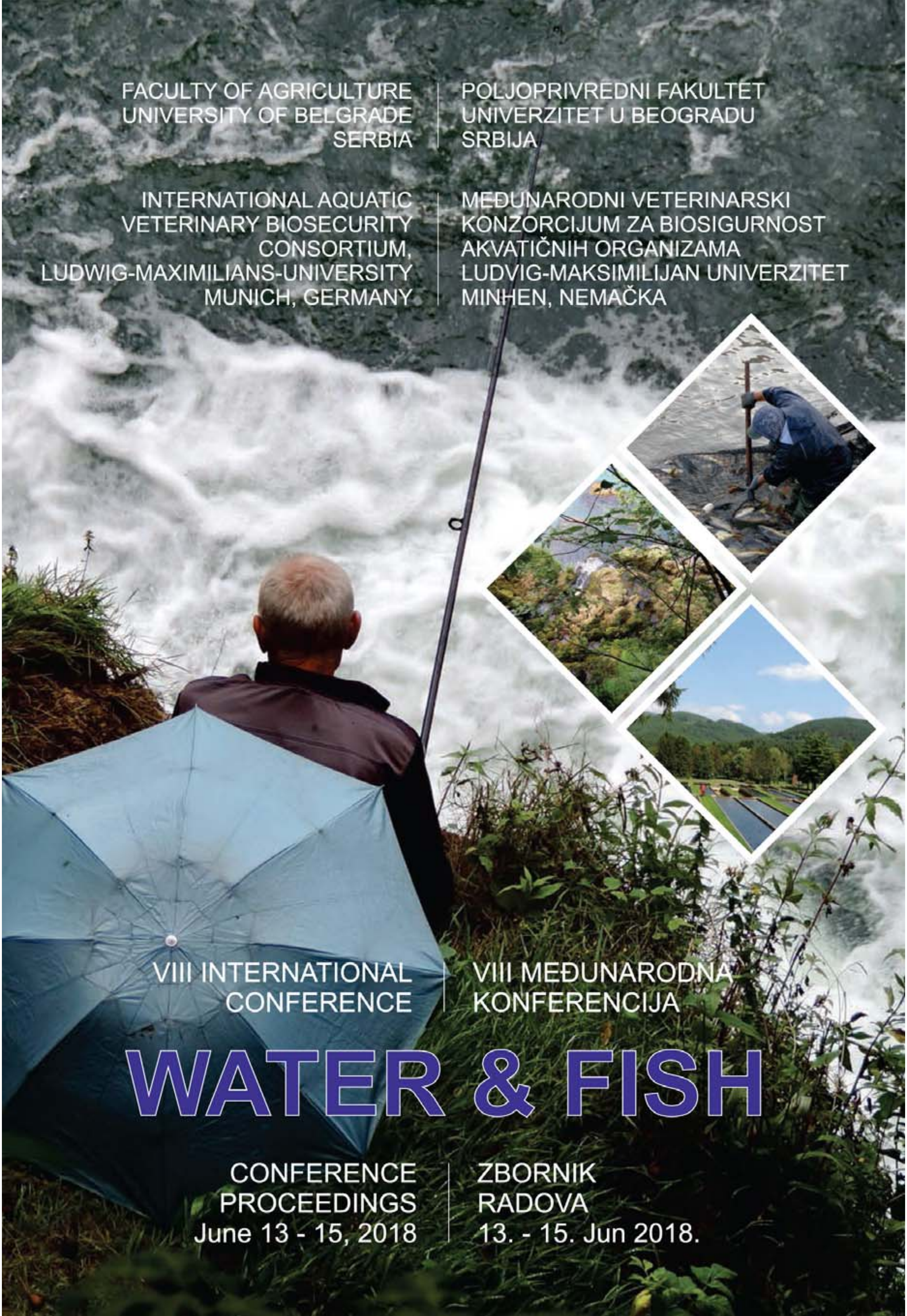

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SRBIJA NA PUTU KA EVROPSKOJ UNIJI: STANJE I OČEKIVANI ISHODI PREGOVORA O POGLAVLJU 13 "RIBARSTVO"

Apstrakt

Republika Srbija je započela pregovore o pristupanju Evropskoj Uniji (EU) 21 januara 2014. To je bio signal za povećanja intenziteta priprema za kompleksni proces pregovaranja, čiji će zaključak omogućiti Srbiji da postane članica EU. Pregovaranje predstavlja proces tokom koga buduće članica prilagođava sopstveni sistem zakonskom, ekonomskom i socijalnom sistemu EU, usvajanjem i implementacijom pravnih tekovina EU („Acquis communautaire"). Sa tačke gledišta EU, predsednik Evropske komisije Jean-Claude Juncker prepoznao je u svom govoru „Stanje Unije" od 13 septembra 2017 strateški značaj budućeg proširenja onda kada zemlja kandidat dostigne pristupne kriterijume. U svojoj novoj ojačanoj Strategiji za Zapadni Balkan, Komisija (2018) je odredila godinu 2025 kao mogući vremenski okvir za pristupanje Crne Gore i Srbije. Put do usvajanja i implementacije pravnih tekovina EU u ribarstvu je opisan u Poglavlju 13 i – kako se sastoji od propisa koji ne zahtevaju pretvaranje u nacionalno zakonodavstvo – to je relativno laka putanja. Ipak, sve ovo zahteva uvođenje mera za pripremu administracije i izvršilaca za učešće u Zajedničkoj politici ribarstva. Ovaj rad će predstaviti pozadinu i okolnosti pregovora o proširenju između EU i Srbije i zasnovano na dovoljno indicija, doneće preporuke o tome kako će EU delati u pregovorima sa Srbijom u Poglavlju 13, kao i o mogućim ishodima ovih pregovora.

Ključne reči: *Evropska Unija, proširenje, Srbija, pristupanje, Poglavlje 13*
Keywords: *European Union, Enlargement, Serbia, Accession, Chapter 13*

INTRODUCTION

The Republic of Serbia started negotiations for its accession to the European Union on 21 January 2014. The beginning of the negotiations was preceded by a decision of the European Council on 28 June 2013. That was the signal for increased preparations for an all-encompassing negotiation process whose conclusion will allow Serbia to become a member of the EU. The analytical examination of Serbia’s alignment to the EU acquis, a.k.a. the “screening” process, began in September of 2013 and it ended in March 2015 (Government of the Republic of Serbia, 2018).

The negotiations represent a process during which the future member states adjust to the legal, economic and social system of the European Union, i.e. they adopt and implement the EU acquis. The negotiations are a process of learning and systematic reform that means the adoption of European values and standards, their implementation and even improvement.

The accession negotiations are divided into 35 Negotiating Chapters. The subject of the negotiations is not which parts of the acquis will be adopted but rather the time frame of alignment to the EU’s legal system. In other words the European Union acquis represents a model through which the state and the society needs to be reformed to be on the same level as other European States.

To complete the Accession Negotiations with Serbia on the negotiating Chapter 13 “Fisheries”, following the submission of Serbia’s Negotiating Position (Government of the Republic of Serbia, 2017), the Commission must submit to the Council a draft Common Position on the above-mentioned chapter to discussed with the Member States to the Working Party on Enlargement and Countries Negotiating Accession to the EU.

For Serbia, to become an EU member state is a strategic goal, as it is a tool towards becoming a more developed and better organized modern democratic state. The same perception of the purpose is not so present in Europe, and that difference shapes many misunderstandings (Miscevic, 2018). This problem goes beyond the case of Serbia and the Western Balkans. The EU has taken steps to protect itself from the risks of expansion. No longer is enlargement considered to be an EU priority, as it was at the time of the accession of the majority of the Central and Eastern European countries.

Indeed, for many member states, enlargement is now seen as contrary to the EU’s interests, and the cause behind this are the problems the EU faced after the last - massive - enlargement with the 10 Member States, before the accession of Croatia, described as not well prepared, having complains between the older Member States and many EU citizen that was promoting only the market, rather than the cohesion and common cultural background and identity.

ENLARGEMENT POLICY OF THE EUROPEAN UNION

EU Western Balkans Strategy

In his “State of the Union” speech of 13 September 2017, European Commission President Jean-Claude Juncker recognised the strategic importance of further enlargement once the candidate countries meet the accession criteria (European Commission, 2017). His declaration was followed by the new and enhanced Western Balkan strategy elaborated by the European Commission, which sets 2025 as a possible time horizon for Montenegrin and Serbian accession (European Commission, 2018).

While the EU could become again larger than 27 Members (having in mind BREXIT, and that United Kingdom will leave EU before 2025), the dynamics of moving forward on their EU paths for all Western Balkans is based on their own merits and at their own speed depending on the concrete results achieved. The Strategy explains the steps that need to be taken by Montenegro and Serbia to complete the accession process in a 2025 perspective. While others could catch up, Montenegro and Serbia are the only two countries with which accession talks are already under way. This perspective will ultimately depend on strong political motivation, the delivery of real and sustained reforms, and definitive solutions to disputes with neighbours (European Commission, 2018).

For Serbia to become a member of the EU by 2025, it will have to meet a series of new conditions (Miscevic, 2018), a result - as we already mentioned - to the recent experience of EU, especially with what happened after achieving membership (problems with democratic backsliding, breach of the rule of law and fundamental values), causing EU governments to be very cautious about enlargement, and reluctant to make strong political statements in support of Western Balkan accession.

The candidate countries are undertaking complex and challenging reforms to harmonize their political, economic and legal systems with those of the EU, and when the support from EU is not strong and clear, accompanied with a lack of assurance about the outcome, makes more difficult for the Governments of the candidate countries to promote to their citizens the benefits from the accession to EU (Figure 1. European Commission, 2018b).

Figure 1. European Commission, 2018b

Progress of Candidate Countries in EU accession

The opportunity for Euro-Atlantic integration was offered to Western Balkan countries in 1999, followed by a long series of agreements. A cooperation agreement, the Stability Pact for Southern and Eastern Europe, was put in place in June 1999. This was an EU initiative but other countries (US, Canada, Japan, Russia, Turkey, Norway and Switzerland) and a number of international organisations, were also involved. The pact had 3 major pillars - democracy, economy and security - and it opened the Stabilisation and Association Process (a first step towards potential EU membership) for the Western Balkans region. The Stability Pact was replaced by the Regional Cooperation Council in 2008 (Dabrowski et al, 2018).

The potential eligibility of the Western Balkan countries to become EU members was confirmed by the Thessaloniki EU summit in June 2003. The European Council expressed "…its determination to fully and effectively support the European perspective of the Western Balkan countries, which will become an integral part of the EU, once they meet the established Criteria" (Council of the European Union, 2003). Subsequently, Stabilisation and Association Agreements, which also include provisions for a Deep and Comprehensive Free Trade Area (DCFTA), were negotiated, signed and ratified by the EU and Western Balkan countries. The agreement with FYRO Macedonia entered into force in 2004, with Croatia in 2005, with Albania in 2009, with Montenegro in 2010, with Serbia in 2013, with Bosnia and Herzegovina in 2015 and with Kosovo in 2016.

Croatia and FYRO Macedonia obtained EU candidate status in 2004, Montenegro in 2010, Serbia in 2012 and Albania in 2014. Croatia was the first to start membership negotiations in 2005, and completed them successfully in 2011, becoming the 28th EU member on 1 July 2013. Montenegro started membership negotiations in 2012 and Serbia started in January 2014. By December 2017, Montenegro had managed to open accession negotiations on 30 out of 35 chapters of the "Acquis Communautaire".

Serbia is less advanced. By December 2017, managed to start negotiation on only 12 chapters, and had provisionally closed only 2 chapters: Chapter 25 "Science & Research", and, Chapter 26 "Education & Culture" (Figure 2. Government of the Republic of Serbia, 2018).

Chapter 13 "Fisheries"

The European Union has exclusive competence in the area of the conservation of marine biological resources under the common fisheries policy as well as for the conclusion of international agreements in that area (Article 3(1) (d), 3(2) of the Treaty on the Functioning of European Union “TFEU”), and this chapter consists of all the necessary actions needed to be taken by the candidate country to implement the acquis of the fisheries & aquaculture sector.

The acquis on fisheries consists of Regulations, which do not require transposition into national legislation. However, it requires the introduction of measures to prepare the administration and the operators for participation in the common fisheries policy, which covers market policy, resource and fleet management, inspection and control, structural actions, and state aid control. In some cases, existing fisheries agreements and conventions with third countries or international organisations need to be adapted.

The EU’s Common Fisheries Policy (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. 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.

The CFP was first introduced in the 1970s and went through successive updates, the most recent of which took effect on 1 January 2014, having also a new fund for EU’s maritime & fisheries policies for the period 2014-2020: the European maritime and fisheries fund (EMFF). The reform also changes the way in which the CFP is managed, giving EU countries greater control at national and regional level, having 4 main policy areas: 1) Fisheries management, 2) International policy, 3) Market & trade policy, 4) Funding of the policy, and includes rules on aquaculture and stakeholder involvement (Chatziefstathiou et al, 2015).

EXPECTED OUTCOME OF THE NEGOTIATIONS

For the Western Balkans, due to their recent turbulent past, regional cooperation is one of the essential criteria for membership, and regional initiatives are growing in numbers (according to some data, there are now more than 70 cross-border initiatives). According to Prof. Tanja Miscevic, Head of the Negotiation Team for Accession of Serbia to the EU (Miscevic, 2018) the introduction of the Berlin Process in 2014 gave rise to a suspicion that regional integration is intended to become a substitute for EU membership.

Meanwhile, in our opinion, there are sufficient indications on how the EU will proceed on the negotiations with Serbia on Chapter 13. Either this concerns the opening of the chapter, taking in mind the latest one, this of Montenegro (Council of the EU, 2016), either this concerns the context of the EU Common Position on this chapter, having in mind also the latest one, this of Croatia (Council of the EU, 2011).

Serbia has submitted negotiating positions for Chapter 13 “Fisheries”, accepting the acquis under Chapter 13 “Fisheries” as in force on 1 January 2017, and declaring that it will be ready to implement it by accession to the EU (Government of the Republic of Serbia, 2017).

However, until the end of 2017 the Member States have not reached consent as regards the opening of this chapter. To successfully implement the “Acquis Communautaire” of Fisheries in Serbia, and close the Chapter 13, as in any other Chapter, all available national capacities in the countries should be employed in the process of envisioning and planning the reforms. In addition, raising capacity of all the stakeholders to understand and adopt the EU and international standards in the area should be a priority.

Draft Common Position

The position of the European Union will be based on its general position for the Accession Conference with Croatia (Council of the EU, 2011), and will be subject to the general negotiating principles therein:

- *any view expressed by either party on a chapter of the negotiations will in no way prejudge the position which may be taken on other chapters;*
- *agreements - even partial agreements - reached during the course of the negotiations on chapters to be examined successively may not be considered as final until an overall agreement has been established;*

The EU probably will remind Serbia the principle of exclusive EU competence over the conservation of marine biological resources under the common fisheries policy, and then will note Serbia’s declaration of acceptance of the acquis under Chapter 13, and encourage Serbia to continue the process of alignment with the acquis and its effective implementation and enforcement, and in general to develop - well before the accession - policies and instruments as close as possible to those of the European Union.

As Serbia does not have access to the sea significant parts of the acquis consisting Chapter 13 “Fisheries” will not require particular implementation efforts. Certain measures will however be needed, in particular as regards marketing standards, aquaculture, data collection, and control measures against illegal, unreported and unregulated fishing:

- *Resource and fleet management*

Serbia plans to perform technical & scientific evaluation to establish whether its river basins are a natural habitat for European eel. This is a key issue for EU, which will ask Serbia to provide information to the Commission as early as possible before the accession, to ensure the correct application of Council Regulation (EC) 1100/2007 for the recovery of European eel. Serbia as an enclosed country has not fishing vessels working in sea, or even to work as long distant fleet under her flag, so, completing the scrutiny of this part will be easy. For EU it is in general of a great importance the existence of a reliable and systematic data collection, in this case will be asked only with regard to aquaculture.

- *Inspection and control*

Serbia has to ensure that will develop the necessary system required by the acquis to control illegal, unreported and unregulated fishing (a.k.a.: IUU fishing).

- *Structural actions*

For Serbia to take part at the European Maritime & Fisheries Fund (EMFF) must put in place as soon as possible all the necessary administrative structure and institutional framework, including the designation of institutional structures with specific tasks and responsibilities.

▪ *Market policy*

Serbia must amend the legal framework on marketing standards for fish and fisheries products in order to ensure full compliance with the acquis. For EU the Common Organisation of the Markets of Fishery and Aquaculture Products (CMO) has an increasingly significant role, and it is important that the administrative capacity will be strengthened and that the collection and monitoring of market information will be improved.

▪ *State aid*

Serbia needs to comply with EU State aid rules in the fisheries sector upon accession, and in particular to ensure that the necessary administrative capacity endowed with appropriate operational independence will be in place. Serbia must provide comprehensive information to the Commission in this regard, before the closure of the Chapter.

▪ *International agreements*

There is a general EU position, that fisheries agreements by acceding countries with third countries need, from the date of accession, to be managed by the EU. Serbia, at the date of accession or the earliest possible date thereafter, will have to withdraw from international fisheries agreements and organisations to which the EU is also a party, unless its membership relates to matters other than fisheries.

When it comes to benchmarking, benchmarks requiring only the adoption of new strategies and plans should be avoided, and replaced by benchmarks clearly defining the key objectives of any action. These should be specified, and also include outcome related indicators, which will help the devoted governments to follow process, and won't allow the "tricky" ones to deliver results and progress reports only in a descriptive way (Abazi Imeri et al, 2018).

The EU usually considers that benchmarks are required to provisionally close a chapter, and these benchmarks include the legislative amendments, as well as the administrative capacity to implement and enforce the relevant "acquis".

For Montenegro to close Chapter 13, the benchmarks to be met are the following:

- *Montenegro adopts legislation that provides a substantial degree of alignment with the EU acquis for fisheries and ensures that Montenegro will be able to fully apply the Common Fisheries Policy upon accession.*
- *Montenegro substantially strengthens the administrative, inspection and control capacity required by the Common Fisheries Policy and ensures that EU requirements will be fully met at the date of accession, in particular as regards inspection and control.*

EU also underlined for Montenegro that it would devote particular attention to monitoring all specific issues mentioned in its Common Position, with a view to ensuring its administrative capacity and its capacity to complete the legal alignment in the relevant areas.

Monitoring of progress in the alignment with and implementation of the "*acquis"* will continue throughout the negotiations. A final assessment of the conformity of Montenegro's legislation with the "*acquis"* and of its implementation capacity can be made only at a later stage of the negotiations. Finally, in any similar case the last comment is that the Conference will have to return to these chapter at an appropriate moment.

CONCLUSIONS

The EU must take full advantage of the accession negotiation process for the promotion of rule of law, and use its “transformative power”. The announced greater political devotion to enlargement by the EU member states has the potential to boost the effectiveness of the EU conditionality mechanisms, but yielded so far limited results (Abazi Imeri et al, 2018).

For the above reason, paired with the well-documented, and well presented, preparation from Serbia on this Chapter, the possibility for the outcome of the negotiations to be the opening, in the near future, and then the swift closure of Chapter 13 “Fisheries”, is very high, as it will give the opportunity to be presented as a case of good practice.

The opening of the chapter will be followed by the Common Position, and by an EU statement, which will declare that if Serbia continues to make progress in the alignment with the implementation of the acquis covered by Chapter 13 “Fisheries” the chapter will be characterised “provisionally closed”, when it is agreed by the EU that a benchmark has been met (Council of the EU, 2011; 2016).

The benchmark that has the predominant possibilities to be asked from EU is that Serbia must present a detailed “Action Plan”, strict on the time schedule of its implementation, which will ensure full compliance with the requirements of the EU legislation by the date of accession, in particular regarding the organisation of the markets, aquaculture, data collection, and control measures against illegal, unreported and unregulated fishing.

The greater involvement of the civil society in this process can reduce the concerns of the citizens, and help speed up the reform process. By communicating step-by-step to the citizens the enlargement mechanism, in a less bureaucratic and technical manner, will permit to easily present the forthcoming benefits from the successful end of the EU integration process.

Monitoring of progress in the alignment with, and implementation of, the acquis described in Chapter 13 “Fisheries” will then continue throughout the negotiations, and particular attention will be on the monitoring of all the specific issues mentioned above, which will ensure the high level’s administrative capacity reached by Serbia, having in mind that there may be new acquis between the 1st of January 2017 and the final conclusion of the negotiations.

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DIFFERENT CONCEPTS OF SUSTAINABLE AQUACULTURE SYSTEMS

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RAZLIČITI KONCEPTI ODRŽIVIH AKVAKULTURNIH SUSTAVA

Apstrakt

Akvakultura je, kao i ostali sektori proizvodnje hrane, u posljednje vrijeme suočena s izazovom održivog razvitka. Tradicionalne uzgojne tehnike se sve više smatraju „zagađujućim“ i „neodrživim“. Većina uzgajivača neprekidno pokušava iznaći načine i sredstva kojima bi unaprijedili postojeću proizvodnu praksu i osigurali njezinu održivost, učinkovitost i isplativost. Održiva akvakultura je dinamični koncept te se održivost takvih sustava razlikuje ovisno o uzgajanoj vrsti, lokaciji, društvenim normama, znanju i tehnologiji. Kako bi sistem bio u potpunosti održiv, mora uključivati ekološku, ekonomsku i društvenu održivost. Uzgoj vodenih organizama ne smije uzrokovati značajne poremećaje ekosustava, negativno utjecati na biodiverzitet ili uzrokovati onečišćenje. Mora biti društveno odgovoran i doprinositi dobrobiti zajednice, ali u isto vrijeme osigurati održivo poslovanje s dugoročnom budućnošću. U tradicionalnoj, ekstenzivnoj akvakulturi, riba se uzgaja u otvorenim vodama, kao što su jezera, estuariji ili obalni zaljevi gdje se hrani prirodno dostupnim nutrijentima; ili pak u ribnjacima gdje može biti hranjena nusproizvodima s farmi. Glavni problem ovog pristupa je da su takvi sustavi neprofitabilni i ekonomski neodrživi. FAO-ov Komitet za ribarstvo (COFI) posebno naglašava unaprjeđenje proizvodnje ribe putem integracije akvakulture i proizvodnje bilja te integrirano korištenje malih i srednjih vodenih površina. Drugi izazov održivosti predstavljaju načini na koje uzgajališta obrađuju otpadnu vodu i ispuštaju organski otpad u okoliš. Krute čestice u kaveznom uzgoju padaju na dno, obogaćuju ga ugljikom, dušikom i fosforom te time negativno utječu na fizičko-kemijska svojstva sedimenta i bentoske organizme ispod kaveza. U svakom slučaju, otpad iz bilo kojeg otvorenog uzgojnog sustava ispušta se direktno u vodeni stupac te se ovakvi sustavi ne mogu smatrati održivim. Međutim, postoje akvakulturni uzgojni sustavi koji zahvaljujući svome dizajnu mogu osigutari efikasan sustav tretiranja otpada. Takve primjere predstavljaju integrirana multitrofička akvakultura (IMTA), recirkulacijski (RAS) i akvaponijski sustavi. Primjer održivih sustava predstavlja i integracija multitrofičke akvakulture koja obuhvaća moderni zatvoreni RAS i hidroponiju, pri čemu se uzgajaju riba i bilje u okviru održivog akvaponijskog sustava. Termin akvaponija odnosi se na bilo koji proizvodni sustav koji predstavlja kombinaciju konvencionalne akvakulture s hidroponijom, tehnologijom uzgoja bilja u nutrijentima obogaćenoj vodi umjesto zemljištu obogaćenom gnojivom. Uspješno funkcioniranje ovakvih sustava može nadomjestiti smanjenu proizvodnju gospodarskog ribolova uzrokovanu prelovom, osigurati okolišno prihvatljivu revitalizaciju poljoprivrede te zapošljavanje kako u urbanim tako i u ruralnim područjima. Nihov dizajn uključuje razvitak programa upravljanja otpadom koji obuhvaćaju kompletnu preradu proizvoda, tretman otpadne vode i recikliranje otpada iz oba sustava, akvakulturnog i hidroponijskog. Ove tehnologije smanjuju potrošnju vode te uklanjaju, obrađuju i recikliraju kruti i tekući otpad putem proizvodnje energije iz obnovljivih izvora i/ili recikliranja organskih hranjivih tvari. Upotrebom inovativnih tehnologija i pravilnim menadžmentom, nus-proizvodi prerade i otpad iz primarne proizvodnje mogu se koristiti za proizvodnju bioplina, biodizela te aktivnih tvari za farmaceutsku i kozmetičku industriju, a ujedno povećavaju financijsku stabilnost i profitabilnost akvakulturne proizvodnje. U ovom radu su opisani osnovni dizajni sustava koji uz socio-ekonomsku i okolišnu, mogu garantirati i energetsku održivost.

Ključne riječi: *održiva akvakultura, RAS, IMTA, akvaponija*

Abstract

Aquaculture, like any other food production practice, is facing challenges for sustainable development. Recently, traditional aquacultural techniques have been labeled "polluting" and "unsustainable". Most fish farmers are continuously pursuing ways to improve their production processes, make them sustainable, more efficient and cost-effective. Sustainable aquaculture is a dynamic concept and the sustainability of an aquaculture system will vary with species, location, societal norms and the state of awareness, knowledge and technology. But for a system to be truly sustainable, it must include environmental, economic, social and community sustainability. Fish farming should not create significant disruption to the ecosystem, cause the loss of biodiversity or result in substantial pollution impact. It must be socially responsible and contribute to community well-being, but at the same time, it must present a viable business with long-term prospects. In traditional aquaculture, fish can be bred in open water such as lakes, estuaries and coastal bays, where they can feed on naturally available nutrients; or in inland farm ponds, where they can be fed with fram by-products. The main problem in such an approach is these systems are usually not profitable and economically viable. COFI (FAO Committee on Fisheries) emphasized need to enhance inland fish production through integrated aquaculture-agriculture farming systems and integrated utilization of small and medium-size water bodies. The second issue of sustainability is the method to which facilities treat and release organic waste so that the resulting discharge does not affect the surrounding ecosystem. The solid waste produced by fish farmed in net pens falls to the seabed below the fish cages are enriched in carbon, nitrogen and phosphorus relative to the natural sediments, hence fish farming may considerably alter the physicochemical composition of the natural sediments in the area below. When the waste byproducts from any open aquaculture system is discharged directly back into the water column, it cannot be considered a sustainable practice or system. There are other aquaculture systems that have effective waste management practices, such as Integrated Multi-Trophic Aquaculture (IMTA), recirculating aquaculture (RAS) or aquaponics systems. Some examples of sustainable systems are IMTA systems, which combine modern closed RAS and hydroponics to cultivate fish and plants within a sustainable aquaponics system. Aquaponics refers to any system that combines conventional aquaculture with hydroponics, the method of growing plants in nutrient rich water instead of fertilized soil. The successful operation of such systems can offset depleted harvests of struggling fisheries, while also providing a significant level of environmentally sound agricultural revitalization and employment in both urban and rural areas. The design results in the development of a waste management program, which defines the complete processing, treatment, and reclamation of wastes generated within their aquaculture and aquaponics production systems. These technologies minimize water use, and remove, treat, and recycle solid and liquid wastes through the co-generation of energy, and the recycling of organic nutrients. With the use of these innovative technologies and proper management, byproducts of food production and waste mitigation can be used to produce biogas, biodiesel, substances for the pharmaceutical and cosmetic industries, while increasing the financial stability and the business profitability. This paper describes the basic designs of the systems that beside socioeconomic and environmental, could guarantee also energy sustainability.

Keywords: *sustainable aquaculture, RAS, IMTA, aquaponics*

INTER AND INTRASPECIFIC INTERACTIONS UNDER EXPERIMENTAL CONDITIONS AFFECT BEHAVIOR AND FEEDING NICHE OF COMMON CARP

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INTER I INTRASPECIFIČNE INTERAKCIJE UTIČU NA PONAŠANJE I HRANIDBENU NIŠU ŠARANA POD EKSPERIMENTALNIM USLOVIMA

Apstrakt

Među mnogobrojnim morskim i slatkovodnim ribama, šaran (*Cyprinus carpio*) je globalno privukao verovatno najviše pažnje. Veliki adaptivni kapacitet, zajedno sa brzim prirastom, rano sazrevanje i visok fekunditet je omogućio šaranu preživljavanje i naseljavanje velikog broja različitih tipova vodenih staništa, čineći ga rasprostranjenim širom sveta (Zhou et al. 2000; Piria et al. 2016). Šaran je jedna od najpopularnijih riba za ishranu u Centralnoj Aziji i Evropi gde se najčešće gaji ili u monokulturi ili polikulturi u ekstenzivnim ili poluintenzivnim sistemima (Adamek et al. 2012; Rahman 2015).

Interakcije vrsta zbog limitiranih resursa u prirodnim sredinama dovode do pojave različitih adaptacija u ponašanju i ishrani dovodeći do ublažavanja ovih efekata (Brannas 2008; Fox and Bellwood 2011). Međutim, istraživanje socijalnog ponašanja i ponašanja u ishrani šarana direktnim posmatranjem u prirodnim ekosistemima, posebno jezerima, akumulacijama i rekama je veoma ograničeno usled visokog turbiditeta i, kao posledice toga, slabe vidljivosti. Inter i intraspecifične interakcije u ishrani i ponašanju mogu da pruže uvid u ponašanje i hranidbene niše šarana i pratećih vrsta riba.

Da bi se razumele interakcije u ponašanju šarana i pratećih vrsta riba, dve studije su izvedene u simuliranim ribnjačkim objektima, u kojima su uspostavljeni polu prirodni uslovi. Da bi se razumeli efekti različitih izvora hrane na (i) hranidbenu nišu i selektivnost u ishrani, i (ii) dnevni ritam ponašanja šarana u hranjenju, plivanju, odmaranju i okupljanju u jata, tri tretmana su poređena (tretman samo sa zooplanktonom, tretman sa zooplanktonom i bentičnim makroinvertebratama i tretman sa zooplanktonom, bentičnim makroinvertebratama i veštačkim hranivom). Da bi se razumele inter i intraspecifične interakcije u ponašanju, ponašanje šarana je praćeno u prisustvu rohua (*Labeo rohita*), vrste ribe koja se hrani u vodenom stubu. U ovom eksperimentu korišćene su dve gustine šarana, sa i bez dodatog veštačkog hraniva.

U tretmanima gde je korišćen zooplankton i bentične makroinvertebrate, šaran se uglavnom kretao u blizini dna sistema, hraneći se pre svega bentičnim makroinvertebratama. U odsustvu bentičnih makroinvertebrata došlo je do promene, i šaran je većinu vremena provodio u vodenom stubu, gde se najviše hranio zooplanktonom. Kada je veštačka hrana bila dostupna, šaran je odmah prelazio na tu vrstu hrane. Međutim, ponašanje i hranidbena niše šarana su bile pod uticajem intraspecifične kompeticije, ali ne i pod uticajem drugih vrsta riba, naročito rohua. Sa druge strane, ponašanje rohua je bilo pod velikim uticajem šarana.

Rezultati inter i intraspecifičnih interakcija u ponašanju bi se potencijalno mogli koristiti u daljem razvoju tehnika gajenja šarana.

Ključne reči: *Cyprinus carpio, Labeo rohita, hranidbena niša, ponašanje, ishrana, plivanje, kompeticija*

Abstract

Among various marine and freshwater fishes, common carp (*Cyprinus carpio*) has perhaps attracted the most attention from humans across the globe. The high adaptive capacity along with the fast growth, early maturity and high fecundity has enabled common carp to persist and proliferate in a wide array of environments, making them widespread throughout the world (Zhou et al. 2000; Piria et al. 2016). Common carp is one of the most popular food fish in Central Asia and Europe, where it is commonly cultured in either in monoculture or polyculture ponds applying extensive or semi-intensive methods (Adamek et al. 2012; Rahman 2015).

Species interaction for limited resources in the natural world leads to various behavioral and feeding adaptations in species to mitigate these effects (Brannas 2008; Fox and Bellwood 2011). However, study on social and feeding behaviours of common carp through direct observation in natural environments particularly in lakes, reservoirs and rivers have largely been restricted due to high turbidity and, in consequence, low visibility. Inter and intraspecific feeding and social interactions can provide more insight on behaviour and feeding niches of common carp and co-cultured fish.

To understand behavioural interactions of common carp and co-cultured fish, two separate studies were conducted in simulated ponds, in which semi-natural conditions were established. To understand the effects of various food resources on (i) feeding niche and food selectivity, and (ii) diel behavioral rhythmicity particularly grazing, swimming, resting and schooling behaviours of common carp, three treatments were compared (treatment with only plankton, treatment with plankton and benthic macroinvertebrates and treatment with plankton, benthic macroinvertebrates and artificial feed). To understand inter and intraspecific behavioral interactions, behaviors of common carp was directly observed in presence of water column feeder fish (rohu *Labeo rohita*). In this experiment, two densities of common carp were used, with and without supplying artificial feed.

In systems containing plankton and benthic macroinvertebrates, common carp mainly lived near the bottom of the system, feeding primarily on benthic macroinvertebrates. In the absence of benthic macroinvertebrates, they shifted from near the bottom to the water column where they spent majority of the time and fed principally on zooplankton.

When artificial feed is available, common carp readily switched to artificial feed. However, behavior and feeding niche of common carp were influenced by intraspecific competition, but not by the presence of other fish particularly rohu. The behavior of rohu was greatly influenced by the presence of common carp. The results of inter and intraspecific behavioral interactions could be potentially used to further the development of common carp husbandry techniques.

Keywords: *Cyprinus carpio; Labeo rohita; Feeding niche, Behaviour, Grazing, Swimming; Competition*

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GOODFISH – AN ACADEMIC PROJECT TO IMPROVE AQUACULTURE PRODUCTION IN HUNGARY

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GOODFISH – AKADEMSKI PROJEKAT UNAPREĐENJA AKVAKULTURE U MAĐARSKOJ

Apstrakt

Četiri vodeće visoko obrazovne i naučne institucije u Mađarskoj su udružile svoje snage u cilju očuvanja genetičkih resursa i razvoja tehnologije proizvodnje tri nativne vrste riba. Cilj konzorcijuma koji je vodio Univerzitet Szent István je unapređenje proizvodnje šarana, smuđa i soma u uslovima i na način koji pogoduje potrošačima. Kao rezultat ovog projekta čiji je ukupni budžet od 987.44 miliona forinti (HUF) (oko 3.3 miliona €) očekuje se značajno poboljšanje proizvodnje kao i potrošnje riba u Mađarskoj. Projekat se implementira u okviru programa Széchenyi 2020 u period 2017-2020. Osnovni cilj članova konzorcijuma (Univerzitet Szent István, Univerzitet Debrecen, Nacionalni poljoprivredni istraživački i Inovativni centar Istraživački institut za ribarstvo i akvakulturu i Fakultet Georgikon Univerziteta Pannonia) je očuvanje resursa i razvoj tehnologije akvakulture tri gorepomenute ekonomski i ekološki značajne vrste u cilju obezbeđenja bolje usluge potrošačima i unapređenja domaće potrošnje riba. Kao rezultat projekta efikasnost gajenja šarana će biti unapređena, trenutna proizvodnja u trogodišnjem ciklusu će biti skraćena, više tržišno orjentisana, dvogodišnja proizvodnja. Ovoj aktivnosti doprinose i veterinarska, biotehnološka i marketinška istraživanja u projektu. Očekuje se i unapređenje efikasnosti tehnologije uzgoja soma, kao i razvijanje varijeteta soma visokih performansi u cilju podsticanja položaja ovih vrsta na domaćim i Evropskim tržištima. Smuđ je nativna vrsta u Mađarskoj sa verovatno najvećim tržišnim potencijalom iako je njegova proizvodnja u intenzivnom sistemu i dalje suočena sa mnogim izazovima. Stoga je istraživački rad u ovom projektu usmeren i na unapređenje proizvodnje smuđa. Povrh pomenutih aktivnosti, očuvanje genetskih resursa prirodnih i gajenih populacija soma i smuđa, takođe je prioritet, pa su tako žive i krioprezervisane banke gena ovih vrsta razvijene u okviru projekta.

Ključne reči: *šaran, som, smuđ, akvakultura, akademija*

Abstract

Four leading institutions of agricultural higher education and research in Hungary have joined their forces in order to preserve the genetic resources and to develop the production technology of three native fish species. The objective of the consortium led by Szent István University is to improve the production of common carp, pikeperch and wels catfish in an environment- and customer-friendly fashion. A significant improvement of Hungarian fish production as well as consumption is expected as a result of this project with a total budget of HUF 987.44 million (approximately € 3.3 million). The project is implemented in the frames of the Széchenyi 2020 program in the period of 2017-2020. The main goal of the consortium members (Szent István University, University of Debrecen, National Agricultural Research and Innovation Center Research Institute for Fisheries and Aquaculture and the Georgikon Faculty of the University of Pannonia) is the preservation of the resources and development of aquaculture technology of the aforementioned three economically and ecologically important fish species in order to provide better services to the customers and improve domestic fish consumption. As a result of this project, the efficiency of Hungarian carp farming will be improved, the current three-year production cycle will be replaced by a faster, more market-oriented two-year production technology. This activity is assisted by the veterinary, biotechnological and marketing research in the project. Improvement of the efficiency of Hungarian wels catfish farming as well as the development of a high-performance catfish variety is expected to boost the market position of this species on the domestic and European markets. Pikeperch is our native species with probably the greatest market potential, yet its production in intensive systems still faces many challenges. Thus, the research work in this project targets the improvement of pikeperch production, as well. In addition to the activities listed above, preservation of the genetic resources of natural and cultured wels catfish and pikeperch populations is also a prioritized topic, thus, live and cryopreserved gene banks are developed of these species within this project.

Keywords: *Common carp, wels catfish, pikeperch, aquaculture, academia*

INTRODUCTION OF AN ALTERNATIVE METHOD FOR ARTIFICIAL FISH PROPAGATION

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UVOĐENJE ALTERNATIVNE METODE VEŠTAČKOG RAZMNOŽAVANJA RIBA

Apstrakt

Veštački mrest - artificial spawning (AS) (ili mrest u tankovima) je jednostavan način razmnožavanja riba. Može da se izazove primenom hormona ili manipulacijama parametrima vodene sredine (tj. temperaturom, svetlosnim režimom, provodljivošću). Mnoge ekonomski važne vrste riba proizvode se AS metodom iz više razloga: prvenstveno u slučajevima poteškoća u predviđanju vremena ovulacije (jegulja, smuđ), male veličine ili osetljivosti (zebrica), ili jednostavnosti (mrest u jezeru ili kavezu). Nasuprot tome in vitro fertilizacija (IVF) je bolja metoda kada su u pitanju odgajivački programi, može se manipulisati i jajima i mlečom (krioprezervacija, proizvodnja triploidnih riba), a moguća su različite kombinacije i ukrštanja različitih linija i vrsta (intra- i interspecijski hibridi).

U praksi Müller et al. (2018a) su koristili metod ovarijalnog ispiranja/veštačkog oplođenja supermom, upotrebom katetera kod šarana (*Cyprinus carpio*). U ovakvom slučaju prikupljeni uzorci sperme su uvedeni u ovarijum, pomoću katetera 12 sati pre ovulacije koja je izazvana primenom hormona. U drugom eksperimentu mešavina sperme i ekstrakta hipofize - carp pituitary extract (CPE) injektovana je u ovarijalni lobus afričkog soma (*Clarias garepinus*). Absorbovani CPE je izazvao ovulaciju i sve ženke su proizvele jaja dobrog kvaliteta i normalno se razvijale posle aktivacije gameta i oplođenja (Müller et al., 2018b). U oba eksperimenta ovarijalna tečnost nije aktivirala spermatozoide koji su zadržali svoju biološku aktivnost tokom 10 – 12 sati. Ovi prikazi principa eksperimenata su pokazali potencijal metode za rutinsku upotrebu kao alternativni za indukovani mrest kod koga sinhronizacija proizvodnje i ovulacija jaja, sa ograničenom raspoloživošću sperme ili indukciju oslobađanja jajnih ćelija ili kada je ceđenje ženki riskantno ili tehnički neizvodljivo.

Ključne reči: *mrest u tankovima, in vitro oplođenje, injektiranje sperme u ovarijum, kateter*

Abstract

Artificial spawning (AS) (or tank spawning) is a simple way of fish propagation. It can be induced by hormonal administration or the manipulation of the ambient water parameters (i.e. temperature, light regime, water conductivity) Many economical important fish species are produced by AS due to several reasons: first of all in case of the hard predictability of the ovulation time (European eel, pikeperch) the small size or sensitiveness (zebra fish), or its simplicity (spawning in pond or cage). In contrast In Vitro Fertilization (IVF) is a better tool for breeding programs, egg and sperm can be manipulated (cryopreservation, producing triploid fish) cross breeding of different lines and species (intra and inter specific hybrids) can be made in several combination.

In practice Müller et al. (2018a) used a method of ovarian lavage/artificial insemination with sperm, by using a catheter in carp (*Cyprinus carpio*). In this case collected sperm samples were introduced to the ovary lobe with the help of a catheter 12 hours before ovulation which was induced through hormonal administration. In another experiment, a mixture of sperm and carp pituitary extract (CPE) was injected into the ovary lobes of African catfish (*Clarias garepinus*). The absorbed CPE induced ovulation and all females produced fertilised good quality eggs that developed normally after activation of gametes and fertilization (Müller et al., 2018b). In both experiments, the ovarian fluid did not activate the spermatozoa which maintained their biological activity for 10-12 hours. These proof of principle experiments demonstrated the potential of these methods for routine use as an alternative to induced spawning, in which synchronising of the production of ovulated eggs with the availability of sperm may be limited or where induction of egg release and or stripping is risky or technically unfeasible.

Keywords: *tank spawning, in vitro fertilization, sperm injection to ovary, catheter*

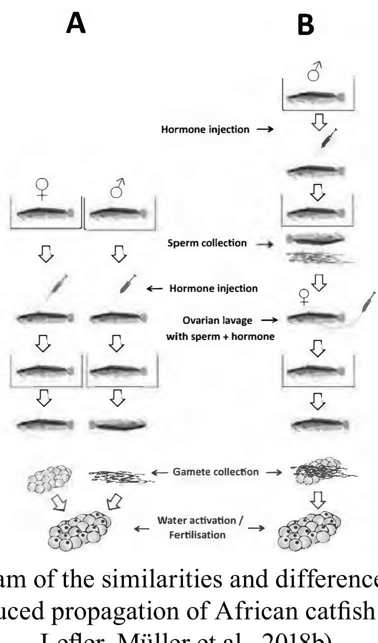

Figure 1. Schematic diagram of the similarities and differences of the traditional (A) and novel method (B) for induced propagation of African catfish (image by Kinga Katalin Lefler, Müller et al., 2018b).

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SURROGATE PRODUCTION OF COMMON CARP (*CYPRINUS CARPIO* L.) FROM SPERMATOGONIAL STEM CELLS

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SUROGAT PRODUKCIJA ŠARANA (*CYPRINUS CARPIO* L.) OD SPERMATOGONIJALNIH STEM ĆELIJA

Apstrakt

Cilj ovog istraživanja bio je razvoj optimalnog protokola za krioprezervaciju spermatogonija šarana – bipotentnih prekursora gameta i testiranje mogućnosti njhovog oporavka kroz interspecijsku transplantaciju. Protokol za zamrzavanje spermatogonija u vidu kriopreezrvaciije testikularnog tkiva je razvijen kroz niz sukcesivnih eksperimenata u kojima su varirani različiti faktori. Prvo je testirana primena šest krioprotektanata od kojih je dimetil sulfoksid (Me_2SO) rezultirao najvećim preživljavanjem spermatogonija. U sledeće eksperimentu, gde su testirane razliite molarne koncentracije dimetil sulfoksida (1 M – 3 M) kao i različite brzine zamrzavanja (0.5 – 10 ˚C/min), najbolje preživljavanje je zabeleženo primenom 2 M Me_2SO i stope zamrzavanja od -1 ˚C/min. Dodavanje četiri različite vrste šećera u kriomedijum (glukoza, fruktoza, trehaloza i saharoza) nije imalo značajan efekat na preživljavanje ćelija. U poslednjem eksperimentu je testiran uticaj različitih veličina fragmenata testikularnog tkiva i dužine perioda inkubacije tkiva u kriomedijumu. Zamrzavanje tkiva mase oko 100 mg inkubiranog 30 minuta je rezultiralo najvećom vijabilnošću ćelija. U toku sukcesivne optimizacije protokola, preživljavanje ćelija se sa 10 % nakon prvog eksperimenta podigla do 41 %. Kako bi se testirala fiziološka aktivnost ćelija nakon krioprezervacije izvršena je interspecijska transplantacija spermatogoniija šarana u larve zlatnih ribica. Embrioni zlatnih ribica (recipijenata) su pre transplantacije teretirani antisens *dead end* morfolino oligonukleotidima kako bi se izvršila njihova sterilizacija. Spermatogonije šarana su transplantirane u sterilne larve zlatne ribice

11 dana nakon oplodnje. Dva meseca nakon transplantacije, recipijenti su žrtvovani s ciljem provere proliferacije injekovanih ćelija i razvoja gonada. Kod oko 50 % proverenih recipijenata su bile jasno razvijene gonade koje su sadržale germinativne ćelije u različitim stadijumima razvoja. Činjenica da nijedna od sterilisanih i neinjektovanih zlatnih ribica nije imala razvijene gonade, ukazuje da su donorske ćelije inkorporirane u gonade recipijenta i uspešno započele proces proliferacije. Ovo zapažanje je potvrđeno i detektovanjem specifičnih PCR produkta karakterističnih ta šarana u okviru gonada zlatnih ribica. Rezultati ovog istraživanja mogu poslužiti kao alternativni način za dugotrajno čuvanje germinativnih ćelija šarana koje mogu biti upotrebljene u tehnologiji surogat proizvodnje.

Ključne reči: *donor, recipijent, zlatna ribica, transplantacija*

Abstract

The aim of the present study was to develop an optimal protocol for cryopreservation of common carp spermatogonia – bipotential precursors of gametes, and test the possibility to their recovery through interspecific germ cell transplantation. A protocol for slow rate freezing of spermatogonia in the form of testicular tissue cryopreservation was developed through varying different factors along a series of subsequent experiments. Firstly, six cryoprotectants were tested, and dimethyl sulfoxide (Me_2SO) yielded the highest survival rates of spermatogonia. In the following experiment, testing different molar concentrations (1 M – 3 M) of dimethylsulfoxide and different cooling rates (0.5 – 10 ˚C/min) resulted in the highest survival using 2 M dimethylsulfoxide and cooling rate -1 ˚C/min. Supplementation of four different sugars (glucose, fructose, trehalose and sucrose) did not have a significant effect on the cryosurvival. The last experiment tested the effect of different sizes of testicular tissue pieces and equilibration time in cryomedia and freezing 100 mg of testicular tissue equilibrated for 30 min resulted in the highest cell viability. The highest viability of spermatogonia was approximately 10% after the first trial, while subsequent optimization improved it up to 41%. To test the physiological activity of the cryopreserved cells, interspecific germ cell transplantation into goldfish recipients was conducted. In order to sterilize goldfish embryos, they were treated with antisense *dead end* Morpholino. Carp spermatogonia were transplanted into sterilized goldfish larvae 11th day post-fertilization. Two months after transplantation, recipients were dissected and checked for cell proliferation and gonadal development. Approximately 50% of the recipients displayed visibly large gonads which contained germ cells at various stages of development, while none of the sterilized controls displayed such gonads which indicated the donor cells incorporated into the recipient gonads and started to proliferate. We confirmed this observation by detecting PCR amplification of common carp-specific amplicon within the recipient goldfish gonads. Results of this study can serve as an alternative way for long-term preservation of germ plasm in carp which can be recovered through surrogate production technology.

Keywords: *donor, recipient, goldfish, transplantation*

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FATTY ACID COMPOSITIONS OF ZOOPLANKTON AND BENTHOS IN FRESHWATER EARTHEN PONDS AS A NATURAL DIET FOR COMMON CARP – COMPARISON WITH COMMERCIAL DIETS

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SASTAV MASNIH KISELINA ZOOPLANKTONA I BENTOSA U SLATKOVODNIM RIBNJACIMA KAO PRIRODNA HRANA ZA ŠARANA – POREĐENJE SA KOMERCIJALNOM HRANOM

Apstrakt

Masne kiseline su među najvažnijim molekulima koji se prenose između biljnih i životinjskih vrsta u lancima ishrane vodenih ekosistema, gde posebno mesto zauzimaju organizmi zooplanktona (*Cladocera* i *Copepoda*). Mnoge rane studije o morskom i slatkovodnom zooplanktonu bile su fokusirane na nutritivne potrebe ovih vodenih organizama, jer se riba hrani zooplanktonom, što je od posebnog značaja kada je reč o ribama gajenim u akvakulturi. S obzirom da je sastav masnih kiselina u mesu šarana zavisi od ishrane, cilj ovog rada je bilo ispitivanje sastava masnih kiselina slatkovodnih beskičmenjaka koje su prirodna hrana šarana i poređenje njihovog masnokiselinskog sastava sa sastavom masnih kiselina komercijalne hrane koje se koriste za ishranu šarana u poluintezivnom sistemu gajenja.

Ključne reči: *zooplankton, Chironomidae, masne kiseline, šaran, komercijalna hrana*
Keywords: *zooplankton, Chironomidae, fatty acids, carp, commercial feed*

INTRODUCTION

In most freshwater organisms, fish and zooplankton lipids are present as fatty acids in triglycerides or phospholipids (Henderson and Tocher, 1987; Olsen, 1999). Fatty acids were used as biomarkers to analyze interactions in various terrestrial and aquatic ecosystems. Fatty acids composition varies between organisms (for example, land-based vegetation, bacteria, cyanobacteria, algae), and consumers often metabolically modify some fatty acids for energy purposes, while others retain because they are structural components of cell membranes (Dalsgaard et al., 2003). Fatty acids are among the most important molecules transmitted between plant and animal species in the aquatic ecosystem chains, and in particular are occupied by herbivores and carnivorous organisms of the zooplankton among Cladocera and Copepoda.

Many early studies of marine and freshwater zooplankton were focused on the nutritional needs of these aquatic organisms because fish feeds with zooplankton, which is of particular importance when it comes to cultivated fish in aquaculture.

The content of fatty acids of zooplankton varies seasonally in relation to food representation and reproductive cycle (Vanderploeg et al., 1992; Arts et al., 1993; Csengeri and Halver, 2006). Zooplankton is usually collected during the production season (spring, summer and beginning of autumn) from different lakes and the content of fatty acids is monitored.

Ballantyne et al. (2003) and Kainz et al. (2004) found that all groups of zooplankton (in relation to body size) contain more essential fatty acids than seston, with the dominance of copepods. Meso-zooplankton (200-500 mm) had a tendency to accumulate DHA, while the Cladocera, which dominate the macro-zooplankton (> 500 mm), had a tendency to accumulate EPA. Also, the zooplankton collection from Lake Otago contained more EPA and less saturated fatty acids and polyunsaturated n-3 and n-6 fatty acids than expected (Burns et al., 2011). Recently, zooplankton organisms have shown that the amounts of essential fatty acids, i.e. ARA and EPA accumulate with increasing plankton size independent of the taxonomic composition of the entire zooplankton community (Kainz et al., 2004). EPA and DHA as well as ALA have been identified as food ingredients that increase somatic growth and reproduction of *Daphnia* in laboratory conditions (Müller-Navarra et al., 2000; Becker and Boersma, 2003; von Elert, 2004).

As the fatty acid profiles of fish lipids are influenced by diet composition (De Silva, 2012) our aim was to investigate the fatty acid composition of some fresh-water invertebrates that are the natural food of common carp to compare their compositions with those of available commercial diets used in carp production (Domaizon et al., 2000; Bell et al, 1994).

MATERIAL AND METHODS

Samples

Zooplankton for fatty acid analysis was sampled at the end of the rearing season in all investigated ponds from fish farm Despotovo by dragging 250 µm plankton net through the water during the movement of the boat. Samples were frozen for further analysis. Chironomidae larvae were sampled with Van Veen grab, grasping area of 260 cm^2. Samples were kept at the temperature of -18 ºC until analysis. Two categories of fish feed (extruded feed 25/7 and pelleted feed 25/7) with two different feed production technologies were analyzed, as well as wheat, barley and maize which are in common use in diet of carp.

Fatty acid analysis by capillary gas chromatography

Total lipids for fatty acids determination were extracted from zooplankton, Chironomidae larvae and feed samples after drying with diatomaceous earth by accelerated solvent extraction (ASE 200, Dionex, Sunnyvale, CA) with a mixture of n-hexane and iso-propanol (60:40 v/v). Fatty acid methyl esters (FAME) in the extracted lipids were prepared by transesterification using 0.25 M TMSH (trimethylsulfonium hydroxide) in methanol (EN ISO 5509:2000) and determined by capillary gas chromatography on GC Shimadzu 2010 (Kyoto, Japan) equipped with flame ionization detector and cyanopropyl HP-88 column. Chromatographic peaks were identified by comparing relative retention times of FAME peaks with peaks in the Supelco 37 Component FAME mix standard (Supelco, Bellefonte, USA).

