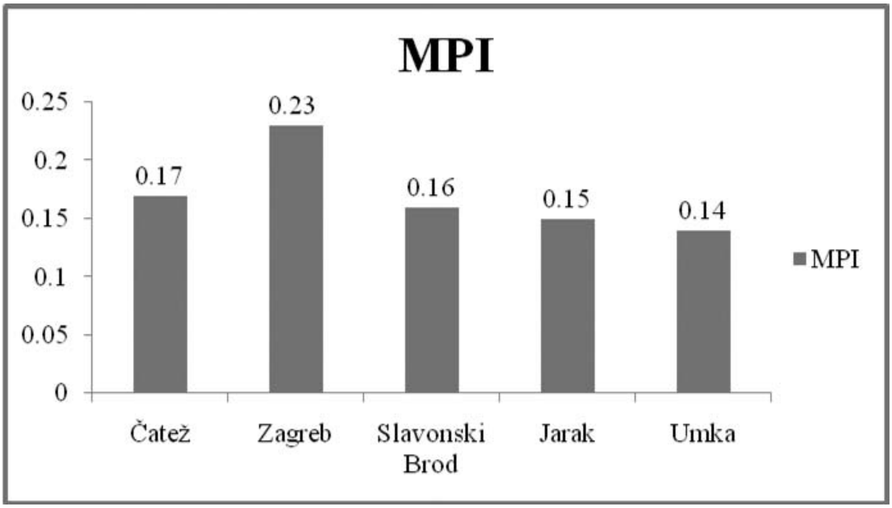

Figure 1. MPI of each examined location from Sava River

The maximum permitted levels (MPC) prescribed by the National Regulation of the Republic of Serbia (28/2011) for Pb, Cd, Hg, Cu, and Zn are 0.3 $mgkg^{-1}$, 0.05 $mgkg^{-1}$, 0.5 $mgkg^{-1}$, 30 $mgkg^{-1}$ (in tin containers), and 100 $mgkg^{-1}$ (in tin containers), respectively (Official Gazette of FRY, No 28/2011) and those prescribed by the EU Regulation (1881/2006) for Pb, Cd, and Hg are 0.3 $mgkg^{-1}$, 0.05 $mgkg^{-1}$, 0.5 $mgkg^{-1}$. This indicates that the bleak samples, except samples from Zagreb where elevated concentration of Cd (0.1 $mgkg^{-1}$) were found, should be safe for utilization in human diet.

ACKNOWLEDGMENT

This investigation was supported by the Ministry of Science and Technological Development of the Republic of Serbia as part of project number 31011.

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MORPHOMETRIC PARAMETERS OF LOCAL CARP WITH DIFFERENT TYPES OF SCALE PATTERNS REARED IN AUTOCHTHONOUS PRODUCTION ECOSYSTEMS

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MORFOMETRIJSKI PARAMETRI LOKALNOG ŠARANA RAZLIČITE LJUSKAVOSTI GAJENOG U AUTOHTONIM PROIZVODNIM EKOSISTEMIMA

Apstrakt

Istraživanje je sprovedeno na ribnjacima u okolini Plovdiva u južnoj Bugarskoj sa šaranima iz lokalnih populacija. Prilikom uzgoja šarana u uslovima monokulture koja se bazira na prirodnim izvorima hrane uz dodatak đubriva (3000 $kg.ha^{-1}$), dvogodišnje jedinke nisu dostigle konzumnu veličinu. Ljuskavi i goli šarani gajeni su na sličan način i na kraju uzgojnog perioda, nije bilo značajne razlike u težini žive ribe. Gajen u monokuluturi, dvogodišnji ljuskavi i goli šarani iz lokalnih populacija, imali su izdužena tela `sazan` tipa, velike glave i nisku vrednost kondicionog indeksa. Vrednosti koje opisuju linearni rast riba i eksteriorne indekse se nalaze u značajnoj korelaciji sa tipom šarana. Goli šaran je imao višu vrednost kondicionog indeksa i kompaktnije telo u poređenju sa ljuskavim šaranom.

Ključne reči: šaran, morfometrijske mere, eksteriorni indeksi
Key words: carp; morphometric measures, exterior indices

Abbreviations: K_1 – 1-year old carp; K_0 – 0-year old carp; TW – total weight; SL – standard length; TL – total length; CL – carcass length; LHwg – head length; D – maximum body width; H – maximum body height; O – girth

INTRODUCTION

There are good opportunities for the introduction of ecological and organic production technologies in Bulgaria, including organic aquaculture in particular (Nikolova, 2013). Local plant varieties and animal breeds are given an advantage when introducing organic farming. Carp species are very suitable for organic farms (Varadi, 2005). Common carp is a traditional species for Bulgarian aquaculture. In 2013 its production increased by almost 89% compared to the previous year and it represented about 35% of the total fish production in the country (MZH, 2014). At the same time there are not enough research studies on the characteristics of the local carp in Bulgaria, especially when reared in extensive and semi-intensive production ecosystems. Morphological characteristics and exterior indices were studied in different carp breeds and populations (Khosrow and Amirkolaie, 2010; Treer et al., 2000 etc.). Growth and body proportions in fish are determined by a complex of genetic and environmental factors (Kapusta et al., 2013; Kirpichnikov, 1979). That is why the individual breeds, local groups and populations should be characterized under the concrete rearing conditions.

The aim of the present study was to establish some morphometrical characteristics of scale and mirror carp of a local population in Bulgaria and the effect of some factors on those characteristics when fish is reared in autochthonous production ecosystem (based on natural nutrient sources in the pond).

MATERIAL AND METHODS

Studies were carried out in four carp ponds on the experimental site of the Institute of Fisheries and Aquaculture in Plovdiv (southern Bulgaria). The ponds were stocked with scale and mirror carps of local populations. Two variants of mixed monoculture were studied: I variant (n=2) – K_1 – 500 pcs.ha^{-1}; K_0 – 15000 pcs.ha^{-1}; II variant (n=2) – K_1 – 500 pcs.ha^{-1}; K_0 – 30000 pcs.ha^{-1}. The mean weight of fish at stocking was 0.045 kg for the one-year old scale carp and 0.048 kg for the mirror carp. In all the ponds the fish was fed only on natural food available in the pond (autochthonous monoculture (by Privezentsev, 1991)). The ponds were supplemented with cattle manure at rates permissible in organic aquaculture (3000 kg.ha^{-1}). During the period of vegetation, the water characteristics of each pond were monitored. Water temperature, pH and oxidability were within the technological limits for carp ponds.

At the end of the vegetation period the fishes were measured following the adopted methods (Kryukov et al., 2007, etc.). Measurements were made with a tape and a caliper with an accuracy of 0.05 mm.

The following parameters were calculated: CFF – Fulton's coefficient (TW*100/SL3); IC – condition index (TW*100/SL*H*O); IHB – high-backed index (SL/H); IBB – broad-backed index (D*100/SL); ILHwg – long-headed index (LHwg*100/SL); IH – hardness index (O*100/SL).

Analysis of variance was used for data processing. The linear equation model was of the following general type: **Yijklmn=μ+Vi+Pj+Tk+VTiк+VTPijk+eijkl,**

where: Yijklmn – the index of the nth individual; μ – general average constant; Vi, Pj, Tk, - fixed effects of the ith variant (2); jth pond (4); kth types of scale patterns (2); VTij and VTPijk chance effect of kth scaling in ith variant; VTPijk chance effect of kth scaling in jth pond in ith variant; e (..) – residual variance.

RESULTS AND DISCUSSION

When reared in monoculture based on natural food available in the ponds, the two-summer old common carp of a local population cannot reach a consumable size (Table 1), the specific conditions in the pond having a significant effect on fish development (Table 2). The scale pattern type also has a significant effect on the linear fish growth (P<0.05), the differences not being dependent on the technological variant and on the pond within the same variant.

Table 1. Exterior measurements and indexes

Indices	I				II			
	Scale		Mirror		Scale		Mirror	
	LS-means	±Se	LS-means	±Se	LS-means	±Se	LS-means	±Se
TW, kg	0.238	13.102	0.287	17.807	0.315	23.614	0.343	34.218
TL, cm	25.61	0.425	27.18	0.577	28.22	0.765	28.54	1.109
SL, cm	20.99	0.348	22.26	0.473	23.11	0.627	23.38	0.909
CL, cm	15.15	0.273	15.87	0.372	16.56	0.493	16.86	0.714
LH, cm	5.84	0.084	6.39	0.114	6.55	0.152	6.52	0.220
D, cm	3.11	0.064	3.48	0.087	3.41	0.115	3.57	0.167
H, cm	6.71	0.115	7.13	0.156	7.22	0.207	7.44	0.300
O, cm	17.49	0.266	18.54	0.361	18.84	0.479	19.27	0.694
IHB	3.14	0.023	3.13	0.031	3.21	0.041	3.13	0.060
IBB	14.82	0.123	15.62	0.168	14.69	0.222	15.26	0.322
ILHwg	27.93	0.186	28.79	0.253	28.41	0.335	28.06	0.486
IH	83.54	0.462	83.41	0.628	81.59	0.833	82.97	1.207
CFF	2.45	0.026	2.56	0.035	2.43	0.047	2.57	0.068
IC	9.17	0.067	9.55	0.091	9.58	0.121	9.71	0.175

Table 2. Effect of the studied characteristics on the body parameters and indices (F-test)

Indices	Variant (V)	Types of scale patterns (Tsp)	Pond (P)	V * Tsp	V*Tsp*P
TW	1.810	2.709	2.185	0.857	0.528
SL	1.308	2.848*	5.079*	0.308	0.458
TL	1.249	2.785*	4.801*	0.289	0.435
CL	0.821	2.212	5.082*	0.214	0.291
LH	3.199*	4.623*	4.002*	0.630	1.118
D	5.108*	2.540	1.592	2.175	1.370
H	0.758	1.623	1.251	0.742	0.423
O	0.834	1.464	4.069*	0.684	0.397
IHB	0.192	0.758	5.837*	0.524	0.791
IBB	8.749**	0.058	2.430	5.088*	2.279*
ILHwg	1.169	0.066	3.285*	0.075	0.317
IH	0.711	3.065*	1.841	0.402	1.596
CFF	0.208	0.121	3.056*	0.273	1.261
IC	0.169	2.933*	1.890	0.189	0.443

* - (P<0.05); **- (P<0.01)

As a whole, it is considered that scale carp has better total productivity compared to mirror carp, however, Katasonov and Gomelskiy (1991) mentioned that mirror carp practically does not fall behind scale carp under favourable rearing conditions.

In a detailed study on pond-reared common carp in Bulgaria, Tsekov (1985) did not establish a significant difference in growth of carp breeds and groups at the age of two summers. No significant difference in growth of local Ukrainian scale and mirror carp fishes was also established by Tovstik (1979). Data about the live weight and the body length, obtained in the present experiment, were close to those published by the author for the two-summer old scale and mirror carp fishes fed only on natural food available in the pond.

Referring to body shape, fishes in our experiment have an elongated body shape (Bogeruk et al., 1997). The established IHB is significantly higher than the indices published for scale carp of Hungarian breeds (Spaho et al., 2012). The index is higher than the established by Tsekov (1985) for two-summer old mirror carp reared under intensive conditions in Bulgaria, the values being close to those defined by the author for Amur carp of the same age. Data obtained in the present study were also similar to IHB established by Tarazevich et al. (2012) for two-summer old mirror and scale Tremlyansky carp.

Hardness index has higher values in the fishes of the I variant (Table 1), however, the effect of the stocking structure is insignificant (Table 2). The scale pattern type has a significant effect on IH, the mirror carp having a more compact body. IH and IBB values established by Pishtenko and Belousov (2003) for two-summer old Altay mirror carp are similar to our results.

The mean ILHwg values in the present study vary from 27.9 to 28.8 but no relationship with the scale pattern was found. The individuals with a long-headed index bigger than 26 belong to the large-headed fishes (Bogeruk et al., 1997). Katasonov and Gomelskiy (1991) mentioned a long headed index of 27 for two-year old scale Ukrainian carp and an index of 31 for mirror carp.

In the present experiment, we established higher values of CFF and IC for mirror carp (Table 1), the scale pattern being a significant source of variation of IC, but not affecting significantly CFF (Table 2). Tsekov (1985) established obvious differences in the carp breeds referring to CFF. CFF is an important index in studies on fish, despite its disadvantages. McPherson et al. (2011) established in their study that the relationship between CFF and mesenteric fat was inconsistent and often non-existent, while CFF was significantly correlated with fatmeter values for the aggregate field dataset and significantly correlated with total muscle fatty acid for the experimental dataset. Kolisnyk et al. (2014) mentioned that CFF is an indicator of the level of nutrition and the natural food quality, as well as an indicator of the ability to absorb available food.

CONCLUSION

When rearing carp of a local population under the conditions of monoculture based on natural nutrient sources in the pond, loaded with manure, the two- summer old fishes cannot reach a consumable size. Carp fishes of different scale patterns grow in a similar way and there is not a significant difference in their live weight at the end of the rearing period. Reared in autochthonous monoculture, the two- summer old scale and mirror carp fishes of a local population have an elongated body of a 'sazan' type, a large head and low IC values. The values characterizing linear fish growth and exterior indices are significantly correlated with the scale pattern type. Mirror carp has a higher IC value and a more compact body compared to scale carp.

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SPHAEROSPORA MOLNARI (MYXOZOA) IN COMMON CARP FINGERLINGS

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SPHAEROSPORA MOLNARI (MYXOZOA) KOD ŠARANSKE MLAĐI

Apstrakt

Sferosporidioza škrga je obolenje riba izazvano parazitom *Sphaerospora molnari* koji napada škrge i kožu. Prvo pojavljivanje sferosporidioze škrga kod šaranskih mladunaca utvrđeno je u Mađarskoj još 1972, zatim u Češkoj i Poljskoj, dok je kod nas obolenje prisutno od sredine osamdesetih godina prošlog veka. Molnar, koji je prvi je izučavao patogeni efekat ovog uzročnika, najpre ga je identifikovao kao *Sphaerospora carassi.* Češki istraživači Lom *et* Dycova detektovali su uzročnika sferosporidioze škrga iz škržnog materijala obolelih mladunaca šarana pomoću histološke sekcije tkiva i predložili da se parazitu da ime *Sphaerospora molnari*. Ovo obolenje je dosta često kod mladunaca ribnjačkog šarana i amura, pri čemu intenzitet infestacije može dostići čak i do 100%. Cilj ovog rada jeste da se utvrdi prisustvo obolenja izazvanog parazitom *Sphaerospora molnari* i da se isprate kliničke i patohistološke promene kod infestiranih mladunaca šarana. Istraživanja su sprovedena na 18 šaranskih ribnjaka u Srbiji, od 2008. do 2012. godine, a u sklopu sistemskog monitoringa najznačajnijih protozooza šarana. Mladunci šarana pregledani su tokom čitavog vegetacionog perioda. Praćene su kliničke promene, i uzimani su uzorci za nativnu mikroskopiju koja je rađena pomoću svetlosnog mikroskopa. Od inficiranih jedinki uzimano je tkivo škrga za patohistološku analizu koja je sprovedena klasičnom metodologijom, fiksiranjem u 10% formalinu, sečenjem 5 µm velikih isečaka koji su kalupljeni u parafin i bojenjem isečaka pomoću H&E. Prisustvo *S. molnari* ustanovljeno je kod mladunaca šarana od 20 dana do 3 meseca starosti. Na škrgama su bili prisutni razvojni stadijumi i zrele spore što se moglo uočiti na stratifikovanom epitelu škržnih filamenata. Spore su invadirale epitel i formirale velike klastere. Akumulacija razvojnih stadijuma i zrelih spora bila je

prisutna je i kod dvostrukog sloja epitelnih ćelija koje pokrivaju sekundarne lamele, i to najčešće između unutrašnjeg i spoljašnjeg omotača izazivajući tako distenziju tkiva. Zaražene lamele podležu nekrozi, što dovodi do kretanja spora prema spolja. Veličina spora iznosila je 10 x 10 µm. Klinički, obolenje se manifestovalo pojavom beličastih depozita na škrgama kao posledica agregacije parazita na njima, pri čemu paraziti mogu da zauzmu i do 80% površine slojevitog epitela, prekrivajući pločice i lukove škrga. Pritisak parazita koji se razmnožavaju je takav da vrši ćelijsku deformaciju tkiva i na kraju se uočava istančanost citoplazme ćelija šktžnog epitela u obliku mreže. Pošto spore prekrivaju najveći deo respiratornog epitela, smanjuju otpornost organizma i stvaraju uslove za razvoj drugih uzročnika obolenja (prvenstveno trematoda), što *Sphaerosporu molnari* svrstava u patogene parazite. Lokalizacija, veličina spora odnosno razvojnih stadijuma *S. molnari*, kao i kliničke i patohistološke promene zabeležene tokom ovog istraživanja odgovaraju rezultatima koje su opisali ostali istraživači koji su se bavili ovom problematikom. Pošto ne postoji ni jedno adekvatno terapeutsko sredstvo, kontrola sferopsoridioze i dalje se bazira na pridržavanju osnovnih sanitarno–profilaktičkih mera, kao što su isušivanje objekata, izmrzavanje, mehanička obrada tla i dezinfekcija krečom.

Ključne reči: Sphaerospora molnari, škrge, mladunci šarana
Keywords: Sphaerospora molnari, gills, young carps

INTRODUCTION

Sphaerosporosis of the gills is a disease of young carps caused by *Sphaerospora molnari* (Lom et al., 1983). The first severe outbreak of sphaerosporosis of the gills in young carps was described by Hungarian researchers Hámory and Molnar (1972). Molnar (1979) studied the pathogenic effects of this causative agent which he named *Sphaerospora carassi*. Later, the same author claimed that sphaerosporosis of the gills, at least in mirror carp, may result in changes in superficial layers of the skin in about 50% of infected fish. Lom et Dycova (1980) detected the causative agent of gill sphaerosporosis in samples of diseased young carps and suggested the name *Sphaerospora molnari*. In Serbia, sphaerosporidiosis of the gills has been present in carp fish ponds since the 1980s (Ćirković, 1986; Ćirković and Novakov, 2013). In the gills, *S. molnari* invades stratified epithelium of gill filaments and secondary lamellae cousing branchial lesions and must be regarded as a serious pathogen of yearling carp. Specific therapeutic measures are not known. The present study deals with the occurance of *Sphaerospora molnari* and with health problems of young carps affected in pond cultures.

MATERIALS AND METHODS

Investigation was carried out in the northern Serbia (Vojvodina province) in 18 fish ponds, until 2008 to 2014, during a systematic survey of protozoan parasites of carp. Common carp *Cyprinus carpio* L. fingerligs were examines during the whole growing season. All investigated fish ponds were traditional soil ponds which provide water from channel network, rivers Tisa, Tamiš, and Danube, and wells. After clinical observations of fish parasitological examination were performed from gill samples using light microscopy. Spores were studied fresh. Samples of infected gills were fixed in 10% neutral formalin and processed, sliced to 5-μm-thick paraffin-wax embedded tissue sections, mounted and stained in haematoxylin and eosine.

RESULTS AND DISCUSION

Sphaerospora molnari parasites were present in young fish at the age of 20 days to 3 months. The development stages and mature spores of *S. molnari* were detected on the epithelium of the gill filaments (Figure 1). They pervaded epithelium diffusely or formed large clusters. In the case of massive infection the parasites may completely destroy the interlamellar cell layer. The accumulation of development stages and mature spores were also present in the double layer epithelium, which cover the secondary lamellae, often between the inner and outer layer, leading to the distension of the tissue (Figure 2). The infected lamellae undergo necrosis, which results in the release of the spores. Size of the spores is 10 x 10 μm.

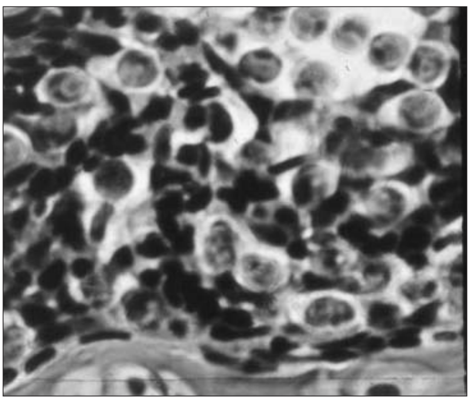

Figure 1. *Sphaerospora molnari in* gill tissue, H&E

Clinically, disease was observed on the gills in the form of whitish deposits as a result of parasites aggregation on them, where spores can take up to 80% of the stratified epithelium covering the gill arches and tiles. Since parasites are covered most of the respiratory epithelium, they reduce resistance and create conditions for the development of other causes of diseases.

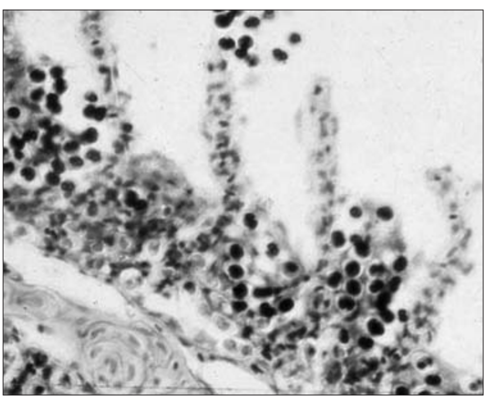

Figure 2. Spores of *Sphaerospora molnari in* gill, H&E

Sphaerospora molnari identified during this investigation is a parasite of carp which is the most present in Hungary, Czech Republic and Poland, and it is a typical myxosporean in intensive aquaculture (Antychowicz, 1985; Dycovà and Lom, 1988; Lom and Dycovà, 1992; El-Matbouli et al., 1992; Yeney and Yeney, 1995; Pojmanska et al., 1998).

Localisation and size of individual developmental forms of *Sphaerospora molnari* was generally the same, as described in the literature (Molnar, 1979; Lom et al., 1983; Antychowicz, 1985; Dycovà and Lom, 1988; Lom and Dycovà, 1992; El-Matbouli et al., 1992).

Covering the majority of the respiratory epithelium, the spores weaken the host immunity and make conditions for the development of other pathogens. Thus, *Sphaerospora molnari* (Molnar, 1979) is rather pathogenic parasite. This is supported by the fact that spores are usually detected in fish invaded with large numbers of dactylogyruses.

CONCLUSIONS

Gill sphaerosporosis manifests on gills in 20 days to 3 months old common carp fingerlings from fish ponds of Serbia. The most prominent clinical signs of the disease were present of whitish deposits on gills as a result of parasites aggregation on them and and the consequently difficulty in breathing. Histological analysis of gill confirms the presence of development stages and mature spores of *S. molnari*. Since *S. molnari* invades stratified epithelium of gill filaments and secondary lamellae cousing branchial lesions this parasite must be regarded as a serious pathogen of yearling carp, especially when it occurs together with other pathogens. Control of gill sphaerosporosis 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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SIMILARITIES AND DIFFERENCES IN THE HABITS OF FISH CONSUMER IN THREE CITIES OF THE REPUBLIC OF SRPSKA

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SLIČNOSTI I RAZLIKE U NAVIKAMA POTROŠAČA RIBE U TRI GRADA REPUBLIKE SRPSKE

Apstrakt

Anketno istraživanje percepcije i navika potrošača u kupovini i potrošnji ribe provedeno je u tri grada Republike Srpske: Banja Luka (BL), Prijedor (PD) i Bijeljina (BN) na gradskoj tržnici, u ribarnici i u gradu. Anketom je obuhvaćeno po 39 ispitanika u BL i PD te 30 ispitanika u BN. Cilj rada je bio analiza kupovnih navika potrošača ribe u tri najveća grada Republike Srpske. Polna struktura ispitanika različita je po gradovima, u BL najviše je anketiranih osoba ženskog pola (56,41%), u PD je više ispitanika muškog pola (51,28%), dok je u BN struktura ispitanika bila podjednaka (50:50%). Dominantan je srednjoškolski nivo obrazovanja ispitanika (BL 53,8%, PD 33,3% i BN 56,7%). U sva tri grada dominantno mjesto kupovine ribe je supermarket, dok se najmanje ribe kupuje od ribolovaca i na ribnjaku. Između gradova postoji razlika u danima najčešće kupovine ribe, što je potvrđeno i χ^2 testom ($p<0,05$). U BL se najčešće kupuje riba utorkom, u PD srijedom i petkom, a u BN subotom. Statističkom analizom je utvrđena značajna razlika između posmatranih gradova u pogledu količine kupljene ribe po jednoj kupovini. Ispitanici po gradovima smatraju da je kvalitet ponuđene ribe na tržištu zadovoljavajući i najveći broj ispitanika je na skali od 1 do 5 dalo ocjenu 4. Ispitanici u sva tri grada smatraju da je cijena ribe na domaćem tržištu visoka. Postoji statistički značajna razlika u pogledu preferencije potrošača po gradovima prema kupovini ribe domaćeg porijekla.

Ključne riječi: anketa, navike potrošača, konzumacija ribe.

Keywords: survey, consumer habit, fish consumption

INTRODUCTION

Consumption, and thus the production of fish in the world is constantly growing. According to FAO (2013), in 2011 the production of fish in the world has reached 156 million tons, i.e. a value of 135 billion USD. Out of the total amounts of consumption fish available to consumers, in fish market in the world, 2/3 refers to the caught fish from open waters, and 1/3 on the fish farming. The annual average in the world is 18.9 kg of consumed fish per capita. In Europe, it is 22 kg, in B&H, it is 5.9 kg (FAO, 2011) and in the Republic of Srpska, it is 6.2 kg per capita (National Bureau of Statistics, 2011).

When buying fish, consumers have different motives, habits and attitudes. The extensive researches have been done in the world on the subject of habits and consumer attitudes toward fish consumption, but in B&H almost none. Cluster regression analysis of consumer attitudes of fish consumers in Greece showed that the choice meal of fish is highly dependent on occupation, level of education and number of children, and poorly dependent on the level of income and sex of the respondents (Batzios et al., 2005). Gaviglio and Demartini (2009) surveyed the attitudes of consumers in Italy to wild-caught fish and farm-raised fish, and found that there is a correlation in terms of occupations, the value of purchase and place of purchase (shop type). Research conducted in Belgium (Verbeke et al., 2005), in addition to confirming that the majority of consumers considered fish as healthy food, confirmed that fish is eaten more by women and older consumers (>40 years), and that younger consumers are better informed about the nutritional characteristics of fish. Fay-Komilus et al. (2012) surveyed the attitudes of consumers of fish in Malaysia and their commitment to purchase fresh fish is mostly defined by taste, freshness and structure of fish. Most of them eat fish once a week or once a month, much more in restaurants than at home. Verbeke and Vackier (2004), as the main factors for the purchase of fish in Belgium identified the taste and image of healthy food, and the main factors for not buying fish are bones and price. Smederevac-Lalic et al. (2013) surveyed sellers of fish in Belgrade and based on their responses, the consumer’s decisions on buying fish are affected by price, type and size of the fish. 63% of fish that they sell comes from ponds, and 23.7% of the fish is caught in the Danube. For B&H, only general attitudes of consumers on food consumption (which includes fish) can be found, which showed that the commitment to purchase food is influenced mostly by taste, health reasons, convenience in purchasing and cost, and less by suitability of preparation, ability to control weight, mood and ethical principles (Stojanović et al., 2014).

MATERIAL AND METHODS

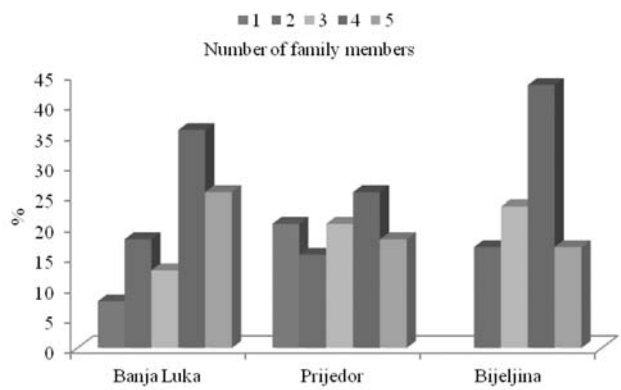
Field research was carried out through the survey („*face to face*”) of consumers in three biggest cities of the Republic of Srpska: Banja Luka (BL), Prijedor (PD) and Bijeljina (BN), with the objectives of determining buying habits and perceptions in fish consumption. The survey was carried out through the structured questionnaire consisting of 25 questions, out of which 2 were open and referred to the expression of views on increasing the consumption of fish and the dominant reason of buying fish of domestic origin. The questionnaire consisted of three parts: socio-demographic data of respondents, motives and buying habits of consumers of the fish in the three largest cities of the RS and characteristics of supply and consumer preferences of fish in the domestic market.

The questionnaire enabled obtaining indicative data on buying habits of domestic consumers of fish. The survey results were analyzed by using analysis of variance and nonparametric tests by using the statistical package SPSS-17.

RESULTS AND DISCUSSION

Socio-demographic indicators

Gender structure of respondents is different in the cities, in BL, the most of the surveyed are females (56.41%), in PD there are more male respondents (51.28%), while in BN, structure of the respondents was equal (50% male and 50% female). In all three cities, in terms of completed school, secondary level education of respondents was dominant (53.8% BL, 33.3% PD and 56.7% BN). In all three cities, in terms of the number of family members (Graph 1), the respondents mostly live in a family of four (35.9% BL; 25.6% PD; and 43.3% BN).



Graph 1. Frequency of family members

The largest number of respondents is in the payment range from 500 to 1.000 KM in PD and BN, while the largest number of respondents has a monthly income of less than 300 KM in BL, which can be linked with the age structure of the respondents. The largest number of respondents (35.9%) in the city of BL was younger than 25 years. In PD, the largest number of respondents was older than 25 and younger than 36 years (28.2%), also a considerable proportion of respondents is in the age group of older than 55 years (23.5%). In BN, the dominant share of respondents aged from 46 to 55 years (40%).

Motives and consumer purchasing habits

In BL, the fish is usually purchased on Tuesdays, in PD on Wednesdays and Fridays, while in BN on Saturday, and the statistical significance was confirmed by χ^2 test ($p<0.05$). In BL and PD consumers usually buy fish on market days, whereas this is not the case in BN. A significant difference exists in volumes purchased in these three cities (p=0.028). By using the Tukey test, statistically significant difference was showed (p=0.021) in the quantities of bought fish from the respondents from BL and BN, while the difference showed in other combinations is a result of incidentally variation.

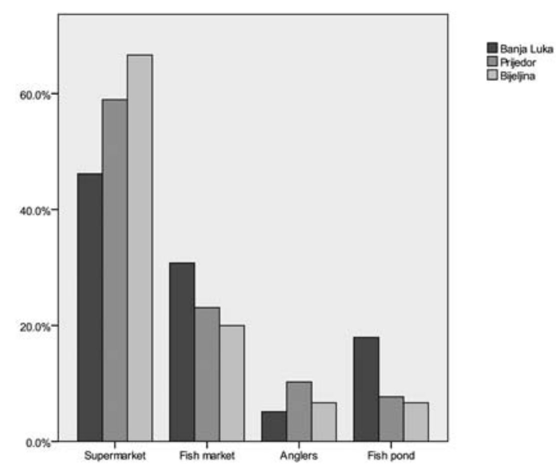
Looking at the impact of the location of the respondents on the preferences of buying fish from domestic producers, χ^2 test showed statistically highly significant difference (p=0.009) in the purchase of fish from domestic and foreign producers. It was shown that consumers in BL mostly prefer to purchase fish from local producers, while it is not the case in PD. It may be noted that the location and preference of buying fish are highly dependent features, i.e. that the location has an impact on the decision of the customers in terms of favouring fish of domestic origin in relation to imported fish.

Table 1. The dependence of buying fish from domestic producers on the respondent locations

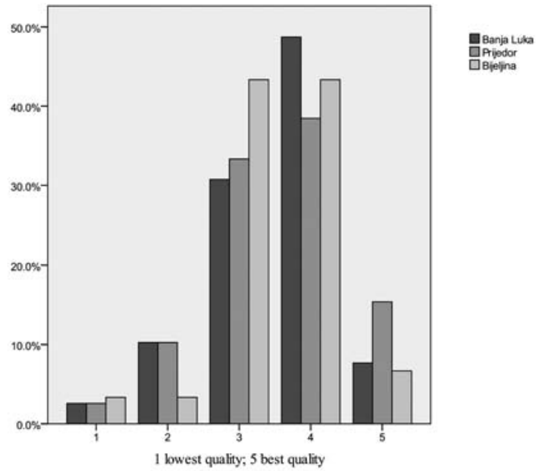
		Do you prefer buying locally produced fish?			Total
		Yes	No	Not important	
Banja Luka	n	28	6	5	39
	%	71.8%	15.4%	12.8%	100.0%
Prijedor	n	15	8	16	39
	%	38.5%	20.5%	41.0%	100.0%
Bijeljina	n	18	1	11	30
	%	60.0%	3.3%	36.7%	100.0%
Total	n	61	15	32	108
	%	56.5%	13.9%	29.6%	100.0%

Characteristics of supply and consumer preferences

By testing differences between the attitudes of consumers in terms of quality of offered fish on the domestic market, it was found that there was no statistically significant difference (p=0.942). When the attitude of consumers toward the quality for fish is analyzed, it shows that the largest number of respondents, on a scale of 1 to 5, gave 4, or it can be said that they were very satisfied with the quality of offered fish. When it comes to the supply of the domestic market with fish, the statistical significance of the observed characteristics was also not found, and the respondents were partially satisfied with the supply of fish markets.



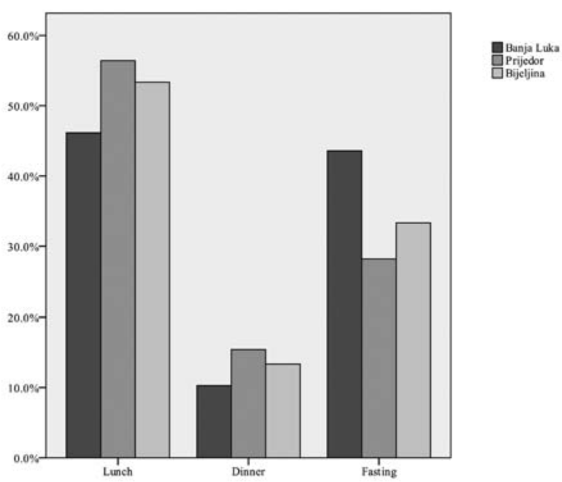
Graph 2. Frequency of fish quality evaluation



Graph 3. Frequency of fish buying place

Respondents in all three cities consider that the price of fish in the domestic market is high. Verbeke and Vackier have come to similar results, and they state that the most common reason for not buying fish are bones and price, i.e. the price is one of the limiting factors of more frequent purchase of fish. Also, Smederevac-Lalic et al. (2013) state that the decision on buying fish is largely influenced by price in addition to other factors.In all three cities the dominant place of buying fish is a supermarket, while the minimum fish is bought from the fishermen and the fishponds. The study of consumer attitudes in Italy (Gaviglio and Demartini, 2009) also states that the fish is most commonly bought in shops. The results indicate that consumers in the surveyed areas prefer to purchase fresh fish, and then frozen fish.

In BL, 59 % respondents has chosen fresh fish, in PD 36%, and in BN 60%. The occasions in which the fish is most commonly consumed are during the fasting season, during the day - for lunch, as the main meal.



Graph 4. Fish consuming occasions

There were two survey questions relating to the similarities and differences in consumer attitudes in the observed cities about information of consumers about the benefits of using fish in the diets and needs of advertising fish consumption in media. By the statistical analysis (χ^2 test) significance of differences has not been established. The majority of respondents in all three cities partly agree that there is sufficient information about the benefits of fish in the diet, but it is still necessary to much more promote the use of fish in the diet because of its health and nutritional importance. The open question of how to increase consumption of fish was answered by the consumers that it is necessary: to improve marketing, improve the quality of offered fish on the market, and work on price competitiveness.

CONCLUSION

Fish consumption in B&H and the Republic of Srpska is significantly below the world average. The habits of consumers in the purchase and consumption of fish in three largest cities in the Republic of Srpska confirmed by the survey that there are the differences in terms of quantity of purchased fish, the days of buying and preferences of the origin of the purchased fish. A statistically significant difference in quantities of purchased fish was found between respondents in BL and BN. In BL, for the majority of the respondents, it is essential whether the fish is of domestic origin or not.

The analysis of consumer attitudes regarding the quality of the fish they buy, the price of purchased fish, supply of fish markets, the place of purchase, type of purchased fish and the levels of awareness of consumers about the importance of fish consumption, and the need for advertising in the media, there were no significant differences in the responses of respondents in BL, PD and BN. The respondents in three largest cities in the Republic of Srpska are satisfied with the quality of offered fish but also considered that the price is one of the limiting factors for the purchase of fresh and primarily freshwater fish.

The research evidenced the attitude of consumers to further improve the quality and supply of fish in the domestic market and the need of producers and retailers for more intensive promotion of fish as a food product, in order to increase consumption of fish in the diet of the local population because of its nutritional and health aspects.

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COMPARATIVE ANALISYS MORPHOMETRIC PARAMETERS BROWN TROUT (*SALMO TRUTTA* MORPHA *FARIO*) FROM LOCALITIES OF RIVERS PLIVA AND VRBANJA

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UPOREDNA ANALIZA MORFOMETRIJSKIH PARAMETARA POTOČNIH PASTRMKI (*SALMO TRUTTA* MORPHA *FARIO*) SA LOKALITETA REKE PLIVE I VRBANJE

Apstrakt

U ovom radu uporedno su analizirani morfometrijski parametri potočnih pastrmki (*Salmo trutta* morpha *fario*) sa dva lokaliteta: izvora reke Plive i reke Vrbanje u avgustu 2010. god. Paralelno sa ovom analizom urađena je i analiza makrozoobentosa kojim su se ribe hranile. Izlovljavanje riba je obavljeno sportskoribolovnom tehnikom, pri čemu je ulovljeno i analizirano ukupno 66 jednki, 33 iz izvora reke Plive i 33 iz reke Vrbanje. Morfometrijska analiza je obuhvatala određivanje: totalne dužine, standardne dužine i mase potočnih pastrmki, kao i dužine i mase digestivnog sistema istih, a ovi paramtri su poslužili za izračunavanje Fultonovog koeficijenta uhranjenosti. Istraživanje je pokazalo promenu u vrednostima parametara potočnih pastrmki sa ova dva lokalitela, kao i promenu istih između polova. Analiza makrozoobentosa koji je služio kao hrana ribama, pokazala je da je najveći udeo u plenu bio iz reda: Ephemeroptera, Trichoptera i Diptera. Larve Diptera iz familije Simuliidae su najčešći plen riba iz rijeke Plive, dok su se ribe iz rijeke Vrbanje najradije hranile larvama Chironomidae. Nije pronadjen ni jedan prazan digestivni sistem potočnih pastrmki u istraživanju. Svi rezultati su statistički obradjeni u programu ANOVA, a razlike izmedju grupa odredjene su stepenom značajnosti pomoću Fiserovog i t-testa.

Ključne riječi: potočna pastrmka, makrozoobentos, morfometrija
Key words: brown trout, macrozoobenthos, morphometric

INTRODUCTION

Natural food plays an important role in the pace fish growth and it depends on the qualitative and quantitative composition of organisms of lower systematic categories in aquatic ecosystems. Very important natural nutritional component of the fish food have organisms from environmental benthic communities, animal organisms belonging to zoobenthos, who settled ichthyofaunal habitats of fish populations. Type *Salmo trutta* morph *fario*, brown trout is wide spread in the waters of Bosnia and Herzegovina. Brown trout lives in the upper reaches of the streams in terms of clean, fast and cold water, rich in oxygen. The values of water temperature at the time of sampling ranged from 8.3°C in the upper reaches of Pliva, to 10.2°C in the upper reaches of the Vrbanja. Concentration of dissolved oxygen is in direct correlation with water temperature, because at higher values of water temperature lower value of dissolved oxygen were recorded. The concentration of oxygen was measured 11.7 gm^{-3}, at Pliva, where was recorded the lowest water temperature. The concentration of oxygen in the river Vrbanja was 11.0 $g\ m^{-3}$. The optimum temperature of water for brown trout ranges from 8 to 12 degrees Celsius, and the concentration of oxygen should be at least 10.0 $g\ m^{-3}$ (Piria, 2007).

Great attention was paid to studying morphological and meristic characteristics of the digestive tract of fishes, brown trout (Bakrač-Bećiraj, 2008; Piria, 2007). Results that were obtained by the Pavlović et al. (2011) give data about diet of brown trout in the upper reaches of Pliva, and give data about state of zoobenthic taxas in source of Pliva. The goal of the research is to determine the basic morphometric characteristics, the mass ratio and Fulton's condition coefficient as well as the analysis of the diet of brown trout from the river Pliva and Vrbanja.

MATERIAL AND METHODS

Brown trouts specimens were collected on rivers Pliva and Vrbanja in the early morning and late evening hours by sport-fishing technique. Dissection, morphometric measurements and determination of sexes of all of 66 brown trouts were performed in the laboratory of Faculty of Natural Sciences and Mathematics, University of Banja Luka. Measured morphometric parameters in fishes were: total body length of fish-TDT, standard body length of fish-SDT, the mass of the fish-MT, the length of the digestive tract-LDT, the weight of the digestive tract-WDT and Fluton's condition coefficient - CF (Markovic, 2012). Morphometric parameters of length were determined by a fixed millimeter scale, and morphometric parameters of body mass with analitic libras with accuracy up to 0.01 grams. Condition factor (CF) is calculated according to the formula:

$CF=\frac{W*100}{TL^3}$, where are: W-total mass of fishes (g), TL- total length of fishes (cm).

Qualitative and quantitative analysis gastro-intestinal of samples of collected macrozoobenthos were performed by stereomicroscope Leica EZ4D. Observed organisms were selected and determined by applying the appropriate keys and manuals (Walace et al., 2003;). Representatives of invertebrates that inhabit the land and which are also found in the digestive tract of collected trouts were also determined (Markovic, 2012). All results are summarized in tables, statistically processed in program ANOVA, presented graphically and discussed.

RESULTS AND DISCUSSION

The mean total body length (TL) in brown trouts (Table, 1.) from the river Pliva was higher than the value of the same parameter for fish that are caught in the river Vrbanja. The difference in the value of this parameter was not statistically significant. The mean standard body length (SL) of brown trouts caught in river Pliva was also higher than those caught in the river Vrbanja, and as well as the previous parameter, nor this difference in values had any statistically significance. Weight of fish specimens found at both locations, in other words the mean body weight (W) shows the difference between fish caught in river Pliva in relation to river Vrbanja and this difference was statistically significant.. Next analyzed morphometric parameter of brown trouts from two locations was the mean total length (LDT) and the weight (WDT) of the digestive system of this fishes. Values of both these parameters were different, and were higher in fishes inhabited river Pliva in relation to fishes from river Vrbanja. The last analyzed parameter was Fulton‘s coefficient, which also showed the difference between fishes from river Pliva, where it was lower than the coefficient from river Vrbanja, and difference was not statistically significant.

Table. 1. Summary values review of morphometric parameters of trouts from localities Pliva and Vrbanja

PARAMETERS		TL	SL	W	WDT	LDT	CF
PLIVA	□	20,40	17,95	95,54	8,20	18,77	1,04
	MIN	14	12,5	29,72	3,09	9,3	0,86
	MAX	29	25,5	224,27	17,33	27,5	1,22
	SD	4,081	3,519	51,482	3,489	4,049	0,099
	KV	13,92	17,06	158,83	12,05	12,39	0,16
VRBANJA	□	18,05	15,88	71,35	5,42	16,81	1,17
	MIN	13,3	11,1	24,16	1,78	8,9	0,93
	MAX	23,9	21,3	163,3	12,04	21,7	1,99
	SD	2,713	2,662	32,713	2,492	3,389	0,166
	KV	6,86	8,21	66,51	6,95	7,776	0,021
	FTEST	0,2370	0,1193	0,0123*	0,0613	0,3187	0,2046
	STEST	0,1075	0,109	0,0261*	0,0741	0,0865	0,1030

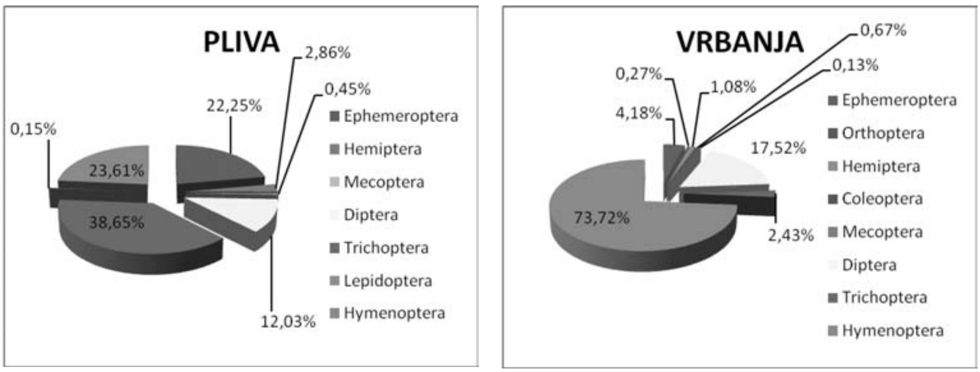
* $p \leq 0,05$ statistically significant

From river Pliva has been collected 14 females and 19 males, and from the river Vrbanja has been collected 18 females and 15 males fishes and they were analyzed.. Analysis of the selected parameters of morphometric measurements by sex can be seen from Table. 2. Brown trouts that were collected from river Pliva had higher values of all parameters except Fulton‘s coefficient in relation to the trouts from river Vrbanja in correlation with sex.