RESULTS AND DISCUSSION

Data for fatty acid profile of freshwater natural diets and commercial feed preparations are presented in Table 1, 2. Major difference found between Chironomidae (C) and zooplankton (Z) and *Daphnia* (D) is due to a much higher diversity and content of n-3 FA in zooplankton. Moreover, three most important FAs: α-linolenic acid (ALA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are 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 EPA in Cladocera and DHA in Copepoda (*Kainz et al*., 2004). Additionally, ALA is a precursor of EPA, thus was also prominent in zooplankton samples. Chironomidae were characterized by lower content of ALA, EPA and DHA than zooplankton samples.

Table 1. Fatty acid profile (g100g-1) of freshwater natural diets

Fatty acid	Daphnia (D)	Zooplankton (Z)	Chironomidae (C)
C18:1n-9	13.84	11.30	12.26
C18:2n-6	3.20	6.66	3.47
C18:3n-3	3.43	6.30	0.20
C20:5n-3	0.75	3.99	0.15
C22:5n-3	nd	1.11	nd
C22:6n-3	0.70	2.20	nd
SFA	58.18	49.23	58.29
MUFA	31.63	27.66	37.92
PUFA	9.19	21.17	3.79
n-3	5.77	13.00	0.37
n-6	3.42	8.14	3.26
n-6/n-3	0.59	0.93	8.06

nd-not detected <0.05%

Table 2. Fatty acid profile (g100g-1) of commercial diets

Fatty acid	Company A Pelleted feed	Company B Extruded feed	Wheat, barley	Maize
C18:1n-9	25.52	24.77	15.71	27.62
C18:2n-6	54.96	54.24	58.03	56.15
C18:3n-3	3.74	5.94	3.37	0.98
C20:5n-3	0.04	0.30	nd	nd
C22:5n-3	nd	0.04	nd	nd
C22:6n-3	nd	0.22	nd	nd
SFA	15.47	16.89	19.19	13.84
MUFA	25.62	25.20	16.91	28.15
PUFA	58.91	57.91	61.77	58.00
n-3	3.78	7.53	3.66	0.98
n-6	55.13	50.38	58.11	57.03
n-6/n-3	14.59	6.69	15.91	58.19

nd-not detected <0.05%

The fatty acid compositions of feed (pelleted and extruded feed) prepared by two of the major fish feed manufacturers in Serbia are broadly similar. Both types of feed had similar content of SFA, MUFA and PUFA. Extruded feed had a higher content of n-3 PUFA and a lower content of n-6 PUFA and therefore, the ratio of n-6/n-3 in extruded feed (6.69) is better than in pelleted one (14.59) (Trbović et al., 2017; Živić et al., 2013). Wheat, barley and maize were characterized by high level of PUFA of n-6 series. Although, wheat and barley contained n-3 fatty acids, particularly ALA (3.37%), maize contained very small amount of this FA (0.98).

A comparison of the major PUFA components of freshwater planktonic crustacean, invertebrates and commercial diets is shown in Table 3. In all invertebrate groups the ratio of 18:2n-6/18:3n-3 is considerably lower than the value in the commercial diets reflecting the abundance of 18:3n-3 in most freshwater invertebrates. The relative abundance of 20:5n-3 over 22:6n-3 in invertebrates is reflected in the ratio of these two PUFAs (ranging from 0.15-1.81) being higher than the value in commercial diets, except extruded feed in which higher ratio probably originated from fish meal used in formulation of feed. The n-3/n-6 PUFA ratio in commercial diets is lower than in all freshwater invertebrates examined. The FA composition show that, compared to extruded and pelleted feed, zooplankton has a higher of 10 fold in n-3/n-6 ratio, while Chironomids have a 3 to 6 fold higher n-3/n-6 ratio.

Table 3. Comparison between invertebrate and commercial diet

Diet/fatty acid	C18:2n-6/C18:3n-3	C20:5n-3/C22:6n-3	n-3/n-6
D n. diet	0.93	1.07	1.69
Z n. diet	1.06	1.81	1.76
C n. diet	10.13	20.78	0.44
Pelleted feed	14.69	0.04	0.07
Extruded feed	9.13	1.36	0.15
Wheat, barley	17.22	0	0.06
Maize	57.34	0	0.02

D- *Daphnia* zooplankton; Z-zooplankton; C-Chironomidae; n. diet-natural diet

CONCLUSION

We believe that changing commercial feed preparations to more closely resemble the natural food might be desirable. The use of various plant oils such as rapeseed and linseed could allow a ratio of 18:2n-6/18:3n-3 more closely resembling the natural food to be achieved, while simultaneously reducing the content of long-chain n-3 PUFA. We believe that diets similar in fatty acid composition to the natural invertebrate food may be beneficial in producing common carp.

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AN ORGANIC GROWTH PROMOTER AND IMMUNOSTIMULANT "POMEGRANATE SEED OIL" FOR COMMON CARP (*CYPRINUS CARPIO*) WELFARE IN AQUACULTURE SYSTEMS

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ULJE IZ SEMENA NARA - ORGANSKI PROMOTER RASTA I IMUNOSTIMULANT ZA DOBROBIT ŠARANA (*CYPRINUS CARPIO*) U AKVAKULTURNIM SISTEMIMA

Apstrakt

Kao bogat izvor antioksidanasa i polinezasićene masne kiseline "punicinske kiseline", ulje iz semena nara (*Punica granatum*) (PSO) predstavlja nutritivno vredan proizvod koji ima široku primenu u medicini i kozmetičkoj industriji. Iz tog razloga, osnovni cilj ove studije je da se analizom vrednosti odabranih parametara krvi ispita uticaj ishrane sa uljem iz semena nara na prirast i status dobrobiti šarana (*Cyprinus carpio*). Za potrebe eksperimenta šaran iz ribnjaka prosečne telesne mase 5,53 ± 0,04 g je smešten u akvarijume zapremine 100 L (20 jedinki po akvarijumu). Tokom 60 dana riba je hranjena hranom u koju je dodato ulje iz semena nara u koncentracijama od 0 ‰ , ‰ 0,5‰ , 1 ‰, 2 ‰ i 4 ‰. Na kraju ispitivanja ustanovljeno je da su vrednosti za RGR i SGR bile značajno veće (P<0.05) u zavisnosti od upotrebljene koncentracije PSO u hrani. Koncentracija PSO u hrani nije uticala na DFI vrednost. U svim ispitivanim grupama šarana ustanovljen je značajan pad FCR vrednosti (P<0.05). Između vrednosti hematoloških parametara ispitivanih grupa šarana nisu ustanovljene značajne razlike (P>0.05) usled upotrebe PSO u ishrani, osim kod HGB vrednosti. Upotreba 0,5 ‰ PSO u hrani povećala je u značajnoj meri koncentraciju hemoglobina u odnosu na kontrolnu grupu šarana (P<0.05). Kod riba koje su hranjene sa PSO vrednosti za GLU, TRIG, CHOL, GOT, GPT i ALP su bile značajno manje (P<0,05), dok su vrednosti za TPROT, ALP i LDH bile značajno veće (P<0,05) u odnosu na kontrolnu grupu. Na osnovu dobijenih rezultata može se zaključiti da upotreba 2 ‰ PSO u ishrani šarana daje najbolje rezultate u pogledu dobrobiti, imunološkog odgovora i prirasta šarana.

Ključne reči: *šaran, biohemijski parametri krvi, prirast, hematologija, dobrobit*
Keywords: *Cyprinus carpio, blood biochemistry, growth performance, haematology, welfare*

INTRODUCTION

Pomegranate (*Punica granatum*) seed oil (PSO) is a product nutritious and rich, which is widely used in medicine and cosmetic because it is a rich source of antioxidants and contains a polyunsaturated oil called "punicic acid", an omega 5 fatty acid, which has strong anti-inflammatory properties (Boussetta et al. 2009). Therefore major aims of this study was to monitor the effects of diet with PSO on growth rate and welfare status of common carp (*Cyprinus carpio*) by assessing blood parameters.

MATERIALS AND METHODS

Healthy cultured carps (5.53 ± 0.04 g) were produced in the Mediterranean Fisheries Research, Production and Training Institute, Turkey. The experiment was performed three times with 300 fish allocated into 100 L aquarium (20 fish aquarium^{-1}). Pomegranate seed oil (PSO) was added to the feed at ‰ 0, ‰ 0.5, ‰ 1, ‰ 2 and ‰ 4. At the end of the feeding trial of 60 days, fish in each aquarium were individually weighed. Growth performance and feed utilization parameters were calculated according to Yigit et al. (2012). Blood samples (fifteen group^{-1}) were collected from caudal vein at the end of the experiment. Haematological parameters were measured with auto haematology analyser (Mindray BC3000) and commercial kits (Bioanalytic Diagnostic Industry, Co.) and spectrophotometer according to the manufacturer instructions were used to determine serum biochemical parameters. One-way ANOVA followed by Tukey tests was used for data analysis after checking the normality of data and homogeneity of variance. Mean values were considered significantly different at P<0.05. Data are expressed as mean values ± SD.

RESULTS AND DISCUSSION

Survival at the end of the experiment was 100% in all experimental groups. RGR and SGR were significantly affected by dietary with PSO (P<0.05). While DFI was not affected by PSO use (P>0.05), FCR significantly decreased in each group where PSO was used (P<0.05). Haematological parameters other than HGB did not show a significant difference due to the use of PSO (P>0.05). Using ‰ 0.5 of PSO increased the haemoglobin ratio substantially compared to control group (P<0.05). Data on the serum biochemical parameters of common carp are shown in Table 1.

Table 1. Serum biochemical parameters of common carps fed diets containing PSO

Parameters	Treatment*					P-Value
	‰ 0	**‰ 0.5**	**‰ 1**	**‰ 2**	**‰ 4**	
GLU (mg/dL)	104.03±14.47^{a}	96.98±21.60^{ab}	83.81±10.73^{b}	82.42±6.30^{b}	91.37±13.39^{ab}	0.013
TPROT (g/dL)	7.72±0.78^{a}	8.78±0.91^{a}	8.17±0.99^{ab}	7.87±0.55^{ab}	7.69±0.74^{b}	0.023
ALB (g/dL)	0.10±0.03^{b}	0.11±0.01^{ab}	0.12±0.02^{ab}	0.12±0.01^{ab}	0.13±0.01^{a}	0.014
TRIG (mg/dL)	38.39±8.47^{a}	27.21±5.50^{b}	27.52±8.59^{b}	26.38±8.05^{b}	25.18±5.39^{b}	0.003
CHOL (mg/dL)	241.00±48.90^{a}	222.69±26.12^{ab}	220.40±31.90^{ab}	181.90±34.60^{b}	191.30±36.40^{b}	0.008
GOT (U/L)	62.34±13.29^{ab}	61.37±7.97^{ab}	55.36±7.31^{b}	70.39±18.28^{ab}	75.11±15.36^{a}	0.023
GPT (U/L)	7.49±0.90^{a}	5.40±0.93^{b}	5.26±0.80^{b}	6.49±1.15^{ab}	7.45±1.65^{a}	<0.001
ALP (U/L)	50.42±6.40^{a}	37.78±10.48^{b}	30.80±7.73^{bc}	25.01±8.43^{c}	29.47±8.99^{bc}	<0.001
LDH (U/L)	530.80±90.20^{b}	532.50±86.60^{b}	553.70±74.00^{b}	674.40±97.00^{a}	713.70±64.90^{a}	0.004

*Values represent, mean ± standard deviation of fifteen fish per treatment. Data in the same row with different superscript are significantly different (P < 0.05). .

Studies conducted by Immanuel et al. (2009) showed that immunostimulants in feed had positive effects on growth performance. Many components in plant products were enhanced growth performance in fish, especially by regulating intestinal microflora. (MacLennan et al. 2002). Changes in hematologic parameters in fish are important to have information about general health status. In addition, the changes in the RBC and HGB parameters can give information on the oxygen delivery capacity (Wells et al., 2005). Our study showed that also HGB level increased in fish treated with PSO at ‰ 0.5 levels. HGB is one of the most well studied proteins to date and together with RBC are key for blood oxygen (O_2) transport in nearly all vertebrates and some invertebrates, as it increases the total O_2 that can be transported in the blood and optimizes tissue O_2 delivery (Rummer and Brauner2015). Serum glucose level was one of the stress symptoms in fish (Morgan and Iwama, 1997). Results showed that use of PSO reduced the glucose level. The increase in the levels of serum protein, albumin and globulins in fish is thought to be associated with a stronger immunity response (Wiegertjes et al. 1996). In our experiment serum protein level was positively affected by PSO. The reduction in serum liver enzyme (GOT, GPT, ALT and LDH) values were among the markers used to monitor liver function (Giannini et al., 2005). liver enzymes were decreased with the amount of PSO in the feed as another study of pomegranate peel extract was applied in tilapia (Ibrahim., 2010).

CONCLUSIONS

Based on our findings, it was concluded that , the use of ‰ 2 PSO in carp fish feeds increase the welfare levels, immune response and growth performance of common carp.

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THE IMPACT OF TOPMOUTH GUDGEON (*PSEUDORASBORA PARVA*, SCHLEGEL 1842) ON THE AQUATIC ENVIRONMENT IN INVADED FISHPONDS

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UTICAJ BEZRIBICE (*PSEUDORASBORA PARVA*, SCHLEGEL 1842) NA VODENU SREDINU INVADIRANIH RIBNJAKA

Apstrakt

Bezribica (*Pseudorasbora parva*) je mala, neautohtona ciprinidaa koja se smatra nepoželjnom vrstom u šaranskim ribnjacima. Ishrana bezribice se preklapa sa ishranom nepredatornih komercijalno gajenih vrsta riba. Njena velika brojnost i način ishrane može dovesti do značajnih promena u životnom okruženju ribnjaka. U ovom radu smo procenjivali uticaj bezribice na kvalitea vode poređenjem šaranskih ribnjaka sa velikim (14 331 ind/ha^{-1}, 8.12 kg/ha^{-1}) i neznatnim (370 ind/ha^{-1}, 2.28 kg/ha^{-1}) prisustvom ove invazivne vrste. Ozbiljno invadiran ribnjak je imao statistični značajno ($p < 0.05$) povećanje BOD_5, N-NO_2, N_{org}, P-PO_4, P_{tot},, brojnosti bakterija i bezbojnih flagelata, dok je neznatno invadiran ribnjak imao značajno veću ($p < 0.05$) gustinu zooplanktona (rotatorija, kopepoda, kladocera i ostrakoda). Crevni sadržaj bezribice u izvodnim kanalima ribnjaka se sastojao uglavnom od detritusa, perifitona (Sphaerotilus, Oscillatoria, Scenedesmus), kladocera (Daphnia, Bosmina) i bentičnih/epifitnih larvi hironomida. Fragmenti makrofita, kopepoda i rotatorija (Brachionus) su takođe konzumirani ali u mnogo manjim količinama.

Ključne reči: *invazivne vrste riba, akvakultura, gajenje šarana, kvalitet vode, kompeticija za hranu*

INTRODUCTION

Topmouth gudgeon (Pseudorasbora parva Temminck & Schlegel, 1846) is a recent example of a successful aquatic invasive species now found in many smaller (< 10 ha) European freshwater lentic ecosystems. Among numerous recent introductions of alien taxa, this species appears to be one of the few that has found suitable conditions for successful establishment (Gozlan et al. 2002). Recently, the topmouth gudgeon was listed under EU Regulation 2016/1141 (EC 2016) as an invasive alien species of Union concern pursuant to Regulation (EU) No 1143/2014, meaning that this species is treated as an invasive alien species of special concern. Information on its introduction, later spread and recent distribution across Europe has been summarised by Gozlan et al. (2010).

In Central European carp ponds, invasion by topmouth gudgeon represents a serious problem for pond management, especially as regards nursery (one-year-old carp) and on-growing (one- to two-year-old carp) ponds. The aims of this study were to evaluate the feeding habits of topmouth gudgeon with respect to natural food resources in heavily and lightly invaded carp ponds and to examine their determinants of environment quality.

MATERIAL AND METHODS

The study was performed at two carp ponds in southern Moravia (joint catchment area; Czech Republic) during the growing season (May to November). The Štítarský horní pond (SH), with the surface area of 6.28 ha was heavily invaded by topmouth gudgeon. Contrarily, the nearby Vracovický pond (VRA, 3.51 ha) was invaded only poorly due to the rare occurrence of bottom depressions enabling topmouth gudgeon survival over the critical period of pond drainage. Both ponds are managed extensively, neither receiving intensive inputs of feed or fertilisers. In addition to common carp (dominant), some other pond fish species were stocked at lower levels. No submerged macrophytes occurred at SH while the beds of *Ceratophyllum demersum* covered 30 % of VRA pond surface at the end of growing season in September.

Physico-hydrochemical and hydrobiological samples were collected using routine sampling protocols bimonthly. Topmouth gudgeon for food analysis were collected using portable battery-powered electrofishing gear downstream of the SH pond dam and their diet analysis was performed according to Hyslop (1980).

At harvesting in November, qualitative (species) and quantitative (numbers and biomass) composition of the pond fish stock was recorded. Statistical evaluation of the results was performed using the non-parametric Wilcoxon test at $p < 0.05$.

RESULTS

Fish stock

Total production (weight gain) from both ponds amounted to 263 $kg.ha^{-1}$ in SH and 258 $kg.ha^{-1}$ in VRA, with 255 and 256 $kg.ha^{-1}$ commercial fish production, respectively. While the density of topmouth gudgeon was 39-times higher at SH, their biomass was only four-times higher, reflecting the ca. three-times higher average TL of topmouth gudgeon at VRA.

Environmental determinants

No significant differences ($p > 0.05$) were recorded in temperature, transparency, dissolved oxygen concentration, chemical oxygen demand and pH values at the two ponds, nor was there any difference in the principal nutrient concentrations ($N\text{-}NH_4$, $N\text{-}NO_3$, P_{org}). On the other hand, significant differences ($p < 0.05$) were observed in BOD_5, $N\text{-}NO_2$, N_{org}, $P\text{-}PO_4$ and P_{tot} concentrations, with higher values at SH.

Bioseston

While total taxa numbers were higher at SH (104 vs. 77 taxa), mean bioseston species diversity was identical at SH and VRA with an average of 32 taxa each. On the other hand, the density of producers, consumers and destruents was significantly higher at SH (p < 0.05).

Zooplankton

Aside from the diversity indices, all zooplankton assemblage values were significantly higher (p < 0.05) in the VRA pond. Mean total zooplankton densities were 193 ind/L^{-1} in SH and 2857 ind/L^{-1} in VRA. Significant differences (p < 0.05) were also recorded in species richness and saprobity index.

Zoobenthos

The average density of zoobenthos in SH was 337 ind/m^{-2} and average biomass 0.82 g/m^{-2}, whilst the respective values in VRA were 658 ind/m^{-2} and 2.73 g/m^{-2}. *Chironomus plumosus* dominated with over 87% in both ponds. Mean zoobenthos taxa richness was 1.3 in SH and 2.3 in VHA, with low indices of diversity in both ponds. No significant differences (p > 0.05) were recorded in quantitative and qualitative zoobenthos determinants between the two ponds. Saprobity index values were significantly higher (p < 0.05) in SH .

Food composition

Of the 89 topmouth gudgeon examined for food preference, 80 had ingested one or more of the 19 dietary items/classes identified, the majority of which (16) were represented by animal prey (i.e. zooplankton, zoobenthos, epiphytic invertebrates and terrestrial insects), the three remaining dietary classes comprising macrophytes or organic detritus. Indices of gut fullness were highest in summer, with maximum values of over 100 $^{o}/_{ooo}$. There was a clear decline in gut fullness in autumn, with 70 % of guts being empty in November.

Detritus was the most important dietary item identified, ranging from 7.5 - 100 % by proportion in gut content, or 43 - 100 % FO. Periphyton, found in the majority of guts, made up 34.2 % of ingested food. Heterotrophic and autotrophic organisms were the most important components of periphyton in topmouth gudgeon diet. Zooplankton contributed 8.2 % (64 % FO) in total to the food bulk. Copepods were the most important planktonic food, occurring in almost half (49 %) of all fish examined (4.2 % by proportion). Cladocerans (4.0 % of food bulk) were represented mainly by *Daphnia*, *Bosmina* and *Chydorus*. Similarly, rotifers made up 0.7 % by proportion of food items ingested. Zoobenthos was found in 22 % of fish, with highest contributions from chironomid larvae (3.0 % proportion, 15 % FO) and oligochaetes (1.8 % proportion, 7% FO).

DISCUSSION

Topmouth gudgeon is an invasive fish species capable of efficient and rapid colonisation of new, suitable habitats. Stillwater bodies, such as small reservoirs and fishponds, are particularly threatened through its overpopulation (Adámek & Siddiqui 1997, Musil et al. 2014). Establishment of new populations in such waterbodies is associated with severe impacts on environmental quality resulting from heavy grazing pressure on the majority of aquatic ecosystem components. In particular, significant negative effects have been recorded on zooplankton assemblages (Chang 2004), with daphnids especially hard hit

(Musil et al. 2014). Furthermore, numerous studies have recorded heavy grazing pressure on epiphytic chironomids (Wolfram-Wais et al., 1999) and water snails (Adámek 2002), as well as serious damage on larger commercial fishes due to nibbling of skin and muscles, often leading to open wounds (Libosvárský et al. 1990, Gozlan et al. 2010).

Fish stock

Total production (fish weight gain) in the pond with a heavy infestation of topmouth gudgeon (SH) reached 263 $kg.ha^{-1}$, compared with 258 $kg.ha^{-1}$ in the less heavily invaded pond (VRA). These figures correspond with the lower limits of natural pond production in carp ponds at altitudes around 400 m a.s.l. (Hartman & Regenda 2014). In our study, therefore, we did not record the heavy negative impacts on carp production from dense populations of topmouth gudgeon recorded in previous studies (Musil et al. 2014); or implied from studies reporting beneficial effects on growth, recruitment and production of roach (*Rutilus rutilus*) and bream (*Abramis brama*) from eradication of topmouth gudgeon (Britton et al. 2009). Note, however, that in the two former studies, however, the carp in question were provided with supplementary feed; whereas those in our SH pond relied on natural food resources only (i.e. extensive management).

The average TL of topmouth gudgeon in VRA was almost three-times bigger than that in the heavily invaded pond (SH), suggesting that the higher carp density in VRA (5.7 times higher), along with a higher density of grass carp [*Ctenopharyngodon idella*] 1.65 $ind.m^{-2}$ vs. 0.3 $ind.m^{-2}$ at SH, was able to provide sufficient predation pressure on topmouth gudgeon recruitment to prevent overpopulation. This hypothesis is supported by the finding of primarily adult topmouth gudgeon (84 ± 4 mm TL) in VRA (SH mostly 0+ fish; 31 ± 12 mm TL), and at much lower densities (VRA: 1 ind per 27 m^2 vs. SH: 1 ind per 0.7 m^2). Moreover, the SH fish were too small to be effectively predated on by the two-year-old pike (*Esox lucius*) and perch (*Perca fluviatilis*) stocked. According to Adámek & Opačak (2005), topmouth gudgeon are positively selected by pike and perch. Furthermore, effective consumption of topmouth gudgeon by pike, perch and zander (*Sander lucioperca*) has also been documented in the wild (Lemmens et al. 2015). Our data would suggest that densities of 80 $ind.ha^{-1}$ two-year-old pike and 16 $ind.ha^{-1}$ perch in SH were insufficient for effective reduction of topmouth gudgeon density. While Lemmens et al. (2015) showed that stocking 0+ pike (4.2 g) at a density of 150 $ind.ha^{-1}$ was effective in suppressing topmouth gudgeon invasion, 0+ topmouth gudgeon (average body weight 0.56 g) were clearly too small prey fish for two-year-old pike with an average body weight of 250 g.

Environmental determinants

Nutrient concentrations (with the exception of $N\text{-}NO_3$) were always higher in the pond with abundant topmouth gudgeon population (SH). High fish recruitment was associated with significantly increased organic loading (expressed as BOD_5 and COD_{Mn}; $p > 0.05$). Increased $N\text{-}NH_4$, $N\text{-}NO_2$ and N_{org} values indicated that the initial phases of nitrification were reduced in SH, while the concentrations of all forms of phosphorus (i.e. $P\text{-}PO_4$, P_{org} and P_{tot}) were also higher in SH. Contrary to our results, Musil et al. (2014) registered significantly higher $N\text{-}NH_4$, N_{tot} and P_{tot} concentrations and increased transparency (Secchi depth) in the absence of topmouth gudgeon.

Bioseston

We were unable to find any previous literature on the impact of topmouth gudgeon on phytoplankton or bioseston in invaded waterbodies. Hence, our data probably represent the first such data available. Our results indicate higher diversity and density of producers, consumers and destruents in the presence of an abundant topmouth gudgeon population. In particular, the density of destruents was almost 100x higher, which would be expected in light of the increased environmental loading with organic matter, $N\text{-}NH_4$ and $N\text{-}NO_2$.

Zooplankton

The density of all zooplankton groups was several times higher in VRA, the pond with lower topmouth gudgeon density. Zooplankton, and particularly planktonic crustaceans, are subjected to heavy grazing pressure by topmouth gudgeon (Musil et al. 2014) and the eradication of large-sized cladocerans (*Daphnia* ssp.) often leads to a preponderance of small- to medium-sized cladocerans and calanoid copepods (Hanazato & Yasuno 1989). We did not observe this, however, as the densities of all zooplankton groups (even small rotifers and copepods) were always significantly ($p > 0.05$) lower in the strong topmouth gudgeon population. Musil et al. (2014) came to similar conclusions as regards South Bohemian carp ponds (N 49°5.97’, E 14°44.88’), where they recorded an increase in the density of almost all planktonic groups following the eradication of topmouth gudgeon. The only exceptions were nauplii of copepods and rotifers, whose numbers were higher in the presence of high topmouth gudgeon numbers. Nagata et al. (2005) also reported that copepod nauplii abundance was not depressed by topmouth gudgeon predation.

The intensity of topmouth gudgeon grazing pressure on cladocerans in stillwater littoral waterbodies (Kotovskaya & Khristenko 2013) depends considerably on the density of littoral reed and submerged macrophytes. As shown experimentally by Priyadarshana et al. (2001), an increasing density of simulated submerged vegetation resulted in a decrease in topmouth gudgeon swimming speed and number of captured prey (*Daphnia pulex*). Since the SH littoral zone was free of macrophyte beds (unlike that of VRA), crustaceoplankton in SH were more vulnerable to topmouth gudgeon predation.

Zooplankton saprobity index values indicated betamesosaprobity in both ponds, though the mean value for VRA was significantly lower than that for SH, indicating lowered organic loading and more favourable environmental conditions.

Zoobenthos

Despite the common opinion that topmouth gudgeon graze on epiphytic invertebrates (Wolfram-Wais et al. 1999) rather than zoobenthos from the pond bottom (Musil et al. 2014), average zoobenthos density and biomass were lower in SH. In the majority of ponds monitored by Musil et al. (2014), macrozoobenthos density tended to decline after topmouth gudgeon eradication. The larvae of *Chironomus plumosus,* which tended to dominate the zoobenthos assemblage on the carp pond bottom, were probably too big to be effectively ingested by small topmouth gudgeon, and hence were less utilised.

Macrozoobenthos saprobiological evaluation revealed significant differences ($p < 0.05$) in water quality, again indicating poorer environmental conditions in the pond with a high topmouth gudgeon population and recruitment (SH).

Food composition

Though preferentially planktonophagous (Hliwa et al. 2002), topmouth gudgeon is omnivorous, consuming zooplankton (Musil et al. 2014) and epiphytic invertebrates (Wolfram-Wais et al. 1999), but also taking zoobenthos (Wolfram-Wais et al. 1999, Declerck et al. 2002) and other less valuable, low-energy food items (Adámek & Siddiqui 1997).

According to Declerck et al. (2002), the diet composition of topmouth gudgeon differs significantly with respect to size and age classes. In the 20-25 mm SL size class, the diet consisted exclusively of cladoceran zooplankton (*Bosmina*, Chydoridae), whilst the gut content of 35-60 mm SL fish comprised > 90 % chironomid larvae, the intermediate group (25-35 mm SL) being characterised by a mixed diet of cladoceran and chironomid larvae. The size of chironomid larvae ingested rose with fish size, though even the largest topmouth gudgeon avoided chironomid larvae with a dry body mass > 0.5 mg.

The majority of food items ingested in fish downstream of the pond was of animal origin (zooplankton, zoobenthos, epiphytic invertebrates and terrestrial insects), though detritus and periphyton prevailed quantitatively, often representing the entire food bulk. In total, detritus constituted more than half of all food ingested (52 %, 88 % FO), with periphyton contributing 34.2 % (59 % FO).

The high abundance of low-energy food items (e.g. detritus, macrophyte debris) is often mentioned but not fully quantified in studies devoted to topmouth gudgeon feeding biology. As mentioned by Declerck et al. (2002), however, a high proportion of low-energy food in fish diet may be indicative of a competition-induced niche shift to suboptimal food sources.

Periphyton, composed mostly of heterotrophic bacteria and fungi and autotrophic organisms (cyanobacteria, diatoms and chlorophytes), appeared in approximately half (59 %) of the gut contents examined. Periphyton in general appears to be inadequately considered in the diet of topmouth gudgeon, often being considered as part of detritus or organic debris. Nevertheless, its nutritional value is quite high and it could conceivably represent a very important source of nutrition for topmouth gudgeon. Periphyton have a protein/metabolisable energy ratio of between 10 and 40 $kJ.g^{-1}$, providing a feed conversion ratio for of between 2 and 3 (van Dam et al 2002). Ble et al. (2007) also recorded favourable nutrition values for periphyton, recording a protein content of 19 % and a proteins/energy ratio of 17 $kJ.mg^{-1}$, values 2 and 3 times above those measured for suspended solids and sediment, respectively, in extensive aquaculture reservoirs stocked with tilapia. Topmouth gudgeon are a small fish with a relatively wide mouth, strengthened mucous membrane and ossified jaws (Libosvárský et al. 1990), adaptations that could prove effective for nibbling periphyton. The ability to efficiently utilise periphyton as a source of nutrition in habitats with critically reduced zooplankton and zoobenthos assemblages could prove important, enabling feeding in waterbodies overexploited by already dense populations.

Gut fullness indices tended to decline with dropping water temperatures, from values exceeding 100 $^{o}/_{ooo}$ in some fish in summer to just 6 to 19 $^{o}/_{ooo}$ in autumn. By early November, 70 % of guts were empty pointing to the dependence of topmouth gudgeon feeding activity on water temperature.

Zooplankton were recorded in the diet at 64 % FO, though its total contribution to the food bulk was much lower at 8.2 %. Its proportion in the diet was undoubtedly influenced by its low abundance in the ponds. Cladocerans were the most affected class, the density of which dropped to one ind. per 10L in SH, a situation similar to that observed in previous studies (Chang et al. 2004), Overall, the contribution of zoobenthos to topmouth gudgeon nutrition was lower than that from zooplankton, and the impact of the topmouth gudgeon population overall less dramatic. Unlike zooplankton, the density of which was reduced 15times, zoobenthos abundance was reduced only twice, and biomass 3times, in the presence of numerous topmouth gudgeon (i.e. in the SH pond).

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EFFECT OF MODIFIED ATMOSPHERE PACKAGING ON SELECTED QUALITY ATTRIBUTES OF CHILLED COMMON CARP (*CYPRINUS CARPIO*) STEAKS

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UTICAJ PAKOVANJA U MODIFIKOVANOJ ATMOSFERI NA ODABRANE PARAMETRE KVALITETA ODREZAKA ŠARANA (*CYPRINUS CARPIO*)

Apstrakt

Činjenica da sveža riba predstavlja veoma kvarljivu namirnicu (pH > 6,0; a_w> 0,98) uticala je na to da fokus proizvođača bude usmeren ka iznalaženju optimalne metode konzerviranja ribe. Poslednjih godina, u svetu, potrošači sve izraženije zahtevaju da u svakom trenutku u ponudi imaju svežu ribu, s obzirom da, kao takva, ima najprihvatljivije senzorne karakteristike. Ovaj trend je uslovio razvoj efikasnog koncepta pakovanja u modifikovanu atmosferu (MAP), koji ribi obezbeđuje duži rok održivosti i očuvanje osnovnih parametara svežine.

Cilj ovog eksperimenta je bio da se ispita rast aerobnih mezofilnih bakterija, sulfitoredukujućih anaerobnih bakterija, enterobakterija i bakterija mlečne kiseline kao i da se konstatuju promene senzornih svojstava odrezaka šarana upakovanih u modifikovanu atmosferu. Uzorci su podeljeni u tri grupe. Prve dve grupe su upakovane u modifikovanu atmosferu sa različitim odnosom gasova: 60%CO_2 + 40%N_2 (MAP 1) i 40%CO_2 + 60%N_2 (MAP 2) dok je kontrolna grupa, upakovana u vakuum. Odnos gas/uzorak u pakovanju bio je 2:1. Svi uzorci su skladišteni pri istovetnim uslovima na temperaturi od +3±0.5°C, a navedena ispitivanja su rađena 0, 4, 7, 9, 12. i 15. dana eksperimenta.

Najmanji broj aerobnih mezofilnih bakterija i enterobakterija ustanovljen je u odrescima šarana koji su pakovani u modifikovanu atmosferu sa 60%CO_2 i 40%N_2, dok je u uzorcima koji su upakovani u vakuum njihov broj bio najveći. U uzorcima odrezaka šarana upakovanim u vakuum i u smešu gasova sa većim udelom ugljen-dioksida rast bakterija mlečne kiseline bio je najveći. Rast sulfitoredukujućih anaerobnih bakterija bio je najveći u odrescima šarana upakovanim u vakuum, dok je u grupi uzoraka koji su upakovani u modifikovanu atmosferu sa većim udelom ugljen-dioksida zabeležen najslabiji rast ovih bakterija.

Na osnovu prosečnih senzornih ocena ukupne prihvatljivosti održivost odrezaka šarana u smeši gasova koja se sastojala od 60%CO_2 i 40%N_2 (MAP 1) bila je 12 dana, dok su uzorci pakovani u smešu gasova sa 40%CO_2 i 60%N_2 (MAP 2) bili održivi 9 dana. Odresci šarana upakovani u vakuum bili su održivi 7 dana.

Ključne reči: *mikroorganizmi, senzorska analiza, održivost, odresci šarana, modifikovana atmosfera*
Keywords: *microorganisms, sensory evaluation, shelf-life, carp steaks, modified atmosphere packaging*

INTRODUCTION

Most of the wild fish and fish from aquaculture consumed in Serbia is marketed for human consumption as fresh or frozen. The fact that fresh fish is a very perishable food (pH> 6.0; a_w> 0.98) has influenced the producers to focus on finding the optimal method for fish preservation. Modern consumers demand high quality food that retains sensory characteristics and nutritive value of raw material from which it is produced; also, it is expected to satisfy very demanding safety standards. This requirement is largely met by packaging the products in modified atmosphere, thus ensuring a longer shelf life of fish and preserving the basic parameters of its freshness (Babić et al., 2015). In the past decade, the attention of researchers engaged in solving the problems of fish packaging in modified atmosphere has been mostly focused to gas mixtures with high concentrations of CO_2 and N_2. While N_2 is an inert gas whose task is to prevent packaging collapse, CO_2 may inhibit the growth of several types of microorganisms, especially those that cause deterioration and unpleasant smells in foods stored at the refrigerator temperature. Modified atmosphere packaging (MAP) extends shelf-life of most fishery products by inhibiting bacterial growth and oxidative reactions (Siverstvik et al., 2002).

Cyprinid species (common carp, bighead carp, grass carp) are most common breed in Serbia. The aim of this research was to monitor changes of selected microbiological and sensory parameters of common carp (*Cyprinus carpio*) steaks packaged in modified atmosphere during the storage at +3±0.5°C, and to determine the shelf life of the products.

MATERIAL AND METHODS

Marketable carp (*Cyprinus carpio*) originated from the fishpond located in the low land region of Serbia, where semi intensive farming was used. In this experiment, two year old carps of average body weight of 2,5 kg were used. Carps were transported live to the fish slaughtering and processing facility, where they were stunned, slaughtered, scale cleared, and carcass was cut in steaks 2 cm thick. Three sample groups of carp steaks were formed. First two groups were packaged in modified atmosphere with different gas ratios: MAP 1: 60%CO_2+40%N_2 and MAP 2: 40%CO_2+60%N_2 whereas the third group of samples were vacuum packaged and used as control. The machine used for packaging of samples was Variovac (Variovac Primus, Zarrentin, Germany), and material used for packaging was foil OPA/EVOH/PE (oriented polyamide/etilene vinyl alcohol/polyetilene, Dynopack, Polimoon, Kristiansand, Norway) with low gas permeability (degree of permeability for O_2 – 3,2 cm^3/m^2/day at 23˚C, for N_2 - 1 cm^3/m^2/day at 23˚C, for CO_2 – 14 cm^3/m^2/day at 23˚C and for steam 15 g/m^2/day at 38˚C). Ratio gas : sample in the package was 2:1. All samples were stored in the same conditions at the temperature of +3±0.5°C and on 0, 4, 7, 9, 12. and 15. day of storage, microbiological and sensory testing was performed.

Microbiological testing

Number of aerobic mesophilic bacteria (TVC), number of anaerobic sulfite-reducing bacteria (SRB), number of bacteria of the family *Enterobacteriaceae* (ENT) and number of lactic acid bacteria (LAB) was determined according to EN ISO 4833-1:2013, SRPS ISO 15213:2011, SRPS ISO 21528-2:2009 and EN ISO 15214:1998, respectively. Microbiological data were expressed as logarithms of the number of colony-forming units *log* cfu/g.

Sensory evaluation

The sensory evaluation was performed by six trained panellists. The samples were evaluated for overall acceptability, with regard to odour, flesh colour and texture using 1-7 intensity scale, with 7 corresponding to the most liked sample and 1 corresponding to least liked sample. The product was defined as unacceptable with score less than 3 points recorded by at least of 50% of the judges.

RESULTS AND DISCUSSION

During the storage of packaged carp steaks at the temperature of +3±0.5°C, there was observed a significant increase of all examined microorganisms. In carp steaks packaged in modified atmosphere with 60% CO_2 and 40% N_2 (MAP 1), growth of aerobic mesophilic bacteria and *Enterobacteriaceae* was slower than in carp steaks packaged in the modified atmosphere with 40%CO_2 and 60%N_2 (MAP 2) or vacuum packaged samples (Fig 1). This can be explained by antimicrobial effect of carbon-dioxide which was most present in percentages in gas mixture used for samples packaged in MAP 1, as well as the fact that carbon- dioxide has inhibitory effect primarily on Gram-negative bacteria, such as the microorganisms of the family *Enterobacteriaceae* and aerobic mesophilic bacteria. Results obtained by Milijašević et al. (2010) show antimicrobial effect of CO_2 , where significantly lower TVC count and enterobacteria count was determined in carp cuts packaged in atmosphere with 100%CO_2 compared to cuts packaged in the gas mixture of 40%CO_2 and 60%N_2. At the end of experiment, TVC in both groups of samples packaged in MAP as well as in vacuum packaged samples had reached value of 7 *log* cfu/g, which is referred to sensory changes of products.

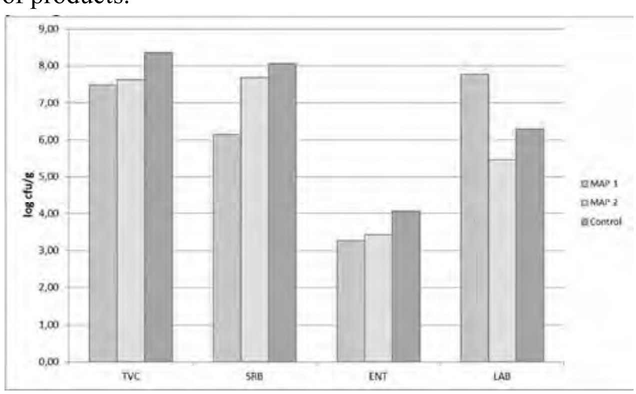

Figure 1. Microbiological status of carp steaks at the end of shelf-life period

In our experiment, at the end of storage period LAB was dominant microflora in samples packaged in MAP 1. These results show the ability of LAB to grow in modified atmosphere with a higher carbon-dioxide content. The Gram-positive bacteria, such as lactic acid bacteria, are not sensitive to the effect of CO_2, hence these become a dominant flora in fish and fish products packaged in gas mixtures (Stamatis et al., 2007). It is considered that these micro-organisms in MAP and vacuum-packed food contribute to prolonged shelf-life by inhibiting other bacteria growth through creating lactic acid and bacteriocine (Gram et al., 1996). This characteristic of LAB can be a reason for lower TVC values and better shelf-life of carp samples packaged in MAP 1 in our experiment.

The highest growth rate of sulfite-reducing bacteria was established in samples packaged in MAP with lower percentage of CO_2 and vacuum packaged samples. The dominance of these bacteria has been reported in salmon fillets packaged in MAP (60% CO_2 i 40% N_2) and vacuum (Hansen et al., 2009) which is in accordance with our results. The growth of these micro-organisms is explained by their ability to use TMAO as the ultimate acceptor of electrons in anaerobic respiration (Gram et al., 1996).

A decrease of scores of the sensory attributes of all examined groups of carp steaks was observed throughout the storage period. Carp steaks packaged in MAP 1 were estimated as unacceptable from the sensory point of view on day 15, samples packaged in MAP 2 on day 12 and vacuum packaged samples on day 9 of experiment.

CONCLUSION

Packaging common carp under MAP 1 and MAP 2 slowed the growth of aerobic mesophilic bacteria as well as enterobacteria. According to those micro-organisms, packaging common carp in MAP 1 and MAP 2 is more suitable compared to vacuum packaging.

Based primarly on odor scores, it was concluded that common carp samples packaged in modified atmosphere with 60% CO_2 + 40% N_2 remained acceptable for up to 12 days of storage period, whereas samples packaged under 40% CO_2+60% N_2 as well as vacuum packaged samples remained unchanged until 9 days ie. 7 days of the experiment.

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FRESHWATER ECOSYSTEMS UNDER ARTIFICIAL LIGHTING: DOES LIGHT POLLUTION MATTER?

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EFEKTI VEŠTAČKOG OSVETLJENJA NA SLATKOVODNE EKOSISTEME: DA LI SVETLOSNO ZAGAĐENJE IMA UTICAJA?

Apstrakt

Sa rastućom urbanizacijom i širenjem električnog osvetljenja, upotreba veštačkog osvetljenja (ALAN) se dramatično povećala tokom poslednjeg veka, menjajući noćna okruženja širom sveta. Sa prosečnom godišnjom stopom rasta od 2-6% na globalnom nivou, ALAN je prepoznat kao jedna od antropogenih promena životne sredine koje se najbrže šire. Upotreba veštačkog osvetljenja može rezultovati svetlosnim zagađenjem – povećanjem nivoa noćnog osvetljenja preko prirodnih vrednosti i poremećajem prirodnih ciklusa svetlosti i tame. Danas, kada je skoro 25% svetskog kopna van polarnih oblasti izloženo svetlosnom zagađenju, ALAN je prepoznat kao faktor koji doprinosi globalnoj promeni životne sredine i opasnost po biodiverzitet. Istovremeno, širom sveta dolazi do zamena tradicionalnih tehnologija (uskog spektra emisije) u uličnom osvetljenju LED tehnologijom (širokog spektra), menjajući tako spektralnu kompoziciju noći. Predviđa se da će ova promena povećati ekološke posledice svetlosnog zagađenja.

Sve je veći broj dokaza koji ukazuju da ALAN utiče na prirodno okruženje, uključujući mikroorganizme, biljke, životinje i ljude. Ipak, naše razumevanje uticaja na slatkovodne ekosisteme je i dalje ograničeno, uprkos činjenici da su ovi ekosistemi često izloženi svetlosnom zagađenju od okolnih naselja. U ovoj prezentaciji, prikazaću dokaze o uticajima ALAN-a na različite nivoe slatkovodnih ekosistema, uključujući mikroorganizme, makrofaunu beskičmenjaka i ribe. Prezentovaću rezultate mog istraživanja koje se fokusira na primarne producente slatkovodnih ekosistema, koristeći zajednice perifitona kao model organizme. Perifiton je kompleksna zajednica algi, bakterija i gljiva koja obrasta podvodne površine; ujedno je važan izvor hrane za primarne konzumente i igra ključnu ulogu u ciklusima kruženja ugljenika i hranjivih materija u vodama.

Simulirali smo svetlosne uslove iz svetlom zagađenih oblasti u dve manipulativne terenske studije, obavljenje u dva različita slatkovodna ekosistema, i analizirali uticaje dva svetlosna izvora (lampi sa natrijumom visokog pritiska (HPS) i belih LED lampi) na biomasu i sastav zajednice perifitona. Rezultati pokazuju da izloženost LED osvetljenju tokom noći smanjuje biomasu perifitona i menja relativne proporcije glavnih fotosintetičkih grupa (dijatoma, zelenih algi i cijanobakterija), često na sezonski i takson-specifičan način. Uticaj LED osvetljenja može biti značajan u novo-osvetljenim oblastima, ali i u oblastima koje su prešle na LED osvetljenje nakon dugoročne upotrebe tradicionalnih tehnologija. Nismo našli dokaze da HPS osvetljenje utiče na perifiton. Smanjena biomasa može omesti primarnu produkciju kao vitalnu funkciju ekosistema, sa posledicama koje se reflektuju na više trofičke nivoe. Kako bi se minimalizovali štetni uticaji veštačkog osvetljenja na vodene ekosisteme, efekti noćnog osvetljenja se moraju uzeti u obzir pri dizajniranju planova osvetljenja u blizini vodenih masa.

Ključne reči: *noćno osvetljenje, stresori urbane sredine, stresori vodene sredine, slatke vode, perifiton*

Abstract

With increasing urbanization and the spread of electrical lighting, the use of artificial light at night (ALAN) has dramatically increased over the last century, transforming nocturnal environments worldwide. With an average global annual increase of 2-6%, ALAN is recognized as one of the fastest-spreading anthropogenic environmental changes. The use of artificial lighting can result in light pollution, namely an increase in nocturnal light above natural levels, and a disruption of natural light-dark cycles. Today, as almost 25% of the world non-polar surfaces experience light pollution, ALAN is recognized as a contributor to global environmental change and a biodiversity threat. Simultaneously, an ongoing global shift from traditional narrow-spectrum outdoor lighting technologies to broad-spectrum LED is occurring, changing the spectral composition of the nightscape. This shift has been predicted to increase ecological impacts of light pollution.

Increasing evidence shows that ALAN affects natural environment, including microorganisms, plants, animals and humans. However, our understanding of its impacts on freshwaters remains limited, despite the fact that these ecosystems are often exposed to light pollution from adjacent human settlements. In my talk I will present rapidly accumulating evidence of impacts of ALAN on different levels of freshwater ecosystems, including microorganisms, macroinvertebrates, and fish. I will present results of my research that focuses on aquatic primary producers, using periphyton as model communities. Periphyton is a complex community of algae, bacteria and fungi that grow attached to underwater surfaces; it is an important food source for primary consumers and plays a key role in nutrient and carbon cycling.

We mimicked light conditions of light-polluted areas in two manipulative field studies performed in two different freshwater ecosystems, and assessed impacts of two light sources (high-pressure sodium (HPS) and white LED) on biomass and community composition of periphyton. We found that LED light at night decreased biomass and altered the relative proportions of major photosynthetic groups (diatoms, green algae and cyanobacteria), often with season-specific and taxon-specific patterns. The impacts of LED illumination can be substantial both in newly-lit areas and in areas that switched to LED illumination after long use of traditional lighting. We found no evidence that HPS light at night impacts periphyton. Decreased biomass may hinder primary production as a vital ecosystem function and have consequences for higher trophic levels. In order to minimize adverse effects of artificial illumination on aquatic ecosystems, the impacts of nocturnal lighting should be considered when designing lighting schemes near water bodies.

Keywords: *artificial light at night, urban stressor, aquatic stressor, freshwaters, periphyton*

POTENTIALLY TOXIC ELEMENTS CONTAMINATION IN RIVER ECOSYSTEMS OF DEBED RIVER CATCHMENT AREA (ARMENIA): ENVIRONMENTAL IMPACT ASSESSMENT

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POTENCIJALNO ZAGAĐENJE TOKSIČNIM ELEMENTIMA U EKOSISTEMIMA SLIVA REKE DEBED (JERMENIJA): PROCENA UTICAJA NA ŽIVOTNU SREDINU

Apstrakt

Istraživanjei mehanizme zagađenja teškim metalima na vodene ekosisteme je važan i hitan zadatak. Usled izraženog rasta sektora rudarstva, zagađenje sliva reke Debed, na severu Republike Jermenije postalo je ozbiljna pretnja vodenim resursima i biodiverzitetu. Cilj istraživanja je bio ispitivanje i procena rizika za životnu sredinu usled zagađivanja teškim metalima ekosistema reka iz sliva reke Debed. Voda i hidrobiološki uzorci (makrozoobentos i ribe) uzeti su sa ugroženih i neugroženih lokacija na rekama sliva reke Debed u aprilu, julu i septembru 2017.godine. Određivanje teških metala (Cu, Mo, Mn, Pb, Cd, Fe, Zn) i hidrobiološke analize urađeni su prema standardnim metodama. Rezultati istraživanja su pokazali da je koncentracija teških metala u vodi ispitivanih vodotokova uslovljena i litogenim i antropogenim izvorima, budući da su koncentracije različitih teških metala na svim lokalitetima prevazilazile nivo koncentracije ovih elemenata u prirodnoj sredini (background level - BL). Na lokalitetima koji su pod rizikom uticaja rudnika, svi ispitivani teški metali su prevazilazili BL, a sadržaj teških metala uglavnom je bio rezultat antropogenog delovanja. U zoni uticaja rudnika, značajan porast sadržaja teških metala zabeležen je u tkivima i organima vodenih organizama (makrozoobentos i ribe). Hidrobiološka ispitivanja su pokazala da je stepen zagađenja vode u oblasti rudnika teškim metalima negativno delovao na rast vodenih organizama. U zoni uticaja rudnika zabeleženo je i opadanje kvantitativnih parametara i diverziteta makroinvertebrata bentosa i riba.

Ključne reči: *teški metali, ekotoksikologija, makrozoobentos, ribe*

Abstract

Investigating the mechanisms behind the impact of heavy metal pollution on aquatic ecosystems is urgently required. Due to increasing growth in the mining sector, pollution in the Debed River catchment area located in the north of the Republic of Armenia has become a serious threat to water resources and aquatic biodiversity. The aim of the present study was to investigate and assess the environmental risks of heavy metal pollution of river ecosystems in the Debed river catchment basin. Water and hydrobiological (macrozoobenthos and fish) samples were taken from the risky and non-risky river sites of the Debed river catchment area in April, July and September of 2017. Heavy metal (Cu, Mo, Mn, Pb, Cd, Fe, Zn) and hydrobiological analyses were done by the standard methods. The results of the study showed that heavy metal concentrations in the waters of the investigated rivers were conditioned by both lithogenic and anthropogenic sources, as the concentrations of different heavy metals in all the investigated observation sites of the rivers exceeded the background level (BL). In the river sites being at the risk of mining impact, all the investigated heavy metals exceeded the BL, and heavy metal content was mainly formed by anthropogenic influence. In the mining impact zone, a noticeable increase in heavy metal content was also registered in the tissues and organs of aquatic organisms (macrozoobenthos and fish). Hydrobiological investigations showed that the degree of heavy metal pollution of the river waters in mining areas negatively affected the growth of aquatic communities. In the mining impact zone, a decrease in the quantitative parameters and diversity of benthic macroinvertebrates and fish was observed.

Keywords: *heavy metals, ecotoxicology, macrozoobenthos, fish*

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DIFFERENT ASPECTS OF SUSTAINABLE USE OF FISH RESOURCES IN SERBIA FOR THE PERIOD 2006-2017

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RAZLIČITI ASPEKTI ODRŽIVOG UPRAVLJANJA RIBLJIM RESURSIMA U SRBIJI ZA PERIOD 2006-2017

Apstrakt

Ovaj rad ima za cilj da ustanovi koja je uloga zakona u dobrom upravljanju ribolovnim resursima. Zaštita i održivo korišćenje ribljeg fonda u Srbiji regulisano je istoimenim zakonom, a odvija se na 17 ribarskih područja i 29 ribarskih područja u zaštićenim prirodnim dobrima. Na 17 osnovnih ribarskih područja upravljaju „javna preduzeća" ili privatne firme u statusu „doo". Kod većih područja, posebno u Vojvodini, postoji više upravljača za jedno ribarsko područje. U zaštićenim prirodnim dobrima upravljači su ujedno i korisnici ribljeg fonda. U periodu od 2006. do 2017. godine Zakon o zaštiti i održivom korišćenju ribljeg fonda menjan je u dva navrata: 2009. i 2014. godine. Podzakonska akta su takođe podložna vrlo čestim promenama. U radu se prate efekti promene zakonske regulative na broj rekreativnih i profesionalnih ribolovaca, broj ribočuvara i trendove u ulovima. Osnovni trendovi su da broj rekreativnih ribolovaca u navedenom periodu opada, broj profesionalnih ribara stagnira, dok broj ribočuvara raste. Ulov ima tendenciju opadanja za sve vrste koje se statististički prate (šaran, som, smuđ i deverika od autohtonih vrsta, i babuška i tolstolobik od alohtonih). Odnos ulova u rekreativnom i komercijalnom ribolovu pokazuje značajne promene u poslednjih desetak godina: osim za tolstolobika, kod koga dominira komercijalni ulov, kod ostalih vrsta je došlo do zamene u smislu da rekreativni ulov preuzima dominaciju u poslednjih nekoliko godina.

Ključne reči: *fisheries management, fishermen, catch, trend*
Keywords: *upravljanje u ribarstvu, ribolovci, ulov, trend*

INTRODUCTION

Use of fish stocks in Serbia has been regulated by the Law of protection and sustainable use of fish resources. Fishing is allowed on 17 common fishing districts and also in the 29 fishing districts in the different protected regions. Appointed public or private companies manage the 17 districts. Larger fishing districts, especially in the Vojvodina province, are each shared by more than one fishery manager. In the period 2006 - 2017, the legal regulation regarding protection and sustainable use of fish stocks was changed twice, in 2009 and 2014. Related legal acts are also subject to the frequent changes. In this study, effects of those changes in legislation on the number of recreational and professional fishermen, as well as on the number of fish guards and trends in catch have been presented. Deterioration in fisheries management in Serbia in last couple of years signaled the failure in administrative management and either erroneous regulation issued by actual law in concern of fishery, or its bad implementation enforced by the administrative organ in charge. The purpose of this paper is to discuss the impacts of legal frame content and fishery administration in the fisheries management. This paper investigates the critical role of the fishery law in practical organization of the fishery in Serbia.

MATERIALS AND METHODS

Data on the numbers of recreational and professional fishermen, their catch, as well as of the number of fish guards engaged at the state-appointed fisheries managers were obtained from the Statistical Office of the Republic of Serbia. Acquired data were processed in Statistica 8. analitical package using bivariate linear regression and piecewise linear regression.

RESULTS

Observed trends in the freshwater fishery in Serbia are that the number of recreational fishermen in the analyzed period decreased, the number of professional fishermen was stable, while the number of fish guards increased (Figure 1). The reported total catch of all fish species decreased (Figure 2). The ratio of catch showed significant changes during the last decade, for all fish species whose catch was recorded, except for the bighead carp (dominant in the commercial catch), while for the rest of the fish species recreational catch was dominant in the last few years (Smederevac-Lalić et al., 2017). In the period from 2006 to 2017, the Law on protection and sustainable use of the fish fund was changed on two occasions: 2009 and 2014. Did these changes in the law contribute to the decline in the number of legally registered commercial and recreational fishermen and reported catch? It is very unlikely that majority of former recreational fishermen who stopped buying licenses for angling quitted fishing. It seems much more likely that they shifted to the illegal fishing with the unreported catch and practicing the unregulated regime of fishing (IUU).

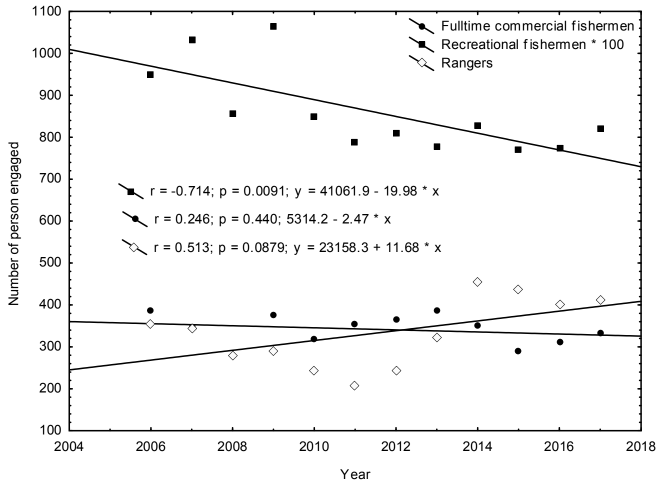

Figure 1. Number of registered fulltime commercial fishermen, anglers and rangers according to Statistical Office of the Republic of Serbia

Striking trend reversal in the total catch statistics appeared between years 2011 - 2012, when fishery districts didn’t have applicable documents for operational management, which lasted until 2017 (Figure 2). Using piecewise linear regression model breakpoint was calculated as 2011.5.

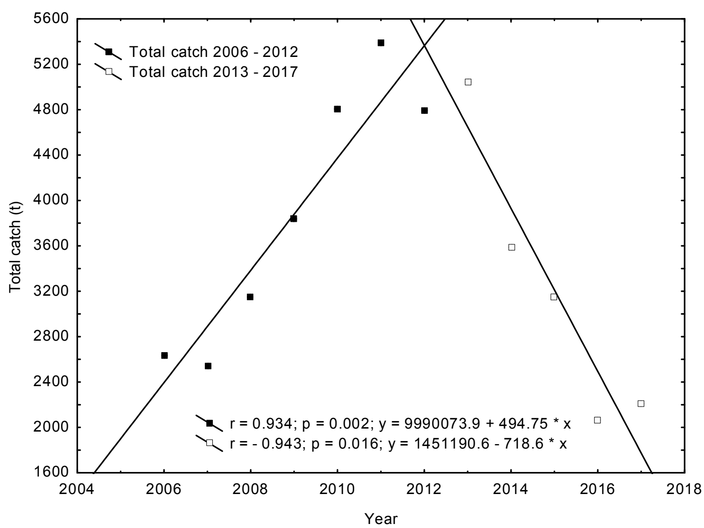

Figure 2. Total fish catch in the Republic of Serbia (Statistical Office of the Republic of Serbia).

As a result of the law established in 2009, there was increase of the catch of registered recreational fishermen, while at the same time their number decreased (Figure 3).

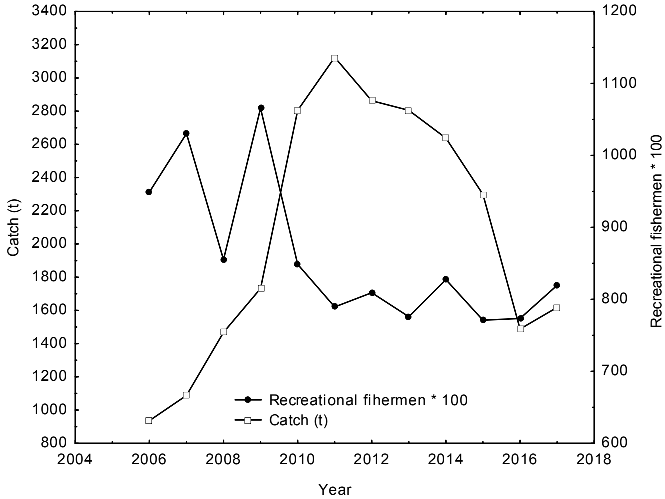

Figure 3. The ratio between recreational fishermen and their catch.

Establishing the law in 2009 commercial catch decreased, while number of commercial fishermen stayed stable (Figure 4).

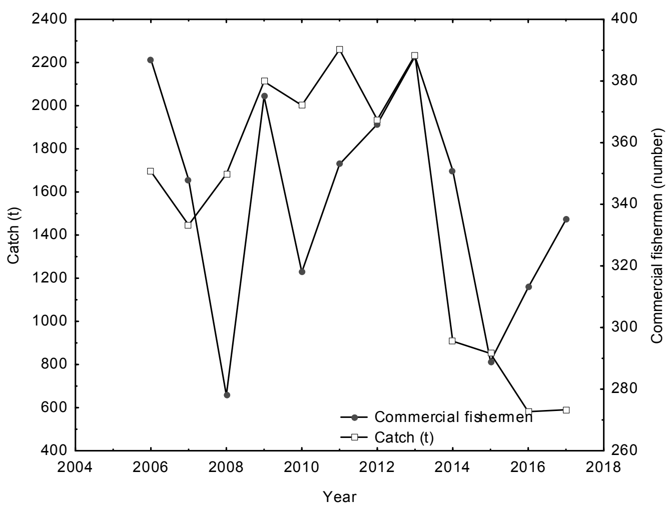

Figure 4. The ratio between commercial fishermen and their catch.

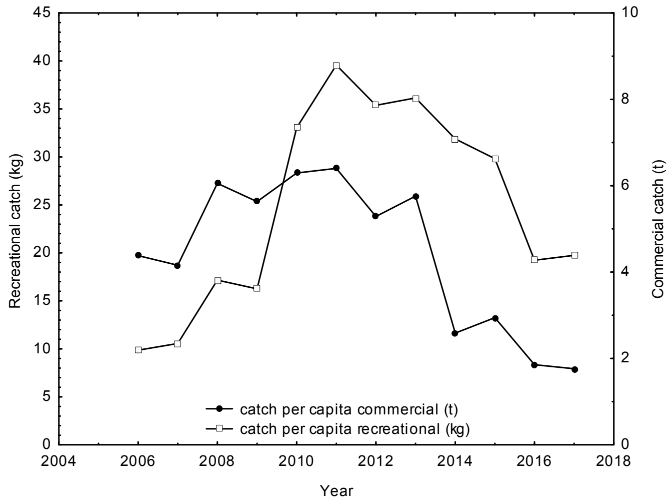

Figure 5. Catch per capita for commercial and recreational fishermen.

Implementation of the Law of protection and sustainable use of fish resources in 2009 reflected on higher catch per capita of the recreational fishermen, while implementation of the Law of protection and sustainable use of fish resources in 2014 resulted on a remarkable decline in legal commercial catch per capita and decreasing trend of legal recreational catch per capita (Figure 5).

DISCUSSION

Frequent changes of the fishery law in Serbia usually do not arise from scientific knowledge, advice and conclusions. These changes generally reflect the need of the current governing leadership to allocate the fishery resources and rights for their exploitation to stakeholders differently compared to the previous period. Observed trends in all analyzed parameters drastically changed in 2012, when the period of confusion has started, because the appointed temporary managers of fishery districts did not have applicable documents for operational management, which has been prolonged for the 5 years period. Decrease of the number of fishermen and of the catch implicates to questionable management. In EU, the waters management system was also acknowledged to be performing poorly in terms of sustainable exploitation (Degnbol and McCay, 2007). Many people involved in the system then looked for a new ways to involve fishery science in a management of fisheries, tracing interactions between knowledge and policy decisions (Degnbol and McCay, 2007; Schwach et al., 2007). Complex relationship between scientific knowledge, fishermen’s experience, politics, and concerned citizens and stakeholders are playing a leading role. The advisory/management framework is undergoing a slow process of change in the direction of more stakeholder participation. It is believed that inland fisheries are doomed because many threats to aquatic ecosystems attributable to anthropogenic activities (water abstraction, habitat degradation, land drainage, dam construction, pollution and eutrophication, alien invasive species, climatic variability, and bad or non-existent fishery management) will inevitably lead to their decline. This is supported by studies and reports that allege that catches are falling and species are disappearing, with many symptoms of chronic overfishing (Welcomme, 2011). The analysis and discussion applies to both capture fisheries supplying food as well as recreational fisheries. Cooke and Cowx (2006) provided evidence that the global fisheries concerns regarding commercial fishing, which can have equivalent, and even in some cases, magnified effects in recreational fisheries. Not all countries report their recreational catch, but it can contribute significantly to the total catch (Cooke and Cowx, 2006; Cowx et al., 2010). In Serbia, threats from the recreational fishing are even wider: introduction of non-native fish species, issues of bycatch, rarely applied catch-and-release, fisheries-induced selection, trophic changes, habitat degradation and advance in fishing technology increased fishing effort. However, the lack of reliable, scientific-based operational documents that had to provide the sustainable fisheries management on which the appointed managers of fisheries districts could rely in the five-year-period after 2012, resulted in only provisional management whose effects were not possible to assess. Likewise, considering that in most countries number of the recreational fishermen increased in last decade (Kohl, 2002) it is not realistic that their number in Serbia has decreased. Hence, we are suspicious that consequences of the laws in 2009 and 2014 in Serbia might have actually decreased only the legal, traceable fishing effort.

ACKNOWLEDGMENTS

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STUDY ON LOWER DANUBE STURGEONS – INFLUENCE OF THE QUALITY OF DANUBE WATERS ON THE CHANCE OF JUVENILE SURVIVAL IN ORDER TO REPOPULATE NATURAL ECOSYSTEMS

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STUDIJA NA JESETRAMA IZ DONJEG DUNAVA – UTICAJ KVALITETA VODE DUNAVA NA PREŽIVLJAVANJE JUVENILA SA CILJEM REPOPULACIJE PRIRODNIH EKOSISTEMA

Apstrakt

Održivi razvoj ribarstva i akvakulture u Rumuniji predstavlja socio-ekonomsku nužnost sa srednjeročnim i dugoročnim implikacijama. U nekim izolovanim oblastima, kao što je delta Dunava i Dunav Meadow, ribarenje je jedna od glavnih delatnosti i izvor prihoda za lokalno stanovništvo. Usled toga ovim oblastima je neophodna održiva eksploatacija ribljeg fonda i podsticaj za razvoj akvakulture radi unapređenja lokalne ekonomije. U poslednjoj deceniji Rumunija je učinila velike napore u zaštiti jesetre i podržala programe poribljavanja jesetri iz donjeg Dunava. Donji Dunav je jedna od poslednjih oblasti, i jedina u Evropskoj Uniji, gde ova vrsta živi i razmnožava se prirodnim putem. Zbog toga je neophodno poznavati mehanizme adaptacije ovih migratornih vrsta na promene faktora okruženja i različitih parametara vodenog okruženja tokom migracije.

U isto vreme, u Rumuniji su osnovane privatne farme sa intenzivnom akvakulturom jesetri, pri čemu je većina sa divljim vrstama uhvaćenim u Dunavu. U tom smislu cilj ovog rada je bio da se dobiju podaci o uticaju toksičnih materija iz okruženja na razvoj juvenilnih stadijuma dobijenih sa farmi za potrebe repopulacije ove vrste u prirodne vode. Obzirom da su jesetre veoma osetljive na variranje faktora okruženja, ugrožena staništa veoma brzo mogu da utiču na mrešćenje, prezimljavanje i ishranu, i na kraju dovedu do nestanka ove vrste. Ova studija će takođe da ukaže na adaptacione mehanizme pri različitim variranjima faktora okruženja kao što su salinitet (osmoregulacija), temperatura, svetlost (ponašanje), kiseonik, što su parametri koji su pod uticajem antropogenog faktora.

Ova studija je bazirana na biohemijskim analizama (modifikacija enzimskog oksidativnog stresa) i promenama histologije ćelija i tkiva kao rezultatima izloženosti juvenila pod eksperimentalnim uslovima.

Ključne reči: *jesetre, donji Dunav, zagađujuće materije, adaptacije, enzimi*

Abstract

The sustainable development of fisheries and aquaculture in Romania represents a socio-economic necessity with medium and long-term implications. In some isolated areas, such as the Delta and Danube Meadow, fishing is one of the main job-producing activities and revenue sources for the local population. Therefore, these areas require a sustainable exploitation of fisheries resources and an incentive for aquaculture to develop the local economy. In the last decade, Romania has made considerable efforts to protect and support sturgeon populations from the Lower Danube by supportive stocking programs. Lower Danube is one of the last areas, the only one in the European Union, where these species grow and reproduce naturally. Thus, is important to know the mechanisms of adaptation of these migratory species to changing environmental factors and various parameters of the aquatic environment during migration.

At the same time in Romania have developed private farms of the intensive aquaculture of sturgeon, most of them with wild breeding species caught from the Danube. Thus, the aim of this study is to obtain data on the toxicity influence of environmental pollutants in the development of juvenile forms, those obtained in farms for the purpose of repopulation of natural waters. Since sturgeons are very sensitive to variation of habitat factors, and the damaged habitats can immediately affect the periods of spawning, wintering and feeding and ultimately, may cause the disappearance of this species. This study will also highlight the adaptation mechanisms at various oscillations of environmental factors such as salinity (osmoregulation), temperature, light (behavior), oxygen, factors that are influenced in the present by anthropogenic disturbance factors.

The study is based on biochemical analysis (modification of oxidative stress enzymes) and on changes in histological cells and organs as a result of exposure of juveniles under experimental conditions.

Keywords: *sturgeon, Lower Danube, pollutants, adaptation, enzymes*

MORPHOMETRIC AND MERISTIC CHARACTERISTICS OF BROWN TROUT (*SALMO TRUTTA* M. *FARIO* LINNAEUS, 1758.) POPULATIONS IN NORTH-WEST CROATIA

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MORFOMETRIJSKE I MERISTIČKE ZNAČAJKE POPULACIJA POTOČNE PASTRVE (*SALMO TRUTTA* M. *FARIO* LINNAEUS, 1758.) SJEVEROZAPADNE HRVATSKE

Apstrakt

Potočna pastrva je široko rasprostranjena vrsta iz porodice Salmonidae, prirodno obitava u Europi i dijelu Azije a unesena je u staništa diljem svijeta. Obitava u hladnim vodotocima i jezerima. U Hrvatskoj je rasprostranjena u gornjim dijelovima tekućica. S obzirom da je ova vrsta izuzetno atraktivna za uzgoj, kao i za sportski ribolov, u prošlosti je zabilježeno mnogo translokacija i poribljavanja s različitih lokacija. Danas se u uzgoju u Hrvatskoj nalazi alohtoni atlantski tip pastrve, a poribljavanja se vrše isključivo ribom iz uzgoja što dovodi do križanja uzgojnih i divljih jedinki te utječe na genotip populacije. Ovim istraživanjem je analizirano nekoliko populacija potočnih pastrva sa Žumberka, Samoborskog gorja i Gorskog kotara (crnomorski slijev, sjeverozapadna Hrvatska). Primarni cilj istraživanja je bio utvrditi postoje li značajne morfološke razlike između ovih populacija. U analizu su uključene i ribnjačke populacije pastrva atlantskog tipa kako bi se utvrdile sličnosti i razlike morfoloških osobina alohtonih i divljih populacija što može ukazati na poribljavanje alohtonim atlantskim genetskim materijalom. Pastrve su uzorkovane metodom elektroribolova na 8 vodotoka i jednom ribnjaku (ukupno 11 lokaliteta) u dva vremenska razdoblja. Prvo uzorkovanje je obavljeno tijekom jeseni 2009.

na potocima Slapnica i Kupčina (Žumberak) te Gradna, Bregana i Rudarska Gradna (Samoborsko gorje). Drugo uzorkovanje je obavljeno tijekom ljeta i jeseni 2017. na potocima Slapnica i Kupčina (Žumberak); Curak, Bresni potok i Mala Lešnica (Gorski kotar) te ribnjacima Vrabac na Žumberku. Ukupno su ulovljene 124 jedinke. Ulovljenim jedinkama je izvagana masa (g) i izmjerena totalna (cm) i standardna duljina (cm). Osim toga, na svakoj jedinki je izmjereno 16 morfometrijskih i 6 merističkih mjera. Prikupljeni podaci su statistički analizani u programu Canoco 5 upotrebom linearne analize kanoničke redundancije (RDA). Testirane su nezavisne morfološke varijable i lokacije kao zavisne nominalne varijable. Analiza morfoloških podataka obrađena je univarijatno, pomoću analize varijance (ANOVA), s time da se testiranje postojanja razlika u morfološkim varijablama provelo Monte Carlo testom s 999 permutacija s povratnom selekcijom. Rezultati ukazuju da geografski bliske populacije nisu fenotipski sličnije u odnosu na geografski udaljenije populacije. Najznačajniji parametri za odvajanje populacija su merističke i morfometrijske vrijednosti peraja. Jedinke analizirane na Samoborskom gorju izdvajaju se od jedinki s ostalih lokacija prema broju tvrdih šipčica u leđnoj peraji, broju mekih šipčica u lijevoj trbušnoj peraji, broju mekih šipčica u lijevoj prsnoj peraji i duljini glave. Jedinke uzorkovane na Kupčini (Žumberak) su morfološki slične jedinkama iz Male Lešnice i Bresnog potoka (Gorski kotar) i to prema broju tvrdih šipčica u lijevoj prsnoj peraji i duljini leđne peraje. Jedinke s ribnjaka Vrabac se razlikuju u najvećem broju morfoloških karakteristika, što se i očekivalo s obzirom da je poznato da se radi o jedinkama atlantskog haplotipa uvezenim iz Danske. Rezultati ukazuju na značajnije poribljavanje atlantskom pastrvom potoka Curak, zatim ostalih vodotokova Gorskog kotara te Samoborskog gorja. Jedinke iz potoka Slapnica (Žumberak) i Rude (Samoborsko gorje) se značajno razlikuju od ostalih analiziranih jedinki pri čemu je Slapnica morfološki sličnija onima u Gorskom kotaru, a Rude ostalima iz Samoborskog gorja. S obzirom na dobivene rezultate, preporuka je da se potakne uzgoj autohtonog dunavskog tipa potočne pastrve usmjerenog upravo za poribljavanje otvorenih voda kako bi se spriječio gubitak izvornih linija potočne pastrve.

Ključne riječi: *morfološke značajke, crnomorski slijev, atlantski tip pastrve, dunavski tip pastrve*

Abstract

Brown trout is a widespread species of salmonid fish that naturally occurs throughout Europe and parts of Asia and it is introduced worldwide. It inhabits upper parts of cold streams and cold lakes. In Croatia, this species has historically been translocated with specimens originating from wide range of locations due to significant sport fishing and farming potential. Today, at fish farms in Croatia, only Atlantic lineage of brown trout is being bred and used for restocking of open waters. However, introduction of such alien lineage leads to hybridization with native strains, alternating the native populations genotype. In this study, several brown trout populations from Žumberak Mountains, Samoborsko Gorje Hills and Gorski Kotar region (Black Sea basin, North-West Croatia) were analyzed. This study aims to determine morphological differences of wild populations of brown trout inhabiting different isolated locations. One population of Atlantic lineage grown on fish farm Vrabac was also included in the analysis with aim to determine the similarities and differences between morphologic features of alien and native lineages. Also, this data could indicate Atlantic lineage presence in each of the investigated waterbodies.. Trout specimens were sampled by electrofishing from 8 streams during two sampling periods and one population was collected at Žumberak Mountain fish farm (11 sampling sites overall). First sampling was performed during fall of 2009 on Slapnica and Kupčina streams (Žumberak Mountains) and Gradna, Bregana and Rudarska Gradna streams (Samoborsko Gorje Hills). Second sampling was performed during summer and fall of 2017 on Slapnica and Kupčina streams (Žumberak Mountains); Curak, Bresni potok and Mala Lešnica streams (Gorski Kotar region) and fish farm pond Vrabac on Žumberak Mountains. Overall, 124 brown trout individuals were caught. All individuals were weighed (g) and measured for total length, stanard length (cm) andadditional 16 morphometric measurments. Also, six meristic counts were taken. Obtained data was analyzed by Canoco 5 software, using linear Redundancy Analysis (RDA). Independent morphological variables and location as dependent nominal variables were tested. Morphological data was analysed univariately, by analysis of variance (ANOVA), while Monte Carlo test with 999 permutations with forward selection was used to determine differences between morphological variables. Results reveal that geographically close populations are not phenotypically more similar than the ones further apart. Meristic and morphometric characteristics of fins represent significant parameter to distinguish different populations. Trouts from Samoborsko gorje hills differ from all other trouts by number of dorsal spines, number of left ventral rays, number of left pectoral rays and head length. Individuals from Kupčina (Žumberak mountain) are morphologically similar to individuals from Mala Lešnica and Bresni potok (Gorski kotar region) by similar number of left pectoral spines and dorsal fin length. Trouts sampled from Vrabac fish farm differ from all other populations in most morphological characteristics. That was expected because it is already known that they belong to Atlantic lineage introduced from Denmark. Results imply that Curak stream is significantly stocked with Atlantic lineage brown trout. Impact of stocking was also observed in the remaining Gorski Kotar Region streams and Samoborsko Gorje Hills streams. Trouts from Slapnica (Žumberak Mountains) and Rudarska Gradna (Samoborsko Gorje Hills) significantly differ from individuals from other locations. Slapnica individuals somewhat morphologicaly resemble Gorski Kotar region trouts, and Rudarska Gradna trouts resemble trouts from Samoborsko Gorje Hills streams. Considering these results, it is advised to enforce breeding of native Danubian lineage of brown trout on fish farms and use native populations for stocking purposes in open waters. Such approach is the only solution to successful conservation of native brown trout lineages.

Keywords: *morphological characteristics, Black Sea Basin, Atlantic lineage brown trout, Danubian lineage brown trout*

WILD AND BREEDING FISH POPULATIONS OF THE *COREGONUS* GENUS AND RESTOCKING

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DIVLJE I GAJENE POPULACIJE RODA *COREGONUS* I PORIBLJAVANJE

Apstrakt

Dve vrste ozimica *Coregonus maraena* i *Coregonus albula* su značajne vrste za Poljsku i Evropsku ihtiofaunu. Istraživanje se odnosilo na jedinke iz prirodne, zaštićene i matične (reproduktivne) populacije. Ono je bazirano na genetičkoj analizi kompozicije haplotipa (PCR-RFLP) kontrolnog regiona (CR) i sekvenciranjem. Sprovedena analiza pokazala je prisustvo 13 i 22 kompozicije haplotipova kontrolnog regiona obe vrste ozimica, respektivno. Najmanja varijacija kompozicije haplotipova (1-2) zabeležena je kod zaštićene populacije DPN, koja duži niz godina nije poribljavana, i u grupi matičnih jedinki. U populacijama koje su u dobrom stanju, poribljavane duži niz godima sa mlađi dobijenom od matica iz prirodnih populacija (*C. maraena* sa jezera Morzycko, Miedwie i reke Reda; i *C. albula* sa jezera Wigry) zabeležena je prednost jedne od kompozicije haplotipova. Ove populacije su korisne i efikasne u programima poribljavanja. Ukupna genetička udaljenost poljskih populacija je 0,4% za svaku vrstu. Zaštićene populacije i dugogodišnja poribljavanja sa mlađi dobijene od matica iz prirode kao i nizak nivo varijabilnosti je dragocen material za obogaćivanje genetskog materijala riba u uzgoju u vodama severnog dela Poljske.

Ključne reči: *Coregonus maraena, Coregonus albula, kontrolni region*
Keywords: *Coregonus maraena, Coregonus albula, control region*

INTRODUCTION

Genetic analysis is an indispensable tool used in the characteristics of species, populations or spawning stocks. Conducted research provides information on the origin of a given species, phylogenetic relationships and the direction of migration. Cisco (*C. albula*) and whitefish (*C. maraena*) are valuable species of European and Polish ichthyofauna. Both species are vulnerable to extinction (Witkowski et al. 2009). For many years, activities have been carried out to support natural grassland populations by growing fish (Turkowski 2002). Activities should be preceded by genetic analysis (Pamminger-Lahnsteiner et al. 2009). It allows to know the genetic pool, to assess genetic diversity, and to analyze the relationship between populations and estimate the genetic potential of individual populations (Bochkarev et al. 2011). In these analysis, mtDNA considered as maternally inherited and do not recombine is very useful.

Whitefish and cisco are species important for maintaining the biodiversity of the aquatic environment, but also harvested for economic purposes (Mickiewicz, Wołos 2012). Supporting existing populations in commercially exploited lakes, strengthening endangered populations, e.g from protected areas such as DPN and Łebsko Lake, are important tasks for lake owners and managers. In the studies of whitefish and cisco conducted so far, populations of these species were analyzed on the basis of several genes (Kirczuk et al., 2015, 2016, 2017). The following is a comparison of genetic resources of two species of Polish fish, whitefish and cisco important for the economy, made on the basis of compositions of haplotypes and the sequence control region mtDNA. The purpose of the following work was to present the differences between wild and farmed populations and to show the influence of the latter on the development of natural populations.

MATERIALS AND METHODS

Study sites and sampling of the biological material

Whitefish from the conducted research comes from Western Pomerania: Dąbie Lake (D) - contact through the Szczecin Lagoon with sea waters, Miedwie Lake (Md), Morzycko Lake (Mr), Middle Pomerania from the river Reda (Rd) and spawning herd from a fish hatchery in Rutki (RuH). Cisco comes from Miedwie Lake (M) - Western Pomerania, Bytyń Lake (B), Żerdno Lake (Z), the strict protection zone of the Drawieński National Park (DPN): Ostrowieckie Lake (T), from the cover of DPN: Szczuczarz Lake (A), Tuczno Lake (U) - Middle Pomerania and Wigry National Park (WPN) - Wigry Lake (W) - North-East Poland. Whitefish from the lake in DPN (consent of MOŚ no. DLPpn-4102-229 / 17717/13 / M). In M and W, it is stocked with material originating from native spawners. In lakes with DPN and the DPN buffer zone (T, U, A) populations are currently not stocked, in a rather poor condition. In the past, Z and B lakes were stocked with material from different hatcheries.

Methods Analysis

Based on the PCR-RFLP analysis, mtDNA control region whitefish (Kirczuk et al. 2017) and cisco (Kirczuk et al. 2015, 2016), a combination of the of haplotypes was made. Analysis was also performed based on the control region (CR, D-loop) sequences of these species.

Data Analysis

Multiple alignment analysis and distance using MEGA7 (Neighbor Joining method, Kimura 2-parameter model, bootstrap 2000). For analysis of affinity based on CR whitefish and cisco from Poland the sequences deposited at GenBank were used.

RESULT AND DISCUSSION

The non-coding control region (CR) of mtDNA is highly variable both intraspecific and between species (Brzuzan et al., 2002). Whitefish (*C. maraena*) and cisco (*C. albula*) from Poland have confirmed that compared to the mitochondrial genes ND1, ND3/4 and ND5/6, the control region has the highest intrapopulation variability (Kirczuk et al. 2017). The control region, as non-coding, is not subject to natural selection, hence mutations are more often accumulated here, which are used in phylogenetic analysis. In our studies in five populations, 13 compositions of haplotypes were shown to be based on CR analysis (Table 1). Four combinations of haplotypes were present in more than one population, while the remaining ones were unique and occurred only in single individuals in one position.

Table 1. Haplotypes compositions in whitefish (Coregonus maraena L.) based on control region analysis (mtDNA PCR-RFLP)

Compositions of haplotypes no	Composite PCR-RFLP haplotype	West Pomerania			East Pomerania		Total
		Lakes			River	Hatchery	
		Dąbie Lake (D)	Miedwie Lake (Md)	Morzycko Lake (Mr)	Reda River (Rd)	Rutki hatchery (RuH)	
1	aaaaa			5			5
2	aaaab			18			18
3	aaaca		8	2			10
4	baaaa		2				2
5	baacf	5					5
6	bbaaa	14	14				28
7	bbaaf		6				6
8	bbaag				28	30	58
9	bbbad	6		5			11
10	bdaag				2		2
11	caaaa	2					2
12	cabae	2					2
13	ccadc	4					4
Total		33	30	30	30	30	153

Populations of Lake Dąbie after stocking in the 1990s, in 2006 and now (data from the Maritime Research Institute, MIR) have rebuilt and are naturally breeding (Czerniejewski, Rybczyk 2010). Therefore, among the studied population of West Pomerania, there is the highest variation in the composition of haplotypes. The possibility of earlier contact of the whitefish population was confirmed by nucleotide sequence analysis. In the tree, sequences from the fish from D group together with sequences from the Md and to farm fish from RuH and Rd, East Pomerania (Fig. 1). Populations of Md and Mr, which for many years are stoc-ked with hatching from native spawners, have 4 composition of haplotypes with a distinct advantage of one combination. In the tree of affinity, the sequences whitefish with Md and Mr create separate clades. All these data may indicate that the populations in tkkhe West Pomerania lakes are genetically stabilized. According to reports, fish management in these lakes are in good condition, which would confirm the effectiveness of previous stocking. The only problem is the quality of water, because the increase in eutrophication, especially Mr, may undermine efforts related to stocking (spoken statement from the employees of PZW). The specimens sown from the culture (RuH) had only one haplotypes composition (8), which occurred in as many as 93% of subjects from Rd. Genetic uniqueness of Rd results from the founder’s effect (Fig. 1) and confirms the effectiveness of the restocking material carried out in this area - unification of the genetic pool of this group.

Table 2. Haplotypes compositions in cisco (Coregonus albula L.) based on control region analysis (mtDNA PCR-RFLP)

Compositions of haplotypes no	Compositions PCR-RFLP haplotypes	DPN Middle Pomerania	DPN Buffer Zone Middle Pomerania		West Pomerania	Middle Pomerania		WPN N-E Poland	Total
		Ostrowieckie L. (T)	Szczuczarz L. (A)	Tuczno L. (U)	Miedwie L. (M)	Bytyń L. (B)	Żerdno L. (Z)	Wigry L. (W)	
1	aaa				4	2		3	9
2	aba	3			2	2	8	2	17
3	abb							7	7
4	acc				2				2
5	ace				2		2	2	6
6	aea					4			4
7	baa	17	30		10	14	16	14	101
8	bab				4				4
9	bac			2					2
10	baf							2	2
11	bbb						2		2
12	bbc					2			2
13	bda				4	4			8
14	bea					1			1
15	beb				2				2
16	bee						2		2
17	beg			10				1	11
18	caa			10					10
19	caf			2					2
20	cba			2		1			3
21	cea			3					3
22	cac			1					1
Total		20	30	30	30	30	30	31	201

In the analyzed cisco populations the presence of 22 composition of haplotypes was shown, Table 2. The smallest variation concerned lakes with DPN (T) and buffer zone DPN (U) - these populations are few in number, breeding naturally without additional restocking. In the rest of the cisco populations there were 5 to 8 haplotypes with a distinct advantage of one composition (marked as 7). The observed variation in the composition of haplotypes may be the result of a large number of founders in populations (B, Z) and/or the persistence of numerous populations over a longer period of time in lakes with large areas and good trophic conditions (M, W). The cisco sequences from Wigry Lake and Hańcza Lake, north-east Poland (Brzuzan, Ciesielski 2002) create a separate clade in the tree of similarity, from which all the sequences obtained by us from middle and west Pomerania originate, (Fig. 2). These analysis show the beneficial effect of restocking on the maintenance of native gene pools associated with adaptation to the local environment.

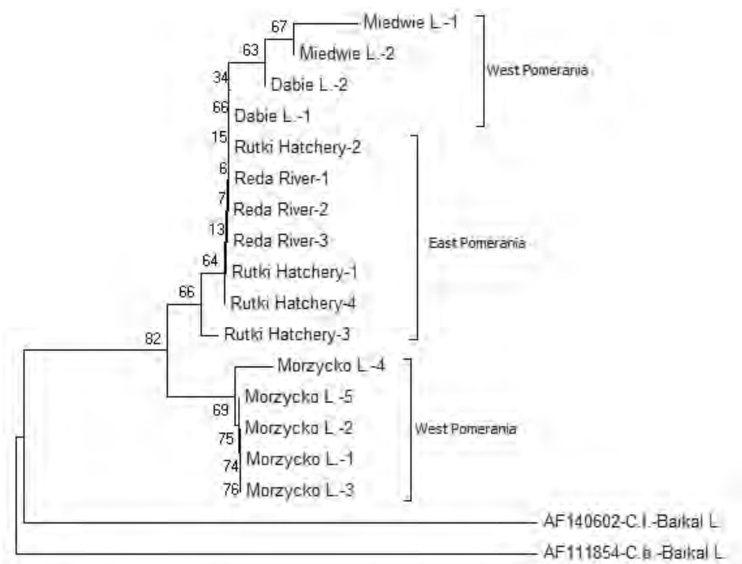

Figure 1. Analysis of the similarity of the Polish C. maraena populations from natural, and rearing reservoirs and the populations based on the sequences of mtDNA fragments (CR). Sequences from GenBank AF140602 (C. lavaretus) and AF111854 (C. baicalensis) from Baikal Lake

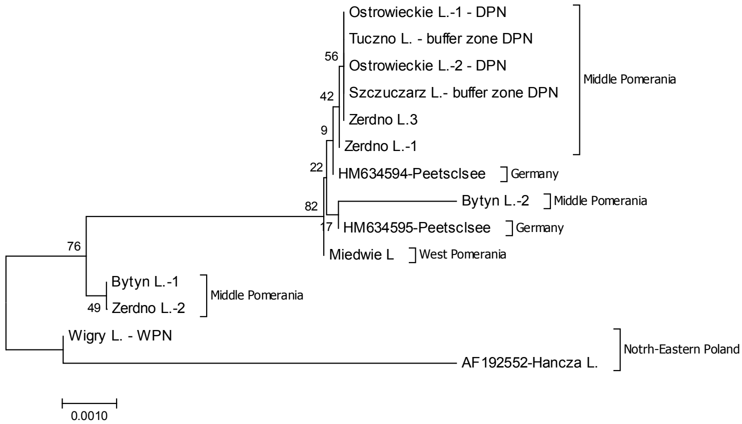

Figure 2. Analysis of the similarity of the Polish C. albula populations from natural, protected and rearing reservoirs and the populations based on the sequences of mtDNA fragments (CR). Sequences from GenBank AF192552, HM634595, HM634595, HM634594, HM634595.

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ASSESSMENT OF PRUSSIAN CARP (*CARASSIUS GIBELIO*) RESPONSES TO WATERS IMPACTED BY THE WASTEWATER TREATMENT PLANT

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PROCENA TKIVNIH ODGOVORA BABUŠKE (*CARASSIUS GIBELIO*) NA ONEČIŠĆENJA OD PROČISTAČA OTPADNIH VODA

Apstrakt

Pročišćene otpadne vode obiluju kompleksnim zagađivalima koji mogu imati značajan utjecaj na akvatične ekosustave. Ribe su optimalni modeli za proučavanje tih učinaka jer pokazuju tkivne reakcije već pri niskim koncentracijama onečišćivača. Biološki odgovori mogu se očitovati na svim razinama biološke organizacije, pa je radi procjene utjecaja pročišćenih otpadnih voda na slobodnoživuću babušku (*Carassius gibelio*) istražen njezin zdravstveni status, kao i niz histopatoloških, hematoloških, biokemijskih i drugih tkivnih parametara. U riba lovljenih u efluentu najčešći histopatološki nalaz bio je fuzija škržnih lamela uz smanjivanje interlamelarnih prostora. U plazmi istih riba uočeno je značajno povišenje vrijednosti uree, ukupnih bjelančevina, albumina i triglicerida, te značajno smanjenje aktivnosti superoksid dismutaze. Uočena je značajna razlika histoloških i hematoloških parametara riba u efluentu u odnosu na nizvodne vode. Histološki, hematološki i biokemijski parametri plazme riba ukazuju na značajne promjene koje se mogu korelirati sa okolišnim stresorima. Istaknuti učinci bili su i na tkivnoj strukturi jetre, dok su kolesterol i trigliceridi iz plazme pokazali najsnažniju korelaciju sa godišnjim dobom i uzvodnom industrijskom aktivnosti. Promjene tkivne morfologije ukazuju na gubitak stanične i tkivne strukture pod utjecajem povišenih koncentracija teških metala u okolišu. Te se promjene mogu korelirati sa razinama oksidativnog stresa i neutrofilijom. Rezultati istraživanja pokazuju da, iako nespecifični za pojedine čimbenike, integrirani histopatološki, hematološki i biokemijski nalazi tkiva babuške mogu služiti kao vrijedni biomarkeri za adaptivne mehanizme riba, kao i za monitoring kakvoće vode i onečišćenje slatkovodnih ekosustava.

Ključne reči: *prečistač otpadnih voda, babuška, Carassius gibelio*
Keywords: *wastewater treatment plant, Prussian carp, Carassius gibelio*

INTRODUCTION

Treated wastewaters are abundant with complex pollutants having a significant impact on aquatic ecosystems. Since fish respond to low concentrations of environmental pollutants, they are ideal models for assessing these effects. The biological effects may affect all levels of biological organization, and it is thus a challenge to identify adequate biomarker indicators of toxic effects, especially since the treated wastewater effluents are chemically complex, constantly changing and biologically active (Topić Popović *et al*., 2016).

Prussian carp (*Carassius gibelio*) is the most dominant fish species in lentic and slowly running aquatic habitats (Lusk *et al*. 2010). Several carp species have previously been included for *in situ* evaluation of effects of wastewater treatment plants (WWTP), but their impact on native species is less investigated, although assessment of biological responses of wild-caught fish in WWTP-related waters will more likely indicate actual ecological effects of treated wastewater. The scope of this work was thus to assess the impact of treated wastewaters on native wild Prussian carp inhabiting respective waters, with a hypothesis that fish would display pronounced biological responses. To that end, general fish health status was determined, as well as an array of selected plasma biochemical, haematological, oxidative stress and tissue histopathological indices.

MATERIALS AND METHODS

The samplings were conducted in spring/fall 2014 throughout the treatment process of a Croatian municipal WWTP serving a city of Virovitica, also receiving hospital and sugar plant wastewaters. Water and fish were sampled from the stream unaffected with the WWTP activities, WWTP effluent-receiving canal, and County canal further downstream. The sampling sites were chosen as the best representatives of their capacity as unaffected, heavily impacted, and possibly impacted by the treated wastewaters. Physico-chemical properties of water and total concentrations of heavy metals were analyzed according to the respective ISO standards.

Fish were caught by nets and angling; they were manipulated by the competent authorized persons in accordance with the provisions of national legislation and the Institute's Bioethical Committee (No. BEP-274/2-2012). A total of 109 Prussian carp (*Carassius gibelio*) were examined.

Wet mounts of gill filaments and skin scrapings were examined under the light microscope. Blood withdrawal and necropsy were performed immediately and tissues (gills, anterior kidney, spleen, and liver) were processed histologically with standard methods. In blood plasma, concentrations of glucose (GLU), urea (URE), creatinine (CRE), cholesterol (CHOL), triglyceride (TRIG), total proteins (TP), albumin (ALB) and activity of alanine aminotransferase (ALT), alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT) were determined by Beckman Coulter commercial kits (Olympus Life and Material Science Europe, Ireland) on the Olympus AU 640 biochemistry analyzer (Olympus, Japan). The activity of superoxide dismutase (SOD), paraoxonase activity (PON 1), and glutathione peroxidase (GSH-Px) were determined by Randox commercial kits on the Olympus AU 640 biochemistry analyzer.

RESULTS AND DISCUSSION

Effluent from the WWTP under this study, discharging into a relatively small receiving canal, had a marked effect on Prussian carp community collected from the effluent canal and a downstream location (County canal). Significantly higher suspended solids, COD-Mn, BODn, and ammonium concentrations were measured in the effluent. Sugar cane processing particularly affected both the effluent-receiving canal and downstream County canal with heavy metal pollutants, namely Cu, Zn, Cr, and Ni. Fish captured at the sampling sites did not vary significantly in external gross signs and necropsy findings. Downstream fish, however, suffered from a heavier parasitic infestation than fish captured in the effluent-carrying canal, which could be attributed to their possible migration over the water bodies in pursuit of the more favorable feeding locations. Monogenean gill flukes were the most prevalent of all parasites found in this study, but showed no site preference. Pollutants such as heavy metals reduce the immunological capabilities of fish host, rendering them more susceptible to parasites (Overstreet 1993), monogenean carp infestations thus serving as auxiliary bioindicator models.

Increased leukocyte (lymphocyte) counts observed in fish collected in County canal during spring sampling (highest overall Cr concentration 12.9 μg/L) were also consistent with increased parasitic loads and gill pathology. Summer samples showing overall decrease of lymphocites, alongside relative increase of granulocytes in the effluent canal, are also indicative of neutrophilia due to heavy metal (Pb and Cd) exposure. Increased numbers of granulocytes in the County canal in summer could suggest transient neutrophilia due to stressful conditions and increased parasite loads (Alvarez-Pellitero 2008).

Elevated heavy metal concentrations (Cr 7.7 μg/L, Pb 5.58 μg/L, Ni 31 μg/L, Zn 90 μg/L, Cu 270 μg/L) might have contributed to increase of some carp plasma metabolites in our study (TRIG, CHOL, ALB, URE, TP in the effluent fish in spring). Plasma CHOL significantly varied in fall (highest in effluent-receiving canal *vs.* lowest in downstream County canal). These parameters represent the main energy metabolites often used in estimation of fish health and condition and assessment of stressors in nature (Čož-Rakovac *et al.* 2005). Therefore, given the decline in these values, the immune suppression and the weakening of the innate, non-adaptive immune defense system of carp in the effluent-receiving waters in fall is very likely. However, since ALT and ALP activities were not elevated in the effluent-receiving canal, the deleterious effects might have been of transient nature, as semi-permeable plasma membranes prevented the leakage of enzymes from damaged (liver) tissues (Topić Popović *et al.* 2006). In spring, a significant decrease in SOD was noted in the fish from the effluent-receiving canal, which can be attributed to the inhibition of the superoxide radical formation implying the reduced ability to protect cells against superoxide radicals.

The severity of gill tissue alterations varied, and increased from spring till fall. Elevated ammonium levels in the effluent-receiving canal (spring 8.4 mg/L, fall 20 mg/L) might have reduced excretion and result in an uptake of ammonia into the tissues, not only gills, but also kidney and liver. Gills of effluent fish exhibited slight to severe changes including epithelial hyperplasia with lamellar fusion, epithelial hypertrophy, and proliferation of mucous cells. Liver is a target organ for lesions by pollutants due to its high metabolic activity in xeno-biotic metabolism, excretion, digestion and storage (Bernet *et al.* 2000). Liver changes in effluent and downstream fish in fall (sugar beet processing) in the form of distension of si-nusoids, blood congestion, leukocyte infiltration, blood cells by hepatocytes, anisokaryosis and picnotic nuclei suggest high hepatocytes metabolic activity in response to the uptake of heavy metals. Most prominent changes in renal tissues, observed in effluent fish in summer (the highest overall Fe concentration, 720 μg/L), were the increased number of basophilic clusters; while in fall (Fe 464 μg/L) newly developed nephrons prevailed, along with epithelial necrosis and atrophy, eosinophilic granules and vacuoles in cytoplasm.

CONCLUSIONS

The results indicate that fish biological responses related to the treated wastewater reach far downstream from the treatment plant and depend on season and/or activity of the sugar processing plant. Prussian carp under investigation demonstrated a high capacity to live in waters of diminished quality. The most prominent effects were on gill and liver cell damage and blood SOD activity, while CHOL and TRIG showed the strongest correlation with the season and sugar plant (beet processing) activity. Alterations of tissue morphology appear to be a consequence of loss of cellular and structural tissue integrity, with increasing concentrations of heavy metals in the environment. These changes can be correlated with the oxidative stress levels and neutrofilia. Integrated histopathological, haematological and biochemical findings, however non-specific to one particular agent, could serve as valuable biomarkers for native fish adaptive patterns and monitoring of water quality and pollution of freshwater ecosystems.

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STATUS OF NATIVE AND INTRODUCED FISH IN RIVER NERETVA RESERVOIRS (BOSNIA AND HERZEGOVINA)

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STANJE AUTOHTONIH I ALOHTONIH RIBA U NERETVANSKIM AKUMULACIJAMA (BOSNA I HERCEGOVINA)

Apstrakt

Izgradnjom brana rijeka Neretva je u cijelom slivu pregrađena što je dovelo do formiranja većeg broja umjetnih vodenih akumulacija. Glavni tok je pregrađen s četiri brane čime su formirane četiri akumulacije: Jablanička, Grabovica, Salakovac i Mostarska akumulacija. Na rijeci Rami je branom formirana velika Ramska akumulacija. U donjem dijelu toka je formirana velika Svitavska akumulacija i manje akumulacije u brdskom području (Vrutak, Mostarsko blato i akumulacija Nuga). Formiranjem akumulacija značajno su promijenjeni izvorni ekosustavi, čime su oni postali biološki izazov za prilagodnu autohtonih vrsta, ali i potencijal za razvoj ribarstva unošenjem novih, uglavnom šaranskih vrsta riba. Praksa unošenja novih vrsta, bilo ciljana, slučajna ili ilegalna, dovela je do značajnih promjena ekosustava akumulacija koje stalno traju. U sliv Neretve je tako unešeno ukupno 25 alohtonih vrsta slatkovodnih riba, bilo iz Dunavskog sliva ili drugih dijelova svijeta (Pavličević i sur., 2016; Glamuzina i sur., 2017), a novi nalazi se stalno bilježe (Dulčić i sur., 2017). U ovom radu će biti opisano stanje ribljih naselja u neretvanskim akumulacija u 2017 godini i uspoređeno sa stanjem zabilježenim prije formiranja ovih akumulacija. Istraživanje ribljih naselja u akumulacijama je obavljeno u 2017 godini, korištenjem ribarskih mreža i elektoragregata. Ribarske mreže su bile tipa troslojne prostrice duljine 300 metara i promjera oka mreže između 24 mm i 72 mm. Mreže su polagane u akumulacije navečer, a dizane ujutro slijedećeg dana. Ribe su identificirane do razine vrste, izmjerene ihtiometrom (mm) i izvagane digitalnom vagom (g). Podaci su statistički obrađeni programom Statistica i Excell. Struktura naselja riba u akumulacijama je pokazala dominantnu brojnost i učestalost alohtonih vrsta. Tako u Ramskom jezeru dominiraju šaran, *Cyprinus carpio*, babuška, *Carassius gibelio*, smuđ, *Sander luciperca* i belica, *Leucaspius delineatus*; u Jablaničkoj akumulaciji šaran, babuška, smuđ i sunčanica, *Lepomis gibbosus*. U Svitavskoj akumulaciji su dominantni linjak, *Tinca tinca*, babuška i sunčanica. Od autohtonih vrsta u akumulacijama su brojni samo klen, *Squalius cephalus* i sval, *Squalius svallize* koji u nekim akumulacijama poput Mostarske i Ramske imaju značajnu ulogu u ukupnom naselju riba. Od endemskih pastrmskih vrsta su rijetko lovljene samo velike jedinke mekousne pastrve, *Salmo obtusirostris* i glavatice, *Salmo marmoratus/dentex*. Većina novih vrsta su rezultat stalnih poribljavanja koji se provode i zakonskom obavezom elektroprivreda, ali i kao rezultat ilegalnih (smuđ, štuka, *Esox lucius*) i slučajnih (belica, sunčanica) aktivnosti. Mala brojnost endemskih pastrmskih vrsta nakon stalnih poribljavanja ukazuje na neučinkovitost ove tradicionalne mjere za ublažavanje posljedica formiranja akumulacija i rada hidroelektrana.

Ključne reči: *reka Neretva, brane, alohtone vrste, poribljavanje*

Abstract

River Neretva catchment is fragmented into several reservoirs by building of dams for energy production. Major flow is reconstructed with four dams forming four reservoirs: Jablanički, Grabovica, Salakovac and Mostarski reservoir. In tributary River Rama large reservoir was also formed. In lower Neretva area, several smaller reservoirs were also formed. Forming of reservoirs led to significant changes of natural ecosystems and this put native fish species in biological challenge to adapt or disappear. However, this change also present significant potential to develop commercial fishery of introduced species, mainly cyprinids. Practice of new species introductions, either targeted, accidentally or illegally, led to significant changes of reservoirs ecosystem. Total number of 25 non-native freshwater fish was introduced, both from River Danube catchment and worldwide (Pavličević et al., 2016; Glamuzina et al., 2017), while new fish are recorded on a yearly basis (Dulčić et al., 2017). This article will describe resent status of ichthyofauna of Neretva reservoirs in 2017 and compare it with historical data. Investigation of fish assemblages in reservoirs was execute during 2017 using fish gillnets and electrofishing device. Gillnets were three layers nylon type of 300 length and mesh size of 24 mm and 72 mm. Nets were deployed during the night, while electrofishing was executed during the morning in shallow coastal areas. Fish were identified to species level, measured with ichthyometer (mm) and weighted (g) with digital balance. Data were processed using Statistica and Excell software. Structure of reservoirs fish assemblages showed dominant number and frequency of non-native species. In Ramski reservoir dominant fish were carp, *Cyprinus carpio*, gibel carp, *Carassius gibelio*, pikeperch, *Sander luciperca* and belica, *Leucaspius delineatus*; in Jablanički reservoir carp, gibel carp, pikeperch and pumpkinseed, *Lepomis gibbosus* dominated. In Svitava reservoir dominant species were tench, *Tinca tinca*, gibel carp and pumpkinseed. Among native fish species in reservoirs, only chubs, *Squalius cephalus* and *Squalius svallize* adapted well to new conditions and in several reservoirs like Mostarski and Ramski dominated in fish assemblage. Native trouts were rarely caught in reservoirs and only bigger specimens of soft-mouth trout, *Salmo obtusirostris* and marble trout, *Salmo marmoratus/dentex*. Most

of the non-native species are results of stock enhancement practice which were conducted due to legal obligation of electro-companies, but also results of illegal (pikeperch, pike, *Esox lucius*) or accidental (belica, pumpkinseed) activities. Low number of endemic trouts which are objects of stock enhancement show low effectiveness of this traditional measure for mitigation of reservoir forming and work of hydro-power station consequences.