Table 2. Summary values review of morphometric parameters of trouts from localities Pliva and Vrbanja in correlation with sex fishes

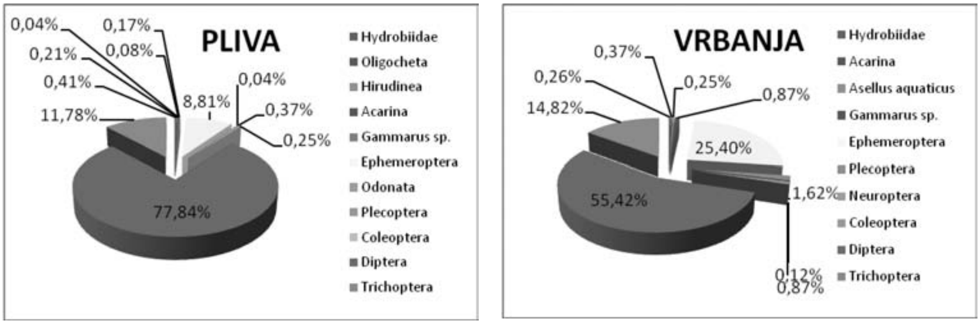
PARAMETRI		TL	SL	MT	WDT	LDT	CF
PLIVA	M□	22,61	19,83	121,13	9,73	19,90	1,01
	F□	18,78	16,57	76,68	7,07	17,94	1,07
VRBANJA	M□	18,43	16,25	75,37	6,17	17,79	1,21
	F□	17,58	15,45	66,523	4,53	15,62	1,12

As in the previous measurement (Tab. 1) difference is in a change of Fulton’s coefficient, more exactly it is higher in brown trouts that were collected from river Vrbanja, and while other morphometric parameters were lower than those in fishes from Pliva.



Graphic 1. Comparative overview of the diversity and presence of terrestrial insects in the gastrointestinal content of brown trouts from rivers Pliva and Vrbanja

Results of the analysis of the gastrointestinal contents, were divided into two categories: terrestrial insects (Graph. 1) and macrozoobenthos (Graph. 2), which brown trouts from Pliva and Vrbanja were fed. From Graph. 1., which shows the diversity and representation of terrestrial insects, it can be seen that the fish that lived in river Pliva mostly chose Trichoptera, Hymenoptera and Ephemeroptera, while those from river Vrbanja mostly chose Hymenoptera and Diptera for their food.



Graphic 2. Comparative overview of the diversity and presence of macrozoobenthos in the gastrointestinal content of brown trouts from rivers Pliva and Vrbanja

Diptera, *Gammarus sp.* and Ephemeroptera larvas were most common prey of brown trouts from river Pliva, and Diptera, Ephemeroptera larvas and *Asellus aquaticus* were also most common prey of trouts from river Vrbanja. Graph. 1. Other taxons are equally represented as food of brown trouts from rivers Pliva and Vrbanja. After comparing the qualitative composition of invertebrate gastro-intestinal content of stream trouts from rivers Pliva and Vrbanja obtained results showed that digestive tract of the fishes from the river Pliva did not contain shrimp species *Asellus aquaticus* and Neuropteras,while that from fishes from river Vrbanja did. Diptera larvas from the family Simulidae were most common prey of the fishes from the river Pliva, while in stomac of fishes that were collected in river Vrbanja Simuliidae were not found. Similar data in the literature came from (Bakrač-Bećiraj et al., 2008).

CONCLUSION

After finishing experimental part in which stream trouts were collected from two locations, the rivers Pliva and Vrbanja, morphometric measurements of the same, analysis of macrozoobenthos that trouts have been fed with, as well as from all the obtained data we can draw the conclusion that the fishes from these two locations, do not differ to a large extent. Water quality at the source of the river Pliva and in the upper reaches of the river Vrbanja is no different, and it was no difference in composition of macrozoobenthos from the bottom of these two rivers as well as in the composition of terrestrial invertebrates which have been feeding component of stream trouts. For this reason, the morphometric parameters of these fishes from two mentioned locations, upon analysis did not differ.

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SIGNIFICANCE OF SOCIAL NETWORKS FOR THE PROCESS OF APPLICATION FOR RURAL DEVELOPMENT SUPPORT

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ZNAČAJ SOCIJALNIH MREŽA U PROCESU PRIJAVLJIVANJA ZA PODRŠKU RURALNOM RAZVOJU

Apstrakt

Za Srbiju kao zemlju u tranziciji, sa značajnim ruralnim resursima, politika ruralnog razvoja je od posebnog značaja za održivi razvoj i kvalitet života seoskog stanovništva. Uticaj i uspeh takve politike zavisi i od mogućnosti poljoprivrednika da dobiju adekvatne informacije i pomoć u procesu prijavljivanja za podršku ruralnom razvoju (PRR). Da bi istražili probleme sa kojima se poljoprivrednici susreću u procesu prijavljivanja za PRR primenili smo teoriju socijalnog kapitala (Coleman1993, Putnam 2000) čije su osnovne komponente: poverenje, društvene norme i društvene (socijalne) mreže. Socijalne mreže olakšavaju protok informacija i pomažu članovima da ostvare pristup resursima koji im mogu doneti (ekonomske) koristi (Lin 2001, Burt 2000, Granovetter 2005, Hoang et al. 2006). Cilj našeg istraživanja je da bio da ispita kakva je uloga formalnih i neformalnih socijalnih mreža u procesu prijavljivanja za PRR.

Istraživanje je urađeno u novembru i decembru 2014. godine, putem intervjua koji su obuhvatili 300 poljoprivrednih gazdinstava u Srbiji (150 u Kragujevcu i 150 u Aleksandrovcu). Rezultati istraživanja pokazuju da je mali broj poljoprivrednika iz uzorka pilot istraživanja koristio podršku za ruralni razvoj (8%), a da su glavne prepreke u procesu prijavljivanja vezane za pripremu dokumenacije, troškovi procedure i nedostatak potrebnih informacija. Ispitanici iz oba regiona od formalnih socijanih mreža (zaduge, nevladine organizacije, strukovna udruženja, savetodavne službe, lokalna samouprava, međunarodni razvojni projekti) doživljavaju samo članove poljoprivredne savetodavne službe, kao kanal informacija o prijavljivanju za PRR. Ove rezultate potvrđuje i niska stopa učešća ispitanika u radu formalnih socijalnih mreža. Istovremeno, rezultati su ukazali da postoji jaka neformalna saradnja među farmerima, i da je pomoć oko tehničke prirpeme aplikacija za

PRR kao razlog nefomalne saradnje nisko rangrana. Zaključak ovog istraživanja je da su neformalne socijalne mreže u istraživanim ruralnim sredinama jake, ali njihov potencijal nije iskorišćen za poboljšanje prijavljivanja za PRR. Ove mreže treba iskoristiti za bolji protok informacija o PRR i za optimalnu mobilizaciju resursa koji proističu iz društvenih veza. Istovremeno, trebalo bi ojačati i kapacitete formalnih socijalnih mreža za bavljenje pitanjima od značaja za PRR.

Ključne reči: socijalni kapital, socijalne mreže, podrška ruralnom razvoju, Srbija
Keywords: social capital, social networks, rural development, Serbia

INTRODUCTION

Rural development is a relatively new concept for countries in transition. For country with significant rural resources, like Serbia, rural development policy is particularly important. The impact and success of such a policy depends on the capabilities of farmers to obtain adequate information and assistance in the process of applying for rural development support (RDP). To investigate the problems that farmers face in the process of applying for RDP, we applied the Theory of Social Capital. Lack of trust in formal institutions and organizations, and strong informal networks are typical features of social capital in the countries in transition (Stiglitz, 1999; Raiser et al., 2002, Tuna 2014). Social networks are useful for information exchange and mobilization of resources which could influence economic development of rural areas. Therefore, the objective of our research is to examine what role formal and informal social networks in Serbia have in process of application for rural development support.

MATERIALS AND METHODS

Social Capital Theory is based on the premise that social structures facilitate actions of individuals who are within the structure (Coleman 1990). Social capital is consisted of social networks, social norms and trust (Putnam 2000). Social networks facilitate flow of information; reduce transaction costs for network members and affect (economic) benefits (Lin 1999, 2001, Burt 2000, Granovetter 2005, Murray 2006). They can be formal or informal. Formal social networks are vertical structures with set of positions linked in authority relations (Lin 2001). Informal social networks are horizontal structures and their members are connected by kinship, friendship, or propinquity (Rose 2000). For poor rural population social networks, especially informal social networks, act as a safety network and help them to gain access to other resources (Hoang et al. 2006).

The paper is focused on the results obtained in November-December 2014 through the survey in rural areas of two municipalities in Serbia (Kragujevac and Aleksandrovac) that belong to the same NUTS II region (Šumadija and Western Serbia), but with entirely different characteristics. Kragujevac (R1) is an example of region with developed professional organizations, regional development agencies, industry and rural economy characterized by intensive farming. Aleksandrovac (R2) is an example of region with less developed organizational structure, and agriculture is the main driver of local economy. Research sample encompassed 300 individuals from farm households in rural areas of R1 (150 individuals in

48 villages) and R2 (150 individuals in 32 villages). Sample selection criteria include rural household that meets the criteria of national statistics to be classified as the farm household and which have a least two members, out of which at least one is younger than 50 years (Kotevska et al, 2015). We have used structured questionnaire which was administered through face-to-face interviews. Questionnaire covered three major research themes: rural development policy; cooperation and networks; and farm household management (Kotevska et al, 2015). Current analysis was focused on questions on application for rural development support and cooperation and networks. These statements were measured through a 5 point Likert scale and yes/no questions. Data from the questionnaire were analyzed by descriptive statistics methods - frequency distributions, mean and standard deviation. Comparative method was used to assess differences in results from two researched regions.

RESULTS AND DISCUSSION

Research results show that small number of farmers in Serbia applied (8%) and used rural development support measures. Answers on set of questions about the application for RDP process indicated obstacles farmers faced in the application process: preparation of documents, related costs, and procedure. In both regions 37-42% respondents consider that it is not easy to access information about RDP.

Data (Table 1) indicate that respondents from both regions perceive extension agency members as primary source of information and assistance with RDP application, while media are ranked on second place. Other formal social networks such as NGO, cooperative, professional organizations are not perceived as important source of information. Although local governments, private consultants and international development projects are low ranked, significant difference exist between two regions ($p<0.05$). In R1 these sources of information exert stronger influence than in R2, which confirms that region selection criteria were good.

Table 1. Best ranked sources of information and assistance with RDP

	National extension agents			Family members			Other people from the village			Media (TV, radio, internet, newspapers)		
	RS	R 1	R 2	RS	R 1	R 2	RS	R 1	R 2	RS	R 1	R 2
Mean	3.360	3.360	3.360	2.810	2.700	2.920	2.513	2.240	2.787	3.143	3.080	3.207
St.dev	1.331	1.313	1.350	1.383	1.432	1.323	1.193	1.164	1.154	1.133	0.860	1.344
Sign. diff			0.500			0.085			0.000			0.167

Results indicate that informal social networks play important role in the process of information and assistance with RDP application. Family members represent and other people from the village are ranked as third and fourth of all formal and informal sources of information and assistance. Some difference is present among the regions, since in R2 other people from the village are stronger source of information ($p <0.05$).

Low ranking of formal social networks as a source of information on the RDP application coincides with low rate of membership of respondents in formal social structures (88

% in R1 and 80% in R2 are not member of organizations (NGO, cooperative, professional organizations). Main reason why farmers do not participate in organizations is that there are no organizations. Second important reason for not being member of organizations in R1 is lack of trust in organizations and in R2 it is lack of time (Table 2). These data indicate that farmers do not recognize existing forms of organizations and that even if organizations do exist they are not functional in the field. Farmers who are members of organizations are members of professional organizations such as associations of wine or fruit producers, cattle or agricultural producers (10.7% in R1, and 17.3% in R2). However, these types of formal social networks do not address issues of application for rural developments support.

Table 2. Reason for not being member of an organization (%)

	Lack of trust	Lack of time	Lack of information	Don‘t see the benefit	Don‘t like the people there	Not functioning well	No such organization	Other
RS	22	26	17	14	3	9	47	2
R 1	31	19	18	12	0	8	47	3
R 2	13	34	16	16	6	11	47	1

High percentage of farmers informally cooperates with other farmers (92% in R1, and 95% in R2). Data also reveal features of such cooperation. Frequency of cooperation is quite regular in both regions. Out of available responses (never, rarely, I‘m not sure, sometimes, and always) in R1 highest percentage of respondents sometimes cooperate, while in R2 highest percentage of respondents always cooperate with other farmers. Main reasons of cooperation in both regions are common problems, followed by non-formal socialization and information exchange. Information exchange about rural development measures in R2 is ranked higher as reason of informal cooperation (47%) than in R1 (19%). This finding isn’t surprising having in mind that majority of households in R2 depend on rural development support (due to investments in increasing perennial crops). Although farmers perceived preparation procedure as a barrier, technical support for RDP application is significantly low ranked as reason for informal cooperation in both regions (3% in R1, 9% in R2). This finding indicates need for adequate formal support in application process.

CONCLUSION

Results indicate that in researched rural areas in Serbia farmers do not perceive formal social networks as support system in RDP application process. Only formal social structure that farmers trust as provider of information and assistance in RDP application process are extension service agents. Therefore, capacities of formal social networks to deal with RDP issues should be strengthen.

On the other side, results indicate that in both regions informal social networks are strong, but their potential is not utilized for the improvement of RDP application and process. These networks should be used for better dissemination of information on RDP and optimal mobilization of resources embedded in social relationships.

ACKNOWLEDGMENTS

The research is done within the regional project "The impact of socio-economic structure of rural population on success of rural development policy", prepared in the framework of the Regional Research Promotion Programme in the Western Balkans (RRPP), which is run by the University of Fribourg upon a mandate of the Swiss Agency for Development and Cooperation (SDC), Federal Department of Foreign Affairs. The views expressed are those of the authors and do not necessarily represent options of the SDC and the University of Fribourg.

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FOREIGN-TRADE EXCHANGE OF BOSNIA AND HERZEGOVINA WITH FISHING PRODUCTS

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SPOLJNOTRGOVINSKA RAZMJENA BOSNE I HERCEGOVINE PROIZVODIMA RIBARSTVA

Apstrakt

Ovim radom je obuhvaćena spoljnotrgovinska razmjena Bosne i Hercegovine (BiH) proizvodima ribarstva za petogodišnji vremenski period (2010-2014). Cilj ovog rada je sagledavanje trgovinske razmjene proizvodima ribarstva (CT 0301-0307) BiH, te najčešćih destinacija u pogledu izvoza i uvoza. Rezultati istraživanja pokazuju da vrijednosti uvoza nadmašuju vrijednosti izvoza čime BiH u okviru glave 3 harmonizovanog sistema carinske tarife bilježi deficit u spoljnotrgovinskoj razmjeni. Analizirajući strukturu navedene glave pojedine carinske tarife i pored toga imaju pozitivne bilanse, ali nedovoljne za pokrivanje ukupnog deficita u okviru ribe i ljuskari, mekušci i ostali vodeni beskičmenjacima. BiH izvozi proizvode ribarstva uglavnom regionalnim partnerima u Srbiju i Hrvatsku, dok je uvoz najvećim dijelom iz zemalja EU.

Ključne riječi: izvoz, uvoz, trend kretanja, destinacija
Keywords: export, import, trend, destination

INTRODUCTION

Fish is highly valued food and it is in demand on the market. It has a major significance in the nutrition of people and its consumption has been increased since 1995 when the world began to realize its nutritional value (Baltić et al. 2009). Fishing, with its full economic, technological, social and other segments can be a significant factor in the common development of local (B&H) food production (Pavličević, 2004). B&H represents a small consumer portion, but that should not be an obstacle to the appearance of many countries with rich offer of fish and fish products in it. B&H has a significant hydro-potential, about 10.000 km of running watercourses, 400 ha of natural lakes, 18.207 ha of water accumulations, 3.300 ha of carp ponds and 10 ha of trout ponds. The length of sea coast is 24 km, and the sea surface covers 1.400 ha (Pavličević et al. 2011, 2014). The aim of this work is to observe trade exchange of the fishing products (CT 0301-0307) of B&H and the most common destinations concerning the export and import.

MATERIALS AND METHODS

For the purposes of this work a research has been conducted by the collection of secondary data from the Indirect Taxation Authority of B&H. This research refers to the five years period (2010-2014). It analyses the structure of export and import of fish, crustaceans and molluscs, as well as the most important destinations of export and import. Considering the type of used data, the method “*desk research*” has been applied. For the tracking of total import and export values a method of trends has been used. The selection of the best adapted trend line has been made on the basis of the value of standard error of the trend. The descriptive statistics for the analysis of the observed phenomena has been applied and the stability of the import and export has been observed through the variation coefficient (C_V). The intensity of the increase or decrease has been analysed with the usage of annual average increase rate. According to the harmonized customs tariff system (CT 01-24) of B&H, the article three „fish and crustaceans, molluscs and other water invertebrates” except from the customs tariff 0308 (“water invertebrates except from the crustaceans and mollusc”) has been analysed for the purposes of this work.

RESULTS AND DISCUSSION

Export of the fishing products

During the analysed period B&H has achieved an average value from the export of fishing products (CT 0301-0307) of 17 million BAM. The intensity of the trend of the observed phenomena is best represented by the parabolic trend $\hat{y}_t = 17829.5429 + 495.8*x - 64.5714*x**2$, whereas the deviation of actual value from the trend line is Sy = 978.6421. The export of the fishing products from B&H has a positive increase rate and during the observed period it has been increased on an average annual rate of 4.41%. During the period from 2010 to 2014 on the level of the article 3 its value was in a range from 15 to 18 million BAM (Table 1).

Table 1. Export of fishing products according to their type from 2010 to 2014 (value is shown in BAM)

CT	Product	Year				
		2010	2011	2012	2013	2014
0301	Live fish	3.642.993	3.660.903	3.421.449	2.688.444	1.932.595
0302	Fresh or cooled fish	5.504.268	6.084.916	6.833.853	6.034.263	6.081.952
0303	Frozen fish	1.723.068	1.461.285	928.132	982.877	721.022
0304	Fish fillets and other fish meat, fresh, cooled or frozen	290.425	53.937	145.539	26.864	19.208
0305	Dried fish, salted or in brine, smoked fish	3.570.426	2.947.745	1.196.359	1.516.009	677.482
0306	Live, frozen or dried crustaceans	245.354	5.275	10.508	94.700	21.105
0307	Live, fresh, frozen, dried molluscs	918.435	4.780.903	4.224.382	6.617.459	9.437.614
	TOTAL	15.894.968	18.994.963	16.760.222	17.960.616	18.890.979

From the analysis of the structure within the article 3 it is evident that B&H has achieved the highest average value of export in terms of customs tariff 0302 with around 6 million BAM. Except from this customs tariff, others had negative trend rates for the observed period. It is interesting that the most intensive increase was registered with CT 0307, that is, with products that are produced in very small amounts in B&H. According to the data of Pavličević et al. (2014), the production of molluscs in B&H is limited to small amounts and its total is around 70 tons pro year.

Table 2. Export of fish, crustaceans and molluscs from B&H (2010 - 2014)

Customs tariff	Average (000 BAM)	Variation interval		C_V (%)	Variation rate (%)
		min	max		
0301	3.069.2	1.933	3.661	24.38	-14.65
0302	6.107.8	5.504	6.834	7.76	2.53
0303	1.163.2	721	1.723	35.59	-19.57
0304	107.2	19	290	106.44	-49.41
0305	1.981.4	677	3.570	61.78	-34.01
0306	75.4	5	245	134.84	-45.89
0307	5.195.8	918	9.437	60.42	79.06

1 € = 1.955830 KM (BAM), Central Bank of Bosnia and Herzegovina on April 28th 2010.

The most intensive decrease of the export from B&H has been registered for the group of products 0304. The export of this group of products has been almost halved, that is, it is decreased for 49%. In the export structure of article 3 the tariff 0302 is dominant with the proportion of 35%.

B&H mostly exports to Serbia and Croatia, and a bit different situation is with the export of „live, fresh, frozen, dried molluscs" which is directed to the countries of EU.

Import of fishing products

During the period 2010-2014 B&H registered higher values of import over export for the observed customs tariffs. Similar to the export, parabolic trend curve best represents the empirical data ($\hat{y}_t$ = 26248.6 + 1247.3*x – 56.5*x*2) with deviation of actual value from the trend line being Sy = 894.1303. Import has increased during the given period on the average annual rate of 5.7%. Average import of fishing products amounted 26 million BAM, namely, its range was from 23 to 29 million BAM, with the C_V of 8.47%.

Table 3. Import of fishing products according to their type from 2010 to 2014 (shown in BAM)

CT	Product	Year				
		2010	2011	2012	2013	2014
0301	Live fish	580.613	870.775	1.785.475	2.215.279	1.975.622
0302	Fresh or cooled fish	2.301.381	2.481.724	1.308.197	1.603.411	1.250.166
0303	Frozen fish	8.990.561	8.970.373	10.249.897	8.991.608	9.493.482
0304	Fish fillets and other fish meat, fresh, cooled or frozen	4.659.424	5.131.761	6.223.730	5.040.249	5.085.860
0305	Dried fish, salted or in brine, smoked fish	471.128	360.677	778.472	409.746	587.501
0306	Live, frozen or dried crustaceans	1.592.089	1.490.974	1.684.350	1.487.847	2.482.577
0307	Live, fresh, frozen, dried molluscs	4.709.076	5.720.947	5.315.587	6.145.762	8.231.392
	TOTAL	23.304.272	25.027.231	27.345.707	25.893.903	2.9106.600

By observing the import structure (Table 4), the greatest value was recorded in terms of customs tariff 0303 (9.3 mill. BAM). The increase of this group of products in terms of the article 3 is very modest and it amounts 1.4%. This is affirmed by the C_V which indicates a pretty stable import trends for this product (5.94%). The most intensive variation rate (35.8%) has been marked with CT 0301 despite the fact that the value of this article in relation to the other customs tariffs is the smallest and it amounts in average of 1.4 million BAM. Negative rate in the increase (-14.2%) of the import was only registered with CT 0302.

Table 4. Import of fish, crustaceans and molluscs to B&H (2010 - 2014)

Customs tariff	Average (000 BAM)	Variation interval		C_V (%)	Variation rate (%)
		min	max		
0301	1.485.6	581	2.215	48.30	35.80
0302	1.788.8	1.250	2.482	31.86	-14.15
0303	9.339.2	8.970	10.250	5.94	1.37
0304	5.228.2	4.659	6.224	11.24	2.22
0305	521.5	361	778	31.98	5.70
0306	1.747.6	1.488	2.483	23.98	11.75
0307	6.024.6	4.709	8.231	22.28	14.98

The most stable import is the one of products from CT 0303 and 0304 with C_V from 5.94% and 11.24%. In the structure of B&H import the most common proportion during the five years period is in average connected to CT 0303 with 35.7%.

Observation of destinations from which the fishing products have been imported to B&H indicates that the destinations from region are not as represented as in export. Dominant place in the countries of region takes Croatia, concerning the import for CT 0301 and 0302. Its proportion when it comes to these two groups is 58% and 70% and this is also supported by the quotes of Knjaza (2007) that Croatia has a positive trend in terms of export of freshwater fish to the countries of EU and Macedonia, B&H, Serbia and Montenegro.

Balance of foreign trade with fishing products (CT 0301-0307)

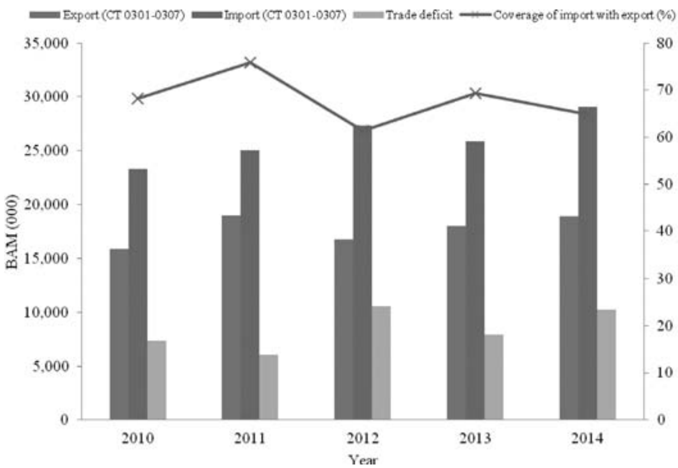

Chart 1. Balance of total foreign exchange of fish, crustaceans and molluscs

B&H has accomplished a negative balance from foreign trade during the analysed period in terms of the article 3. Average deficit for the period 2010-2014 amounted around 8 million BAM. It can be concluded that both the trend of export (Cv_E=7.63%) and import Cv_I= 8.47%) were stable, when observed on the whole level. Export and import have positive average increase rate pro year, but the variation rate of the import (5.72) is more intensive in relation to that of export (4.41). This data explains the deficit due to the fact that the import of fish and crustaceans, molluscs and other water invertebrates to B&H is more intensive than the export (Chart 1).

According to the shown data, B&H had a constant deficit during the observed period in terms of foreign exchange with the fishing products (CT 0301-0307). The increase of deficit in 2014 in relation to 2013 for almost 30% is worrying. This sector has a high level of coverage of imports by exports considering the situation of B&H in frames of agro-industry sector, which shows the more significant deficit according to the results of Ostojić et al. (2010). Vlahović et al. (2011) believe that the exchange deficit could be eliminated also by the improvement of the products quality, by applying the marketing concept and by improvement of technical-technological production, what can affect the price competitiveness of agricultural and food products. The level of coverage of imports with exports was averagely 68%, and maximal value of 75% was realized in 2011 (Chart 2).

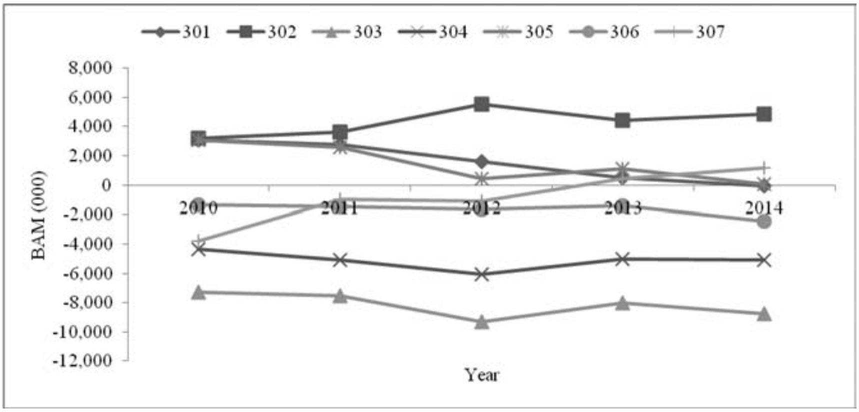

Chart 2. Balance of foreign trade exchange according to CT (0301-0307)

During the whole period CT 0302 have positive balance and the average surplus was around 1.5 mill BAM. Also, the CT 0305 indicates a positive exchange trend with a tendency of constant decrease and if that continues it will most likely have a negative trend in the following period. It can be generally observed that CT 0301 had a tendency of decrease and a negative trend in the last year of the observed period, CT 0302 had a positive trend, CT 0303, 0304 and 0306 indicate a permanent deficit with increase tendency and CT 0307 had a negative balance until 2012, which became positive since 2013 with an increase tendency of export in relation to the import. This is considered interesting due to the fact that this production is on a very modest level in B&H.

CONCLUSION

Generally, it can be concluded that B&H had a more intensive import of fishing products than export during the observed period for CT 0301-0307.The value of import increases on a higher average annual level than the value of export. Analysis of the observed time period, on the basis of standard errors established that the parabolic trend expresses the long-term development tendency in trends of export and import in general (“fish and crustaceans, molluscs and other water invertebrates”). In the total export structure the highest proportion take products from the group “fresh and cooled fish”, whereas in the import the group „frozen fish”. Certain customs tariffs from the article 3 of the harmonised system also indicate surplus in the foreign exchange, mostly the products from the group “fresh and cooled fish”; however this is not enough to compensate the decrease trends of exports in relation to the imports for other groups of products. We can determine a high level of coverage of imports with exports for the fishing products (average 68%) in relation to other agro-industry products. B&H exports most of its products to the countries in region, mostly to Serbia and Croatia, whereas the proportion of the import from the countries of region is negligible. The existing trade exchange deficit should be decreased through the improvement of the quality of export products, through the improvement of technical-technological production and marketing.

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NUTRITION OF COMMON CARP WITH FEED CONTAINING BY-PRODUCTS FROM THE SLAUGHTERHOUSE

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ISHARANA ŠARANA SA HRANOM KOJA SADRŽI NUSPROIZVODE IZ KLANICE

Apstrakt

Nusproizvodi klanične industrije čine više od 50% prinosa prilikom klanja u zavisnosti od vrste životinje i korišćenje ovih sirovina, što je veoma važno za održivost klanične industrije. Pojedini nusproizvodi se koriste i u ishrani ljudi jer sadrže esencijalne hranjive materije. Cilj ovog rada je bio da se formuliše i proizvede hrana za ribe koja sadrži svinjske i goveđe jetre, pluća, slezine i želuce i da se ispita njen nutritivni kvalitet u ishrani šarana. Proizvodnja šarana je vršena tokom sezone 2014. na ribnjaku površine 3,5 ha koji je sagrađen u okviru objekata klanice „Agropapuk“ u Kukujevcima. 2100 kg jednogodišnjeg i dvogodišnjeg šarana je nasađeno, pri čemu je prosečna težina jednogodišnje ribe bila 60 g, a dvogodišnje 600 g. Tokom proizvodnog ciklusa u ishrani riba je korišćenja komercijalna smeša sa 32% proteina i 10% masti. Pored toga, korišćena je hrana koja je pripremljena kao smeša jestivih iznutrica dobijenih iz klanice u kombinaciji sa žitaricama. Na kraju sezone gajenja ukupna proizvodnja na ribnjaku je bila 7500 kg. Konverzija je iznosila 0,9, upotrebom komercijalne hrane i 0,8 sa hranom pripremljenom od jestivih iznutrica i žitarica. Dobijeni rezultati mogu doprineti povećanom iskorišćavanju nusproizvoda iz klanične industrije u proizvodnji hrane za ribe. Ishrana riba u tradicionalnom poluintenzivnom sistemu gajenja u Republici Srbiji je uglavnom zasnovana na upotrebi žitarica, pri čemu je lizin prva limitirajuća amino kiselina i inkorporacija nusproizvoda koji su bogati sa ovom amino kiselinom je od neosporivog značaja. Pored toga, korišćenje slezina u hrani za ribe doprinosi smanjenju pojave anemije kod ribe. Jestive iznutrice su veoma bogat izvor proteina, minerala, vitamina i drugih hranjivih materija. Iako broj zaklanih životinja raste, u mnogim evropskim zemljama se zapaža trend smanjenja upotrebe jestivih iznutrica pogotovo posle pojave goveđe spongiformne encefalopatije (BSE), sa druge strane efikasno iskorišćavanje jestivih iznutrica je krucijalno kako za ekonomsku održivost klanične industrije, a isto tako i za očuvanje životne sredine. Postoji veoma malo podataka o korišćenju iznutrica u hrani za ribe. Dobijeni rezultati ukazuju na veoma dobre proizvodne parametre prilikom gajenja šarana sa upotrebom hrane koja sadrži nusproizvode klanične industrije. Imajući u vidu sve navedeno, jasno je da su dalja istraživanja o korišćenju nusproizvoda iz klanične industrije u ishrani riba neophodna. Efikasno iskorišćavanje nusproizvoda iz klaniče industrije ima direktne pozitivne ekonomske efekte, kao i efekte na smanjnje zagađenja, dok njihovo ne-korišćenje može dovesti do ekonomskih i zdravstenih posledica.

Ključne reči: šaran, jestive iznutrice, parametric prirasta, ishrana
Key words: common carp, edible offal, growth parameters, nutrition

INTRODUCTION

In Republic of Serbia, the slaughterhouse waste management system is generally very poor and only several measures are being taken for the effective management of wastes generated from slaughterhouses (Pelić, 2014). Meat by-products constitute about or more than 50% of the yields of slaughtering, depending upon the animal species slaughtered, and usage of such by-products is important for the viability of the meat industry (Subba, 2002). These by-products are also used for human nutrition, and one of the most important factor both in the utilization of meat by-products for human consumption is sensory acceptability. Edible meat by-products contain many essential nutrients. Liver is used as a fresh for preparing meals or in the industrial manufacture of sausage and different meat products. Spleen and lung are also edible but they are not highly acceptable by consumers, so porcine and bovine lung and spleen are mostly wasted. It is noteworthy that liver, lung and spleen are good sources of protein and of the essential amino acid lysine (Kitts and Weiler, 2003) while bovine spleen is a very rich source of iron (Lönnerdal et al., 2006). General, porcine and bovine liver represent rich sources of vitamin A (Ikeda et al., 2005). The objective of the present study was to develop a fish feed containing processed porcine and bovine lung, spleen liver and stomach and to evaluate its acceptability and nutritional quality for cyprinid fish species.

MATERIAL AND METHODS

Production of carp was carried out during 2014 growing season at fish pond of 3.5 ha which was built near the slaughterhouse in Kukujevci. Hydrated lime was used in amount of 1000 kg/ha in April, May and June, while in July, August, September and October the amount of lime was 2000 kg/ha. 2100 kg of one-and two-year old common carp were stocked in the fish pond (Table 3). The average weight of a one year old carp was 60 g and two-year old carp was 600 g. During the production, commercial extruded fish feed, with 32% of proteins and 10% of fat, were given to fish. Besides that, feed in form of dough prepared as mixture of by-products obtained in a slaughterhouse with the addition of cereals was added. Fresh porcine and bovine spleen, lung, liver and stomach were obtained from the slaughterhouse „Agropapuk“, Kukujevci, Republic of Serbia. Covering capsules and adhering fats were removed from the spleen. Trachea, aorta, bronchial tubes and adhering fat were removed from lung and the lung lobes were cut into slices. Spleen, lung liver and stomach were cooked in water at 90°C. The offal was then minced with grains in the coater to obtain the dough. Formulation of feed is given in Table 1. Feeding was carried out twice a day in 8 am, and the 3 pm. Moisture, protein, ash and crude fat were determined by the standard analytical methods of Scientific Veterinary Institute „Novi Sad“ (Sl. list SFRJ, 15/87; SRPS EN ISO 16634-1:2010; Sl. List SFRJ, čl 28/18; Sl. List SFRJ, 15/87, čl. 29/12). Chemical composition of fish feed (on dry matter basis) is given in Table 2.

RESULTS

At the end of the growing season, the average weight of the two-year old carp was 900 g, respectively 3.8 kg for a three-year old carp. Harvesting weight was 7500 kg. FCR was 0.9 for commercial feed and 0.8 for additional dough feed prepared as mixture of by-products obtained in a slaughterhouse with the addition of cereals (Table 3). Mortality ranged 5% in two-year old carps, and 10% in one-year old carps.

Table 1. Formulation of feed (dough)

Ingredients	**%**
Porcine and bovine spleens	40
Porcine and bovine livers	10
Porcine and bovine lungs	10
Porcine and bovine stomach	10
Soybean meal	10
Wheat flour	10
Corn	6
Bran	2
Yeast	2

Table 2. Chemical composition of fish feed (on dry matter basis)

Nutrient	**Content**
Crude protein, %	29.82
Dry matter, %	46.62
Crude fiber, %	4.40
Crude fat, %	11.33
Crude ash, %	3.39

Table 3. Production parameters of common carp

Total stocking density	600 kg/ha
Stocking density of one year carp	200 kg/ha
Stocking density of two year carp	400 kg/ha
Harvesting density	2150 kg/ha
FCR for comercial feed	0.9
FCR for dought	0.8

DISSCUSION

Obtained results could help to increase the consumption of slaughterhouse’ offal and its utilization in fish feed processing. The traditional fish nutrition in semi-intensive culture systems in Republic of Serbia is cereal-based (Ljubojević et al., 2013ab). Lysine is the first limiting amino acid in cereals (Boisen et al., 2000) and the incorporation of lysine-rich by-products, such as lung, spleen and liver, in a cereal-based formulated fish feed can improve lysine intake of the cyprinids fish. Moreover, use of spleen in a formulated feed can be helpful in reducing anaemic health problems in fish (Adams and Thompson, 2006). According to results of Florek et al. (2012) the offal of livestock are abundant sources of protein comparable with protein from muscle tissue and macro- and micronutrients and such results help increase offal utilization in fish feed industry. Despite of the fact that the number of slaughtered animals has increased, the use of edible by-products for human consumption has declined (Florek et al., 2012). In majority European countries this trend has been strengthened by the appearance of bovine spongiform encephalopathy (BSE) that has given consumers a negative image of edible by-products, which consequently led to restriction of the use of edible offal in pet food (Selmane et al., 2008). On the other hand, efficient utilization of edible offal is essential in order to support economical and viable meat production systems (Kurt and Zorba, 2007). Only a limited number of studies are available on the composition of by-products from the slaughterhouses (Kurt and Zorba, 2007; Florek et al., 2012). Furthermore, the available literature concerning the composition and nutritional value of offal for fish nutrition is scarce. The favorable weight of the carp at harvesting is the result of good environmental conditions that were based on the use of well water, adequate flow, aeration, use of hydrated lime, quality of complete feed and feed supplements from the slaughter industry. Bearing in mind all the above mentioned facts, it is clear that further researches on the use of by-products from slaughterhouses in the diet of fish are required.

CONCLUSION

Efficient utilization of by-products from slaughterhouse has direct positive impact on the economy and environmental pollution of the country, while non-utilization of animal by-products in a proper way may create major economical and catastrophic health problems. Besides hazard and pollution aspects, edible offal (by-products) from the slaughterhouses have a great potential for conversion into valuable sources of proteins and other nutrients in fish feed. Utilization of edible offal from the slaughterhouse leads to favourable growth of fish and satisfactory fish production.

ACKNOWLEDGMENTS

This work was supported by grants from the Ministry of Education, Science, and Technological Development of the Republic of Serbia (project no. TR31011).

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CONDITION OF FISH, SEA BREAM (*SPARUS AURATA*) AND SEA BASS (*DICENTRARCHUS LABRAX*) FROM FISH FARM IN BOKAKOTORSKA BAY (SOUTH-EAST ADRIATIC)

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KONDICIONO STANJE RIBA, ORADA (*SPARUS AURATA*) I BRANCIN (*DICENTRARCHUS LABRAX*), SA FARME U BOKOKOTORSKOM ZALIVU (JUGOISTOČNI JADRAN)

Apstrakt

U ovom istraživanju obrađeno je 210 jedinki orade (*Sparus aurata* Linnaeus, 1758) i 210 jedinki brancina (*Dicentrarchus labrax* Linnaeus, 1758) sa uzgajališta COGIMAR koje se nalazi na lokaciji Ljuta u Bokokotorskom zalivu. Istraživanja su započeta u decembru mesecu 2014. godine i u ovom radu biće predstavljeni preliminarni podaci o dužinsko-težinskom odnosu i kondicionom faktoru ovih vrsta.

Totalna dužina (TL) jedinki orade u uzorku kretala se od 19,9 do 31,1 cm, sa prosečnom vrednošću od 28,1 ± 1,8 cm (prosečna dužina ± standardna devijacija, SD), dok se raspon ukupnih težina kretao od 130 do 485 g, sa prosečnom vrednošću od 337,56 ± 55,75 g (prosečna dužina ± SD). Kod brancina totalna dužina jedinki u uzorku kretala se od 20,8 do 54 cm, sa prosečnom vrednošću od 33,7 ± 3,8 cm (prosečna dužina ± SD), dok se raspon težina kretao od 150 do 1945 g, sa prosečnom vrednošću od 397,84 ± 171,85 g.

Analiza dužinsko-težinskog odnosa kod obe vrste pokazala je da se vrednost parametra b razlikuje od idealne izometrijske vrednosti 3. Kod orade vrednost parametra b iznosi 2,4851, dok je kod brancina vrednost parametra b iznosi 3,0591. Studentov t-test pokazao je da se parametar b dužinsko-težinskog odnosa kod ukupnog uzorka orade i brancina nije statistički značajno razlikovao od idealne izometrijske vrednosti 3.

Koeficijent kondicije kod obe vrste određen je pomoću Le Cren-ove i Fulton-ove formule po mesecima istraživanja i prema dužinskim klasama. Prosečan koeficijent kondicije orade (Slika 3.) iznosi Ka=0,9467± 0,099 (Le Cren, 1951), odnosno Ka=1,4607 ± 0,1875 (Fulton, 1904), a od istraživanih meseci najniža vrednost zabeležena je u februaru, Ka=0,947 (Le Cren), odnosno Ka=1,4196 (Fulton). Kod brancina prosečan koeficijent kondicije (Slika 4.)

za ceo ispitivani uzorak iznosi Ka=1,0164±0,0627 (Le Cren), odnosno Ka=1,0041±0,0721 (Fulton), a od istraživanih meseci najniža vrednost zabeležena je u februaru mesecu Ka=0,9656 (Le Cren), odnosno Ka=0.9512 (Fulton).

Ključne reči: orada, brancin, odnos dužina-širina, faktor kondicije, bokokotorski zaliv
Keywords: Gilthead sea bream, European sea bass, length-weight relationship, condition factor, Bokakotorska Bay

INTRODUCTION

Gilthead sea bream (*Sparus aurata*, Linnaeus 1758) and European sea bass (*Dicentrarchus labrax*, Linnaeus 1758) are demersal species distributed in Eastern Atlantic, entire Mediterranean and Adriatic Sea. Due to its euryhaline and eurythermal habits, both species are found in both marine and brackish water environments, such as coastal lagoons and estuarine areas, in particular during the initial stages of its life cycle (Jardas, 1996). *S. aurata* is protandric hermaphrodite species, maturing first as male (during the first or second year of age, at length 20-30 cm) and after the second or third year of age, at length 33-40 cm, as female. Spawning of this species happens generally from October to December, with sequenced spawning during the whole period (Bauchot et al., 1981; Buxton and Garratt, 1990). *D. labrax* reaches sexual maturity at 23-30 cm at males (second year of life), and 31-40 cm at females (third year of life). Spawning of *D. labrax* happens between November and March (Jardas, 1996).

Gilthead sea bream and European sea bass are the most cultivated species in aquaculture at Mediterranean, and only two fish species cultivated in marine aquaculture in Montenegro. At this moment there are two marine fish farms in Montenegro, both located inside Bokakotorska Bay, and both of them Gilthead sea bream and European sea bass are cultured.

MATERIALS AND METHODS

Sampling of Gilthead sea bream (*Sparus aurata*, Linnaeus 1758) and European sea bass (*Dicentrarchus labrax*, Linnaeus 1758) from fish farm COGIMAR started in December 2014, and continued until May 2015. Each month 50 individuals of both species were sampled. The length was measured to the nearest mm, and the total body weight (using a electronic balance) to the nearest 1 g. Length-weight relationship was determined for the entire sample, according to the formula $W = a \cdot L^b$. Parameters *a* and *b* were estimated using ordinary least–square regression after transforming the data in natural logarithms ($\ln W = \ln a + b \cdot \ln TL$). Modified Student's *t*-test was used to test whether there was a statistically significant difference between the value of slope (parameter *b*) of the length-weight relationship and the ideal, isometric value of 3.

The allometric condition index was determined according to the formula: $Ka = W/aLt^b$ (Le Cren, 1951) where *a* and *b* are the coefficient and exponent of the length–weight relationship, respectively. Additionally, Fulton's condition factor, Ka, was calculated according to the formula $Ka = 100(W/L^3)$, where *W* is the whole body wet weight in grams and *L* is the length in centimeters; the factor 100 is used to bring K close to a value of one.

RESULTS AND DISCUSSION

A total of 210 individuals of Gilthead sea bream (*S. aurata*) and 210 individuals of European sea bass (*D. labrax*) were processed. Total length of *S. aurata* ranged from 19.9 to 31.1 cm, with mean value 28.1 ± 1.8 cm (Figure 1), while weight ranged from 130 to 485 g, with mean value 337.56 ± 55.75 g. For sampled individuals of *D. labrax*, total length ranged from 20.8 to 54 cm, with mean value 33.7 ± 3.8 cm (Figure 1, while total weight ranged from 150 to 1945 g, with mean value 397.84 ± 171.85 g.

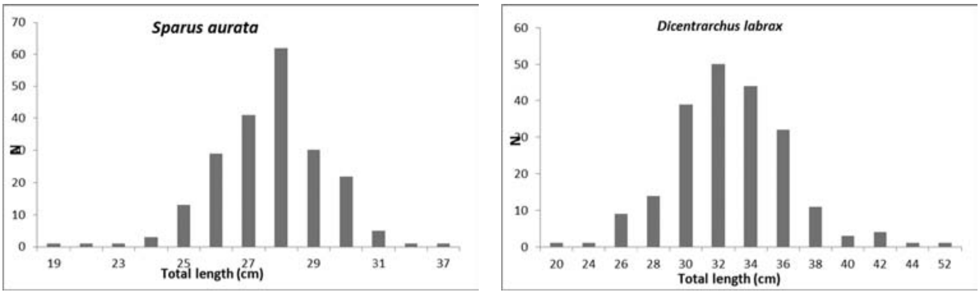

Figure 1. Total length frequency distribution of *S. aurata* and *D. labrax*

The length-weight relationship parameters for the total sample of *S. aurata* show that the parameter *b* (slope) has a value lower than the ideal, isometric value of 3, implying a faster growth in length compared to the gain in weight, or, alternatively, the body assuming a more elongated shape with growth. On the contrary, at *D. labrax* parameter *b* (slope) has a value higher than the ideal, isometric value of 3 (Table 1). Student's *t*-test showed that there is no statistically significant difference between the estimated value of coefficient *b* and the isometric value of 3 at both examined species.

Table 1. Length-weight relationship parameters of *S. aurata* and *D. labrax* total sample (*a* – intercept, *b* – slope, R^2 — coefficient of determination, *t* – t-statistic)

Species	Number	*a*	*b*	R^2	*t*
S. aurata	210	0.0842	2.4851	0.7968	0.524*
D. labrax	210	0.008	3.0591	0.9195	0.0665*

* No statistically significant difference for $p \geq 0.05$

Length-weight relationships of *S. aurata* and *D. labrax* are presented in Figure 2. The highest value of parameter *b* for *S. aurata* is recorded in December, b = 2.8209, and the lowest in March, b = 2.3286. For *D. labrax* highest value of parameter *b* is recorded in March, b = 3.4172, and the lowest in December, b = 2.5653.

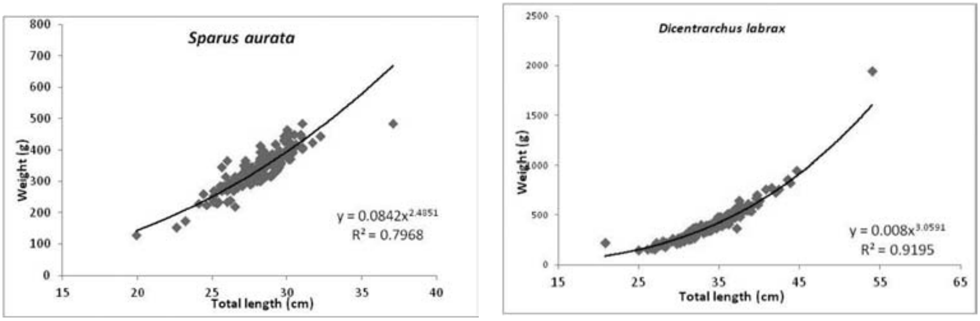

Figure 2. Length-weight relationship of *S. aurata* and *D. Labrax*

Similar results for parameter *b* of length-weight relationship for Gilthead sea bream was reported for some other parts of Mediterranean: $b = 2.736$ (Ceyhan et al., 2009), $b = 2.835$ (Sangun et al., 2007), $b = 2.985$ (Cicek et al., 2006), while for Adriatic Sea reported value is $b = 3.052$ (Kraljevic and Dulcic, 1997). For Adriatic area reported value of *b* parameters for European sea bass are $b = 3.065$ (Dulcic and Glamuzina, 2006) and $b = 3.146$ (Dulcic and Kraljevic, 1996), while for North – West Aegean Sea are reported values $b = 3.158$ and $b = 3.200$ (Koutrakis and Tsikliras, 2003).

Condition factor of Gilthead sea bream by length and by month is presented in Figure 3. Highest value of condition factor is reported in December, $Ka = 1.0504$ and $Ka = 1.5868$ (Le Cren and Fulton respectively), while the lowest value is recorded in February, $Ka = 0.947$ and $Ka = 1.4196$ (Le Cren and Fulton respectively). Lowest values of condition factor are recorded at lengths of 22 and 37 cm, which coincides with length of first maturity (20-30 cm for males, and 33-40 cm for females), when reserves are used for the development and maturation of the gonads , rather than to the growth and development of individuals, for what would normally be used.

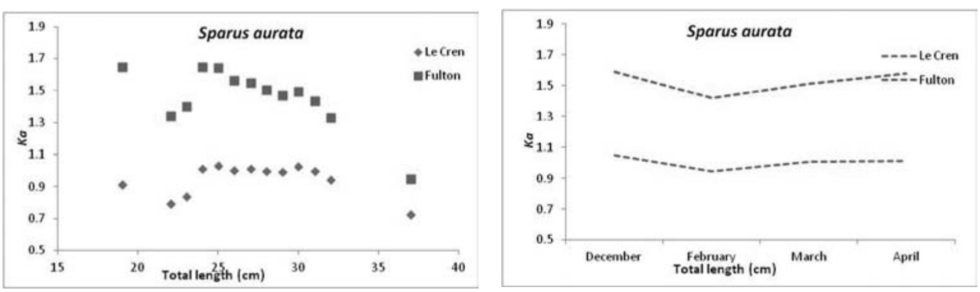

Figure 3. Condition factor of *S. aurata* (Le Cren and Fulton) by length and by month

Condition factor of European sea bass (Figure 4) shows lowest value in February $Ka = 0.9655$ and $Ka = 0.9512$ (Le Cren and Fulton respectively), while the highest values are recorded in April, $Ka = 1.0579$ and $Ka = 1.0466$ (Le Cren and Fulton respectively). Similar to Gilthead sea bream, value of *Ka* is decreasing at the length of reaching sexual maturity.

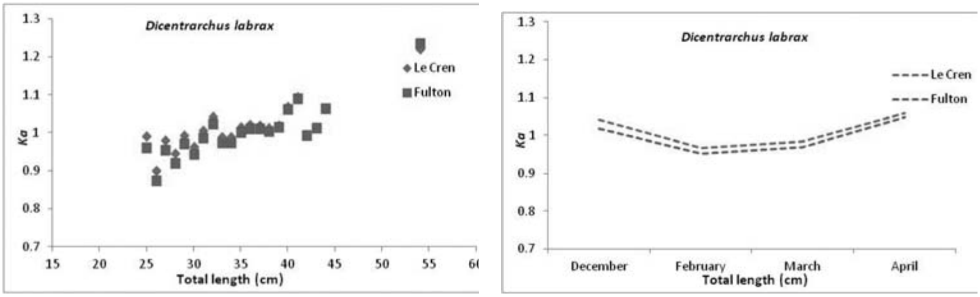

Figure 4. Condition factor of *D. labrax* (Le Cren and Fulton) by length and by month

Earlier reported results of condition factor for *S. aurata* shows similar values: *Ka* = 1.29 – 1.49 (Deguara et al., 1999), or much higher values *Ka* = 1.72 – 2.59 (Faggio et al., 2014). For *D. labrax* reported values for *Ka* are *Ka* = 1.0713 – 1.5862 (Erguden and Turan, 2005). Condition factor is a measure of the condition of fish. If *Ka* < 1 fish is in a poor condition, if *Ka* > 1.4 a fish is in good to excellent conditions. Our results showed that, even if in some months *Ka* values are next to 1, this factor is mostly >1.

ACKNOWLEDGEMENT

This work has been supported by the Ministry of Science of Montenegro and the HERIC project through the BIO-ICT Centre of Excellence (Contract No. 01-1001).

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POSSIBLE CONTRIBUTION OF CASA TEMPUS PROJECT TO AQUACULTURE EDUCATION AND PRACTICE

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DOPRINOS CASA TEMPUS PROJEKTA OBRAZOVANJU I PRAKSI U OBLASTI AKVAKULTURE

O projektu CaSA

Izgradnja kapaciteta srpskog obrazovanja u oblasti poljoprivrede radi povezivanja sa društvom (CaSA) 544072-TEMPUS-1-2013-1-RS-TEMPUS-SMHES (2013 – 4604 / 001 - 001) je projekat iz grupe Strukturnih Mera (SM) finansiran od strane EACEA (Izvršna agencija za obrazovanje, medije i kulturu) u pozivu za TEMPUS projekte iz 2013. godine. Prioriteti za poslednji poziv za TEMPUS projekte su bili: visoko obrazovanje i društvo, obuka ne- univerzitetskih nastavnika i celoživotno učenje (lifelong learning, LLL).

Projekat je osmišljen da obezbedi neophodne obuke glavnih aktera poljoprivrednog obrazovanja : univerzitetskih nastavnika (UT), nastavnika stručnih predmeta u srednjim poljoprivrednim školama (AMS) i savetodavaca u poljoprivrednim stručnim savetodavnim službama (PSSS). Nadalje, predviđeno je osnivanje Nacionalnog repozitorijuma za poljoprivredno obrazovanje, NaRA (Poleksić et al. 2014). U takvom repozitorijumu/riznici znanja će biti smešteni i dostupni svi, za poljoprivredu, relevantni sadržaji. NaRA će biti interaktivna platforma jer će, osim dokumenata sadržati i kurseve, klasične i onlajn, koji će biti na raspolaganju nastavnicima i savetodavcima. Kreiranje kurseva predviđenih za NaRA je aktivnost pripremljena kroz obuke za savremene metode aktivnog učenja/nastave i e–učenja za univerzitetske i nastavnike srednjih škola. Takođe, akademske i komunikacione veštine su uključene u obuke za profesionalce u poljoprivrednom obrazovanju.

Pod «relevantnim sadržajem« koji će biti pohranjen na NaRA podrazumeva se čitav spektar dokumenata - baza podataka i informacija vezanih za savremenu i održivu praksu u svim oblastima poljoprivrede, kao što su: naučni i stručni časopisi koje izdaju poljoprivredni fakulteti učesnici CaSA projekta, zbornici radova i apstrakata skupova koje pomenuti fakulteti organizuju, izveštaji nacionalnih projekata koje finansira Ministarstvo prosvete, nauke i tehnološkog razvoja i Ministarstvo poljoprivrede i zaštite životne sredine, kao i sadržaji relevantni za razvoj profesionalnih veština – akademskih, učenja/nastave, komunikacijskih i upravljačkih.

U projektu CaSA učestvuje 13 partnerskih institucija: 5 univerziteta (4 državna i 1 privatni), udruženja srednjih škola područja rada poljoprivrede, proizvodnje i prerade hrane, Institut za primenu nauke u poljoprivredi, 2 organizacije koje obavljaju obuku, Ministarstvo prosvete, nauke i tehnološkog razvoja i 3 partnerska univerziteta iz EU (Maribor, Temišvar i Foggia). Projektom rukovodi Univerzitet u Beogradu, Poljoprivredni fakultet. CaSA ima 11 radnih paketa koji uključuju: obuke, nabavku opreme, uspostavljanje NaRA (stvaranje virtuelne platforme sa onlajn kursevima i širokom bazom podataka), osnivanje Savetodavnog Odbora NaRA, kreiranje kurseva, njihova implementacija i podnošenje kurseva za akreditaciju/sertifikaciju i na kraju obavezne radne pakete: obezbeđenje kvaliteta, diseminacija rezultata projekta i upravljanje projektom.

Potrebe i mogući doprinos CaSA oblasti akvakulture

Odgovor na pitanje « na koji način CaSA TEMPUS projekat može da doprinese obrazovanju i praksi akvakulture?« leži u samoj ideji repozitorijuma – riznice znanja svih oblasti poljoprivrede i mogućnosti stručnog usavršavanja/osvežavanja znanja koje projekat nudi tokom i posle njegove realizacije.

Činjenica je da je akvakultura u Srbiji, kao i u celom svetu, delatnost koja je u usponu. U našoj zemlji postoje šaranski i pastrmski ribnjaci (Marković i Poleksić, 2009). Ukupna proizvodnja riba je oko 15000 tona. Proizvodnja šarana u Srbiji je u poslednjoj dekadi porasla od 5500 tona do preko 12000 t (Marković i sar. 2013). Iako proizvodnja šarana u Srbiji po podacima Zavoda za statistiku Republike Srbije predstavlja samo 1 – 1,5 % ukupnog obrta u proizvodnji u poljoprivredi, šumarstvu i akvakulturi, postoji ogroman potencijal za značajno veći doprinos proizvodnje riba srpskoj (poljo)privredi. Povrh toga tehnologija i proizvodnja hrane za ribe, prerada ribe, promet i druge aktivnosti vezane za akvakulturu zahtevaju obrazovane stručnjake sposobne da prate najnovija dostignuća nauke i prakse u akvakulturi.

Nažalost, mora se istaći da akvakultura praktično ni ne postoji u ponudi kurseva za stručno usavršavanje nastavnika srednjih stručnih poljoprivrednih škola. Što se može videti na sledećim stranicama: http://katalog2015.zuov.rs/Program2015.aspx?katbroj=891&godina=2014/2015, http://katalog2015.zuov.rs/Program2015.aspx?katbroj=896&godina=2014/2015

Takođe, akvakultura je gotovo “nevidljiva” na web portalu Savetodavne stručne službe: prosečno se pojavljuje samo 1 post godišnje koji se tiče bilo kakvog gajenja riba (po jedan post u 2015, 2013, 2012, 2009 i 2 posta u 2011. godini) (http://www.psss.rs/e107_plugins/forum/forum_viewforum.php?4.125).

Pomenuti prioriteti za TEMPUS projekte se u potpunosti mogu primeniti i na oblast akvakulture. Obrazovanje i njegove veze sa proizvodnjom vodenih organizama mogu biti značajno unapređeni u Srbiji, tako da aspekti upotrebe sistema koji uspostavlja CaSA projekat i NaRA, nacionalni repozitorijum, mogu da se iskoriste za unapređenje sektora akvakulture. Među oko 60 kurseva koji se, tokom projekta, pripremaju od strane univerzitetskih nastavnika, oko 20 kurseva mogu biti korisni za oblast akvakulture, tj. proizvodnju vodenih organizama, njihovu preradu, promet i/ili kvalitet vode.

Ključne reči: TEMPUS projekat, akvakultura, NaRA, stručno usavršavanje

About the CaSA project

Building Capacity of Serbian Agricultural Education to Link with Society, CaSA, 544072-TEMPUS-1-2013-1-RS-TEMPUS-SMHES (2013 – 4604 / 001 - 001) is a Structural Measures (SM) project funded by EACEA (Education, Audiovisual and Culture Executive Agency) under the 2013 Call for TEMPUS projects. Priorities for this, last call of TEMPUS projects, were: Higher Education (HE) and society; training of non university teachers; and lifelong learning (LLL).

The project is conceived to make necessary trainings of main players of agricultural education: university teachers (UT), agricultural vocational secondary/middle school (AMS) teachers and advisors in Agricultural advisory services; and subsequently to establish a National Repository for Agricultural education, NaRA (Poleksić et al. 2014). In such a repository all relevant content for agriculture will be placed, and NaRA will be an interactive platform since it will contain courses, classical and online that will be taken by AMS teachers and advisors. Creation of courses foreseen for NaRA is the activity prepared by trainings: in modern teaching methodology of active teaching/learning (ATL) and eLearning. These trainings were provided to UT and AMS teachers. In addition academic and communication skills were also in the list of trainings for professionals involved in agricultural education.

Under "relevant content" to be placed in NaRA, a broad spectrum of data bases and information, all linked to modern and sustainable practice in all areas of agriculture is identified: scientific and professional journals published by agricultural faculties participating CaSA, Proceedings and Books of Abstracts from meetings organized by these faculties, reports from national projects supported by Ministries of Education, Science and Technological Development and of Agriculture and Environmental Protection, as well as content relevant to development of professional skills - academic, teaching, communication, and management.

There are 13 partner institutions in CaSA: 5 Universities (4 state and one private), the Association of Agricultural Middle Schools, the Institute for Science Application in Agriculture, 2 training organizations, the Ministry of Education (ME), and 3 EU partners Universities (Timisoara, Maribor and Foggia). The project is coordinated by the University of Belgrade. CaSA consists of 11 work packages that include: trainings; equipment purchase; establishment of NaRA, both construction of the virtual platform and its functionalities; NaRA Advisory Board establishment, courses creation, implementation and submission for accreditation/certification; and finally compulsory work packages: quality assurance - QA, dissemination and project management.

Needs and possible CaSA contribution to Serbian aquaculture sector

The answer to the question "How can CaSA TEMPUS project contribute to aquaculture education and practice?" lays in the very idea of knowledge repository for all areas of agriculture and the possibilities of knowledge refreshment offered by the project during and after its lifetime.

The fact is that aquaculture is in Serbia as well an emerging agricultural practice. In Serbia there are trout and carp farms (Marković and Poleksić, 2009). Total fish production is approximately 15 000 tons. Carp production in Serbia increased from 5 500 t to over 12 000 tons in the past decade (Markovic et al. 2013). Although carp production in Serbia represents only 1 – 1.5 % in the total turnover of agricultural production, forestry and aquaculture, according to the Statistical Office of the Republic of Serbia, there is a great potential for significantly higher contribution of fish production in Serbian agriculture. In addition fish feed production and technology, fish processing, trade, and other aquaculture related activities need educated professionals able to follow newest achievements of aquaculture research and practice.

Unfortunately, it must be emphasized that aquaculture is practically nonexistent among courses offered to teachers teaching professional subjects in secondary agricultural schools. http://katalog2015.zuov.rs/Program2015.aspx?katbroj=891&godina=2014/2015, http://katalog2015.zuov.rs/Program2015.aspx?katbroj=896&godina=2014/2015.

Aquaculture is as well almost not visible at all at the web portal of Serbian Agricultural Advisory Service: a single post concerning aquaculture and/or fishery appears approximately once a year (one in 2015, 2013, 2012, 2009, and 2 in 2011) (http://www.psss.rs/e107_plugins/forum/forum_viewforum.php?4.125).

All the above mentioned priorities for TEMPUS projects call apply for the aquaculture sector. Both education and its link to production in aquaculture could be improved in Serbia, therefore aspects of the use of a system established by the CaSA project and NaRA creation will be used for improvements in the aquaculture sector. Among approximately 60 CaSA courses under preparation by UTs, there are about 20 courses that might be useful for aquaculture i.e. linked to aquaculture production, processing or trade, and/or water quality.

Keywords: TEMPUS project, aquaculture, NaRA, in-service training

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GENETIC STUDIES PROBLEMS OF EUROPEAN GRAYLING (*THYMALLUS THYMALLUS*) OF RUSSIAN EUROPEAN NORTH

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GENETSKA STUDIJA LIPLJANA (*THYMALLUS THYMALLUS*) NA SEVERU EVROPSKOG DELA RUSIJE

Apstrakt
U ovom radu je izložen komercijalni i akvakulturni značaj porodice lipljana (*Thymallidae*) na teritoriji Rusije. Posebna pažnja je poklonjena genetičkim studijama ove familije, uzimajući u obzir da populacije evropskog lipljana u Rusiji još uvek nisu u dovoljnoj meri istražene. Rad zapravo predstavlja polaznu genetičku studiju evropskog lipljana u svrhu konzervacije ove vrste. Studija uključuje prikupljene uzorke od 5 jedinki evropskog lipljana (*T. thymallus*) iz slivova reka Severna Dvina i Kuloj, 2 sibirska lipljana (*T. arcticus*) iz sliva reke Ob i jednog uzorka lipljana iz gornjeg toka reke Jenisej. Analiza MtDNA je urađena koristeći 3 fragmenta gena ATP6, CR i COI. Po prvi put su dobijeni genetički podaci riba iz reke Kuloj. Od uzoraka svih riba, jedino su identifikovani haplotipovi evropskog lipljana.

Ključne reči: Thymallus thymallus, CR, ATP6, COI, mtDNA haplotipovi
Keywords: Thymallus thymallus, CR, ATP6, COI, mtDNA haplotypes

INTRODUCTION

On the territory of Russia *Thymallidae* are widespread almost in all water basins of Siberia, Far East and European North. But in Central and Southern Russia, European grayling (*T. thymallus*) inhabits the Volga and the Ural Rivers’ basins and some populations are included in the regional Red Books. Scientific concerns are connected both with commercial use and protection of the grayling. Detailed genetics aspects of European grayling conservations are formulated in Gum *et al.* (2009). Morphologic and systematics analyses of graylings were conducted in Russia for a long time (Svetovidov, 1936; Tugarina & Khramtzova, 1980; Zinoviev, 1980; Knizhin, *et al*. 2004, etc.). At the same time, systematics of genus *Thymallus* was constantly being reconsidered and revised. Grayling populations from Asian part of Russia (east of the Ural Mountains) are regarded as the most important ones for theoretical studies due to diversity of their forms.

The commercial use of grayling in Russia has many aspects. Grayling is highly appreciated for its taste qualities, and conserved by salting, smoking or drying. Grayling is one of favorite objects of recreational angling, its presence being important factor of regional tourism strategies. Grayling is perspective fish for water bodies stocking; the hatcheries exist in Sayan, Baikal, Siberia and Ladoga. Also, grayling is frequently reared on trout farms. At the moment, principles and techniques of grayling farm rearing is sufficiently developed. But the real practice of grayling used as an aquaculture object and fishery object still needs more attention in Russia.

The ichthyological studies (as well as fisheries) of European North of Russia have traditionally concentrated on the most valuable commercial species, namely Atlantic salmon (*Salmo salar* L.) and Brown trout (*S. trutta* L.), while European grayling populations of large territories stay underexplored, though in the Yemtsa, a tributary of the Northern Dvina, were caught up to 2 tons of grayling. While grayling populations of Asian part of Russia were extensively studied, both morphologically and genetically (Knizhin, *et al*., 2004; Froufe *et al*., 2005; Weiss *et al*., 2006), genetic studies of European grayling of Russia are still based on small quantity of samples (Koskinen *et al*., 2000; 2002). Only one sample from the territory of Archangelsk region (the Sjamzhenga River, eastern tributary of the Northern Dvina) was studied, mtDNA haplotypes characteristic for Siberian grayling (*T. arcticus*) were found at two specimens (Koskinen *et al*., 2000). It was suggested that hybridization zone of European and Siberian grayling is situated more to the west than was being supposed earlier. But the question of hybridization zone border is still not resolved. The objective of our study involves Koskinen *et al.* (2000) statement: „Molecular investigation, including assessment of nuclear loci, for a dense sample from the Archangelsk region and east of the Urals needs to be conducted to conclusively resolve this issue”.

MATERIALS AND METHODS

Samples were collected according to our study objectives (Table 1). ATP6 gene and control region (CR, D-loop) of mtDNA were chosen because of using in phylogenetic *Thymallidae* studies (Froufe *et al*., 2005; Weiss *et al*., 2006; Marić *et al*., 2011) and mtDNA COI, as more conservative fragments which used for DNA barcode project (Ivanova *et al*., 2007).

Sample from the Toimushka River (the Northern Dvina basin) was taken from archived scales, containing degraded DNA less than 700 bp. As a result of short DNA fragments, PCR products were either weak or undetected. Hence internal primers were designed for amplification. PCR conditions are being optimized to gain stable amplification products.

Table 1.

Samples	Basin	N	Year	Tissue
Toimushka river	Northern Dvina	24	1999	archived scales
Ustya river	Northern Dvina	1	2014	fin (ethanol)
Oboksha river	Northern Dvina	2	2015	fin (ethanol)
Kelda river	Kuloy river	10	2104	fin (ethanol)
Laka river	Kuloy river	14	2015	fin (ethanol)
Bolshoe Shchuchye Lake	lower Ob` river	10	2007	liver (ethanol)
Bolshoe Hadata Lake	lower Ob` river	16	2007	liver (ethanol)
Maimamysh river	upper Enisey river	3	2013	fin (ethanol)

RESULTS AND DISCUSSION

The preliminary results of CR and ATP6 sequences for tributaries of the Northern Dvina and the Kuloy rivers were obtained. Only one haplotype was found within CR sequences and three haplotypes were found within ATP6 gene. A cladogram is constructed, based on obtained and GeneBank sequences. Our samples were displayed in red color (Fig. 1 and 2).

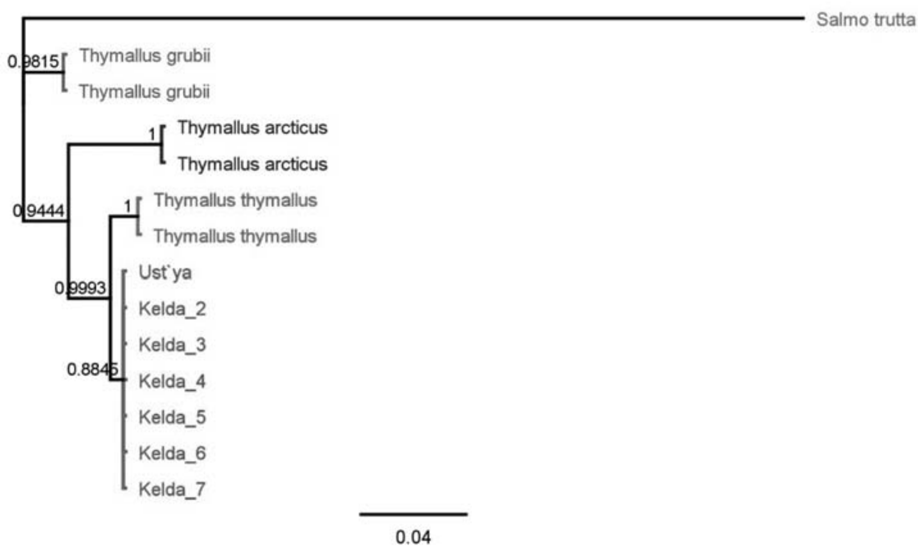

Figure 1. Consensus tree of mtDNA control region (CR) haplotypes for *Thymallus sp.* based on algorithm MrBayes 3.2.1., HKY model. The tree is rooted with haplotype from European brown trout (*S. trutta*). Haplotypes of our specimens are in red color.

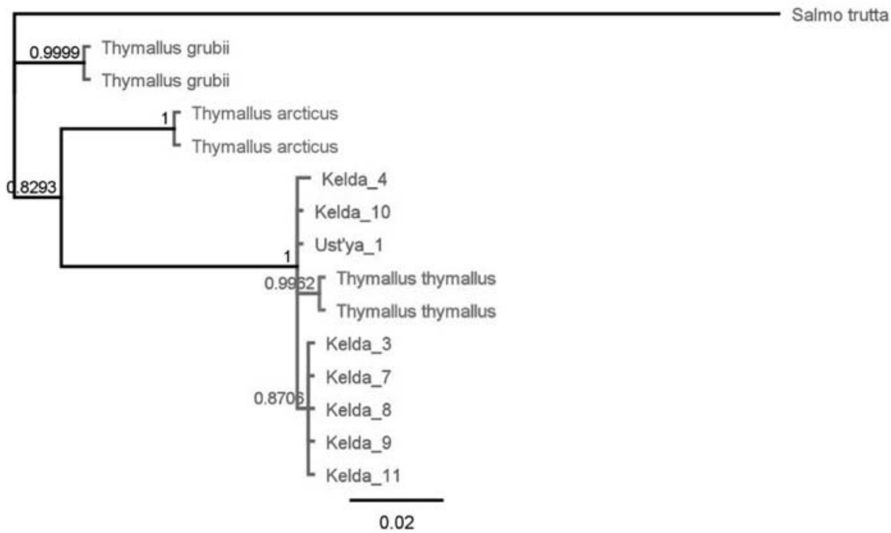

Figure 2. Consensus tree of mtDNA ATP6 gene haplotypes for *Thymallus sp.* based on algorithm MrBayes 3.2.1., HKY+G model. The tree is rooted with haplotype from European brown trout (*S. trutta*). Haplotypes of our specimens are in red color.

Genetic data from the grayling population living in the Kuloy River were obtained for the first time. Though it could seem more probable to find Siberian grayling haplotype in rivers which, like Kuloy, are situated closer to the Urals Mountains, haplotypes close to Siberian grayling were not found in studied specimens. To obtain further genetic information, microsatellite analysis is planned.

ACKNOWLEDGMENTS

We express our thanks to Yu.G. Vedernikov for help in collecting samples and to N.V. Gordeeva for granted samples.

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WATER QUALITY ASSESSEMENT OF JABLANICA RIVER BASED ON AQUATIC MACROINVERTEBRATE COMMUNITY

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ANALIZA KVALITETA VODE REKE JABLANICE NA OSNOVU ZAJEDNICE VODENIH MAKROINVERTEBRATA

Apstrakt

Cilj rada je da se prikažu rezultati ocene kvaliteta vode reke Jablanice (sliv Kolubare) zasnovane na prisutnoj zajednici vodenih makroinvertebrata. Jablanica je relativno kratka (25 km) desna sastavnica, koja u blizini Valjeva sa Obnicom (leva sastavnica) formira Kolubaru. Kolubara, kao poslednja veća pritoka Save, protiče kroz područje sa izraženim i različitim antropogenim uticajima. Sama Jablanica nastaje spajanjem većeg broja potoka sa obronaka planina Jablanik i Medvednik. U skladu sa zahtevima EU prilikom ocene stanja rečnih sistema neophodno je utvrđivanje referentnih uslova. Da bi se utvrdilo recentno stanje, i usaglašenost sa zahtevima za dostizanje referentnog statusa, izvršeno je ispitivanje ove reke u aprilu 2013. godine na tri lokaliteta gornjeg toka. Kao parametri za ocenu kvaliteta vode, korišćeni su sledeći pokazatelji: ukupan broj taksona, saprobni indeks (SI; Zelinka & Marvan), BMWP i ASPT skorovi, broj taksona grupa Ephemeroptera, Plecoptera and Trichoptera (EPT) i indeks diverziteta (SWI; Shannon-Wiener’s indeks). Zabeležena su ukupno 72 taksona makroinvertebrata. Kao najraznovrsnije grupe izdvajaju se Trichoptera (16 taksona) i Ephemeroptera (15 taksona). U odnosu na saprobnu vrednost, utvrđeno je da je većina prisutnih taksona osetljiva na organsko zagađenje (ksenosaprobni i oligosaprobni organizmi). Dobijene vrednosti praktično svih korišćenih pokazatelja kvaliteta vode, upućuju na veoma dobar status (klasa I), čime su ispunjeni uslovi da se ispitivani deo toka Jablanice može smatrati referentnim stanjem, za potrebe istraživanja sliva Kolubare. Puštanje u rad akumulacije u Rovnima (srednji tok Jablanice) može izmeniti zatečeno stanje, zbog čega je neophodan redovni monitoring.

Ključne reči: Saprobni indeksi, referentni uslovi, kvalitet vode, reka Jablanica, Srbija
Key words: Saprobic Indices, Reference conditions, Water Quality, Jablanica River, Serbia

INTRODUCTION

The Jablanica River is a relatively short right branch of the Kolubara River, which along with the left branch (the Obnica River), near the city of Valjevo (western Serbia) forms the Kolubara River (at elevation of 190 m.a.s.l.). The Jablanica itself is formed by numerous mountain brooks, flowing from the northern slopes of Mt. Jablanik (1274 m.a.s.l.) and from the eastern slopes of Mt. Medvednik (1244 m.a.s.l.). The river is 25 km long, with a drainage area of 150 km². In its middle course, near the settlement of Rovni there is dam construction ongoing, which is not yet in operative state. The future reservoir will alter the aquatic biota and the water status of this river. Because the Jablanica is a short watercourse and is situated in relatively unpolluted surroundings, without high influence of settlements, except the city of Valjevo, it is considered as a clean mountainous river. As one of the constituencies of the Kolubara, it could be regarded as a spring part of this river. Knowing that the Kolubara basin is densely populated, and under various anthropogenic pressures, for the purpose of ecological monitoring and restoration in the basin, it is important to have referent null point for comparison. For the upper stretch of the Jablanica River the water quality assessment based on aquatic macroinvertebrates was done in year 2013, in order to check if this part of river could be considered as the “referent” point (referent conditions).

MATERIALS AND METHODS

The sampling was performed in April of 2013 (high water level regime) at three sampling sites (Table 1). Semi-quantitative samples were taken with a standard benthological hand net (25x25 cm, 500 µm mesh size), in accordance with the AQEM protocol (2001). All samples were preserved with 60-80% ethanol solution and further processed in the laboratory. Identification of macroinvertebrates was done using appropriate taxonomic keys.

Table 1. Sampling localities of the Jablanica River

Locality	N lat	E long	Altitude (m.a.s.l.)
JAB 1	44° 11’ 31’’	19° 41’ 37’’	498
JAB 2	44° 12’ 23’’	19° 42’ 23’’	370
JAB 3	44° 12’ 57’’	19° 43’ 15’’	321

As water quality indicators following metrics were used: Total number of taxa, Saprobic Index (SI; Zelinka and Marvan, 1961), BMWP (Biological Monitoring Working Party) Score, ASPT (Average Score Per Taxon), number of Ephemeroptera, Plecoptera and Trichoptera (EPT) taxa (Armitage et al, 1983) and Diversity Index (Shannon, 1948). Saprobiological analysis were carried out using a list of bioindicator organisms according to Moog (1995). The AQEM software was used for all calculations (AQEM, 2002). Water quality assessment was performed in accordance with the national legislation (Official Gazette of the R. of Serbia 74/2011), based on ecological status classes for small and medium-sized streams, altitude up to 500 m, with the dominance of large substrates.

RESULTS AND DISCUSSION

A total of 72 aquatic macroinvertebrate taxa were identified. Having in mind that some groups, most notably chironomids, were not identified to the species level, the diversity is probably higher. Trichoptera (16 taxa) and Ephemeroptera (15 taxa) were found to be the most diverse groups. The highest diversity was recorded at the site JAB 1, with 50 identified taxa, while the lowest was found at the locality JAB 2 with 38 recorded taxa. The recorded diversity is slightly higher than in some previous investigations (57 taxa; Stefanović et al., 2009). Marković et al. (1998) during early 1990s recorded 100 taxa in total in the Jablanica River, but this research covered a prolonged period of time (three years). With regard to taxonomic composition, the dominance of Trichoptera and Ephemeroptera corresponds to results of Stefanović et al. (2009) where the dominance of the same groups was recorded (Ephemeroptera – 19 taxa, Trichoptera – 14 taxa), while in the case of Marković et al. (1998), besides Ephemeroptera (19 taxa), dominance of Oligochaeta (21 taxa) were also found. These differences in diversity and taxonomic composition, in comparison to previous research, could be explained by selection of sampling sites – in our study, where only upper river stretch was investigated.

Regarding the percentage share of the groups, Ephemeroptera were found to be the most abundant (38,3%), while Coleoptera (19,3%), Diptera (16,1%) and Trichoptera (13,2%) were also significant members of the macroinvertebrate assemblages.

In relation to ecological classification of taxa regarding its saprobic valence (Moog, 2002) the majority of taxa are sensitive to organic pollution, with 21% of identified taxa belonging to xeno- and oligosaprobic organisms. Taxa tolerating the low degree of organic pollution (β –mesosaprobic) were present with 17,5%, while taxa adapted to higher organic load (α –mesosaprobic and polysaprobic organisms) with only 5% of total community. However, it should be mentioned that for the majority of taxa (56,5%) there is a lack of data regarding its saprobic preferences. Assemblages in all three localities have the same structure regarding sensitivity to organic pollution (Figure 1).

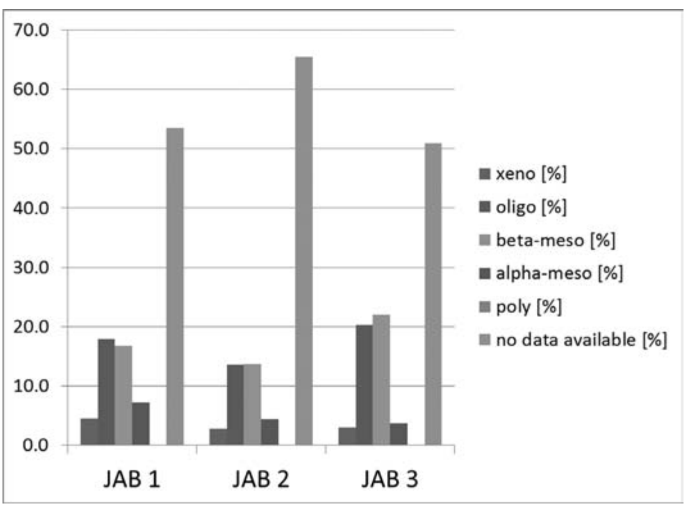

Figure 1. Percentage participation (%) of main saprobic groups in the community of the Jablanica investigated localities

Table 2. Values of calculated metrics and water quality classes for upper stretch of the Jablanica River

Locality	JAB 1		JAB 2		JAB 3	
Total Number of Taxa	50	I	38	I	46	I
SI	1.523	I	1.536	I	1.539	I
BMWP	162	I	142	I	169	I
ASPT	7.043	I	6.762	II	7.348	I
SWI	3.101	I	2.574	I	3.283	I
EPT	24	I	19	I	26	I

In regard to obtained values of used metrics, water quality at all sampling sites, is assessed as very good (class I), except in the ASPT at site JAB 2, whose value corresponds to II quality class (Table 2). Having in mind results of some previous investigations of this river (Marković et al. 1998; Stefanović et al. 2009) it could be noted that water quality of this river remained of high quality for longer period of time. Considering this, the Jablanica River, and particularly its upper part could be used as reference point for the assessment of the Kolubara River. Future forming of the Rovni reservoir lake could affect this upper part of the river as well, so it is important to maintain continuous monitoring.

CONCLUSIONS

During the investigation of the Jablanica River 72 macroinvertebrate taxa were identified. In regard to species richness, Trichoptera and Ephemeroptera were found to be dominant groups. Number of taxa per locality was high, and varied from 38 to 50, corresponding to very good water quality (class I).

Obtained values of saprobic index (SI), BMWP, SWI and EPT indicate very good water quality at investigated sites. The ASPT values, except at site JAB 2 (class II) also corresponds to very good water quality. In relation to ecological classification of taxa regarding its saprobic valence, it was found that the majority of taxa are sensitive to organic pollution. Overall good water quality of the River Jablanica in this study, confirms that this river has potential to be used as referent point for monitoring of its recipient river Kolubara.

ACKNOWLEDGEMENTS

The study was supported by the Ministry of Education and Science of the Republic of Serbia, Projects Grants No. III43002 and ON173025.

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WATER QUALITY ASSESSEMENT OF MLAVA RIVER BASED ON AQUATIC MACROINVERTEBRATES

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ANALIZA KVALITETA VODE REKE MLAVE NA OSNOVU VODENIH MAKROINVERTEBRATA

Apstrakt

Cilj rada je da se predstave rezultati ispitivanja kvaliteta vode reke Mlave, zasnovani na prisutnoj zajednici vodenih makroinvertebrata. Mlava, sa dužinom toka od 78 km i površinom sliva od 1885 km², jedna je od značajnijih pritoka Dunava u Srbiji. Kao najvažniji izvori komunalnog i industrijskog zagađenja mogu se izdvojiti gradovi Požarevac (70000 stanovnika; u donjem toku) i Petrovac na Mlavi (8000 stanovnika; srednji tok), kao i Žagubica (2600 stanovnika; gornji tok). Slivno područje, naročito u srednjem delu toka, je i pod uticajem spiranja sa poljoprivrednih površina, kao i komunalnih otpadnih voda iz brojnih manjih naselja.

Negativni efekti, ovih antropogenih uticaja mogu biti još izraženiji, zbog specifične hidrologije, odnosno preovlađivanja stanja niskih voda u slivu ove reke. Uzorkovanje je izvršeno u julu 2013. godine. Kao parametri za ocenu kvaliteta vode, korišćeni su sledeći pokazatelji: Saprobni indeks (SI; Zelinka & Marvan), BMWP i ASPT skorovi, broj taksona grupa Ephemeroptera, Plecoptera and Trichoptera (EPT) i indeks diverziteta (SWI; Shannon-Wiener’s indeks). Ukupno 72 taksona makroinvertebrata su identifikovana. Insekti su bili najraznovrsnija komponenta, a u okviru njih najbrojnije su bile grupe Ephemeroptera (13 taksona) i Trichoptera (11 taksona). Najveća raznovrsnost zabeležena je na lokalitetu Leskovac (33 taksona). Većina taksona je tolerantna na određeni stepen organskog zagađenja (β –mezosaprobni i α–mezosaprobni organizmi), naročito u donjem delu toka (Rašanac i Požarevac). Vrednosti SI na svim lokalitetima upućuju na veoma dobar i dobar kvalitet vode (klase I i II). Vrednosti BMWP i ASPT ukazuju na veoma dobar kvalitet vode samo na lokalitetima u srednjem i donjem delu toka (Leskovac i Požarevac), dok je u gornjem delu toka, kvalitet vode, ili loš (BMWP), ili umeren do dobar (ASPT). I preostali pokazatelj (EPT), takođe, ukazuje na bolji kvalitet vode u donjem delu toka. Na osnovu svih korišćenih pokazatelja, kvalitet vode reke Mlave u julu 2013. godine se može oceniti kao dobar (klasa II).

Ključne reči: Saprobni indeksi, biotički indeksi, kvalitet vode, reka Mlava, Srbija
Key words: Saprobic indices, biotic indices, water quality, Mlava River, Serbia

INTRODUCTION

The Mlava River is one of the larger tributaries of the Danube in Serbia. The river is 78 km long, the river basin is 1885 km², and with an average annual discharge of 13,3 m³/s (maximum in April – 28,2 m³/s, minimum in September with 3,1 m³/s) at the confluence into the Danube, near settlement of Kostolac (Gavrilović and Dukić, 2002). The Mlava springs (Vrelo Mlave) in Žagubica settlement (Kučajske Mountains) in eastern Serbia is at 325 m a.s.l..

The Thermal Power Plant „Kostolac” (TPP „Kostolac”) and surface coal mine “Drmno” are situated in the lower river stretch, near its confluence with Danube, affecting the river with wastewater and coal waste. The main pollution sources of communal and industrial wastewater, are the city of Požarevac (70000 inhabitants; in the lower course), the city of Petrovac na Mlavi (8000 inhabitants; middle course) and the Žagubica settlement (2600 inhabitants; upper course). Besides, the river basin and the river itself, especially in its middle course, are affected with agricultural land washout, and communal discharge from numerous smaller settlements. Such anthropogenic pressures are particularly serious because of predominance of low waters in the basin (Manojlović et al., 2003).

The aim of the paper is to present data of the water quality assessment based on present macroinvertebrate assemblages at selected sites along the Mlava River.

MATERIALS AND METHODS

The samples were collected in the July of 2013 (low water level regime) at five sampling sites (Table 1). AQEM protocol was applied (AQEM, 2001) with semi-quantitative sampling performed using a standard benthological hand net (25x25 cm, 500 µm mesh size). Samples were preserved using 60 - 80% ethanol solution and further processed in the laboratory. Identification to the species level for the main taxonomic groups was done by using appropriate taxonomic keys.

Table 1. Location of sampling sites at the Mlava River

Locality	N lat	E long	Altitude (m.a.s.l.)
Mlava Spring	44° 11.498‘	21° 47.033‘	325
Gornjak Gorge	44° 15.593‘	21° 33.05‘	192
Leskovac	44° 21.321‘	21° 26.170‘	128
Rašanac	44° 27.418‘	21° 20.126‘	103
Požarevac (downs.)	44° 41.606‘	21° 12.907‘	74

For the assessment of water quality following metrics were used: Saprobic Index (SI; Zelinka & Marvan, 1961), BMWP (Biological Monitoring Working Party) Score, ASPT (Average Score Per Taxon), Number of Ephemeroptera, Plecoptera and Trichoptera (EPT) taxa (Armitage et al., 1983) and Diversity Index by Shannon-Wiener's (SWI; 1948). Saprobiological analyses were carried out using a list of bioindicator organisms according to Moog (1995). All calculations were done in AQEM software (AQEM, 2002). Water quality assessment, was performed according to the national legislation (Official Gazette 74/2011) for Type 2 (large rivers with medium grain-size mineral substrates, except Pannonian plain rivers) and Type 3 (small and medium-sized streams, altitude up to 500 m, the dominance of large substrates) rivers.

RESULTS AND DISCUSSION

A total of 67 aquatic macroinvertebrate taxa were identified. Insects were the most diverse macroinvertebrates component, with Ephemeroptera (13 taxa) and Trichoptera (11 taxa) as the most diverse groups. The highest diversity was recorded at the site Leskovac (middle course), with 33 identified taxa, while the lowest was found at the Gornjačka Gorge with only six recorded taxa. Ephemeroptera and Mollusca, with 17% and 15% participation in the community were the most abundant groups. The majority of recorded taxa at the Mlava River, could be classified as tolerant to a certain degree of organic pollution. According to the ecological classification of taxa in relation to saprobic valence (Moog, 2002), 26.7% of identified taxa belongs to β -mesosaprobic group of organisms, while 9.8% of taxa could be described as α -mesosaprobic. Relatively high proportion of taxa (22.4%.) are sensitive to organic pollution (xeno-saprobic and oligo-saprobic species). On the other hand, species adapted to the high organic load (polisaprobic taxa) are represented with only 0.37% of the total number of taxa. For 40.7% of recorded taxa there is no data in relation to its saprobic tolerance. As it could be expected, the dominance of taxa sensitive to organic pollution (xeno-saprobic and oligo-saprobic) was recorded at the sites of the upper river stretch (the Mlava Spring and Gornjak Gorge). In the lower part of the river course (Rašanac and Požarevac), the most abundant were taxa tolerant to some degree of organic pollution (α and β -mesosaprobs) (Figure 1).