Keywords: *River Neretva, reservoirs, non-native species, stock enhancement*

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INTERSPECIFIC GERM CELL TRANSPLANTATION AS A NOVEL TOOL FOR CONSERVATION OF ENDANGERED TROUT GENETIC RESOURCES

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TRANSPLANTACIJA GERMINATIVNIH ĆELIJA KAO NOVA METODA U KONZERVACIJI UGROŽENIH PASTRMSKIH RIBA

Apstrakt

Transplantacija germinativnih ćelija potočne pastrmke *Salmo trutta m. fario* i lipljena *Thymallus thymallus* u kalifornijsku pastrmku *Oncorhychus mykiss* kao recipijenta je urađena sa ciljem očuvanja ugroženih populacija pastrmskih vrsta Balkanskog poluostrva. Trenutne strategije konzervacije se najviše oslanjaju na *in situ* konzervacione programe kao što su čuvanje čistih matičnih jedinki u izolovanim potocima i repopulacija određenih lokaliteta sa genetički čistim larvama. Međutim, uvođenje novih *ex situ* strategija je neophodne kako bi konzervacioni planovi mogli biti bolje sprovedeni. Početni koraci neophodni za transplantaciju kao što su izolacija velikog broja živih germinativnih ćelija i njihovo fluorescentno obeležavanje moraju se optimizovati pre samog procesa transplantacije. Izolovane i obeležene ćelije su intraperitonealno transplantirane u larve kalifornijske pastrmke stare u periodu tri do pet dana nakon izleganja. Preživljavanje injektovanih larvi je bilo slično preživljavanju netretiranih kontrolnih jedinki. 60 dana nakon transplantacije, fluorescentno obeležene ćelije su vizualizovane unutar gonada recipijenata što je ukazivalo na uspešnu inkorporaciju donorskih germinativnih ćelija (spermatogonije i oogonije potočne pastrmke – 27%; spermatogonije lipljena – 28%; oogonije lipljena – 23%). PCR amplifikacija fragmenata donorske mtDNK CR unutar gonada recipijenata je dodatno potvrdila uspešnu inkorporaciju donorskih ćelija. Transplantacija urađena u ovoj studiji predstavlja prvi korak ka aplikaciji tehnologije surogat produkcije potomstva kod pastrmskih vrsta Balkanskog poluostrva sa ciljem konzervacije i revitalizacije genetičkih resursa ugroženih i endemičnih pastrmskih vrsta.

Ključne reči: *spermatogonije, oogonije, transplantacija, Salmo trutta, Thymallus thymallus*

Abstract

Interspecific transplantation of germ cells from the brown trout *Salmo trutta m. fario* and the European grayling *Thymallus thymallus* into rainbow trout *Oncorhychus mykiss* recipients was carried out in order to improve current practices in conservation of genetic resources of endangered salmonid species in the Balkan Peninsula. Current conservation methods mainly include *in situ* efforts such as the maintenance of purebred individuals in isolated streams and restocking with purebred fingerlings, however additional *ex situ* strategies such as surrogate production are needed. Steps required for transplantation such as isolation of high number of viable germ cells and fluorescent labeling of germ cells which are to be transplanted have been optimized. Isolated and labelled brown trout and grayling germ cells were intraperitoneally transplanted into three to five days post hatch rainbow trout larvae. Survival of the injected larvae was comparable to the controls. Sixty days after transplantation, fluorescently labelled donor cells were detected within the recipient gonads indicating successful incorporation of germ cells (brown trout spermatogonia and oogonia – 27%; grayling spermatogonia – 28%; grayling oogonia – 23%). PCR amplification of donor mtDNA CR fragments within the recipient gonads additionally corroborated the success of incorporation. Overall, the transplantation method demonstrated in this study presents the first step and a possible onset of the application of the germ cell transplantation technology in conservation and revitalization of genetic resources of endangered and endemic species or populations of salmonid fish and thus give rise to new or improved management strategies for such species.

Keywords: *spermatogonia, oogonia, transplantation, Salmo trutta, Thymallus thymallus*

MICROALGAE AS FEED INGREDIENTS FOR ATLANTIC SALMON – AN UPDATE ON ONGOING RESEARCH AT NORD UNIVERSITY

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MIKROALGE U HRANI ZA ATLANSKOG LOSOSA – NOVOSTI O TEKUĆIM ISTRAŽIVANJIMA NA UNIVERZITETU NORD

Apstrakt

Makro i mikronutrijenti sadržani u novim morskim resursima kao što su mikroalge od interesa su za industriju hrane za ljude i životinje. Mikroalge mogu da predstavljaju održivu alternative za riblje brašno i riblje ulje. Mikroalge su primarni producenti morskih ekosistema i ovi mikroorganizmi sposobni za fotosintezu koriste svetlosnu energiju, CO_2 $_I$jone rastvorene u void za sintezu kompleksnih molekula koji čine njihovu biomasu. Biomasa mikroalgi je izvor protein I funkcionalnih sastojaka kao što su polinezasićene masne kiseline, polisaharidi, pigmenti, minerali, vitamini, enzimi i bioaktivni peptidi. Nekoliko varijeteta mikroalgi su široko komercijalno prihvaćene; koriste se u hrani za ljude i životinje. Industrija akvakulture bi, zbog održivosti, mogla da zavisi od ovog novog izvora kao sastojka hrane. Brašno I ulje od mikroalgi se smatraju više održivim nego sastojci, hraniva poreklom od suvozemne biljne mase zato što mikroalge brže rastu, proizvode veću koncentraciju nutrijenata po kg suve materije, imaju veliku fotosintetsku efikasnost i bolji kapacitet usvajanja CO_2, a imaju I sposobnost rasta na neobradivom zemljištu ili u jezerima.

Istraživanja mikroalgi su započela na Univerzitetu Nord 2009.godine radi ispitivanja potencijala obezmašćene biomase - koja je bila nus proizvod proizvodnje biogoriva – kao sastojak hrane za ribe i školjke. Od tada, pomoću nacionalnih i međunarodnih sredstava, ispitivali smo potencijal obezmašćenih (*Nanofrustulum* sp., *Desmodesmus* sp. i *Nannochloropsis* sp.) i celih mikroalgi (*Nannochloropsis* sp., *Tetraselmis* sp. i *Scenedesmus* sp.) za upotrebu kao sastojka hrane za vodene životinje. Naša istraživanja do sada su pokazala da mikroalge mogu biti obećavajuća alternative ribljem brašnu u umerenim količinama (Gong et al., 2018; Kiron et al., 2012; 2016; Sørensen et al., 2016; 2017). Naši trenutni napori su usmereni prema povećanju udela mikroalgi u hrani za Atlanskog lososa.

Da bi se razumeli efekti obrade mikroalgi ili dodataka hrani na iskoristivost mikroalgi predstavljeni su rezultati dva eksperimenta sa Atlanskim lososom. U prvom eksperimentu ispitivali smo prirast ribe hranjene obrađenim i neobrađenim mikroalgama (*Nannochloropsis* sp. ili *Tetraselmis* sp.). Prirast i iskoristivost riblje hrane na bazi ribljeg brašna, kao kontrola, poređen je sa ovim parametrima kod riba koje su hranjene hranom sa 30% jedne od gorepomenutih vrsta algi kao zamena osnovnih komponentti kontrolne hrane. U drugom eksperimentu ispitivali smo da li dodaci hrani mogu da povećaju svarljivost i iskoristivost *Nannochloropsis* sp od strane Atlanskog lososa.

Biće izloženi efekti obrade i upotreba aditiva u cilju bolje iskoristivosti mikroalgi.

Ključne reči: *alternative ribljem brašnu i ulju, hrana za ribe, losos*

Abstract

Macro and micronutrients in novel marine resources such as microalgae are of interest to the food and feed industry. Microalgae may represent sustainable alternatives to fishmeal and fish oil. Microalgae are primary producers in the marine ecosystem, and these photosynthetic microorganisms make use of light energy, CO_2 and dissolved ions in the water to synthesize complex molecules that constitute their biomass. Microalgal biomass is a source of protein and functional ingredients such as polyunsaturated fatty acids, polysaccharides, pigments, minerals, vitamins, enzymes and bioactive peptides. Several varieties of microalgae are gaining widespread commercial acceptance; they are used in foods and feeds. The aquaculture industry could depend on these new sources of feed ingredients to maintain sustainability. Microalgal meal and oil are perceived as more sustainable than terrestrial plant-derived feed ingredients because microalgae have the ability to grow faster, produce higher nutrient concentration per kg dry matter, have greater photosynthetic efficiency and better CO_2 capture capacity, and they have the ability to grow on non-arable land or in ponds. Research on microalgae was initiated at Nord University in 2009 to examine the potential of defatted biomass—derived as a co-product of biofuel production—as ingredients in the feeds for fish and shellfish. Ever since, through national and international funding, we have been examining the potential of defatted (*Nanofrustulum* sp*., Desmodesmus* sp. and *Nannochloropsis* sp*.)* and whole microalgae (*Nannochloropsis* sp., *Tetraselmis* sp. and *Scenedesmus* sp.) to be used as components in aquafeeds. Our research so far has shown that microalgae are promising alternates to fishmeal, at moderate inclusion levels (Gong et al., 2018; Kiron et al., 2012; 2016; Sørensen et al., 2016; 2017). Our current efforts are directed towards increasing the utilization of higher inclusion levels of microalgae by Atlantic salmon. Here we present the results of two experiments–to understand the effects of microalgae preprocessing or feed additives on microalgae utilization–that were carried out with Atlantic salmon. In the first experiment, we investigated the growth of the fish fed either pre-processed or non-processed microalgae (*Nannochloropsis* sp. or *Tetraselmis* sp.). The growth and feed utilization of fish fed a control fishmeal-based diet was compared with those of fish that received test diets that contained 30% of one of the above-mentioned algae types, which replaced the basal components in the control diet. In the second experiment, we examined if feed additives can increase the nutrient digestibility and utilization of *Nannochloropsis* sp. by Atlantic salmon.

Effect of pre-treatment and use of feed additives to improve utilization of microalgae will be presented.

Key words: *fish meal and oil alternative, fish feed, salmon*

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REARING OF LEECHES IN ABANDONED PONDS OF FISH FARMS AND SPECIALIZED REARING FACILITIES

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GAJENJE PIJAVICA U ZAPUŠTENIM RIBNJAČKIM JEZERIMA I SPECIJALIZOVANIM UZGAJILIŠTIMA

Apstrakt

Medicinske pijavice su jedna od najpoznatijih i najbolje proučenih vrsta beskičmenjaka, posebno u smislu njihove primene u medicini, biologiji i fiziologiji. Primena u medicini bila je poznata još za vreme Rimskog carstva, a nastavljena je i do današnjih dana zbog činjenice da sekreti medicinske pijavice sadrže malu «fabriku» biološki aktivnih supstanci.

Međutim, nekontrolisano i masovno prikupljanje pijavica u 19. veku, kao i smanjenje površina močvarnih staništa (njihovim potpunim ili delimičnim isušivanjima i pretvaranjem u poljoprivredna zemljišta, kao i uređenjem rečnih korita, čime se gube plavne zone), kao i zagađenje ovih vodenih staništa, izazvali su dramatičan pad brojnosti jedinki u populacijama dve vrste evropskih pijavica *Hirudo medicinalis i H.verbana* širom njihovih areala te im je opstanak doveden u pitanje.

Jedan od načina očuvanja, ali i održivog korišćenja ovih vrsta u medicini i biologiji, jeste akvakultura, to jest formiranje farmi pijavica i njihovo gajenje u napuštenim jezerima šaranskih ribnjaka, a koji odgovaraju karakteristikama prirodnih staništa pijavica (močvarama, barama...). Gajenjem ugroženih vrsta kakve su medicinske pijavice i njihovim kontrolisanim vraćanjem u prirodne ekosisteme vrši se zaštita i omogućava uključivanje ovih vrsta u lance ishrane vodenih ekosistemasa jedne strane, a istovremeno se obezbeđuje dovoljan broj visoko kvalitetnih pijavica za upotrebu u medicini. U Srbiji je, 2012. godine formirana prva farma za proizvodnju i prodaju medicinske pijavice «Hirudofarma MB» (Šabac). Pored ove, podnet je zahtev za formiranja još dve farme na teriroriji Srbije.

Ključne reči: *medicinske pijavice, gajenje, šaranski ribnjaci, farme pijavica*
Keywords: *medicinal leeches, rearing, fish farms, leech farms*

INTRODUCTION

The European continent is home to two species of medicinal leeches (*Hirudo medicinalis* Linnaeus 1758 and *Hirudo verbana* Carena 1820), which are typical dwellers of marshes, swamps, and eutrophic lakes with dense shoreline macrophytic vegetation and high summer temperatures (Elliott and Kutschera, 2011). In addition to these places, where they achieve their greatest abundance, they also live in lentic habitats of typical highland streams, where they are usually concealed under rocks covered with fallen leaves or overgrown with moss. In the mentioned aquatic ecosystems, they inhabit muddy and sandy substrates. In nature and under laboratory conditions, favourable water temperatures range from 22 to 25^{0}C for their growth, and from 22.5 to 27.5^{0}C for reproduction (Kutschera and Elliott 2014).

Medicinal leeches are strict haematophages and prefer the blood of mammals. Young individuals can also feed on the blood of insect larvae and smaller molluscs, as well as on that of amphibians and fish (Elliott and Kutschera, 2011). They feed infrequently, but take large quantities of the host’s blood, from 3 to 10 times more blood than their body weight (a leech of average weight in one meal sucks from 5 to 10 ml of blood), which is enough to keep it alive for a year and sometimes for a year and a half (Brajković, 2004).

Medicinal leeches are among the best-known species of invertebrates, and their biology and physiology have been thoroughly studied due to their being used for medical and therapeutic purposes. Their use in medicine is known from the time of the Roman Empire (the physician Galen mentions them already in the 2nd Century), while doctors in ancient Egypt considered leech treatment to be capable of working miracles and used leeches for a wide variety of purposes, from conventional bleeding to therapy for systemic diseases, including skin afflictions, problems of the urinary tract and reproductive system, and abscess teeth (Abdualkader *et al*., 2013).

Throughout history, there have been periods when leeches were used extensively, for example in France during the 19th Century, but there have also been periods when their use was prohibited and even a punishable offense. The reason for their use lies in the fact that the secretions of medicinal leeches represent a little “factory” of biologically active substances (Rigbi *et al*., 1987). Attaching themselves to the skin of a human being, leeches with their saliva inject into the blood more than 100 enzymes (hirudin, alpha-chymotrypsin, heparin, hyaluronidase, bufrudin, theromacin, theromizin, chymazin, suptilizin…) with different action. Because of the action of these substances, above all their anticoagulative and anti-inflammatory effects, they are used even today (Sohn *et al.,* 2001; Markwardt, 2002; Abdualkader *et al*., 2013). Medicinal leeches have found wide application in fundamental sciences because they are used as model organisms in neurophysiological and genetics research (Zapkuviene and Petrauskiene, 2000; Petrauskiene, 2001, 2008; Baskova and Isakhanyan, 2004; Utesky and Trontelj, 2005). They also have a significant role in microsurgery and reconstructive operations during transplantation of tissues and amputated parts of the body (fingers, auricles…) and after surgical operations (Whitaker *et al*., 2005; Knobloch, 2010; Abdualkader *et al*., 2013).

DISTRIBUTION OF MEDICINAL LEECHES AND THEIR STATUS

Two species of medicinal leeches (*H. medicinalis* and *H. verbana*) are distributed on the European continent. These two closely related species were often confused or both were designated “*Hirudo medicinalis*” until data on their life cycle and molecular analyses showed that they belong to different, reproductively isolated taxa (Kutschera, 2007a, 2007b; Siddall *et al.*, 2007), even though they inhabit the same types of aquatic ecosystems and have virtually the same medicinal significance. Such erroneous determination was also present on the territory of Serbia (Živić *et al.*, 2015), where identification of medicinal leeches was performed solely on the basis of morphological characters and certain findings of *H. medicinalis* (Marković, 1998; Živić, 2005; Simić *et al.*, 2007) in fact were referable to *H. verbana* (Živić *et al.*, 2015).

Intensive collection of leeches from nature in the 19th Century, which is continuing today (primarily in the countries of Southeast Europe and Turkey, from which they are placed on the West European market), has led to a dramatic decline in the abundance of individuals in populations of medicinal leeches (above all in populations of *H. medicinalis* throughout its range). Apart from collecting, the decline of abundance is also a consequence of loss of wetland habitats (due to complete or partial drainage and conversion to agricultural land, but also owing to loss of flood zones alongside rivers and embankment building, as well as increasingly intensive pollution of aquatic habitats).

On the territory of Serbia, the range of the medicinal leech (*H. verbana*) is fragmented and consists of habitats that are isolated from each other and have relatively small area, except in the province of Vojvodina (Živić *et al.*, 2015). However, massive and uncontrolled collecting for export from nature in the province (especially in the Srem and South Banat districts) has brought about drastic shrinkage of the range and abundance of this species on the territory of Serbia (Živić *et al.*, 2015). Due to inadequate education of the collectors, collecting is very often done during the period of reproduction (June-September), thereby leading to additional impoverishment of the populations in Serbia, since sexually mature individuals left no progeny.

DEGREE OF PROTECTION OF MEDICINAL LEECHES

The problem of erroneous identification of *H. medicinalis* and *H. verbana* brought with it the problem of adequate protection of those species because only the species *H. medicinalis* was awarded the status of a protected taxon on the international level (IUCN; CITES) and in Europe [Bern Convention and the Habitat Directive, which protect it in many European countries, above all the countries of Southeast Europe, although Utewsky *et al.* (2010) assert that its range is not confined to those regions]. Legal regulation of the status of leeches on the territory of Serbia exists only for the species *H. medicinalis*, which was declared a protected species (Official Gazette, No. 47/2011). Moreover, on the basis of an export quota of 500 kg annually (http://trade.cites.org/§), the responsible ministry of the Republic of Serbia issued a decree (Official Gazette, No. 24/2012) prohibiting the collection and export of medicinal leeches from natural populations (but not from nurseries or for scientific purposes). However, this decree applied only to the species *H. medicinalis* (which does not live on the territory of Serbia), not to *H. verbana.*

In view of the fact that *H. verbana* has much greater commercial significance than *H. medicinalis* (Utewsky *et al.*, 2010) and is today exported intensively from the two largest centres (Turkey and Russia), there is a need to place it under a certain degree of protection as soon as possible, both on the territory of Serbia and over its entire range, so as to prevent it from suffering the fate of many populations of *H. medicinalis* throughout Europe. In this connection, it should be noted that the species *H. verbana* in 2010 was mentioned in Appendix II of the CITES Convention (CoP15, Convention of Parties), although its status on the territory of Serbia has still not been legally regulated.

REARING OF MEDICINAL LEECHES

Due to ever increasing use in modern medicine and cosmetics and continuing decline in the abundance of natural populations, the demand for medicinal leeches in the past few decades has been constantly on the increase. A solution that suggests itself is the rearing of leeches in already existing facilities, i.e., **abandoned ponds of fish farms**, and in **artificial specialized rearing facilities (farms and laboratories)**.

That the use of such facilities represents a good solution is indicated by the fact that reproduction can in this way be increased up to 300-fold. To be specific, one leech in natural conditions usually gives from 1 to 8 cocoons and the number of eggs in cocoons varies from 12 to 16. Under laboratory conditions, on the other hand, one leech can produce from 3 to 30 eggs per cocoon (Kutschera and Elliott, 2014). The number of offspring that one leech gives in the course of a year can vary in a wide range from 5 to more than 200 individuals, depending on conditions of the habitat. Young specimens weigh from 0.12 to 0.18 g at the time of hatching, attain a weight of 0.5 to 0.6 g after one year of growth, usually weigh from 1.4 to 1.8 g at the end of the second year of life, and attain a weight of 2.4 g at the end of the third year (Kutschera and Elliott, 2014). However, leech farms on the other hand represent a danger because they make possible the introduction of many exotic species (from Turkey, for example) that can escape into natural habitats. In East Germany, small populations of the species *H. verbana* were found which could be the progeny of leeches which were disposed of by being thrown out into marshes and rivers after use in hospitals.

REARING IN ABANDONED PONDS OF FISH FARMS

Places where leeches are reared (marshes, canals alongside carp farms, abandoned ponds on carp farms, winter ponds, start-up ponds…) are formed so as to resemble their natural habitat, with aquatic plants and peat on the bottom of the basin (Figs. 1, 2, and 3). A muddy (peaty) bottom enables leeches to dig themselves into it easily when the temperature becomes too cold or warm. Aquatic plants ensure adequate amounts of oxygen in the water and protect the leeches, which most often hide among plants. The bottom of the pond can be covered with plastic foil, to which a thick layer of sludge and peat can then be applied. The water needs to be clean, not chlorinated, and similar to natural lake water (most favourable are water of quality class II, pH values close to neutral, and temperatures ranging from 22 to 25^{0}C). In the rearing facility, it is necessary to maintain a constant level of water (with a depth of 50 to 60 cm) using a system of intake/outlet canals or pipes. This is especially important during the period of reproduction in order to prevent sinking of cocoons if the water level rises. Care should be taken to prevent the pond’s shoreline vegetation from contacting and destroying deposited cocoons.

To prevent medicinal leeches from leaving the rearing facility, it should be completely surrounded by a wire fence just above the water level (the wire being stretched between metal or wooden support poles, see Figure 1, 2, and 3). Also, the pond/basin should be covered by a protective net so as to provide additional protection from birds that feed on leeches. The fenced-off rearing facility should be further protected by low-voltage electrical current (using “electric shepherdesses”) along the fence to prevent the leeches from leaving the pond. Such a prepared and protected rearing facility is stocked with the start-up school of leeches (the size of which is determined in accordance with the facility’s productive capacity). The start-up school is obtained from already existing rearing facilities or by collecting from nature after obtaining the required permits.

In the rearing of leeches (both those of the start-up school and young leeches), special attention must be paid to feeding them in such a way as to satisfy their needs for growth and reproduction. According to some authors (Dickinson and Lent, 1984), the best food for leeches is the blood of warm-blooded animals or human blood, but such feeding carries certain risks due to the possibility of spreading of viruses and bacteria from leeches to the host (Al-Khleif *et al.,* 2011).

Feeding with particles of fish, fresh pieces of horse meat or beef, and fresh blood (delivered in pork guts filled with blood from a slaughterhouse) should be avoided due to rotting of the meat and the risk of infection. For the indicated reasons, the best food consists of green frogs and fish (Prussian carp) lowered into the rearing facility in a cage or fishermen's net.



Figure. 1, 2 and 3. Fenced-in and covered natural facility for rearing of medicinal leeches.

"Harvesting" (collection) of leeches is done with hydrobiological benthos nets or even manually by collectors using their fingers to remove leeches clinging to their boots (Figure 4 and 5). Leeches weighing between 1 and 2 g are best for sale and are deposited in moist cotton bags measuring 15 x 30 cm (which hold about 1 kg of leeches). Leeches weighing about 2.4 g can be deposited in bags for sale or in most cases used for reproduction. The cotton bags are put in foam plastic boxes (70 x 60 x 30 cm) on whose bottoms water-soaked moss is employed to maintain humidity in the boxes.

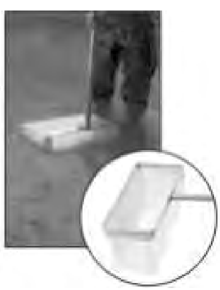


Figure 4 and 5. Collection of medicinal leeches by net and manually.

REARING IN SPECIALIZED FACILITIES (ON FARMS AND IN LABORATORIES)

Medicinal leeches are today successfully reared in specialized enclosed nurseries (**farms**) in many countries (Malasia, China, Turkey, Taiwan…). The farms are set up in different ways, depending on the wishes of their investors, but they all have in common a constructed barracks or residential building housing basins for rearing of leeches (Figs. 6, 7, and 8).



Figure. 6, 7 and 8. Barracks housing basins for rearing of medicinal leeches.

The basins can be of variable form (Figure 6, 7, and 8) and constructed of different materials: concrete, plastic, impregnated cloth, glass (aquariums). They are filled with sludge from a natural habitat, after which water is added. The water is changed/discharged through intake and outlet pipes so as to maintain a constant level (from 30 to 50 cm) and heated with the aid of a thermostat to the desired temperature (from 22 to 25^{0}C). Seeding with leeches is then performed, and the basins (which are marked to indicate those containing young specimens and ones holding leeches for reproduction or sale) are covered with gauze permitting circulation of air (Fig. 8) or even with a lid that is opened from time to time. Farmers are most often advised to rear leeches in water tanks (basins) since it is easy to find a suitable location for them, which saves time in construction of the farm and thereby ensures the lowest expenditures. It is recommended that farms always have one polyethylene basin holding about 500 “mother” leeches and that sites for egg laying be provided in it (in the guise of water lilies, duckweed, and other plants in whose roots eggs can be laid).

Many companies in France, Russia, England, and Germany have developed and optimized methods of rearing leeches under **laboratory conditions**. They hold certificates that cover the entire program, from rearing to selling of leeches. All of these phases are strictly controlled (for example, blood from poultry slaughterhouses possessing an ISO certificate is used for feeding).

In laboratories for rearing of leeches, glass jars filled with water (or moist soil) containing leeches in various phases of development are kept on high metal shelves in special rooms (Figure 9, 10, and 11). The water in the jars containing leeches should be dechlorinated and changed every 3-6 days, depending on the number of leeches in the jar. The laboratories also have specially designed “rooms for mating”, where the process of mating of leeches is monitored, as well rooms in which the whole life cycle is followed, from splitting of the cocoon full of young individuals to preparation of mature specimens for medicinal use.



Figure 9, 10 and 11. Rearing of medicinal leeches under laboratory conditions.

When they attain the desired size, leeches are starved for at least three months and after that transported and used for therapeutic purposes. Leeches ready for delivery are stored in glass jars (up to 50 leeches per 4-litre jar). Even though the leeches are reared under strictly controlled conditions, they are still sterilized before being packed for sale so as to remove symbionts that dwell on leeches in nature. Sterilization is done with sodium hypochloride, and the leeches are inspected by a veterinarian before every delivery. Moreover, modern companies (in France, Russia, and Germany) have optimized the technology of reproduction in such a way as to make it possible to obtain cocoons throughout the whole year by cultivation in special laboratories, thereby freeing the process of reproduction from the periodicity associated with the natural biological cycle of leeches, with the result that mating can occur whenever the market demand is high.

REVIEW OF THE PROCEDURE INVOLVED IN SETTING UP A REARING FACILITY AND THE ECONOMICS OF LEECH PRODUCTION

The rearing process itself entails a small investment and ensures a high profit. Rearing of leeches requires little space, it gives off no unpleasant odours, and the food (frogs, Prussian carp…) is inexpensive. Farm maintenance is minimal.

In Serbia, the first farm for production and sale of medicinal leeches, "Hirudofarma MB" (Šabac), was formed only after 2012, when the decree prohibiting collection and export of leeches from natural populations went into effect. This farm's production capacity is 350 kg annually. In addition to the indicated farm, permission for construction of two more farms with projected production capacities of 12 and 56 kg of leeches with average weight of from 1.5 to 2.0 g has been sought from the Nature Protection Institute of Serbia.

In order to obtain a permit from the Serbian Ministry of Environmental Protection, an investor who wants to farm leeches in specialized natural rearing facilities must present a detailed proposal developed by experts. This proposal should include analysis of the following factors: the type of substrate; proximity to roads and railways; protection of the facility from floods; chemical and physical parameters of water in the ponds/basins where the rearing process will take place; results of biological research on the diversity of surrounding plant and animal life; and information about the possible presence of predators. In addition, it should contain a blueprint of the proposed rearing facility, a production plan (size of the start-up stocking school, feeding details, harvesting schedules), and instructions for transport of leeches. As an integral part, the proposal must include accompanying documentation providing proof of ownership or rental of the parcel where the rearing facility will be located, in addition to the contract for construction of a rearing facility for production of medicinal leeches.

The expenses and profits of a leech farm will depend on the area and capacity of the facility for production. Initial expenses for the investor include those required for development of his proposal, installation of a wire fence around the facility (including the pouring of concrete for the support poles and their emplacement), and installation of a protective net (across the metal poles above the basin) in the event that the facility is an abandoned pond of an already established fish farm. If the planned facility is to be formed at a locality outside an existing fish farm, then the initial expenses will also include the cost of construction work (mechanical excavation of a basin of certain depth, removal and dispersion of the excavated soil, and formation of a dirt embankment around the basin). In addition to the indicated initial expenses, the investor must also make allowance for expenses incurred in the course of production: for feeding of the leeches (two or three times a year with frogs or fish), monitoring of their condition, and collection, packing, and transport of leeches to the buyer. Except for the first year (when he has to meet the costs of construction), the investor in other years has only to deal with the expenses of production. The leech producer can count on his first profit only after two years of rearing leeches, the period of time needed for his start-up stocking school to produce young individuals with average weight of 1.5 to 2.0 g, which is the age category most sought on the market.

In order to obtain an orientational idea of the financing needed to farm leeches, it is necessary to have information about certain important factors, namely the number of leeches that can be produced per unit of area and their financial value. The maximal production in our region is 2.5-3 kg of leeches per 100 m^2 or 250 to 300 kg per hectare of production area. One kilogram (500-600 individuals) of leeches in Serbia costs between 450 and 600 euros, i.e., the value of leeches is between 45,000 and 60,000 euros per hectare. Depending on supply and demand on the foreign market, this profit can be significantly greater, in view of the fact that one leech (weighing about 1.8 g) can cost as much as 5 euros. On the basis of what has been said, it is clear that the investment (including money spent on production) is returned very soon (in the course of up to four years), which naturally will depend primarily on the type of rearing facility and the farmer's ability to make contact with good buyers.

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OPTIMIZATION OF ARTIFICIAL REPRODUCTION OF WILD PIKEPERCH (*SANDER LUCIOPERCA*) UNDER CONTROLLED CONDITIONS – INFLUENCE OF TEMPERATURE ON FINAL OOCYTE MATURATION (FOM)

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OPTIMIZACIJA VEŠTAČKOG RAZMNOŽAVANJA SMUĐA (*SANDER LUCIOPERCA*) U KONTROLISANIM USLOVIMA – UTICAJ TEMPERATURE NA ZAVRŠNO SAZREVANJE OVOCITA (FOM)

Apstrakt

Smuđ *Sander lucioperca* (L.) je visokovredna vrsta sa velikim potencijalom za evropsku slatkovodnu akvakulturu (Blecha et al. 2015). Jedan od najproblematičnijih stadijuma proizvodnje je njegova veštačka reprodukcija (Kucharczyk et al. 2007; Muller-Bellecke and Zienert 2008; Blecha et al. 2015). Procedure dobijanja gameta visokog kvaliteta, oplođenje i uklanjanje lepljivosti rezultirale su visokom stopom izvaljivanja, preživljavanja embriona, kvaliteta larvi (Żarski et al. 2015). Međutim, za sada, neki elementi ovih procesa moraju biti bolje ispitani i unapređeni. Jedan od njih je povezanost između stadijuma sazrevanja ovocita, temperature i vremena ovulacije u poređenju sa kvalitetom ovocita (Kucharczyk et al. 2008). U ovom radu su predstavljeni rezultati 4 godine ispitivanja. Za to vreme su ispitivani različiti termalni uslovi: stabilna temperature, kao i fluktuirajuće vrednosti temperature (u opsegu od 9 do 17 °C). Utvrđena je veza između stadijuma zrelosti ovocita (opisano od strane Żarski i sar. 2012), temperature i latentnog perioda (vreme ovulacije). Promena temperature vode (fluktuacija) direktno je uticala na kvalitet ovocita. Rast temperature, naročito u opsegu 15-17 °C dovodio je do pada kvaliteta gameta, nekada i dramatičnog. Sa druge strane, opadanje temperature je obično dovodilo samo do produžavanja latent-

nog perioda. Procedura je primenjena tokom 2 sezone mresta na komercijalnim farmama. Pokazalo se da je moguće dobiti veliki broj jaja sa odličnim preživljavanjem embriona i visokim kvalitetom larvi

Ključne reči: *veštačka reprodukcija, ovocite, temperatura, vreme ovulacije*

Abstract

Pikeperch *Sander lucioperca* (L.) is a highly valued species with great potential in European freshwater aquaculture (Blecha et al. 2015). One of the most problematic stages of production is its artificial reproduction (Kucharczyk et al. 2007; Muller-Bellecke and Zienert 2008; Blecha et al. 2015). Obtaining high quality gametes, fertilization and unsticking-batch procedures resulted of high embryos survival rate, hatching rate and larvae quality (Żarski et al. 2015). But for now, some elements of these processes should be studied and improved. One of them is relationship between oocyte maturity stage, temperature and time of ovulation in comparison with oocyte quality (Kucharczyk et al. 2008). In present study, the results of 4 years study were presented. During this work, different thermal stable and fluctuated conditions (in range 9 to 17 °C) were tested. It was found a relationship between oocyte maturity stage (described by Żarski et al. 2012), temperature and latency (ovulation time). Water temperature changes (fluctuation) strictly affect to oocyte quality. Increasing temperatures, especially to level 15-17 °C caused on decreasing gametes quality, sometimes dramatically. On the other hand, decreasing temperature, usually only elongated latency time. The procedure was applied during 2 spawning seasons in commercial fish farm. It was shown that it is possible in mass scale obtaining huge number of pikeperch eggs with excellence embryo survival and high larvae quality.

Keywords: *artificial reproduction, oocyte, temperature, time of ovulation*

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IDENTIFICATION AND DETERMINATION OF DISEASE HAZARDS, RISKS, AND IMPACTS ON AN AQUACULTURE OPERATION

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IDENTIFIKACIJA I ODREĐIVANJE OPASNOSTI, NIVOA RIZIKA I UTICAJA BOLESTI NA RIBNJAKE

Apstrakt

Biosigurnosni programi su namenski dizajnirani i prilagođeni specifičnoj epidemiološkoj jedinici, ali takođe i za bolesti koje su (ili mogu biti) prisutne na tom specifičnom području (ribnjaku, zoni, regionu, državi…). Razmatranje bolesti koje se nikad neće pojaviti na vrsti koja se gaji (na primer, razmatranje posledica izbijanja neke od bolesti školjaka kada su u pitanju šaranski ribnjaci) ili bolesti kojih dokazano nema (ili ih ne može biti) u državi gde se nalazi ribnjak, ne spade u efikasno korišćenje raspoloživih resursa. S obzirom da je u najvećem broju slučajeva za pripremu i implementaciju biosigurnosnih programa potrebno odvojiti određena materijalna i nematerijalna sredstva (ljude, vreme, opremu, i ostale troškove), očekuje se da se biosigurnosni planovi fokusiraju na bolesti koje imaju najveći potencijal da negativno utiču na proizvodne kapacitete i operativne troškove ribnjaka. Ukoliko je potrebno, bolesti koje su egzotične ili se tek javljaju, je bolje obraditi u planovima za slučaj nužde. Sem toga, očekivano je da se situacija na ribnjaku menja tokom vremena, tako da je potrebno da se i biosigurnosni programi i planovi podvrgnu povremenoj reviziji. Tada se, u slučaju potrebe, mogu promeniti i bolesti od značaja za ribnjak.

Proizvodjači su u velikom broju slučajeva već dobro upoznati sa najznačajnijim bolestima koje izazivaju probleme na njihovom ribnjaku. Međutim, takođe je moguće da nisu sve od tih bolesti u kategoriji "ozbiljne potencijalne opasnosti". Tako da je neophodno da proizvodjač, zajedno sa nadležnim veterinarom, dobije informaciju od državne uprave (npr. veterinarskog instituta) nadležne za tu regiju o trenutnoj epizootioloskoj situaciji i bolestima od posebnog interesa za ribnjak. Kada se identifikuju sve bolesti koje predstavljaju postojeću ili potencijalnu opasnost za epizootiološku jedinicu, potrebno je odrediti konkretne opasnosti i posledice za svaku bolest na listi.

Više različitih pristupa se može koristiti kod kvalitativnog ili kvantitativnog procenjivanja rizika. U velikom broju slučajeva, polu-kvantitativni pristup je adekvatan za primenu u biosigurnosnim planovima vezanim za određeni ribnjak ili više ribnjaka u neposrednoj blizini. U OIE Zdravstvenom kodu za vodene životinje, rizici kod određenih bolesti su klasifikovani po kriterijumima koji omogućavaju da se pojedine bolesti evaluiraju i rangiraju po potencijalu da izazovu pad proizvodnje i prema potencijalu da se prošire na druge regije ili na divlje populacije. Dodatni kriterijumi su zasnovani na dostupnosti pouzdanih dijagnostičkih testova korišćenih za definitivnu dijagnozu bolesti koja se ispituje. Važno je napomenuti da bolesti koje jesu ili mogu biti od posebnog značaja za ribnjak, ne moraju da istovremeno ispunjavaju uslove da budu stavljene na OIE listu, kao i da svaka država, ili čak regija u okviru države, može da putem pravilnika ili zakona reguliše različite bolesti, kao i da zahteva upotrebu kontrolnih mera u zavisnosti od epizotiološke situacije i očekivanog ili stvarnog uticaja bolesti na lokalnu proizvodnju ili trgovinu.

Usled gore navedenog, svaka epizootiološka jedinica (država, region, ribnjak, itd) mora da bude evaluirana na osnovi njene specifične situacije, i potrebi da se identifikuje koje bolesti predstavljaju specifične opasnosti i imaju najveću verovatnoću da se pojave. Identifikacija i procena verovatnoće pojave određene bolesti se može izvesti putem metode profilisanja rizika. Koristeći relativno jednostavni polu-kvantitativni pristup, rizik od izbijanja bolesti na ribnjaku može se predstaviti kao verovatnoća pojave bolesti pomnoženoj sa težinom posledica u slučaju izbijanja bolesti (t.j. rizik = verovatnoća x posledice). Ovaj pristup nam omogućava da dobijemo objektivnu informaciju o riziku vezanom za specificnu opasnost (u ovom slučaju, opasnost bi bila zarazna bolest riba). Najvažniji elementi koji se koriste prilikom analize biosigurnosnih rizika i posledica su:

1. Odrediti koje bolesti su od značaja za, i mogu da ozbiljno ugroze zdravlje životinja u specifičnoj epizootiološkoj jedinici (ribnjaku).
2. Koristeci polu-kvantitativni ("težinski") pristup*, proceniti rizike i posledice svake bolesti kako bi se napravila prioritetna lista koja će se koristiti u biosigurnosnom planu; i,
3. Odrediti koje "nove" bolesti mogu biti od značaja za ribnjak i da li ih treba uvrstiti u biosigurnosni program ribnjaka.
4. Na osnovu kumulativnog skora (zbira numeričke vrednosti dodeljene riziku i posledicama) se izaberu najviše rangirane bolesti koje se zatim uključe u biosigurnosni plan.

*U kompleksnijim epizootiološkim jedinicama, (većim farmama/ribnjacima, kompartmanima, zonama, ili državama) je verovatno neophodno uraditi formalnu procenu rizika da bi se dobile bolje informacije neophodne za prioritizaciju i akcije kontrole i prevencije zaraznih bolesti.

Koristeći dostupne informacije (izveštaje o bolestima i epizootiološkoj situaciji) i ekspertna mišljenja o varijabilnosti i verovatnoći prenosa i posledica zarazne bolesti, proces procene rizika ce doprineti da se ostvari uvid u različite korake neophodne da bi se sprečio ulaz zaraze na ribnjak, ali istovremeno i sprečilo širenje bolesti sa ribnjaka. U toku ovog koraka prilikom pripreme biosigurnosnog plana, relativna "težina" svake identifikovane bolesti od značaja se određuje na osnovu iskustva proizvođača i veterinara, uz moguću konsultaciju sa kompetentnom državnom službom. Kada se obavi rangiranje i prioritizacija bolesti, sledeći korak je da se evaluiraju kritične tačke na ribnjaku ili u procedurama upravljanja gazdinstvom preko kojih bi prioritetne bolesti mogle da uđu ili izađu sa ribnjaka, kao i da se odluči koje bi bile najprimenljivije preventivne mere da se umanji rizik od introdukcije i transmisije

Evaluacija, ublažavanje (mitigacija) i ispravljanje (remedijacija) kritičnih kontrolnih tačaka (KKT): Saznanje kako bolesti mogu da uđu na ribnjak, i koje procedure ili rutine mogu da dozvole ulaz bolestima, je od izuzetne važnosti u prevenciji introdukcije i širenja bilo koje zaraze. Od jednake važnosti su procedure ili mesta koja bi mogla da dozvole zarazi da se proširi sa ribnjaka (prioriteti za bioiskljičivanje i biozadržavanje). Na nivou ribnjaka, primenom procesa evaluacije, ublažavanja, i ispravljanja KKT, smanjuje se odgovornost proizvodjača u slučaju širenja zaraznih bolesti, a u slučaju da su KKT protokoli u skladu ili akreditovani od strane nacionalnih programa biosigurnosti, postiže se usaglašenost sa sanitarnim i fitosanitarnim dogovorom Svetske trgovinske organizacije, i sa OIE standardima.

Sve bolesti (infektivni patogeni) koji mogu da uđu na ili izađu sa ribnjaka, su neizbežno povezani sa vektorima (životinjama ili ljudima) ili neživim objektima (fomitima). Vektori I fomiti se takođe smatraju kritičnim tačkama koje se mogu kontrolisati. Uopšteno, KKT pokazuju veliku raznolikost, i uključuju (ali ne samo): životinje, ljude, štetočine, ptice, vodu, hranu, opremu i veliki broj raznih ostalih stvari. Razvijene procedure i procesi za korekciju, ublažavanje ili eliminaciju KKT značajno doprinosi zaštiti epizootiološke jedinice (ribnjaka). Prioritizacija KKT u smislu koliko je lako ili tesko za bolesti ili patogene da uđu ili izađu sa ribnjaka, takođe pomaže prilikom prioritizacije korišćenja resursa. Važno je razmišljati u pravcu razvoja procesa ili procedura koje mogu da umanje (ili potpuno eliminišu) verovatnoću ulaska/izlaska bolesti ili patogena u ili iz ribnjaka, kao o polisi osiguranja koja štiti ribnjak, industriju ili državu. Nakon analize informacija dobijenih od: nacionalne procene rizika od zaraznih bolesti, preliminarnog upitnika popunjenog od strane proizvodjača, i sa prioritizovane liste rizika i opasnosti od bolesti specifičnih za taj ribnjak, sledeći ciljevi bi trebali da budu ostvareni:

1. Procedure, lokacije, vektori i fomiti (kritične tačke) gde bolesti mogu da uđu ili napuste ribnjak su identifikovane.
2. Nivo rizika za svaku kritičnu kontrolnu tačku je određen i prioritizovan, tako da i resursi za korekcije, ublažavanje ili eliminaciju rizika mogu biti adekvatno raspodeljeni.
3. Odeređeni su zadaci i aktivnosti neophodni za korekcije, ublažavanje ili eliminaciju kritičnih kontrolnih tačaka.

Krajnji rezultat gore navedenih aktivnosti u okviru pripreme biosigurnosnog plana je detaljna lista svih kritičnih tačaka (vektora, fomita, i procedura) preko kojih bi zarazne bolesti ili patogeni mogli da dospeju do, ili se raširе sa ribnjaka. Integralni deo biosigurnosnog plana je kompletna lista aktivnosti i preduzetih ili planiranih mera u cilju korekcije, ublažavanja ili eliminacije problema za svaki vektor, fomit, ili proceduru, to jest kritičnu tačku na ribnjaku. S obzirom da svaka aktivnost ili preduzeta mera može zahtevati dodatne resurse, preporučljivo je da se takođe: 1) donese odluka o tome koliko resursa (ljudskih, vremenskih, ili finasijskih) će biti potrebno da se ulozi, i 2) rangiraju akcije za svaki vektor, fomit i proceduru na osnovu nivoa rizika i resursa/troškova potrebnih da se uradi korekcija, ublažavanje ili eliminacija problema.

Ključne reči: *biosigurnosni programi, bolesti, epizootiološke jedinice*

Abstract

Biosecurity programs are best designed and tailored for a specific EpiUnit, and *for* diseases that are, or may be, encountered on the particular EpiUnit. Including diseases that will never be encountered in the animals used on the farm (i.e. considering mollusk disease on a finfish farm), or diseases that are documented not exist in the country where the farm is located, is not a necessary first step. In many cases, because developing and implementing biosecurity programs will require resources (typically personnel time, equipment, and cost), developing

biosecurity programs for those diseases that have the greatest potential to negatively impact the farm is a prudent approach. If necessary, emerging disease(s) found elsewhere are better addressed in contingency plans. Furthermore, because a number of issues change over time, overall biosecurity program will need to be periodically revised and new diseases can be addressed at that time.

The aquaculture producer is likely familiar with diseases that cause problems on the farm. However, it is possible that not all of those diseases are considered a "serious potential hazard". Therefore, producer and the attending veterinarian should obtain information from a regulatory authority of the region/country about the current epidemiological situation of important or reportable diseases that could be of concern to the farming operation. When a list of diseases that would be hazardous to the Epidemiological Unit (EpiUnit) is identified, their respective risks and impacts need to be determined.

A number of different approaches can be used to perform qualitative or quantitative risk assessments. In most cases semi-quantitative approaches are adequate for developing a farm-based biosecurity plan. In the OIE Aquatic Animal Health Code disease risks are classified based on several criteria so that each disease can be evaluated and ranked according to its potential to cause production loss and possible spread to other areas or animals in the wild. Additional criteria are based on the availability of reliable diagnostic tests used to definitively identify any disease in question. It is important to note that not all diseases of concern for every farm or EpiUnit fulfil the requirements for being listed as reportable diseases with OIE and that each country or local area might regulate diseases and require control measures depending on the epidemiology and the impact of a disease on their local production impact or trade situation. Therefore, each EpiUnit (country, region, farm etc.) has to be evaluated based on its own specific situation, and the need to identify which diseases pose specific hazards and have the highest probability of occurring. Such identification and probability estimation may be done by risk profiling. Using a relatively simple semi-quantitative approach, the risk of each disease to a farm can be defined through estimating the probability of it occurring and the consequences of the occurrence (i.e. risk = probability x consequences). This approach can provide objective information on the risk associated with a specific hazard (an infectious disease in this case). Important elements used in a biosecurity risk-impact analysis.

1. Determine what diseases might be hazards, and severely impact the EpiUnit;
2. Using a semi-quantitative (weighted) approach*, estimate the risks and impacts of each disease, in order to prioritize the diseases for inclusion in the specific biosecurity plan; and,
3. Determine what important "emerging" diseases might be considered in a biosecurity program for the EpiUnit.
4. Using cumulative scores (sum of risk and impact scores) select the highest ranking diseases to include in the biosecurity plan for this EpiUnit or farm.

*In more complex EpiUnits such as larger farms, compartments, zones, or countries/regions, a more formal risk assessment process is likely needed in order to provide better estimate of priorities and associated actions to be developed for disease control and prevention program.

Using available information (disease reports and epidemiological information available) and expertise including associated variability and uncertainty, the risk assessment process will provide insight on various steps needed to prevent the hazardous diseases being introduced or released from the farm or EpiUnit. During this step of developing a biosecurity program, the relative "weight" for each identified disease needs to be determined based on the experience of the producer and veterinarian, with possible input from the government. Once the disease hazards are prioritized, the next step would be to evaluate critical points on the farm or in the farms management where these diseases may enter or leave the EpiUnit, and decide on best applicable preventive measures.

Critical Control Point (CCP) evaluation, mitigation, and remediation: Identifying how diseases might enter the EpiUnit, and what procedures/policies might allow this to happen, are important to preventing the introduction (or spread) of any unwanted diseases. Equally important are procedures or places that might allow a disease to leave the EpiUnit (bioexclusion and biocontainment priorities). At farm level, applying the process of CCP evaluation, mitigation and remediation reduces farmer's liability for being responsible for spreading disease, and when associated with national aquatic biosecurity plan/program, it provides compliance with the SPS agreement and OIE standards.

Inevitably all diseases or infectious pathogens that can enter and leave the EpiUnit are associated with vectors (animals or people), or inanimate fomites (non-living objects). Vectors and fomites should also be considered as critical points that can be controlled. Overall, critical control points may be very diverse, and include (but are not limited to): animals, people, pests, birds, water, feed, equipment, and a large number of other things. Developing processes or procedures to correct, mitigate, or eliminate these, contributes substantially to protecting the EpiUnit. Prioritizing these in terms of how easy or difficult it will be for diseases or pathogens to enter or leave the EpiUnit, helps prioritizing where to allocate resources. It is important to think about developing processes or procedures that can reduce (or even eliminate) the probability of diseases or pathogens entering or leaving EpiUNit, as an insurance policy to protect the operation, or country. After evaluation the information obtained from the national risk assessment and preliminary producer/operation biosecurity questionnaire, as well as prioritized disease risk and hazard list that is specific for the epidemiological unit the following objectives need to be met:

1. Identify procedures or locations, and vectors and fomites (critical points) where diseases may enter or leave this farm;
2. Determine and prioritize the level or risk for each critical control point, so resources for correcting, mitigating, or eliminating risks, can be allocated; and
3. Determine what needs to be done to correct, mitigate or eliminate these critical control points.

A completed output of this step should include a detailed list of all Critical Points (vectors, fomites, and procedures) that might allow a disease or pathogen to enter or leave this EpiUnit. An integral part of documenting this in the biosecurity plan, is a list of actions or measures that have or will be implemented in order to correct, mitigate or eliminate the problem for each vector, fomite and procedure. As these actions may use or require additional resources it is also advisable to: 1) decide how much effort (manpower, time or money) will be required; and, 2) prioritize (rank) the actions for each vector, fomite and procedure based on the level of risk, and the resources needed to correct, mitigate or eliminate the problem.

Keywords: *biosecurity programs, diseases, EpiUnit*

BIOSECURITY IN ORGANIC AQUACULTURE: ADDITIVES TO PREVENT AND CONTROL DISEASES

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BIOLOŠKA SIGURNOST U ORGANSKOJ AKVAKULTURI: ADITIVI ZA SPREČAVANJE I KONTROLU BOLESTI

Apstrakt

Poslednjih godina postoji povećan interes za farmaceutsku upotrebu prirodnih supstanci, kao što su ulje origana i ulje ruzmarina, koja imaju značajno antimikrobno, antiparazitsko, antitumorno i antiinflamatorno dejstvo. Ulje origana je ranije korišćeno za tretiranje miksosporidija kod orade (*Sparus aurata*) i pica (*Puntazzo puntazzo*) (Athanassopoulou et al., 2004, b, Golomazou i sar., 2006). Korišćen je za lečenje *Aeromonas sobria* infekcija šarana (*Cyprinus carpio*) (Yiagnisis et al., 2009), i kao dodatak hrani za tretiranje mešovitih parazitskih i *Vibrio* spp. infekcija kod brancina (*Dicentrarchus labrax*) (Yiagnisis i sar., 2009). Ruzmarin je poznat po antioksidativnim i farmaceutskim svojstvima i koristi se kao začin u hrani. Dodavanje 0,5% ruzmarinovog se ulja koristi se kao faktor rasta kod živine. Nedavno je naša laboratorija izvršila istraživanje ulja origana, ulja ruzmarina i mešavine bilja, ulja i biljnih ekstrakata dopunjene hranom, kako bi proučili njihov uticaj na smrtnost i prevalenciju različitih infekcija:

1. Upotreba smeše esencijalnih ulja origana i ruzmarina od 0,5% u hrani za ribe je rezultirala smanjenjem mortaliteta nakon 10 dana kod brancina eksperimentalno inficiranog sa *V. anguillarum*. Istovremeno su korišćeni antibiotici (oksitetraciklin, sulfadiazin, trimetoprim, enrofloksacin) kako bi se uporedili efekti biljnih ulja i antibiotika.
2. Ulje origana je korišćeno kod brancina eksperimentalno inficiranog Nodavirusom. Cilj je bio da se ispita sposobnost ulja da smanji mortalitet uzrokovan virusom, kao i da se prouči prirodni i stečeni imunitet riba. Rezultati su bili veoma ohrabrujući.
3. *Artemisia annua* eksperimenti u cilju eliminacije ektoparazitoza morskih riba. *Artemisia annua* je korišćena od strane drugih autora u *in vitro* i *in vivo* eksperimentima sa oradom inficiranom sa *Sparicotyle chrysophrii.*

4. RepaXOL sa 22% koncentracijom specifične mešavine eteričnih ulja, karvrakola, citrusnih ulja, kapsikuma i cinamaldehida i dodatnih biljnih ekstrakata. Preparat se koristi kod živine za tretiranje crevnih protozoa. Koristili smo ga kod 20g orade kako bismo procenili njegov uticaj na *Microcotyle sp.* i *Furnestinia sp.* ektoparazite, crevne parazite i *Enteromyxum leei* koji uzrokuju probleme u akvakulturi. Rezultati su obećavajući kod crevnih parazita, prevalenca je smanjena za 25%, a mortalitet za 35%. Stopa konverzije hrane bila je vrlo dobra.

Ključne reči: *ulje ruzmarin, oregano, mešavina bilja, orada, delovanje i upotreba u organskom uzgoju*

Abstract

In the recent years there is an increased interest for the pharmaceutical use of natural substances such as oregano oil and rosemary oil that have important antimicrobial, antiparasitic, antitumor and anti-inflammatory properties. Oregano oil has already been used for treatment of Myxosporean parasites in sea bream (*Sparus aurata*) and sharp snout sea bream (*Puntazzo puntazzo*) (Athanassopoulou et al., 2004 a,b; Golomazou et al., 2006). It has been used in the treatment of *Aeromonas sobria* infections in carp (*Cyprinus carpio*) (Yiagnisis et al., 2009). In sea bass (*Dicentrarchus labrax*) as a dietary supplement against mixed infections of parasites and *Vibrio* spp. (Yiagnisis et al., 2009). Rosemary is well known for its antioxidant and pharmaceutical properties and is used as aromatic in food. Addition of 0.5% of its oil has been used as a growth factor in chicken.

Recently our lab has carried out research of oregano oil, rosemary oil and mixed herbs, oils and plant extracts supplemented in food in order to study their effect on mortality and prevalence of various infections:

1. The use of a mixture of essential oils of oregano and, rosemary at 0.5% in feed had as result the decrease of mortality in 10 days in sea bass experimentally infected with *V. anguillarum.* In the same time antibiotics were used (oxytetracycline, sulphadiazine, thrimethoprim, enrofloxacin) in order to compare the effects of the plant oils and the antibiotics.