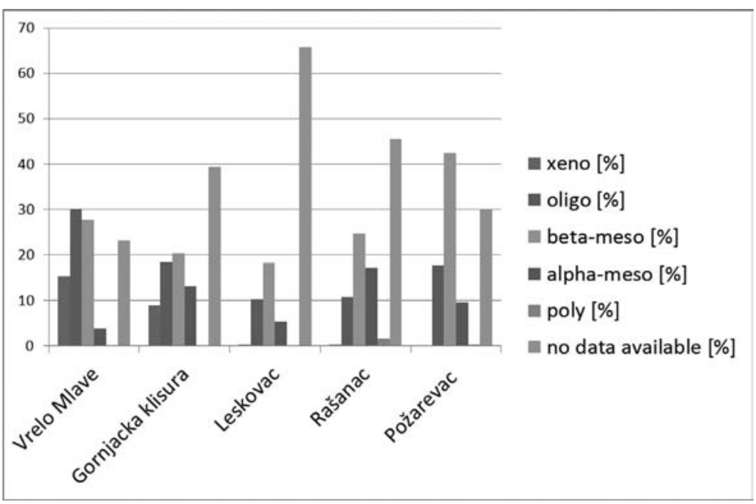

Figure 1. Percentage participation (%) of the main saprobic groups at investigated localities of the Mlava River

Saprobic index ranged from 1.304 (Gornjak Gorge) to 2.161 (Rašanac). Values of BMWP and ASPT metrics ranged from 34 (Gornjak Gorge) to 132 (Leskovac) and from 4.778 (Mlava Spring) to 7.33 (Leskovac), respectively (Table 2).

For all localities obtained values of SI indicate very good and good water quality (water quality classes I and II) (Table 2). High values of BMWP (>60) were recorded at localities situated at the lower river course (Leskovac and Požarevac), while corresponding ASPT (>6.00) were detected at the same sites and at Gornjak Gorge, as well (Table 2).

Values of BMWP and ASPT parameters indicate very good water quality at this lower river stretch (sites Leskovac and Požarevac), while the water quality, according to BMWP, in the upper stretch is poor, or moderate to good, according to ASPT (Table 2). The EPT metric, also, indicates better water quality in the lower stretch, with very good status (class I) at localities Leskovac and Rašanac, and moderate status at locality downstream of Požarevac. Contrary, at localities in the upper river course, a low number of EPT taxa indicate very poor water quality (Table 2).

According to all used biotic metrics the water quality of the Mlava River in July of year 2013 could be assessed as good (class II). Surprisingly, the water quality was better in the lower river stretch, because of higher values of BMWP, ASPT and EPT metrics.

Table 2. Values of calculated metrics and water quality classes for investigated localities

Locality	Mlava Spring		Gornjak Gorge		Leskovac		Rašanac		Požarevac (down-stream)	
Water Body	ML_7		ML_4		ML_3		ML_2		ML_1	
River Type	Type 3		Type 3		Type 2 (HMWB)		Type 2 (HMWB)		Type 2 (HMWB)	
Total No. of Taxa	12		6		33		23		16	
SI	1.507	I	1.304	I	1.825	I	2.161	II	1.864	I
BMWP	43	IV	34	IV	132	I	56	II	69	I
ASPT	4.778	III	6.8	II	7.333	I	5.091	II	6.273	I
SWI	1.953	II	1.626	II	2.866	I	2.42	I	2.49	I
EPT	2	V	4	V	15	I	12	I	3	III
Average	III		III		I		II		II	

CONCLUSIONS

During the investigation of the Mlava River 67 macroinvertebrates taxa were identified. Ephemeroptera and Trichoptera were found to be dominant groups regarding species richness. Number of taxa per sampling site varied from only 6 to 33.

Calculated saprobic index (SI) indicates very good water quality at majority of investigated localities. The resulting BMWP and ASPT scores were in compliance with the obtained SI only for the middle and the lower stretch, while for the upper course BMWP and ASPT indicated significantly lower water quality (comparing to SI). The values of used diversity index (SWI) were in accordance with SI, indicating good and very good water quality along the investigated river course. The EPT metric, on the other hand, indicates very good water quality only in the middle course, while in the upper course water quality is found to be very poor. This could be explained by the fact that springs in general are known for their lower diversity due to homogenous and oligotrophic habitat. The low quality of water at site Gornjak (low diversity) could also be consequence of above mentioned. This discordance, especially in the case of the upper river stretch (type 3, according to 74/2011), reveals need for further improvements of this evaluation system. Overall good water quality of the River Mlava in this study could be partly explained by the decreased discharge of industrial waste waters in recent years in this poorly developed part of Serbia.

ACKNOWLEDGEMENTS

The study was supported by the Ministry of Education and Science of the Republic of Serbia, Projects Grants No. III43002 and ON173025.

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THE OCCURRENCE AND POPULATION CHARACTERISTICS OF INVASIVE FISH AMUR SLEEPER (*PERCCOTTUS GLENII*) IN OXBOW LAKES IN SOUTH-EASTERN POLAND

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PRISUSTVO I POPULACIONE KARAKTERISTIKE INVAZIVNE VRSTE AMURSKI SPAVAČ (*PERCCOTTUS GLENII*) U MRTVAJAMA U JUGOISTOČNOM DELU POLJSKE

Apstrakt

Amurski spavač (*Perccottus glenii,* Dybowski 1877) je vrsta ribe koja se u toku poslednjih godina smatra alohtonom vrstom za prirodne vodene ekosistemime Centralne Evrope. U Poljskoj je prvi put zabeležena 1993. godine u srednjem delu reke Vistula. Zbog njenih morfoloških i bioloških predispozicija ona predstavlja pretnju za prirodne vodene ekosisteme. Ova vrsta ribe se karakteriše velikom proždrljivošću, otpornošću na nepovoljne faktore sredine i brojnim drugim odlikama karakterističnim za invazione vrste. Cilj ove studije je bio da se odredi prisustvo, abundanca i procentualni udeo amurskog spavača u fauni riba u mrtvajama koje se nalaze u jugoistočnom delu Poljske.

Istraživanja su obavljena u toku dva dana 2012. godine i obuhvatila su šest mrtvaja (No 1-6) koje se nalaze u basenu reke Wieprz. Izlov ribe je izvršen korišćenjem aparata za elektroribolov (IUP-12, 220-250V, 7A). Svi izlovljeni primerci riba su određeni do nivoa vrste i izmerena je njihova totalna dužina (Lt; ± 1 mm) i totalna težina (W; ± 1 g). Određen je ihtiofaunistički sastav mrtvaja i abundanca amurskog spavača, kao i dužinska i težinska struktura i gustina (kao CPUE, tj. ind.*100m^{-2}*h^{-1} izlovaljavanja) ove vrste.

Ukupno je nađeno 14 vrsta riba u istraživanim mrtvajama. Amurski spavač je nađen u 4 od 6 ispitivanih mrtvaja. Totalna dužina amurskog spavača je bila u rangu od 34 mm do 128 mm. (u proseku 55 ± 26 mm) i varirala je u zavisnosti od istraživanog lokaliteta. Prosečna biomasa amurskog spavača je bila 5.4 ± 7.3 g i varirala je od 1 g to 12 g. Proporcionalni udeo amurskog spavača u abundanci riba je varirao od 40% u mrtvaji u blizini lokacije Latyczów (No 2) do 67.3% u mrtvaji u blizini grada Wał (No 6). Gustina ove alohtone vrste je varirala od 4.0 do 35.6 CPUE i zavisila je od istraživanog lokaliteta. U dužinskoj strukturi populacije amurskog spavača dominirale su jedinke sa totalnom dužinom manjom od 50 mm.

U cilju identifikacije i određivanja puteva širenja amurskog spavača u nove oblasti, potrebno je vršiti kontinualni monitoring ihtiofaune. Štaviše, bilo bi korisno pronaći biološke karakteristike ove vrste koje bi omogućile njenu eliminaciju iz slatkovodnih ekosistema.

Ključne reči: amurski spavač, Perccottus glenii, invazivne vrste riba, populacione karakteristike

Abstract

Amur sleeper (*Perccottus glenii,* Dybowski 1877) is fish which in recent years has been regarded as an alien fish species in natural water ecosystems of Central Europe. In Poland, it was first recorded in 1993 in the middle part of the Vistula River. Because of its morphological and biological predispositions it is a threat to natural water ecosystems. This fish is characterized by high voracity, resistance to adverse environmental factors and a number of features conducive to invasion. The aim of this study was to determine the presence, abundance and percentage contribution of Amur sleeper in the fish fauna of oxbow lakes located in south-eastern Poland.

The study was conducted on two different terms (June and August) in 2012 in six oxbow lakes (No 1-6) located in the Wieprz River basin. Control fishing was carried out using electric fishing gear (IUP-12, 220-250V, 7A). All caught fish were identified to species and their total length (Lt; ± 1 mm) and weight were measured (W; ± 0.1 g). The species composition of the fish fauna of the oxbow lakes and the abundance of Amur sleeper were determined, as well as the size structure and density (in CPUE, i.e. ind.*$100m^{-2}*h^{-1}$ of fishing) of Amur sleeper.

A total of 14 fish species were found in the oxbow lakes. Amur sleeper was reported in four of the six surveyed oxbows. The total length of Amur sleeper ranged from 34 mm to 128 mm (on average 55 ± 26 mm) and varied depending on the study site. The average biomass of Amur sleeper was 5.4 g (± 7.3 g), ranging from 1.3 g to 11.8 g. The proportional contribution of Amur sleeper in the abundance of fish ranged from 40% in the oxbow in the vicinity of Latyczów (No 2) to 67.3% in the oxbow lake near the town of Wał (No 6). The density of this alien species ranged from 4.0 to 35.6 CPUE and depended on the study site. The size structure of the Amur sleeper population was clearly dominated by individuals with a total length of less than 50 mm.

To identify and determine the routes of dispersion of Amur sleeper into new areas, continuous fish fauna monitoring should be conducted. Moreover, it would be useful to find biological features of this species that would enable its elimination from freshwater ecosystems.

Key words: Amur sleeper, (Perccottus glenii), invasive fish, population characteristics

INJURIES AND DEFORMITIES IN COMMON CARP FINGERLINGS GROWN IN DIFFERENT STOCKING DENSITIES – PRELIMINARY RESULTS

RENATA RELIĆ, BOŽIDAR RAŠKOVIĆ, DALIBOR VUKOJEVIĆ, MARKO STANKOVIĆ, ZORKA DULIĆ, ZORAN MARKOVIĆ, VESNA POLEKSIĆ

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POVREDE I DEFORMITETI KOD ŠARANSKE MLAĐI GAJENE U RAZLIČITIM GUSTINAMA NASADA - PRELIMINARNI REZULTATI

Apstrakt

Povrede i deformiteti predstavljaju pokazatelj narušene dobrobiti jer ukazuju na neodgovarajuće uslove gajenja i utiču na ponašanje, zdravstveno stanje i životni vek riba. Ove promene kod potrošača izazivaju odbojnost i mogu da smanje iskoristljivost ribljeg mesa. U ovom istraživanju utvrđeni su vrsta i karakter povreda i deformiteta na telu šaranske mlađi posle 96 dana boravka u tankovima sistema sa recirkulacijom vode (RAS). Mlađ je bila raspoređena u četiri gustine nasada: 1,31 kg/m^3, 2,62 kg/m^3, 3,94 kg/m^3 i 5,24 kg/m^3 (I, II, III i IV grupa). Grupa sa najmanjom gustinom nasada imala je najveću stopu preživljavanja (100%) i najmanje povreda i deformiteta. Povrede su najčešće uočene bočno, na sredini tela, kao i na repnom peraju. Prosečno su obuhvatale do 1% tela i na njihovu površinu, kao i na broj riba sa deformacijama nije uticala gustina nasada, ($p > 0,05$). Pojava deformitetea u različitim gustinama nasada kretala se od 1,60 to 6,92% ($p < 0,05$), a najčešće su zapažene lordoza i skraćenje gornje vilice. Detaljnije istraživanje karaktera i uzroka promena na telu može da doprinese pronalaženju optimalne gustine nasada za šaransku mlađ u tankovima, a time i poboljšanju proizvodnih rezultata i pružanju maksimalne zaštite njihove dobrobiti.

Ključne reči: šaran, RAS, gustina nasada, povrede, deformiteti, dobrobit
Key words: *common carp, RAS, stocking density, injuries, deformities, welfare*

INTRODUCTION

Injuries and deformities can be often observed in fish in intensive rearing systems that are characterized by increased stocking density. In such condition the potential for injuries caused by other fish such as skin lesions, scale loss, split fins and broken fin rays also increases, and they have been used as indicators of poor welfare (EFSA, 2008). Injuries are a precursor to subsequent infection and fish mortality, and healed wounds reduce the commercial value of afflicted fish. Skeletal deformities also represent an important economic problem in aquaculture. Deformed fish are less acceptable to consumers and it may be difficult to fillet. Deformities may have the physiological and behavioral implications, and they reflect an imbalance in the fish environment thus giving them importance in the terms of welfare (Kocour et al., 2006; Jaward et al., 2014). They may be associated with a degeneration of the bone structure with age, or genetic, nutritional, and environmental factors and management, or relate to each other, making difficult to determine a single causative agent (Argüello-Guevara et al., 2014).

Optimal stocking density for carp in tanks is not fully defined. In study by Karakatsoulis et al. (2010) 1.22 kg/m^3 was low and 4.89 kg/m^3 was high stocking density. Increased stocking density can deteriorate water quality and thus adversely affect the survival, growth and development of young carps. The aim of this study was to determine the incidence and character of injuries and deformities observed in common carp fingerlings in different stocking densities.

MATHERIAL AND METHODS

The study was carried out in recirculation aquaculture system (RAS) of the CEFAH (Centre for Fishery and Applied Hydrobiology of the Faculty of Agriculture, University of Belgrade, Serbia). In period of 96 days 450 specimens of common carp (*Cyprinus carpio*) with the initial weight of 10.48±0.02 g were held in 120-litre plastic tanks with constant water flow of 0.5 l/min. They were distributed in four groups in three replicate tanks according to the stocking density: 1.31 kg/m^3, 2.62 kg/m^3, 3.94 kg/m^3, 5.24 kg/m^3 (I, II, III and IV group, respectively). The groups were formed of apparently healthy, normal shaped fish with no injuries and damages of body and fins. Fish were fed 3% of the commercial extruded mixture (38% of proteins and 12% fat, "VZ Subotica"- Serbia), in relation to the ichthyomass. In tanks, the average water temperature was 21.69 °C, pH 8.34 and dissolved oxygen 4.52 mg/l.

On 96th day of the study all carps were harvested and fish with any kind of changes on their body were recorded by Nikon Coolpix P90 12.1MP Digital Camera. In digital images' analysis localization and character of the changes were determined. Bodily injuries are identified as: (1) *fresh lesion* - light red skin defect, or bleeding, (2) *older lesion* - lack of the skin or deeper tissues with signs of healing process at the edges, (3) *the scar* - a mark left on the skin or within body tissue where a wound has not healed completely and fibrous connective tissue has developed, and (4) *missing scales* - area without scales, with no signs of skin inflammation. The size of wounded area on the fish body was determined according to Adamek et al. (2007). Fin injuries were divided in three categories: (1) *splitting*, (2) *erosion* and (3) *thickening* (Noble et al., 2012). Deformities were classified as spine deformities - *lordosis* (1) and *scoliosis* (2), and mouth deformities - *shortened upper jaw* (3) and *shortened lower jaw* (4). Survival rate was determined as: the final number of the fish per group x 100 / the initial number of the fish per group. Data were analyzed by STATISTICA 8.0 Software (StatSoft, Inc. 2007), and Microsoft Office EXCEL 2007.

RESULTS AND DISCUSSION

The highest survival rate and the lowest occurrence of injuries and deformities in the lowest stocking density (Group I) were recorded. Differences in the number of surviving fish are not statistically significant ($P > 0.05$), but differences in the number of fish with bodily changes are significant ($P < 0.05$) (Table 1).

Table 1. Survived, injured and deformed fish at the end of the study (%)

Group	Survived fish* (%)	Injured fish (%)			Deformed fish (%)		Fish affected by any type of change** (%)
		body + fins	body only	fins only	injuries + deformities	deformities only	
I	100	-	2.22	-	2.22	-	2.22
II	92	2.41	7.23	-	1.20	1.20	12.05
III	93	10.40	4.00	1.60	0.80	0.80	17.60
IV	88	1.89	4.40	2.52	1.89	4.40	15.09

* $P > 0.05$ (Chi-Square test); ** $P < 0.05$ (Chi-Square test)

Affected fish have had single or multiple lesions on the body and/or on the fins, as well as single or multiple deformations along with some injury or separately. In Table 2 the incidence of injuries at certain areas on the body and their categorization by character of the process is shown.

Table 2. Localization and category of lesions in the fish with injuries

Type	Ranking according the incidence		Category*			
	Localization	%	1	2	3	4
body injuries	lateromedial	26.53	4.08	8.16	-	**16.33**
	tail base	18.37	2.04	12.24	4.08	-
	dorsolateral	16.33	2.04	4.08	4.08	6.12
	ventrolateral	16.33	2.04	12.24	2.04	-
	dorsocranial	10.20	4.08	2.04	2.04	2.04
	upper lip	8.16	-	6.12	2.04	-
	lower lip	6.12	4.08	2.04	-	-
	behind operculum	4.08	2.04	2.04	-	-
	laterocranial	4.08	-	2.04	2.04	-
	behind dorsal fin	2.04	-	2.04	-	-
fin injuries	tail fin	28.57	-	24.49	2.04	/
	dorsal fin	26.53	-	26.53	-	/
	anal fin	12.24	-	8.16	4.08	/
	pelvic fin	2.04	-	2.04	-	/

* *body injuries*: (1) fresh lesion, (2) older lesion, (3) the scar, and (4) missing scales; *fin injuries:* (1) splitting, (2) erosion and (3) thickening

Most frequently, the lack of scales in the middle of the fish body i.e. lateromedial and erosion of the tail i.e. caudal fin were observed. At all localizations there were lesions in regeneration process (category 2), followed by fresh injuries and scars (categories 1 and 3). Erosion was the most common change of fins followed by thickening, while splitting was not observed. Erosion results in loss of both the epithelial fin tissue and the whole or part of the fin ray. Thickening refers to nodular, opaque thickening along the distal edge of an affected fin. (Noble et al., 2012).

The integrity of the skin-scale complex provides a relatively impermeable barrier to water and electrolytes. Epidermal damage such as scale loss, wounds and ulcers can result in a loss of body water and changed ion balance, which produces an osmotic stress that potentially can be life threatening. Ulceration affecting as little as 10% of the body surface area can result in high acute mortality. The degree of mortality is directly related to the amount of skin damage (Bouck and Smith, 1979). In this study, average size of skin area under lesions (%) was (Mean ± SD): 0.90 ±0.00 (Group I), 0.66 ± 0.33 (Group II), 1.04 ± 0.68 (Group III) and 0.77 ± 0.75 (Group IV). According to Kruskal-Wallis test stocking density did not affect the size of surface under injuries, and Mann-Whitney U test showed no significance in results of the groups (both $P > 0.05$). In Table 3 the incidence of deformities in affected fish is showed, as well as the incidence of deformities in the each group.

Table 3. The incidence of deformities

<table>
<tr><th rowspan="2">Type</th><th colspan="2">Ranking according the incidence</th><th colspan="4">Incidence in groups** (%)</th></tr>
<tr><th>Category</th><th>%</th><th>I</th><th>II</th><th>III</th><th>IV</th></tr>
<tr><td rowspan="2">Spine deformities</td><td>lordosis</td><td>8.77</td><td rowspan="5">2.22^{ab}</td><td rowspan="5">2.41^{ab}</td><td rowspan="5">1.60^a</td><td rowspan="5">6.92^b</td></tr>
<tr><td>scoliosis</td><td>5.26</td></tr>
<tr><td rowspan="2">Mouth deformities</td><td>shortened upper jaw</td><td>8.77</td></tr>
<tr><td>shortened lower jaw</td><td>5.26</td></tr>
<tr><td colspan="2">Total deformed among affected fish</td><td>26.32*</td></tr>
</table>

* $P < 0.05$ (Kruskal-Wallis ANOVA); ** $P > 0.05$ (Chi-Square test, calculated on the total number of fish/group); No significant difference between values marked with the same letter ($P > 0.05$); Significant difference between values marked with different letters ($P < 0.05$) (Mann-Whitney U test)

Almost one third of all affected fish have had some deformity ($P < 0.05$), and the most common were abnormal, V-shaped curvature of the caudal vertebral column (lordosis), and shorten upper jaw. Spinal and mouth deformities were equally represented (both 14.03% of cases). Al-Harbi (2001) has found spinal deformity in 18.2% of deformed fish and predominant types of spinal deformities were lordosis and scoliosis. Kocour et al. (2006) included a possibility that some mouth deformities could be a consequence of pathogen infestations.

Group III had the lowest percent of deformed fish and Group IV the highest (1.60 and 6.92%, respectively) ($P < 0.05$). The occurrence of deformities was not significantly influenced by the treatment ($P > 0.05$).

CONCLUSSIONS

In this study, most frequently the lack of scales in the middle of the body and erosion of the tail fin were observed. The most common deformities were lordosis and shorten upper jaw. Differences between the groups in the number of fish with bodily changes are significant ($P < 0.05$). Stocking density did not show the influence on average size of damaged skin area and number of fish with deformities per group ($P > 0.05$). This study provides a basis for further research with more detailed analysis of the changes and their causes in different stocking densities. Finding the optimum density for carp fingerlings in tanks is important for achieving good production results and maximum protection of fish welfare.

ACKNOWLEDGMENT

The study was supported by the Ministry of Education, Science and Technological Development, Republic of Serbia, project No. TR-31075.

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LAKE LUDAS SPECIAL NATURE RESERVE – BACTERIOLOGICAL POINT OF VIEW

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STANJE SPECIJALNOG REZERVATA PRIRODE "LUDAŠKO JEZERO" SA BAKTERIOLOŠKOG ASPEKTA

Apstrakt

U ovom radu su prikazani rezultati bakteriološkog ispitivanja vode i sedimenta severnog i južnog dela jezera Ludaš. Jezero Ludaš je deo parka prirode Palić-Ludaš, a zbog velikog značaja u očuvanju ornitološkog diverziteta ovog područja od 1977. godine je zaštićeno i Ramsarskom konvencijom. Uzorci su sakupljani od novembra 2013. do marta 2015. godine i ukupno je analizirano 10 uzoraka vode i sedimenta. Kvalitet vode je ocenjen na osnovu ukupnog broja heterotrofnih bakterija (mezofila i psihrofila), fakultativnih oligotrofa, ukupnog broja koliformnih i fekalnih koliformnih bakterija, broja *E.coli* i fekalnih enterokoka. Ocenjivanje je izvršeno prema ekološkom i sanitarnom kriterijumu koristeći kriterijume Uredbe o kvalitetu površinskih voda i sedimenta Republike Srbije. Rezultati ukazuju na veliko variranje mikrobnog diverziteta tokom ispitivanog perioda. Na osnovu ukupnog broja bakterija, voda se može svrstati u II - IV klasu voda na severnoj strani, dok je na južnom delu voda u kategoriji III - V klase kvaliteta. Međutim, na osnovu indikatora fekalnog zagađenja, voda jezera na južnoj strani je značajno boljeg kvaliteta i u većini merenja odgovara kvalitetu I i II klase vodotoka. Razlike u sastavu saprofitne i potencijalno patogene grupe mikroorganizama zastupljene u različitim delovima jezera se mogu objasniti geografskim karakteristikama i vodi koja se kanalom Palić-Ludaš uliva u severni deo jezera, što utiče na broj bakterija indikatora fekalnog zagađenja. Veliko organsko opterećenje u južnom delu jezera se ogleda u značajno većem broju saprofitnih heterotrofa, a manjem autohtonih oligotrofa čime se smanjuje sposobnost autopurifikacije vode i ukazuje na ekološku ugroženost i ubrzan process eutrofikacije jezera Ludaš.

Ključne reči: jezero Ludaš, heterotrofne bakterije, indikatori fekalnog zagađenja, autopurifikacija vode
Key words: Lake Ludas, bacterial density, indicator bacteria, autopurification

INTRODUCTION

Lakes provide water for numerous uses ranging from recreation and fisheries to power generation, industry and waste disposal. As a result of the last two uses, most lakes suffer from water quality degradation to some extent. The primary targets representing fecal contamination in temperate waters are now considered to be *E. coli* and enterococci (Ashbolt et al., 2001). Coliform bacteria originate from different sources (e.g. birds, boats, recreational swimmers, etc.), whose participation in pollution is difficult to assess, especially in urban areas where additional sources may exist, as industrial or urban wastewater (Canale et al., 1973). Activities like disposal of biological waste, including fertilization with manure, may, at the same time, increase the load of phosphorous, nitrogen and coliform bacteria in surface waters (Hofmann & Beaulieu, 2001). Both, suspended sediment at the bottom of the lake and the sediment of the tributaries that recharge the lake represent a source of *E. coli* (Struffolino, 2010). Prolonged survival of fecal coliforms and *E. coli* in freshwaters, as well as survival of *E. coli* in sediments and soils over extended periods of time has already been indicated in several studies (Ksoll et al., 2007). Pathogens that reach the watercourses as a consequence of wastewater discharge, can also influence aquatic organisms (Harvell et al., 2004), as well as birds that live in the vicinity (Anza et al., 2014), which is especially important for protected areas, such as nature reserves. The Lake Ludas is a shallow lake in the province of Vojvodina in northern Serbia. In 1977 this lake was added on a list of Ramsar sites, while it is a centre of biological diversity, where numerous rare, endemic and relict species exist. The aim of the paper is to highlight the current state of this nature reserve from the bacteriological and ecological point of view, considering both water and sediment quality of the central part of the reserve, the Lake Ludas.

MATHERIAL AND METHODS

The Lake Ludas lies on a sandy terrain between the Danube and the Tisza River. It covers area of 3.17 km^2, with the depth up to 2 m (average 0.9 m). The canal "Palic-Ludas", which carries a mixture of atmospheric water collected by open-canal network, wastewater and water from Lake Palic, represents an important water source for Lake Ludas.

Water and sediment samples were collected in the period November 2013 - March 2015 (10 samples of each). Sampling was performed at the South and North side of the Lake (near the school and near Visitor's centre). Water samples were collected at 20-50 cm beneath the water surface and the sediment from the surface layer of sediments. Samples were transported in cool containers at 4°C and tested within 12-24 hours of collection.

Detection of fecal contamination indicators (total coliforms, fecal coliforms, *Escherichia coli* and *Enterococcus*) was done by the most probable number (MPN) method using three tubes in each dilution. Presumptive test for total and fecal coliforms were done using the Mac Conkey (lactose) broth with inverted Durham tubes at 37°C/48 h. Detection of *Escherichia coli* and other fecal coliform strains were done by inoculation Endo agar from positive tubes and incubation at 44°C/24h. Presumptive test of *Enterococcus* were done by using azide–dextroze broth (37°C/48h), and the confirmation by inoculation on the Bile Esculin Agar (37°C/24h). Results are reported as MPN per 100 mL of water, or 1 g dw sediment.

Total number of heterotrophic bacteria was estimated by Pour Plate method, using Nutrient agar; incubation of mesophile was ensured at 37°C/48h and psychrophile at 20°C/3-5 days. Assessing facultative oligotrophs were done using diluted Nutrient agar (1:10), 20°C/3-5 days. Present results demonstrate total number of viable bacteria cells as counted colonies (CFU) per 1 mL. Petrović et al. (1998) suggest using density ratio of facultative oligotrophs and heterotrophic bacteria (FO/H) to obtain more in-depth ecological information about water quality: FO/H <1 pour, ≥1 satisfactory and >10 is a sign of good autopurification efficiency.

RESULTS AND DISCUSION

Nation Regulation of surface water quality (Official gazette RS 50/2012) differs five water categories regarding bacterial density, from I category - excellent ecological status, to V category – very polluted. Qualitative and quantitative composition of bacterial population in the lake reflects all seasonal and physico-chemical changes. High eutrophication level and intensive agricultural and anthropogenic impact to the lake intensify bacterial activity in numerous natural decomposition processes. These conditions are favorable to many human, animal and plant pathogens or potentially pathogen microorganisms.

During investigated period, high fluctuation of bacteria number was noticed. Total number of mesophile heterotrophs at South varied between $30x10^1$ and $77x10^4$ cfu·mL^{-1}, while the number of psychrophiles ranged from $3x10^3$ to $7.2x10^6$ cfu·mL^{-1}. At North mesophiles ranged from $32x10^1$ to $11.1x10^4$ cfu·mL^{-1}, and psychrophiles from $1x10^3$ to $19.2x10^4$ cfu·mL^{-1} (Fig.1). High number of psychrophile heterotrophs (August) indicates high organic load. Nevertheless, the autopurification process was intensive (FO/H ≥1). Water is mainly categorized in II-IV category at North and III-V in August at South (Fig.1).

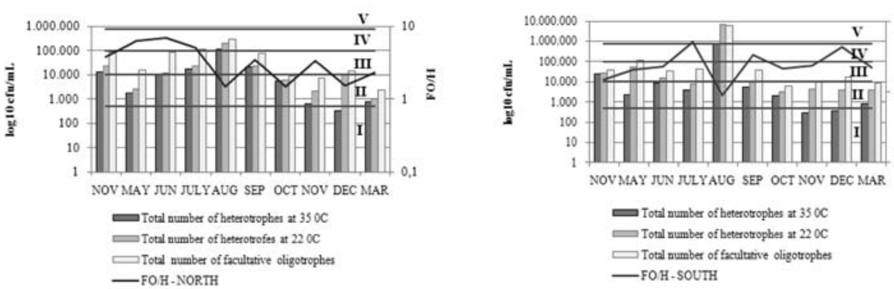

Figure 1. Total number of bacteria and autopurification capacity: South (left) and North (right)

Number of total coliforms was in 60% (South) and 50% (North) samples within I category (Fig.2). MPN of fecal coliforms was one log10 lower then coliforms. Maximal MPN was in July 29.870/100 mL at South and 20.140/100 mL in September at North. The lowest levels of fecal coliform contamination were detected in November and March. Number of *E.coli* ranged from $3x10^0$ to $3x10^3$ at North and notably decreased at South ($3x10^0$ - $3x10^2$/100 mL). Number of *E.coli* was one log10 lower than fecal coliforms, respectively (Fig.2c). The content of *Enterococcus* mostly refer to moderate lake pollution and water belongs to I category according to this parameter (Fig.2d).

Content of coliforms was significantly higher in sediment of northern spot, with extremes in July, December and March (Fig.3a). MPN value of fecal coliforms was one log 10 higher than in water and ranged between $18x10^1$ and $1.8x10^3$/1g dw at North. At South, in almost 50% of investigated samples MPN<10/1g dw (Fig.3b). During the investigated period, the MPN of *E. coli* was obviously higher at North, ranging from 18 to 1860/1g dw (Fig.3c). The MPN values of *Enterococcus* varied from 2 to 979/1g dw at the South and from 15 to 544/1g dw at the North. Occasional absence of Enterococci was registered in October, August and November.

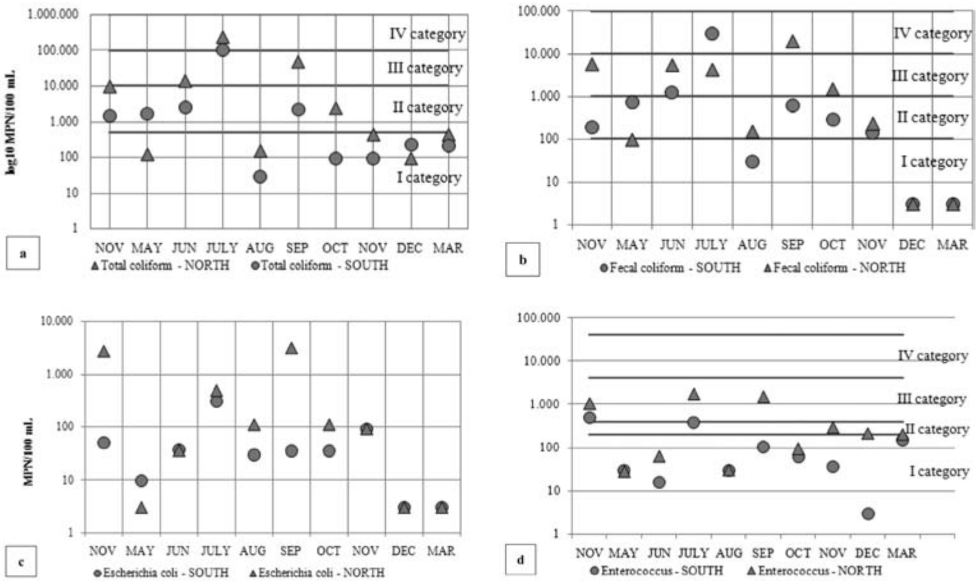

Figure 2. Water quality: a. total coliforms, b. fecal coliforms, c. *E. coli* and d. *Enterococcus*

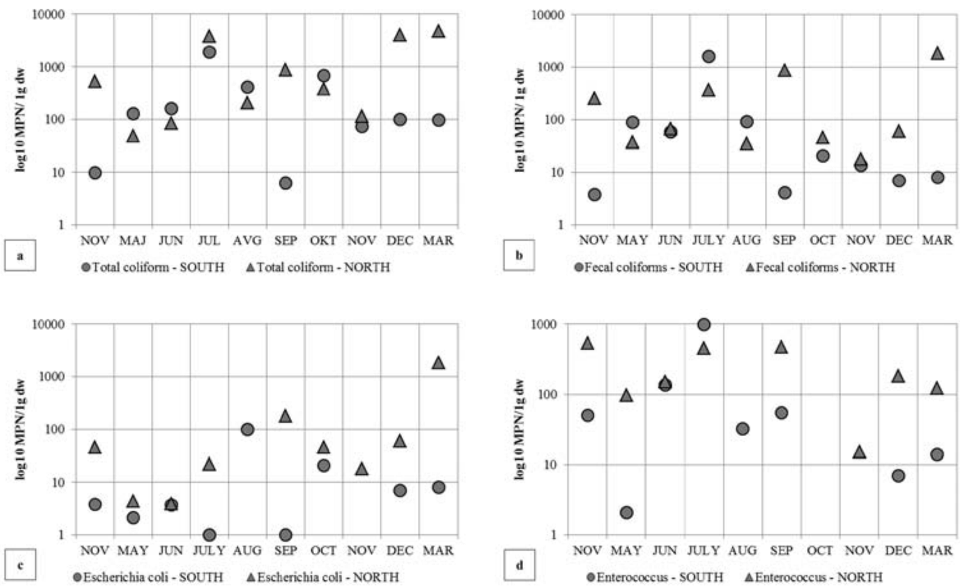

Figure 3. Sediment quality: a. total coliforms, b. fecal coliforms, c. *E. coli* and d. *Enterococcus*

CONCLUSIONS

Obvious differences in water and sediment quality exist between southern and northern parts of the Ludas Lake. Water and bottom sediments at the South were less bacteriologically polluted, when considering average values of indicator bacteria. But content of heterotrophic, saprophyte bacteria in water were higher as a result of higher organic water pollution and poor autopurification capacity in this part of the lake. Content of fecal indicators in most samples of water and sediment taken at the North were significantly higher. This indicates that water from the canal "Palic-Ludas" has a considerable impact on the sanitary quality of water and bottom sediment of Lake Ludas, since the confluence of the canal is approximately 800 m away from the northern sampling point.

ACKNOWLEDGEMENTS

This research was partially supported by the Ministry of education and science of Republic of Serbia (grant number TR 31080) and EU Commission project AREA, contract No. 316004.

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THE INFLUENCE OF DIFFERENT NUTRITION LEVELS ON THE GROWTH CHARACTERISTICS AND MEAT TEXTURE OF THE RAINBOW TROUT (*ONCORHYNCHUS MYKISS* WAL.)

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UTICAJ RAZLIČITIH NIVOA ISHRANE NA KARAKTERISTIKE RASTA I TEKSTURU MESA DUŽIČASTE PASTRMKE (*ONCORHYNCHUS MYKISS* WAL.)

Apstrakt

Eksperiment je realizovan u laboratoriji za akvakulturu Poljoprivrednog fakulteta Univerziteta u Banjoj Luci. Ukupno je naseljeno 100 jedinki u 5 eksperimentalnih grupa, prosječne individualne mase 91.09±1.37 g, totalne dužine tijela 20.08±0.10 cm i dužine tijela do račve repnog peraja 19.37±0.10 cm (M±SEM). Cilj rada je bio praćenje efekata različitih nivoa ishrane na karakteristike rasta i teksturu (tvrdoću) mesa dužičaste pastrmke (*Oncorhynchus mykiss*). Dužičasta pastrmka u svim eksperimentalnim grupama hranjena je istom hranom, sa različitim nivoima ishrane: 20% (G_{-20}) i 10% manje (G_{-10}) u odnosu na standardni nivo ishrane, standardni nivo ishrane (G_{100}) (preporuka proizvođača hrane), 10% (G_{+10}) i 20% više (G_{+20}) u odnosu na standardni nivo ishrane. Statistički značajna razlika sredina ($p<0.05$) mase i dužine tijela između posmatranih eksperimentalnih grupa javlja se u drugom periodu. Najmanja potrebna sila (kg) za presijecanje mesa dužičaste pastrmke konstatovana je na početku eksperimenta kod jedinki prosječne mase oko 90 g. Najtvrđe meso je kod riba iz eksperimentalnih grupa G_{+10} i G_{-10}. Između posmatranih eksperimentalnih grupa konstatovana je statistički značajna razlika sredina ($p<0.05$) potrebne sile za presijecanje mesa.

Ključne riječi: ishrana, rast, tekstura, meso, dužičasta pastrmka
Keywords: nutrition, growth, texture, meat, rainbow trout

INTRODUCTION

Rainbow trout (*Oncorhynchus mykiss*) is widely farmed in many countries around the world due to its rapid growth and high nutritional value (Fallah et al., 2011). The feeding cost of farmed rainbow trout is high, over 50% of total production costs (Hardy & Barrows, 2002). Therefore, there is a need of continued research in the field of salmonid's nutrition and study of the effects of different types of feed and nutrition level in different environmental conditions on the growth characteristics of rainbow trout (Savić et al., 2013).

In addition, an important issue is the quality of meat of farmed trout in terms of chemical composition and texture of meat (Andersen et al., 1997). The texture of fish meat depends on the physical factors (fish species; age and size etc.), chemical factors (the content and arrangement of the water etc.) and the applied treatment (freezing, cooling, etc.) (Cheng et al., 2014).

The aim of the study was to analyze the effects of different diets on growth performance and meat texture of the rainbow trout (*Oncorhynchus mykiss*) starting with initial weight around 90 g and final weight of approximately 200 g.

MATERIALS AND METHODS

The experiment was carried out at the laboratory of Aquaculture, Faculty of Agriculture, University of Banja Luka. It lasted 48 days. A total of 100 individuals were placed in five experimental groups. The average weight was 91.09 g, total body length 20.08 cm and fork length 19.37 cm. The fish were distributed in 5 experimental groups, 20 fish per experimental group. Each aquarium had a volume of 55 l with constant water flow. Rainbow trout was fed with the same feed in all the experimental groups with different levels of nutrition: 20% (G_{-20}) and 10% less (G_{-10}) than the standard level of the diet, a standard diet level (G_{100}) (recommended by the feed producers), 10% (G_{+10}) and 20% higher (G_{+20}) than the standard level of the diet. Fish were fed twice a day, with feed having the following composition: crude protein 42%, crude fat 24%, fiber 3%, ash 6%, total phosphorus 0.9% and digestible energy of 19.4 MJ/kg.

Water temperature (°C) and dissolved oxygen (mg/l) in water were measured every day before the first feeding with digital oxy-meter (Oxi 330i/SET 2B20-0011 WTW), and the pH value with digital pH-meter (pH 330i/SET 2A20-1011 WTW). Body mass of each individual was measured by scales Denver DL-501, capacity of 0.5 kg. The body length of each individual was determined with ichthyometer. The following formulas were used to analyze the growth characteristics and other parameters: The growth of body mass (%)=((FBW-IBW)/IBW)x100; $CF=(BW/L^3)x100$; SGR=((lnFBW-lnIBW)/D)x100; Survival (%)=(N_t/N_0)x100; $TGC=((FBW^{1-3}-IBW^{1-3})/\Sigma(TxD))x100$; FCR=F/G. (FBW-final body weight (g); IBW-initial body weight (g); CF-condition factor; BW-body weight (g); L–fork length (cm); SGR-specific growth ratio; ln-natural logarithm; D-the number of feeding days; N_0-the number of fish at the beginning (n); N_t-the number of fish at the end (n); TGC-thermal-unit growth coefficient; T-water temperature (°C); FCR-feed conversion ratio; F-feed consumption; G-gain weight).

The texture of fresh raw fish meat was determined by sampling the muscle *Musculus lateralis maior*. Eight fish from each experimental group, a total of 40 fish, were taken for determination of the meat texture at the end of the experiment. The thickness of meat samples was 10 mm and length about 60 mm. Measurements of hardness of fish meat were carried out using instrument for analysis of textures, Texture Analyser TA.XT Plus (Stable Micro Systems, England) with the help of Warner-Bratzler knife and HDP/BSK platform. The load cell of the instrument was 25 kg, the speed of the knife was 5.0 mm/s during the test and distance 20 mm.

The statistical program SPSS-17 was used to calculate the average value, the SEM, CV, the univariate analysis of variance and LSD test.

RESULTS AND DISCUSSION

Basic physical and chemical water quality parameters (Table 1) for fish farming were in a narrow range of variation and there were no significant differences between the experimental groups.

Table 1. Average values (Mean±SEM) and the coefficients of variation of water temperature, dissolved oxygen, and pH values during the experimental period

Experimental groups	Water temperature (°C)		Dissolved oxygen (mg/l)		pH value	
	Mean±SEM	CV	Mean±SEM	CV	Mean±SEM	CV
G_{-20}	16.29±0.03	1.10	7.17±0.08	6.72	7.32±0.02	1.57
G_{-10}	16.32±0.02	0.80	6.73±0.11	9.58	7.27±0.02	1.48
G_{100}	16.33±0.02	0.74	6.62±0.10	8.63	7.22±0.01	0.90
G_{+10}	16.31±0.02	0.69	7.08±0.11	9.14	7.28±0.02	1.11
G_{+20}	16.34±0.01	0.53	6.96±0.14	12.35	7.18±0.02	1.31

A statistically significant difference ($p < 0.05$) of weight and body length in the observed experimental groups appeared in the second period (Table 2). The largest variations in body mass were presented in the experimental group G_{+10} with a tendency of growth, and variations of body weight in the group G_{+20} a continuous upward trend. This is understandable, considering that the group was given the largest amount of feed per day.

Table 2. Growth of weight and body length (Mean±SEM) and coefficients of variation (CV) of rainbow trout

Parameter	Eksperimental groups									
	G_{-20}		G_{-10}		G_{100}		G_{+10}		G_{+20}	
	Mean±SE	CV	Mean±SE	CV	Mean±SE	CV	Mean±SE	CV	Mean±SE	CV
Mass (g)										
Beginnig	91.6±2.78^{ns}	13.56	89.7±3.55^{ns}	17.69	94.3±2.78^{ns}	13.18	91.2±2.89^{ns}	14.19	88.8±3.42^{ns}	17.22
I period	115.1±3.13^{ns}	12.16	114.1±4.37^{ns}	17.14	122.6±3.35^{ns}	12.21	118.7±3.87^{ns}	14.58	118.0±4.66^{ns}	17.68
II period	142.6±4.44^{a}	13.93	145.2±6.81^{ab}	20.98	160.4±5.01^{b}	13.98	155.0±6.40^{ab}	18.45	157.7±6.30^{ab}	17.87
III period	176.3±5.64^{a}	14.31	183.3±8.41^{a}	20.52	208.7±6.22^{b}	13.34	209.0±8.70^{b}	18.14	209.5±9.12^{b}	19.47
Totall lenght (cm)										
Beginnig	20.1±0.16^{ns}	3.64	19.8±0.29^{ns}	6.67	20.2±0.19^{ns}	4.20	20.2±0.20^{ns}	4.38	20.2±0.26^{ns}	5.74
I period	21.6±0.18^{ns}	3.63	21.3±0.37^{ns}	7.86	21.9±0.20^{ns}	3.99	21.7±0.24^{ns}	4.95	21.8±0.29^{ns}	6.06
II period	22.8±0.21^{ab}	4.11	22.6±0.41^{a}	8.18	23.5±0.23^{b}	4.36	23.1±0.33^{ab}	6.34	23.3±0.34^{ab}	6.45
III period	24.1±0.24^{a}	4.36	24.3±0.46^{a}	8.56	25.4±0.24^{b}	4.15	25.3±0.34^{b}	5.78	25.3±0.40^{b}	7.16
Fork length (cm)										
Beginnig	19.5±0.18^{ns}	4.01	19.0±0.29^{ns}	6.74	19.5±0.21^{ns}	4.77	19.5±0.19^{ns}	4.32	19.4±0.27^{ns}	6.17
I period	20.7±0.18^{ns}	3.99	20.4±0.37^{ns}	8.13	21.0±0.20^{ns}	4.33	20.7±0.25^{ns}	5.39	20.9±0.30^{ns}	6.51
II period	21.9±0.21^{a}	4.23	21.8±0.40^{a}	8.30	22.8±0.23^{b}	4.61	22.3±0.32^{ab}	6.40	22.4±0.35^{ab}	6.91
III period	23.4±0.24^{a}	4.65	23.5±0.46^{a}	8.74	24.7±0.25^{b}	4.47	24.5±0.34^{b}	6.06	24.5±0.39^{b}	7.06

Values with different superscripts are significantly different (p<0.05<) ^{ns}not significant

In general it can be said that the CF was fairly uniform during the observation period in all experimental groups and it had a growth trend from the beginning to the end of the experiment.

Total FCR is the same in experimental groups G_{100} and G_{-20}, unlike experimental groups with higher consumption (G_{+10} and G_{+20}) which had higher FCR. This is in accordance to the research of Savić et al. (2013). Bitaraf et al. (2012) suggests that the FCR in rainbow trout with similar size, diet with a protein content of 36% and 14% fat, grown in brackish water (salinity 8.5 gl^{-1}, temperature 10-15°C, O_2 5.2-6.6 mg/l) ranged from 1.27 to 1.70.

Table 3. Characteristics of growth, conversion coefficient and survival of rainbow trout

Parameter	Experimental groups				
	G_{-20}	G_{-10}	G_{100}	G_{+10}	G_{+20}
Condition factor (CF)					
Beginning	1.23	1.31	1.28	1.24	1.22
I period	1.30	1.34	1.32	1.33	1.30
II period	1.36	1.41	1.36	1.40	1.39
III period	1.37	1.42	1.38	1.41	1.43
The level of nutrition (%)	-20	-10	100	+10	+20
Feed conversion ratio (FCR)					
I period	0.77	0.81	0.80	0.87	0.87
II period	0.87	0.86	0.84	0.93	0.93
III period	0.87	0.89	0.86	0.95	0.95
Total FCR	0.84	0.86	0.84	0.89	0.92
Specific growth ratio (SGR)					
I period	1.53	1.60	1.75	1.76	1.89
II period	1.43	1.61	1.79	1.78	1.93
III period	1.41	1.55	1.76	1.99	1.89
Total SGR	1.46	1.59	1.77	1.84	1.91
Thermal-unit growth coefficient (TGC)					
I period	0.122	0.127	0.142	0.141	0.151
II period	0.147	0.166	0.190	0.187	0.203
III period	0.157	0.174	0.205	0.231	0.220
Total TGC	0.141	0.154	0.176	0.183	0.189
Weight gain - WG (%)					
I period	25.72	27.22	30.01	30.17	32.83
II period	23.89	27.27	30.79	30.64	33.66
III period	23.60	26.25	30.15	34.84	32.86
Total weight gain	92.52	104.41	121.31	129.29	135.87
Survival (%)	100.00	100.00	100.00	95.00	100.00

SGR and TGC are expected and in accordance with levels of nutrition. SGR ranged from 1.46 to 1.91, which is significantly higher compared to the results obtained from Guler & Yildiz (2011) in their study, carried out in five treatments (SGR was ranged from 0.82 to 0.92), at a temperature between 10°C and the experiment duration of 60 days. Ustaoğlu & Alagille (2009) reported that rainbow trout of similar weight, for a period of 60 days, at a similar temperature, with a slightly lower content of dissolved oxygen in water and fed different diet frequencies achieved a SGR of 1.02 and 1.07.

Table 4. The hardness of fresh raw meat of rainbow trout

Period	Experimental group	The maximum force (kg) required for cutting of fish meat			
		Mean±SEM	CV	Min.	Max.
Initial		0.75^{a}±0.039	28.29	0.47	1.22
Final	$\mathbf{G_{-20}}$	1.15^{bd}±0.030	13.85	0.75	1.39
	$\mathbf{G_{-10}}$	1.25^{d}±0.038	17.23	0.92	1.84
	$\mathbf{G_{100}}$	1.12^{b}±0.041	20.59	0.77	1.73
	$\mathbf{G_{+10}}$	1.34^{c}±0.031	13.73	1.06	1.71
	$\mathbf{G_{+20}}$	1.17^{bd}±0.024	11.49	0.90	1.37

Values with different superscripts are significantly different (P<0.05)

Minimum needed force (kg), for cutting the meat of rainbow trout was found at the beginning of the experiment when the average weight of the fish was about 90 g. This is to be expected considering the size of the fish and the water content, according to the results of Hultmann & Rustad (2002). They reported that higher water content in muscle reduces the required force for cutting the meat. Cheng et al. (2014) also suggested that meat texture depends of the physical and chemical factors. Among the observed experimental groups was found statistically significant differences (p<0.05) required maximum force for cutting meat. The biggest variations in needed force for cutting fish meat were found in experimental group G_{100} (from 0.77 to 1.73 kg). Hardest meat is found in fish from experimental groups G_{+10} and G_{-10} (G_{+10} = 1.34 kg and the G_{-10} = 1.25 kg).

CONCLUSION

The effects of different levels of nutrition on growth characteristics demonstrate the presence of a statistically significant difference. Significant differences were found in the groups fed with 10 and 20% less feed, and groups fed with standard and higher (10 and 20%) feed. Age or size of the fish had a significant influence on the texture (hardness) of meat, which means that less force is required for cutting meat of fish with smaller mass. There is a statistically significant difference of meat texture in fish with larger mass as a direct consequence of the different levels of nutrition.

Analysis of the growth performance of rainbow trout during the breeding cycle was significant, particularly in terms of achieving optimal or maximal mass growth while preserving the good health status of farmed fish, good feed consumption and better results, and consequently achieving good quality of meat in reared rainbow trout.

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BIOLOGICAL AND ECOLOGICAL CHARACTERISTICS OF THE EUROPEAN MUDMINNOW *UMBRA KRAMERI* AS A BASIS FOR *IN-SITU* AND *EX-SITU* CONSERVATION

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BIOLOŠKE I EKOLOŠKE KARAKTERISTIKE CRNKE *UMBRA KRAMERI* KAO OSNOV ZA *IN-SITU* I *EX-SITU* ZAŠTITU

Apstrakt

Na osnovu analize ekoloških karakteristika i morfološko-genetičke diferencijacije populacija crnke (Umbra krameri Walbaum, 1792) sa lokaliteta Lugomir i Bakreni Batar u Srbiji kao i sa lokaliteta Gromiželj u Bosni i Hercegovini, dat je osnov za buduće aktivnosti na in-situ i ex-situ zaštiti jedne od najugroženijih vrsta riba na ovim prostorima.

Analiza fizičko-hemijskih parametara vode i kvalitativnog sastava zajednice biljaka, riba, akvatičnih beskičmenjaka iz mulja i submerzne vegetacije pokazala je da su na svim istraživanim lokalitetima rezultati u skladu sa literaturnim podacima koji ukazuju na to da crnka naseljava tipična staništa u okviru svog areala. U odnosu na dužinu i težinu, pol i uzrast uzorkovanih jedinki, analizirana je struktura populacija, koja je pokazala da su na svim istraživanim lokalitetima srednje vrednosti dužinskih i težinskih parametara kod najfrekventnijih uzrasnih klasa (1^+ i 2^+) prilično ujednačene, da su na Gromiželju evidentirane i mlađe (0^+) i starije (3^+ i 4^+) jedinke, kao i da mužjaci dominiraju u uzorcima iz Lugomira i Bakrenog Batara, a ženke u uzorku iz Gromiželja. Analizom dužinskog i težinskog rasta, najveći apsolutni i relativni priraštaj ustanovljen je kod jedinki sa lokaliteta Gromiželj, a konstatovano je i da rast crnke varira i zavisi od više faktora, kao što su fizičko-hemijski parametri sredine, dostupnost hrane, gustina populacija, prisutni predatori itd. Na osnovu želudačno-crevnog sadržaja utvrđen je kvalitativno-kvantitativni sastav ishrane crnke, koji ukazuje na to da crnka predstavlja zoofagnu vrstu i oportunističkog predatora.

Metodama geometrijske morfometrije analizirana je varijabilnost spoljašnje morfologije, odnosno varijabilnost veličine i oblika tela istraživanih populacija, a upotrebom molekularno-genetičkih metoda (mikrosateliti i mitohondrijalna DNK), utvrđena je i genetička raznovrsnost analiziranih populacija. Uz visok stepen podudarnosti dobijenih rezultata korišćenjem navedenih metoda, utvrđeno je jasno razdvajanje dunavske populacije sa lokaliteta Lugomir u odnosu na savske populacije sa lokaliteta Bakreni Batar i Gromiželj, koje pokazuju znatno veći stepen međusobne sličnosti i srodnosti. Svi dobijeni rezultati ovih istraživanja od izuzetne su važnosti za preduzimanje odgovarajućih mera aktivne zaštite, očuvanja i unapređenja stanja recentnih populacija, od kojih su najznačajniji repopulacija, reintrodukcija i akvakultura crnke u *ex situ* uslovima i u kontrolisanim prirodnim plodištima na samim staništima.

Ključne reči: crnka, staništa, struktura populacija, geometrijska morfometrija, genetička raznovrsnost

Abstract

The analysis of ecological characteristics and morphological-genetic differentiation of populations of the European mudminnow (*Umbra krameri* Walbaum, 1792) from the localities Lugomir and Bakreni Batar in Serbia and the locality Gromiželj in Bosnia and Herzegovina provides a basis for future activities related to *in situ* and *ex situ* conservation of one of the most endangered species in the region.

Physical and chemical habitat parameters, aquatic plant and fish species composition, as well as invertebrate assemblages from mud and submerged vegetation at all investigated localities are consistent with the literature data which indicates that European mudminnow lives in typical habitats within its range. Population structure was analysed with regard to measured body lengths and weights, sex ratio and the age of the sampled individuals. The results show that the mean lengths and weights of the most frequent age classes (1^+ and 2^+) are rather uniform at all investigated localities. Younger (0^+) and older (3^+ and 4^+) individuals were recorded in Gromiželj; males outnumber females in samples from Lugomir and Bakreni Batar, while females outnumber males in samples from Gromiželj. Individuals from Gromiželj show the highest absolute and relative growth. The growth of this species varies and depends on several factors, such as physical and chemical environmental parameters, food availability, population density, presence of predators, etc. Qualitative and quantitative composition of European mudminnow diet was determined on the basis of its gastrointestinal contents. The obtained results indicate that the European mudminnow is a zoophagous species and an opportunistic predator.

The morphological variability (body size and shape) was analyzed using the methods of geometric morphometrics, while the genetic diversity of the analyzed populations was determined by applying the molecular-genetic methods (microsatellites and mitochondrial DNA). These analyses indicate high level of compatibility between the morphological variability and the genetic diversity. Namely, the Danube River population from Lugomir differs from the Sava River populations from Bakreni Batar and Gromiželj, the latter two having a significantly higher level of similarities and relatedness. All obtained results are extremely important for implementing the adequate measures of active protection, conservation, and improvement of recent populations, most notably repopulation, reintroduction, and aquaculture of the European mudminnow in *ex situ* conditions and at controlled natural spawning sites within their habitats.

Keywords: European mudminnow, habitats, population structure, geometric morphometrics, genetic diversity

THE ROLE AND IMPORTANCE OF CENTERS FOR GENETIC IMPROVEMENT IN CONSERVATION AND SUSTAINABLE EXPLOITATION OF SALMONID FISH STOCKS

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ULOGA I ZNAČAJ CENTARA ZA GENETSKO UNAPREĐENJE U OČUVANJU I ODRŽIVOM KORIŠĆENJU RIBLJEG FONDA SALMONIDA

Apstrakt

Cilj ovog rada je da ukaže na potrebu znatno većeg učešća naučno zasnovanih principa u procesu poribljavanja salmonidnih ribolovnih voda na području Srbije. Rad ukazuje na potrebu za izgradnjom i unapređenjem objekata za uzgoj riblje mlađi u kojima će se ona proizvoditi u adekvatnim uslovima, odgovarajućim biotehnološkim procesima i bez genetičke kontaminacije. Rezultati rada dobijeni su na osnovu proučavanja podataka o poribljavanju ribolovnih voda koji su prikazani u Programima upravljanja ribarskim područjima na teritoriji Srbije. Kao primer uzeta su područja u zaštićenim oblastima kao što su: Nacionalni park “Kopaonik”, Park prirode “Stara planina” i Predeo izuzetnih odlika “Vlasina”. Dosadašnja praksa poribljavanja voda u Srbiji neadekvatnom ribljom mlađi doprinela je narušavanju prirodne ravnoteže i potiskivanju autohtonih vrsta riba što se odrazilo na potencijal ribolovnih voda, kako u biološkom tako i ekonomskom smislu. Do poboljšanja bi moglo da dođe ukoliko bi se u Srbiji proizvodila kvalitetna riblja mlađ u dovoljnim količinama u centrima za reprodukciju (reprocentrima). Reprocentri za razliku od postojećih ribnjačkih mrestilišta obezbeđuju riblju mlađ koja je adekvatna za ekosisteme koji se poribljavaju.

Osnovne razlike između reprocentara i ribnjaka jesu što reprocentri uzgajaju ribu sa proverenim genetskim poreklom i što na odgovarajući način pripremaju riblju mlađ za život u prirodi.

Ključne reči: centri za reprodukciju, poribljavanje ribolovnih voda Srbije, konzervacija, salmonidni riblji fond
Keywords: centers for genetic improvement of fish stock, restocking of Serbian fishery waters, conservation, salmonid fish stock

INTRODUCTION

As populations of exploited fish continue to decline worldwide (Food and Agricultural Organization of the United Nations, l999), fishery managers have adopted three general strategies for rebuilding depleted stocks: regulating harvests, restoring or preserving essential habitats, and increasing recruitment through the use of hatcheries (Blankenzhip & Leber, 1995).

Conservationist in early 80’s of the last century represented scientifically based attitude that restocking of natural waters in the classical way has no ecological justification and generally has a negative impact on wild populations. In order to reduce the negative effects of classical hatchery practice of restocking natural waters, conservationists propose a different approach to the introduction of cultivated populations in natural ecosystems (Van Dyke, 2010). The basis for this approach is different production technology of cultivated organisms, in this case, fish fry, based on the genetic characteristics of natural populations and aimed at preserving genetic diversity and natural ecological habits of cultivated populations. Facilities of this kind of controlled breeding and growing organisms are called reproduction centers or centers for genetic improvement of fish stock.

Fish produced in this kind of centers were reared and selected on a scientific manner prescribed. These fish do not lead to genetic contamination of natural populations, and can reduce the harmful effects of existing genetic contamination in the water. In this way is provided stability of ecosystems and their greater cost-effectiveness in terms of use as fishing waters (Sevä, 2010).

The aim of this paper is to point out the need for much greater participation of science-based principles in the process of restocking salmonid fishery waters in Serbia.

MATERIAL AND METHODS

Results and materials were obtained from the data of the restocking of fishing waters, which is displayed in the management of fishing areas in Serbia. As example are represented parts of protected areas such as National Park «Kopaonik», Nature Park «Stara Planina» and «Vlasina», nature preserve of special interest at category I. We examine the relationship between potential production and real production of *Salmo trutta* in fishing waters of protected areas as well as the relationship between the predicted amount and optimal amounts of juvenile fish for restocking.

In addition to the management plans also were investigated the origin of parental flocks and technological production process of fry of *Salmo trutta* in hatcheries in Serbia.

RESULTS AND DISCUSSION

Results of relation between real and potential production and optimal quantity of fry for restocking of fishing waters of some protected areas in Serbia, are summarized in the Table 1.

The example illustrated in Table 1. shows the following: the estimated potential production of *Salmo trutta* in all fishing waters is more than real, which gives the possibility of stocking salmonid waters with suitable quantity of fry of this species.

From the above examples can be seen that for stocking of only those fishing waters annually there is need to produce around three million fingerlings of *Salmo trutta*.

Our research shows that the current hatchery produces about 200 000 pieces of two-year *Salmo trutta* that can be used for restocking.

Because of this apparent lack of the required amount of adequate fish fry on the Serbian market, which would be genetically and phenotypically appropriate, these amounts are in management programs significantly reduced (Simić & Simić, 2012, 2012a, Simić et al. 2012).

Ideal restocking is that which does not change the genetic, its age and sex structure of natural populations of indigenous fish. Appropriate stocking would be considered that removal of the deficits of all fishing important species (Simonović, 2010).

Table 1. Relationship between real and potential production of *Salmo trutta* in some fishing waters of Serbia and the optimum quantity of fingerlings for restocking

Fishing water	Fish species	R.P[1]	P.P[2]	Est restocking	Realistically optimally restocking
NP “KOPAONIK”[1]	*Salmo trutta*	Pieces / km / ha total for the period 2012-2020			
Samokovska river	*Salmo trutta*	13.6	19.83	10 000 (2+)	25 000 (2+)
Gobeljska river		2.4	15.4	15 000 (2+)	30 000 (2+)
“VLASINA”[2]	*Salmo trutta*	2.34	27.8	125 000 (2+)	700 000 (2+)
Vlasina lake					
NP “STARA PLANINA“[3]	*Salmo trutta*	2.5-7.8	10-40	500 000 (2+)	2 000 000 (2+)
Zavojsko Lake Rivers: Visočica, Toplodolska, Crnovrška, Golema					

[1]Simić & Simić, 2012; [2]Simić & Simić, 2012a; [3]Simić et al., 2012

Once a decision has been made to incorporate artificial propagation into a recovery plan, its implementation involves several important considerations. The intent of such a plan should be to facilitate recovery of the natural population, minimize its risk of further decline, and restrict genetic changes resulting from artificial propagation. To reduce the potential for these risks to arise, the use of artificially propagated fish to supplement a listed natural population should be held to the minimum necessary for sustained recovery. As part of a recovery plan, artificial propagation might require the collection of natural broodstock, the culture of progeny from those adults, and the release of the progeny at appropriate localities to supplement the natural population. Without adequate precautions, these activities may have negative effects on listed species, including deleterious ecological and genetic interactions between hatchery fish and natural fish (Hard et al.,1992).

By eliminating the many causes of natural mortality, hatcheries do offer scope for increasing the survival of progeny to any life stage. The potential gains are substantial and the gains increase in proportion to the length of time for which the fish are retained before their release. After release, further gains in the survival rate of stocked fish compared with wild fish are possible, using judicious stocking protocols to ensure that levels of competition in receiving streams are kept low (Youngson, 2007).

Potential gains from hatcheries:

1. Potential spawners removed from rivers as brood stock are subsequently protected from mortality due to predation, angling or poaching.
2. Survival rates of progeny in hatcheries are potentially much greater than in the wild. Depending on how long hatchery-reared fish are kept before their release, they are not susceptible to natural mortality due to the following causes : Any destruction of eggs caused by over-cutting of redds, egg predation by parr at spawning time, washout of redds during incubation, egg mortality due to siltation of, or groundwater intrusion to redds, the effects of any acid episodes in geologically and geographically susceptible catchments, natural mortality among free-swimming stages, including predation of fry or parr by their own or other species.
3. By judicious stocking, natural levels of competitive mortality among fry or parr, after their release can be reduced by the following means: hatchery fish can be stocked into areas of stream that do not contain potential competitors or contain sub-optimal numbers of natural progeny, any given number of hatchery fish can be stocked at low average density, over correspondingly large areas of stream, in any given area of stream, any given number of hatchery fish can be stocked out at low initial densities by using a correspondingly large number of planting or introduction sites (Youngson, 2007).

The previous practice of restocking the waters of Serbia, as inadequate fingerlings contributed to the disruption of the natural balance, suppressing native species of fish which reflected on the potential of fishing waters, both in biological and economical terms. The improvement may come if the Serbia-quality young fish produced in sufficient quantities in the centers for genetic improvement of fish.

There, unlike the existing hatchery ponds, are provided juvenile fish that is adequate for the ecosystems that are stocked. The main differences between the reproduction center and ponds are that first bred fish with proven genetic background and properly preparing juveniles to life in nature.

CONCLUSION

It is necessary to secure funding increased investment, personnel training, and better information to the public, especially to users of fishing areas on the importance of stocking water in Serbia with quality fish fry obtained under controlled conditions which allows centers for genetic improvement of fish stock. Better supply of fish for stocking would contribute to the work of the users of fishing areas and allow them to have a greater economic benefit from the issuance of a number of licenses at profitable prices.

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ELEMENTS CONCENTRATION IN TISSUE OF CHUB (*SQUALUS CEPHALUS*) FROM RESERVOIRS OF NATIONAL PARK "TARA"

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KONCENTRACIJE ELEMENATA U TKIVIMA KLENA (*SQUALUS CEPHALUS*) IZ AKUMULACIJA NACIONALNOG PARKA „TARA"

Apstrakt

Planinsko područje Tare nalazi se na krajnjem zapadu Srbije. Sa severne i zapadne strane ograničeno je dolinom Drine, sa jugozapada dolinom Rzava, sa juga plitkom kremanskom udolinom koja ga odvaja od zlatiborske površi. Ceo prostor Nacionalnog parka obuhvata: planinu Taru, Crni vrh, Zvezdu, Stolac, kanjon Drine sa Perućcem i okolinu Bajine Bašte. Nacionalni park „Tara" i njegova uža zaštitna zona raspolaže rekama i potocima koji pripadaju uglavnom gornjim i delimično srednjim pastrmskim regionima. Najznačajnije reke su Rača, Derventa sa pritokama, Brusnički potok sa pritokama, Karaklijski Rzav i Baturski Rzav i reka Jarevac. U Nacionalnom parku „Tara" formirano je nekoliko veštačkih jezera različitog tipa. Akumulacija „Perućac" je veštačko jezero nastalo u rečnom koritu reke Drine, njenim pregrađivanjem betonskom branom. Na osnovu lokacije, pripada nizinskom tipu. Akumulacija „Zaovine" je po lokaciji visinskog tipa, a po načinu nastanka reverzibilna. Nastaje izbacivanjem vode iz akumulacije „Perućac" i sakupljanja vode od Karakliskog i Baturskog Rzava i drugih manjih pritoka. Akumulacija „Spajići" je visinska sabirna akumulacija koja nastaje od proceđene vode iz jezera „Zaovine", te reke Zmajevačke i Popovića potoka. Jezero „Kruščica" nastaje od Karaklijskog i Baturskog Rzava i koristi se kao pijaća voda (Hegediš, 2012).

Ribolovne vode NP „Tara" naseljava 28 vrsta riba iz sedam familija, a šaranske vrste (Ciprinidae) dominiraju po brojnosti sa 17 vrsta. Klen (Squalius cephalus) je ciprinidna vrsta ribe, široko rasprostranjena u Srbiji južno od Save i Dunava dok je u Vojvodini redak. U Srbiji je popularna sportsko-rekreativna ribolovna vrsta i lovi se različitim tehnikama ribolova. U akumulacijama Zaovine, Spaići i Kruščica klen je najzastupljenija vrsta, dok je i u akumulaciji Perućac prisutan u značajnom broju (Hegediš, 2012).

Tokom terenskih istraživanja 2014. godine ispitivan je nivo akumulacije 17 elemenata u tkivima (mišić, jetra, škrge) klena iz četiri akumulacije – Perućac, Zaovine, Spaići i Kruščica. Ribe se nalaze na vrhu lanaca ishrane u vodenoj sredini i 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).

Ribe se često koriste kao bazični organizmi po pitanju pozicije u lancima ishrane, kao i u ishrani ljudi, zbog njihovog potencijala za bioakumulaciju toksičnih materija i njihove osetljivosti na čak i male koncentracije mutagena (Szefer et al., 1990; Višnjić-Jeftić et al., 2010). Jedinke klenova sa Perućca su se na osnovu analize glavnih komponenata (PCA – Principal Components Analysis) izdvajale po višim koncentracijama Cu i Zn u jetri, kao i B i Cu u mišićima; jedinke sa Zaovina se izdvajaju po koncentracijama Sr, Mo, Fe, Cr, Al, Hg u jetri višim nego u jedinkama sa drugih akumulacija, Mn, Sr, Hg, Mo i Cr u mišićima i Al, Sr, Li i Hg u škrgama; jedinke sa akumulacije Kruščica se izdvajaju po koncentracijama Pb, Mn i Ni u jetri, kao i, Mo, Mn, Fe, Pb i Cr u škrgama; jedinke sa akumulacije Spaići su bile grupisane između jedinki sa drugih analiziranih akumulacija, nisu se izdvajale po koncentracijama bilo kog analiziranog elementa u bilo kom tkivu, a najsličnije su bile jedinkama sa Perućca.

Ključne reči: klen, teški metali, koncentracija, akumulacija, Nacionalni park „Tara“
Key words: Chub, Heavy metals, Concentrations, Reservoir, National park "Tara"

INTRODUCTION

Mountain Tara is located on the far west Serbia. Limited with the Drina valley, from the southwest with valley of River Rzav, from the south limited with valley Kremansko that separates it from Zlatibor surface. The teritory of the National Park includes the mountain Tara, Crni vrh, Zvezda, Stolac, canyon of River Drina with Perućac Lake and surrounding of Bajna Bašta. National Park "Tara" and its narrower protection zone has rivers and streams that belong mostly to the upper and middle part trout regions. The most important rivers are the Rača, Derventa and its tributaries, bilberry stream with its tributaries, Karaklijski Rzav and Baturski Rzav and the River Jarevac. The National Park "Tara" is composed of several artificial lakes of different types. Accumulation "Perućac" is an artificial lake created in the riverbed of the Drina River. Based on the location, it belongs to the lowland type. Accumulation "Zaovine" is reversible, upper-level type, created by removing water from the reservoir "Perućac" and collecting water from Karaklijski and Baturski Rzav and other smaller tributaries. Accumulation "Spajići" collects drained water from the lake "Zaovine" and Zmajevačka River and stream Popović. Lake "Kruščica" arises from Karaklijski and Baturski Rzav and it is used as source of drinking water. Fishing areas of the NP "Tara" are inhabited with 28 fish species (7 families), while Cyprinids are dominating with 17 species. The Chub (*Squalius cephalus*, Cyprinidae) is widely distributed south of Sava and Danube in Serbia, while in Vojvodina province it is rare. In Serbia, chub has considerable recreational-fishing importance and catches with various techniques of fishing. In reservoirs Zaovine, Spaići and Kruščica chub is the most common species, significantly presented in Perućac (Hegediš, 2012).

Fish, as situated at the top of the food chain, accumulate large amounts of metals and trace elements (Yilmaz et al., 2007). Also, fish are considered as one of the most sensitive aquatic organisms on the presence of the toxic elements in the water (Alibabić i sar., 2007).

Fish are often used as sentinel organisms due to their role in the food webs, human nutrition, their potential for bioaccumulation of toxic substances, and their sensitivity to even low concentrations of mutagens (Szefer et al., 1990; Višnjić-Jeftić et al., 2010).

The Chub, is ubiquitous fish species widely distributed all over Europe, which provides the possibility to explore a variety contexts of pollution (Durand et al., 1999). Monitoring of the concentrations of heavy metals in fish tissues has an important role as an early warning indicator of the problems related to the water and sediment quality, and it also enables detection of toxic chemicals in fish as a source of nutrition supply for the humans. Thus, such monitoring allows taking appropriate and timely measures to protect public health and environment (Lenhardt et al., 2012).

MATERIAL AND METHODS

The Chub specimens were collected between July and September 2014, from four reservoirs: Perućac (N 43°59”54‘, E 19°16”20‘), Zaovine (N 43°52”03‘, E 19°24”13‘), Kruščica (N 43°54”13‘, E 19°23”04‘) i Spajići (N 43°51”34‘, E 19°24”21‘) located at National park “Tara”. Sampling was performed with benthic and pelagic gillnets (dimension 20-30m x 1-2m, 20-50mm mesh size). Gillnets were left over night. Total weight (g) and total body length (cm) of each fish were measured. The samples of gills, liver and dorsal muscles were removed, washed with distilled water and stored at -20 ºC prior to analysis.

Samples were dried by Freeze Dryers Rotational-Vacuum-Concentrator, GAMMA 1-16 LSC, Germany, and sample portions between 0.2 and 0.4 g dry weight were subsequently processed in a microwave digester (speedwave TM MWS-3þ; Bergof productsþInstruments GmbH, Eningem, Germany), using 6 ml of 65% HNO3 and 4 ml of 30% H2O2 (Merck suprapure) at a food temperature program (100–170 ºC). Potential presence of analyzed elements in chemicals used in digestion was resolved by using a number of blank samples. Following 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).

The principal component analysis (PCA) was applied to assess the differentiation among the analyzed fish from four reservoirs, based on elemental concentrations. The untreated data for elemental concentrations in each tissue were used as input variables.

RESULTS AND DISCUSSION

During this study 10 individuals of chub from each reservoir have been analyzed. Mean body weight of individuals from Perućac reservoir was 223.1 g (range 120 – 392 g), total body length 27.4 cm (range 23 - 34 cm); from Zaovine reservoir mean body weight was 250.4 g (range 130.9 – 414.5 g), total body length 28.3 cm (range 23 - 33 cm); from Kruščica reservoir mean body weight was 212.1 g (range 140.7 – 330.4 g), total body length 26 cm (range 22.4 – 31.2 cm); from Spaići reservoir mean body weight was 279.1 g (range 221.7 – 388.7 g), total body length 28.3 cm (range 25 – 31 cm).

A total of 17 elements of heavy metals and trace elements (Al, As, B, Ba, Cd, Co, Cr, Cu, Fe, Hg, Li, Mn, Mo, Ni, Pb, Sr and Zn) were analyzed in three tissues of chub. Mean values and standard deviation are shown in Tables 1, 2 and 3.

At the locality Perućac elements that were not detected are: As, Cd, Co Li, Mo and Ni; at the locality Zaovine elements that were not detected are: As, Ba, Cd and Co; at the locality Spajići elements that were not detected are: As, Cd, Co and Li; on the accumulation Kruščica in all three tissues were not detected: As, Cd and Co.

In the muscle of the chub, in samples taken on the locations Zaovine and Perućac, largest number of heavy metals and trace elements had the highest concentrations. On the locality Zaovine the highest concentrations had Al, Cr, Hg, Mo and Sr. On the locality Perućac highest concentrations had B, Cu, Fe, Pb and Zn. Locality Krušcica was distinguished by the highest concentrations of Ba and Mn (Table 1).

Table 1. The mean value of element concentrations in the muscle of chub (*S. cephalus*) from reservoirs NP „Tara”

Elements	Perućac	Zaovine	Spajići	Kruščica
Al	3.69	11.14	6.53	8.99
B	0.94	nd	nd	nd
Ba	0.50	nd	0.17	0.61
Cr	0.25	3.01	2.19	2.82
Cu	1.33	0.50	0.84	0.40
Fe	21.75	16.63	19.49	13.67
Hg	0.15	0.70	0.23	0.25
Mn	0.87	0.85	0.67	1.01
Mo	nd	2.74	2.54	2.16
Pb	0.18	nd	nd	nd
Sr	1.19	4.48	1.14	1.60
Zn	49.20	30.06	28.57	20.74

* (As, Cd, Co, Li and Ni – not detected in all specimens)

Liver had the highest concentrations of the most analyzed elements at the locality Zaovine (Al, Cr, Fe, Hg, Mo and Sr). On the locality Perućac B, Cu and Zn had the highest concentrations and on the locality Kruščica the elements with the highest concentrations were Mn, Ni and Pb. On the locality Spajići no heavy metal and trace element had the highest concentration (Table 2).

Table 2. The mean value of element concentrations in the liver of chub (*S. cephalus*) from reservoirs NP „Tara"

Elements	Perućac	Zaovine	Spajići	Kruščica
Al	4.34	17.83	7.86	7.87
B	2.37	2.17	0.46	0.77
Cr	0.26	7.25	3.89	4.56
Cu	28.46	21.33	27.99	7.95
Fe	265.33	761.92	431.92	652.50
Hg	0.24	0.56	0.26	0.31
Mn	3.79	3.77	5.24	14.40
Mo	nd	5.78	2.85	2.81
Ni	nd	nd	nd	0.56
Pb	0.5	nd	1.83	1.90
Sr	0.37	0.73	0.49	0.61
Zn	125.65	104.6	125.14	65.89

* (As, Cd, Co. Ba and Li – not detected in all specimens)

In the gills at the locality Spajići, most of the elements had the highest concentrations (Cr, Fe, Mn, Mo, Ni, Pb). Locality Zaovine had four elements with highest concentrations (Al, Hg, Li, Sr). Perućac and Spajići, each, had two heavy metals and trace elements (B and Ba; Cu and Zn) (Table 3).

Table 3. The mean value of element concentrations in the gills of chub (*S. cephalus*) from reservoirs NP „Tara"

Elements	Perućac	Zaovine	Spajići	Kruščica
Al	9.51	27.49	7.76	9.26
B	1.06	0.61	0.17	0.23
Ba	13.09	nd	8.93	10.44
Cr	0.69	2.80	2.38	3.27
Cu	1.12	1.17	1.36	1.28
Fe	87.70	126.49	129.24	153.75
Hg	nd	0.25	0.11	0.15
Li	nd	5.14	nd	1.93
Mn	13.43	12.86	10.55	26.07
Mo	nd	3.07	2.86	7.36
Ni	nd	0.04	0.33	0.72
Pb	nd	1.80	1.66	3.27
Sr	41.36	121.83	47.94	63.62
Zn	294.54	293.81	463.34	228.39

* (As, Cd and Co – not detected in the all specimens)

Results of the PCA analysis showed that the grouping can be performed based on the concentrations of heavy metals and trace elements in the muscle, liver and the gills of the chub (Figure 1).

In the liver of the chub there is a separation on all four localities. Perućac stands out based on the concentrations of the Cu and Zn; Zaovine based on the concentrations of Sr, Mo, Fe, Cr, Al, Hg; and Kruščica based on the concentrations of Pb, Mn and Ni (Figure 1a). According to the results of PCA analysis for chub muscle, separation can be observed: for locality Perućac based on the concentrations of B and Cu; for locality Zaovine based on the concentrations of Mn, Sr, Hg, Mo and Cr; both locations Kruščica and Spajići stand out based on the concentrations of Al and Ba (Figure 1b). In the case of the results of PCA analysis for chub gills, separation can be observed for the locality Kruščica based on the concentrations of Ni, Mo, Mn, Fe, Pb and Cr; for the locality Zaovine based on the concentrations of Al, Sr, Li and Hg (Figure 1c).

Monitoring of the concentrations of metals and trace elements in fish tissues has an important role as an early warning indicator of the problems related to the water and sediment quality and it also enables detection of toxic chemicals in fish as a source of nutrition supply for the humans. Thus, such monitoring allows taking appropriate and timely measures to protect public health and the environment (Lenhardt et al., 2012). Current status of metal and trace element concentrations in tissues of one of the common fish species in the waters of National park ‘’Tara’’ was analyzed. In Serbia chub has considerable recreational-fishing importance and thus it is commonly used in the human diet. Using fish tissues as a bioindicator of water quality is good method for screening waterways for the presence of heavy metals. The results can be used for the environmental managers and policy makers to improve management measures of the observed reservoirs which are currently under the status of the protected area of the National park ‘’Tara’’.

Basic hypothesis of the research from which we started was to analyze represented fish species from the reservoirs of National park ‘’Tara’’, as a source of cleaner water without obvious pollutants and to compare results of fish tissue contamination with other lowland water bodies, expecting that ‘’Tara’’ can be used as a reference locality. The significant differences between two types of the reservoirs (mountain-Tara vs. lowland-Medjuvršje reservoir, Skorić & Djikanović, 2013) were not found after one sampling.

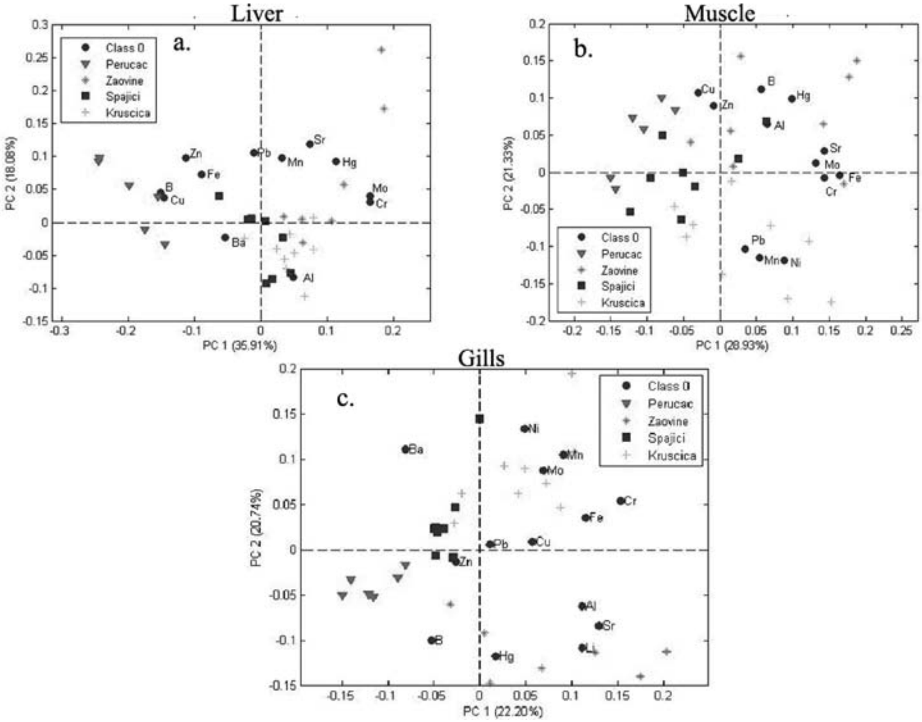

Figure 1. PCA analysis results for the chub liver (a), muscle (b) and gills (c) based on the heavy metals and trace elements from all four localities

Although, the differences between concentrations of some elements in analyzed tissues from different localities can be observed, all concentrations remained bellow MAC (maximum allowed concentrations, Službeni glasnik RS 25/2011, 28/2011).

ACKNOWLEDGMENTS

This research was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia, Project No. 37009.

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ELEMENT CONCENTRATIONS IN TWO FISH SPECIES WITH DIFFERENT HABITAT AND FEEDING PREFERENCES (CARP AND BURBOT) IN THE DANUBE RIVER NEAR BELGRADE

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KONCENTRACIJE ELEMENATA KOD DVE VRSTE RIBA SA RAZLIČITIM PREFERENCIJAMA STANIŠTA I ISHRANE (ŠARAN I MANIĆ) U DUNAVU KOD BEOGRADA

Apstrakt

Akvatični ekosistemi se uobičajeno smatraju krajnjim recipijentima zagađenja toksičnim metalima, čiji izvori mogu biti prirodni ili, najčešće, antropogeni. Ova klasa zagađivača predstavlja značajnu pretnju za životnu sredinu i vrste koje žive u njoj, kao i potencijalnu pretnju za ljudsko zdravlje. Osim toksičnih elemenata, i elementi u tragovima, kao i esencijalni mikronutrijenti, mogu pokazivati toksična svojstva ako su prisutni u većim koncentracijama. Dunav je recipijent zagađenja oslobođenog oko industrijskih gradova koji se nalaze duž toka ove reke. Koncentracije metala u tkvima riba zavise od većeg broja faktora, među kojima veliki značaj imaju ishrana i stanište. Koncentracije 15 elemenata (Al, As, B, Ba, Cd, Co, Cr, Cu, Fe, Hg, Mn, Mo, Se, Sr i Zn) analizirane su upotrebom ICP-OES u uzorcima jetre i škrga jedinki šarana i manića uhvaćenih u Dunavu u blizini Beograda. Elementi Li, Ni i Pb nisu detektovani u uzorcima. Mann-Whitney U test je ukazao na postojanje značajne razlike između šarana i manića u odnosu na koncentracije nekoliko elemenata, i u jetri i u škrgama. PCA analiza je pokazala da šaran i manić formiraju dve odvojene grupe na osnovu koncentracije elemenata u analiziranim tkivima. Jetra šarana karakteriše se visokim koncentracijama Cd, Cu i Zn, a škrge visokom koncentracijom Zn, dok se kod manića i jetra i škrge karakterišu visokim koncentracijama As. U škrgama šarana detektovana je viša koncentracija Zn. Šaran živi na svim dubinama i preferira staništa sa gustom akvatičnom vegetacijom i potopljenim drvećem, a u pogledu ishrane predstavlja omnivornu vrstu koja konzumira i detritus. Odrasli manić je riba bentosa koj preferira kamenitu podlogu i oportunistički je predator. Različita ishrana šarana i manića može biti uzrok razlika u koncentracijama elemenata u jetri, dok razlike u preferencijama staništa mogu ukazati na razlike u škrgama.

Ključne reči: šaran, manić, ICP-OES, toksični metali
Keywords: carp, burbot, ICP-OES, toxic metals

INTRODUCTION

Environmental pollution can be defined as the introduction of a pollutant into the air, water, or soil, which may be poisonous or toxic and is hence harmful to organisms in that environment (Nagayoti *et al*. 2010). The aquatic environment is generally the ultimate sink for metal pollutants (Velma *et al*. 2009), which is pronounced in the Danube River basin, because industrial facilities around the cities of Novi Sad, Belgrade, Pančevo, and Bor discharge various pollutants into the environment (Stanić *et al*. 2006). Metals can have negative effects on fish biology and health due to their toxicity, persistence, and bioaccumulation (Alhashemi *et al*. 2012). Gills and liver can be used as indicators for the assessment of metal accumulation (Alhashemi *et al*. 2012), because these tissues generally have higher concentrations of elements than the muscle tissue (Dural *et al*. 2007; Yilmaz 2009).

MATERIALS AND METHODS

Fourteen carp (*Cyprinus carpio*) and twenty burbot (*Lota lota*) specimens were caught at two locations in the Danube River near Belgrade, between October and December 2010. Carp was caught with portable fish nets, while burbot was caught with traps. Caught specimens were dissected with a plastic laboratory set and samples of liver and gills were taken, rinsed with distilled water, and stored at -18 ºC prior to analysis. Samples were freeze-dried using a rotational vacuum concentrator (GAMMA 1-16 LSC Germany) and sample portions between 0.2 and 0.5 g were processed in a microwave digester (speedwave™ MWS-3+; Berghof Products +Instruments GmbH, Eningem, Germany), using a 6 ml of 65% HNO_3 (Suprapur®, Merck) and 4 ml of 30% H_2O_2 (Suprapur®, Merck) at a food temperature program (100-170 ºC). After cooling to room temperature, the digested samples were diluted with distilled water to a total volume of 25 ml. The analysis of concentrations of Al, As, B, Ba, Cd, Co, Cr, Cu, Fe, Hg, Mn, Mo, Se, Sr, and Zn in samples was performed by ICP-OES (Spectro Genesis EOP II, Spectro Analytical Instruments DmbH, Kleve, Germany). The results are presented as μg/g dry weight.

To test whether variables were normally distributed, the Shapiro-Wilk test was used. Since the assumption of a normal distribution of variables was not satisfied, we applied the non-parametric Mann-Whitney test for two independent samples to compare the element levels between the two fish species (in both liver and gills). Statistical tests were done using the SPSS 20.0 software. We used the principal component analysis (PCA) as an unsupervised statistical method that summarizes the variation of a data set between samples to a set of uncorrelated components (each component is a particular linear combination of the original variables), in order to assess the differentiation among the analyzed fish tissues, based on the element level. We tried to present, in an approximate manner, clusters of individuals within smaller dimension subspaces. The untreated data for element concentrations in each tissue were used as input variables for Eigenvector Solo 7.0 software. The Varimax method was used for orthogonal factors rotation.

RESULTS

Elements Li, Pb, and Ni were not detected in the analyzed tissues. The Mann-Whitney test showed significant differences between carp and burbot with regard to element concentrations in liver for As, B, Cd, Cu, Fe, Mn, Mo, Se, Sr, and Zn, and no significant differences for Al, Ba, Co, Cr and Hg. In gills, there are significant differences between these two species in concentrations of Al, As, Ba, Cr, Mn, Se, Sr, and Zn, and no significant differences for B, Cd, Co, Cu, Fe, Hg and Mo (Table 1).

Table 1. Mean concentrations and standard deviations of 15 elements in liver and gills of carp (*C. carpio*) and burbot (*L. lota*) (in µg g^{-1} dry weight). Significant difference in concentrations of elements between species is marked by different statistical letters (*a*, *b*).

Element	Tissue	*C. carpio* (carp)	*L. lota* (burbot)
Al	liver	3.86 ± 1.96	6.36 ± 9.46
	gills	14.62 ± 16.79 *a*	31.66 ± 32.62 *b*
As	liver	0.49 ± 0.24 *a*	1.06 ± 0.52 *b*
	gills	0.29 ± 0.26 *a*	2.71 ± 1.35 *b*
B	liver	0.31 ± 0.28 *a*	0.13 ± 0.51 *b*
	gills	0.52 ± 0.66	0.60 ± 1.19
Ba	liver	0.16 ± 0.48	0.07 ± 0.19
	gills	12.61 ± 2.79 *a*	5.00 ± 3.33 *b*
Cd	liver	0.28 ± 0.40 *a*	0.03 ± 0.09 *b*
	gills	0.03 ± 0.09	0.01 ± 0.01
Co	liver	0.0001± 0.00	0.002 ± 0.005
	gills	0.0001 ± 0.00	0.0001 ± 0.00
Cr	liver	0.01 ± 0.03	0.005 ± 0.01
	gills	0.01 ± 0.03 *a*	0.11 ± 0.12 *b*
Cu	liver	33.49 ± 13.50 *a*	8.21 ± 5.02 *b*
	gills	1.90 ± 0.56	1.74 ± 0.41
Fe	liver	141.44 ± 65.23 *a*	36.97 ± 18.90 *b*
	gills	139.26 ± 38.88	162.55 ± 47.30
Hg	liver	1.63 ± 0.18	1.65 ± 0.21
	gills	0.89 ± 0.37	1.12 ± 0.31
Mn	liver	2.21 ± 0.80 *a*	0.49 ± 0.61 *b*
	gills	10.05 ± 2.74 *a*	7.21 ± 2.19 *b*
Mo	liver	0.17 ± 0.13 *a*	0.03 ± 0.07 *b*
	gills	0.06 ± 0.13	0.01 ± 0.03
Se	liver	0.36 ± 0.32 *a*	0.15 ± 0.26 *b*
	gills	0.21 ± 0.21 *a*	0.66 ± 0.50 *b*
Sr	liver	0.18 ± 0.08 *a*	0.14 ± 0.10 *b*
	gills	86.75 ± 28.58 *a*	42.96 ± 9.37 *b*
Zn	liver	325.37 ± 107.21 *a*	18.78 ± 7.71 *b*
	gills	1186.37 ± 419.46 *a*	71.09 ± 9.98 *b*

The PCA showed that carp is differentiated by the concentrations of Cd, Cu, and Zn in liver and by the concentration of Zn in gills, while burbot is differentiated by the concentration of As in both liver and gills (Figure 1).