2. Oregano oil was used in experimentally infected sea bass with Nodavirus. The aim was to investigate the ability of the oil to decrease the mortalities caused by the virus and also to study the natural and acquired immunity of the fish. Results have been very encouraging.

3. *Artemisia annua* trials for the elimination of ectoparasitosis of marine fish. *Artemisia annua* has been used by other authors in vitro and in vivo experiments of seabream infected with *Sparicotyle chrysophrii.*

4. RepaXOL contains a 22% concentration of a specific blend of essential oils, of carvracol, citrus oils, capsicum and cinnamaldehyde and additional plant extracts. It is used in poultry for intestinal protozoa are present such as blackhead. We used it in 20g seabream in order to assess its effect on the ectoparasites *Microcotyle sp.* and *Furnestinia sp.*, common flukes and for *Enteromyxum leei* parasites that cause problems in aquaculture. The results were promising for the enteric parasite, prevalence was reduced by 25% and mortality by 35%. The food conversion rate was very good.

Keywords: *oregano oil, rosemary oil, mixed herbs, sea bream, effects and use in organic culture*

ANTIMICROBIAL RESISTANCE AND TREATMENT OPTIONS IN BIOSECURE AQUACULTURE

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REZISTENCIJA MIKROORGANIZAMA U BIOSIGURNOJ AKVAKULTURI: ALTERNATIVNI I DOPUNSKI TRETMANI

Apstrakt

Akvakultura je industrija koja se ubrzano razvija od 1970. godine i postaje izuzetno važan izvor hrane za ljude. Uprkos svim zabranama, u nekim delovima sveta, antibiotici značajni za upotrebu u humanoj medicini i dalje se rutinski primenjuju u hrani za životinje kako bi se povećao profit i sprečile potencijalne bakterijske infekcije koje mogu nastati kao posledica stresa usled prenaseljenosti u ribnjacima. Na taj način antibiotici se puštaju u prirodne ekosisteme, gde mogu imati uticaj na populaciju bakterija u okruženju. Trenutne količine upotrebljenih antimikrobnih sredstava u akvakulturi širom sveta nije lako odrediti, jer različite zemlje imaju različite sisteme distribucije i registracije upotrebe antibiotika.

Svi ranije otkriveni mehanizmi rezistencije bakterija na antibiotike identifikovani su i među bakterijama koje potiču od riba. Velika pažnja je posvećena nalazu bakterija otpornih na antibiotike koji se koriste samo kod ljudi. Posledice primene antibiotika u akvakulturi nisu u potpunosti objašnjene, ali ako uzmemo u obzir sve faktore i načine širenja bakterija rezistentnih na antibiotike, u epidemiologiji rezistencije, riba i vode ribnjaka mogu biti najopasniji izvor gena rezistencije za ljude i druge vrste životinja. Rizik za ljude i druge vrste životinja sastoji se od mogućeg horizontalnog transfera gena rezistencije sa bakterija tipičnih za ribe (*Aeromonas hidrophila*, *Aeromonas salmonicida*, *Vibrio* spp., *Stenotrophomonas maltophilia*, *Pseudomonas fluorescens*) na neke humane patogene (*E. coli*, *Pseudomonas aeruginosa*) ili u direktnoj infekciji ljudi i životinja sa multirezistentnim patogenim bakterijama tipičnim za stanište riba (*Vibrio, Salmonella, Shigella*).

Iako se moderna akvakultura više oslanja na vakcinaciju i poboljšanje upravljanja ribnjacima kako bi se izbegle infekcije, još uvek postoji mnogo bakterijskih infekcija riba koje se tretiraju antibioticima u hrani i kupanjem. Najčešće korišćeni antibiotici su: fluorohinoloni, florfenikol, oksitetraciklin i sulfonamidi.

Povećanje nalaza bakterija rezistentnih na antibiotike i rezidua antibiotika izaziva globalnu zabrinutost. Postoji potreba za razvijanjem alternativnih terapija bakterijskih infekcija u akvakulturi. U cilju zaustavljanja i kontrole pojave antimikrobne rezistencije predlaže se nekoliko rešenja koja imaju za cilj razvoj održive akvakulture, kao što su ona koja uključuju terapiju bakteriofagima, korišćenje probiotika i esencijalnih ulja za povećanje imunog statusa riba, kao i usvajanje mera koje mogu da garantuju brzo smanjenje rezidua antibiotika u životinjskom otpadu.

Potrebno je uložiti globalni napor da se prekine preterana upotreba i/ili zloupotreba antimikrobnih sredstava u akvakulturi i podstaknu zainteresovane strane da usvoje druge mere za sprečavanje bolesti.

Ključne reči: *akvakultura, antimikrobna rezistencija, riba, mehanizmi rezistencije*

Abstract

Aquaculture has been rapidly growing industry since 1970' and is becoming extremely important source of food for human consumption. Despite all bans, in some parts of the world, antibiotics important for use in human medicine are still routinely delivered in feed to feed animals in order to increase profit and prevent potential bacterial infections that could be caused by stress due to overcrowding in fish farms as well as in ponds ecosystems. As consequence of this action, antibiotics are released into natural ecosystems, where they can have impact on the environmental bacterial populations. Current levels of antimicrobial use in aquaculture worldwide are not easy to determine because different countries have different distribution and registration systems.

All previously discovered mechanisms of resistance have also been identified among bacteria originating from fish. Great attention is drawn to the results of the discovery of bacteria originating from fish resistant to antibiotics that are used only in humans. Thus, the consequences of antibiotics application in aquacultures are not partially perceived, but if we take into consideration all factors and ways of spreading resistance on antibiotics with bacteria, in epidemiology of resistance, fish and waters of fishponds can be the most dangerous source of resistance gene for people and other types of animals. The risk for people and other types of animals consists of possible horizontal transfer of resistance gene from bacteria typical for fish (*Aeromonas hydrophila, Aeromonas salmonicida, Vibrio* spp., *Stenotrophomonas maltophilia, Pseudomonas fluorescens*) to some humane pathogen (*E. coli, Pseudomonas aeruginosa*) or in direct infection of people and animals with multi-resistant pathogen bacteria typical for fish habitat (*Vibrio, Salmonella, Shigella*).

Although modern aquaculture is more relying on vaccination and improvement of management to avoid infections, there are still many bacterial fish infections that are treated with antibiotics in feed and by baths. The most used antibiotics are: fluorochinolones, florfenicol, oxytetracycline and sulphonamides.

The rise in bacterial antibiotic resistance and antibiotic residues has become global concern, and there is a need to develop alternative therapies for bacterial pathogens in animal production, especially in aquaculture. In order to contain and manage the emergence of antibiotic resistance, several solutions can be suggested, that aim at the development of sustainable aquaculture practices, such as those including the use of probiotics, phage therapy and essential oils to increase fish immune status as well as the adoption of measures able to warrant the fast abatement of antimicrobial residues in animal wastes. Global effort must be made to cease antimicrobial overuse in aquaculture and encourage stakeholders to adopt other disease prevention measures.

Keywords: *aquaculture, antibiotic resistance, fish, resistance mechanisms*

AUDITING, ASSURING AND CERTIFYING DISEASE FREEDOM OF AQUACULTURE OPERATIONS

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INSPEKCIJA I SERTIFIKACIJA BIOSIGURNOSNOG STATUSA RIBNJAKA

Apstrakt

“Kako da dobijem nezavisnu potvrdu o zdravstvenom statusu mog ribnjaka?’. Primarni cilj implementacije biosigurnosnog plana ili programa je da se uspostavi i održava visoka verovatnoća da je određena epidemiološka jedinica (ribnjak, farma, zona, država, itd.) slobodna od specifičnih bolesti, to jest da nije inficirana sa specificnim patogenima. Utvrđivanje da je ribnjak slobodan od specificnih patogena ili bolesti (SPF) ima očigledne prednosti. U komercijalnoj akvakulturi, prednosti mogu biti povecana proizvodnja, uvećana ekonomska vrednost proizvoda, kao i značajna prednost u trgovini.

Razvijanje, implementacija, pregled i sertifkacija bilo kog sveoubuhvatnog biosigurnosnog programa je relativno kompleksno i uključuje određene troškove. Odnos troškova i dodate vrednosti treba pažljivo proceniti, što je od velike važnosti za veliki broj malih i srednjih ribnjaka koji čine značajan deo svetske akvakulture. Inicijativa za implementaciju, pregled i sertifikaciju biosigurnosnih programa može poteći od industrije ili od državne uprave, ali neizbežno je da najpraktičniji i najizvodljiviji okvir za biosigurnosne programe uključuje državno-privatnu saradnju i zajedničko učešće u troškovima razvoja programa. Globalno se razvija nekoliko dobrovoljnih programa za sertifikaciju biosigurnosti ili zdravstvenog statusa koji su bazirani na privatnim inicijativama a imaju implikacije u biosigurnosti ribnjaka: SPF program za morske škampe (SAD), Akvakultuni dijalozi (Svetski fond za zaštitu životinja, WWF), Globalno Partnerstvo za Najbolje poljoprivredne metode (GLOBALGAP), kao i Najbolja praksa u akvakulturi (Globalna alijansa za akvakulturu, GAA BAP).

Trenutno, ni jedna šema za dobrovoljnu biosigurnosnu sertifikaciju nije potpuno integrisala principe veterinarske biosigurnosti, kao ni pristupe i infrastrukturu neophodnu da bi se efikasno ostvarili ciljevi biosigurnosnih programa. Inspekcija biosigurnosnih procedura i dokumentacije, kao i utvrđivanje odsustva određenih bolesti (zdravstvenog

stanja) bi trebalo da se obavi putem kredibilne, iskusne i nezavisne "treće strane", usled toga što su sve navedene dužnosti oko izdavanja potvrde o zdravstvenom stanju praćene zakonskom i profesionalnom odgovornošću. Zbog gore navedenog, izdavanje potvrde o zdravstvenom stanju (često nazivana i inspekcijskim, karantinskim procedurama ili sertifikatima zdravlja) je tipično obaveza jedne ili više osoba (veterinara, ili neke druge kvalifikovane i iskusne osobe) koja:

1. Ima kompletan uvid i znanje o svim biosigurnosnim procedurama na ribnjaku.
2. Poseduje stručnost da pregleda i potvrdi da se biosigurnosni zadaci izvršavaju i naročito da je u stanju da ispita svu dokumentaciju i zapise, uključujući tu i rezultate dijagnostičkih testova.
3. Ima licencu/akreditaciju od strane odgovarajuće državne institucije (veterinarske službe ili odgovarajućeg ministarstva) zadužene za brigu o zdravsvenom stanju životinja; i
4. Da ima pravnu i materijalnu odgovornost za tačnost izdatih potvrda ili sertifikata.

U pojedinim zemljama, organi državne uprave nisu u mogućnosti da zaposle dovoljan broj veterinara za vršenje gore navedenih dužnosti. U takvom slučaju, veterinari iz privatne prakse mogu da prođu kroz državni program za akreditaciju ili licenciranje i dobiju pravo da vrše kontrolu zdravstvenog stanja i izdaju potvrde u ime državnih agencija. U Evropi se takva stručna lica zovu "zvanični veterinari" dok se u SAD zovu "akreditovani veterinari". U slučaju da su životinje ili proizvodi životinjskog porekla, namenjeni izvozu u druge zemlje, potvrde o zdravstvenom stanju (cesto nezvanično nazivani i "zdravstveni sertifikati") moraju da budu izdati ili overeni/potvrđeni od strane državnog upravnog organa nadležnog za veterinu odnosno zdravlje životinja.

Validacija, verifikacija (kontrola) i utvrđivanje da su biosigurnosni programi i planovi pripremljeni i u upotrebi, su vrlo često u osnovi procedura za izdavanje veteirnarskih sertifikata. Biosigurnosni sertifikati izdati od strane veterinara iz privatne prakse koji su dodatno pregledani odobreni od strane odgovornog državnog organa (npr. službe za veterinu) nadležnog za zdravlje vodenih životinja (ili za akvakulturu), na taj način postaju zvanična potvrda da su ciljevi biosigurnosnog plana ispunjeni. Od velike je važnosti da je dovoljan broj akreditovanih veterinara ili paraveterinara na raspolaganju industriji i državnim organima, kako bi zdravstvena zaštita ribljeg fonda u akvakulturi bila u skladu sa direktivama nacionalnih i međunarodnih organizacija i samim tim olakšala promet robe i usluga u međudržavnoj trgovini. U pojedinim zemljama kapaciteti veterinarske službe, uključujući i kapacitet za zdravstvenu zaštitu vodenih životinja u akvakulturi, mogu da se evaluiraju putem "OIE PVS" ili "OIE PVS AQUATIC" procesa. U tom slučaju, postojanje državnog sistema za akreditaciju veterinara za obavljanje poslova izdavanja biosigurnosnih potvrda za akvakulturu bi značajno povećalo kapacitete države da podrži rast akvakulture i međunarodne trgovine.

Ključne reči: *razvijanje, implementacija, sertifkacija, biosigurnost*

Apstract

"How do I get third-party recognition of disease freedom?" The primary objectives of implementing a biosecurity plan or program is to establish and maintain a high level of assurance that a selected epidemiological unit (tank/pond, farm, zone, country, etc.) is not diseased, or infected with specific infectious and contagious pathogens. Certifying an

operation, or other epidemiological unit, as specific pathogen free (SPF) offers a number of obvious benefits and advantages. For commercial aquaculture advantages include greater animal production, increased economic value of saleable products, and a distinct trade advantage.

Developing, implementing, auditing and certifying any comprehensive biosecurity program may be complex and therefore comes at a cost. The benefits and costs need to be carefully assessed and this assessment is of particular importance for the large number of resource-limited, small-scale producers who make a considerable share of the world's aquaculture production. Implementing, auditing and certifying biosecurity programs may be driven by industry and/or governmental needs, but inevitably the most palatable, feasible and practical will be those that involve government-industry partnerships and cost-sharing. Several voluntary, industry-driven biosecurity or certification programs are evolving that may have some application to animal health and biosecurity: U.S. Marine Shrimp Farming SPF Program; World Wildlife Fund-led Aquaculture Dialogues; Global Partnership for Good Agricultural Practice (GLOBALGAP); and Global Aquaculture Alliance's Best Aquaculture Practices (BAP) certification standards.

Currently, no voluntary biosecurity certification schemes have fully integrated the veterinary biosecurity principles, approaches and infrastructure required to effectively meet biosecurity objectives. The auditing of biosecurity procedures and records, and certifying disease-freedom for any specific pathogen needs to be undertaken by a credible, knowledgeable and experienced independent third-party, as these responsibilities are accompanied by legal and professional liability. Therefore, certifying disease or pathogen freedom (often referred to as "health certification, "quarantine," or "inspection" procedures) is typically required to be done by one or more individuals (the attending veterinarian, or other knowledgeable and experienced individual), that:

1. Have full knowledge of all biosecurity procedures implemented on the farm;
2. Is able to audit and verify these biosecurity processes are in place and, in particular, examine all documentation and results of diagnostic tests;
3. Is given the authority to certify disease/pathogen-freedom by the governmental regulatory agency (veterinary or competent authority) that has jurisdiction over animal health; and,
4. Is held accountable for the accuracy of his/her certification.

Some countries or government agencies are unable to employ sufficient numbers of veterinary or other personnel to perform this work, and have developed programs to accredit or approve private practice veterinarians who are allowed to perform animal health audits and certify disease/pathogen freedom on behalf of the government agency. In Europe, these veterinarians are referred to as "Official Veterinarians"; other countries give them the "Accredited Veterinarian" designation. However, when animals or animal products are moved between countries the Certificates of Veterinary Inspection (often called "health certificates"), require endorsement by a National government official from the agency with responsibility over animal health (veterinary or competent authority.

Validation, verification (auditing) and certification that effective biosecurity plans or programs have been developed are also the basis for issuing Certificates of Veterinary Inspection, often referred to as "health certificates". When endorsed by the government agency with regulatory authority over aquatic animal health (competent authority), biosecurity certificates provide the official credibility that the primary biosecurity objectives

have been met. Having a sufficient experienced and credentialed workforce to provide these services to support aquaculture is imperative, particularly if done with government oversight or involvement and if used for international trade purposes. Evaluating this workforce capacity using the OIE PVS Tool may be warranted in some countries, and a system for competent authorities to accredit this workforce would substantially expand national capacities to support aquaculture industry growth and trade.

Keywords: *developing, implementing, certifying, biosecurity*

BIOSECURE AQUACULTURE: CHALLENGES OF TRADING AND MOVEMENT OF ANIMALS, COMMODITIES AND BIOMASS

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IZAZOVI ODRŽIVE AKVAKULTURE: OPASNOSTI POVEZANE UZ TRGOVANJE I PREMJEŠTANJE ŽIVOTINJA, ROBA I BIOMASE

Apstrakt

Zemlje uključene u međunarodnu trgovinu obvezne su opravdati eventualne restrikcije trgovanja temeljene na mogućim izazovima po zdravlje ljudi, životinja ili biljaka. Velik je broj zemalja potpisnika međunarodnih trgovinskih sporazuma koji uključuju odredbe o zaštiti biodiverziteta koji bi se mogao narušiti unošenjem ili premještanjem raznih akvatičnih vrsta. Priroda svjetske trgovine akvatičnim životinjama takva je da je gotovo nemoguće da svaka zemlja obavi detaljnu zdravstvenu kontrolu ili karantenu svih uvezenih životinja. Očekivani rast prometa robom i biomasom riba i rakova za sve veću populaciju ljudi, u budućnosti će trebati podržati odgovarajućim rastom akvakulturne proizvodnje. Nebrojena količina akvatičnih životinja godišnje se premješta iz raznih razloga, od kojih su najznačajniji trgovina ukrasnim ribama i razvoj akvakulture. Gotovo sva premještanja roba podrijetlom od akvatičnih životinja uključuju elemente ekološke, patogene ili genske opasnosti. Istovremeno, razvoj održive akvakulture suočava se sa brojnim izazovima. Rizici u akvakulturi mogu se odraziti na financijsko i gospodarsko dobrostanje, zdravlje ljudi, fizičkog i biološkog okoliša. Stoga integrirani pristup analizi rizika pomaže sektoru akvakulture u smanjivanju opasnosti, a istovremeno može pomoći u zaštiti okoliša i društva od nepoželjnih i nepredvidljivih utjecaja. To može voditi do povećane profitabilnosti i održivosti sektora, a istovremeno i poboljšati sliku javnosti o akvakulturi kao odgovornoj i održivoj aktivnosti koja vodi računa o okolišu.

Ključne reči: *trgovinski sporazum, biodiverzitet, akvakultura*

Abstract

With the liberalization of international trade, countries need to justify any restrictions on international trade based on challenges to human, animal or plant health. Most countries are also signatories of one or more international agreements that include provisions for the protection of biodiversity from the impacts of ill-considered introductions and transfers of aquatic species. The nature of world trade in aquatic animals is such that it is impossible for most countries to conduct detailed health inspections or quarantine all the animals being imported. Future growth in commodities and biomass of fish and shellfish for an expanding human population will have to be met by continued aquaculture growth. Uncountable numbers of aquatic animals are moved annually for a wide variety of reasons, the ornamental trade and aquaculture development being the two most important. Almost all movements of aquatic animal commodities involve an element of ecological, pathogen or genetic hazard. Also, sustainable aquaculture development faces many challenges, and these can be viewed from various perspectives depending on the individual stakeholder. Aquaculture risks are manifold and can be reflected to financial and economic well-being, to human health, to social well-being, to the physical environment, to the biological environment etc. An integrated approach to analysis of challenges will assist the aquaculture sector in reducing hazards to successful operations from both internal and external ones and can similarly help to protect the environment, society and other resource users from adverse and often unpredicted impacts. This could lead to improved profitability and sustainability of the sector, while at the same time improving the public’s perception of aquaculture as a responsible, sustainable and environmentally friendly activity.

Keywords: *agreements, biodiversity, aquaculture*

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EMERGING FISH DISEASES IN SERBIAN AQUACULTURE

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NOVE I PRETEĆE VIRUSNE BOLESTI RIBA U SRBIJI

Apstrakt

Na svetskom nivou, intenzivna akvakultura je najbrže rastući sektor za proizvodnju hrane životinjskog porekla. Šta više, to je jedini sektor koji raste brže od ljudske populacije i obezbeđuje značajnu dopunu i zamenu izlovljavanju riba iz otvorenih voda. Na žalost ovaj trend je praćen pojavom brojnih bolesti riba na globalnom nivou, što je dovelo do velikih ekonomskih gubitaka u akvakulturi i pretnji populacijama riba u otvorenim vodama. Ekspanzija akvakulture, koja se u velikoj meri oslanja na trgovinu i promet riba, uključujući i vrste koje ranije nisu korišćenje u akvakulturi je dovela i do istovremenog širenja patogena. U poslednjih nekoliko godina u populacijama riba u Srbiji otkriveno je nekoliko novih bolesti, a uočena je i ponovna pojava ranije prisutnih bolesti. U radu su prikazane nove i preteće bolesti riba sa kojima se suočava akvakultura u Srbiji i primena raspoloživih mera kontrole.

Ključne reči: *akvakultura, bolesti, ribe*
Keywords: *Aquaculture, fish, emerging disease*

INTRODUCTION

Increased aquaculture production is accompanied by increased disease risk and emerging disease epizootics which frequently cause substantial mortalities among populations of fish in aquaculture and threats to valuable stocks of wild fish. The aquaculture is the only food-producing sector (food of animal origin) growing faster than the human population and provides an acceptable supplement to fish catching and substitute for wild fish. Intensive growth and inability to precisely control the aquatic environmental parameters brings specific challenges unlike any other intensive animal production system. Expansion of aquaculture production and associated changes in production systems, in particular the trend toward high-density farming systems, has resulted in an environment favorable to the emergence and spread of infectious diseases (Baldock et al. 2006). High fish population density in aquaculture, together with other stress factors, increase possibility for the emergence of diseases caused by pathogens that may be harmless under natural conditions (Kurath and Winton, 2011).

In addition, presence of farmed and wild aquatic animals in the same water column, as in semi-intensive carp production, may result in increased pathogen challenge (Kurath and Winton, 2011; Saksida et al., 2014; Jones et al., 2015).

Wild fish usually acts as a natural reservoir of the aquatic viruses, but the presence of the virus usually do not cause the disease, due to the fact that they are not sufficient to sustain the natural transmission cycle density (Garver et al., 2013; Taranger et al., 2015). On the other hand, in aquaculture, presence of the pathogenic virus in the fish population will probably cause the episode of the disease with mass mortality. This disease pressure is additionally increased with intensive, usually not adequately controlled, trade in live fish, which enables huge geographical redistribution of fish species and their pathogens.

Complete information about presence of the viral pathogens in wild fish is hard to get and most information comes from episodes of large-scale die-offs. And even in such case, u cause of mortality is not adequately and completely determined. The wild aquatic animals that overcome the disease may become asymptomatic carriers, exhibiting light viral loads and constantly excreting viruses (Gozlan et al., 2006).

Most recently, emerging aquatic animal viruses are grouped into eight categories: a virus introduced in a new host and new geographic area; a virus infecting the same host but introduced in a new geographic area; enzootic virus with increased pathogenicity in same host (manifesting as increased incidence or more severe disease); enzootic virus with increased host range; enzootic virus with expanded geographic range; increased awareness; improved diagnosis and surveillance and newly discovered viral cause of existing disease (Kibenge, 2016).

Over the last decade, several emerging diseases of 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. The World Organization for Animal Health (OIE) Aquatic Animal Health Code (2015) (Aquatic Code) defines an emerging disease as a disease, other than listed diseases, which has a significant impact on aquatic animal or public health resulting from a change of known pathogenic agent or its spread to a new geographic area or species; or a newly recognized or suspected pathogenic agent. The Aquatic Code mandates Member Countries to report emerging diseases in aquatic animals, as does the European Fish Health Legislation for EU member states.

MATERIALS AND METHODS

Samples of different fish species (mostly cyprinids and salmonids) were aquired from fish farms (56 carp and 52 trout farms) and open waters, and examined for the presence of fish viral and bacterial diseases. 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, PCR, RT-PCR, RealTimePCR 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

In the last few years several emerging or re-emerging fish diseases have been detected in fish populations in Serbia.

Herpesviral haematopoietic necrosis (HVHN) caused by CyHV-2 is detected in a wild population of Prussian carp in Grliško Lake in eastern Serbia. This episode lasted for 1 week, at a water temperature of 26°C, and did not affect other fish species.

Episodes of disease characterized by a typical sleepy behaviour, enophthalmia, generalized oedematous condition and gill necrosis, leading to hypoxia and mortality of up to 20% were observed during 2015 and 2016 in spring time on many carp farms, but causative agent was not detected.

Carp edema virus disease (CEVD), caused by poxvirus (family *Poxviridae*), an emerging disease of concern to carp aquaculture around the world, was diagnosed during April and May 2017, in common carp at water temperatures between 9 and 15°C, in two carp farms. In both farms, diseased fish showed similar clinical signs and experienced about 20 percent mortality.

Sleeping Disease (SD), caused by salmonid alphavirus (SAV) subtype 2, a contagious disease of rainbow trout, is characterized by a "sleeping" behaviour. Trout populations with characteristic signs of the disease were noted in Serbian trout farms since 2014, but virus was detected in 2016, and again in 2017.

Epizootic hematopoietic necrosis caused by European catfish virus (ECV) was found in Serbia in 2008 in brown bullhead. Since then, serious outbreaks of the disease have been noticed in brown bullhead (*Ameiurus nebulosus*) in fish farms but also in wild populations.

Infectious pancreatic necrosis (IPN) was detected in Serbia in 1989 (Jeremic et al. 1989) and did not emerge until 2007, when the disease was confirmed at a trout farm in Mačva district, in diseased rainbow trout. The number of IPN affected localities in 2016 and 2017 was similar to previous years i.e. slightly over 10. The disease is almost certainly under-diagnosed and therefore under-reported.

Koi Herpes Virus (KHV)

KHV is present in the region since 2011 (Toplak et al. 2011; Láng et al., 2014).

Spring Viremia of Carp (SVC)

The disease was last time diagnosed in 2004, but reoccurrence of OIE listed disease in Romania in May 2016 warns on possible spread of the disease to susceptible fish populations in Serbia.

Control of emerging infectious diseases is challenging goal on many levels. Crucial factor for limiting any emerging disease is fast execution of adequate control measures which will prevent dissemination of the disease.

'Control' is a general term describing the reduction in morbidity and mortality from disease, and encompasses all preventive and treatment measures taken to interfere with disease appearance (Thrusfield, 2005). The management of infectious diseases in aquaculture can be classified into three basic strategies:

i) controlling by eradication, i.e. eliminating the infectious agent from the area or reducing the infectious agent to minute levels

ii) controlling the disease, but living with the infectious agent

iii) no control, but handling the recurrent costs associated with the disease (Thrusfield, 2005).

The choice of strategy depends on a number of factors, such as the resources available, likelihood of success, support among stakeholders, disease characteristics and legislation (Thrusfield, 2005). The choice between strategies requires an understanding of the differences between them, in terms of knowledge requirements, costs and cost-sharing protocols. Emergency diseases are often controlled through the first of these strategies, which means that control measures are aimed at eradicating the infectious agent and the associated disease and reducing the biological impacts to zero or as near zero as possible.

Unfortunately, for successful control, as for the emergence of the disease, simultaneous action of several factors is necessary. There is a need for the cooperation of all stakeholders, which have different views on the problem itself, and on the ways of solving it. With such relationships, it is difficult to make a solution that will be seen by all stakeholders as appropriate, efficient and cost-effective. Open-water or flow-through aquaculture or fishing sites are subject to impacts from multiple users with widely differing socio-economic values and priorities (McGladdery and Zurbrigg, 2006). Aquaculture farm owners are often driven primarily by economic parameters. On the other hand, public administration must pay attention to the preservation of the environment, the maintenance of wild fish populations in nature and the maintenance of the entire branch of agriculture. Insufficient knowledge of disease characteristics and the importance of proper implementation of control measures can additionally discourage the fish farmers in making painful and economically demanding decisions. Especially when regulations dictate an eradication strategy, this is associated with huge costs for the individual producer.

Fish farmers are obliged to report to competent authority any mortality of fish with signs of disease. However, it is sometimes difficult to determine in a timely manner the presence of diseases in the pond with lakes of tens or hundreds of hectares. It may take time for a fish farmer to understand the situation and receive answers from veterinarians and diagnostic laboratories. Also, lack of adequate regulative and procedures leads to delayed response. Usually, when a disease is suspected or diagnosed, fish farmer make and execute a decision focusing only on his own farm. If we want to limit negative impact of emerging diseases, there is a need for wider perspective and fish farmer needs to consider how other farms in the vicinity or the external environment might be infected by a disease, and the farmer's decision about control measures needs to be consistent with statutory regulation of the disease or its treatment. Any other reaction will amplify transmission and spread of disease. A disease control program should include clearly defined objectives and rationale, an implementation plan, effective surveillance programs and outbreak investigations, an emergency action plan, and measures for monitoring, evaluating and, if necessary, adapting the program (OIE, 2015). Shared factors, however, in the success of any disease control include effective effluent and upstream influent controls, as well as control of human activities on and among farms (McGladdery and Zurbrigg, 2006). Developing 'carrot and stick' approaches to disease control that combine the promise of compensation for those that invest in good biosecurity measures with a schedule of fines for those that fail to report disease promptly could potentially enhance control efforts, and could be more effective than simply providing compensation alone (Pettersen et al. 2015).

CONCLUSIONS

The confirmation of outbreaks of many emerging diseases in Serbia is in line with the actual epidemiological situation in Europe, in which fish viruses are found to be more widespread than supposed earlier. A number of important infectious diseases have emerged in intensive aquaculture and this trend is likely to continue. Close contact with wild fish, hydrodynamic and management links between farms, and movements of the fish are the main drivers for disease emergence. The consequences of some disease epidemics have been severe for the fish industry and the public sector. As the aquaculture sector continues to grow, the potential impacts of disease epidemics on the industry, society and environment will increase accordingly. Control of these diseases is thus essential in developing a sustainable industry. Improved methods for laboratory diagnosis and pathogen surveillance, and more extensive molecular analyses of viruses from farmed and wild aquatic organisms, will improve our understanding of the many complex factors that underlie viral disease emergences in aquaculture and fisheries. Technical solutions do exist, but fostering cooperation between the public and private sector, together with development of a mechanism for cost-sharing with the private sector, would be more favourable for disease control and aid in overcoming the limitations of the current institutional system.

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ANTIBIOTICS RESISTANCE IN BACTERIAL STRAINS ISOLATED FROM FISH COLLECTED FROM DIFFERENT ENVIRONMENTS IN SERBIA

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ANTIBIOTSKA REZISTENCIJA BAKTERIJSKIH SOJEVA IZOLOVANIH OD RIBA IZ RAZLIČITIH ŽIVOTNIH SREDINA U SRBIJI

Apstrakt

Samo Gram-negativni izolati iz briseva kože, škrga i rektuma klinički zdravih riba sa ribljih pijaca, akavarijuma i ribnjaka izabrani su za ispitivanje njihove otpornosti na karbapeneme, ureidopeniciline (sa i bez inhibitora beta-laktamaze), cefalosporine treće i četvrte generacije, aminoglikozide, kolistin, fluorohinolone, sulfametoksazol sa trimetoprimom, tertaciklin i hloramfenikol.

Cilj je bio utvrditi prisustvo multi-rezistentnih sojava bakterija kod riba, sa mehanizmima rezistencije na antibiotike koji su značajni u humanoj i veterinarskoj kliničkoj praksi.

Identifikacija ispitivanih sojeva bakterija vršena je primenom metoda PCR, sekvenciranjem gena za 16S rRNK i MALDI-TOF metodom. Osetljivost svakog izolata na antibiotike ispitivana je disk difuzionim i E-testom. Prisustvo gena rezistencije utvrđivano je metodom PCR.

Od 134 izolata, 48 je pripadalo rodu *Pseudomonas,* 22 rodu *Aeromonas* i 6 rodu *Acinetobacter.* 18 izolata je identifikovano kao *Stenotrophomonas maltophilia* a 40 je pripadalo familiji *Enterobacteriaceae.* Posmatrano na ukupan broj ispitanih sojeva, 56,7% je osetljivo na sve antibiotike a kod 20,2% sojeva otkrivena je rezistencija na 3do 16 antibiotika. Kod 23,1% zabeležena je rezistencija na 1do 2 antibiotika. Koristeći E test, rezistencija na kolistin nađena je kod 3 soja *Pseudomonas* spp. izolovana iz šarana (*Cyprinus carpio*) sa vrednostima MIK od 4 µg/mL. Kod jednog soja *Aeromonas hydrophila* izolovanom iz akvarijumske ribice gupi (*Poecilia reticulata*) potvrđen je mehanizam rezistencije na aminoglikozide - 16S rRNK metiltransferaza detekcijom gena *rmt*B. *Pseudomonas* sojevi rez-

istentni na karbapeneme, ureidopeniciline, cefalosporine treće i četvrte generacije testirani su i bili negativni na različite gene rezistencije primenom PCR metode.

Pošto se većina otkrivenih fenotipova rezistencije na antibiotike nije mogla potvrditi pronalaskom relevantnih gena, neophodna su dalja istraživanja zbog mogućih intrinzičnih mehanizama rezistencije na antibiotike u bakterijama izolovanim iz riba.

Ključne reči: *Aeromonas hydrophila, kolistin, ribe, Pseudomonas spp., rmtB gen*

Abstract

Only Gram-negative isolates from swabs of the skin, gills and rectum from clinically healthy fish originating from live fish markets, ornamental fish stores and aquaculture ponds were selected in order to test their resistance to carbapenems, ureidopenicillins (with and without inhibitor of beta-lactamase), 3rd and 4thgeneration cephalosporins, aminoglycosides, colistin, fluoroquinolones, sulfamethoxazole/trimethoprim, tetracycline and chloramphenicol.

The aim was to determine the presence of multi-resistant strains of bacteria in fish, with the mechanisms of resistance to antibiotics of significant importance in human and veterinary clinical practice.

Identification of tested strains of bacteria was performed by PCR, 16S rRNA gene sequencing and MALDI-TOF. The susceptibility of each isolate to antibiotics was determined by disk diffusion and E-test. The presence of resistant genes was conducted by PCR.

Out of the 134 isolates, 48 belonged to the genus *Pseudomonas,* 22 to *Aeromonas*. 6 to *Acinetobacter,* 18 were identified as *Stenotrophomonas maltophilia*, and 40 isolates belonged to the *Enterobacteriaceae* family. Observing the total number of tested strains, 56.7% were sensitive to all antibiotics, and in 20.2% of strains resistance was detected to 3-16 antibiotics. In additional 23.1% of strains the resistance to 1 or 2 antibiotics was recorded. Using E test, resistence to colistin was found in 3 strains of *Pseudomonas* spp. isolated from common carp (*Cyprinus carpio*) with MIC value of 4 µg/mL. In one *A. hydrophila* strain isolated from a guppy (*Poecilia reticulata*) 16S rRNA methyltransferase was confirmed by identifying gene *rmt*B. *Pseudomonas* strains resistant to carbapenems, ureidopenicillins, 3rd and 4th generation cephalosporins, were tested and found negative for different resistance genes by PCR.

Because most of the discovered antibiotic resistant phenotypes could not be confirmed by finding relevant genes further studies on the resistance mechanisms are necessary, due to possible intrinsic resistance mechanisms to antibiotics in bacteria isolated from fish.

Keywords: *Aeromonas hydrophila, colistin, fish, Pseudomonas spp., rmtB gene*

PARASITES OF CYPRINIDS - MONITORS OF ENVIRONMENTAL HEALTH IN ROMANIAN IMTA SYSTEM *VERSUS* TRADITIONAL AQUACULTURE SYSTEM

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PARAZITI CIPRINIDA KAO POKAZATELJI STANJA OKRUŽENJA U DVA SISTEMA UZGOJA: U INTEGRISANOM MULTITROFIČNOM SISTEMU U ODNOSU NA TRADICIONALNI SISTEM

Apstrakt

Ciljevi prikazanog rada su identifikacija parazitofaune ciprinidnih riba koje potiču iz dva različita sistema uzgoja, tradicionalnog sistema (TS) i integrisanog multitrofičnog sistema (IMTR) u skladu sa osnovnim parametrima okruženja, kao što su: temperatura (°C), pH, rastvoreni kiseonik DO (mg/L), nitrati N-NO_3 (mg/L) i ukupan broj mezofilnih aerobnih bakterija (log CFU/mL) iz uzoraka vode, tokom vegetativne sezone (jul – septembar) 2016. godine.

U akvakulturi, kvalitet vode i sedimenta direktno utiču na produktivnost i zdravlje riba. Rezultati dobijeni u ovoj studiji pokazuju da se u oba sistema akvakulture zapažaju slične strukture faune nađenih parazita. Ipak, intenzitet infekcije ciprinidne ribe domaćina iz tradicionalnog sistema ribnjaka je bio veći od ciprinidne ribe domaćina iz integrisanog multitrofičnog sistema.

Ključne reči: *ciprinidne ribe; tradicionalni sistem, multitrofični system; akvakultura; paraziti.*
Keywords: *cyprinids; traditional system; integrated multitrophic system; aquaculture, parasites.*

INTRODUCTION

In aquaculture there is a wide range of systems, from small ponds to large-scale commercial systems. Successful pond fish farming depends on the water physical, chemical and biological characteristics and on the species nutrition management. In pond fish farming all these factors are inter-related and require careful and constant monitoring to avoid contamination and/or degradation of the aquaculture environment (Gorlach-Lira et al., 2013).

The parameters which play a vital role in the identification and establishing the indicators for the most adequate habitual environment for the good development of cyprinids species are the physico-chemical and bacteriological parameters. The aims of the present study was to identify the parasite-fauna of cyprinids originating from two different rearing systems, traditional system (TS) and integrated multitrophic system (IMTA) corelated with the main parameters of the technological environment, such as: temperature T (°C), pH, dissolved oxygen DO (mg/L), nitrates N-NO_3 (mg/L) and total number of mesophilic aerobic bacteria (log CFU/mL) from water samples, during vegetative season (July - September) from 2016.

MATERIALS AND METHODS

The researches were conducted in the Movileni Fish Farm, Iaşi district, Romania. The water entrance and outlet from the ponds are made gravitationally, by using „monk" type hydraulic constructions. The experiment used two ponds with an area of 0.45 ha each and an average water depth of 1.5 m. The first pond (P1) has been divided by using a net as follows: first part with an area of 0.15 ha dedicated to carp monoculture and the second part with an area of 0.30 ha dedicated to cyprinid polyculture. The second pond (P2) was used for rearing polyculture common carp (*Cyprinus carpio*) with grass carp (*Ctenopharyngodon idella*), bighead carp (*Hypothalmichthys nobilis*) and silver carp (*Hypophthalmichthys molitrix*). The parasitological examination consisted in wet mounts (scrape mounts and squash mounts) studied at the microscope using lenses with magnification starting from 5x to 100x. The scrape mounts were made both superficial and profound from gills, tegument, flippers and intestinal mucus. The squash mounts were made from cellular tissue of organs such as: liver, reins and swim bladder. The water and soil samples were collected from each of the two ponds, P1 pond with four sampling points coded - I.1.1., O.1.1., I.1.2, O.1.2. and P2 pond with two sampling points coded - I.2.1., O.2.1. The instruments used for water physico-chemical parameters measurements were: the HQ40d Portable Multi-Parameter (HACH) for pH, Dissolved Oxygen, respectively Spectroquant photometer, Nova 400 for nitrate, with Merck kits. The number of total mesophilic aerobic bacteria were carried out in pour plate using plate count agar followed by incubation at 37°C for 48h, method provided by the Romanian Standard STAS 3001-91. All the measurements were performed in July, August and September, 2016, followed by the data analyses.

RESULTS AND DISCUSSIONS

The results of the microbiological parameter of the water samples from the nine sampling points on the two cyprinid ponds, integrated multitrophic system (P1) and traditional system - (P2) are presented in Figure 1.

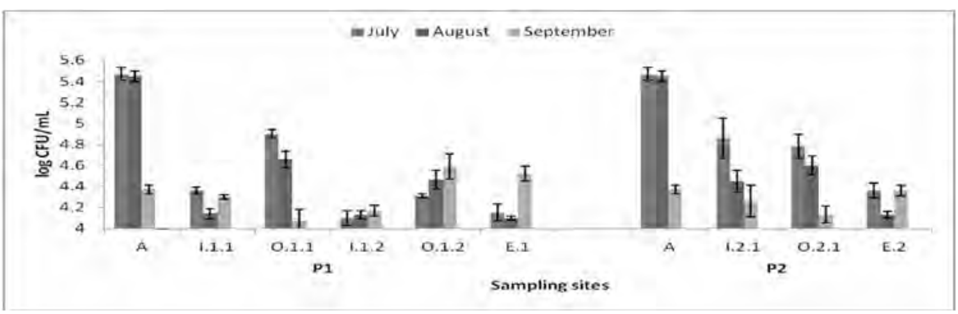

Figure 1. The total number of mesophilic aerobic bacteria in vegetative season

Theoretically, aquaculture in eutrophic environments is predisposed to pH changes and leads to the possibility of increasing the toxicity of the non-ionized ammonia and nitrite. High water temperatures can catalyse those processes turning them into evident and frequent processes.

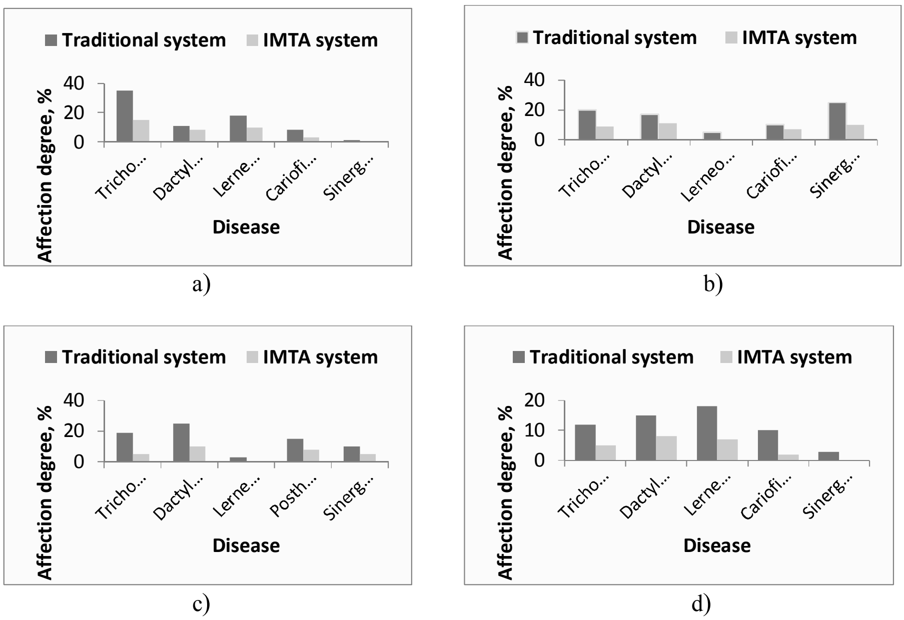

Figure 2. Diseases and affection degree (%) at: a) Cyprinus carpio, b) Hypophthalmichthys molitrix, c) Hypothalmichthys nobilis, d) Ctenopharyngodon idella

The interactions of these environmental factors can affect fish production by reducing the rate of food conversion, reducing the weight gain, due to the parasitic fauna. All examined fish were low contaminated with biological agents in what regards the number of parasites (Figure 2). The biological contaminants of the cultured cyprinids were represented by the following types of parasites: protozoans, worms and crustaceans (Ash et al., 2011).

CONCLUSIONS

In aquaculture, the water and soil quality directly influence the productivity and the health of the fish. The results obtained in this study demonstrate that the both aquaculture systems registered the similar parasitic fauna structure. However, the infestation intensity of fish host cyprinids from the traditional pond system has been higher than the fish host cyprinids from the integrated multitrophic system.

ACKNOWLEDGEMENTS

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HUMAN DIETARY EXPOSURE TO TRACE ELEMENTS VIA THE CONSUMPTION OF MUSSELS (*MYTILUS GALLOPROVINCIALIS*) AND OYSTER (*OSTERA EDULIS*) FROM THE BOKA KOTORSKA BAY

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IZLAGANJE LJUDI ELEMENTIMA U TRAGOVIMA PREKO KONZUMIRANJA DAGNJI (*MYTILUS GALLOPROVINCIALIS*) I KAMENICA (*OSTERA EDULIS*) IZ BOKOKOTORSKOG ZALIVA

Apstrakt

Bokokotorski zaliv predstavlja vrlo povoljno područje za rast školjki. Još od prvih istraživanja sprovedenih 60tih godina prošlog veka, utvrđeno je da je kvalitet vode, dostupnost hrane, dotok slatke vode, kao i niz drugi faktora i kriterijuma životne sredine, veoma pogoduju za industrijski razvoj sektora mariklulture. Proizvodnja školjki u Crnoh Gori je na relativno niskom nivou i pored prirodnih predispozicija i potencijala. Trenutno, u okviru Bokokotorskog zaliva postoji 20 farmi, sa godišnjom proizvodnjom dagnji od oko 170 tona i 13 tona kamenica.

Školjke su organizmi koji hranu uzimaju filtriranjem vode i predstavljaju biološke filtere koji prečišćavaju vodu u kojoj žive. Takođe imaju značajnu ulogu u funkcionisanju morskog ekosistema kroz kontrolu primarne proizvodnje, organskih i hranljivih materija, uklanjanje fitoplaktona, mikroorganizama i zagađujućih materija iz vodene kolone.

Školjke su odličan izvor esencijalnih elemenata i njihovo konzumiranje izuzetno je korisno za ljudsko zdravlje, ali u isto vreme mogu biti i zagađene što može prouzrokovati trovanje i bolesti. Zbog toga je poznavanje sadržaja elemenata u tragovima, posebno potencijalno toksičnih, u dagnjama i kamenicama od suštinske važnosti kako bi se smanjili potencijalni štetni efekti koji mogu nastati konzumiranjem kontaminiranih školjki.

Ključne reči: *dagnje, kamenice, elementi u tragovima, rizik po zdravlje, Bokokotorski zaliv*
Keywords: *mussels, oysters, trace elements, health risk, Boka Kotorska bay*

INTRODUCTION

Boka Kotorska bay is very favorable area for shell growing. From the first scientific studies in the field of marine aquaculture which were conducted in 60’s of the last century, to date, it has been established that water quality, food availability, fresh water inflows, organographic conditions, as well as other environmental factors and criteria’s are very suitable for the industrial development of the mariculture sector (Mandić et al., 2016; Stjepčević, 1974).

Marine aquaculture in Montenegro includes fish farming (sea bass and sea bream) and farming of two species of bivalves–mussels (*Mytilus galloprovincialis*) and oysters (*Ostrea edulis*). Commercial mussel farming began to develop in the second half of 1980s and it implied introduction of the long-line methodology, used even today, while commercial oyster farming began as late as in 2009. Bivalve farming in Montenegro is at a relatively low level, considering the natural potential available. On 20 farming sites, all concentrated in the area of the Boka Kotorska bay, the current annual mussel production is somewhat below 170 tons, while the quantity of farmed oyster is about 13 tons (MONSTAT, 2016).

Mussels and oysters are filter feeders - organisms which play an important role in the functioning of coastal ecosystems through the control of primary production, bacterial components, organic matter and nutrients, removing phytoplankton, sediments, pollutants and microorganisms from the water column (Stankovic et al., 2012). They represent biological filters which in a certain way purify the water in which they live, and the breeding of these species can be proposed in order to improve the water quality of certain areas, especially in integral multi-trophic aquaculture in order to reduce the negative impact of fish farming on marine environment. Individual mussel filters about 17 L of water per hour, while individual oysters filter about 10 L per day, so these organisms can reasonably be considered ecosystem and environmental engineers.

Bivalves are an excellent source of essential elements and are, therefore, extremely beneficial for human health, but at the same time can be contaminated and can cause poisoning and promote diseases (Tanaskovski et al., 2016). Therefore, knowledge of the contents of the elements in bivalves *M. galloprovincialis* and *O. edulis* is essential in order to minimize potential adverse health effects which may arise from the consumption of contaminated bivalves.

MATERIALS AND METHODS

The mussel *M. galloprovincialis* and oysters *O. edulis* were collected from two locations in the Boka Kotorska bay during the winter of 2018: Orahovac and Sv Nedjelja. In order to obtain a representative sample at the each location more than 5 kg of mussels and oysters of similar length were collected, placed in plastic bags with seawater and transported to the laboratory. The mussels and oysters were cleaned, rinsed with seawater and dissected fresh. Mussel and oyster soft tissues were rinsed with Milli-Q deionized water to remove any remaining sand and/or other particles. The soft tissue of mussels and oysters from each location were pooled and subjected to lyophilization and then pulverized and homogenized using a planetary mono mill with agate milling balls. The resulting homogeneous fine powders were stored for subsequent analysis. Samples were weighed before and after lyophilization to determine the water content. Element concentrations were measured by ICP-MS model Thermo iCAP Q. Tissue samples and certified reference material SRM 2976 (Mussel homogenate, NIST) were dissolved in 7 mL HNO_3 and 2 mL H_2O_2 in a MARS 5 microwave oven and the digestate was diluted with Milli-Q deionized water.

The provisional tolerable weekly intake (PTWI) of toxic elements through consumption of the mussel and oyster soft tissues was calculated by following equation:

PTWI=(C×WC)/BW

where C is the concentration of element (mg/kg ww), WC is the average mussel weekly consumption (125 g/week) and BW is the individual's body weight (70 kg) (Jović and Stanković, 2014). The non-carcinogenic (THQ) and carcinogenic risks (CR) have been calculated according to the equations:

THQ=$((C\times EF\times ED\times IR\times CF)/(AT_n\times BW))/RfD$

TCR=$((C\times EF\times ED\times IR\times CF)/(AT_c\times BW))\times CSF$

where C is the concentration of element (mg/kg ww), EF is exposure frequency (365days/year), ED is exposure duration (70 years), IR is ingestion rate (17.9 g/day), CF is conversion factor (10^{-3} kg/g), AT_n is the averaged exposure time (365 days/year×70 years), ATc is the averaged exposure time to the carcinogen (365 days/year×70 years), BW is body weight (70 kg), RfD is reference dose, element-specific toxicological value and CSF is a carcinogenic slope factor, element-specific value (Risk Assessment Information System, 2018).

The hazard index (HI) is the sum of the individual target hazard quotients of the trace elements assessed for each sample. If the HI is >1 there is the potential for adverse non-carcinogenic health effects (Jović and Stanković, 2014).

RESULTS AND DISCUSSION

The results of the investigated trace elements in the soft tissue of mussels and oysters are given in Table 1.

Table 1. Mean concentrations of Cd, Pb, Hg and As (mg/kg dry weight) and standard deviation in the soft tissues of M. galloprovincialis and O.edulis collected from the Boka Kotorska bay

Location	**Orahovac**		**Sv Nedjelja**	
Species	***M. g.***	***O. e.***	***M. g.***	***O. e.***
Water content (%)	85.1	81.6	86.1	77.8
Cd	1.20±0.05	2.80±0.11	1.10±0.04	1.60±0.07
Pb	1.50±0.08	0.50±0.02	1.70±0.09	0.70±0.02
Hg	0.26±0.02	0.17±0.01	0.28±0.02	0.15±0.01
As	35±3	27±2	33±2	24±1

In comparison with the permissible limits set by the European Union (EC, 2006) for Cd, Pb and Hg (1.0, 1.5 and 0.5 mg/kg wet weight, respectively) all concentrations of investigated trace metals in mg/kg wet weight were lower than the prescribed limits.

Table 2 shows the PTWI of analyzed trace elements considering the average per capita consumption of mussels and oysters (125 g per week i.e. one meal of mussels/oysters once every two weeks, Jović and Stanković, 2014), expressed as % of the established PTWI values. To calculate the PTWI and THQ of methyl Hg (MeHg) and TCR of inorganic As (iAs), 36% of the total Hg and 10% of the total As were used (Tanaskovski et al., 2016).

Table 2. The provisional tolerable weekly intake (PTWI) of trace elements considering the average per capita mussels/oysters consumption

Trace elements	Established PTWI (μg/kg bw)	PTWI expressed as % of the established PTWI			
		M.g. Orahovac	*O.e.* Orahovac	*M.g.* Sv Nedjelja	*O.e.* *Sv Nedjelja*
Cd	10[a]	3.2	7.5	2.9	4.3
MeHg	1.6[b]	1.5	0.9	1.6	0.8

[*] PTWI for Pb and As were withdrawn. Not possible to establish a new PTWI that would be considered health protective (JECFA, 2014); [a] (JECFA, 2014); [b] (JECFA, 2004).

A THQ < 1 signifies that the level of exposure is lower than the reference dose, which assumes that a daily exposure at this level is not likely to cause no negative health effects during a lifetime in a human population (Jović and Stanković, 2014).

The THQ values for all trace elements in mussels and oysters were lower than 1 (Table 3) indicated that no health risk was present. Hazard index (HI) indicates that there is a slight risk for human health from eating mussels and oysters since for analyzed samples HI falls within the range of 0.53-0.74. Higher HI was obtained for *M. galloprovincialis* then for *O. edulis* for both sampling locations. The dominating trace element, the one that has the greatest THQ values in all samples, was iAs (Table 3). The iAs carcinogenic risks in all samples were above $1x10^{-6}$ which is considered as the lowest acceptable level of risk. For all samples the calculated TCR values were about 10^{-4} and these results should not be neglected.

Table 3. Target hazard quotients (THQ), Hazard index (HI) and Target cancer risk (TCR) values

	Orahovac		Sv Nedjelja	
	M.g.	*O.e.*	*M.g.*	*O.e.*
THQ				
Cd	0.05	0.11	0.04	0.06
MeHg	0.03	0.02	0.04	0.02
iAs	0.66	0.51	0.62	0.45
HI	0.74	0.64	0.70	0.53
TCR				
iAs	0.00013	0.00010	0.00012	0.00009

CONCLUSIONS

The trace elements concentrations in mussels *M. galloprovincialis* and *O. edulis* from the Boka Kotorska bay, didn't exceed the European Union requirements. Risk assessment of the investigated elements through bivalves consumption suggested that weekly exposure to 125 g/week of mussels and oysters during a human lifetime is not likely to cause negative health effects. However, for all samples the calculated TCR values were about 10^{-4} and these results should not be neglected and should continue to be monitored.

ACKNOWLEDGEMENTS

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EFFECT OF COOKING ON FLORFENICOL RESIDUE IN RAINBOW TROUT MEAT

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EFEKAT KUVANJA NA REZIDUE FLORFENIKOLA U MESU KALIFORNIJSKE PASTRMKE

Apstrakt

Prekomerna upotreba florfenikola (FF) na farmama kalifornijskih pastrmki dovodi do prisustva rezidua u jestivim tkivima riba, uzrokujući zdravstvene rizike za potrošače. Istraživanje termalne stabilnosti rezidua florfenikola u ribljem mesu obezbeđuje korisne podatke vezane za zdravlje potrošača. Stoga je ovo istraživanje imalo za cilj da proceni efekat različitih metoda kuvanja na ostatke florfenikola u kalifornijskoj pastrmki. Zdrave kalifornijske pastrmke (500 ± 18,5 g) 5 dana oralno su dobijale 10 mg/kg florfenikola. Koncentracije FF u sirovom i kuvanom mesu određene su pomoću HPLC-a. Prema rezultatima, ostaci FF u sirovim, kuvanim u ključaloj vodi, grilovanim, kuvanim na pari i prženim filetima bili su redom: 0,88, 0,45, 0,51, 0,61 i 0,51 µg/g. Rezultati pokazuju da kuvanje može smanjiti florfenikol za 30 do 50%. Upoređivanje metoda kuvanja pokazalo je da je kuvanje u ključaloj vodi značajno efikasnije od drugih metoda u smanjenju florfenikola ($p \leq 0.05$). Nalazi ove studije, kao prve, otkrili su da su rezidue FF iz filea kalifornijske pastrmke poreklom iz tretirane ribe delimično uklonjeni kuvanjem, dok je kuvanje u ključaloj vodi značajno smanjilo nivo ostatka leka.

Ključne reči: *Florfenikol, kalifornijska pastmka, kuvanje, rezidue antibiotika*
Keywords: *Florfenicol, rainbow trout, cooking, antibiotic residue*

INTRODUCTION

In recent years, along with the development of aquaculture industry, using antibiotics has been increased. Large amounts of antibiotics are used every year for prevention and treatment of bacterial diseases in intensive fish culture. The use of antibiotics has the potential to generate residues in fish meat. It not only threatens human health but also causes antibiotic resistance even when used at moderate doses for long periods of time (Filazi et al., 2005).

Florfenicol is an effective antibiotic which is widely used in the treatment of bacterial diseases in fish. It has been demonstrated to be effective against most bacterial pathogens of fish, including *Photobacterium damsela* (subsp. *Piscicida*), *Vibrio anguillarum*, *Aeromonas hydrophila*, *Aeromonas salmonicida*, *Edwardsiella ictaluri*, *Edwardsiella tarda*, *Pasteurella piscicida*, *Streptococcus iniae,* and *Lactococcus garvieae* (Inglis et al. 1991; Nordmo et al. 1994; McGinnis et al. 2003; Ansari et al. 2014). Furthermore, other studies have proved its effectiveness on the treatment of several bacterial diseases in rainbow trout, Atlantic salmon, channel catfish, yellowtail and goldfish (Yasunaga and Yasumoto, 1988; Inglis et al. 1991; McGinnis et al. 2003; Ansari et al. 2014).

Florfenicol has been approved for use in many countries including European countries, South Korea, China, Japan, and Canada for several fish species (Gaunt et al. 2003). Florfenicol is commonly used along with the fish feed either before pelleting or covering the pellets and is administered at the amount of 10 mg kg^{-1} body weight (Nordmo et al. 1994). Florfenicol is the most commonly used antibiotic against bacterial diseases in Iran (Ansari et al., 2014). Residues of florfenicol in rainbow trout have been reported before by Ansari et al. (2014). Their results showed that the level of florfenicol changed from 0.4 to 3.6 µg g^{-1} with an average of 0.8 µg g^{-1}. Florfenicol residue in 12.5% of samples has been reported higher than the maximum permissible amount (1 mg kg^{-1}). Considering the high use of florfenicol in fish farms and it's consequent accumulation in meat, this is important to know whether the antibiotic residue decreases after cooking or not.

This study was carried out to evaluate the effect of different cooking methods on florfenicol residue in rainbow trout meat for the first time.

MATERIAL AND METHODS

Healthy rainbow trout (500±18.5 g) were obtained from a commercial farm and reared in a 1000-liter tank with a continuous flow of aerated freshwater. The water temperature was 14.5±1.2°C, and the mean dissolved oxygen, pH, salinity, ammonia, and nitrite were 8.7 mg lit^{-1}, 7.3, 0 g lit^{-1}, 0.006 mg lit^{-1}, and 0.012 mg lit^{-1}, respectively. The experiment began after a one-week acclimation period.

Fish were individually given the suggested oral dose of florfenicol taken daily, 10 mg kg^{-1} body weight for 5 days. The medicated feed was then fed to each fish by inserting the syringe connected to a tube down the esophagus into the stomach without applying anesthetics. Each fish was then taken to a separate pond to be monitored for possible regurgitation during a period of about 5 min. Fish were harvested 6 h after oral treatment in order to determine FF residues in fillets. Fillets of each fish were cooked using 4 methods. Boiling 100±2°C for 30 minutes, steaming at 102±3°C for 30 minutes, frying in canola oil at the temperature of 180±5°C for 30 minutes and grilling at 150±2°C for 30 minutes were applied (Du et al., 1997). The internal temperature of samples was measured by an iron-constantan thermocouple.

Extraction and analysis of florfenicol were performed in accordance with methods by Park et al. (2006). Briefly, one gram of meat was mixed with 4 ml of 0.1 M phosphate buffer (pH 7.0) in a test tube. Prior to adding 4 ml of ethyl acetate, it was homogenized. After that, it mixed for about 10 min then was centrifuged at 1500 g for 10 min.

Nitrogen gas was utilized to evaporate organic layer. The remainder was injected into the HPLC (20 µl) after reconstituted with 500 µl of the mobile phase.

Analyses were performed on a Hewlett Packard 1090 HPLC system (Agilent, USA), consisting of an L-4200 UV–VIS detector at 223 nm wavelength. The chromatographic column was Eclips Plus C_{18} reverse phase column (3.5 µm, 4.6 mm×150 mm). The limit of quantitation was reported to be 50 ng ml^{-1}, the precisions of inter and intra-day (CV, %) were less than 15%, and linear calibration curves (rN0.99) from 0.05 µg ml^{-1} to 50 µg ml^{-1} were observed.

Microsoft Excel was used to analyze the achieved data. For ANOVA and Tukey tests, SPSS software (ver. 18.0) was utilized. The discrepancies were perceived to be significant at $p<0.05$.

RESULTS AND DISCUSSION

The antibiotic residues in raw, boiled, grilled, steam-cooked and fried fillets were 0.88, 0.51, 0.51, 0.61 and 0. 45 µg g^{-1}, respectively. Our results revealed that cooking for 30 min could lead to a reduction of florfenicol contents in rainbow trout fillets. Comparison of cooking methods showed that boiling is more effective than other methods in reducing florfenicol. Boiling was shown to result in approximately 50% degradation of florfenicol in fillets. The reduction rate was 30% for steam-cooked and 41% for grilled and fried fillets for the same duration of time.

Degradation of antibiotics including florfenicol after cooking depends on many factors including cooking time and temperature, nature and type of food and method of cooking (Javadi, 2011). It can explain the differences which are observed in different studies.

Table 1. Florfenicol contents (µg g-1) in raw and cooked fish fillets

Sample	mean±SD (µg g^{-1})	Minimum (µg g^{-1})	Maximum (µg g^{-1})
raw fish	0.88 ± 0.22^{a}	0.68	1.12
boiled	0.51 ± 0.10^{ab}	0.41	0.62
grilled	0.51 ± 0.11^{ab}	0.44	0.65
steam-cooked	0.61 ± 0.11^{ab}	0.49	0.71
fried	0.45 ± 0.12^{c}	0.58	0.34

Concerning the effect of cooking method, boiling has been reported as the most effective method on degradation of the antibiotic residue in many researches (Franje et al., 2010; Tian, 2017). It is reported that boiling water at 100 °C for one hour results in about 15.6% to 27% degradation of florfenicol (Tian, 2017). In this study, the internal temperature of the fillets did not exceed 100°C in any method. The highest temperatures were about 97°C during boiling, 99°C during grilling and 98°C during frying and 90°C for steaming while more degradation was observed. Franje et al. (2010) reported that boiling for 30-60 min and microwave cooking for 5 min was resulted in only 2-3% degradation of florfenicol in water, while boiling for 30 min reduces 32% of the antibiotic residue in meat. This finding is in agreement with our results. Decreasing the antibiotic residue in meat after boiling may be due to the meat of a low water binding capacity after being heated as Clarke et al. (1987) indicated that the degradation of antibiotic could be increased by the low water binding capacity.

On the other hand, Abou-Raya et al. (2013) indicated that degradation of tetracycline in chicken meat was increased by increasing cooking time. Boiling for 20 min has led to 57.3% reduction in the antibiotic residue, while it has reached to 73.6 and 91.5% after 30 and 40 min, respectively (Abou-Raya et al., 2013). High degradation of ampicillin in tissues following long-time roasting is also previously reported (O'Brien et al., 1981). It is also reported that sterilization results in the remarkable decrease of β-lactam antibiotics in both water and milk (Roca et al., 2011; Hsieh et al., 2011).

CONCLUSIONS

According to the findings of this study, boiling is the most effective cooking method to reduce florfenicol residue in fish meat. However, it does not guarantee consumer's health so it is necessary to control florfenicol usage in fish farms and consideration of withdrawal time.

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COULD THE PESTICIDE ALDRIN COMPROMISE THE HEALTH STATUS AND REPRODUCTION OF THE FRESHWATER FISH *CLARIAS GARIEPINUS*?

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MOŽE LI PESTICID ALDRIN UGROZITI ZDRAVSTVENO STANJE I REPRODUKCIJU SLATKOVODNE RIBE *CLARIAS GARIEPINUS*?

Apstrakt

Cilj istraživanja je bio određivanje efekata pesticide aldrina na afričkog soma *C.gariepinus* u laboratorijskim uslovima. Odrasli primersi *C.gariepinus* su bili podvrgnuti koncentraciji aldrina koja je relevantna za životnu sredinu (0.14 µg/L), kao i povišenoj koncentraciji od 1.4 µg/L (28.7°C; pH 8) tokom 96 sati. Procena zdravstvenog stanja zasnovana na histološkom pregledu uključivala je: (1) uzorkovanje krvi iz dorzalne aorte u cilju određivanja broja belih i crvenih krvnih zrnaca, hematokrita, leukokrita i koncentracije hemoglobin (2) disekciju za utvrđivanje mogućih unutrašnjih i spoljašnjih makroskopskih abnormalnosti i (3) kvalitativnu i kvantitativnu histološku procenu jetre i bubrega. Organi su fiksirani u 10% neutralno buferovanog formalina i obrađeni korišćenjem standardne histološke tehnike. Parametarska i neparametarska statistička analiza urađena je korišćenjem IBM SPSS softvera. Melano makrofagni centri (MMC) bili su prisutni u 78%, a vakuolizacija u 41% ispitanih uzoraka jetri riba izloženih organohlornom pesticidu. U jetri riba izloženih 1.4 µg/L aldrina preovlađujući histopatološki nalaz je bio pojava MMC u 97% i vakuolizacija u 74% uzoraka. Ipak, MMC (74%) i vakuolizacija (91%) su bili prisutni i u bubregu riba izloženih koncentraciji od 0.14µg/L aldrina. U bubregu riba izloženih 1.4 µg/L, utvrđeno je 94% MMC i 97% vakuolizacije. Može se zaključiti da koncentracija aldrina koja je viša od one relevantne za životnu sredinu nepovoljno utiče na zdravlje *C. gariepinus*.

Ključne reči: *Histopatologija, Reprodukcija, Pesticidi, Hematologija, Endokrini disruptori*
Keywords: *Histopathology, Reproduction, Pesticides, Haematology, Endocrine disrupting chemicals*

INTRODUCTION

The organochlorine pesticide aldrin were recently detected in the water of the Albasini Dam, Limpopo Province in South Africa (Nibamureke et al, 2016). The presence of aldrin came as a surprise as the use of this chemical has been banned under the UNEP Stockholm Convention of 2004 and withdrawn in South Africa since 1992. The presence of aldrin in the Albasini Dam is a cause of concern as this dam has recreational value for the surrounding community and the local communities use the fish from the dam as a food source (Barnhoorn et al, 2015). As aldrin bio-accumulate in fish tissue, fish consumed from the Albasini Dam may be a human-related health risk (Nibamureke et al, 2016). Aldrin is known to be carcinogenic, slightly toxic and may act as an endocrine disruptor (ATSDR, 2002; WHO, 2008).

Aldrin have oestrogenic properties and is therefore considered as an endocrine disrupting chemical (EDC) and listed in the initial twelve persistent organic pollutants (POPs) known as the “dirty dozen”. Once exposed to the environment, aldrin is rapidly broken down into dieldrin. Therefore, its presence in the Albasini Dam is an indication of recent exposure raising concern of possible illegal use.

The aim of the study was to determine the health and reproductive effects of aldrin on the sharptooth catfish *C. gariepinus* under controlled laboratory conditions. The health assessment includes a histology-based fish health assessment protocol and the effects on the reproductive effects include the maturity stages, testes index and the sperm quality of mature male *C. gariepinus*. An environmentally relevant concentration (0.14 µg/L) and an increased concentration (1.4 µg/L) aldrin were used to induce more severe effects during the exposure of the study species.

MATERIAL AND METHODS

Ethical clearance was obtained from the Faculty Ethical Committee, University of Johannesburg. Mature catfish (n =88, 80 male and 8 female) between the ages of 8 to 10 months old, were obtained from the Hartbeespoort Dam Fisheries. The fish were acclimated in a single large holding tank for three months. Mature catfish males were acutely (96 hours) exposed to the 0.14 µg/L and a higher concentration, 1.4 µg/L, aldrin under controlled laboratory conditions (28°C ± 1°C and 11:13 day/night cycle). Control and solvent control tanks were run concurrently with each exposure group. Following the exposure, data of each fish was recorded and included: weight and length, papillae measurements and an external and internal necropsy performed to determine any macroscopic abnormalities. Blood was drawn from the caudal vein for haematological analyses, namely; red blood cell (RBC) counts, white blood cell (WBC) counts, haematocrit (Hct) and leukocrit (Lct). The fish were killed and dissected and the liver, kidney and testes removed. The liver and testes were weighed to determine the hepatosomatic index (HSI) and the gonadosomatic index (GSI). The livers and kidneys were then removed and placed in to 10% neutrally buffered. The gonads and gills were also removed and placed into Bouin’s solution. The right testis of each fish was fixed, sectioned and stained to microscopically determine the maturity stage of each specimen and to note any possible abnormalities or changes at histological level using a semi-quantitative histological assessment protocol. The protocol of Bernet et al (1999) was used to determine the histological changes and to determine the liver, kidney and gonad index. The left testis was used for sperm quality analysis by means of computer assisted sperm analysis (CASA) (Das Neves et al., 2018). The results were statistically analyzed using IBM SPSS software where normality amongst the data was tested using the Shapiro-Wilk test. If there was normality amongst the data parametric statistical analyses were done to indicate any statistical significant differences using the independent t-test. If there was no normality amongst the data the Mann-Whitney U test was done to determine any statistical significant differences.

RESULTS AND DISCUSSION

A statistical significant difference in the condition factor of the catfish was observed between the fish exposed to 0.14 µg/L aldrin (n = 12) and the fish exposed to 1.4 µg/L aldrin (n =12) with $p = 0.001$ lower than α, which was set to $\alpha = 0.05$. The haematological parameters served as an early indicator of the effect of the pollutant after acute exposure of fish to chemical pollutants and provided rapid evaluation of the toxicity of the chemical. The results showed that although not statistically significantly different, there was a potential trend of a decrease in the RBC count of fish exposed to 0.14 µg/L aldrin when compared to the control fish. These results showed evidence of changes in the haematology of fish at low environmentally relevant concentrations.

Histology as an assessment tool was successful in showing histopathological changes in the liver, kidney and testes of *C. gariepinus* at an early stage, even after 96 hour exposure to aldrin. Qualitative histological assessment showed melano-macrophage centres (MMC's) at 78% and vacuolation at 41% were present in the livers of fish exposed to the environmentally relevant concentrations of aldrin. In the livers of fish exposed to the higher concentrations of aldrin the most prevalent histopathology was MMC's at 97% and vacuolation at 74%. The MMC's (74%) and vacuolation (91%) were present in the kidneys of the fish exposed to the environmentally relevant concentration of aldrin. In the kidneys of fish exposed to the higher concentration of aldrin, MMC's (94%) and vacuolation (97%) were also present.

There was an increase in the percentage prevalence of vacuolation of the germ cells during the early stages of spermatogenesis of fish exposed to aldrin. This may be indicative of disruption to these cells caused by the endocrine disrupting effects of the pesticides. This would affect the process of spermatogenesis thus the formation of mature spermatozoa. However, the CASA results showed no significant differences in the sperm motility parameters of *C. gariepinus* in the control, solvent control and aldrin exposure groups. Therefore, there was no immediate effect on the reproductive health of male *C. gariepinus* after the acute exposure to environmentally relevant concentrations of aldrin (Das Neves et al., 2018).

Table 1. Percentage prevalence of histological changes and quantification of the severity of the changes represented as the mean testes index (IT) of Clarias gariepinus after exposure to aldrin 0.14 µg/L and 1.4 µg/L

	Control			Aldrin	
	Control	S Control 0.14	S Control 1.4	0.14 µg/L	1.4 µg/L
LIVER					
MMCs	33.3	100	100	50	83
Vacuolation	83	67	67.7	58	58
Atrophy	0	0	67.7	17	8.3
Necrosis	16.7	0	33.3	25	8.3
Hepatocellular pleomorphism	33.3	0	67.7	17	25
Mean Liver Index	9	9.3	22	12.3	12.3
KIDNEY					
MMCs	100	67	100	81	100
Vacuolation	100	33.3	100	54	100
Atrophy	0	0	0	0	0
Necrosis	0	0	0	0	0.08
Hepatocellular pleomorphism	0	0	0	0	0
Mean Kidney Index	6	8	6	6.4	10
TESTES					
Disorganisation of lobules	0	0	0	0	0
MMCs	50	16.7	0	0	0
Spermatogonia vacuolation	0	0	0	25	0
Sparmatocyte vacuolation	16.7	0	0	33.3	0
Mean Testes Index	1.7	0.7	0	1	0

CONCLUSIONS

The higher concentrations of aldrin caused a decrease in the condition factor of the catfish and an increase in the haematocrit of the catfish which could indicate acute stress. It can be concluded that the higher concentrations aldrin adversely affected the health status of *C. gariepinus*.

With regards to the reproduction, it can be concluded that the acute exposure of male *C. gariepinus* to low concentrations aldrin does not hinder the function of the reproductive system. However, the results of this study do indicate some initial whole-body response effects in changes to the haematology of the fish as well as in the early stages of the development of spermatogonia. Therefore, these initial effects should be taken into consideration with regards to the effects after long-term exposure of aldrin on *C. gariepinus* and population stability. It is therefore assumed that an acute exposure of fish to aldrin could not have caused a decline in fish numbers in the Albasini Dam (Das Neves et al., 2018). It can also not conclusively be argued, that prolonged exposure of fish to aldrin will not affect the reproductive health of the fish in a way that could lead to decreased fish populations in the Albasini Dam until the long-term effect of exposure has been investigated.

This protocol could in future studies used to assess the health and reproductive effects of EDCs on fish that were exposed to environmental pollutants.

In conclusion better legislation, regulation and enforcement of pesticide import and application is needed in South Africa because aldrin is a banned substance and its presence in the Albasini Dam is unacceptable. The research confirms that at environmentally relevant concentrations and at higher concentrations of aldrin in the aquatic environment may have adverse effects on fish health.

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POPULATION STRUCTURE OF SOCKEYE SALMON (*ONCORHYNCHUS NERKA*) OF THE RUSSIAN FAR EAST INFERRED FROM DATA ON SINGLE NUCLEOTIDE POLYMORPHISM (SNP)

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POPULACIONA STRUKTURA ALJASKOG CRVENOG LOSOSA (*ONCORHYNCHUS NERKA*) NA RUSKOM DALEKOM ISTOKU NA OSNOVU PODATAKA O POLIFORMIZMU POJEDINAČNIH NUKLEOTIDA (SNP)

Apstrakt

Ispitivana je populaciona struktura Aljaskog crvenog lososa sa poluostrva Kamčatka, Šukotka i sa severo zapadne obale Ohotskog mora. Ispitivana je varijabilnost 45 SNP lokusa u 17 uzoraka sa 10 najvažnijih slivova u kojima se mreste lososi na Azijsko Pacifičkoj obali. Opšti obrazac genetske heterogenosti crvenog lososa odgovarao je prostorno-geografskoj strukturi vrste. Svi uzorci su bili podeljeni na južnu i severnu grupu. Populacije iz subperifernog sliva obale Ohotskog mora (Ohotska regija, reka Palana) su više diferencirane od takozvane nuklearne populacije (glavni populacioni kompleks Azijske obale) reka Ozernaja, Kamčatka i grupu numerički sekundarnih stokova jezersko rečnog sistema Karjak brdski i Šukotka. Klasterizacijom u programu STRUCTURE 2.3.4 identifikovano je pet grupa populacija. Među njima uzorci iz reka Palana i Ohota, kao i Kamčatka grupa sa jugozapada i grupe Severoistočna Kamčatka i Šukotka i sliv reke Kamčatke su bili odvojeni. Karakter klasterizacije je odgovarao rezultatim AMOVA (hijerarhijska analiza molekularne varijanse). Razmatraju se verovatni uzroci visoke divergencije populacija reka Palana i Ohota kao i sličnosti između jugoistočne Kamčatske populacije. Najverovatniji faktor izdvajanja populacije reke Palana je lokalna adaptacija kao rezultat direkcionalne selekcije nekih lokusa u specifičnoj reproduktivnoj okolini. Reka ima hiperplimsko ušće (mlađ tokom transformacije/smoltifikacije mora da savlada 10-20 km slane zone velike zamućenosti i nizvodni tok gde provodi nekoliko dana). Ušće se nalazi na severnoj obali Ohotskog mora (mlađ migrira tako što dugo pliva duž obale zapadne Kamčatke da bi stigla do Pacifika), dakle treba da bude dovoljno jaka i zrela da bi preživela ovakve ekstremne uslove. Tako je uzrast migranata u donjem toku reke Palana pretežno 2+. U prilog našem zaključku je i statistički značajnan pravac selekcije za dva SNP locirana na genima MHC kompleksa i hormon glikoprotein (GPH), odgovoran za imune procese i humoralnu regulaciju rasta, sazrevanja i temperaturne tolerancije. Sa druge strane za populacija reke Ohota pretpostavlja se različit obrazac. Značajan pad genetičkog diverziteta u ovom uzorku verovatno ukazuje na nedavno opadanje veličine populacije, pre nego efekat direkcionalne selekcije. Populacija lososa iz reke Ohota se karakteriše oštrom fluktuacijom brojnosti koja se povezuje sa vodnim režimom reke Ohota. Test neutralnosti nije pokazao akciju selekcije ni na jednom SNP lokusu u uzorku. Svi naši zaključci su podržani rezultatima analize sa neutralnim markerima (mikrosateliti, mtDNK).

Ključne reči: *Aljaski crveni losos, polimorfizam pojedinačnih nukleotida (SNP), struktura populacije*
Keywords: *sockeye salmon, single nucleotide polymorphism (SNP), population structure.*

INTRODUCTION

Sockeye salmon, *Oncorhynchus nerka* Walbaum, is one of the three the most numerous species of Pacific salmons. The Asian part of its range is almost completely located on the territory of the Far East of Russia. The largest stocks, providing more than 95% of the Russian catch of sockeye salmon, reproduce in Kamchtka peninsula. Some commercially important minor stocks of sockeye salmon inhabit rivers of Chukotka and the continental coast of the Sea of Okhotsk.

Characteristic features of this species are strong phylopatry and extremely complex hierarchic population structure. Integrative approach to the study of such a structure with the involvement of both neutral and selective genetic markers seems to be the most promising as providing exhaustive information on the demographic and historical processes in populations, as well as on the effect of local selection, which determines their divergence. This is especially important for the species with multilevel intraspecific organization and recurrent history of range colonization, that sockeye salmon is. The objective of this study was an analysis of adaptive and neutral population structure of sockeye salmon from Kamchatka, the north-west coast of the Sea of Okhotsk, and Chukotka inferred from variability of 45 single nucleotide polymorphism (SNP) loci.

MATERIALS AND METHODS

The material was collected in 2004 through 2008 in the rivers of Kamchatka, Chukotka, and the continental coast of the Sea of Okhotsk (Fig. 1). Tissue samples were fixed in 96% ethanol. Genomic DNA was extracted from liver and fin fragments by standard methods. The polymorphism of 45 SNP loci described earlier (Habicht et al., 2010) was examined; three of them are localized in the mitochondrial genome, while the others are mostly found in nuclear genes, dispersed repeats, and EST sequences. The study presents original data on the genetic variation of 45 SNP loci of sockeye salmon from the Far East of Russia obtained with TaqMan-PCR. Molecular genetic analysis was carried out at the Laboratory of Ecological Genomics, School of Aquatic and Fishery Sciences, University of Washington. The genotyping method and statistical approaches are described in detail in (Khrustaleva et al., 2017).

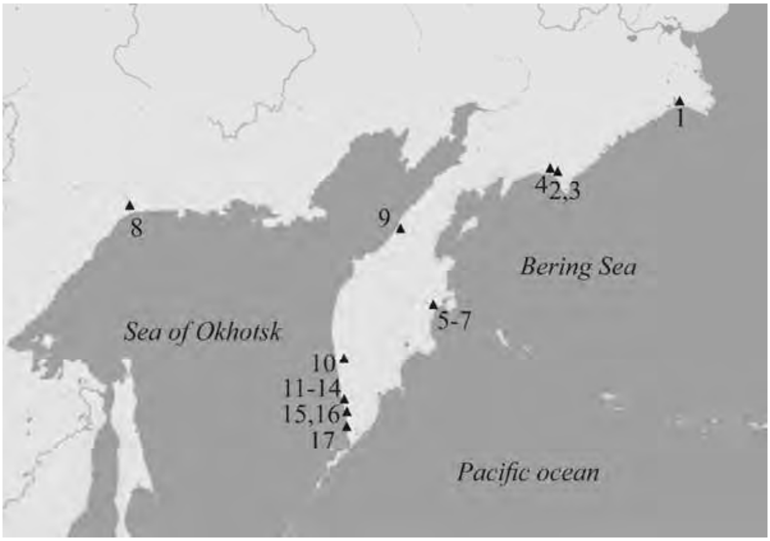

Figure 1. Schematic map of the study area showing sampling locations. The sample numbers are: 1 - Meinypilgin lake–river system, Lake Vaamochka (Ch), 2 - Apuka River (early sockeye salmon) (KAe), 3 - Apuka River (late sockeye salmon) (KAl), 4 - Pakhacha River (KPh), 5 - Kamchatka River (late-run sockeye salmon) (KK-04), 6 - Kamchatka River (early-run sockeye salmon) (KK-05), 7 - Azabach'e Lake, Bushuika River (early rheophilic sockeye salmon) (KKa), 8 - Okhota River (Okh), 9 - Palana River (KP), 10 - Bol'shaya Vorovskaya River (KV), 11 - Bol'shaya River (broodstock) 2003 (KB-03), 12 - Bol'shaya River (broodstock) 2004 (KB-04), 13 - Bol'shaya River basin, Bystraya River (juvenile) (KBb), 14 - Bol'shaya River basin, Plotnikova River (juvenile) (KBp), 15 - Opala River 2007 (KOp-07), 16 - Opala River 2008 (KOp-08), 17 - Ozernaya River (KO).

RESULTS AND DISCUSSION

Forty-three of the examined 45 SNP loci were polymorphic. Tests for linkage disequilibrium were not significant except for mitochondrial loci in all samples. For this reason allelic variants of these loci were combined into joint haplotypes. Thus, after excluding the monomorphic loci and combining mitochondrial SNPs, the number of analyzed loci was reduced to 41.

In a number of samples, deviations from the Hardy–Weinberg equilibrium at some loci were revealed (in 35 tests from 680). In all positive tests, statistically significant heterozygote deficiency was detected. The most probable causes of the observed disequilibrium in mixed samples from the mouths of large lake–river systems, in our opinion, are the Wahlund effect and the action of selection at some of nonequilibrium loci.

Genetic diversity among the samples, estimated by F_{ST}, averaged 0.0904 ($p = 0$). Detection of selectively loaded SNPs showed that the main histocompatibility complex (MHC) loci (MHC2_190v2 and MHC2_251v2) ($p < 0.01$) and glycoprotein hormone locus GPH-414 ($p < 0.01$) were the candidates for the effect of diversifying selection; the candidate for the effect of balancing selection was anonymous locus U504-141 ($p < 0.01$).

In the samples of sockeye salmon from the examined major spawning watersheds of this species in Asia, statistically significant heterogeneity of the allele and genotype frequencies of SNP loci was observed. The differences were not statistically significant (after sequential Bonferroni correction) for only the three pairs of samples from the watersheds of North-Eastern Kamchatka (Pakhacha and Apuka rivers), and between the samples of adjacent years from the mouth of the Kamchatka River, as well as for all pairs of samples from South-Western Kamchatka except the Ozernaya River sample.

The traces of recent reduction of effective population size (bottleneck) based on the results of the corresponding tests (sign test, standardized difference criterion, and Wilcoxon test) were detected in all samples at neutral SNP loci ($p < 0.05$).

In general, genetic differentiation of sockeye salmon in the examined part of the range closely corresponds to the spatial-geographic structure of this species. On both multidimensional scaling (MDS) plots, constructed using both neutral and selective SNPs and neutral loci only, the division into northern and southern groups is clearly seen (Fig. 2a, b). As it can be noticed from the diagrams populations associated with the watersheds located at the border of the range (North coast of the Sea of Okhotsk) are considerably differentiated from the so-called population nuclei of this species, that is, the largest population complexes of the Asian coast, the Ozernaya and Kamchatka rivers, and the group of numerous secondary stocks of the Koryak Upland lake–river systems and Chukotka. Using the STRUCTURE 2.3.4 clustering, five groups of populations were identified, among which the samples from the Palana and Okhota rivers were independently differentiated, as well as the groups of populations from Southwestern Kamchatka, Northeastern Kamchatka and Chukotka, and the basin of the Kamchatka River (Fig. 2c). The results of cluster analysis correlate well with the MDS-plots and with the estimates obtained using hierarchical analysis of molecular variance.

The high level of divergence of populations from the Palana and Okhota rivers deserves special attention. As for the first of them, the most likely explanation for this may be the effect of diversifying selection at some of the SNP loci due to the peculiarities of geographic location of this river (the subperiphery of the range) and to the peculiarities of the biology of the Palana sockeye salmon. The river has hypertidal estuary (juveniles during smoltification are forced to overcome a 10-20 km salt zone with high turbidity and flow rate in the lower course, where they spend up to several days) and is located at the north of the Sea of Okhotsk coast (juveniles have to make an extensive migration along the coast of Western Kamchatka to access the Pacific Ocean), consequently smolts should be enough strong and mature in other to survive in such extreme conditions. Thus the age of downstream migrants in Palana River is dominantly 2+. In support of our conclusions statistical evidences of directional selection were revealed for two SNPs located in genes of MHC complex and glycoprotein hypophysis hormone, responsible for immune processes and humoral regulation of growth, maturation, and temperature tolerance. According to another version, the reason for the pronounced differences of the Palana River sample could be a decrease in genetic diversity and a shift in the gene frequencies because of the relatively recent reduction of effective population size (bottleneck), but it wasn't evident based on neutral markers data.

On the other hand, for the Okhota River population a different pattern can be hypothesized. Considerable decrease in genetic diversity in this sample is likely to indicate a recent decline in the effective population size, rather than the effect of directional selection. The Okhota sockeye salmon population is characterized by rather sharp fluctuations in abundance, associated with the Okhota River water regime. The tests on neutrality did not reveal the action of selection at any of SNP loci in this sample. All our inferences are sustained by neutral markers (microsatellites, mtDNA) data analysis.

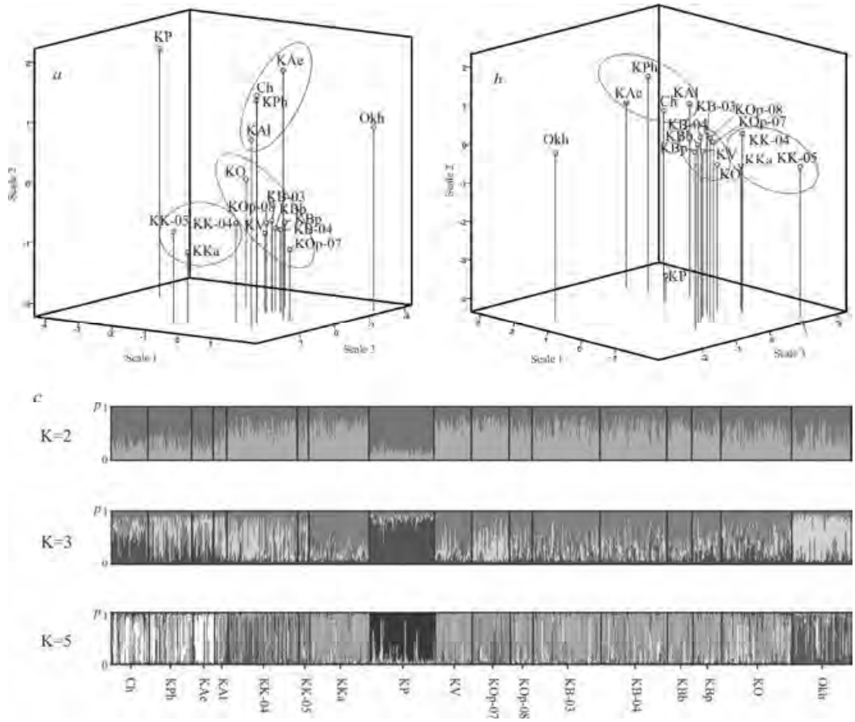

Figure 2. MDS-diagramms plotted by the Cavalli-Sforza chord genetic distances. (a) based on the analysis of 41 SNP loci polymorphism; (b) based on the analysis of 35 neutral SNP loci polymorphism, and clusterization of the sockeye salmon samples on the basis of STRUCTURE 2.3.4 algorithm. Different gradations of grey color are equal to two (K=2), three (K=3), and five (K=5) clusters and represent probability (p) of individual belonging to the clusters. Sample key as in Fig. 1.

CONCLUSIONS

We can conclude that high differentiation of Palana and Okhota River sockeye salmon is likely caused by mutual impact of adaptive (local selection) and demographic (gene drift, effective number decrease) processes occurred in these populations.

ACKNOWLEDGEMENTS

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BIOCHEMICAL AND HEMATO-IMMUNOLOGICAL PARAMETERS IN STERLET (*ACIPENSER RUTHENUS*) JUVENILES FED DIETS SUPPLEMENTED WITH SEA-BUCKTHORN AND THYME EXTRACT

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BIOHEMIJSKI I HEMATO-IMUNOLOŠKI PARAMETRI MLAĐI KEČIGE (*ACIPENSER RUTHENUS*) PRI ISHRANI OBOGAĆENOJ EKSTRAKTIMA PASJEG TRNA I MAJČINE DUŠICE

Apstrakt

Jedan od stalnih interesa u svetskoj akvakulturi je smanjenje troškova proizvodnje u uslovima poboljšanja prirasta u najkraćem roku. Postoje podaci da prirodni biljni ekstrakti ili esencijalna ulja pospešuju različite aktivnosti kao što prirast, stimulacija apetita i imunostimulacija u sistemima akvakulture. Poznavajući korisne osobine ulja pasjeg trna i majčine dušice, cilj ovog istraživanja bio je procena efekata ovih ekstrakata na biohemijske, hematološke i neke imunološke parametre mlađi kečige. Mlađ kečige (11,06 ± 1,73 g) sa farme jesetri „S.C. Danube Reasearch - Consulting S.R.L." (Tulcea, Rumunija) nasumično je raspoređena u četiri grupe: (1) kontrola, ne tretirane ribe, uobičajeno hranjene, (2) ribe hranjene sa 1% pasjeg trna/kg hraniva, (3) ribe hranjene sa 1% majčine dušice/kg hraniva i (4) ribe hranjene mešavinom 0,5% pasjeg trna i 0,5% majčine dušice/kg hraniva. Uzorci krvi uzeti su šezdesetog dana od sedam riba iz svake grupe i analizirani na: broj crvenih (RBC) i belih krvnih ćelija (WBC), hematokrit (Ht), hemoglobin (Hb), prosečnu koncentraciju hemoglobina na litar eritrocita (MCHC), prosečnu količinu hemoglobina u eritrocitu (MCH), prosečni volumen eritrocita (MCV), koncentraciju lizozima, malonilaldehida i ukupan antioksidativni kapacitet. Albumini, alanin aminotrasferaza, aspartat aminotransferaza, holesterol, glukoza, ukupni proteini i trigliceridi analizirani su uz pomoć autoanalizatora za hemiju krvi. Statistička analiza hematoloških podataka pokazala je da nema značajnih razlika u vrednostima Hb, Htc, MCV, MCH i MCHC posle davanja različitih uljnih ekstrakata u hrani u poređenju sa kontrolnom grupom. Istovremeno, broj RBC, WBC i neutrofila pokazao je značajan porast kod grupe hranjene dodatkom 1% majčine dušice u poređenju sa kontrolnom grupom. Boj eritrocita i leukocita povećan je uz administraciju majčine dušice, što može da ukaže na imunostimulirajući efekat. Statistička analiza

biohemijskih parametara ukazala je da nema promena u koncentraciji albumina, holesterola i aspartat aminotransferaze kod svih oglednih grupa u poređenju sa kontrolnom. Postoje značajne razlike u koncentraciji glukoze, ukupnih proteina i alanin aminotransferaze kod riba koje su hranjene dodatkom 1% majčine dušice, 1% pasjeg trna i 1% mešavini majčine dušice i pasjeg trna. Količina ukupnih proteina povećana je u svim oglednim grupama u poređenju sa kontrolnom grupom, ali je najveća količina utvrđena u grupi hranjenoj dodatkom 1% majčine dušice (1,98 ± 0,21 g·dl-1), što se smatra da je povezano sa poboljšanjem nespecifičnog imunološkog odgovora. U poređenju sa kontrolnom grupom, vrednost glukoze pokazala je značajno postepeno smanjenje, što može da bude u vezi sa hipoglikemijskim efektima karvakola, glavnom antioksidativnom komponentom majčine dušice. Aktivnost lizozima i ukupni antioksidativni kapacitet nisu bili promenjeni posle ishrane sa različitim uljnim ekstraktima, ali koncentracija malondialdehida bila je značajno smanjena samo u grupi čija je ishrana bila dopunjena sa 1% pasjeg trna ukazujući njegov antioksidativni efekat. Ovo istraživanje pruža nove informacije za upotrebu lekovitog bilja kao dodatak u ishrani kečiga. Njihova upotreba može da poveća otpornost na bolesti putem poboljšanja hematoloških i imunoloških indeksa.

Ključne reči: *kečiga, hemato-imunološki i biohemijski parametric*

Abstract

One of the constant interests in world aquaculture is to reduce production cost in the conditions of improving growth performance in the shortest time. Natural plant extracts or essential oils have been reported to promote various activities like growth promotion, appetite stimulation and immunostimulation in aquaculture systems. Knowing the beneficial properties of sea buckthorn and thyme oil, the objective of present study was to evaluate the effects of these extracts on biochemical, hematological and some immunological parameters in juvenile sterlet. The juvenile sterlets (11.06 ± 1.73 g) from the sturgeon farm of S.C. Danube Research - Consulting S.R.L. (Tulcea, Romania) were randomly distributed in four groups: (1) control, untreated fish, fed with normal diet, (2) fish fed with 1% Sea buckthorn/kg feed, (3) fish fed with 1% thyme/kg feed and (4) fish fed with mix 0.5% Sea buckthorn and 0.5% thyme/kg feed. Seven fish per group on 60th day were used for blood sampling and were analyzed to: red (RBC) and white blood cells (WBC) counts, hematocrit (Ht), hemoglobin (Hb), mean cell haemoglobin concentration (MCHC), mean cell haemoglobin (MCH), mean cell volume (MCV), lysozyme, malondialdehyde and total antioxidant capacity. Albumin, alanine aminotrasferase, aspartate aminotransferase, cholesterol, glucose, total protein, triglycerides were analyzed using a blood chemistry autoanalyser. Statistical analysis of hematological data showed that were no significant differences of Hb, Htc, MCV, MCH, and MCHC after feeding with different oil extracts compared to the control group. At the same time the RBC, WBC and neutrophil cells counts showed a significant increase in 1% thyme enriched diet feeding group compared to the control. The erythrocyte and leucocyte counts increased with the administration of thyme, which might indicate an immunostimulant effect. Statistical analysis of biochemical parameters indicated that were no changes in albumin, cholesterol and aspartate aminotransferase in all treatment groups compared to the control. There were significant changes in the glucose, total protein and alanine aminotrasferase of fish feed 1% thyme, 1% sea buckthorn and 1% mix thyme and sea buckthorn. The total protein content increased in all experimental groups compared to the control group but the highest content of total protein was determined in group 1% thyme (1.98 ± 0.21 g·dl-1) which is thought to be associated with a enhancement of the innate immunity response. Compared to the control group, the glucose value showed a gradual significant decrease, which might be associated with hypoglycemic effects of carvacrol, the main antioxidant components of the thyme. Lysozyme activity and total antioxidant capacity were not changed after feeding with different oil extracts, but concentration of malondialdehyde was significantly decreased only in the group which diet was supplemented with 1% Sea buckthorn indicating its antioxidant effect. This study provides a new information for the use of medicinal plants as supplementation to sterlet diet. Their use can increase disease resistance by improving the hematological and immunological indices.

Keywords: *sterlet, haemato-immunological and biochemical parameters*

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THE OPTIMIZATION OF THE LARGE-SCALE CRYOPRESERVATION AND THE MOTILITY ASSESSMENT IN WELS CATFISH (*SILURUS GLANIS*) SPERM

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OTIMIZACIJA MASOVNE KRIOPREZERVACIJE I ANALIZE POKRETLJIVOSTI SPERMATOZOIDA SOMA (*SILURUS GLANIS*)

Apstrakt

Som (*Silurus glanis*) je ekonomski značajna vrsta ribe. Veštački mrest soma se suočava sa određenim poteškoćama koje utiču na smanjenje efikasnosti proizvodnje (loš kvalitet sperme, asinhrona produkcija gameta itd.) (Bokor i sar., 2010). Krioprezervacija sperme omogućava sinhronizaciju dostupnosti gameta tokom praktičnog rada (Cabrita i sar., 2010). Ovom metodom vrši se dogotrajno skladištenje i omogućava transport sperme što može dovesti do značajnog smanjenja troškova prilikom rada sa mužjacima u period mresta (Bokor i sar., 2010, Cabrita i sar., 2010). Cilj ovog rada je bio poređenje uspešnost primene cevčica zapremine 5 mL i kriotubica zapremine 10 mL tokom masovne krioprezervacije sperme soma. U radu je takođe izvršeno i poređenje pokretljivost spermatozoida nakon odmrzavanja i aktivacije sa četiri različita aktivaciona rastvora.

U ovom istraživanju korišćeni su mužjaci (*N*=17) iz Szegedfish Ltd. (Segedin, Mađarska). Jedinke su bile anestezirane u rastvoru etilen glikol monofenil etra (u odnosu 1:200). Spermiacija je bila hormonski indukovana sa 4.5 mg/telesne mase kg^{-1} 24 časa pre sprovođenja eksperimenta. Testisi svih jedinki su disekovani i izmerena im je masa (37±24 g). Nakon uzorkovanja, celih testisi su isečeni na manje delove i procеđeni kroz mrežu promera 200 µm. Sperma prikupljena na ovaj način je skladištena na 4 °C. Parametri pokretljivosti (progresivna pokretljivost – pMOT (%), krivolinijska brzina – VCL (µm/s) i linearnost -STR (%)) sveže i otopljene sperme su određivani pomoću CASA sistema (Computer-assisted Sperm Analisys, Sperm VisionTM v. 3.7.4., Minitube of America, Venture Court Verona, USA). Sperma je bila aktivirana pomoću četiri različita aktivaciona rastvora sa dodatkom BSA (albumin iz seruma govečeta). Uzorci su, prema eksperimentalnom dizajnu, bili krio-

prezervirani pomoću dve različite metode. U oba slučaja, korišćen je ekstender koji se sastojao od 6% fruktoze, 10% metanola (kao krioprotektant) i 20 mM $NaHCO_3$ u odnosu 1:1 (Bokor i sar., 2010). Krioprezervirana sperma je bila topljena u vodenom kupatilu na 40 °C.

Eksperiment 1: Poređenje primene cevčica zapremine 50 ml i kriotubica zapremine 10 ml

Sperma 11 mužjaka je zamrzavana u cevčicama zapremine 5 mL u trajanju od 7 minuta (ukupna zapremina u tubici: 4 mL) na stiropornom ramu, 3 cm iznad nivoa tečnog azota (Bokor i sar. 2010). Drugi deo uzoraka je krioprezerviran pomoću aparata za kontrolisano zamrzavanje (CRF, IceCube 14s, IceCube Series v. 2.24, Sy-Lab, Neupurkersdorf, Austria) u kriotubicama zapremine 10 mL (ukupna zapremina u tubici: 8 mL). Program hlađenja (od 4 °C do -160 °C, stopa hlađenja: 15 °C/min) je bio meren pomoću termokapilare (Digi-Sense DualLogR®, Eutech Instruments Pte Ltd, Singapore) na 3 cm iznad tečnog azota tokom 7 minuta (Bokor i sar., 2010). Sperma je bila aktivirana pomoću fiziološkog rastvora (50 mM NaCl, 30 mM Tris, pH: 8, Lahnsteiner 2011).

Eksperiment 2: Poređenje četiri različita rastvora za akzivaciju otopljene sperme

U ovom eksperimentu, uzorci sperme 6 mužjaka su krioprezervirani u kriotubicama zapremine 10 mL pomoću prethodno pomenute metode. Parametri motiliteta spermatozoida su mereni nakon njihove aktivacije pomoću 4 različita rastvora i to 10, 20, 30, 60, 90 i 120 sekundi nakon aktivacije.

1. 50 mM NaCl, 30 mM Tris, pH: 8 (NaCl rastvir-**Ns**, Lahnsteiner 2011).
2. 75 mM NaCl, 2 mM KCl, 1 mM $MgSO_4 \times 7 H_2O$, 1 mM $CaCl_2 \times 2 H_2O$, 20 mM Tris, pH: 8 (modifikovani aktivacioni rastvor prema Lahnsteiner-**La**, Lahnsteiner 2011).
3. 5.5 g NaCl, 3.76 g glicin, 2.42 g Tris, pH: 8 (Billard rastvor-**Bs**, Billard 1977).
4. 4 g/L NaCl, 3 g/L urea pH 8,0 (Woynárovich rastvor-**Ws**, Woynárovich i Woynárovich 1980).

U Eksperimentu 1, značajno niži pMOT je izmeren nakon topljenja obe test grupe u odnosu na svežu kontrolu (84±5%). Značajno viši pMOT je zabeležen kod uzoraka krioprezerviranih u kriotubicama zapremine od 10 mL (36±7) nego od 5 mL (11±10%). VCL i STR nisu pokazaali značajan pad u vrednostima u krioprezerviranim grupama u odnosu na kontrolne uzorke niti međusobno. U Eksperimentu 2, značajno niži pMOT je zabeležen prilikom korišćenja Bs u odnosu na La i Ws i periodu od 10 do 120 sekundi dok je slična tendencija primećena i u poređenju sa Ns na 10 do 90 sekundi. Značajno niža vrednost pMOT je zabeležena primenom Ws na 10 i 30 sekundi u poređenju sa Ns. Značajno viša vrednost VCL je izmerena u slučaju La nego Bs na 20 i 90 sekundi. BS je takođe pokazao i smanjenje u VCL u poređenju sa Ws na 20, 90 i 120 sekundi. Slično ovome, značajno niža vrednost STR je zabeležena sa Bs u poređenju sa La i značajno smanjenje je takođe zabeleženo u poređenju sa Ws na 20 i 90 sekundi.

Može se zaključiti da je nova metoda (kriotubice zapremine 10 mL) uspešno testrana za primenu u masovnoj krioprezervaciji sperme soma. Rastvor 50 mM NaCl, modifikovan Lahnsteiner imobilizacioni rastvor i Woynárovich rastvor su pogodni za aktivaciju odmrznute sperme soma.

Ključne reči: *som, masovno, krioprezervacija, analiza prokretljivosti, sperma*

Abstract

Wels catfish (*Silurus glanis*) is an economically important species. Its propagation faces with several difficulties (low quality of stripped sperm, harvesting males during propagation, asynchronous gamete production etc.) which reduce the efficiency of the production (Bokor et al 2010). Sperm cryopereservation allows the synchronization of gamete collection during the hatchery process (Cabrita et al 2010). The method provides the long-term storage and transportation of sperm which can reduce the costs originated from the harvest of wels catfish males (Bokor et al 2010, Cabrita et al. 2010). The aim of our study was to compare the 5 mL straws and 10 mL cryotubes during large-scale sperm cryopreservation in wels catfish. Our objective was also to compare the sperm motility using 4 different activating solutions following thawing.

Males (*N*=17) were purchased from the broodstock of the Szegedfish Ltd. (Szeged, Hungary). Fish were anaesthetized using ethylene glycol monophenyl *ether dissolved in hatchery water (in a ratio 1:200)*. Spermiation was hormonally induced with 4.5 mg/body weight kg^{-1} 24 hours prior to the experiments. Testis from all individual was dissected and measured (37±24 g). Following sampling, the whole testis was cut into small pieces and was squeezed using a 200 µm net. The collected milt was stored at approximately 4 °C. Motility parameters (progressive motility-pMOT (%), curvilinear velocity-VCL (µm/s) and straightness-STR (%)) of fresh and thawed sperm was recorded using a CASA system (Computer-assisted Sperm Analisys, Sperm VisionTM v. 3.7.4., Minitube of America, Venture Court Verona, USA). Sperm was activated using 4 different activating solutions in a mixture of BSA (bovine serum albumin). Samples were cryopreserved using two different methods also according to the experimental design. In both techniques, an extender composed of 6% fructose, 10% methanol (cryoprotectant) and 20 mM $NaHCO_3$ in a ratio 1:1 was used (Bokor et al. 2010). Frozen sperm was thawed using a water bath at 40 °C.

Experiment 1. The comparison of 5mL straw and 10 mL cryotubes

Sperm from 11 males was frozen for 7 minutes using 5 mL straws (total volume in a straw: 4 mL) on a Styrofoam frame 3 cm above the liquid nitrogen (Bokor et al. 2010). The second portion of the samples was cryopreserved with a controlled-rate freezer (CRF, IceCube 14s, IceCube Series v. 2.24, Sy-Lab, Neupurkersdorf, Austria) in 10 mL cryotubes (total volume in a cryotube: 8 mL). The cooling program (from 4 °C to -160 °C, cooling rate: 15 °C/min) was measured at 3 cm above the liquid nitrogen using a thermo couple (Digi-Sense DualLogR®**, Eutech Instruments Pte Ltd, Singapore**) for 7 minutes (Bokor et al. 2010). Sperm was activated using a simple saline solution (50 mM NaCl, 30 mM Tris, pH: 8, Lahnsteiner 2011).

Experiment 2. The comparison of 4 different activating solution in thawed sperm

In this experiment, samples from 6 males was cryopreserved in 10 mL cryotubes using the above mentioned method. Motility parameters of thawed sperm were recorded using 4 different activating solutions following activation at 10, 20, 30, 60, 90 and 120 seconds:

1. 50 mM NaCl, 30 mM Tris, pH: 8 (NaCl solution-**Ns**, Lahnsteiner 2011).