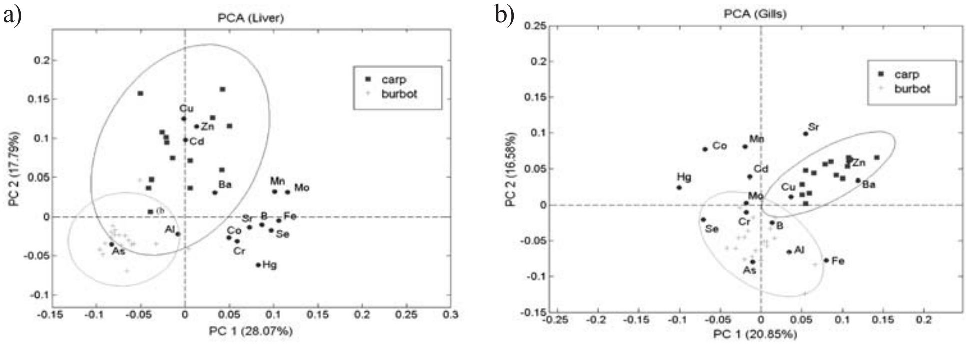

Figure 1. PCA plot of element concentration in (a) liver and (b) gills of analyzed fish species.

DISCUSSION

A higher concentration of As in liver in comparison to other fish species was also reported by Allen-Gill *et al.* (2003). On the other hand, carp is characterized by higher concentrations of Zn, which could be attributed to the tendency of Zn to inversely correlate with the trophic position of the fish (Papagiannis *et al.* 2004), and also to carp physiology and the presence of Zn-binding proteins (Liao *et al.* 2006).

The results show that carp and burbot form two distinctive groups based on the concentration of some elements in the liver, which can indicate their different feeding habits. Carp has a broad diet spectrum and is considered an omnivore/detritivore (García-Berthou 2001; Koehn 2004), while adult burbot is a predatory fish that mostly feeds on other fishes with a small portion of invertebrates in its diet (Pääkkönen and Marjomäki 2000).

These two species form two distinctive groups based on the concentration of some elements in gills as well. Because metal concentrations in gills well reflect concentrations of metals in water (Alhashemi *et al.* 2012), this separation could be explained by different habitat preferences of these species. Carp is a habitat generalist and can be found in all major aquatic habitat types (Smith 2005), while burbot utilizes several habitats during its ontogenesis – juveniles live in the littoral and adults prefer cold profundal waters (Hofmann and Fischer 2002).

CONCLUSIONS

Statistical differences were found between carp and burbot, both in liver and in gills, with regard to concentrations of As, Mn, Se, Sr, and Zn. There were no statistical differences with regard to concentrations of Co and Hg.

The PCA analysis showed that carp and burbot form two distinctive groups based on the concentration of several elements in liver and gills. Differences in concentration of elements in liver could reflect the different feeding habits of analyzed fish species, while differences in gills could reflect the different habitat preferences.

ACKNOWLEDGEMENTS

We acknowledge the support provided by the Project No. 173045, funded by the Ministry of Education, Science and Technological Development of the Republic of Serbia.

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EVALUATION OF RIVER WATER GENOTOXICITY WITH COMET ASSAY IN DIFFERENT TISSUES OF EUROPEAN CHUB

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PROCENA GENOTOKSIČNOSTI KORIŠĆENJEM KOMETA TESTA U RAZLIČITIM TKIVIMA KLENA IZ REČNE VODE

Apstrakt

Cilj ovog rada je bila analiza oštećenja molekula DNK primenom komet testa (engl. *Single Cell Gel Electrophoresis,* SCGE) na klenu (*Squalius cephalus* L.) kao potencijalnom model organizmu za procenu genotoksičnosti vode Kolubarskog basena. Kolubarski basen je bogat nalazištima lignita pa kao takav trpi veliki pritisak rudarskog postrojenja, “Kolubara”, pored već postojećeg uticaja komunalnih voda kao i spiranja sa obradivih površina. Uzorkovanja su vršena mesečno tokom sezone 2011/2012 god. Jedinke klena sa Zlatarskog jezera, Uvac, su korišćene kao prirodna kontrolna populacija.

Komet test je osetljiva i relativno jednostavna metoda primenljiva na velikom broju različitih organizama i tkiva. Za detekciju oštećenja DNK molekula u ovom radu korišćena su tri tkiva: krv, jetra i škrge.

Ključne reči: *ekogenotoksikologija, komet test, klen, reke*
Keywords: ecogenotoxicology, comet assay, European chub, river

INTRODUCTION

The European chub, *Squalius cephalus*, an ubiquitous fish species displaying a wide ecological niche, feeding on a low trophic level and widely distributed all over Europe, offers possibilities to investigate various polluted contexts (Durand et al., 1999). The use of chub as bioindicator has already been reported in field studies assessing the environmental quality of inland waters differently impacted by anthropogenic activities (Winter et al., 2005; Randak et al., 2006; Krca et al., 2007; Pavlica et al., 2011). Several biomarkers have also been characterized in this species in response to chemical pollutants, including levels of DNA strand breaks (SB), ethoxyresorufin *O*-deethylase (EROD) activity, polycyclic aromatic hydrocarbon (PAH) metabolites in bile, DNA adducts, erythrocyte micronucleation, inhibition of acetylcholinesterase (AChE), activities of glutathione *S*-transferases (GST) and vitellogenin gene induction (Devaux et al.,1998; Flammarion et al., 2000; Machala et al., 2001; Winter et al., 2005; Randak et al., 2006; Krca et al.,2007, Frenzilli et al., 2008).

The comet assay, also referred to as the single cell gel electrophoresis assay (SCGE), is arapid, visual, and quantitative technique for measuring DNA damage in eukaryotic cells (Singh et al., 1988).

The aim of the present research was to evaluate the application of the comet assay on different tissues of *Squalius cephalus* as a model organism for monitoring the pollution of Kolubara basin.

MATERIAL AND METHODS

Field sampling was conducted at two sites (N 44◦26'05.26'' E 20◦15'22.95'', N 44◦29'40.67'' E 20◦17'07.88'') at Kolubara basin. Kolubara basin has rich deposits of lignite and hence the whole area is under intensive mining activity. Total of 65 specimens of chub were caught by electrofishing device ELEMAX SHX 2000 (SAWAFUJI). Average body length and weight were: 22.75±7.22 cm and 163.27±164.92 g.

Uvac River "Zlatar" reservoir was used as a reference site. This is a protected natural area of great importance with very low anthropogenic impact. Total of 11 specimens were used for analysis. Average body length and weight were: 23.89 ± 6.97 cm and 194.7 ± 217.87 g, respectively. The alkaline comet assay procedure was performed in accordance with the method described by Singh et al. (1988) but with some modifications (Sunjog et al., 2014). Results of the comet assay were compared by analysis of variance, one-way ANOVA (Stat Soft, , 2007). Results that yield $p < 0.05$ are considered border-line statistically significant.

RESULTS AND DISCUSION

Results presented in Fig. 1 represents DNA damage expressed with TI (tail intensity or % the DNA in tail), and shows the difference in level of DNA damage in three tissues (blood, liver and gills) at Kolubara basin compared with reference site, Uvac. All tissues were significantly different compared to reference site. Gills showed the best response as compared to other tissues. Gills may be more prone to injury than other tissues, due to a high respiratory blood flow and permanent contact with the water environment. Blood was less sensitive in comparison to other tissues. This might be due to circulation of blood cells in the bloodstream, which indicates that blood could be used as a biomarker only for acute contaminations. It was determined that the turnover rate of fish erythrocytes is approximately 100 days (Devaux et al., 1998; Fischer et al., 1998; Buschini et al., 2004).

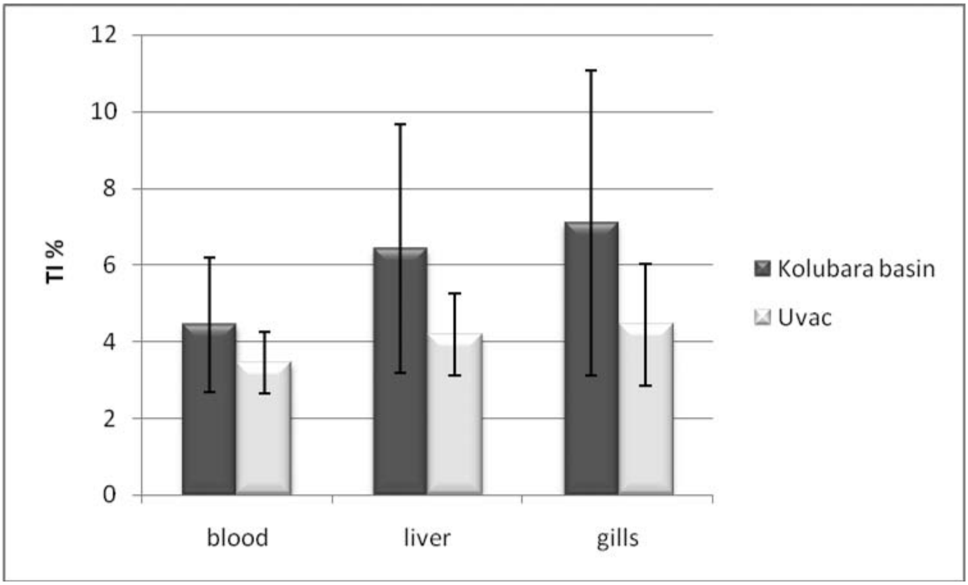

Figure 1. DNA damage of blood, liver and gills at Kolubara basin compared with reference site

Tissue specific responses are expected because of variations in alkali-labile sites and cell types with different background DNA single-strand break levels, due to variations in excision repair activity, metabolic activity, antioxidant concentrations, or other factors (Lee & Steinert, 2003). However, although the blood gave the lowest response to DNA damage compared to other tissues, it was still possible to observe the significant difference compared to Uvac.

CONCLUSION

Overall the results obtained in this study confirmed that *S. cephalus* can be used for the assessment of river basin genotoxicity based on comet assay. All tested biomarkers are sensitive and suitable for this type of research.

ACKNOWLEDGEMENT

his study represents a part of activities within the Project No. 173045, funded by the Ministry of Education, Science and Technological Development of the Republic of Serbia.

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COMPARATIVE ANALYSIS OF THE PRODUCTION OF DIFFERENT STOCKING CATEGORIES OF CARP IN SEMI-INTENSIVE SYSTEMS

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UPOREDNA ANALIZA PROIZVODNJE RAZLIČITIH NASADNIH KATEGORIJA ŠARANA U POLUINTENZIVNOM SISTEMU GAJENJA

Apstrakt

Šaran (*Cyprinus carpio*) je kao slatkovodna vrsta široko zastupljena kada je u pitanju farmsko gajenje riba u centralnoj i istočnoj Evropi. Na području Srbije, "klasična" poluintenzivna proizvodnja je dominantan tip gajenja riba, sa preko 95% ukupno proizvedenog šarana, a zasnovana je na kombinaciji prirodne i dodatne hrane (žitarica i smeša koncentrata). Kada je reč o gajenju različitih kategorija šarana u okviru jednog proizvodnog objekta, uglavnom se pristupa gajenju samo jedne kategorije. Razlog tome su jednostavniji i manji manipulativni procesi prilikom izlova kao i sama ishrana riba, naročito kad su u pitanju žitarice i ručno hranjenje. Cilj ovog istraživanja je bio da se analiziraju mogućnosti gajenja mešanog nasada različitih kategorija šarana u odnosu na gajenje samo jedne kategorije.

Eksperiment je realizovan na proizvodnom ribnjaku OZZ "Despotovo" iz Despotova. U toku jedne proizvodne sezone, na ribnjačkim površinama A, B i C, analiziran je uticaj kombinovanog i nezavisnog gajenja dvogodišnjeg (S1+) i konzumnog šarana (S2+) na proizvodne karakteristike u poluintenzivnom sistemu gajenja, upotrebom peletirane hrane.

Na osnovu dobijenih podataka o masi riba i količine potrošene hrane, dobijeni su rezultati ostvarenog prirasta (BWG) i koeficijenta konverzije hrane (FCR). Gajenjem jedne kategorije šarana ostvaren je bolji BWG nego kombinovanim gajenjem dve uzrasne kategorije riba u okviru istog proizvodnog objekta. Rezultati ostvarenog prirasta pokazali su razlike između jezera (A = 2 287 kg/ha; B = 1 399 kg/ha; C = 988 kg/ha). Razlog tome je potencijal prirasta nasađenih kategorija riba, jer se kod S1+ ostvaruje oko 3 puta veći prirast nego pri gajenju starije kategorije (S2+). FCR je takođe bio najniži pri gajenju samo S1+ (2.26). Pri kombinovanom gajenju S1+ i S2+ vrednost FCR bila viša (2.41) nego u jezeru A, ali i znatno niža nego pri gajenju S2+ u jezeru C (3.2).

Korišćenje peletirane hrane sa višim sadržajem proteina (35/7), dodatno je uticalo na postizanje boljih rezultata kod S1+ gajene u jezeru A, nego kod S1+ i S2+ gajene u jezerima B i C. Ovo potvrđuju i drugi istraživači, koji ukazuju da je prirast riba bolji ukoliko je riba hranjena smešom sa većim sadržajem proteina, posebno onih životinjskog porekla. Razlike u FCR-u se pripisuju bržem metabolizmu koji je prisutan kod mlađih kategorija riba.

Na osnovu vrednosti ostvarenog prirasta riba, gajenjem samo S1+ (A = 2 287 kg/ha) ili S2+ (C = 988 kg/ha), postiže se za 10% niža vrednost nego pri kombinovanom gajenju S1+ i S2+ (B = 1 399 kg/ha). Razlog tome je što mlađe kategorije riba (usled zbijenijih branhiospina) bolje iskorišćavaju sitnije organizme koji čine prirodnu hranu, kao i sitnije frakcije dodatne hrane koja se brže raspada u vodi kada se u ishrani riba koristi peletirana hrana.

Moglo bi se zaključiti da se bolji rezultati BWG i FCR ostvaruju ukoliko se gaji samo jedna kategorija riba. Međutim, na osnovu preračunatog odnosa u nasadu S1+ i S2+ (20 : 80) u okviru istog jezera i ostvarenog prirasta (kg/ha) u individualnom gajenju, može se zaključiti da je prirast viši za oko 10% pri gajenju mešanog nasada S1+ i S2+.

Ključne reči: kategorije šarana, ribnjački objekti, poluintenzivna proizvodnja, peletirana hrana
Keywords: carp categories, fishponds, semi-intensive production, pelleted feed, natural food

INTRODUCTION

Common carp (*Cyprinus carpio*) is one of the most widely cultured freshwater fish species in Central and Eastern Europe (Masilko et al., 2014). Semi-intensive and extensive production systems are favoured in order to provide economically feasible production, with low investments in technological processes and accordingly low production.

In Serbia, semi-intensive production is the dominant type of production. Over 95% of carp is produced on the basis of the combination of natural food and supplemental feed (wheat and/or formulated compound feed) (Marković et al., 2013). In recent years, in order to intensify carp production, row cereals are substituted up to different levels with compound pelleted or extruded feed formulations. This type of supplemental feeding is applied to all fish categories, from fingerlings to marketable size fish. As a result, higher production per surface area is achieved, from 1500 kg/ha up to over 3000 kg/ha (Marković et al., 2009).

Common carp is usually reared in polyculture with other freshwater fish: Chinese carps (grass carp, silver carp, and bighead carp) and predatory fish (pike perch, pike, catfish). This type of fish farming has advantages compared to monoculture since fish more efficiently use space and food available in the pond. Additionally, positive interactions between compatible fish species with different diets and ecology are intensified (Lin and Peter, 1991).

In carp production, usually, one stocking category of fish is farmed in a single pond. The reason for this is in its simplicity, easier handling during harvest and feeding (when fish are fed by hand). The aim of this study is to analyze possibilities for combined culturing of different stocking categories of carp and their comparison with the production of only one stocking category.

MATERIALS AND METHODS

The experiment was carried out at the fish farm OZZ “Despotovo“ (Despotovo, Serbia). During one production season, the effects of combined and single production of two years old and marketable size carp (S1+ and S2+) in three fish ponds were analyzed. Ponds A and C were stocked with one stocking category, S1+ and S2+, respectively, while fish pond B was stocked with combined stocking categories S1+ and S2+ in 20:80 ratio (Table 1).

Table 1. Stocking densities and average fish weight in fish ponds

Fish pond	A	B	C
Fish pond production area (ha)	35	25	16
Total number of stocked fish (kg)	11 464	10 465	6 902
Stocking density (kg/ha)	327.5	418.6	431.4
Average fish weight S1+ (kg)	0.103	0.210	/
Average fish weight S2+ (kg)	/	0.710	0.780

During the production season, fish from ponds B and C were fed pelleted feed containing 25% of proteins and 7% of lipids, manufactured by ”Bafi” (Novi Sad, Serbia). Later, during the season, when the water temperature dropped under 20^{o}C, fish were fed pelleted feed containing 20% of proteins and 7% of lipids. Fish from pond A were fed with pelleted feed with 35% of proteins and 7% of lipids, while during the colder period, with feed containing 25% of protein and 7% of lipids. Fish were fed at 2-2.55% of the total fish biomass, twice a day, around 9 h and 13 h to increase the natural food utilization. In order to monitor fish health and determine the total biomass, fish were sampled every 15 days. For detailed adjustment of the amount of supplemental feed added to ponds, development of natural food (zooplankton and zoobenthos) was determined, and environmental variables were measured once per month.

Based on obtained results, average fish weight, fish growth rate and the amount of utilized feed were determined.

Following growth parameters were calculated:

Body weight gain (BWG) (kg) = final body weight (kg) – initial weigth (kg);

Obtained gain (kg/ha) = (total final weight (kg) – total initial weight (kg)) / production area (ha);

Feed conversion ratio (FCR) = total feed consumed / total weight gain

RESULTS AND DISCUSSION

Measured environmental parameters in ponds were in the acceptable range for culturing of common carp. Additionally, there were no major differences between ponds in these parameters.

The values of fish growth rate and feed conversion ratio are shown in Table 2.

Table 2. Production parameters at the end of the rearing season

Fish pond		A	B	C
Carp fry S1+	Total harvest (kg)	91 520	20 554	/
	Average biomass (kg)	1 036	1 050	/
	BWG (kg)	0.933	0.840	/
Marketable size carp S2+	Total harvest (kg)	/	24 890	22 715
	Average biomass (kg)	/	2 610	3 150
	BWG (kg)	/	1.900	2.370
Harvest per surface areas (kg/ha)		2 615	1 817	1 420
Total obtained growth rate (kg)		80 056	34 979	15 813
Growth rate per surface area (kg/ha)		2 287	1 399	988
Feed conversion ratio		2.26	2.41	3.2

Higher BWG was obtained in carp categories reared separately (A S1+ = 0.933 kg; C S2+ = 2.37 kg) than in carp categories reared together (B S1+ = 0.84 kg; B S2+ = 1.9 kg). Total obtained growth rate of fish was relatively different between ponds (A = 2 287 kg/ha; B = 1 399 kg/ha; C = 988 kg/ha). This is probably due to differences in growth potential of reared fish, where S1+ stocking category achieves approximately three times higher growth rate that older fish category - S2+. FCR was also the lower in pond (A) stocked with S1+ (2.26). In the pond with combined rearing of S1+ and S2+, FCR value was slightly higher (2.41) than in pond A, but considerably lower than in C stocked with S2+ (3.2).

Pelleted feed with higher protein content (35/7) additionally positively influenced the growth rate of S1+ fish in pond A. These results are in line with the study of Rai and Bista (2001) concluding that fish growth rate is higher in fish fed diet with higher protein level, especially of animal origin. Singh et al. (2011) reported that differences in FCR can be attributed to higher level of metabolism that characterizes younger individuals.

Based on the obtained values of fish growth rate, by rearing only one fish category S1+ (A = 2 287 kg/ha) or S2+ (C = 988 kg/ha), 10% lower values are achieved compared to combined rearing of S1+ and S2+ (B = 1 399 kg/ha). One of the possible reasons for these differences is better utilization of small size classes of natural food (<250 μm) i.e. Rotifers, Copepods, smaller Cladocerans, zoobenthos, due to smaller inter-gill raker spacing in younger carp (Budy and Haddix, 2005), as well as utilization of small particles of pellets present in the water due to high dissolving properties of this type of feed.

CONCLUSION

In semi-intensive fish production as a dominant type of fish farming, higher amounts of supplemental feed are used during natural food depression, in order to provide good growth and health of fish. According to our results, it can be concluded that higher BWG and FCR are obtained when only one stocking category of fish is reared. However, comparing the ratio in stocking densities of S1+ and S2+ from the same fishpond (20 : 80) and the obtained growth rate (kg/ha) in individual rearing of S1+ and S2+, it can be concluded that 10% higher growth rate is attained in combined fish rearing. An additional reason for favoring combined rearing of different stocking categories of carp is the lower price of pelleted feed with inferior protein levels that compensates for a slightly higher FCR value.

ACKNOWLEDGMENTS

The study was supported by the Ministry of Education, Science and Technological Development, the project Improvement of production capacities of carp (*Cyprinus carpio*) through nutrition and selective breeding programs (TR 31075) and FP7 project AREA (316004).

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CONCENTRATION DATA FOR 30 ELEMENTS IN THE MUSSELS *MYTILUS GALLOPROVINCIALIS*

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PODACI O KONCENTRACIJAMA 30 ELEMENATA U DAGNJAMA *MYTILUS GALLOPROVINCIALIS*

Apstrakt

Priobalni deo jugoistočnog Jadrana prima velike količine zagađujućih materija koje se u morsku sredinu unose putem različitih anropogenih i prirodnih izvora kao što su luke, industrija, turizam, poljoprivredna aktivnost, erozija zemljišta, atmosfersko taloženje, itd. Bokokotrski zaliv je prirodno podeljen na četiri manja zaliva: hercegnovski, tivatski, risanski i kotorski zaliv. Svaki od unutršnjih 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. Bokokotorski zaliv se smatra jednim od glavnih priliva slatke vode južnog Jadrankog mora. Reljefne karakteristike stimulišu razvoj naselja duž obale zaliva i upravo se pogodne poljoprivredne zone i nalaze u neposrednoj blizini velikih naseljenih gradova Kotora, Herceg Novog i Tivta. Poluzatvoren sistem zaliva, slabo strujanje i cirkulacija vode, utiče na veće vreme potrebno da se određene zagađujuće materije prirodnim putem uklone iz samog zaliva.

Dagnja *M. galloprovincialis* je nativna vrsta ove oblasti i ovog zaliva i danas se komercijlno uzgaja i proizvodi, ali i kao nativna vrsta može se naći duž cele obale Bokokotorskog zaliva. Dagnje su veoma važne za ljudsku ishranu i njihovim konzumiranjem u organizam se unose proteini visoke biološke vrednosti, minerali i vitamini. Sa aspekta hranljivosti, školjka je važan izvor hrane bogate bitnim elementima (Ca, Fe, Se, Zn, I, itd.), kao i određenim vitaminima (A, C, B1, B2, B3, B9, B12). Minerali i mikroelementi predstavljaju značajne komponente u ljudskoj ishrani, a njihov nedostatak kao i višak može izazvati ozbiljne zdravstvene probleme. Poznavanje elementarnog sastava dagnji od suštinskog je značaja za procenu dostupnosti hranjivih materija, jer one predstavljaju jeftinu visoko proteinsku hranu sa niskim sadržajem masti i kalorija, ali i sa apekta procene i smanjenja potencijalno negativnih efekata po zdravlje ljudi, jer je ova vrsta plodova mora poznata po sposobnosti da akumulira velike količine prisutnih zagađujućih materija.

Cilj ovog rada je bio da se ispita elementarni sastav mekog tkiva dagnji *M. galloprovincialis* iz Bokokotorskog zaliva, Crna Gora, jugoistočni Jadran. Energetski disperzivnom rendgenskom fluoroscentnom metodom (ED-XRF) analizirani su sledeći elementi: Cl, Si, S, K, P, Ca, Fe, Br, Zn, Mn, Sr, I, As, Cr, Ti, Ce, Ba, Cu, Cs, Rb, Pb, Ni, Th, Sb, Sn, Cd, V, Co, Zr i Hg , na osnovu izmerenih vrednosti, izvršena je procena zastupljenosti makro, mikro, neesencijalnih i toksičnih elemenata u mekom tkivu dagnji.

Ključne reči: Mytilus galloprovincialis; Makro, mikro i elementi u tragovima; ED-XRF; Crna Gora.
Keywords: Mytilus galloprovincialis; Makro, mikro and trace elements; ED-XRF; Montenegro.

INTRODUCTION

Natural weathering and human activities are the sources contributing to element contamination of the marine coastal environment through rivers and atmospheric transport. The Southeastern coastal part of the Adriatic Sea receives large amounts of contaminants which are introduced by marine harbors, domestic, industrial, touristic and agricultural activities, by land erosion and through atmospheric deposition (Jović et al., 2011). The Boka Kotorska Bay is naturally divided into four smaller bays: the Herceg Novi, Tivat, Risan and Kotor bay. They are connected and interact with the open sea through narrow straits and the Bay can be considered as one of the main freshwater inputs into the southern Adriatic Sea. The semi-enclosed systems such as Boka Kotorska Bay, are very sensitive to contamination, and their structure attributes to the longer removal time of the contaminants (Jović and Stanković, 2014).

The mussel *M. galloprovincialis* is a native species for this area. Commercial farming of these mussels dates back 30 years in this area, and today these mussels are cultivated on several farms along the whole coast of Boka Kotorska Bay. In addition to cultivated mussels, wild mussels are widespread throughout the Bay, which are hand-collected for personal consumption (Jović et al., 2012). In the same time mussels are an important and excellent source of proteins, some vitamins and essential elements for humans, but they can potentially be toxic. Mussels are well known to accumulate a wide range of metals in their soft tissues and increasingly frequently are used as an indicator of marine pollution (Jović et al., 2011; Stanković et al., 2011, Stanković and Jović, 2013).

The aim of this work was to investigate the element composition of mussels *M. galloprovincialis* from Southeastern Adriatic by measuring Cl, Si, S, K, P, Ca, Fe, Br, Zn, Mn, Sr, I, As, Cr, Ti, Ce, Ba, Cu, Cs, Rb, Pb, Ni, Th, Sb, Sn, Cd, V, Co, Zr and Hg with the energy dispersive X-ray fluorescence method, and also to identify the contribution of macronutrients, micronutrients, nonessential and toxic elements to the total mussel elemental concentrations.

MATERIALS AND METHODS

Boka Kotorska Bay is located in Montenegro, Adriatic-Mediterranean country. Mussel samples were collected at seven locations: Krašići, Kukuljina, Tivat, Opatovo, Sv Stasija, Perast and Herceg Novi. At each sampling site about 2 kg of mussels were collected, placed in nylon bags with seawater and transported to the laboratory. The biggest 25–30 mussels, of approximately the same size, were washed and cleaned out, opened raw and the flesh scraped out of the shells, which was then freeze-dried at -40 °C for 48 h, weighed, homogenized and ground to a fine powder. The powdered sample was pressed with a hydraulic press by applying a pressure of 7 t for 20 s. No binder material was applied. The resulting pellets had a diameter of 32 mm and a uniform mass of 400 ± 3 mg. The samples were prepared in this manner, in the form of pressed pellets, for energy dispersive X-ray fluorescence analysis (ED-XRF). In this work a MiniPal 4 ED-XRF spectrometer (PANalytical, Almelo, Netherlands) was used to determine major, minor and trace elements (Cl, Si, S, K, P, Ca, Fe, Br, Zn, Mn, Sr, I, As, Cr, Ti, Ce, Ba, Cu, Cs, Rb, Pb, Ni, Th, Sb, Sn, Cd, V, Co, Zr and Hg) concentrations.

RESULTS AND DISCUSSION

Minerals are inorganic substances present in all body tissues, and their presence is necessary for the maintenance of certain physicochemical processes which are essential to life. Every form of living matter requires minerals for their normal life processes (Soetan et al., 2010). In relation to their concentrations minerals may be classified as macro (major), micro (trace) and ultra trace elements (Stanković et al., 2012). The significance and importance of mineral elements for human and animal nutrition is well clarified in this century, and modern analytical techniques tend to their exact quantification. Element composition of mussels *Mytilus galloprovincialis* from Boka Kotorska Bay was determined by measuring Cl, Si, S, K, P, Ca, Fe, Br, Zn, Mn, Sr, I, As, Cr, Ti, Ce, Ba, Cu, Cs, Rb, Pb, Ni, Th, Sb, Sn, Cd, V, Co, Zr and Hg with the relatively new nondestructive method ED-XRF. Results of the investigated macro, micro and ultra trace elements in the mussels’ soft tissue are shown in Table 1.

Table 1. Elements concentrations in the mussels soft tissue (wet weight basis expressed as mg/kg) at seven location in Boka Kotorska Bay, Montenegro

	Sampling locations						
	Krašići	**Kukuljina**	**Tivat**	**Opatovo**	**Sv Stasija**	**Perast**	**H. Novi**
Cl	6402.9	5665.9	7511.8	6454.5	3822.0	3809.5	5399.0
Si	4541.3	4797.4	4648.1	4530.4	4548.3	4520.8	4566.4
S	2155.7	2026.8	2414.4	2041.0	1841.3	1696.0	1904.0
K	1321.6	1398.4	1497.1	1349.3	1270.2	1219.9	1226.3
P	978.7	933.0	1116.6	935.6	1085.1	965.4	885.1
Ca	485.5	468.7	1028.4	373.7	249.8	268.7	825.3
Fe	59.86	179.9	97.75	34.78	22.84	22.84	31.66
Br	63.49	50.52	56.06	67.47	42.04	42.21	65.05
Zn	14.71	22.15	42.04	24.05	14.01	15.92	27.85
Mn	13.84	15.05	16.96	15.57	14.01	14.19	16.78
Sr	9.00	7.44	15.57	6.06	8.48	5.88	7.96
I	7.44	6.75	4.67	7.44	6.06	4.67	7.79
As	5.88	4.15	4.15	4.15	4.50	4.33	4.67
Cr	4.33	4.84	4.33	4.50	4.50	4.33	4.50
Ti	3.63	10.0	0.52	2.60	1.38	0.35	2.25
Ce	2.60	2.60	2.77	2.60	2.25	3.46	2.94
Ba	2.42	2.77	2.25	1.38	1.73	1.90	1.56
Cu	1.52	1.44	2.16	1.45	1.07	1.38	1.38
Cs	1.63	1.57	0.90	1.11	1.28	1.31	1.02
Rb	1.09	1.49	1.07	1.16	0.95	0.88	1.06
Pb	0.66	0.69	1.28	0.80	0.59	0.62	0.69
Ni	0.43	1.04	0.76	0.52	0.36	0.40	0.54
Th	0.381	0.398	0.381	0.450	0.329	0.294	0.502
Sb	0.381	0.398	0.294	0.363	0.415	0.415	0.381
Sn	0.311	0.294	0.450	0.260	0.242	0.706	0.260
Cd	0.242	0.173	0.208	0.277	0.173	0.156	0.190
V	0.104	0.104	0.156	0.087	0.017	0.398	0.069
Co	0.090	0.149	0.140	0.131	0.090	0.045	0.156
Zr	0.190	0.294	0.069	0.035	0.035	0.035	0.035
Hg	0.003	0.016	0.016	0.019	0.043	0.019	0.003

In the investigated mussels the Cl, Si, S, K, P and Ca concentrations are significantly higher than the remaining tested elements, which is understandable since these are macronutrients. These elements have many important roles in the organisms (Soetan et al., 2010). An anthropogenic influence on obtained concentrations is possible and expected since these elements are present in many manufacturing processes and products (Jović et al., 2011). The Cl, K and Ca are dominant elements in seawater, and high concentrations of these elements in mussels are expected. The measured contents of Si in mussels from all sampling locations were in the close range. It is known that Si is the main constituent in marine organisms as a biogenic mineral and in the sediment clay of the Bay bottom (Tanaskovski et al., 2014). Similar distribution of this element in the soft tissue of mussels at all locations primarily indicates its natural origin. Both elements, S and P, are a natural and anthropogenic component of an aquatic environment, but fluctuation of mussels concentrations by locations indicates possible increased anthropogenic influence of these two elements. Fluctuations of individual micronutrients, nonessential and toxic elements concentrations in the investigated mussels from different sampling locations, besides the natural origin of elements, can also indicate the possible increase in anthropogenic input. The specificity of the Bay geo-hydrology can also influence the level and a natural origin of these elements in mussels (Tanaskovski et al., 2014). In relation to the wet matter the mussel soft tissue contained 98.7 % macronutrients, 0.76 % micronutrients and 0.54 % nonessential and toxic elements.

CONCLUSION

Element composition of mussels *Mytilus galloprovincialis* was determined with the relatively new nondestructive method - the energy dispersive X-ray fluorescence. In relation to the wet matter, the mussel soft tissue contained 98.7% macronutrients (Cl, Si, S, K, P and Ca), while the remaining 1.3% refers to all the remaining elements: 0.76% micronutrients (Fe, Zn, Mn, I, Cr, Cu, Ni, V and Co) and 0.54% nonessential and toxic elements (As, Ba, Br, Cd, Ce, Cs, Hg, Pb, Rb, Sb, Sn, Sr, Th, Ti and Zr). Fluctuations of individual elements' concentrations in the investigated mussels' soft tissue from different sampling locations besides the natural origin of elements can also indicate to the possible increase in anthropogenic input. The geo-hydrology impact of investigated area can also influence the level and a natural origin of these elements in mussels.

ACKNOWLEDGEMENTS

The authors acknowledge funding from the Ministry of Education, Science and Technological Development of the Republic of Serbia, Contract No. III43009.

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ELEMENT CONCENTRATIONS IN MUSCLE TISSUE OF TWO FISH SPECIES FROM DIFFERENT TROPHIC LEVELS (BLEAK AND PIKE) IN THE DANUBE NEAR BELGRADE

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KONCENTRACIJE ELEMENATA U MIŠIĆNOM TKIVU DVE VRSTE RIBA RAZLIČITOG TROFIČKOG NIVOA (UKLIJA I ŠTUKA) U DUNAVU KOD BEOGRADA

Apstrakt

Industrijski i komunalni efluenti uzrok su visokih koncentracija nekih elemenata u rečnoj vodi. Procenjuje se da se u Srbiji oko 90% industrijskih otpadnih voda ispušta u vodotokove bez prethodne obrade. Koncentracije elemenata u tkivima riba pod uticajem su kako prisustva zagađenja staništa, tako i trofičkog položaja i veličine ribe. Koncentracije 15 elemenata analizirane su upotrebom ICP-OES u uzorcima mišićnog tkiva uklije i štuke uhvaćenih na ušću Save u Dunav, između oktobra 2011. i marta 2012. godine. Elementi Cd, Co, Li i Pb nisu detektovani u uzorcima. Jednofaktorska ANOVA je pokazala da postoje značajne razlike između mišićnog tkiva uklije i štuke u odnosu na koncentracije Ba, Cu, Fe i Zn. U mišićnom tkivu štuke, koja predstavlja piscivornu vrstu, detektovana je viša koncentracija Hg nego kod uklije, što ukazuje na biomagnifikaciju kroz lance ishrane, dok je kod uklije, kao obligatnog zooplanktivora, detektovana viša koncentracija Zn, što potvrđuje tendenciju negativne korelacije Zn sa trofičkim nivoom ribe. U mišiću uklije, od analiziranih elementa samo je kod Se uočena pozitivna korelacija sa totalnom dužinom tela (TL), dok je kod štuke uočena negativna korelacija Se sa težinom (W) i pozitivna korelacija koncentracije Al sa TL i W. Kod obe vrste ukupno je nađeno 20 korelacija (14 pozitivnih i 6 negativnih) između koncentracija elemenata u mišićnom tkivu. Kod uklije, najveći broj korelacija sa drugim elementima uočen je kod Mn i Sr. Kod štuke, pozitivna korelacija uočena je između Fe, Se i Zn, dok su sva tri elementa bila negativno korelisana sa Al, a Se i Zn pozitivno sa Mn i Sr.

Ključne reči: uklija, štuka, ICP-OES, mišićno tkivo, toksični metali
Keywords: bleak, pike, ICP-OES, muscle tissue, toxic metals

INTRODUCTION

High concentrations of pollutants in the aquatic environments are often the result of industrial and municipal effluent discharges into the surface waters (Asuquo and Ewa-Oboho 2004). In the Danube River basin, this is especially pronounced around industrial facilities in the vicinity of the cities of Novi Sad, Belgrade, Pančevo, and Bor (Stanić *et al*. 2006), as it is estimated that approximately 90% of all industrial wastewaters in Serbia are discharged into waterbodies without a previous treatment (Matijašević *et al*. 2010). Element concentrations in fishes depend not only on the contamination of the environment, but also on several ecological factors, such as trophic position (Kehrig *et al*. 2013) and size (Al-Yousuf *et al*. 2000; Canli and Atli 2003) of the fish.

MATERIALS AND METHODS

Nine bleak (*Alburnus alburnus*) and six pike (*Esox lucius*) specimens were caught at the confluence of the Sava River with the Danube River, from October 2011 to March 2012. Caught specimens were dissected with a plastic laboratory set and samples of muscle tissue were taken, rinsed with distilled water, and stored at -18 ºC prior to analysis. Samples were freeze-dried using a rotational vacuum concentrator (GAMMA 1-16 LSC Germany) and sample portions between 0.2 and 0.5 g were processed in a microwave digester (speedwave^{TM} MWS-3+; Berghof Products +Instruments GmbH, Eningem, Germany), using a 6 ml of 65% HNO_3 (Suprapur®, Merck) and 4 ml of 30% H_2O_2 (Suprapur®, Merck) at a food temperature program (100-170 ºC). After cooling to room temperature, the digested samples were diluted with distilled water to a total volume of 25 ml. The analysis of concentrations of Al, As, B, Ba, Cd, Co, Cr, Cu, Fe, Hg, Mn, Mo, Se, Sr, and Zn in samples was performed by ICP-OES (Spectro Genesis EOP II, Spectro Analytical Instruments DmbH, Kleve, Germany). The results are presented as μg/g dry weight.

We tested the normality of variable distribution with the Shapiro-Wilk test as well as the homogeneity of variance with the Levene's test. Since the assumption of a normal distribution of variables and the homogeneity of variances was satisfied, we applied the parametric one-way ANOVA test to compare the element concentrations in muscle tissue between the two fish species. A correlation analysis (bivariate correlations with Pearson's correlation coefficients) was used to check for significant relationships between the fish total length (TL) and weight (W) with element concentrations. Statistical tests were done using the SPSS 20.0 software.

RESULTS

The mean total length of the analyzed bleak individuals was 16.3 ± 1.0 cm and weight was 35.9 ± 8.4 g, while the mean total length of the analyzed pike individuals was 56.6 ± 4.5 cm and weight was 1263.2 ± 289.6 g.

Elements Cd, Co, Li, and Pb were not detected in both bleak and pike muscle samples. The concentrations of B and Ni were below the detection threshold in the bleak muscle, while the concentration of Mo was below the detection threshold in the pike muscle. The one-way ANOVA test showed significant differences between the bleak and pike muscle tissue with regard to the concentrations of Ba, Cu, Fe, and Zn (Table 1).

Table 1. Mean concentrations and standard deviations of 11 elements in muscle tissue of bleak (*A. alburnus*) and pike (*E. lucius*) (in µg/g dry weight). Significant difference in concentrations of elements between species is marked by an asterisk.

Element	*A. alburnus* (bleak)	*E. lucius* (pike)
Al	2.60 ± 3.38	4.58 ± 3.09
As	1.06 ± 0.21	1.03 ± 0.32
Ba	0.90 ± 0.42 *	0.16 ± 0.13 *
Cr	0.15 ± 0.32	0.26 ± 0.19
Cu	1.71 ± 0.25 *	0.48 ± 0.28 *
Fe	22.30 ± 7.77 *	8.89 ± 5.25 *
Hg	1.47 ± 0.45	2.32 ± 1.15
Mn	1.37 ± 0.46	0.99 ± 1.01
Se	0.34 ± 0.19	0.49 ± 0.21
Sr	3.81 ± 2.44	2.03 ± 3.45
Zn	69.52 ± 24.67 *	18.87 ± 12.26 *

The results of the correlation analysis are presented in Tables 2 and 3. Correlations significant at 0.01 level (two-tailed) are indicated by bold numbers and correlations significant at 0.05 level (two-tailed) are indicated by italic numbers.

Table 2. Correlation matrices between the total length (TL) and weight (W) and element concentration in bleak (*A. alburnus*) muscle (0.01 level significance in bold; 0.05 level significance in italic).

	TL	W	Al	As	Ba	Cr	Cu	Fe	Hg	Mn	Se	Sr	Zn
TL	1												
W	**0.96**	1											
Al	0.06	0.12	1										
As	0.69	0.67	- 0.56	1									
Ba	- 0.21	- 0.05	0.29	- 0.11	1								
Cr	- 0.43	- 0.31	- 0.19	- 0.29	- 0.28	1							
Cu	- 0.24	- 0.28	- 0.58	0.22	- 0.44	0.44	1						
Fe	- 0.49	- 0.39	- 0.39	- 0.15	- 0.29	**0.96**	0.65	1					
Hg	- 0.47	- 0.29	0.56	*- 0.75*	0.43	0.48	- 0.41	0.29	1				
Mn	- 0.20	- 0.11	*0.76*	*- 0.77*	0.30	0.16	- 0.52	- 0.06	**0.86**	1			
Se	*0.73*	0.67	- 0.02	0.62	- 0.07	- 0.27	- 0.05	- 0.27	- 0.42	- 0.30	1		
Sr	- 0.35	- 0.22	*0.83*	*- 0.74*	0.63	- 0.01	- 0.54	- 0.17	*0.83*	**0.88**	- 0.31	1	
Zn	- 0.69	- 0.63	0.22	- 0.63	0.61	0.19	- 0.06	0.19	0.62	0.51	- 0.26	0.66	1

Table 3. Correlation matrices between the total length (TL) and weight (W) and element concentration in pike (*E. lucius*) muscle (0.01 level significance in bold; 0.05 level significance in italic).

	TL	W	Al	As	Ba	Cr	Cu	Fe	Hg	Mn	Se	Sr	Zn
TL	1												
W	**0.96**	1											
Al	*0.88*	**0.94**	1										
As	0.52	0.54	0.38	1									
Ba	- 0.40	- 0.39	- 0.23	- 0.55	1								
Cr	- 0.62	- 0.60	- 0.61	- 0.36	- 0.38	1							
Cu	0.20	0.06	- 0.01	0.55	- 0.61	0.10	1						
Fe	- 0.63	- 0.71	*- 0.85*	- 0.43	0.37	0.34	- 0.30	1					
Hg	- 0.49	- 0.40	- 0.51	- 0.30	0.39	0.21	- 0.73	0.74	1				
Mn	- 0.64	- 0.62	- 0.77	0.06	0.26	0.22	- 0.18	0.77	0.75	1			
Se	- 0.76	*- 0.82*	*- 0.90*	- 0.15	0.41	0.23	0.00	*0.83*	0.52	*0.89*	1		
Sr	- 0.62	- 0.60	- 0.74	0.11	0.29	0.16	- 0.15	0.73	0.71	**1.00**	*0.89*	1	
Zn	- 0.64	- 0.69	*- 0.85*	- 0.19	0.33	0.27	- 0.22	**0.95**	0.76	**0.92**	*0.91*	*0.90*	1

DISCUSSION

The higher concentration of Hg was detected in pike muscle tissue than in bleak. The same result was obtained by Zrnčić *et al*. (2012) in the Danube River in Croatia, which is consistent with the observed biomagnification of this element through the food chain (Barbosa *et al*. 2003), considering that pike is a piscivorous predator and bleak an obligatory zooplanktivore. On the other hand, a higher concentration of Zn was detected in bleak muscle tissue. The trend of a higher Zn concentration in tissues of non-predatory than in predatory species is also shown by Mazej *et al*. (2010), which can be attributed to the tendency of Zn to correlate inversely with the trophic position of the fish (Papagiannis *et al*. 2004).

In bleak muscle, only Se is significantly and positively correlated with TL, while in pike muscle Se is significantly and negatively correlated with W, and Al is significantly and positively correlated with both TL and W. Although Zn is negatively correlated with both TL and W, which is consistent with observations made by Hogstrand (2011), this correlation was not significant.

In total, 20 correlations (14 positive and 6 negative) were observed between elements in muscle tissues of bleak and pike. In bleak, Mn and Sr had the largest number of correlations with other elements (four). In pike, a significant positive correlation was observed between Fe, Se, and Zn, while all three were negatively correlated with Al and Se and Zn positively with Mn and Sr. Klavins *et al*. (2009) found a positive correlation between Fe and Zn in muscle tissue of another piscivorous fish (perch, *Perca fluviatilis*), which was also observed in pike samples in our study.