2. 75 mM NaCl, 2 mM KCl, 1 mM $MgSO_4 \times 7 H_2O$, 1 mM $CaCl_2 \times 2 H_2O$, 20 mM Tris, pH: 8 (modified Lahnsteiner’s activating solution-**La**, Lahnsteiner 2011).

3. 5.5 g NaCl, 3.76 g glicin, 2.42 g Tris, pH: 8 (Billard’s solution-**Bs**, Billard 1977).

4. 4 g/L NaCl, 3 g/L urea pH 8,0 (Woynárovich solution-**Ws**, Woynárovich and Woynárovich 1980).

In *Experiment 1*, a significantly lower pMOT was measured following thawing in both groups in comparison with the fresh control (84±5%). In the 10 mL cryotubes (36±7%), a significantly higher pMOT was recorded than in the 5 mL straw group (11±10%). VCL, and STR did not show a significant reduction in the cryopreserved groups compared to the control group or each other. *In experiment 2*, a significantly lower pMOT was recorded using the Bs than in the La and Ws in a range at 10 to 120 seconds where a similar tendency was observed in comparison with the Ns at 10 to 90 seconds. A significantly lower pMOT was recorded using the Ws at 10 and 30 seconds compare to the Ns. A significantly higher VCL was measured in La than in Bs at 20 and 90 seconds. Bs showed also a decrement in VCL compare to Ws at 20, 90 and 120 seconds. Similarly, a significantly lower STR was recorded in Bs in comparison with La and a significant reduction was also observed compare to Ws at 20 and 90 seconds.

In conclusion, a new method (10 mL cryotube) for the large-scale cryopreservation of wels catfish sperm was efficiently tested. The 50 mM NaCl solution, modified Lahnsteiner's immobilizing solution and Woynárovich solution are suitable as activating solution for thawed wels catfish sperm.

Keywords: *wels catfish, large-scale, cryopreservation, motility assessment, sperm*

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SOCIOECONOMIC IMPACT AND MICROBIOLOGICAL STUDIES OF SOME WATER SPRINGS IN UTTARAKHAND, INDIA

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SOCIOEKONOMSKI UTICAJ I MIKROBIOLOŠKA STUDIJA IZVORA U UTTARAKHANDU U INDIJI

Apstrakt

Izvori se smatraju važnim nalazištima vode za vodosnabdevanje stanovništva. Na putu hodočašća u Utarakhandu, u Indiji, postoji nekoliko izvora koji služe kao važna odmorišta za hodočasnike i putnike. Međutim, mnogi izvori vode su mikrobiološki kontaminirani usled različitih antropogenih uticaja. U ovom radu opisuje se značaj mnogih izvora koji se nalaze duž nacionalnog autoputa kao izvora vode za piće, a i naglašava se njihov uticaj na socioekonomski razvoj lokalnih zajednica u Utarakhandu u Indiji. U ovoj studiji je takođe utvrđeno ukupno kultivisano bakterijsko opterećenje i prisustvo štetnih patogena u pomenutim izvorištima.

Ključne reči: *izvori vode, kontaminacija, patogeni, antropogeni uticaj, socioekonomski razvoj*

Abstract

Springs are considered as an important source of water for human consumtion. There are several water springs on the pilgrimage route in Uttarakhand, India, serving as a significant resting area for pilgrims and travelers. Many of the water sources however are getting microbial contamination due to a variety of anthropological influences. The present study describes the importance of many springs along national highway, as a source of drinking water, as well as highlights their impact on socioeconomic development of local people in Uttarakhand, India. The total cultivated bacterial load and presence of harmful pathogen in those water sources were also determined in this study.

Keywords: *water sources, contamination, pathogen, anthropological impact, socioeconomic development*

PROTECTION OF SLOVENIAN FISH POPULATIONS AGAINST INTRODUCTION OF VIRUS HEMORAGIC SEPTICEMIA (VHS) AND INFECTIOUS HEMATOPOIETIC NECROSIS (IHN)

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ZAŠTITA SLOVENAČKIH POPULACIJA RIBA OD UNOŠENJA VIRUSNE HEMORAGIČNE SEPTIKEMIJE (VHS) I ZARAZNE HEMATOPOEZNE NEKROZE (IHN)

Apstrakt

Slovenija je jedna od najbogatijih evropskih zemalja u pogledu količine vode. Ukupno, preko 9000 ha vode pokriva njenu površinu. U našim rekama i jezerima živi 96 vrsta riba, od kojih su 33 vrste zaštićene. Upravljanje ribarstvom u Sloveniji vrše ribarske porodice (FF), koje upravljaju sa 90% slovenačkih voda, dok sa preostalih 10% upravlja Institut za ribarstvo (FRIS). Trenutno ima 64 FF u našoj zemlji, od kojih 24 imaju sopstvena mrestilišta ili ribnjake za gajenje autohtonih vrsta riba u kojima su pretežno uzgaja potočna pastrmka (*Salmo trutta*). Intenzivno gajenje salmonidnih vrsta u ribnjacima je doprinelo i širenju zaraznih bolesti, poput virusne hemoragične septikemije (VHS) i infektivne hematopoezne nekroze (IHN). VHS i IHN uzrokuju virusi koji pripadaju istom rodu, Rhabdoviridae. Obe bolesti nastaju u hladnom periodu godine i obično se javljaju od oktobra do maja, ili kada su temperature vode ispod 14°C (de Kinkelin 1983). Iako ove bolesti izazivaju srodni virusi, način njihovog delovanja je drugačiji. IHN virus uglavnom izaziva oboljenje kod mlađi kalifornijske pastrmke (*Oncorhynchus mykiss*) i lososa, dok virus VHS-a zahvata sve starosne kategorije, a posebno je osetljiva konzumna kalifornijska pastrmka, potočna pastrmka, lipljan (*Thymallus thymallus*) i štuka (*Esox lucius*). Jedinke prijemčivih vrsta koje prežive infekciju ostaju doživotni nosioci virusa (Jorgensen 1980, de Kinkelin i Castric 1982, Castric i de Kinkelin 1984, de Kinkelin 1983). Kada se životinje nadju u stresnim uslovima, one oslobađaju i prenose virus na neinficirane populacije, pa iako su klinički normalne, predstavljaju stalnu pretnju za širenje bolesti (Traxler et al., 1997).

1995. godine u Sloveniji je uvedena sistematska kontrola obe bolesti ispitivanjem ovarijalne i semene tečnosti matičnih zapata pastrmke i lipljena u svim registrovanim

mrestilištima koristeći izolaciju VHSV i IHNV na kulturi ćelija, i identifikaciju pomoću metoda zasnovanih na antitelima (test indirektnog fluorescentnog antitela) i molekularnih tehnika (OIE, 2017). Pre nekoliko godina, ova kontrola je proširena i na ribnjake koja se bave prodajom žive ribe, gde se godišnje vrši uzorkovanje organa 30 prijemčivih vrsta.

Već više od dve decenije ova metoda obezbedjuje dobar pregled prisustva obe bolesti i predstavlja dobru polaznu tačku za primenu odredbi nacionalnog zakonodavstva prema kome će, nakon 1.1.2019., unošenje ribe u otvorene vode biti dozvoljeno isključivo iz ribnjaka sa zdravstvenim statusom «slobodan od bolesti». Kada se FF ili ribarska farma prijave za dobijanje zdravstvenog statusa « slobodan od bolesti», Nacionalni veterinarski institut priprema Program nadzora u skladu sa evropskim zakonodavstvom. Program pokriva potreban broj kliničkih pregleda i uzoraka u određenom periodu. Nakon uspešnog završetka programa, Uprava za bezbednost hrane Republike Slovenije, Uprava za veterinu i zaštitu bilja dodeljuje zdravstveni status « slobodan od bolesti». Do 1.1. 2019., riba sa farme se može uvesti u otvorene vode, kada je ribnjak najmanje istog statusa kao i otvorena voda, ali ni u kom slučaju iz zarazne akvakulture. Trenutno ima 22 IHN i 4 VHS pozitivna ribnjaka u Sloveniji, dok su 22 ribnjaka dobilo status «VHS i IHN slobodna farma ili kompartment». Očekujemo da će do 1.1.2019. još 18 dobiti taj status.

Većina slovenačkih ribarskih proizvođača koji proizvode ribu za repopulaciju otvorenih voda priključili su se Programima za dobijanje statusa «VHS i IHN slobodan ribnjak» pa stoga možemo očekivati da se pastrmske vrste riba mogu slobodno uvoditi u Slovenačke otvorene vode od naredne.

Ključne reči: *upravljanje ribolovom, IHN virus, VHS virus, ovarijalna i semena tečnost*

Abstract

Slovenia is one of the richest European countries concerning water quantity. In total, more than 9000 ha of water cover its surface. In our rivers and lakes live 96 fish species, of which 33 are protected. Fish management in Slovenia is organized by fishing families (FF), which manage 90% of Slovenian waters, while the remaining 10% are managed by Fisheries Research Institute (FRIS). Currently there are 64 FF in our country, of which 24 have their own hatcheries or fish farms for the cultivation of the indigenous fish species where brown trout (*Salmo trutta*) is mostly breed and introduced in open water at various stages of development. Intensive breeding of salmonid species in farms has also contributed to the spread of contagious infectious diseases such as viral haemorrhagic septicemia (VHS) and infectious haematopoietic necrosis (IHN). VHS and IHN cause viruses of the same genus, Rhabdoviridae. Both diseases arise in the cooler part of the year and usually occur from October to May, or when the water temperatures are below 14 ^{o}C (de Kinkelin 1983). Although diseases cause related viruses, their mode of action is different. The IHN virus is characterized mainly by affecting the fingerling of *rainbow trout* (*Oncorhynchus mykiss*) and salmon, and the VHS virus affects all categories, especially susceptible are fish species of consume size i.e. rainbow trout, brown trout, grayling (*Thymallus thymallus*) and pike (*Esoxlucius*). Fish susceptible to the disease that survive infection remain virus carriers for lifetime(Jorgensen 1980, de Kinkelin and Castric 1982,Castric and de Kinkelin 1984, de Kinkelin 1983). When animals are in in stressful situations they release and transmit the virus to the uninfected population, although clinically normal, they represent a permanent threat for spreading of the disease (Traxler et al., 1997).

In 1995 a systematic control of both diseases was introduced in Slovenia by examining ovarian and seminal fluid of trout and grayling broodstock in all registered hatcheries using isolation of VHSV and IHNV in cell culture followed by identification using antibody-based methods (indirect fluorescent antibody test) and molecular techniques (OIE, 2017). A few years ago, this control extended also to the fish farms engaged in the sale of live fish, where the sampling of organs of 30 susceptible species is carried out annually.

For more than two decades this method provide a good overview of both diseases and is a good starting point for the implementation of the provisions of national legislation according to which after 1.1.2019 introduction of fish in open water will be allowed exclusively from fish farms with the «disease free» health status.When the FF or fish farm apply for achieving "disease free" health status, the National Veterinary Institute prepare a Surveillance Program in accordance with European legislation. The Program covers the required number of clinical examinations and samplings in a definite period. After the successful completion of the program, the Administration of the Republic of Slovenia for Food Safety, Veterinary Sector and Plant Protection, assigns the «disease free» health status. Before 1.1. 2019, fish from farms may be introduced into open waters, when fish farm have at least the same status as open water, but in no case from infected aquaculture. Currently there are 22 IHN and 4 VHS positive fish farms in Slovenia, while 22 fish farms already achieved the status «VHS and IHN free farm or compartment». We expect that by 1.1.2019 another 18 will obtain this status.

Most Slovenian fish farmers who produce fish for the repopulation of open waters have joined the Programs for obtaining the status «VHS and IHN free farm» therefore we can expect that trout fish species can be freely introduced in Slovenian open waters from next year onwards.

Keywords: *Fish management, IHN virus, VHS virus, ovarian and seminal fluid*

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EFFECTS OF DIETARY VITAMIN E ON GROWTH AND ANTIOXIDANT STATUS OF RAINBOW TROUT UNDER HIGH CULTURE DENSITY

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UTICAJ VITAMINA E U ISHRANI KALIFORNIJSKE PASTRMKE NA RAST I ANTIOKSIDATIVNI STATUS U USLOVIMA VELIKE GUSTINE NASADA

Apstrakt

Gustina nasada ima tendenciju rasta u sistemima za gajenje pastrmki (*Oncorhynchus mykiss*), uglavnom u cilju povećanja profitabilnosti. Ranija istraživanja su ukazala da uzgoj riba u velikoj gustini nasada može izazvati hronični stres. Stres izaziva oksidativni stres i umanjuje antioksidativni status gajenih riba. Ovo rezultuje stvaranjem reaktivnih kiseoničnih vrsta (reactive oxygen species, ROS). Kada je stepen proizvodnje ROS veći od stepena eliminacije, dolazi do oksidativnog stresa koji dovodi do peroksidacije nezasićenih masti ćelijskih membrana. Studija je ispitivala uticaj vitamina E u hrani na rast i antioksidantni odgovor kalifornijske pastrmke u uslovima velike gustine nasada. Komercijalna hrana za pastrmke sa 104.75 mg kg^{-1} vitamina E, korišćena je kao osnovna u ovom ispitivanju. Ribe su bile podeljene u 2 grupe i hranjene: osnovnom i hranom "vitamin E". Hrani za grupu vitamin E je dodavano 500 mg vitamina E po kg hrane. Mlađ kalifornijske pastrmke (mase 42.6±2.3 g) nasađena je u 6 tankova u gustini od 80 kg m^{-3}. Svaka grupa se sastojala iz 3 tanka. Ribe su hranjene jednom od eksperimentalnih hrana, ručno, do zasićenja, 3 puta dnevno u periodu od 60 dana. Posle 60 dana izračunati su: prirast (weight gain, WG), specifična stopa rasta (specific growth rate, SGR) i koeficijent konverzije hrane (feed conversion ratio, FCR). Za određivanje enzimskih antioksidanasa uzorkovano je tkivo jetre od po 3 ribe po tanku. Parametri rasta riba dati su u Tab. 1. Uticaj vitamina E u hrani na oksidativni stres i odgovor antioksidanasa u tkivu jetre pastrmke predstavljeni su na Tab. 2.

Tabela 1. Uticaj vitamina E u hrani na parameter rasta kalifornijske pastrmke u uslovima velike gustine nasada u periodu od 60 dana (srednja vrednost ± SD, n = 3).

Grupa[a]	Kontrola	Vitamin E
Početna težina - Initial weight (g)	42.60 ± 2.30	42.60 ± 2.30
Završna težina - Final weight (g)	91.57 ± 1.43[b]	108.66 ± 3.30[a]
Prirast - Weight gain (g)	48.97 ± 1.43[b]	66.06 ± 3.30[a]
FCR ($g\ g^{-1}$)	1.13 ± 0.00[a]	1.06 ± 0.03[b]

Srednje vrednosti u istom redu sa različitim slovima u superskriptu su značajno različite ($P < 0.05$). [a] Ribe su bile podeljene u 2 grupe: kontrolna hranjena osnovnom hranom i grupa vitamin E hranjena osnovnom hranom sa dodatkom 500 mg kg^{-1} vitamina E. FCR, koeficijent konverzije hrane, feed conversion ratio.

Tabela 2. Uticaj vitamina E u hrani na aktivnost enzimskih antioksidanasa i nivo malondialdehida (malondialdehyde, MDA) pastrmke u uslovima velike gustine nasada u period od 60 dana (srednja vrednost ± SD, n = 3).

Grupa	Kontrola	Vitamin E
CAT (U mg^{-1} protein)	4.23 ± 0.24	4.61 ± 0.22
GPX (U mg^{-1} protein)	3.50 ± 0.04	3.59 ± 0.13
SOD (U mg^{-1} protein)	4.23 ± 0.30[b]	5.49 ± 0.32[a]
MDA (nmol mg^{-1} protein)	4.74 ± 0.22[a]	3.43 ± 0.27[b]

Srednje vrednosti u istom redu sa različitim slovima u superskriptu su značajno različite ($P < 0.05$). CAT, catalase; GPX, glutathione peroxidase; SOD, superoxide dismutase; MDA, malondialdehyde.

Najbolje rezultate u uslovima velike gustine nasada ostvarile su ribe hranjene hranom sa dodatim vitamina E . Antioksidansni sistem odbrane koji uključuje SOD, CAT I GPx ključan je za eliminaciju ROS u uslovima oksidativnog stresa izazvanog ROS. TBARS test se smatra indeksom proizvoda lipidne peroksidacije i niži nivo MDA se povezuje sa dužim životom i boljim kvalitetom mesa. Najviša aktivnost SOD je zabeležena u grupi Vitamin E. Najniži nivo MDA je takođe konstatovan u grupi Vitamin E. Rezultati ukazuju da vitamin E može da poboljša antioksidativni status i smanji lipidnu peroksidaciju kod kalifornijske pastrmke u uslovima velike gustine nasada.

Ključne reči: *kalifornijska pastrmka; velika gustina nasada; vitamin E; antioksidativni status*

Abstract

In rainbow trout (*Oncorhynchus mykiss*) aquaculture systems, fish densities tend to increase, mostly to improve farm profitability. Previous works revealed that rearing fish at high density may act as a chronic stressor. Stressors may cause oxidative stress and reduce antioxidant status of farmed fish, resulting in the generation of reactive oxygen species (ROS). When ROS production rate exceeds that of their removal, oxidative stress occurs, leading to the peroxidation of unsaturated lipids from cell membranes. The present study was undertaken to examine the effects of dietary vitamin E on growth and antioxidant response of rainbow trout under high density conditions. A commercial rainbow trout diet

containing 104.75 mg kg^{-1} vitamin E was used as the basal diet in this study. The fish were divided into two groups according to diet: control (basal diet) and vitamin E. The diet of the vitamin E group was supplemented with an additional 500 mg vitamin E per kg diet. Juvenile rainbow trout (average weight of 42.6±2.3 g) were stocked into 6 tanks at a density of 80 kg m^{-3}. Each group consisted of three tanks. The fish were fed one of the two experimental diets by hand to apparent satiation three times a day for 60 days. After 60 days, weight gain (WG), specific growth rate (SGR), and feed conversion ratio (FCR) were calculated. The liver tissue was also collected from 3 fish per tank for determination of antioxidant enzymes. Growth performances of the fish are presented in Table 1. Effects of dietary vitamin E on oxidative stress and antioxidant responses in liver tissues of rainbow trout are presented in Table 2.

Table 1. Effects of dietary vitamin E on growth performance of rainbow trout under high culture density for 60 days (mean ± SD, n = 3).

Group^a	Control	Vitamin E
Initial weight (g)	42.60 ± 2.30	42.60 ± 2.30
Final weight (g)	91.57 ± 1.43^b	108.66 ± 3.30^a
Weight gain (g)	48.97 ± 1.43^b	66.06 ± 3.30^a
FCR (g g^{-1})	1.13 ± 0.00^a	1.06 ± 0.03^b

Means in the same row with different superscripts are significantly different ($P < 0.05$). ^{a} The fish were divided into two groups: Control group fed with basal diet and Vitamin E group fed with basal diet supplemented with 500 mg kg^{-1} vitamin E. FCR, feed conversion ratio.

Table 2. Effects of dietary vitamin E on antioxidant enzyme activities and MDA levels in rainbow trout under high culture density for 60 days (mean ± SD, n = 3).

Group	Control	Vitamin E
CAT (U mg^{-1} protein)	4.23 ± 0.24	4.61 ± 0.22
GPX (U mg^{-1} protein)	3.50 ± 0.04	3.59 ± 0.13
SOD (U mg^{-1} protein)	4.23 ± 0.30^b	5.49 ± 0.32^a
MDA (nmol mg^{-1} protein)	4.74 ± 0.22^a	3.43 ± 0.27^b

Means in the same row with different superscripts are significantly different ($P < 0.05$). CAT, catalase; GPX, glutathione peroxidase; SOD, superoxide dismutase; MDA, malondialdehyde.

The best performance under high density condition was observed in fish fed on vit E-supplemented diet. Under oxidative stress induced by reactive oxygen species (ROS), antioxidant defense system including SOD, CAT and GPx are essential for scavenging the ROS. TBARS test is considered as an index of lipid peroxidation products and lower MDA level is associated with longer life and better meat quality. The highest SOD activity was observed in the vitamin E group. These results coincided with the lowest levels of MDA in the vitamin E group as compared to the control, suggesting that vit E can improve the antioxidant status and reduce lipid peroxidation in rainbow trout under high density conditions.

Keywords: *Rainbow trout; High density; Vitamin E; Antioxidant status*

DIFFERENTIAL ACCUMULATION OF HEAVY METALS IN THE TISSUES OF *CLARIAS GARIEPINUS* FROM ASA RIVER, ILORIN NIGERIA

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DIFERENCIJALNA AKUMULACIJA TEŠKIH METALA U TKIVIMA *CLARIAS GARIEPINUS* IZ REKE ASA, ILORIN, NIGERIJA

Apstrakt

Opasan otpad i ostali industrijski sporedni proizvodi su glavni izvor zagađenja u Nigeriji. Većina organskih i neorganskih zagađujućih materija koje se ispuštaju u životnu sredinu su u rastvorenom, suspendovanom ili nerastvorljivom obliku. Poznato je da ribe bioakumuliraju teške metale u mišićima i drugim organima. Ovo istraživanje je rađeno u cilju procene biokumulacije teških metala u određenim organima i tkivima afričkog soma u reci Asa Ilorin, Kwara State Nigerija. Riba je uzorkovana na 3 lokaliteta (A, B, C) zavisno od mesta ispuštanja otpadne vode. Ribe vrste Afrički som (mase 129.4±1g dužine 25.2±2.00cm) su uzorkovane sa svakog lokaliteta (uzvodno, sa mesta ispusta otpadne vode i nizvodno) upotrebom ribarskih mreža. Uzorci su obrađeni i pripremljeni za analizu upotrebom atomskog apsorbcionog spektrofotometra (Atomic Absorption Spectrophotometer (Unicam 969, Analytical Technology Inc. Cambridge, United Kingdom). Statistička analiza rađena je pomoću programa SPSS verzija 5 a dvofaktorijalna ANOVA korišćena je radi poređenja lokaliteta i organa. Studija je pokazala neželjene efekte na živi svet, naročito ribu. Teški metali iz vodene sredine mogu da se kumuliraju u telu i organima riba, tako da je voda i riba iz reke Asa nisu pogodne za ljudsku upotrebu.

Ključne reči: *reka Asa, teški metali, Clarias gaiepinus, tkiva*
Keywords: *River Asa, heavy metals, Clarias gariepinus, tissues*

INTRODUCTION

With the increased urbanization and industrialization in Nigeria, there has been a rapid increase in the industrial effluents, hence environmental pollution, which affected aquatic organisms directly or indirectly. Heavy metal ions do not degrade into harmless end products and will be toxic to many life forms (Ajayan *et al.,* 2011; Paulami and Banerjee, 2012). Due to their toxicity and accumulation in the biota, determination of the levels of heavy metals in fish species has received considerable attention (Olowu et al., 2010; Wariaghli *et al.,* 2013). Fish species are known for their innate potential to bioaccumulate heavy metals in their muscles and various organs (Ayotunde and Offen. 2012). However, there is paucity of information on the bioaccumulation of metals in *African catfish* from different sites of Asa River. This study primarily assessed the bioaccumulation status of heavy metals in *African catfish* from three sampling sites in Asa river Ilorin, Kwara State Nigeria.

MATERIALS AND METHODS

Asa River is endowed with a surface area of 302 hectare and a maximum depth of 14m, located approximately 4km south of Ilorin, township Kwara state (Adekeye, 2004). The river lies between latitude $8^{o}28^{I}$ and 8^{o} 52N and longitude $4^{o}45^{o}$ E. Some people in Ilorin and its environs depend on this river as their major source of water for agricultural activities such as irrigation of farmland and fishing. Three sampling sites (A, B and C) were selected in this study in relation to the industrial effluents that enter the stream. Site A is upstream along Osere stream, about 1.5m from the discharge site (site B), it occasionally receives refuse and runoffs water from land and other effluents, while site C is downstream about 1.5m from discharge site. The choice of the sampling sites was based on the accessibility, the rate at which they receive effluents from soap and detergent industry, the extent of their pollution and the distance from Global soap and detergent industry.

African catfish (mean weight 129.4±1g and mean length 25.2±2.00cm) were randomly harvested from each site, using fishing net to catch the fish from the three sites (upstream, point of discharge and downstream). There were no fishes in the point of discharge throughout the sampling period. Fishes caught from site A and C were stored in clean coolers packed stocked with ice blocks in order to maintain optimum temperature and immediately taken to laboratory for dissection using stainless steel scalpels. The fish organs (gills, head, bone and muscles) were dried separately for 24 hours to constant weight in an oven at 80^{0}C. Each dried sample were pooled and milled with mortar and pestle. They were kept in dry labeled foiled paper and stored in desiccators until digestion.

The samples were digested by adding 3ml of nitric acid (65%) and 1ml hydrogen peroxide (35%) (Taghipoura and Aziz, 2010). Hydrogen peroxide was added to the nitric acid as it reduces nitrous vapours and accelerates the digestion of organic matters by raising the temperature (Dig-Acids, 2001). The microwave was adjusted for 20mins at150^{0}C and left for 35 mins to cool in the microwave until they reached room temperature. The samples were then transferred to clean volumetric flasks and diluted to 50ml with deionized water. Then, the samples were filtered using Whatman filter paper. Concentrations of Zn, Mn, Fe, Pb, Cu, Cd, and Cr were then determined using Atomic Absorption Spectrophotometer (Unicam 969, Analytical Technology Inc., Cambridge, United Kingdom). Statistical analysis of data was carried out using program SPSS version 5 and two way ANOVA to compare data among sites and organs.

RESULTS

The mean and the statistical comparisons of zinc, manganese, iron, lead, copper, cadmium and chromium concentrations in: the gills, head region, bones, and muscles of *C.*

gariepinus from the sampling sites in River Asa is shown in figures 1 to 5. Zinc, Manganese, Iron and Lead concentrations varied significantly; in the four tissues with exception of head regions and bones, in all the months and the sampling sites examined.

High concentrations of Zn, Mn, Fe and Pb were recorded in fish samples from Site C in the month of February while least concentrations were predominant in the month of September. Gill tissues were implicated to serve as the highest reservoirs of the metals followed by bones, head regions and muscles. Thus, the prevalent order of the bioaccumulation of the metals in the tissues of African catfish from Site A and C was in the order of; gills > bones> head regions and muscles.

The general level of copper concentrations in the tissues of the fish samples from Site A and C showed a different trend from other metals. The bone tissues accumulated high concentrations of metals followed gill tissues > head regions > muscles. Site C also had the highest metal concentration compared to that of Site A. It was observed that the least metal concentrations were recorded in the month of September while February had the highest concentrations.

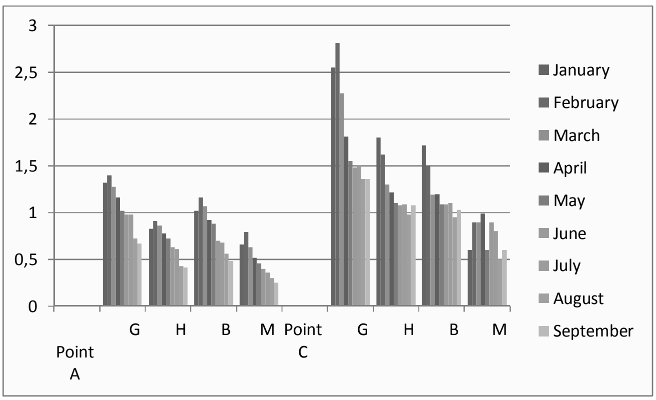

Figure 1. Mean monthly value of zinc in selected organs of C. gariepinus (Adults) from points A and C (mgkg-1)

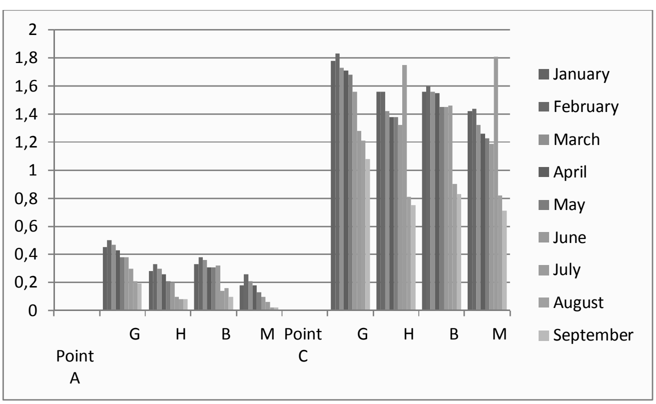

Figure 2. Mean monthly value of manganese in selected organs of C. gariepinus (Adults) from points A and C (mgkg-1)

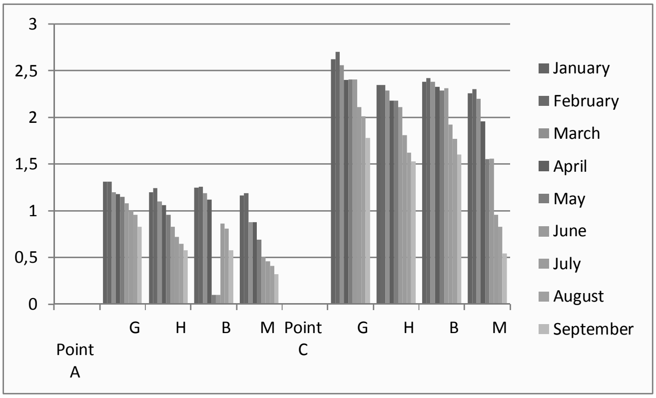

Figure 3. Mean monthly value of iron in selected organs of C. gariepinus (Adults) from points A and C (mgkg-1)

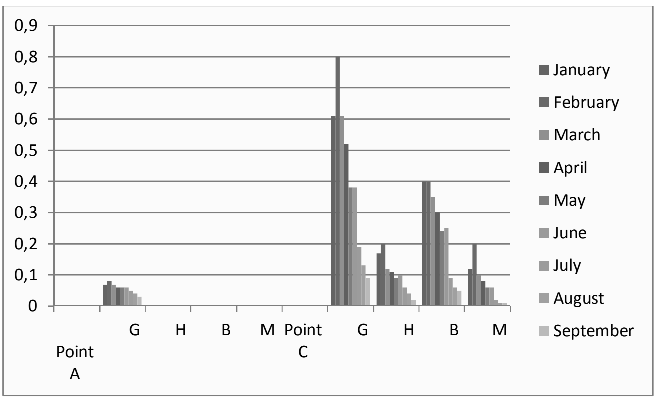

Figure 4. Mean monthly value of lead in selected organs of C. gariepinus (Adults) from points A and C (mgkg-1)

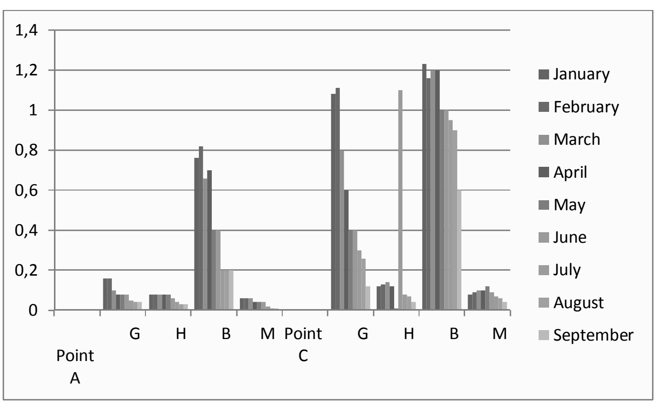

Figure 5. Mean monthly value of copper in selected organs of C. gariepinus (Adults) from points A and C (mgkg-1)

DISCUSSION

The recorded significant ($P < 0.05$) differences in the bioaccumulation of metals in the tissues of African catfish from the two sites (A and C) of Asa River and the seasonal variations may be linked to the observed behavioral differences and metabolic responses of the fish to varying concentrations of the effluent in the water, a factor that controls mortality as the concentration increases. Variations in the accumulation of metals in fish tissues could arise from factors which may be dependent on different ion accumulation in the species, season of the year and or fish habitat (Obasohan and Eguavon, 2012).

A prominent trend in the bioaccumulation pattern of metals at the two sites (A and C) was always evident. African catfish collected from the two sampling sites accumulated high concentrations of zinc, manganese, iron, lead and chromium in the gills followed by the bones, head regions and muscles except the deviation recorded in copper with high concentration in bones followed by gills, head regions and muscles. *C. gariepinus* was implicated to bioaccumulate high concentrations of these metals at site C compared to the bioaccumulation at site A. This trend further confirmed that Site C suffers point pollution while that of Site A could be non-point pollution.

High concentrations of zinc, manganese, iron, lead, copper and chromium in the tissues of *C. gariepinus* from the two sites in the month of February; a period that corresponded to the peak of dry season while low concentrations were recorded in the month of September; a period of flood. These observations conformed to the findings of Coetzee *et al.,* (2002) that *C. gariepinus* bioaccumulated high concentration of metals in the month of February and May, while liver was implicated to bioaccumulate high concentration of metals. Possibly, the observed trend could be linked to the absence of dilution of water in dry season since the river experienced low flows during this period as a result of evaporation. While the low values recorded in the month of September was probably due to dilution as a result of large volume of water in the river during flood.

The gills, due to their proximity to the external environment could serve as a depot tissue, since metal uptake may exceeds elimination. In freshwater fishes, the gills are the primary route for the uptake of waterborne pollutants while the gut plays a secondary role. Zinc has twofold influences on the gill, namely bioconcentration of metals and the structural cellular alterations as noted on the gills of *Tilapia sparmanii* (Wariaghli et al., 2013). The high level of zinc in the gill tissues can possibly be due to the fact that fish gills play a distinct role in metal uptake from the environment.

Concentrations of manganese and iron showed a similar sequence with that of zinc. The gill bioaccumulated high concentration of manganese and iron followed by bone, head region and muscles (G > B >H > M). This is similar to the opinion of Ekeanyahun *et al*.,(2011) that the high manganese concentration in the gills could be attributed to the role of gill as the main route of uptake of manganese as little absorption of this metal occurs through the gut from food. The high iron concentrations in the gill tissues may be due to iron containing enzymes and the extensive vascular system of the gill, as haemoglobin in the blood binds approximately three quarters of the iron in the body.

The muscle accumulated only small amounts and similar concentrations of lead. Fishes are said to accumulate very little lead in edible tissue. While the low concentrations of lead in head region and bone could be linked to the low binding rate of lead to sulphydryl groups Lead could possibly interfere with the action of gonadotrophins by binding to the sulphydryl groups on the cell membrane receptor sites for these molecules (Sorensen, 1991).

The levels of copper in the bone during this study were usually slightly higher than that of the gill, tissues, head region and bone. This however disagreed with the trend and observation of Seymore *et al.,* (1994) and Allen *et al.,* (1998) that gills are target organs for copper toxicity in fish and there is usually a positive correlation between the copper concentrations in the tissues of the gill and in the environment. The trend (Bone > Gills > Head region and muscle) could be ascribed to the absorption properties of copper unto suspended particles and skeletal parts.

In this study, all the tissues examined accumulated insignificant concentrations of cadmium and chromium. This is in line with the observations of Allen, *et al.,* (1998) and could be ascribed to the chemical form of cadmium and chromium differences in exposure period and the composition of the tissues. The observation recorded in this study could be as a result of the alteration in the morphology of the gills and other tissues thus a reduction in the cadmium and chromium – binding capacity and this probably led to low accumulation of cadmium and chromium

CONCLUSIONS

The present study indicated significant accumulation of heavy metals in African catfish from Asa River. Overall, the total means of heavy metal concentration of all fish species in this study were concordant with the surrounding waters of the Asa river reported by the Adewoye (2013). Therefore, the results in this study demonstrated that fish species caught in the Asa River were contaminated with heavy metals. Nevertheless, the heavy metal concentration in the fish tissues did not exceed the WHO, USFDA OR FAO guidelines. The regular monitoring of heavy metal concentrations in fish tissue is necessary. Therefore, there is an urgent need for continual assessment of the level of pollution of the Asa waters with metals from the mentioned sources, with a view to reducing the level of pollution via education and public enlightenment.

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GROWTH METRICS OF JUVENILE RAINBOW TROUT AS INFLUENCED BY FEED STORAGE CONDITIONS (FROZEN OR NON-FROZEN)

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MERENJE PARAMETARA RASTA KALIFORNIJSKE PASTRMKE POD UTICAJEM NAČINA ČUVANJA HRANE (ZAMRZNUTA ILI NEZAMRZNUTA)

Apstrakt

Kod gajenih riba se najbolja konverzija hrane i rast postižu ishranom peletama dobrog kvaliteta. Kvalitet hrane može značajno da se pogorša ukoliko se ne čuva na određeni način. Kvalitet hrane se održava zamrzavanjem na niskim temperaturama. Cilj istraživanja je bio određivanje efekata načina čuvanja hrane za ribe na stopu rasta kalifornijske pastrmke. Ukupno 240 riba težine 8.7–10.5 g raspoređeno je po principu slučajnosti u 4 grupe. Svaka grupa je podeljena na 3 replikacije od po 20 riba. Hranjene su 2 puta dnevno do zasićenja sa jednom od eksperimentalnih dijeta: zamrznuta hrana (čuvana na 10° C, -1.1° C i -15° C) i nezamrznuta (čuvana na sobnoj temperaturi) tokom 42 dana. Rezultati ukazuju da uslovi čuvanja ne utiču značajno na unos, FCR i stopu rasta kod kalifornijske pastrmke.

Ključne reči: *stopa rasta, FCR, način čuvanja, Oncorhynchus mykiss*

Abstract

The best food conversion efficiency and growth in cultured fish can be obtained by feeding them with good quality commercial pellets. The quality of the feeds can rapidly deteriorate if they are not properly stored. The nutritional quality of feed can be maintained by frozen them at low temperature. The aim of this study was to determine the effects of feed storage on the growth rate of rainbow trout. Total 240 fish weighing 8.7–10.5 g were randomly distributed into four groups. Each group was further subdivided into three replicates with 20 fish in each. They were fed twice a day to apparent satiation with one of the experimental diets: frozen feed (stored at 10° C, -1.1° C and -15° C) and non-frozen (stored at room temperature) for 42 days. The results data suggest that feeds storage condition do not significantly affect the ability of food intake, FCR and growth rate in rainbow trout.

Keywords: *growth rate, FCR, feed storage, Oncorhynchus mykiss*

DETERMINATION OF TOXINS PRODUCTION OF *CLOSTRIDIUM DIFFICILE* STRAINS ISOLATED FROM FISH, SHRIMP, CRAB AND LOBSTER USING ELISA

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ODREĐIVANJE PRODUKCIJE TOKSINA SOJA *CLOSTRIDIUM DIFFICILE* IZOLOVANOG IZ RIBE, ŠKAMPA, KRABA I JASTOGA UPOTREBOM ELISA TESTA

Apstrakt

Cilj ispitivanja je bio određivanje prisustva toksigenog *C. difficile* u uzorcima sirovih riba, škampa, kraba i jastoga u Iranu. Od marta do decembra 2017, ukupno je analizirano 418 uzoraka riba, škampa, kraba i jastoga kupljenih od slučajno određenih dobavljača u provinciji Bushehr u Iranu. Analizirani su na prisustvo *C. difficile* korišćenjem selektivnog obogaćivanja kulture *C. difficile,* zatim šok tretmanom alkoholom i zasađeni na *C. difficile* selektivni medijum. U izolatu *C. difficile* određivana je produkcija toksina A i B pomoću enzim vezanog imunoabsorbentnog testa. Od 418 ispitivanih uzoraka 11 (2.64%) je bilo pozitivni na prisustvo *C. difficile*. Među 11 *C. difficile* izolata samo 8 (1.92%) uzoraka su bili toksigeni i pozitivni za toksine A i/ili B. ispitivanje je pokazalo da plodovi mora mogu biti izvor CA-CDI.

Ključne reči: *Clostridium difficile, toksični sojevi, ribe, škampi, kraba, jastog*
Keywords: *Clostridium difficile, toxigenic strains, fish, shrimp, crab, lobster*

INTRODUCTION

Clostridium difficile - associated diarrhea (CDAD) is a very common nosocomial infection. This is probably due to greater awareness of the disease among physicians, improved diagnostic methods, and a truly increased incidence of CDAD. The clinical expression of *C. difficile* infection ranges from asymptomatic infection to diarrhea (the most frequent clinical symptom) to pseudomembranous colitis, severe abdominal pain, toxic megacolon, bowel perforation, and death (Weese 2010; Keessen *et al.* 2011).

Clostridium difficile strains associated with the two conditions produce either toxin A (an enterotoxin) or toxin B (a cytotoxin), or both. Some strains do not produce toxins and do not cause diarrhea or pseudomembranous colitis. Much is known about the epidemiology of *C. difficile* in the hospital environment (Simango 2006).

Little is known about the prevalence of *C. difficile* in the environment such as in domestic animals, soil and water in rural communities. Food animals are an important source of human enteropathogenic micro-organisms and can be spread to humans through consumption of foods of animal origin. Moreover, molecular typing of *C. difficile* isolates from calves and humans has shown similarities in PCR ribotypes from the two species including two PCR ribotypes associated with outbreaks of severe disease in humans (Rodriguez-Palacios *et al.* 2006; Songer 2010), suggesting that sea food may be reservoirs of *C. difficile* for humans (Metcalf et al. 2011).

In recent studies *C. difficile* has been isolated from several food animal species and variety of foodstuff, sharing high level of genetic similarity with those of human isolates (Simango 2006; Rodriguez-Palacios *et al.* 2006; Rupnik *et al.* 2009; Songer *et al.* 2009; Weese 2010). The mechanisms of transmission of *C. difficile* between animals and humans are not yet fully understood (Jhung *et al.* 2008). If animals are a potential source of *C. difficile*, food could be one of the transmission routes from animals to humans. Though there is currently no clear evidence that *C. difficile* contamination of food can cause CDI in humans, it is important to collect information on the possible exposure of humans by *C. difficile*-contaminated food (de Boer *et al.* 2011).

The epidemiology of *C. difficile* infection (CDI) in Iran is essentially unknown. Although toxicogenic *C. difficile* isolation has been reported from Iranian hospitals (Jalali *et al.* 2012), to the authors' knowledge, the prevalence rate of *C. difficile* in foodstuff in Iran has never been reported. The present study was conducted to determine the prevalence of toxin producing of *C. difficile* strains isolated from fish and other sea foods in Iran.

MATERIALS AND METHODS

Sample Collection

A total of 418 samples, including fish, shrimp, crab and lobster, were collected from the retail trade in the period March to December 2017 from Bushehr province of Iran (Table 1). The samples were transported and stored at a temperature about 4 °C and analyzed within 48 hours from the time of sampling.

Isolation and identification of *C. difficile*

The detection and isolation method used was based on the method described by Rodriguez-Palacios *et al.* (2007) and de Boer *et al.* (2011). Briefly, 5 g of each sample was transferred to 20 mL of *C. difficile* broth (CDB), containing *C. difficile* selective supplement (Oxoid SR0173) and 5% (v/v) defibrinated sheep blood. After inoculation at 37 °C for 10 to 15 days in an anaerobic condition, 2 mL of the enriched broth was added to 2 ml of 96% ethanol in a centrifuge tube and homogenized during 50 min on a shaker. After centrifugation (3800×g for 10 min), a loopful material from the sediment was streaked onto *C. difficile* agar base (Oxoid CM0601) supplemented with an antibiotic supplement for the selective isolation of *C. difficile* (Oxoid SR0174) and 7% (v/v) defibrinated sheep blood and the plates were incubated for 24 to 48 h at 37 °C, under anaerobic conditions. Up to three colonies per plate were subcultured onto tryptone soya agar (Oxoid CM0131) and tested by standard microbiological and biochemical procedure (Harvey *et al.* 2011). Crudely extracted DNA (boiling, 10 min) was used for PCR confirmation (housekeeping tpi gene detection) of isolates as performed in previous studies (Rodriguez-Palacios et al. 2006; Rodriguez-Palacios *et al.* 2009).

Determination of toxins A or B production

The isolates confirmed as *C. difficile* were cultured on sheep blood agar under anaerobic conditions at 37 °C for two days. After culture, a thick bacterial cell suspension was prepared in 1 ml universal stool buffer (RIDASCREEN, R-Biopharm AG, Darmstadt, Germany) and centrifuged at 3000 rpm for 10 min. The supernatants were tested for the presence of *C. difficile* toxins A or B by enzyme linked immunosorbent assay (ELISA) detection kit (RIDASCREEN, R-Biopharm AG, Darmstadt, Germany) according to the manufacturer's instructions. Positive and negative controls were included in each batch.

RESULTS AND DISCUSSION

The present study determined the occurrence and ability of toxins production of *C. difficile* in sea food Iran to show how sea food may be reservoirs of this organism. The results of the prevalence testing are summarized in Table 1.

Table 1. Prevalence of Clostridium difficile detected in fish, shrimp, crab and lobster samples in Iran

Meat products	No. of samples	No. of *C. difficile*-positive samples	No. of isolates positive for toxins A and/or B
Fish	140	4 (2.86%)	3 (2.15%)
Shrimp	118	4 (3.39%)	4 (3.39%)
Crab	80	1 (1.25%)	1 (1.25%)
Lobster	80	2 (2.50 %)	0 (0.00%)
Total	418	11 (2.64%)	8 (1.92%)

*Results expressed as the number of *C. difficile* -positive samples / number of samples analyzed (%).

In total, 11 of 418 (2.64%) samples were *C. difficile* positive. Among 11 *C. difficile* isolates only eight strains were found to be toxigenic for toxin A and/or B. Recent studies have shown the presence of *C. difficile* in food animals and their products such as sea food and fish (Metcalf et al. 2011), poultry and sheep (Simango & Mwakurudza 2008; de Boer *et al.* 2011), pigs (Songer & Anderson 2006), chickens, goats, calves and cattle (Rodriguez-Palacios *et al.* 2006; Bouttier *et al.* 2010; de Boer *et al.* 2011) as was observed in fish, shrimp, crab and lobster in our study. *C. difficile* has also been isolated in ground beef, summer sausage, ground pork, ground veal, chorizo, pork sausage, ground turkey and salads (Rodriguez-Palacios *et al.* 2007; Bakri *et al.* 2009; Songer *et al.* 2009; Jöbstl *et al.* 2010) as well as in commercial turkey-based raw diets intended for cats and dogs (Weese *et al.* 2005). Studies that compared human and animal *C. difficile* isolates and revealed the presence of *C. difficile* in foods of animal origin suggest that animal reservoirs and transmission via foods are possible sources for community-acquired human infections (Rupnik 2007).

However, further studies are required to determine whether meat and other foodstuff could be an important route of transmission to humans.

This and other published studies show that there is a potential for transmission of *C. difficile* to humans via the food chain. Rates of contaminated food product vary strongly and different *C. difficile* types are found in different countries. However, until now there are no documented cases of *C. difficile* infection that resulted from eating food that contained this bacterium.

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THE LENGTH-WEIGHT RELATIONSHIP OF CULTURED BLACK SEA TROUT AFTER STOCKING IN NATURE

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DUŽINSKO TEŽINSKI ODNOS GAJENE CRNOMORSKE PASTRMKE POSLE PORIBLJAVANJA

Apstract

Dužinsko-težinski odnos (LWR) riba donosi vredne informacije o opštem zdravstvenom stanju ribljih vrsta. Crnomorska pastrmka (*Salmo coruhensis*, Syn: *Salmo trutta labrax*) je endemična vrsta rasprostranjena u severoistočnim obalama i rekama Turske. Populacije ove vrste su ugrožene zbog ilegalnog ribarenja. Takođe su izložene zagađenju, zamućenju vode i građevinskim radovima u okolini reka. U cilju oporavka populacija i pravilnog iskorišćavanja prirodnih populacija, Ministarstvo šumarstva i vodoprivrede Turske je započelo poribljavanje ovom vrstom u prirodi u cilju ublažavanja gubitaka. U ovom istraživanju ispitivan je LWR crnomorske pastrmke kojima je prethodno poribljena reka Çağlayan River, Rize, u cilju određivanja opšeg stanja posle nasađivanja u prirodi. Istraživanje je utvrdilo da poribljavanje ne utiče na alometrijski rast koji je iznosio $W= 0.016\ L^{2.767}$ i bio u saglasnosti sa rastom riba gajenih u mrestilištu.

Ključne reči: *slatkovodna sredina, dužinsko-težinski rast, Salmonidae*

Abstract

The length-weight relationship (LWR) of fish provides valuable information about the general health of fish species. Black Sea trout (*Salmo coruhensis*, Syn: *Salmo trutta labrax*) is an endemic species distributed in North-East coasts and rivers of Turkey. Their population has been far much exploited due to illegal fishing. Also, they are exposed to several external drivers such as pollution, siltation, and infrastructure construction around the rivers. For population recovery and natural recruitment of this species, the Ministry of Forestry and Water Affairs of Turkey has been started to restock this fish in nature to mitigate for past losses. In this study, the LWR of Black Sea trout which were previously released to Çağlayan River, Rize was studied in order to understand the general well-being of this fish after stocking in nature. The results evince that the stocking of this fish in nature does not affect their allometry growth which was W= 0.016 $L^{2.767}$ and in accordance with the observation obtained from hatchery reared stock.

Keywords: *freshwater, length-weight relationship, Salmonidae*

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FISH FEED MICROBIOLOGICAL STATUS

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MIKROBIOLOŠKI STATUS RIBLJE HRANE

Apstrakt

Riba kao i druge životinje imaju potrebu za esencijalnim hranjivim sastojcima kako bi se pravilno razvijale. Kompletna ponuda svih sastojaka (proteina, ugljenih hidrata, masti, vitamina i minerala) je neophodna za optimalan rast i zdravlje ribe. U prirodnim uslovima i na toplovodnim ribnjacima sa poluinterzivnom proizvodnjom, ribe zadovoljavaju svoje potrebe za vitaminima uzimanjem prirodne hrane (fitoplankton, zooplankton i bentos). Pri intenzivnim oblicima proizvodnje bilo kojih vrsta riba (pastrmka, lososi, šarani i dr) gde je prirodna hrana, praktično, u potpunosti izostavljena, zadovoljenje ribljeg organizma vitaminisko-mineralnim premiksima ima vitalni značaj. Veštačka ishrana može biti ili potpuna ili dodatna. U ovom istraživanju istražili smo sledeće grupe mikroorganizama u hranjenju ribe: ukupan broj bakterija, poroznih bakterija, kvasca i koliformi, *Escherichia coli* i Proteus vrsta, kao i fekalne streptokoke i stafilokoke i sulfitoreduktivne klostridije. Istraživanjem je dokazano odsustvo ispitivanih bioloških hazarda.

Ključne reči: *sigurnost riblje hrane, mikrobiološki status, zagađivači*
Keywords: *fish feed safety, microbiological status, contaminants*

INTRODUCTION

A feed hazard is defined as any biological, chemical (including radiological), or physical agent in feed with the potential to cause illness or injury to animals or humans. It is when controls are not adequate at feed establishments that these same agents may cause the feed to be a much greater risk to animal or human health. Good nutrition in fish production system is essential to economically produce healthy, high quality fish products. Fish feeds are constantly in contact with environmental organisms and become readily colonized by various microbial species. According to FAO (1987), environmental factors during storage predispose the fish feeds to microbial spoilage. The presence of bacteria in feeds causes their decomposition and subsequently, fish diseases. Bacteria such as *Salmonella* spp., *E. coli* and other bacteria strains have been reported to contaminate fish feeds (Zmyslowska and Lewandowska,1999, Ciceron *et al.*, 2008, Kaarine, 2010). Fungal contamination of fish feed has been reported to result in aflatoxicosis (Ashley, 1970). Aflatoxins are chemical produced by fungi like *Aspergillus flavus* and *A. parasiticus* commonly known as mold (Russso and Yanong, 2006). Mold-infested fish feeds have been reported to impact negatively on the growth of *Heterobranchus bidorsalis* fish (Effiong and Alatise, 2009). Aflatoxins in fish have been known to be capable of having carcinogenic effects on human consumers of contaminated fish (Brown, 2009). The occurrence of these microbial strains in fish feeds has been reported to depend on the storage conditions of the feeds, particularly temperature. The quality of fish feeds and the hygienic levels of technological process employed during feed formulation determine the level of risk of microbial contamination aided by temperature. According to Zmyslowska (2000), storage conditions especially temperature and humidity are important factors affecting microbial quality of fish feeds. Improper storage temperature may prolong survival of the microorganism in fish feeds by enhancing their multiplication and production of toxic substances which may be injurious to fish. Objective of this study is to show hygienic wholesomeness and safety of animal feed manufactured in region of Serbia and from import.