CONCLUSIONS

Statistical differences between bleak and pike muscle tissue were observed with regard to concentrations of Ba, Cu, Fe, and Zn, and bleak had higher concentrations of these elements. A higher concentration of Hg in muscle tissue of the predatory species (pike) than in non-predatory (bleak) was also observed, which indicates the biomagnification of this element through the food chain. On the other hand, a higher concentration of Zn was detected in bleak muscle tissue, which can be attributed to the tendency of Zn to correlate inversely with the trophic position of the fish. In bleak muscle, only Se is significantly and positively correlated with TL, while in pike muscle, Al was positively correlated with TL and W, and there were similarities between Se and Zn regarding the correlation with other elements.

ACKNOWLEDGEMENTS

We acknowledge the support provided by Project No. 173045, funded by the Ministry of Education, Science and Technological Development of the Republic of Serbia.

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PROTECTION MODEL OF NATURAL RESOURCES BY INTEGRATION OF MANAGEMENT FUNCTIONS OF FISHING AND HUNTING AREAS

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MODEL ZAŠTITE PRIRODNIH RESURSA INTEGRACIJOM UPRAVLJAČKIH FUNKCIJA RIBARSKOG I LOVNOG PODRUČJA

Apstrakt

Ribarska i lovna područja u zakonodavnom, organizacionom i funkcionalnom smislu ispoljavaju veliku sličnost, koja proistiće iz zakonskih definicija. Kvalitet upravljanje obnovljivim resursima u oblasti ribarstva i lovstva uslovljen je političko-zakonodavnim, ekonomskim, socijalnim i tehnološko-ekološkim parametrima (kategorijama) određenim širokim spektrom različitih interesa i potreba. Zato je veoma važno odabrati model upravljanja pomenutim prirodnim resursima koji može zadovoljiti više ciljnih grupa, a da pri tome bude efikasan i ekonomičan.

Cilj ovog rada je da se na realan i praktičan način prikažu kriterijumi i alternative za poboljšanje upravljačkih funkcija kroz model zaštite prirodnih resursa integracijom upravljačkih funkcija ribarskog i lovnog područja.

Kao polazni materijal u ovom radu korišćeni su zakonska i planska dokumenta iz oblasti održivog korišćenja ribljeg fonda i lovstva. Izvršena je višekriterijumska analiza na osnovu kriterijuma PEST analize u odnosu na moguće alternative (posebno ribarsko, posebno lovno, integralno upravljanje ribarsko-lovnim područjem). PEST analiza se zasniva na analizi eksternih uticaja (Političko-zakonodavna, Ekonomska, Sociološka i Tehnološko-ekološka analiza) koji utiču na izbor organizacionog oblika upravljanja ribarskim i lovnim područijima. Kao kontrolni metod korišćen je analitičko hijerarhijski proces (AHP), sa namerom da se utvrdi u kojoj meri eksterni kriterijumi okruženja deluju na izbor ponuđenih alternativa (Saaty, 1980).

Prema većini autora u Srbijii postoji potreba integralnog upravljanja vodnim resursima i vodoprivrednim objektima, koja bi kroz adekvatnu pravnu regulativu ostvarila svoj puni tehnološko-tehnički kapacitet, ekonomičnost, efikasnost i efektivnost. Srbija raspolaže velikim brojem reka, prirodnih i veštačkih akumulacija. Integralno upravljanje rečnim slivom pretpostavlja plansko upravljanje i izradu monitoringa baziranih na ekološkim karakteristikama i kontinuiranom praćenju biotičkih i abiotičkih faktora (Simonović i sar., 2005).

U Srbiji je ustanovljeno oko 300 lovišta, u kojima su uočeni kao najčešći nedostaci: nedosledna primena i nepoštovanje zakonskih propisa, monopolizam određenih organizacija i interesnih grupa, nizak nivo tehničko-tehnološkog i institucionalnog razvoja, nepostojanje dokumenta strateškog planiranja u lovstvu, nerazvijeni monitoring divljači i njihovih staništa Sa aspekta održivog upravljanja lovnim područjima osnovni ciljevi su: povećanje brojnosti populacije sitne i krupne divljači, poboljšanje polne i starosne strukture populacija, očuvanje retkih i ugroženih vrsta lovne divljači (Medarević i sar., 2008).

Radovi i dokumenta, kao i iskustva drugih zemalja upućuju na potrebu objedinjenog upravljanja pomenutim obnovljivim resursima.

Može se zaključiti da bi integralno gazdovanje ribarskim i lovnim područjima poboljšalo upravljačke funkcije, smanjilo troškove i povećalo efikasnost poslovanja. Sigurno bi pozitivno uticalo i na socijalni aspekt i promenu svesti u javnosti da ribolov i lov nisu samo socijalna već i značajna privredna kategorija. Integralnim upravljanjem ribarskim i lovnim područjima bio bi olakšan monitoring sa jasnijim i uporedivim rezultatima, a kontrola i korišćenje finansijskih sredstava bila bi neuporedivo bolja i racionalnija. U pogledu funkcionisanja stručnih i čuvarskih službi potrebno je izvršiti detaljnu analizu geografskog rasporeda, veličine i broja lovnih tj. ribolovnih područja, sa ciljem optimizacije upravljanja istim na ekonomskim i ekološkim principima.

Potencijalna korist za državu se vidi u smanjenju troškova uprave, pri čemu bi se mogli formirati regionalni centri na nivou jednog ili više područja, a koji bi služili za obuku kadrova, organizaciju monitoringa, reagovanja u akcidentnim situacijama i slično. Pre toga potrebno je zakonsku regulativu, a potom ribarska i lovna područja učiniti međusobno kompatibilnim.

Ključne reči: ribarsko i lovno područje, upravljanje.
Keywords: fishing and hunting areas, integration

INTRODUCTION

Fishing and hunting areas in the legislative, organizational and functional terms manifest great similarity that is derived from the legal definitions. The quality of renewable resources management in the field of fishery and hunting is conditioned by political and legislative, economic, social, technological and ecological parameters (categories) determined by a wide range of different interests and needs. Therefore, it is very important to choose the model of managing natural resources mentioned above that can satisfy many target groups and by doing so to be efficient and economical.

Besides legal formulations, for unified management of fishing and hunting areas it is necessary that the idea and functions of river basin are presented as the framework for spatial areas formation within the management of fishing and hunting areas. The aim of this work is to show, in a realistic and practical way, the criteria and alternatives for improving management functions through the protection model of natural resources by integration of management functions of fishing and hunting areas.

MATERIAL AND METHODS

As a starting material in this work we used legal and planning documents in the field of sustainable use of fish stock and hunting. Multi-criteria analysis was performed on the basis of PEST criteria analysis in relation to possible alternatives (separately fishing, separately hunting, integrated management of fishing and hunting area). PEST analysis (political and legislative, economic, social, technological and ecological analysis) is based on external influences analysis that affects the choice of organizational forms of managing fishing and hunting areas. Analytical hierarchy process (AHP) was used as a control method in order to determine at what extent external environmental criteria affects the choice of given alternatives (Saaty, 1980).

RESULTS AND DISCUSSION

In the Law on the protection and sustainable usage of fish stock and The Law on game and hunting it is defined: **A fishing area** as "natural or artificial fishing waters or its part which makes hydrological, biological and economic entity for protection and sustainable use of fish stock. Fishing area is assigned for using by a tender for a decade. User is required to obtain and bring the approval on the Program of fishing area management…"

Hunting area as "physically rounded geographical and natural environment which is established for the purpose of implementation of unique hunting policy, long-term rational management of certain game species populations and efficient in taking appropriate measures in the hunting grounds. The right on the hunting ground is given for a period of ten years, except in cases where the law provides otherwise. The implementation of objectives and measures for development of hunting and improving the condition of game populations is done on the basis of the program of hunting areas development…"

In the law, safeguarding of fishing area is defined as "taking preventive and repressive measures of fish guard service, which prevents performing actions on the fishing waters that are in accordance with the provisions of the law, **and for game guarding service**

to perform the protection and regulation of hunting ground, as well as breeding and protection of game in the hunting ground…"

According to the most authors (Bajčetić and Stojanović 2011) in our country there is a need for integrated water resources management and water management facilities, which would through appropriate legal regulations, achieve its full technological and technical capacity, economy, efficiency and effectiveness. Serbia has a great number of rivers, natural and artificial accumulations. Integrated management of river basin assumes planned management and creation of monitoring based on ecological characteristics and continuous monitoring of biotic and abiotic factors (Simonović et al , 2005.)

About 300 hunting grounds were established in Serbia. The most common deficiencies identified in those hunting grounds were: inconsistent compliance and contempt of legislation, monopolism of certain organizations and interest groups, low level of technical as well as institutional development, lack of strategic planning document in hunting, underdeveloped monitoring of game and their habitats.(Professional basis for the development strategy of hunting in Republic of Serbia, 2011). In terms of sustainable management of hunting areas the main goals are: to increase number of small game population, improvement of age and gender structure of population and preservation of endangered and rare species of hunting game. (Medarević et al., 2008.)

Papers and documents mentioned above, as well as experiences of other countries refer to the need of unified management of fishing and hunting areas. According to the experiences of the USA Federal Agency, natural resources in hunting and fishing should be interconnected and that is the reason why the integrated management plans for natural habitats of game, fish and wild flora are prepared.

Based on the results and studies of other authors, legally defined objectives in fishing and hunting, as well as experiences of other countries in the integrated management of these significant natural resources, the PEST analysis was performed in order to evaluate: political and legislative, economic and organizational, sociological, technological and ecological influence on organizational alternatives; fishing, hunting and integrally fishing and hunting area. In this analysis potential opportunities are marked as plus sign (+), threats minus sign (-), and influences of certain factors were estimated from 1 to 10. The importance of factors was evaluated with marks from 1 to 5 (Pfeifer, 2002). In this way we defined weights of influence in relation to offered alternatives.

Table 1. PEST analysis of influence on organizational alternatives of fishing and hunting areas.

FACTORS OF INFLUENCE	Possibility of threat (+ / -)	Influence of factors (1 do 10)	Importance of factors (1 do 5)	Weights of influence factors
ALTERNATIVE A - SEPARATED FISHING AREA				
Political and legislative		6	4	24
Economic and organizational		5	5	25
Sociological		-6	4	-24
Technological and ecological		4	3	12
			TOTAL	37
ALTERNATIVE B – SEPARATED HUNTING AREA				
Political and legislative	+	8	4	32
Economic and organizational	+	6	5	30
Sociological	-	-8	4	-32
Technological and ecological	+	6	3	18
			TOTAL	48
ALTERNATIVE C - INTEGRATED HUNTING AND FISHING AREA				
Political and legislative	+	8	5	40
Economic and organizational	+	7	5	35
Sociological	-	-4	4	-16
Technological and ecological	+	7	3	21
			TOTAL	80

According to the results shown in Table 1, the minimum value of weighting factors of influence are determined at the option of separate management of fishing areas (37). The maximum value of the weighting factors (80) is calculated for the option of integrated management of fishing and hunting areas. Limitations of external factors are the most prominent in the social segment, due to untapped opportunities in economic and organizational, technological point of view and especially the legislative-political, dominates the view that fishing and hunting are social , not economic categories ,whereby limiting impacts are the largest in hunting, and slightly smaller in fishing. It should be pointed out that insufficient influence of political and legislative solutions have not given an adequate response to economic and financial problems so far (Šarčević, et al., 2013.)

Table 2. Results of multi-criteria analysis of comparing alternatives in relation to criteria

ALTERNATIVES - Areas	CRITERIA - FACTORS OF INFLUENCE			
	Political - legislative	Economic- organizational	Sociological	Technological and ecological
Fishing area	0.2857	0.1593	0.3338	0.2721
Hunting area	0.5714	0.2519	0.1416	0.1199
Hunting and fishing area	0.1429	0.5889	0.5247	0.6080
TOTAL	**1.0000**	**1.0000**	**1.0000**	**1.000**

Table 2 shows the results of comparison, by AHP method, of management alternatives in relation to the set of criteria i.e. the results of criteria influence to the management concept of fishing and hunting areas. Regarding political-legislative criteria , integrated management of fishing and hunting areas has a very small weighting value (Wv = 0.1429), which can be interpreted as a consequence that lack of influence of the experts has on political and legislative as well as on administration. Inertness of traditional understanding of the significance of these natural resources has contributed to such a small value.

Paradoxically, the option of integrated management of fishing and hunting areas, has expressed values in three main components of the criteria above, but in political and legislative terms, that significance is negligible. It can be seen that the economic and organizational as well as technological and organizational criteria strongly and positively affect the social component, so political structures and legislator should pay more attention to that. In a similar way, by using AHP as a method, the analysis of influence of factors on work of professional and guarding services in fishery are performed. The results are shown in table 3.

Table 3. Vector of weighting value (Vwv) of the basic matrix of criteria

Criteria	Vector of weighting value (Vww)	Vector of weighting value (Vww%)
Number of sold licenses	0,4656	46,56%
Number of orders	0,1885	18,85%
The length of water flow–km	0,2772	27,72%
Accessibility of terrain	0,0687	6,87%
Total	1,0000	100,00%

From table 3 it can be seen that the percentage of vector weighting values (Vwv%) for the number of sold licenses is 46.56%, for the length of water flow 27.72% , for the number of orders it‘s 18.85% and 6.87% for the accessibility of terrain. Foregoing vectors of weighting value (Vww) in relation to particular objective, clearly position key factors for work of professional and guarding services in fishery. The analysis of influence of certain factors on work of professional and guarding services in hunting has been performed and the results are shown in table 4. From table 4 it can also be seen that the percentage of vectors of weighting values (Vwv%) for the criteria hunting area is 52.32%, for the number of hunters 22.89%, orographic conditions 15.53% and for the openess of the road network it is 9.26%.

Table 4. Vector of weighting value (Vwv) of the basic matrix of criteria

Criteria	Vector of weighting value (Vwv)	Percentage of vector of weighting value (Vwv%)
Hunting area	0.5232	52.32%
The number of hunters	0.2289	22.89%
Orographic conditions	0.1553	15.53%
The openess of the road network	0.0926	9.26%

Table 4 show that the number of direct beneficiaries (hunters and fishermen), then hunting area i.e. the length of water flow, has a significant influence on work of professional and guarding services and that when legislator i.e. political structures make decisions of organization these factors should be considered as determined criteria.

CONCLUSIONS

From these results we can conclude that integrated management of fishing and hunting areas would improve management functions, reduce costs and increase business efficiency. That would certainly have a positive influence on the social aspect and it would change public awareness that fishing and hunting are not only social but also significant economic category. By integrated management of fishing and hunting areas the monitoring would be facilitated with clearer and more comparable results, and the control and usage of funding would be incomparably better and more rational. In terms of functioning of professional and guarding services it is necessary to perform a detailed analysis of geographical distribution, size and number of hunting i.e. fishing areas, with the aim of optimizing its management in the economic and ecological principles.

The potential benefit for the state can be seen in reducing the cost of management, where regional centers at the levels of one or more areas could be established and which would be used for stuff training, organization of monitoring, reacting in accidental situations etc. Before all that, it is necessary to make legislation and afterward fishing and hunting areas, mutually compatible.

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CHANGES OF THE FATTY ACID COMPOSITION AND TOTAL LIPID CONTENT IN CULTURED CARP (*CYPRINUS CARPIO* L.) CORRELATED TO SUPPLEMENTARY DIET

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PROMENE SASTAVA MASNIH KISELINA I SADRŽAJA LIPIDA U MESU ŠARANA (*CYPRINUS CARPIO* L.) U ZAVISNOSTI OD DODATNE HRANE

Apstrakt

U ovom radu razmatrane su promene grupa masnih kiselina (zasićenih, mononezasićenih, polinezasićenih n-3 i n-6, kao i njihovog odnosa n-3/n-6) u zavisnosti od sadržaja lipida u mesu šarana sa dve vrste prihranjivanja (ekstrudirane hrane i kukuruza). Vrsta korišćene hrane značajno je uticala na sadržaj lipida deponovanih u tkivu ribe. Korelacija lipida sa sastavom masnih kiselina pokazala je, da se sa povećanjem sadržaja lipida u mesu šarana, masnokiselinski sastav značajno menjao: povećavale su se mononezasićene masne kiseline, sa dominantnom oleinskom kiselinom (18:1n-9), a istovremeno smanjivale n-3 i n-6 polinezasićene masne kiseline. Istraživanja koja su prikazana u ovom radu su ukazala na opravdanost prihranjivanja šarana ekstrudiranom hranom u cilju poboljšanja kvaliteta mesa ribe.

Ključne reči: šaran, dodata hrana, masnokiselinski sastav
Key words: common carp, supplementary feed, fatty acid composition

INTRODUCTION

Production of freshwater fish dominates in the world with 71% of cyprinid family (FAO, 2010). Carp (*Cyprinus carpio)* is one of the most cultivated species and accounted for 18% of total production of cyprinids in the world (Takeuchi et al. 2002). Recently, more than 50% of the carp additionally fed by complete, primarily extruded feed which enabled more intensive carp production and the development of aquaculture in Serbia (Marković, 2010; Marković, Poleksić, 2013) what follows trend in the world carp production (De Silva, 2012).

The main objective of this study was to implement the relevant test for the influence of diet in the semi-intensive carp farming system, primarily on lipid content and fatty acid composition.

MATERIALS AND METHODS

Fish samples

The total of 64 two-year old carp samples were taken during the autumn/winter 2009 from two ponds with the semi-intensive farming, Ečka a.d. and Živača a.d., according to the breeding season and fish farm productivity. Except naturally occurring food, fish was additionally fed extruded feed (25/7), from the first fish farm and maize from the second fish farm. 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

Total lipids for the fatty acid determination were extracted with hexane/isopropanol mixture by accelerated solvent extraction (ASE 200, Dionex, Sunnyvale, CA) and further converted to fatty acid methyl esters (FAMEs) by transesterification with trimethylsulfonium hydroxide (EN ISO 5509:2000). FAMEs were analyzed by gas-liquid chromatography (GLC, Shimadzu 2010) using the strongly polar column in the temperature range from 125 °C to 230 °C. The duration of the analysis was 50.5 min. (Spirić et al., 2010). Results were expressed as mass of fatty acid (g) in 100 g of fatty acids.

Statistical analysis

Correlation analysis was performed using JMP 8.0.1 software (SAS Institute Inc. NC, USA) to determine significance of the lipid content increase on the fatty acid composition during carp breeding in two fish ponds, (groups of saturated, monounsaturated and polyunsaturated n-3 and n-6 fatty acids and n-3/n-6 ratio).

RESULTS AND DISCUSSION

The share of lipids was significantly higher in carp fillets supplemental fed maize (8.6-11.6%) than supplemental fed extruded feed (3.2-4.7%) which is in accordance with the results of other researches (Fajmonova et. al, 2003; Vacha et al., 2007; Trenovszki et. al. 2011).

Correlation of the groups of fatty acids and total lipids content in carp fillets with corresponding levels of significance are given in table 1.

Table 1. Correlation of the groups of the fatty acids (% of total fatty acids) and total lipids (g/100 g of fillet) in carp suplemental fed extruded feed and maize

Fatty acids	Lipids (extruded feed)		Lipids (maize)	
	r	P	r	P
SFA	-0.820	<0.0001*	-0.241	0.192
MUFA	0.702	<0.0001*	0.447	0.012*
PUFA	-0.465	0.0127*	-0.453	0.010*
n-3	-0.596	0.0008*	-0.369	0.041*
n-6	-0.358	0.0612	-0.482	0.006*
n-3/n-6	-0.634	0.0003*	-0.383	0.033*

r – coefficient of correlation, P – level of significance

The studies of Trenovszki et al. (2011) have shown that the different types of supplementary carp feeding lead to significant variation in the contents of fat, monounsaturated and n-6 and n-3 polyunsaturated fatty acid in the carp meat.

Monounsaturated fatty acids were dominated with an increase in lipid content in carp fillet (Table 1). Variability in the content of monounsaturated fatty acids were closely related to changes in the share of lipids in fish, which was influenced by the type and amount of food, the growing season, the diversity of individuals etc (Geri et al., 1995; Kiessling et al., 2001; Fajmonova et al., 2003). The opposite was the effect on the content of saturated fatty acids, which are considerably reduced with the increase in the proportion of lipids in the fish (Table 1), which is a possible consequence of the enzyme desaturation of saturated to monounsaturated fatty acids (Henderson, 1996). The total lipids content in carp supplemental fed maize was from 8% to 11%, which makes, probably, neutral lipids and also due to less differentiation of carp specimens than were the lower correlation coefficients of the fatty acids.

Polyunsaturated n-6 fatty acids were not strongly correlated with the lipid content (r = -0.358; -0.482). However, the proportion of n-3 polyunsaturated fatty acids with an increase in the lipid content in fish significantly decreased (r = -0.596; -0.369) as similarly reported for carp by Geri et al., 1995, Fajmonova et al., 2003, Mráz and Pickova, 2009. Correlation of total lipids content with oleic acid and the most important content of n-3 and n-6 in carp supplemenatal fed extruded feed and maize were presented in Figures 1 and 2.

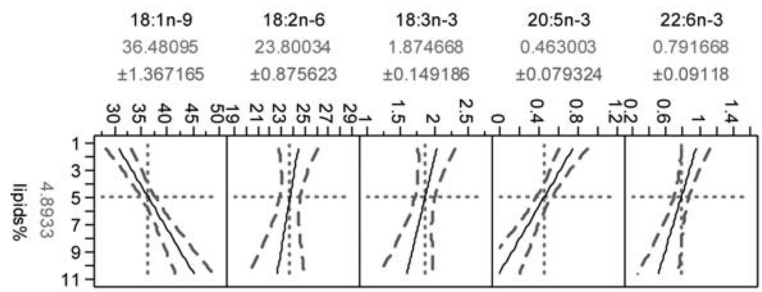

Figure 1. Correlation of total lipids content with oleic acid and the most important content of n-3 and n-6 in carp supplemenatal fed extruded feed

Since the content of the total lipids strongly influences on the composition of lipid classes, the change in the lipid composition were more impact on the reduction of n-3, which were less represented than n-6 (Mráz and Pickova, 2009, Mráz et al., 2012). With the increase of the total lipids increased the share of neutral lipids in which are less present n-3 fatty acids (Kiessling et al., 2001; Wood et al., 2008, Henderson, 1996).

The changes in the content of n-3 fatty acids with the change in the lipid content have resulted in reduction of the n-3/n-6 ratio.

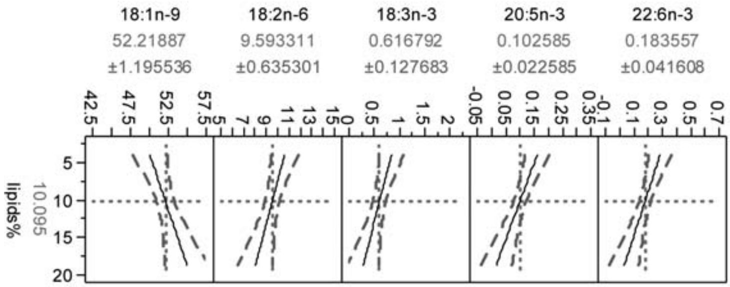

Figure 2. Correlation of total lipids content with oleic acid and the most important content of n-3 and n-6 in carp supplemenatal fed maize

CONCLUSION

Carp, regardless of the type of additional feed, shows similar metabolic changes of fatty acids as well as other fish species. The type of feed which was used on the farm significantly influenced the content of lipids which were deposited in the tissues of fish. The correlation of lipids to fatty acid composition showed that by the increase in the content of lipids, the fatty acid composition of carp meat significantly changed: the monounsaturated fatty acids increased, with the predominant oleic acid (18:1n-9), and, at the same time, the n-3 and n-6 polyunsaturated fatty acids decreased. The results presented in this work have indicated the justification for supplementary feeding of carp with extruded feed in order to improve the quality of meat. The need to improve the composition or quality of the supplementary feed and feed efficiency is essential to the continuous improvement of aquaculture.

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ACKNOWLEDGEMENT

This work was supported by the projects TR 31075 and TR 31011 funded by the Ministry of Education, Science and Technological Development of the Republic of Serbia.

EFFECT OF DIETARY BETAINE SUPPLEMENTATION ON SOME PRODUCTIVE TRAITS OF COMMON CARP (*CYPRINUS CARPIO* L.) CULTIVATED IN RECIRCULATION SYSTEM

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EFEKTI BETAINA KAO DODATKA U ISHRANI NA NEKE PROIZVODNE OSOBINE ŠARANA (*CYPRINUS CARPIO* L.) GAJENOG U RECIRKULACIONOM SISTEMU

Apstrakt

Cilj ovog istraživanja bio je da se ustanove efekti betaina kao dadatka u ishrani na stopu preživljavanja, prirast i konverziju hrane kod šarana (*Cyprinus carpio* L.), gajenog u recirkulacionom sistemu. Četrdeset i osam jedinki šarana čija je prosečna početna težina bila 1238.13±39.19 - 1241.25±29.73 g podeljeni su u tri grupe. Eksperiment je rađen u duplikatu. Ribe su gajene u betonskim tankovima zapremine 0.8 m^3, koji su pripadali recirkulacionom sistemu. Riba je hranjena ekstrudiranom hranom ”Aqua VITAL“, koju proizvodi “Aqua garant“. Veličina peleta bila je 6 mm. 1% betaina dodat je hrani šarana koji je pripadao eksperimentalnoj grupi EG1, dok je hrana za drugu eksperimentalnu grupu (EG2) sadržala 3% betaina. Šaran iz kontrolne grupe (CG) nije dobijao betain u ishrani. Hrana kojom je riba hranjena predstavljala je 2% težine ribe. Ogled je trajao 60 dana, a kontrolni ulov obavljen je 30-og dana da bi se ispitao uticaj betaina kao dodatka u ishrani na prirast i odnos konverzije hrane kod šarana (*Cyprinus carpio* L.), koji se gaji u recirkulacionom sistemu. Težina ribe (g) na kontrolnom ulovu ustanovljena je tako što je svaka jedinka pojedinačno merena. Finalna težina šarana iz eksperimentalnih i kontrolnih grupa iznosila je: EG_1 – 1727.50±40.52 g, EG_2 – 1749.39±33.45 g and CG – 1646.88±26.51 g. Vrednosti ovog parametra kod ribe iz druge eksperimentalne grupe EG2 bile su više nego kod jedinki iz EG1 (1.27%) i CG (6.22%), međutim razlike nisu bile značajne (P>0.05). Prosečni individualni prirast kod šarana iz dve replikacione kontrolne grupe bio je 406.25±20.61 g, što je 20.46% i 25.08% niže nego kod riba koje su dobijale 1% i 3% betaina, dok su razlike bile značajne (P<0.001). Najbolji koeficijent konverzije hrane bio je kod šarana iz EG2 - 1.49. Ova osobina je imala niže vrednosti nego kod riba iz EG1 (3.36%) i CG (24.16%)

(P<0.001). Kada je reč o stopi preživljavanja riba iz svih grupa, diskrepance nisu uočene. Ekonomska efikasnost grupe koje je dobijala 1% betaina bila je 1.66, što je niže nego kod EG2 (10.34%) i CG (15.66%).

Ključne reči: šaran, Cyprinus carpio L., betain, osobine prirasta, koeficijent konverzije hrane.
Key words: common carp, Cyprinus carpio L., betaine, growth traits, feed conversion ratio.

INTRODUCTION

The use of various supplements in fish feeds has been increasing in the recent years. In addition to improve the quality of the feed, these compounds are intended to enhance the growth of the cultivated species, while at the same time decreasing the feed conversion ratio and augmenting the survival rate. The digestibility of the feeds is also expected to improve, due to the presence of these additives. A lot of experiments have been carried out investigating different supplements and betaine has proved to show a great potential (Polat and Beklevik, 1999).

Betaine (glycinebetaine, trimethylglycine) is highly water soluble and hence diffusing compound, capable of stimulating the olfactory organs of fish. It is contained in high amounts in the sea invertebrates, microorganisms and some plants (Meyers, 1987).

The effect of betaine supplementation has been studies mainly in species like rainbow trout (*Onchorynchus mykiss*) by Rumsey (1991) and Virtanen et al. (1994), however their results were contradictory. Rumsey (1991) reported that exogenous betaine supplementation did not augment the weight of the rainbow trout, while on the other hand Virtanen et al. (1994), found that the weight of the same species was increased by 12 % and the mortality was decreased by 60 % after dietary betaine supplementation in amount of 1 %. Betaine in feed led to enhanced feed consumption and growth in rainbow trout larvae (Can and Sener, 1992; Polat and Beklevik, 1999). Rahimabadi et al. (2012) concluded that betaine might improve the quality of the feed, used for zander larvae (*Sander lucioperca*), as they become easily adapted, when shifting the nutrition from live food to extruded pellets.

Shankar et al. (2008) reported, that betaine supplementation in amount of 0.25 % to the feed of Rohu (*Labeo rohita*) improved considerably the growth, when compared to fish that did not receive the dietary supplement.

Przybyl et al. (1999) concluded that the addition of 0.20 % betaine in the diet of carp larvae enhanced the productive traits.

The aim of the study was to determine the effect of the dietary betaine supplementation on the survival rate, weight gain and feed conversion ratio of common carp (*Cyprinus carpio* L.), cultivated in recirculation system.

MATERIAL AND METHODS

Forty eight common carps with an initial live weight of 1238.13±39.19 - 1241.25±29.73 g were divided in three groups (control, CG and experimental groups: EG_1, EG_2), consisting of eight fish, as each of them had two replicates. The fish were cultivated in concrete tanks with a volume of 0.8 m^3, which were part of the recirculation system. Carps were fed with extruded feed "Aqua VITAL", a product of "Aqua garant", with 6 mm size of the pellets. Betaine in amount of 1 % was sprayed in the feed of the fish from the experimental group EG_1, while these ones from the second experimental group (EG_2) received 3 % betaine, added to the diet. The carp from the control group (CG) received feed without added betaine. The content of nutrients in the extruded feed for the groups is presented in Table 1. Fish from all groups were fed 3 times/daily, based on 2 % of biomass. The trial period was 60 days.

Hydrochemical Analysis

The hydrochemical parameters in the recirculation system of the carp (*Cyprinus carpio* L.) were determined, using methods, adapted for fish farming (Bessonov and Privezentsev, 1987; Todorov, 1992). They are as follows:

- Water temperature, °C;
- Quantity of the dissolved oxygen, $mg.l^{-1}$ – MultiLine P4;
- pH – MultiLine P4;
- Electrical conductivity, $\mu S.cm^{-1}$ – MultiLine P4 and BDS EN 27888;
- Quantity of nitrates, $mg.l^{-1}$ – BDS 17.1.4.12:1979;
- Quantity of nitrites, $mg.l^{-1}$ – BDS ISO 26777:1997.

Intensity of fish growth

In order to study the betaine influence on the weight gain and feed conversion ratio in the carp (*Cyprinus carpio* L.), cultivated in recirculation system, control catch was carried out at 30th day. The average live weight (g) at the control catch was determined as the fish were weighed individually. At the end of the trial the final body weight (g), the weight gain (g), survival rate (%) and the feed conversion ratio in fish were determined.

Table 1. Nutrient content in the extruded feed for a common carp (*Cyprinus carpio* L.)

№	**Item**	**Groups**		
		CG	**EG_1**	**EG_2**
1	Crude protein, %	34.00	34.00	34.00
2	Lipids, %	10.00	10.00	10.00
3	Fiber, %	4.00	4.00	4.00
4	Moisture, %	8.74	8.74	8.74
5	Lysine, %	1.43	1.43	1.43
6	Methionine+cysteine, %	0.80	0.80	0.80
7	Ca, %	1.23	1.23	1.23
8	P, %	1.60	1.60	1.60
9	Chlorides, %	0.78	0.78	0.78
10	Betaine, %	-	1	3
11	ME, MJ/kg	17.00	17.00	17.00
12	ME, kcal/kg	4063	4063	4063

** 1 kg feed contains: vitamin A – 10000 IE; vitamin D_3 – 1500 IE; vitamin E – 200 mg; vitamin K – 3 mg; thiamin – 10 mg; riboflavin– 15 mg; pyridoxine – 8 mg; vitamin B_{12} – 0.02 mg; nicotinic acid– 40 mg; folic acid – 3 mg; biotin – 0.3 mg.*

*** 1 kg feed contains: Fe – 145 mg; Mn – 67 mg; Cu – 16 mg; Zn – 68 mg; I – 1.5 mg; Co – 0.5 mg; Se – 0.6 mg*

Economic analysis

In order to analyse the economic efficiency of the betaine supplementation in the diet of carp (*Cyprinus carpio* L.), cultivated in recirculation system, data for feed conversion ratio, weight gain and survival rate were used. Comparisons of these traits were made between the fish of the different experimental groups and the costs for the extruded feed were determined. The price cost for 1 kg weight gain of the fish, cultivated in recirculation systems was determined. The economic conversion ratio (ECR) was calculated, using the following equation (Piedecausa et al., 2007):

ECR = Cost of Diet x Feed Conversion Ratio (FCR)

Statistical evaluation of the data was done by STATISTICA 6.0 software (StatSoft Inc., 2002).

RESULTS

Hydrochemical analysis

During the trial period the hydrochemical parameters of the recirculation system were maintained in the optimal limits for carp. The temperature, dissolved oxygen, pH, nitrates, nitrites and the electric conductivity of the water were daily measured.

The water temperature during the trial is presented in Table 2. For the different groups it was 23.50ºC - 24.50ºC, which is optimal for this species. The amount of the dissolved oxygen was optimal as well. In the different experimental groups the values of this parameter varied between 6.30 mg.l^{-1} and 6.96 mg.l^{-1} /Table. 2/. The pH of the water in the recirculation system for the different experiments was within the range of 7.58-7.73, that was optimal for the common carp (Table 2). The quantity of nitrates during the trial period varied between 0.51 mg.l^{-1} and 0.63 mg.l^{-1}, which is below the maximal value, specified in the Regulation №4/20.10.2000. (Table 2). The content of nitrites in the water of the different experimental groups is shown in Table 2 as well. It was within the range of 0.021 mg.l^{-1} - 0.030 mg.l^{-1}, which is also below the maximal value, cited in the above regulation. The electric conductivity of the water during the trial period is presented in Table 2 and varied within 635.00 µS.cm^{-1} and 714.00 µS.cm^{-1}, which is optimal for the cultivated species.

Table 2. Water parameters in the recirculation system during the experiment with common carp

Parameter	n	Min.	Max.	Optimum values (Zaykov and Staykov, 2013)
Temperature, ºC	60	23.50	24.50	22.0-26.0
Dissolved oxygen, mg.l^{-1}	60	6.30	6.96	> 5
pH	60	7.58	7.73	6.5-8.5
Nitrates, mg.l^{-1}	60	0.51	0.63	< 2.0
Nitrites, mg.l^{-1}	60	0.021	0.030	< 0.05
Electric conductivity, µS.cm^{-1}	60	635.00	714.00	-

Growth of the common carp

The average initial live weight of the carp from both replicates of the control and experimental groups were 1240.63±27.31 g, 1238.13±39.19 g and 1241.25±39.19 g, respectively as there were no significant differences between values of different variants (P>0.05) /Table 3/.

In the middle of the experimental period a trend towards higher live weight in the fish, receiving 1 % and 3 % betaine was observed (1456.88±38.64 g and 1473.13±31.86 g when compared to the control group - 1420.60±34.21) (Table 3). The same tendency existed at the end of the trial, as the live weight was the highest in the carp from EG_2 - 1749.39±33.45 g, followed by EG_1- 1727.50±40.52 g. The fish from the control group displayed the lowest live weight.

The survival rate of the carp during the trial is presented in Table 3. The values of this trait in the fish, fed betaine supplemented diet were 100 %. Such was the survival rate in the control group.

At the end of the experiment the weight gain was higher in the fish from the betaine supplemented groups. The average individual weight gain of the carp from the two replicates of CG was 406.25±20.61 g, which is 20.46 % and 25.08 % lower, than the fish, receiving betaine in amounts 1 % and 3 %, as the difference was significant (P<0.001) (Table 3).

During the experimental period the carp were fed three times per day. The analysis of the feed at the replicates of the control and the experimental groups was done. The feed conversion ratio of the carp, receiving 3 % betaine was 1.49 and it was 3.36 % lower, than that of the fish, fed 1 % betaine, while 24.16 % lower in the individuals from the control group (Table 3). The differences were significant (P<0.001) between the experimental groups and this one without betaine supplementation.

Table 3. Fish production parameters

Parameter	n	CG	EG_1	EG_2	Significance
		$\bar{x} \pm SD$	$\bar{x} \pm SD$	$\bar{x} \pm SD$	
Initial body weight, g	16	1240.63±27.31	1238.13±39.19	1241.25±29.73	NS
Body weight in the middle of the trial, g	16	1420.60±34.21	1456.88±38.64	1473.13±31.86	NS
Final body weight, g	16	1646.88±26.51	1727.50±40.52	1749.39±33.45	NS
Survival rate, %	16	100	100	100	NS
Average individual weight gain, g	16	406.25±20.61[a]	489.37±34.15[b]	508.14±48.61[b]	***
FCR	16	1.85±0.09[a]	1.54±0.11[b]	1.49±0.15[b]	***

*** P≤0.001; ** P≤0.01; * P≤0.05; NS – non significant.

Economic analysis

The price of the extruded feed for a common carp was 1038.80 BGN/t (VAT excluded). Liquid betaine was sprayed in the pellets of the fish from two experimental groups, which made the feed more expensive. The increase of the feed price in the group, fed 1 % betaine was 45 BGN/t, while in the group receiving 3 % betaine, it was 135 BGN/t.

The calculated economic conversion ratio for the carp in the group, fed 1 % dietary betaine supplementation was 1.66, which was lower, when compared to these ones of the fish from groups CG and EG_2, respectively by 10.34 % and 15.66 %.

Table 4. Economic efficiency of the betaine supplementation in the feed

Item	**CG**	**EG_1**	**EG_2**
Price, BGN/t feed (VAT excluded)	1038.80	1083.80	1173.80
Price, BGN/kg feed (VAT excluded)	1.04	1.08	1.17
ECR	1.92	1.66	1.74

DISCUSSION

The analysis of the data, concerning the hydrochemical traits (temperature, oxygen, dissolved in water, pH and electric conductivity) during the trial period showed that they were within the optimal range for the particular species. The same could be said for the maximal concentrations of the nitrates and nitrites in the water. For the carp farms these parameters must be up to 2 mg/l and 0.05 mg/l respectively and they were considerably higher, than those, maintained in the water during the experimental period (Regulation № 4/ 20.10.2000; Zaykov and Staykov, 2013). The optimal values of the traits for all the studied groups are due to the fact that the carp were cultivated in optimized technical and technological conditions of the recirculation system. The tanks were cleaned three times per day and fresh water in amount of the total volume of the recirculation system was daily added. For the maintenance of the optimal hydrochemical traits in the system during the experimental period the mechanical filter and particularly biofilter were of critical importance.

The supplementation of betaine to the carp diet in amounts of 1 % and 3 % did not affect the survival rate of the fish. The data obtained at the end of the experiment, as stated above, showed that the values of this variable were 100 % in the individuals of the replicates in the betaine fed groups. The same was observed for the fish from the control group /Table 3/. This was due to the maintenance of the optimal hydrochemical parameters that are required for the cultivation of the species at optimized technological conditions – stocking density, daily diet, feeding frequency.

The analysis of the data for the weight gain of the carp revealed, that it was 406.25±20.61 g in the control group, which was 20.46 % and 25.08 % lower, than these ones of the fish in the experimental groups, fed 1 % and 3 % betaine, as the differences were significant ($P<0.001$) /Table 3/.

At the end of the trial period, the feed conversion ratio of the carp, cultivated in recirculation system and fed 3 % betaine was 1.49. This value is 3.36 % lower, than that of the fish, receiving 1 % betaine in the diet and 24.16 % lower in the individuals from the control group /Table 3/. The differences of this parameter were significant between the experimental groups and the control one ($P<0.001$). The results obtained in this study are due to the improved metabolism of the nutrients in the fish receiving betaine, since the latter is a donor of methyl groups. This is confirmed by many other studies, reporting that the increase of the methyl groups after betaine supplementation in the diet, enhance the live weight of the fish, achieved with the same quantity of feed (Virtanen et al., 1994; Przyby et al., 1999; Polat and Beklevik, 1999; Rahimabadi et al., 2012).

The better conversion of the extruded feed with betaine, added in amount of 1 % and 3 %, affects positively the growth of the fish from the experimental groups, cultivated in recirculation system. At the beginning of the trial the carp were made equal in weight (P>0.05). In the middle of the experimental period the live weight of the fish from the supplemented groups tended to be higher, when compared to this one of fish from the control. The average live weight of the carp from both replicates of the group, fed 3 % betaine was 1473.13 g and it was 1.12 % higher, than this one of the fish, receiving 1 % betaine and 3.70 %, than the values of this parameter of carps from the control group (P>0.05) /Table 3/. This trend was kept until the end of the experimental period. The average live weight of the carp in the two replicates of EG_2 was 1749.39 g and it was 1.27 % higher, that the EG_1 and 6.22 %, than CG (P>0.05) /Table. 3/. These results confirm the findings of other studies, concerning experiments with different betaine concentrations in different fish species. After an experiment with dietary betaine supplementation in amount of 1.5 % in the feed of rainbow trout, Polat and Beklevik (1999) reported significant influence on the feed, consumed and the growth of the fish. Similar results were reported for betaine, added in the feed of rainbow trout (Can and Sener 1992), red seabream (Goh and Tamura, 1980), *Solea solea* and *Anguilla anguilla* (Mackie and Mitchell, 1982). According to these authors, this was a result of the betaine, which is a donor of methyl groups, used for synthesis of methionine, carnitine, phosphatidylcholine and creatine. These substances are important for the metabolism, although betaine might be synthesized by choline in the mitochondria. Usually the synthesis is not sufficient for the needs of the fast growing hydrobionts (Stekol et al., 1953).

Although the group, receiving betaine in amount 3 % displayed the best feed conversion ratio, the data of the economic analysis showed that the group, fed 1 % betaine had the best economic conversion ratio. In regards of this trait our results were confirmed by those of Virtanen et al. (1994) and Przybyl et al. (1999).

The dietary betaine supplementation in amount of 1 % and 3 % to the extruded feed for carp, cultivated in recirculation system affected positively the following traits:

- Increased the weight gain of the fish from the EG_2 and EG_1 groups, when compared to this one from the control CG, respectively by 25.08 % and 20.46 %.
- Decreased the feed conversion ratio in the carp from EG_2 and EG_1, respectively by 24.16 % and 20.13 %, in comparison to the values of this trait, determined in the fish from the control group;
- Had no effect on the survival rate of the fish in all experimental groups;
- Decreased the economic conversion ratio in the carp from EG_1 and EG_2, respectively by 15.86 % and 10.34 %, when compared to the values of this trait in the fish from the control group.

CONCLUSION

The study showed that the betaine might successfully be used as a feed additive to the diet of common carp. Its supplementation to the extruded pellets influenced positively the growth, did not have negative effect on the survival rate of the fish, enhanced the weight gain, reduced the feed conversion ratio, as well as the economic conversion ratio. The best economic conversion ratio had fish from the group, fed 1 % betaine.

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APPLICATION OF PREBIOTIC MOS IN TROUT NUTRITION

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PRIMENA PREBIOTIKA MOS-A U ISHRANI PASTRMKI

Apstrakt

Ispitivan je uticaj manan oligosaharida (MOS) kao aditiva hrane, primenjenog u koncentraciji od 0,2% (O-I grupa riba) i 0,3% (O-II grupa riba) na morfometrijske karakteristike i osnovne proizvodne pokazatelje gajenja kalifornijske pastrmke. Ogled je sproveden na 450 riba podeljenih u tri grupe sa po 150 jedinki u svakoj grupi i trajao je 40 dana. Analizom dobijenih rezultata, ustanovljen je povoljan efekat primenjenog aditiva na morfometrijske pokazatelje tempa rasta riba, kako njihove završne komadne mase i završnih dužinskih mera, tako i njihovog prirasta, ali bez utvrđenih statistički značajnih razlika (p>0,05). Najveću prosečnu telesnu masu i dužinu tela kod ispitavanih grupa, ostvarile su pastrmke O-II grupe (118,49 g i 19,59 cm), zatim pastrmke O-I grupe (118,04 g i 19,58 cm), a najmanju K-grupa riba (115,94 g i 19,56 cm). Dodavanje MOS-a u smeše za ishranu pastrmki je imalo povoljan uticaj (p>0,05) na ostvarivanje niže konverzije hrane (HK) i boljih vrednosti osnovnih proizvodnih pokazatelja: faktora kondicije (FK), koeficijenta proteinske efikasnosti (PER), specifične stope rasta (SGR) i proizvodnog indeksa (PI) u odnosu na ribe K grupe, koje su hranjene smešama bez dodatka mananoligosaharida. Najbolje rezultate su ostvarile ribe O-II grupe, a iskazano u relativnim pokazateljima u odnosu na K grupu riba: bolju konverziju hrane za 5,61%; bolju vrednost iskorišćenja proteina hrane za 5,94%; veću vrednost faktora kondicije za 1,73%; specifične stopa rasta za 2,26% i bolju vrednost proizvodnog indeksa za 8,27%.

Ključne reči: manan oligosaharid, kalifornijska pastrmka, proizvodni pokazatelji
Keywords: mannan oligosaccharides, rainbow trout, production indicators

INTRODUCTION

Prebiotics mannan oligosaccharides (MOS) and supplements based on it are complex carbohydrates derived from the cell wall of yeast *Saccharomyces cerevisiae* and are non-digestible food ingredients. The form present in the cell wall (α-1, 3 and α-1, 6 branched mannans) is particularly effective in binding to pathogens in the digestive tract of animals and fish (Spring et al., 2000). Their use contributes to the increased vitality of animals and fish, reduces mortality, stimulates production of immunoglobulins that enhance the immune system, and also improves the conversion and absorption of food, which leads to good production results and positive economic effect (Ferket et al., 2002) mannan oligosaccharides are now the most used in monogastric animal nutrition.

Considering the above facts relating to the applicability of biologically active substances as food additives, the aim of this paper is to show the potential of mannan oligosaccharides as a food additive on production characteristics of yearling rainbow trout (1 + years).

MATERIAL AND METHODS

The experiment was conducted on 450 of sorted and approximately uniform specimens of rainbow trout aged 1^+ years which formed three experimental groups of 150 fish each. Studied groups of fish were distributed into 3 individual pools of 20 m^3 volume, with the same water inlet of 24 l/sec, and the flow of water comprising of 69 changes within 24 hours. The experiment lasted 40 days.

Fish in all three experimental groups were fed with standard mixture for feeding fattening trout with 40% of the total protein. The control group of fish was fed feed without additives, and other experimental groups were fed diets supplemented with mannan oligo saccharides (MOS) as follows: O-I group was fed mixture containing 0.2% MOS and O-II group mixture containing 0.3% MOS.

The amount of food and number of daily meals were determined on a daily basis according to pre-determined food tables, adjusted to the water temperature and body weight of fish during the test (Phillips, 1970). Consumed food was chemically analyzed at the beginning of the experiment using standard testing methods (AOAC, 1990), except that the energy content was obtained by calculation. Ingredients and chemical composition of the mixture used for feeding fish in the experiment is shown in Table 1.

Table 1. Ingredients and chemical composition of the used mixture

Component ,%	K	O-I	O-II
Corn	11,30	11,10	11,00
Fish meal	48	48	48
Soybean meal	19	19	19
Sunflower meal 33%	3	3	3
Lime	1,9	1,9	1,9
Mono-Ca-phosphate	0,5	0,5	0,5
Iodized salt	0,3	0,3	0,3
Premix	1	1	1
Soybean oil	15	15	15
Mannan oligo saccharides (BioMos)	-	0,2	0,3
Average chemical composition of used mixtures (%) VSM			
Water	9,33	9,31	9,30
Ash	11,00	11,10	11,14
Proteins	40,39	40,41	40,35
Fibre	2,66	2,64	2,70
Dry matter (DM)	90,67	90,69	90,70
Metabolic energy ME MJ/kg (calculation)	15,06	15,10	15,08

Composition of used premix/kg mixture: Vitamin A 20000 IJ/kg; Vitamin D_3 3000;Vitamin E 80 mg/kg; Vitamin K_3 5 mg/kg; Vitamin B_1 15 mg/kg; Vitamin B_2 25 mg/kg; Vitamin B_6 15 mg/kg; Vitamin B_{12} 0,04 mg/kg; Vitamin C 500 mg/kg; Niacin 100 mg/kg; Ca-pantotenat 50 mg/kg; Biotin 1 mg/kg; Folic acid 4 mg/kg; Cholin chloride 100 mg/kg; Fe 40 mg/kg; Cu 10 mg/kg; Mn 40 mg/kg; Zn 40 mg/kg; J 10 mg/kg; Se 0,05 mg/kg; Co 1 mg/kg; Mg 50 mg/kg; Antioxidant BHT 100 mg/kg.

Based on the shown chemical composition of the mixture it can be concluded that the quality of the mixture was such that it meets the optimal nutritional requirements of rainbow trout and meets the requirements that are placed in the design of the experiment (NRC, 1993, Official Gazette of RS No.4/2010).

Control measurements of body mass and body length of fish were performed at the beginning, middle and end of the experiment. In order to determine the production characteristics of fish from each group two hours after feeding 20 individuals were caught, according to the method of random sampling. Individual body weight of fish was determined by measuring on a decimal technical scale (accuracy to 10-2 g) and body length using the ichthyometer (accuracy to 0.1 cm).

Based on the determined measurement results in order to determine the effect of mannan oligo saccharides as additive to the mixtures, the following product parameters were analyzed: total body weight gain, average individual weight gain and total body length, feed conversion ratio (FC), condition factor (FC), specific growth rate (SGR), protein efficiency ratio (PER) and production index (PI). In order to determine the hygienic conditions in the pools, indices of fish stocking density (Ig) and flow (Ip) were determined.

These parameters were calculated using the following forms:

$HK = H / TM_2 – TM_1$

$FK = TM_2$ (g) / L_2^3 (cm)

$SGR = [(TM_2 - TM_1) / (T_2 - T_1)] \times 100$

PER = Ptm / Up

Where: H-consumed food; TM2 - Final body weight (g); TM1 - initial weight (g); L2 - Final total body length (cm); T2-T1 - the number of days the experiment; Ptm - gain of body weight (g); Up - consumption of protein (g).

According to Piper et al. (1982) the following was calculated:

Ig = [TM / L] x Q and Ip = [TM / L] x P

Where: TM-weight (g); L - total body length (cm); Q - volume of the fish pool (m3), P - the water flow (l/sec).

Statistical analysis was performed using analysis of variance with assessment of statistical significance using the t-test.

RESULTS AND DISCUSSION

The results on the impact of adding different concentrations of mannan oligo saccharides in the feed mixture of rainbow trout on the performance are presented in Tables 2 and 3.

Upon completion of the experiment, i.e. 40 days, the highest body weight values were recorded in O-II fish group (118.49 g), followed by trout of O-I group (118.04 g), and the lowest in K-group (115.94 g). Average values of body length were highest in fish of O-II group (19.59 cm) and lowest in group K (19.56 cm). The differences of the final body weight and total body length of fish between the groups (Table 3) were not statistically significant ($p > 0.05$). Realized values of morphometric parameters analyzed directly influenced the results in the gain. Expressed in relative terms, O-II group fish achieved a higher average weight gain compared to fish in K group and 7.58% of the total body length of 7.50%, while the fish of O-I group for the same parameters had better results by 6.54% and 5.00%, respectively. For these two parameters between the groups statistical analysis did not establish a significant difference ($p > 0.05$).

Table 2. Comparative overview of the values of some production traits

Indicator	Groups		
	K	O-I	O-II
Initial body weight (beginning of trial), g	82,30	82,20	82,30
Average total body length (beginning of trial), cm	19,16	19,16	19,16
Production indicators at the end of the trial			
Average body weight (TM), g	115,94	118,04	118,49
Average total body length (L), cm	19,56	19,58	19,59
Total fish weight, kg	17,39	17,71	17,77
Total gain of body weight, kg	5,05	5,38	5,43
Average individual gain of TM, g	33,64	35,84	36,19
Difference (%)		6,54	7,58
Average individual increase of L, cm	0,400	0,420	0,430
Difference (%)		5,00	7,50
Total feed consumption, kg	9,06	9,15	9,20
Average daily feed consumption per fish, g	1,510	1,525	1,533
Difference (%)		0,99	1,55
Feed conversion ratio (FC)	1,795	1,702	1,695
Difference (%)		-5,21	-5,61
Protein efficiency coefficient (PER)	1,392	1,469	1,475
Difference (%)		5,49	5,94
Condition factor (FK)	0,0155	0,0157	0,0158
Difference (%)		1,50	1,73
Specific growth rate (SGR)	2,10	2,24	2,26
Difference (%)		6,54	7,58
Production index (PI)	2,421	2,601	2,622
Difference (%)		7,40	8,27

Table 3. Average values of body weight (g) and total body length (cm), 40 days of the experiment

Group	n	$\bar{x}$	min	max	$S\bar{x}$	Sd	t- test
Average values of body weight							
K- group	20	115,94	108,20	124,30	1,040	4,649	p>0,05
O-1 group	20	118,04	113,50	127,60	0,950	4,248	
O-2 group	20	118,49	110,10	128,30	1,032	4,634	
Average values of total body length							
K- group	20	19,56	19,18	19,91	0,061	0,273	p>0,05
O-1 group	20	19,58	19,16	19,93	0,063	0,283	
O-2 group	20	19,59	19,15	19,95	0,066	0,287	

Obtained results are in agreement with the data reported by researchers who established that the addition of MOS to mixtures for various species of fish, has stimulating effect on increase of growth and weight gain, but without statistical significance (Binh et al., 2008; Dimitroglou et al. , 2011; Sara et al. 2011).

Based on the obtained production results (Table 2) it can be concluded that the presence of MOS in the mixtures for fattening trout, O-I and O-II group of fish, caused realization of greater feed intake and better feed conversion value and the ratio of protein efficiency. The lowest total food consumption had fish in group K (9.06 kg), and the highest trout in O-II group (9.20 kg) and average daily feed intake of fish in the O-II group was by 1.55%, and O-I group by 0.99% higher than in the K group.

In the analysis of feed conversion (FC) as the interaction of growth and food consumption, it is evident that the best value for this production parameter was achieved by O-II fish group (1.695), followed by O-I group (1.702), and the worst results of the value of this parameter were established for the fish group K (1.795). As for the relative values of utilization of food protein/protein efficiency (PER), it was found that the O-II group fish performed better for this parameter compared to the group K by 5.94%, while the O-I group of trout by 5.49% in comparison to the K group. Significance test for differences in determined average values for feed intake, conversion and utilization of proteins in food pointed to the absence of a statistically significant difference ($p> 0.05$).

The results in terms of feed conversion are in accordance with the results of Hossu et al. (2005*)* who found that the addition of MOS had positive impact on feed conversion in the experiment with gilthead (*Sparus aurata*), but without significant differences. Similar results have been presented by Peterson et al. (2010): the addition of MOS (0.2% and 0.4%) had no significant effect on feed conversion. However, the results obtained in the present study are somewhat lower than the data of Staykov et al. (2005), who reported that the addition of prebiotics to trout food induced increase of the efficiency of utilization of food by 9.01% and 10.16% ($p <0,05$). Also, Čuljak et al. (2006) indicate that the addition of MOS of 0.6% to carp food contributed to a better feed conversion ratio values by 22.81% ($p <0.05$) and PER by 22.49% ($p <0. 05$).

Average values of condition factor (FC) as an indicator of relations of body weight and total body length of fish, indicate that the groups of fish where the MOS was applied as a diet supplement, achieved better value of the condition factor. Compared with the K group of fish, O-II group had better values of this parameter by 1.73%, and trout of O-I group by 1.50%, which justifies the above statement that the application of 0.3% MOS in food achieved the best weight and length gain of the tested fish.

According to the data presented in Table 2, it can be concluded that the fish of O-II group compared to K group achieved better values of fish specific growth rate (SGR) by 7.58% and O-I group of fish by 6.54%. Statistically significant differences between the groups were not observed ($p > 0.05$). The obtained results were in line with that of other researchers Binh et al. (2008), Sarah et al. (2011), while slightly lower in terms of significance were results reached by Čuljak et al. (2006), Ognean et al. (2009).

The value of production index (PI) ranged from 2.421 (K group) to 2.622 (O-II group). The highest value of this ratio was observed in fish of O-II group, and as a result more vitality and better feed conversion in this group.

Stocking index values ranged from 0.0044 (K group) to 0.0045 (O-I and O-II group), while flow index values ranged from 0.0556 (K group) to 0.0567 (O-II group). The recorded values of Ip and Ig are in accordance with standards of sanitation for raising trout (Piper et al. 1982, Klontz, 1991). From the results of fish mortality in this study, it can be concluded that the use of MOS in the mixtures had no effect on mortality, and that the applied stocking density and carried hygienic measures caused low percentage of deaths and good health of the fish.

CONCLUSIONS

Based on the conducted research, it can be concluded that the use o mannan oligo saccharides (MOS) added to mixtures for rainbow trout had a positive effect on the tested production results: morphometric indicators of fish growth rate, feed conversion, condition factor, protein efficiency ratio, specific growth rate and production index.

This especially relates to best production results achieved in the O-II grou fish, which used MOS in concentration of 0.3%. Slightly lower values of recorded production parameters of fish in O-I group, which were fed mixture containing 0.2% MOS, while the lowest studied parameters were established in the K group of trout. Despite the favourable effect, the differences in average values of production parameters between studied groups were not statically significant ($p > 0.05$).

For this reason, there is a need for further research in order to find the optimal dose of mannan oligo saccharides as food additive, which would allow a wider application in intensive fish farming.

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COMPARATIVE ANALYSIS OF THE FATTY ACID COMPOSITION OF NATURAL FOOD AND SUPPLEMENTAL FEED IN CARP FROM A FISH FARM

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KOMPARATIVNA ANALIZA SASTAVA MASNIH KISELINA U PRIRODNOJ I DODATNOJ HRANI ŠARANA SA RIBNJAKA

Apstrakt

Rezultati prikazani u radu ukazuju na daleko veći značaj prirodne hrane kao izvora polinezasićenih masnih kiselina u odnosu na peletiranu hranu u ishrani šarana u poluintenzivnom sistemu gajenja. Utvrđeno je da su od organizama koji su činili prirodni obrok šaranu: predstavnici familija Chironimidae sa dominaciojm vrste *Chironomus plumosus* iz faune dna i zooplanktonski organizmi (sa dominacijom Cladocera) imali znatno veći sadržaj omega 3 i omega 6 masnih kiselina nego peletirana hrana. Istraživanja su obavljena u 4 ribnjačka jezera šaranskog ribnjaka „Despotovo“ sa poluintezivnim sistemom gajenja baziranom na prihrani peletiranom hranom.

Peletiranu hranu odlikuje najmanja raznovrsnost masnih kiselina i izrazita dominacija linolne i oleinske kiseline. Larve Chironomidae i zooplankton karakteriše veća zastupljenost zasićenih masniih kiselina(SFA: 49.36 ± 1.92 i 38.70 ± 0.67) u odnosu na peletiranu hranu (15.47±0.41), a zooplankton odlikuje i najveća raznovrsnost i zastupljenost n-3 masnih kiselina. Odnos n-3/n-6 masnih kiselina deset puta je veći kod zooplanktona nego kod Chironomidae, a sedam puta veći kod Chironomidae nego kod peletirane hrane.

Ključne reči: šaran, prirodna hrana, peletirana hrana, masne kiseline
Keywords: carp, natural food, pelleted feed, fatty acids

INTRODUCTION

The analysis of fatty acid (FA) composition of carp meat showed that different rearing methods and diets provide considerable differences in the contribution of n-3 and n-6 polyunsaturated fatty acids (PUFA). Carp fed with row cereals contains less n-3 PUFA (Steffens and Wirth, 2007) compared to carp fed with extruded and pelleted feed. The n-3/n-6 ratio of PUFA in carp fed commercial pellets is very similar to those in the feed. Numerous studies demonstrated that the fatty acid composition of food has significant effects on the fatty acid composition of fish meat (Steffens and Wirth, 2007). Food richer in n-3 PUFA in the same fish production conditions, substantionaly improves the n-3/n-6 ration in fish tissues.

The natural food of carp in semi-intensive production systems mainly consists of zooplankton and bottom fauna (Marković, 2010). The significance of these organisms for carp culture is in their nutritive value. Except proteins, these organisms are a source of lipids, fatty acids, vitamins and enzymes for all age classes of carp (Dulić et al., 2011, Živić et al., 2012). Deficiency of natural food leads to the deficiency of vitamins, minerals, amino acids and fatty acids in carp diet. This can affect the immunity and fitness of fish, increase diseases and fish mortality (Marković, 2010). Therefore the enhancement of natural food (zooplankton and Chironomidae larvae) development in carp fish ponds is one of the feasible ways to improve the meat quality of produced carp (Steffens and Wirth, 2007; Živić et al., 2012). The aim of this investigation was to analyze the composition of PUFA in natural food and pelleted feed for fish.

MATERIALS AND METHODS

Site location

The fish farm “Despotovo” is located in the village of Despotovo, in Vojvodina Province. The carp farm consists of six earthen fishponds that cover a surface area of about 200 ha. For the study, four fish ponds (J1, J3, J4 and J6) were used with surface area of: 100ha, 25ha, 25ha and 16ha, respectively. Fish were fed daily with commercial pelleted feed “BAFI” (Futog, Serbia) with 25% proteins (plant origin) and 7% fat.

Sample collection and preparation

Samples of Chironomidae larvae (*Chironomus plumosus*) and zooplankton were taken monthly from June to October 2012 from three points in every pond (inflow, middle, and outflow). Chironomidae larvae were sampled with Van Veen grab, grasping area of 260 cm^2. In order to analyze fatty acid composition of *Chironomus plumosus* larvae, 279 individuals were collected from the pond J1, J3, J4 and J6 (fourth and fifth larval instar). Zooplankton samples were collected with 250 μm mesh plankton net. Samples were kept at the temperature of -18 ºC until analysis. Fatty acid content and total lipid analysis of of Chironomidae, zooplankton and pelleted feed were completed at the Institute of Meat Hygiene and Technology in Belgrade (Serbia) using standard methods.

Statistical Methods

Correspondence analysis (CA) was used to analyze relationship between fatty acids in Chironomidae, zooplankton and pelleted feed. CA was performed using the Brodgar program (Highland Statistics Ltd, UK). To obtain differences between mean values of the two samples, t-test with statistical importance of $P<0.05$ was used.

RESULTS AND DISCUSSION

We analyzed 20 fatty acids from three main sources: carp feed, zooplankton, Chironomidae and pelleted feed. Data were collected monthly during four months from four neighboring fish ponds. ANOVA showed that there was no significant difference between lakes in analyzed zooplankton, Chironomidae and pelleted feed fatty acids composition, thus the results were pooled, and average values used in further analysis. Average values of all investigated fatty acids are presented in Table 1.

Correspondence analysis of studied fatty acids from all samples is presented on Fig 1. The two axes describe 100% of variance. F1 axis explains the difference between the fatty acid concentrations in supplemental and natural food. F2 axis explains the difference between two types of natural food. Since F1 axis explains most of the variability (78.68%) it is clear that there is a bigger difference between supplemental and natural food than between zooplankton and bottom fauna. The main cause for these differences is the domination of linoleic and to a lesser extent oleic acid. Pelleted feed contain up to 80.48% of these acids, while Chironomidae and zooplankton contained only 27.78% and 14.53% respectively (Tab. 1). In pelleted feed only palmitic (10.96%), stearic (3.73%) and linolenic (3.74%) acids were represented in larger amounts, while the rest of the fatty acids were either absent (six of them) or accounted for less than 0.32% of total amount of fatty acids (Tab. 1). Particularly evident is the lack of long chain PUFA n-3 fatty acids in pellet feed.

Contrary to this, the fatty acid content of natural food, especially zooplankton, was considerably more balanced compared to pelleted feed. This is especially evident in the case of monounsaturated fatty acids (MUFA) with four of them (C16:1, C18:1cis-9, C18:1cis-11 and C22:1n-9, Tab. 1) significantly represented in zooplankton and Chironomidae.

Table 1. Average values (±1SE) of fatty acids representations in zooplankton, Chironomidae and pelleted feed.

	Zooplankton	Chironomidae	Pelleted feed
C14:0	3.07 ± 0.23	4.39 ± 0.05	0.070±0.004
C15:0	1.61 ± 0.11	1.66 ± 0.06	0.030±0.004
C16:0	23.03 ± 0.53	27.91 ± 1.68	10.96±0.41
C16:1	8.94 ± 0.92	9.71 ± 1.08	0.100±0.009
C17:0	3.31 ± 0.18	2.18 ± 0.30	0.080±0.004
C18:0	7.67 ± 0.37	11.95 ± 0.24	3.73±0.01
C18:1cis-9	7.21 ± 0.48	11.41 ± 0.29	25.52±0.46
C18:1cis-11	6.79 ± 0.45	6.75 ± 0.71	0.00
C18:2n-6	7.33 ± 0.40	16.36 ± 0.92	54.96±0.47
C20:0	0.14 ± 0.02	1.26 ± 0.17	0.28±0.01
C18:3n-6	0.51 ± 0.04	n.d	0.00
C18:3n-3	10.68 ± 0.67	3.90 ± 0.94	3.74±0.05
C20:2	0.42 ± 0.05	n.d	0.050±0.009

	Zooplankton	Chironomidae	Pelleted feed
C20:3n-6	0.21 ± 0.02	n.d	0.130±0.009
C20:3n-3	0.46 ± 0.08	n.d	0.00
C22:1n-9	3.61 ± 0.25	0.94 ± 0.26	0.00
C20:5n-3	7.86 ± 0.42	1.56 ± 0.18	0.040±0.009
C22:0	n.d	n.d	0.32±0.022
C22:5n-3	0.66 ± 0.09	n.d	0.00
C22:6n-3	6.70 ± 1.08	n.d	0.00
SFA	38.70 ± 0.67	49.36 ± 1.92	15.47±0.41
MUFA	22.93 ± 1.20	27.87 ± 1.50	25.62±0.27
PUFA	34.76 ± 1.30	21.82 ± 0.16	58.91±0.25
n-3	26.30 ± 1.22	5.46 ± 0.92	3.78±0.06
n-6	8.46 ± 0.40	16.36 ± 0.76	55.13±0.43
n-3/n-6	3.19 ± 0.20	0.34 ± 0.06	0.070±0.008

Fatty acid composition of zooplankton and Chironomids are clearly separated along the F2 axis. This is mainly due to the higher level of SFA in Chironomids that are, with the exception of the C22:0, grouped near Chironomids (Fig. 1). High level of SFA in Chirnomids (*Chironomus plumosus*) from fish pond has been observed in earlier studies (Bogut *et al.,* 2007, Živić, et al., 2013) therefore it can be considered as their important characteristic. Major difference found between Chironomids and zooplankton is due to a much higher diversity and content of n-3 FA in zooplankton (Tab. 1, Fig. 1). Moreover, three most important FA: α linolenic (ALA), eicosapentaenoic (EPA) and docosahexaenoic (DHA) are equally distributed in samples of zooplankton. Numerous studies observed the high content of EPA and DHA in freshwater mesozooplankton (>250 µm), with the prevailing content of eicosapentaenoic acid in Cladocerans and docosahexaenoic in Copepods (Kainz et al., 2004). In our study the Cladocerans were dominant in zooplankton. Additionally, ALA is a precursor of EPA (Von Elert, 2002), thus was also prominent in zooplankton samples. The FA composition found in samples show that, compared to pelleted feed, zooplankton has a 10 fold higher n-3/n-6 ratio, while Chirnomids have a 7 fold higher n-3/n-6 ratio (Tab.1).

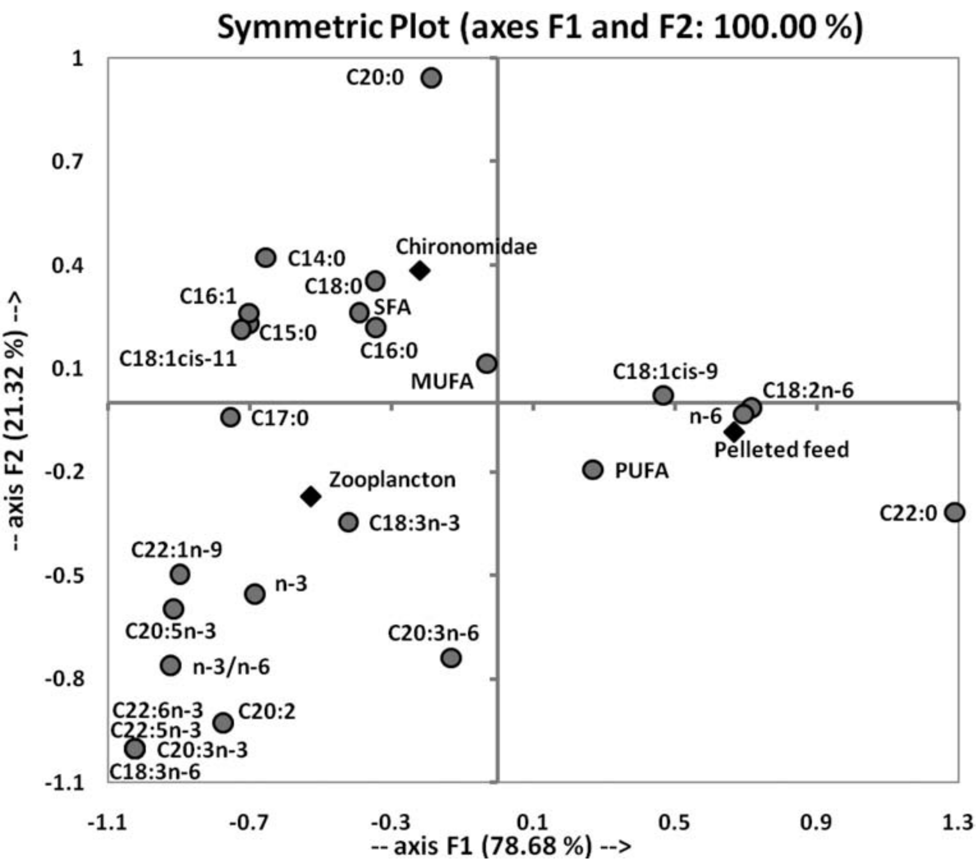

Figure 1. The CA biplot showing relationships between zooplankton, Chironomidae and pelleted feed based on their fatty acids concentrations.

Feeding carp mainly with supplemental feed would probably lead to the domination of oleic acid, already dominant in carp meat regardless of diet type, and linoleic acid and unfavorable n-3/n-6 ratio. Conversely, enhancement of natural food in fish ponds would provide a more diverse and balanced fatty acid composition with a highly favorable n-3/n-6 ratio, and especially increased level of long chain n-3 fatty acids (Bogut et al., 2007). It should be taken into account that supplemental feed has a dominant effect on the FA composition in carp compared to natural food (Živić et al., 2013), since in the presence of supplemental feed carp switches to easily accessible artificial food, while depending on the natural food availability, carp will preferably feed on bottom fauna or zooplankton (Rahman, 2010).

CONCLUSIONS

Comparative analysis of FA content of natural food and pelleted feed from the fish farm “Despotovo” showed that the dominant natural food for carp were Chironomids and Cladocerans with a significantly higher level of n-3 and n-6 fatty acids than in pelleted feed. Zooplankton is characterized by a higher diversity and content of n-3 FA compared to bottom fauna, with the domination of the most valuable essential fatty acids, EPA, DHA and ALA.

ACKNOWLEDGEMENT

The present study was supported by the Serbian Ministry of Education, Science and Technological Development (project No. TR 31075)

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MORFOLOGICAL DEFORMITIES OF MOUTHPARTS IN GENUS *CHIRONOMUS* (DIPTERA: CHIRONOMIDAE) INDUCED BY HEAVY METALS

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MORFOLOŠKI DEFORMITETI USNOG APARATA RODA *CHIRONOMUS* (DIPTERA: CHIRONOMIDAE) IZAZVANI TEŠKIM METALIMA

Apstrakt

U slatkovodnim ekosistemima larve *Chironomidae* su izložene svim prisutnim toksičnim materijama u vodi i sedimentu i kao takve mogu se koristiti kao idealni biotest organizmi. Kod larvi hironomida često se razvijaju deformacije usnih delova, posebno mentuma, za koje se pretpostavlja da su uglavnom prouzrokovani neorganskim hemikalijama. Kod gajenih hironomida (*Chironomus*) na sedimentu iz šaranskog ribnjaka utvrđen je stepen i tip deformiteta u zavisnosti od koncentracije i tipa teških metala. Larve *Chironomus*-a (prikupljene iz Jelenačkog potoka) bile su izložene olovo (II) acetat-3 hidratu i bakar (II) sulfat-pentahidratu u koncentracijama 30, 120 i 200 µg/g suve mase sedimenta. Za procenu stepena deformiteta korišćen je indeks toksičnosti TSI po Lenatu. Kod larvi *Chironomus*-a, izloženih olovu primećen je porast učestalosti deformiteta sa povećanjem koncentracija i to najčešće deformitet medijalno-lateralnih zuba, koji su kraći, i nedostatak zuba. Larve *Chironomus*-a izložene bakru pokazuju pad stope deformiteta od najniže ka najvišoj koncentarciji, a najčešći tipovi deformiteta su kraći i podeljeni medijalni zubi i kraći medijalno-lateralni zubi.

Ključne reči: deformitet usnog aparata, teški metali, olovo, bakar, sediment, Chironomidae
Key words: mouthpart deformity, heavy metals, lead, copper, sediment, Chironomidae

INTRODUCTION

In developing countries, a large number of aquatic environments is exposed to degradation which also includes trace metal pollution. Benthic community represents one of the most sensitive community in aquatic ecosystems, which reacts on different type of processes in these ecosystems. Only by simultaneous monitoring of trace metals content in aquatic ecosystems and benthic communities we can fully understand into which extant aquatic environment is contaminated. Chironomid larvae are considered to be ideal biotest organisms, because they are exposed to toxic material in water and sediment. Chironomid larvae represent good candidates for ecotoxicological studies because of their relatively short life cycle and because they are mainly grazers (Beneberu and Mengistou, 2014). During feeding, these larvae can metabolize different type of compounds in water and sediment. These compounds can induce deformities in some body parts, firstly in mouthparts (Al-Shami et al., 2010). With the goal to determine the toxicity level of trace metals in *Chironomus* larvae, based on the deformities of mouthparts, as well as to establish the degree and the type of the deformity depending on the concentration and the type of trace metals, an experiment which results are shown in this essay was realized.

MATERIAL AND METHODS

Chironomid larvae were collected in the Jelenački brook. Material was sampled with Surber net. The sediment for the experiment was taken from the fish pond and dried in sterilizer for four hours at 100°C. Further on, the sediment was mixed with lead (II) trihydrate and copper (II) sulfate pentahydrate in concentrations: 30, 120 and 200 μg/g of dry weight for both metals. The experiment was carried out using 3 concentrations with 3 replicates (3x3) for each metal. Water and sediment were placed for two days to achieve the toxic equilibration of suspended firm substances (OECD, 2004). At the beginning of the experiment, 25 chironomid larvae were put in each aquarium. Larvae were fed with finely ground extruded feed used for carp nutrition. After seven days, larvae were separated from the sediment. Microscopic mouthparts slides were made according to Namiotko et al. (2011).

Toxic Score Index (Lenat, 1993) was used in order to estimate the level of deformities in chironomid larvae. TSI index can be calculated as follows: [No. of Class I+ 2 (No. of Class II) + 3 (No. of Class III)] × 100/ Total No. of larvae. According to TSI index, deformities of mentum are divided into three classes: Class I – larvae with slight noticeable deformities, Class II – larvae with noticeable deformities: extra teeth, missing teeth, teeth with large gaps, Class III – larvae with significant deformations, including at least two deformities from the Class II.

RESULTS AND DISCUSSION

The mentum (labium) of *Chironomus* larvae consists of 15 dark pigmented teeth (Al-Shami et al., 2010): median which is tripartite (M), two larger medio-lateral (ML) and four smaller lateral teeth (L) each side (Figure 1).

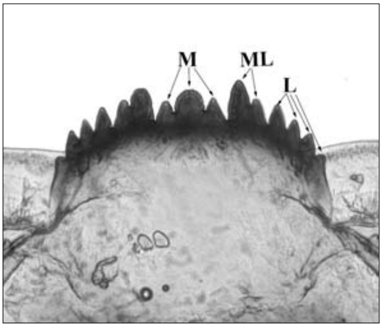

Figure 1. Mentum structure: M – median tooth; ML – medio-lateral teeth; L – lateral tooth

The appearance of the deformities is classified according to teeth position. Larvae in our experiment were exposed to copper and lead, and the following deformities were noticed: shorter median tooth and both medio-lateral teeth, missing third lateral tooth, missing median tooth, shorter medio-lateral teeth, median tooth split, missing first lateral tooth (Figure 2. A-F) Shorter teeth (Fig. 2, A, B) belong to the Class I, missing one tooth (Fig. 2 C) and split tooth belong to the Class II while Class III includes deformities with more teeth in mentum (Fig. 2, E, F). With the increase of lead (II) trihydrate (Fig.3) in the organisms exposed to lead, an increase of deformity frequency was noticed.

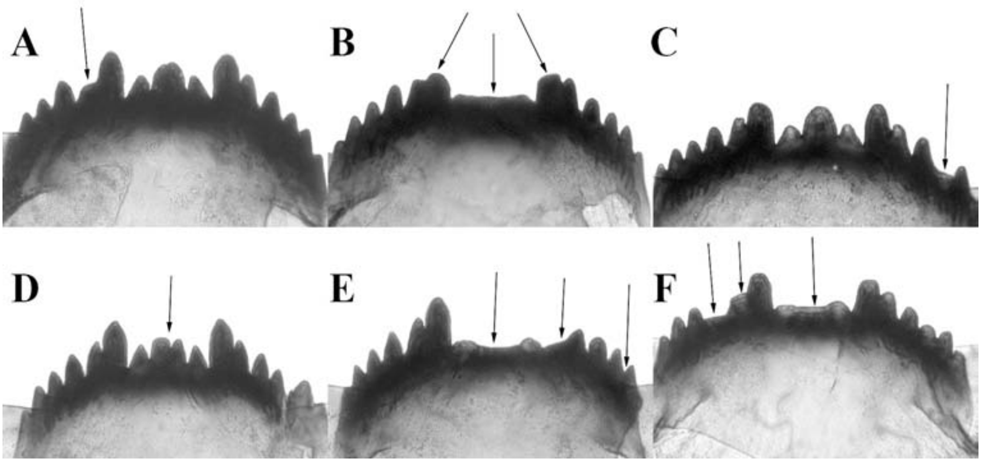

Figure 2. Deformity types: A: shorter tooth; B: shorter median and mediolateral teeth; C :missing teeth; D: split median tooth; E: shorter teeth and missing lateral tooth; F: more shorter teeth and missing teeth

Chironomus larvae exposed to copper showed decrease in rate of deformities from the lowest to the highest concentration (Fig. 3) Larvae exposed to the lowest, average and the highest Cu and Pb concentrations showed significantly higher rate of deformities than larvae reared in control aquariums (Fig. 3)

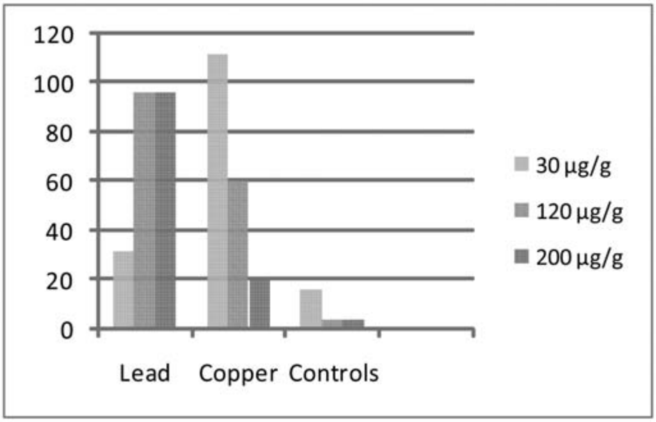

Figure 3. TSI index for the larvae exposed to different Pb and Cu concentrations

In larvae reared in sediment with the increasing lead concentrations, higher frequency of deformities can be noticed. This can be explained by the fact that lead in the sediment acts directly on organisms, causing appearance of deformities, or acts indirectly through bioacumulation in organisms, entering in food chains.

Deviation of the results in our experiment was a consequence of high mortality of individuals exposed to concentrations of 120 µg/g and 200 µg/g of $CuSO_4$x $5H_2O$ (Fig. 3).

Martinez et al. (2003) used the same concentrations of copper like in our experiment, and significant difference of rate of deformities was noticed between larvae exposed to various copper concentrations and those from the control. However, the linear relationship between metal concentrations and rate of deformities hasn’t been established.

In our experiment, the lowest copper concentration induced the highest deformity percentages like shorter median and shorter lateral tooth (Fig. 4) As copper concentrations increased, the number and type of deformities decreased which can be explained by high mortality of larvae on higher concentrations

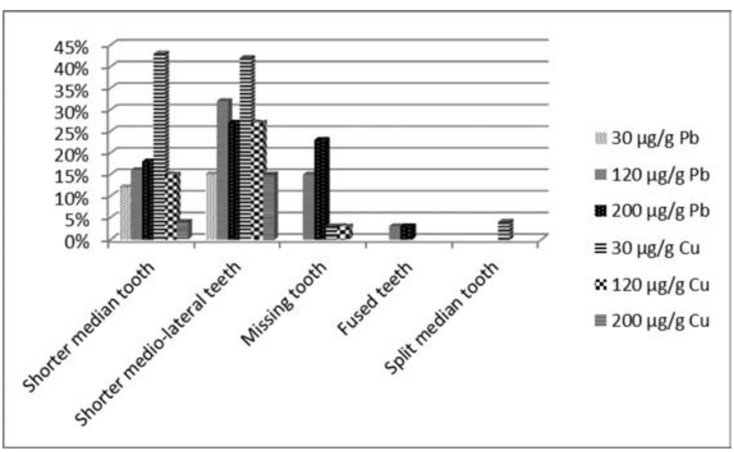

Figure 4. Percentage of deformities type in relation to different Cu and Pb concentrations

Larvae exposed to average concentration of lead had the highest percentage of deformities with shorter medio-lateral teeth and missing teeth as dominant (Fig. 4). Larvae exposed to the highest lead concentration had the lowest pecentage and deformity types which can be explained by high mortality of larvae, as well as by the presence of different pollutants in natural sediment, which can influence the deformity types.

Comparing with the results published by Martinez et al (2003), the copper concentrations in our experiment were the same. Deformity types at the lowest concentration were missing teeth, fused teeth and split median tooth, while in the experiment conducted by our team the most common deformities were shorter median tooth, missing teeth, split median tooth and absence of fused teeth. Comparing to their results at average concentration, the most common deformities were missing and fused teeth, while in our experiment prevailed deformities on medio-lateral teeth. Martinez et al. (2003) showed, when using the highest concentration of copper, the most common deformities were: missing teeth, split median teeth and fused teeth, while in our experiment, we could not define the most common deformity type at the highest copper concentration because of high mortality.

CONCLUSION

Larvae of *Chironomus* exposed to lead showed increase in frequency of mouthpart deformities with increase of lead concentration, while larvae exposed to copper showed decrease in frequency of mouthpart deformities from the lowest to the highest lead concentration. The possible reason could be due to high mortality of individuals on higher concentrations. The most common deformity of *Chironomus* larvae exposed to lead were shorter medio-lateral teeth and missing teeth, while in *Chironomus* larve exposed to copper, the most common deformities were observed on medial tooth and medio-lateral teeth.

ACKNOWLEDGMENTS

The study was supported by Serbian Ministry of Sciences and Technological Development (project TR 31075).

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639.2/.3(082)

INTERNATIONAL Conference "Water & Fish" (7 ; 2015 ; Zemun)
Conference Proceedings / VII International Conference "Water & Fish", Belgrade-Zemun, Serbia June, 10-12. 2015. ; [editors in chief Vesna Poleksić and Zoran Marković]. - Belgrade : University, Faculty of Agriculture, 2015 (Novi Sad : Graphic). - 572 str. : ilustr. ; 30 cm

Na spor. nasl. str.: Zbornik predavanja / VII Međunarodna konferencija "Voda i riba", Beograd-Zemun, Srbija, 10-12. jun, 2015. godine. - Tiraž 400. - Bibliografija uz svaki rad. - Apstrakti.

ISBN 978-86-7834-224-0

a) Рибарство - Зборници
COBISS.SR-ID 215521292

ISBN 978-86-7834-224-0

9 788678 342240

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Portabl oksimetar
Terenski i laboratorijski aparati za merenje:
• Rastvorenog kiseonika
• pH
• Provodljivosti
• Temperature
CyberScan PCD 650
Portabl multi-parametarski uređaj za merenje: rastvorenog kiseonika, pH, provodljivosti i temperature

Tempus

CaSA

Building Capacity of Serbian Agricultural Education to link with Society, CaSA 544072-TEMPUS-1-2013-1-RS-TEMPUS-SMHES (2013 – 4604 / 001 - 001)

Sub programme Structural Measures, Action Higher Education and Society

CaSA foresees creation of the National Repository for Agricultural Education (NaRA) - a repository of online courses and teaching resources for in-service vocational training and other resources relevant for lifelong learning (LLL) and professional development of all participants in agricultural education.

Timing of the project: 01/12/2013 - 30/11/2016

Project partners

P1 University of Belgrade Faculty of Agriculture UB
– coordinating institution
P2 University of Novi Sad Faculty of Agriculture UNS
P3 University of Kragujevac Faculty of Agronomy Cacak UNIKG
P4 University EDUCONS Faculty of Ecological Agriculture
P5 State University Novi Pazar SUNP
P6 Association of Middle Agricultural Schools AMS
P7 Institute for Science Application in Agriculture IPN
P8 Educational Forum (NGO) EF
P9 Balkan Security network, (NGO) BSN
P10 Ministry of Education, Science and Technological Development ME
P11 Banat University of Agricultural Sciences and Veterinary Medicine USAMVBT
P12 University of Maribor UM
P13 University of Foggia UNIFG

Izgradnja kapaciteta srpskog obrazovanja u oblasti poljoprivrede radi povezivanja sa društvom, CaSA 544072-TEMPUS-1-2013-1-RS-TEMPUS-SMHES (2013 – 4604 / 001 - 001)

Podprogram Strukturne mere, akcija Visoko obrazovanje i društvo

Projekat predviđa stvaranje Nacionalnog repozitorijuma za obrazovanje u poljoprivredi u kome će se nalaziti online i klasični kursevi i drugi resursi za stručno usavršavanje, kao i podaci i dokumenti relevantni za celoživotno učenje (LLL) i profesionalno usavršavanje svih učesnika poljoprivrednog obrazovanja.

Trajanje projekta: 01/12/2013 - 30/11/2016

Partneri na projektu

P1 Univerzitet u Beogradu Poljoprivredni fakultet UB
– institucija kordinator
P2 Univerzitet u Novom Sadu Poljoprivredni fakultet UNS
P3 Univerzitet u Kragujevcu Agronomski fakultet Čačak UNIKG
P4 Univerzitet EDUCONS Fakultet za ekološku poljoprivredu
P5 Državni Univerzitet Novi Pazar SUNP
P6 Udruženja srednjih škola područja rada poljoprivrede, proizvodnje i prerade hrane AMS
P7 Institut za primenu nauke u poljoprivredi IPN
P8 Obrazovni Forum OF (NVO) EF
P9 Balkan Security network, (NVO) BSN
P10 Ministarstvo prosvete, nauke i tehnološkog razvoja ME
P11 Banatski Univertitet Poljoprivrednih nauka i Veterinarske medicine USAMVBT
P12 Univerzitet Maribor UM
P13 Univerzitet Foggia UNIFG

Project web site: http://casa.polj.uns.ac.rs/casa/
Internet stranica projekta: http://casa.polj.uns.ac.rs/casa/

AREA

UNAPREĐENJE ISTRAŽIVANJA U POLJOPRIVREDNIM I PREHRAMBENIM NAUKAMA NA POLJOPRIVREDNOM FAKULTETU UNIVERZITETA U BEOGRADU

www.area.agrif.bg.ac.rs

This project has received funding from the European Union's Seventh Framework Programme for research, technological development and demonstration under grant agreement no 316004

Naziv projekta: "UNAPREĐENJE ISTRAŽIVANJA U POLJOPRIVREDNIM I PREHRAMBENIM NAUKAMA NA POLJOPRIVREDNOM FAKULTETU UNIVERZITETA U BEOGRADU"

Finansijer i program: European Commission, Seventh Framework Programme, SP-4 Capacities, Coordination and Support Action, Support action

Oznaka programa: FP7-REGPOT-2012-2013-1

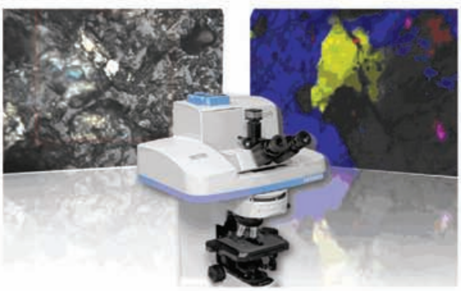
Broj projekta: 316004

Učesnik: Poljoprivredni fakultet - Univerzitet u Beogradu

Koordinatora projekta: Prof Radmila Stikić

Sažetak projekta

Cilj AREA projekta je da ojača i unapredi istraživački kapacitet trinaest ekselentnih naučnih grupa na Poljoprivrednom fakultetu u Beogradu. Te grupe vode naučnici čija je ekspertiza iz oblasti anatomije i fiziologije gajenih biljaka, biodiverziteta, herbologije, hortikulture, patologije biljaka, akvakulture, biohemije hrane, prehrambene tehnologije i biotehnologije jedinstvena u Srbiji. Cilj AREA projekta je da koherentnim aktivnostima unapredi naučni kapacitet svake grupe i da dovede do napretka njihovih savremenih istraživačkih programa, kao i da stimuliše interakcije među istraživanjima implementacijom postojećih DNK marker tehnologija i uvođenjem inovativne Ramanove mikroskopije/spektroskopije, kao tehnologijama relevantnim za svaku istraživačku grupu.



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