MATERIALS AND METHODS

To evaluate microbiological safety of feed stuffs, 31 samples were analyzed during 2013-2017, fifteen fish feed samples, seven trout feed samples and nine carp feed samples. A part of samples was collected from domestic producers and part form imports. Samples were analyzed in the microbiological laboratory, Department of food and feed safety in Institute of Veterinary Medicine of Serbia. Horizontal method for the detection of Salmonella spp. according to the ISO 6579:2008 standard was used for Salmonella isolation. *Salmonella* remains the pathogen of focus in animal feed due to the organisms’ ability to infect food-producing animals and thereby potentially contributing to human foodborne disease (Crump *et al.*, 2002). International standard method “Microbiology of food and animal feeding stuffs – Horizontal method for the enumeration of microorganisms – Colony-count technique at 30°C” (ISO 4833-1:2008) was used for enumeration. Horizontal method for the enumeration of Clostridium perfringens Colony-count technique according to the ISO 7937:2010. Horizontal method for the enumeration of yeasts and moulds; Part 2: Colony count technique in products with water activity less than or equal to 0,95 according to the ISO 21527-2:2008. Perform isolation and identification as specified in the Regulation of the Rules on the quality of animal feed, art. 101,102 (Official Gazette of the Republic of Serbia, No. 4/2010).

RESULTS AND DISCUSSION

Total of 31 analyzed samples of feedstuffs is in accordance with the legal regulations of the Republic of Serbia. There is absence of microbiological parameters which confirms good manufacturing practices addressing manufacturing, processing, packing, and holding animal food.

Each link in the food production chain has a share in the state of public health, proves that feed quality is a part of food quality (Davides *et al.* 1999; Geiser, 2001). The animal health status is mainly determined by factors, such as nutritional value of the feed and its microbiological quality. However, it is important to control the quality of the feed materials and compound feed. The sources of microorganisms in the plant materials are soil, dust, water, animals (insects, vertebrata), or secondary contamination during processing, storage, and dispersal of the product (Maciorowski *et al*. 2007). *Salmonella* sp., one of the most important bacterial zoonotic agents, is an essential bacterium in the assessment of microbiological quality of feed. In the second place, ubiquitous sporeforming microorganisms, resistant to adverse conditions of processing (high temperature, pressure), should be mentioned. These include anaerobic bacilli of *Clostridium* sp., aerobic bacilli of *Bacillus* sp., and abundantly sporulating moulds (Pirttijärvi *et al*. 2000). A quality assessment of feed also comprises hygiene indicators as *Enterobacteriaceae* family, total aerobic bacteria, fungi, and total plate counts. The total plate count (TPC) defines how many aerobic, mesophilic microorganism colonies, such as bacteria, yeast, and mould fungi will grow for 72 h on an agar plate that was normalised for microbiological testing at 30°C. Microbiological requirements, currently being in force in the EU, exclude the presence of *Salmonella* sp. in 25 g in all feed materials. Besides, in accordance with EU Regulation (EC) No. 142/2011, *Enterobacteriaceae* count cannot exceed 300 colony forming units (cfu)/g in five batch samples of feed material derived from animal by-products.

CONCLUSION

Prevention is the cornerstone of any feed safety control plan. Established verification procedures in a feed safety system are used to confirm that products are safe and that they comply with regulatory requirements. Manufacturers for animal food and ingredient producers to comply with good manufacturing practices addressing manufacturing, processing, packing, and holding animal food. It also establishes hazard analysis and risk-based preventive controls for many animal food facilities. These measures will provide a greater assurance that animal food will not cause illness or injury to animals or to humans who handle animal food or consuming food derived from animals. During feed production, a lot or batch can become contaminated from a variety of sources including ingredients themselves, unclean silos and machinery, dust, birds, rodents and the general environment, etc. Grinding and adding liquid ingredients results in hot and moist conditions, which may favour bacterial or fungal growth (ICMSF, 2005). Heat treatment is the most common antimicrobial treatment for feeds and when performed as a critical control point should kill *Salmonella* and other pathogens. Feed quality and safety are important prerequisites for sustainable development of livestock production and this study show that.

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HEALTH STATUS OF THE BLACK BULLHEAD POPULATION (*AMEIURUS MELAS*) IN SAVA LAKE

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ZDRAVSTVENO STANJE POPULACIJE CRNOG AMERIČKOG PATULJASTOG SOMA (*AMEIURUS MELAS*) U SAVSKOM JEZERU

Apstrakt

Crni američki patuljasti som *Ameiurus melas* (Rafinesque, 1820) je jedna od najzastupljenijih i najuspešnijih alohtonih vrsta riba koja naseljava kopnene vode Evrope. Istraživanja koja su sprovedena u proteklih nekoliko godina ukazuju na to da je ovo dominantna invazivna vrsta i u mnogim kopnenim vodama u Srbiji, uključujući i Savsko jezero (Jaćimović, 2015).

Zdravstveno stanje populacije crnog američkog patuljastog soma u Savskom jezeru procenjeno je na osnovu analize prevalencije i intenziteta parazitiranosti kod 2349 jedinki u uzorcima prikupljenim tokom 2011. i 2012. godine. Prevalencija parazitiranosti predstavljena je kao broj zaraženih jedinki, tj. procentualni udeo zaraženih jedinki, dok je intenzitet parazitiranosti predstavljen kao broj parazita u svakoj analiziranoj jedinki.

Osim kod jedne jedinke kod koje je nađena cista Trematoda, svi uočeni endoparaziti pripadali su razdelu Nematoda. Najverovatnije se radilo o vrsti *Philometra rischta.* Zdravstveno stanje populacije tokom 2012. godine bilo je znatno bolje u odnosu na 2011. godinu. Jedinke starosti 3+ bile su najzaraženije endoparazitima, kako u svakoj pojedinačnoj godini, tako i u celom uzorku. Iako bi se moglo očekivati da nezaražene jedinke imaju veće vrednosti Fultonovog faktora kondicije (K) u poređenju sa zaraženim, rezultati istraživanja u Savkom jezeru pokazali su da takva pravilnost ne postoji.

Ključne reči: *crni američki patuljasti som, zdrastveno stanje populacije, prevalencija parazitiranosti, intenzitet parazitiranosti, endoparaziti*

Keywords: *black bullhead, population health status, parasitism prevalence, parasitism intensity, endoparasites*

INTRODUCTION

Species invasion is one of the leading mechanisms of global environmental change, and the most frequently introduced aquatic species in Europe are freshwater fishes (García-Berthou et al., 2005). Non-native fishes can threat all forms of aquatic environments and affect native ecosystems in various ways, including predation, competition, habitat alteration, hybridization, and disease transmission (Ribeiro and Leunda, 2012).

The black bullhead *Ameiurus melas* (Rafinesque, 1820) is a North American ictalurid catfish introduced in Europe (Copp et al., 2016). Its capability to live in ecosystems with poor water quality (Ribeiro et al., 2008), as well as a high flexibility of life history traits (Novomeská & Kováč, 2009), enabled this species to invade and establish viable populations in new areas (Copp et al., 2016). Several studies indicated that the black bullhead is one of the dominant invasive species in Serbian waters, including Sava Lake (Lenhardt et al., 2011; Jaćimović, 2015).

Studies on the health status of invasive species are important because when free-living non-native species are introduced into new environments, their parasites can also be introduced (Williams et al., 2013). These parasites can potentially spillover into native species, causing various changes, with probable adverse consequences for growth, survival, and fitness (Britton, 2013). The aim of this research was to assess the health status of black bullhead specimens in Sava Lake by estimating the prevalence and intensity of parasite infestation.

MATERIALS AND METHODS

Sava Lake is a former right-hand branch of the Sava River in Belgrade (Serbia) near the Ada Ciganlija river island. Twenty fish species from 19 genera, belonging to 8 families, inhabit the lake. Based on the total biomass, the most significant species are the common carp *Cyprinus carpio* (21.2%), pikeperch *Sander lucioperca* (13.5%), and common bream *Abramis brama* (8.4%); the total biomass of the black bullhead is 5.7% (Hegediš et al., 2008).

Black bullhead samples were collected monthly from April 2011 to December 2012 using double fyke nets (length 85 cm, diameter 50 cm, 8 mm mesh-size). Specimens were measured for their total length (TL) and mass (W) to the nearest 0.5 cm and 1 g, respectively, and their organs were examined for endoparasites. The prevalence of parasite infection was estimated as the percentage of infested individuals during the sampling period, and the intensity as the number of parasites in each fish. The analyses were done on a sample of 2349 specimens of the black bullhead.

Fulton's condition factor (K) was calculated to assess the general condition of individuals during different sampling seasons, using the formula $K = W / L^3 \times 100$ (Ricker, 1975). Age of the black bullhead specimens was determined by combining otolith examination (Secor et al., 1992) and Bhattacharya's method to split the age-classes from the length-frequency data, using FiSAT II software package (Bhattacharya, 1967; Gayanilo et al., 2006).

RESULTS

All detected endoparasites were from the phylum Nematoda, probably the species *Philometra rischta* Skrjabin, 1917, except for one black bullhead individual that had an endoparasite of the class Trematoda, in the cyst stage. The parasites were found both in the abdominal cavity and inside the stomach and intestines of the examined fish.

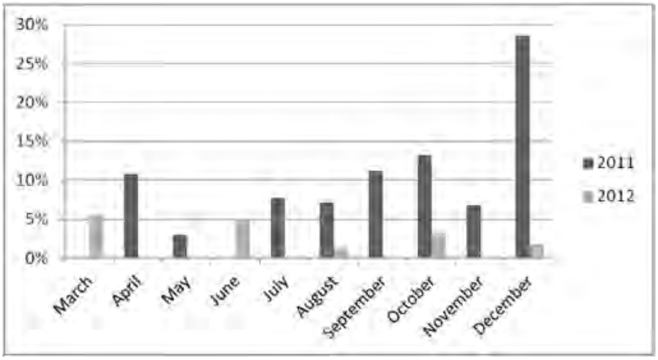

Figure 1. The prevalence of parasite infestation of the black bullhead in Sava Lake in 2011 and 2012.

The prevalence of parasite infestation of the black bullhead in Sava Lake was highest in December of 2011 (29%) and lowest in June 2011 (0%) (Figure 1). The mean intensity was highest in April of 2011 and lowest in December of 2012 (Table 1). Overall, the health status of the population in 2012 was much better compared to 2011.

Table 1. The intensity of parasite infestation of the black bullhead in Sava Lake in 2011 and 2012 – minimum, maximum, mean, and standard deviation (SD) values.

Year	Month	Min	Max	Mean	SD
2011	April	1	7	5.00	2.83
	May	1	1	1.00	0.00
	July	1	3	2.00	1.00
	August	1	4	1.55	0.93
	September	1	3	1.43	0.65
	October	1	7	2.17	1.70
	November	1	3	1.40	0.70
	December	1	4	2.17	1.17
2012	March	2	2	2.00	0.00
	June	1	3	1.40	0.89
	August	1	2	1.21	0.43
	October	1	9	1.67	2.31
	December	1	1	1.00	0.00

Black bullhead individuals 3+ years old were the most infested age class in both years (36.51% in 2011 and 41.18% in 2012), as well as in the whole sample (38.14%).

Comparing the values of the Fulton’s condition factor (K) of infested and non-infested specimens during 2011, it was observed that they were higher in non-infested specimens in April, May, November, and December (Figure 2). During all other months in 2011, the K values were higher in infested individuals, particularly in September. In 2012, K values were higher in non-infested individuals only during June. In March and especially in August, the values of *K* were higher in infested individuals, and in October and December these values were identical.

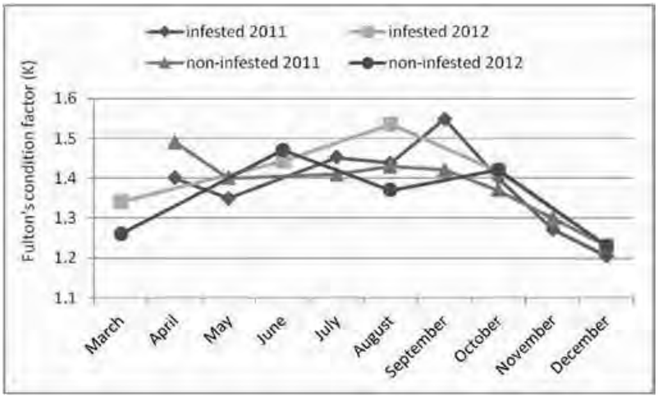

Figure 2. Fulton's condition factor of infested and non-infested black bullhead in Sava Lake in 2011 and 2012.

DISCUSSION

Fishes can be infested with a particularly diverse array of parasites at all stages of their development (Anderson, 2000). Nematodes are one of the most common groups of parasites in fish (Moravec, 2004), which was also shown in black bullhead specimens in Sava Lake.

In a study on parasites of non-native invasive fish species in Slovakia, Košuthová et al. (2009) have determined the presence of only one parasite, *Acanthocephalus anguillae*, in black bullhead specimens. The prevalence of parasite infestation was 6.3% and the intensity was 1 to 2 parasites per specimen. The prevalence in Sava Lake was 7.6% and this result is in line with the result from Slovakia, although the parasite was of a different species. Another similarity can be noticed – a low diversity of parasites. It has been observed that the parasite fauna of invasive animal species, including fish, is less diverse in non-native ecosystems, which may be due to several factors, including the reduced probability of parasites being introduced with allochthonous species, as well as the absence of other necessary hosts in a new location (Torchin et al., 2003). On the other hand, the release of introduced species from their native parasites, in addition to other factors, may contribute to their invasive success and improve their demographic performance (Torchin et al., 2003; Košuthová et al., 2009).

Individuals aged 3+ were the most infested age class in both years. A study conducted on *Paralabrax humeralis* (Serranidae) in Chile has shown that the nematode *Philometra* sp. infested the host only after it reached full sexual maturity (Oliva et al., 1992). A study on parasitism of a commercially important fish *Sciaenops ocellatus* (Sciaenidae) by *Philometra floridens* has shown that the intensity of infestation and size and age of infested fish are positively correlated (Bakenhaster et al., 2014).

Although it could be expected that non-infested individuals would have higher values of the Fulton's condition factor (*K*) compared to infested ones, the results have shown that such regularity does not exist in Sava Lake. Many studies confirm that the relationship between *K* and the level of parasitism can be very complex and uneven. In Parana River (Brazil), it was observed that the values of the relative condition factor were lower in individuals infested with ectoparasites and higher in those infested with endoparasites, which may be due to the fact that fish that consume larger quantities of food may also ingest more infective forms of parasites transmitted through trophic routes (Guidelli et al., 2011). A study on *Lepomis macrochirus* in England showed that the values of *K* and the density of parasites in infested specimens were negatively correlated (Neff and Cargnelli, 2004), and the results showed that the parasite mass was more highly correlated with *K* than parasite numbers.

Studies on non-native fish parasites can be useful in invasion ecology, considering that introduced fish species represent suitable hosts for both native and non-native parasites that can spillover into native host species, which consequently represents a potential threat for their populations.

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THE IMPACT OF ANTIDEPRESSANT ON GENE EXPRESSION IN *DANIO RERIO* EMBRYOS

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UTICAJ ANTIDEPRESIVA NA EKSPRESIJU GENA U EMBRIONA *DANIO RERIO*

Apstrakt

Za procenu uticaja antidepresiva venlafaksin (venlafaxine) na ne-ciljne organizme korišćeni su embrioni zebrice (*Danio rerio*) u raznim stadijumima razvića. Izabrane su dve različite koncentracije venlafaksina: niska, od 300 ng/l koja je koncentracija koja se nalazi u životnoj sredini i visoka, 100x viša od prethodne, radi procene efekta dozne zavisnosti. Za ekspresiju gena izabrani su geni CYP1A i GST kao i dva proteina mnogostruke rezistentnosti kodirana genima ABCC1 i ABCB4, svaki od njih predstavlja gen jedne od 4 faze biotransformacije, od 0 do III. Rezultati su pokazali da je uticaj venlafaksina na embrione zebrice najočigledniji u vreme izvaljivanja (96h posle oplođenja). U ovom trenutku razvića rezultati ekspresije gena su pokazali povećanje količine mRNK za gene ABCB4 i GST u obe testirane koncentracije venlafaksina. CYP1A i ABCC1 geni su pokazali povećanje u visokoj koncentraciji antidepresiva; nasuprot tome, u slučaju niske koncentracije za ove gene došlo je do sniženja količine mRNK. Drugo povećanje genske ekspresije je zabeleženo 144h posle oplođenja za obe koncentracije venlafaksina i to za sve gene. Istraživanje je pokazalo da venlafaksin utiče na gensku ekspresiju enzima biotransformacije embriona zebrice u koncentracijama koje se nalaze u životnoj sredini.

Ključne reči: *zebrica, biotransformacija ksenobiotika, real-time PCR.*
Keywords: *zebrafish, xenobiotics biotransformation, real-time PCR.*

INTRODUCTION

The consumption of antidepressants in developed world is on a rise, the effects on non-target organisms living in surface water is still not fully understood. Aquatic organisms have developed a multixenobiotic resistance defence mechanism against environmental micropollutants, which eliminates emergent contaminants from cells. This detoxifying mechanism includes ABC transporters (ATP-binding cassette transporters), biotransformation enzymes and antioxidant enzymes (Bard, 2000; van der Oost et al., 2003).

The aim of this study was to assess the potential impact of the antidepressant venlafaxine on biotransformation enzymes in the non-target organism *Danio rerio* (zebrafish), which serves as a highly used ecotoxicological model (Scholz et al., 2008). The effect of venlafaxine was assessed by means of the expression of detoxification genes in different embryonic life stages. As appropriate genes for the evaluation of gene expression, the ABCC1, ABCB4, CYP1A and GST genes were chosen, each of them represents one of the phase of biotransformation (phase 0 to III). According to Baker and Kasprzyk-Hordern (2013) source, the first concentration of venlafaxine in our study was chosen to be 300 ng/L in order to represent the environmentally relevant concentration. The second tested concentration was established as being 100x higher, namely 30 µg/L, for evaluation of the dose-dependent effect of venlafaxine.

MATERIAL AND METHODS

The experimental design of this study was, in its first part, focused on exposure of the embryos to the tested antidepressant. The 1200 eggs in total were divided into three groups – the first one was exposed to the low (L) concentration of venlafaxine, 300 ng/L, selected as environmentally relevant. The second group was exposed to the concentration which was 100x higherthan the first one – 30 µ/L (concentration H), for evaluation of dose-dependent effect. The third group served as a control (concentration C) and was exposed only to dilution water prepared according to ISO 7346 (ISO, 1996). After 24, 96 and 144 hpf these embryos were divided into eight cryotubes, with 10 mg of embryo sample in each; thus the eight replicates for each time and concentration group. The samples were stored in liquid nitrogen at -80°C.

The second part of our study was focused on RNA extraction and reverse transcription into cDNA. After homogenisation of samples, the TRI Reagent (Molecular Research Center, USA) and silica beads were added. For total RNA extraction the RNeasy Mini kit (Qiagen, Germany) was used according to the manufacturer’s protocol. RNA was reverse-transcribed using Moloney Murine Leukemia Virus Reverse Transcriptase (Invitrogen, UK) and oligo-dT primers at 37°C for 1.5 h. As negative samples for the evaluation of genomic contamination and correctly performed reverse transcription, samples of DNAse/RNAse free water with exactly the same master mix as used for samples of cDNA were employed. Samples of cDNA, including the negative control, were stored at -20°C until quantitative real time PCR (qRT-PCR).

The third part of study was the qRT-PCR. The primers were selected with regard to the literature sources. The EF1A1 was selected as housekeeping gene and for evaluation of relative gene expression. The SYBR Green Master (Qiagen, Germany) was used as mastermix and the LighCycler® 480 (Roche, Germany) as a device in all qPCRs. The obtained data were analysed using LightCycler ® 480 SW 1.5 programme (Roche, Germany) within Ct value (threshold cycle) and comparative Δ Ct method.

Statistical analysis of results was performed using the Unistat 5.6 fo Excel software. All data were tested for criteria of normality using the Shapiro-Wilk normality test. The data were subjected to a two-way analysis of variance test (ANOVA) with concentrations (C, L, H) and time of exposure (24 hpf, 96 hpf, 144 hpf) for multiple comparisons in order to assess the significance of differences between all possible pairs of groups. The difference between groups was assessed as significant at $p < 0.05$.

RESULTS AND DISCUSSION

Aquatic organisms are exposed to an extensive range of xenobiotics occurring in surface water which can induce adverse effects in these organisms already at environmentally relevant concentrations (Santos et al., 2010). Once a xenobiotic enters a cell, the detoxification pathway is started to prevent the adverse effect of this undesirable compound in the organism (van der Oost et al., 2003).

In our study, we wanted to verify the hypothesis of whether venlafaxine could influence gene expression of biotransformation enzymes even in environmentally relevant concentrations and also whether the effect is dose-dependent. The evaluation of gene expression was carried out using *Danio rerio* embryos. The mRNA amount of selected genes was detected in different stages of embryonic development, so the impact of time could be observed as well. This mRNA amount was expressed in relation to the reference gene EF1A1 as relative gene expression. Two experimental concentrations of venlafaxine were used: 300 ng/L, representing the environmental concentration, and 30 µg/L for the evaluation of the dose-dependent effect.

Firstly, the ABCB4, CYP1A, GST and ABCC1 gene were selected for phase 0, I, II and III, respectively. ABCB transporters acting in phase 0 are non-specific and their main function is to protect cells against toxicants (Fischer et al., 2013). Regarding phase 0, we observed a significant increase of mRNA amount at 96 hpf in all of the groups. A significant effect of time was detected in the C group in which a decrease of the mRNA amount was noted at 24 hpf.

In phase I and II of biotransformation, the non-polar xenobiotic is biotransformed to a more soluble product. The CYP1A gene was selected to represent the cytochrome P450 family. An effect of the concentration at 144 hpf was observed: mRNA values for H were significantly higher than for L and C.

For phase II, the relative expression of GST revealed an influence of concentrations at 144 hpf, when a significant decrease of the mRNA amount was detected in C. Moreover, the effect of time was observed for every concentration group. A significant increase of the mRNA amount was found in C at 96 hpf. Furthermore, in L and H, the same tendency was observed – a significant increase between 24 and 96 hpf and also between 96 and 144 hpf.

In phase III, the ABCC transporters play a major role in the biotransformation of xenobiotics. Regarding the concentration impact, a significant increase of the ABCC1 gene expression was observed at 96 hpf in C. Regarding the time effect, a significant increase was observed at 96 hpf in the C group.

To sum up the results, we observed some similar trends in the expression of mRNA amount of the same genes during the same time periods and for the same concentration groups. In the matter of time influence, we could observe an increase in C and H at 96 hpf for all genes. In the case of L at 96 hpf, the increase was evident only for ABCB4 and GST. In contrast, a decrease was observed in CYP1A and ABCC1. Regarding *Danio rerio* embryonic development, 96 hpf is the time of hatching, which is the first time when the species is exposed to an exogenous environment and therefore also to xenobiotics in water. That is possibly why we can mostly observe an increase in relative gene expression, because the biotransformation enzymes are probably the most active at this time in order to prevent the adverse effect of a xenobiotic which entered the cell.

A similar tendency was also observed at 144 hpf. In C group, the mRNA amount decreased in comparison with 96 hpf for all of the genes. In contrast, in L and H, an increase of mRNA amount was observed for all of the genes at 144 hpf in comparison with 96 hpf. 144 hpf is the time post-hatching, when the embryo is in contact with external environment. However, during the first 6 days of development, the embryo is still being nourished by the egg yolk. At 144 hpf, the species proceeds to exogenous nutrition. At this stage of development, the embryo faces the second most serious threat to its existence because it is exposed to external conditions. In this case, the embryos were fully exposed to antidepressant venlafaxine in water and we observed an increase in relative gene expression.

We hypothesized that environmental concentration (L) of antidepressant venlafaxine caused stress to the embryos leading to the inhibition of expression of all genes, except ABCB4 and GST, at the time of hatching (96 hpf). In addition, gene expression increased again at 144 hpf for L concentration, meaning that the biotransformation enzymes, after a while, can re-establish their function if they are exposed to lower concentrations. In contrast, in H concentration, the situation is quite the opposite: the relative gene expression seems to be stimulated by higher concentrations of antidepressant and increases not only at 96 hpf, but also at 144 hpf.

CONCLUSIONS

Venlafaxine is an antidepressant commonly found in surface waters. The aim of this study was to assess gene expression and mRNA amount of ABCB4, CYP1A, GST and ABCC1 gene in relation to the reference gene EF1A1. Results showed that venlafaxine in environmentally relevant concentrations can affect gene expression of biotransformation enzymes in *Danio rerio* embryos. This effect is most evident at 96 hpf (at the time of embryo hatching) and then at 144 hpf (at the time of the first exogenous nutrition).

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THE APPLICATION OF DIATOM INDICES FOR WATER QUALITY ASSESSMENT – CASE STUDY OF JOVAC AND ROČNJAK STREAMS

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UPOTREBA DIJATOMNIH INDEKSA ZA PROCENU KVALITETA VODE – STUDIJA SLUČAJA POTOKA JOVAC I ROČNJAK

Apstrakt

U ovom istraživanju predstavljeni su rezultati analize epilitskih zajednica silikatnih algi iz potoka Jovac i Ročnjak, dve pritoke akumulacije Vrutci. Uzorci su sakupljani mesečno u periodu od decembra 2014. do oktobra 2015. godine. Identifikovano je ukupno 118 taksona silikatnih algi. Najbrojnije populacije u okviru epilitske zajednice silikatnih algi, u oba potoka, grade *Achnanthidium minutissimum* var. *minutissimum* i *Cocconeis placentula* var. *lineata*. Rezultati izračunavanja dijatomnih indeksa, uz pomoć softverskog paketa OMNIDIA, ukazuju da je ekološki status vode oba potoka u ispitivanom periodu dobar, sa veoma niskim organskim opterećenjem i uz odsustvo antropogene eutrofikacije.

Ključne reči: *bentosne silikatne alge, dijatomni indeksi, kvalitet vode, potoci Jovac i Ročnjak*

Keywords: *benthic diatoms, diatom indices, water quality assessment, Jovac stream, Ročnjak stream*

INTRODUCTION

Benthic diatoms are so ecologically important that they are used as bioindicators in water quality assessment (Ács et al. 2004). They have been used in a number of countries as bioindicators of river pollution (Kelly and Whitton 1995, Blanco et al. 2004, Gosselain et al. 2005, Solak 2011). However, in Serbia it is still a new topic (Krizmanić et al. 2013, 2015, Vasiljević et al. 2014, 2017, Jakovljević et al. 2016a, 2016b).

The Jovac and Ročnjak streams, tributaries of the Vrutci accumulation, are located at western part of Serbia. Streams have a torrential character and are built from limestone.

Catchment area is characterized by mountainous and hilly terrain on altitude from 570 to 1250 m.a.s.l. (KRO "Bioktoš" 1986).

The aim of this study is the use of diatom indices as a tool for estimating the stream water quality.

MATERIAL AND METHODS

The material used in the present study was collected between December 2014 and October 2015 from Jovac and Ročnjak streams, tributaries of the Vrutci accumulation. Epilithic samples were scraped from the surface of gravel and boulders by using scalpel blade and brush. *In laboratory the field samples were treated with standard method with cold acid (Krammer and Lange-Bertalot 1986) to prepared permanent slides.* 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 was estimated by counting 400 valves present on each permanent slide. The biological assessment of water quality was performed by OMNIDIA 5.3 software. The ranges of diatom indices were used together with a water quality classes and ecological status according to the regulation of the Ministry of Environmental Protection (Sl. Glasnik 74/2011).

RESULTS AND DISCUSSION

In this study, a total of 118 diatom taxa were identified, of which 54 are joint taxa for both streams. The most numerous were taxa of the genera *Gomphonema*, *Nitzschia* and *Navicula*. In Jovac stream taxa *Achnanthidium minutissimum* var. *minutissimum* and *Cocconeis placentula* var. *lineata* dominated in epilithic diatom communities of both streams. These two taxa dominate in all months 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 oligo- to eutrophic waters, and develop numerously in mountain streams with no anthropogenic impact (van Damm et al. 1994, Cantonati et al. 2017). In Ročnjak stream, in addition to these two taxa, 6 other taxa were defined as dominant, those whose percentage participation was 10% or more.

Table 1. Dominance in diatom communities in Jovac and Ročnjak streams between December 2014 and October 2015.

Stream	Jovac									Ročnjak								
Taxon	dec	mar	apr	may	jun	jul	aug	sep	oct	dec	mar	apr	may	jun	jul	aug	sep	oct
Achnanthidium latecephalum	0	0	0	0	0	0	0	0	0	<10	10-20	0	21-35	<10	<10	<10	<10	<10
Achnanthidium minutissimum	>35	>35	>35	21-35	<10	<10	10-20	10-20	10-20	<10	21-35	<10	10-20	10-20	10-20	10-20	<10	<10
Achnanthidium pyrenaicum	0	0	<10	<10	0	0	<10	<10	<10	10-20	>35	<10	<10	10-20	10-20	>35	<10	<10
Amphora inariensis	0	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	<10	21-35	10-20	>35	>35
Amphora pediculus	0	<10	<10	<10	<10	<10	<10	<10	<10	>35	<10	<10	0	<10	<10	<10	<10	<10
Cocconeis placentula var. *lineata*	0	<10	<10	>35	>35	>35	10-20	>35	>35	<10	0	10-20	<10	<10	<10	<10	10-20	<10
Cocconeis pseudolineata	0	<10	10-20	21-35	21-35	>35	<10	10-20	21-35	<10	0	<10	0	<10	<10	<10	<10	<10
Cymbella excisa	0	0	0	<10	0	0	21-35	<10	<10	<10	0	<10	<10	<10	<10	<10	<10	<10
Fragilaria ulna	10-20	<10	<10	0	0	<10	0	0	0	0	0	10-20	<10	<10	0	0	0	0
Gomphonema elegantissimum	<10	<10	10-20	0	0	0	<10	<10	<10	0	<10	<10	<10	<10	<10	<10	<10	<10
Gomphonema olivaceum	10-20	<10	<10	0	0	0	0	0	<10	0	<10	<10	0	0	0	0	0	0
Meridion circulare	<10	0	<10	<10	0	0	0	0	0	0	0	21-35	<10	0	<10	<10	0	0

[%] occurrence:

0	<10	10-20	21-35	>35

Dominant and frequent species identified in the epilithic communities of the Jovac and Ročnjak streams, such as *Achnanthidium pyrenaicum*, *Cocconeis pseudolineata*, *Amphora pediculus* and *A. inariensis*, were also found in other water ecosystems with a good water quality, e.g. Baryczka stream (Noga et al. 2013).

Rivers and streams with 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 Jovac and Ročnjak streams. Further, a substantial part of the diatom species identified in these streams are species that prefer water with low level of pollution, β-mesosaprobic zones, according to indicator values after the OMNIDIA 5.3 database.

In order to determine the water quality of Jovac and Ročnjak streams, 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 rivers in Serbia.

The results of a diatom indices analysis (Tab. 2) in general show the excellent (first class) ecological status of Jovac and Ročnjak streams in the investigated period, with the absence of organic pollution and anthropogenic eutrophication. However, ecological status of Jovac stream in December 2014 for both indices is classified as good (second class). The presence of *Gomphonema olivaceum* and *Fragilaria ulna* with high abundance (Tab. 1) could give explanation in changes of ecological status in December 2014. Those taxa are tolerant to high concentration of nutrients and eutrophic conditions (van Damm et al. 1994).

Table 2. Diatom indices values of Jovac and Ročnjak streams in the investigated period. Blue color – excellent ecological status (first class); green color – good ecological status (second class)

Stream	**Jovac**									**Ročnjak**								
Index	dec	mar	apr	may	jun	jul	aug	sep	oct	dec	mar	apr	may	jun	jul	aug	sep	oct
CEE	13,7	16,2	18,3	19,2	18,5	17,5	18,1	18,9	17,9	17,2	17,7	17,2	17,7	17,7	17,9	17,5	18,1	16,0
IPS	14,6	18,3	18,2	17,4	16,7	17,0	16,4	16,8	17,1	15,9	17,0	16,3	15,8	17,1	17,5	17,0	18,6	17,5

In the Ročnjak stream the values of CEE index were the whole time inside the boundaries of first class, while IPS showed lower ecological status in December 2014 and May 2015. *Amphora pediculus* and *Achnanthidium pyrenaicum* were the most dominant taxa (Tab. 1) in December 2014. Their tolerance on eu- to mesotrophic conditions (van Damm et al. 1994) is clearly reflected in the reduced index value. Bioindicator caracteristics of *A. latecephalum*, the most dominant taxon in May 2015 (Tab. 1), was obtained for IPS index only. Lack of ecological information in OMNIDIA softwer excludes the influence of this taxon on the values of other indices.

According to the Serbian legislations, IPS and CEE indices together leads to the conclusion that both streams have good ecological status and belonged to the second class of water quality.

CONCLUSIONS

A total of 118 diatom taxa were identified in Jovac and Ročnjak streams. The most dominant in taxa were *Cocconeis placentula* var. *lineata* and *Achnanthidium minutissimum* var. *minutissimum*.

Based on the obtained values of diatom indices can be concluded that both streams have no major variations of water quality during the observed period. The water had good ecological status and belonged to the second class of water quality. Water quality monitoring based on diatom indices is still a new topic in Serbia; and will be getting more improved with new research in the near future.

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INFLUENCE OF THE AMOUNT OF ESSENTIAL FATTY ACIDS IN MUSCLE TISSUE OF SILVER CARP AND COMMON CARP FRYING IN SUNFLOWER OIL AND PORK FAT

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UTICAJ PRŽENJA U SUNCOKRETOVOM ULJU I SVINJSKOJ MASTI NA SASTAV MASNIH KISELINA U FILETIMA TOLSTOLOBIKA I ŠARANA

Apstrakt

Utvrđene su promene u sastavu masnih kiselina tolstolobika (*Hypophthalmichthys molitrix*) i šarana (*Cyprinus carpio*) posle dubokog prženja u suncokretovom ulju i svinjskoj masti. Korišćenjem suncokretovog ulja i svinjske masti povećavao se udeo polinezasićenih i zasićenih masnih kiselina, respektivno. Rezultati su pokazali povećanje sadržaja linolne kiseline (C18:2n-6) u ribama prženim u suncokretovom ulju i smanjenje n-3 masnih kiselina u prženim ribama, naročito C20:5n-3 (EPA) i C22:6n-3 (DHA). Očigledna retenciona vrednost (ARV - apparent retention value) n-3 masnih kiselina bila je viša kod tolstolobika (69-78%) nego kod šarana (21 do 43%), koje su pržene u suncokretovom ulju - što je veći broj ARV, uticaj prženja je lošiji. Prženjem ribe u svinjskoj masti, rezultati su pokazali povećanje zasićenih masnih kiselina, naročito palmitinske i stearinske kiseline. Sa stanovišta javnog zdravlja važno je napomenuti da se odnos n-6/n-3 u filetima tolstolobika i šarana povećao posle dubokog prženja u suncokretovom ulju (2,61; 28,50), za razliku od prženja u svinjskoj masti (1,20; 7,99).

Za filete tolstolobika, duboko prženje u suncokretovom ulju povećalo je ukupan sadržaj masti u ribi za oko 54%, sa 4,16% na 7,68%, dok je prženje u svinjskoj masti povećalo ukupan sadržaj masti u ribi za oko 56%, sa 4,16% na 7,39%. Za filete šarana duboko prženje u suncokretovom ulju povećao je ukupan sadržaj masti u ribi za oko 23%, sa 2,58% do 11,42%, dok je prženje u svinjskoj masti povećalo ukupan sadržaj masti u ribi za oko 34%, sa 2,58% na 7,50%. Rezultati do kojih smo došli jasno uakzuju da izbor kulinarskih ulja/masti utiče na ukupan sadržaj masnih kiselina i sastav pripremljenog fileta ribe.

Ključne reči: *prženje, suncokretovo ulje, svinjska mast, tolstolobik, šaran*
Key words: *frying, sunflower oil, pork fat, silver carp, common carp*

INTRODUCTION

Fish species, from different ecosystems, are known to differ in their fatty acid (FA) composition; therefore, studies of polyunsaturated fatty acid (PUFA) contents of diverse fish from various locations are of great importance for revealing their potential value as sources of the essential n-3 acids for human nutrition (Ćirković et al., 2011; Trbović et al., 2013; Ahlgren et al., 1994). Meanwhile, information about PUFA contents of raw fish may have limited value for a conclusion on their food quality. Several studies were undertaken to determine the effects of different cooking methods on the FAs of fish species, in particular boiling, pan-frying, deep fat-frying and oven-baking (Candela et al., 1998; Al-Saghir et al., 2004; Sioen et al., 2006; Gladyshev et al., 2007). The quality of the heating medium is of great concern, since most oils used for this purpose are now vegetable oils containing linoleic (18:2n-6) acid and lesser amounts of linolenic (18:3n-3) acid. Such PUFA are susceptible to oxidation and to thermal damage from local excess heating (Sebedio et al., 1993; Sioen et al., 2006). Less attention has been paid to the transfer of lipids between the food item and the frying medium (Peers and Swoboda, 1982; Thompson and Aust, 1983; Sebedio et al., 1990). Frying is a frequently applied method and gives flavour characteristics to the food that are highly appreciated by the consumers. In Serbia, carp is the most eaten species of medium fatty fish (Marković and Poleksić, 2011; Ćirković et al., 2011). Effects on the FA composition of silver carp (*Hypophthalmichthys molitrix*) and common carp (*Cyprinus carpio*) after frying in sunflower oil and pork fat were determined.

MATERIAL AND METHODS

Fish samples

Commercially available fish were collected at the same time from the fish farm ''Ečka'' (Zrenjanin, Serbia). The fish species were silver carp (*Hypophthalmichthys molitrix*) and common carp (*Cyprinus carpio*). Fish, upon arrival to laboratory, were held in frozen storage cabinets below -20 °C before the following treatment: three fishes of each species were used in each analysis, i.e., were sampled under each treatment: control (raw) and frying. Therefore, six fishes were analyzed. Muscle tissues (fillets) below the dorsal fin were taken as the samples. The tissues of raw fish were thawed at room temperature during about one hour prior to analyses. The fried fish were sampled for the following analyses within in one hour after frying. Frying in sunflower oil, as the most common cooking oil in Serbia, as well as pork fat at 150–170 °C during 15–20 min was used.

Chemical analysis and FAs analysis

To measure moisture content, fillets from the same fish samples of about 10–15 g of wet weight were taken and dried to constant weight at 105 °C. Lipid extraction after acid hydrolysis of sample was performed using petroleum ether. Fatty acid methyl esters (FAMEs) were performed using conditions that have been previously reported (Spirić et al., 2010). Briefly, total lipids for FAs determination were extracted from fish muscle tissues by accelerated solvent extraction (ASE 200, Dionex, Sunnyvale, CA) with a mixture of n-hexane and iso-propanol (60:40 v/v) in 33 ml extraction cell at 100 °C and nitrogen pressure of 10.3 MPa. The solvent was removed under the stream of nitrogen in solvent cabinet (Dionex SE 500, Sunnyvale, CA) at 50 °C until dryness. The fat extract was further used for FAs determination.

FAMES were prepared by transesterification by using trimethylsulfonium hydroxide, according to SRPS EN ISO 5509:2007 procedure. The GC instrument Shimadzu 2010 (Kyoto, Japan) used for FAMEs determination was equipped with fused silica cianopropyl HP-88 column (length 100 m, i.d. 0.25 mm, film thickness 0.20 µm, J&W Scientific, USA), and flame ionization detector. The column temperature was programmed. Injector temperature was 250 °C and detector temperature was 280 °C. The carrier gas was nitrogen at a flow rate of 1.33 ml/min and injector split ratio 1:50. Injected volume was 1 µl and total analysis time was 50.50 min. The chromatographic peaks in the samples were identified by comparing relative retention times of FAME peaks with peaks in a Supelco 37 Component FAME mix standard (Supelco, Bellefonte, USA).

RESULTS AND DISCUSSION

The FA content of sunflower oil and pork fat is presented in Table 1.

Table 1. FA composition of sunflower oil and pork fat (mean ± SEM)

Fatty acids	Sunflower oil	Pork fat
SFA	9.75±0.14	39.07±0.92
MUFA	28.87±0.76	45.33±0.33
PUFA	54.77±2.10	15.32±0.64
n-3	0.06±0.00	0.62±0.13
n-6	54.71±2.10	13.80±0.50

SFA – saturated fatty acids, MUFA – monounsaturated fatty acids, PUFA – polyunsaturated fatty acids

The FA contents of raw fish and after deep fat-frying in sunflower oil and pork fat is shown in Table 2 and Table 3. The most abundant fatty acids in the raw silver carp fillets in this study were MUFAs (51.66%) and n-3 PUFAs (12.78%). The most abundant fatty acids in the raw common carp fillets in this study were MUFAs (49.01%) and n-6 PUFAs (19.29%). Using sunflower oil in frying of silver carp and common carp fillets resulted in decreases of the amounts of saturated fatty acids (SFA) (apparent retention values (ARV) were from 66 to 79%), decrease of monounsaturated fatty acids (MUFA) (ARV were from 82 to 96%, respectively) and in an increase of the amount of PUFA (ARV were from 154 to 203%, in fish species). The obtained result showed alteration of n-6 PUFAs in frying fish using sunflower oil (179 to 748%) and thus a decrease in n-3 PUFAs (31 to 70%). The lowest retention values of n-6 and n-3 fatty acids were obtained for common carp. Hence, from a public health point of view, it is important to mention that the n-6/n-3 ratio in the carp's fillets (silver carp and common carp) altered after frying when using sunflower oil (23.34 and 28.50, respectively).

Frying of silver carp in pork fat resulted in a significant increase in the total SFAs and n-6 PUFAs (ARV were 117 and 237, respectively). Frying in pork fat also changed the FA profile of common carp. While the n-6 PUFA accounted for 19.29% of the total FAs in raw common carp, frying in pork fat resulted in increase of the total SFAs and MUFAs (ARV were 125 and 112%, respectively) and decreasing of n-6 and n-3 PUFAs (ARV were 58 and 36% respectively). The n-6/n-3 ratio altered from 4.98 in raw to 7.99 after frying in pork fat.

Table 2. FA compositions of silver carp before and after deep fat-frying in sunflower oil and pork fat and apparent retention values of fish

Fatty acids	Silver carp (n=3)				
	raw	fried in sunflower oil	fried in pork fat	ARV% fried in sunflower oil	ARV% fried in pork fat
SFA	29.59	23.26	34.57	79	117
MUFA	51.66	42.46	50.51	82	98
PUFA	15.9	32.27	13.53	203	85
n-3	12.78	8.93	6.15	70	48
n-6	3.12	23.34	7.39	748	237
n-6/n-3	0.24	2.61	1.2		

SFA – saturated fatty acids, MUFA – monounsaturated fatty acids, PUFA – polyunsaturated fatty acids

Table 3. FA compositions of common carp before and after deep fat-frying in sunflower oil and pork fat and apparent retention values of fish

Fatty acids	Common carp (n=3)				
	raw	fried in sunflower oil	fried in pork fat	ARV% fried in sunflower oil	ARV% fried in pork fat
SFA	25.48	16.9	31.88	66	125
MUFA	49.01	46.81	54.88	96	112
PUFA	23.16	35.71	12.51	154	54
n-3	3.87	1.21	1.39	31	36
n-6	19.29	34.49	11.11	179	58
n-6/n-3	4.98	28.5	7.99		

SFA – saturated fatty acids, MUFA – monounsaturated fatty acids, PUFA – polyunsaturated fatty acids

Table 4. Mean moisture content (MC %), fat content (FC %) and apparent retention values (ARV %) of raw and fried freshwater fish

Nutrient	Silver carp (n=3)				
	raw	fried in sunflower oil	fried in pork fat	ARV% fried in oil	ARV% fried in pork fat
MC %	74.39	71.46	69.96	96	94
FC %	4.16	7.68	7.39	54	56

Table 5. Mean moisture content (MC %), fat content (FC %) and apparent retention values (ARV %) of raw and fried freshwater fish

Nutrient	Common carp (n=3)				
	raw	fried in oil	fried in pork fat	ARV% fried in oil	ARV% fried in pork fat
MC %	77.98	67.41	71.07	86	91
FC %	2.58	11.42	7.5	23	34

Silver carp and common carp in our study belong to medium fatty fish species containing less than 4 g fat per 100 g fish (Huss, 1995). For silver carp fillets, frying in sunflower oil increased the fat content (4.16 to 7.68 g/100 g) for about 54%, whereas, for common carp fillets, it increased (2.58 to 11.42 g/100 g) for about 23%, Table 4 and 5). Using pork fat in frying of silver carp increased fat content (4.16 to 7.39 g/ 100 g) for about 56% while for common carp it increased (2.58 to 7.50 g/100 g) for about 34%, Table 4 and 5). The moisture content varied in both fish samples from about 74% to 78% (Table 4 and Table 5). Higher moisture contents were characteristic of common carp, and lower contents were found for silver carp. An explicit tendency to decrease of moisture contents in both fish species due to frying occurred (Table 4 and Table 5).

CONCLUSION

Comparing the use of culinary fats with different FA profiles is relevant to investigate their influence on the FA profile of the food after preparation. Using sunflower oil significantly increased the total PUFAs content in both fish species, whereas using pork fat resulted in a increasing trend in the SFAs of silver carp and common carp fillets. Hence, control over the FA composition of the consumed fried fish can be achieved to some extent by the selection of the culinary fat. Consequently, this study showed that the uptake of FA from the culinary fat into the fried samples was inversely correlated with the total FA content of the fish.

In conclusion, the culinary oil/fat selection affects the total FA content and composition of the prepared fish fillet.

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FYKE NETS SELECTIVITY FOR BLACK BULLHEAD (*AMEIURUS MELAS*) IN SAVA LAKE

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SELEKTIVNOST VRŠA U IZLOVU CRNOG AMERIČKOG PATULJASTOG SOMA (*AMEIURUS MELAS*) U SAVSKOM JEZERU

Apstrakt

Istraživanje populacione dinamike crnog američkog patuljastog soma *Ameiurus melas* (Rafinesque, 1820) u Savskom jezeru realizovano je u periodu od avgusta 2009. do decembra 2012. godine. U cilju pronalaženja načina za smanjenje i kontrolu brojnosti populacija ove invazivne alohtone vrste (pre svega u stajaćim vodama), važno je ispitati i selektivnost ribolovnog alata (vrša). Selektivnost vrša procenjena je na osnovu odnosa ulovljenih jedinki crnog američkog patuljastog soma i drugih vrsta riba, za svaki mesec pojedinačno. Ukupno je uhvaćeno 13866 jedinki, od čega su jedinke crnog američkog patuljastog soma činile 94.2%, grgeča (*Perca fluviatilis*) 3.2% i sunčice (*Lepomis gibbosus*) 2.1%. Ulov štuke (*Esox lucius*), deverike (*Abramis brama*), babuške (*Carassius gibelio*), šarana (*Cyprinus carpio*), bodorke (*Rutilus rutilus*)), smuđa (*Sander lucioperca*) i glavoča (Gobiidae) bio je na nivou statističke greške (< 1%). Rezultati istraživanja pokazuju da se vrše mogu primenjivati u kontroli brojnosti populacija crnog američkog patuljastog soma, jer osim što su selektivne, efikasne i lake za rukovanje, imaju minimalan negativni uticaj na druge vrste riba.

Ključne reči: *crni američki patuljasti som, selektivnost ribolovnog alata, vrše, kontrola populacija*

Keywords: *black bullhead, fish gear selectivity, fyke nets, population control*

INTRODUCTION

The impacts of non-native invasive species are immense and usually irreversible, and their adverse effects to native species and ecosystems can be compared to those caused by the loss and degradation of habitats (IUCN, 2000). Introduction of non-native species can have a serious negative impact on the environment, diversity of native species, economic resources, and human health (Clavero & García-Berthou, 2005; Shirley & Kark, 2006). A large number of non-native fish species were introduced into freshwater ecosystems on a global scale (Leprieur et al., 2008), and at least 134 exotic/translocated fish species are introduced in Europe (Holčík, 1991). The black bullhead *Ameiurus melas* (Rafinesque, 1820), native to North America, is one of the most abundant and successful non-native fish species in European freshwater ecosystems (Cucherousset et al., 2006b). It poses a major problem for fisheries management throughout Europe, which has led to many attempts to apply selective fishing of this species (Cucherousset et al., 2006b; Louette & Declerck, 2006). The first record of the black bullhead in Serbia was in 2005 (Cvijanović et al., 2005), and according to Lenhardt et al. (2011), it is one of the dominant non-native species in Serbian waters.

The aim of this research was to investigate the efficiency of fyke nets as a sampling tool for population assessment, as well as their selectivity for population control and mass removal of this species, considering that there are only a few studies published on the efficiency of black bullhead trapping and mass removal (Cucherousset et al. 2006a; Louette & Declerck, 2006).

MATERIALS AND METHODS

Sava Lake is a reservoir formed in 1967 by damming a right-hand branch of the Sava River in Belgrade (Serbia), near the Ada Ciganlija river island, as a facility for water supply, water sports, and recreation. It is a mesotrophic to eutrophic lake in the process of succession on the site of a former river ecosystem (Janković & Janković, 1987). The lake has the status of a special fishing waterbody ("catch-and-release fishing").

Based on the total biomass, among 20 fish species currently present in the lake, the most notable are the common carp *Cyprinus carpio* (21.2%), pikeperch *Sander lucioperca* (13.5%), common bream *Abramis brama* (8.4%), Eurasian perch *Perca fluviatilis* (6.0%), and European catfish *Silurus glanis* (4.3%). The non-native species with the highest biomass are the silver carp *Hypophthalmichthys molitrix*, bighead carp *Hypophthalmichthys nobilis*, black bullhead (5.7% each), and Prussian carp *Carassius gibelio* (5.0%) (Hegediš et al., 2008).

Black bullhead samples were collected monthly from August 2009 to December 2012 using double fyke nets (length 85 cm, diameter 50 cm, 8 mm mesh-size). Nets were positioned in three rows, with five nets placed in each row, at 3 m, 10 m, 18 m, 25 m, and 35 m distance from the shore, at depths of 1.5 m, 4 m, 5.5 m, 7.5 m, and 8 m, respectively. The distance between the rows was 15 m. Nets were left in water for 3 nights each month and checked daily.

The selectivity of fyke nets was estimated based on the ratio of the captured individuals of black bullhead and of other fish species in the total catch for each month: northern pike (*Esox lucius*), common bream, Prussian carp, common carp, roach (*Rutilus rutilus*), Eurasian perch, pikeperch, pumpkinseed (*Lepomis gibbosus*), and gobies (Gobiidae).

RESULTS

The results in 2009 and 2010 were similar, and the percentage of black bullhead individuals in the total catches was 94.6% ± 5.0 and 99.9% ± 0.17 (mean ± SD), respectively. Different results were observed in 2011. May was the month in which the percentage of black bullhead individuals was the lowest (17.3%), and it was the month when a mass mortality of the bullhead occurred in the lake. At that time, the Eurasian perch comprised the largest percentage in the catch (55.6%), followed by pumpkinseed (23.5%), common bream (2.5%), and Prussian carp (1.2%). The next month with the lowest number of black bullhead individuals in the total catch was November 2011 (64.6%), followed by pumpkinseed (24.8%) and Eurasian perch (10.6%). In July 2011, the percentage of black bullhead individuals in the total catch was 67.7%, followed by Eurasian perch (22.9%), gobies (7.3%), and pumpkinseed and common bream (1% each). The catch in other months of 2011 was uniform, and the average percentage of black bullhead individuals was 88.1% ± 7.3, and of all other fish species it was 5.6% ± 5.6. Not a single northern pike or common carp were caught during 2011.

March and June 2012 are characterized by a lower percentage of black bullhead in the total catch (11.5% and 36.9%, respectively). In March, individuals of Eurasian perch were dominant (87.8%), and in June, the percentage of pumpkinseed and Eurasian perch individuals were 34.7% and 27.6% respectively. During all other months of 2012, black bullhead was dominant in the total catch and its average percentage was 96.9% ± 2.9, while of all other fish species it was 0.8% ± 1.1. No common carp or roach were caught in 2012.

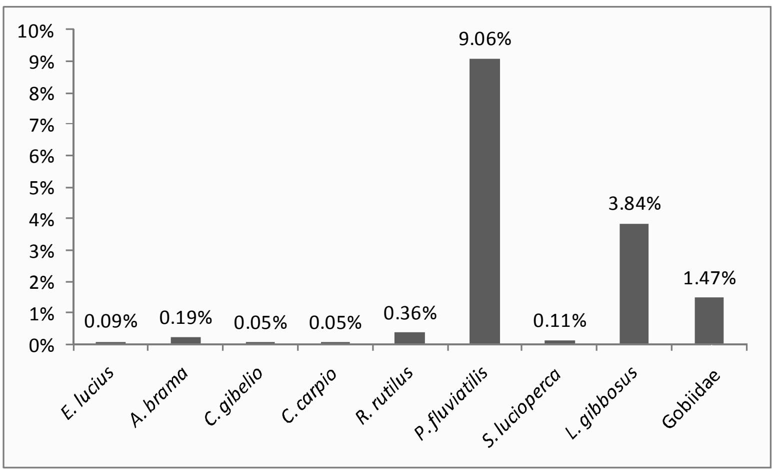

Figure 1. The average percentage of other fish species (black bullhead excluded) in the catch from August 2009 to December 2012.

The average percentage of black bullhead individuals caught during the entire sampling period was of 84.8%, and for other species it was 15.2%. The average percentage of other species was the highest for the Eurasian perch (9.1% ± 18.9), followed by pumpkinseed (3.8% ± 8.5), and gobies (1.5% ± 3.6) (Figure 1). High values of standard deviation (SD) indicate large differences between months. The average percentage in the total catch of the northern pike, common bream, Prussian carp, common carp, roach, and pikeperch were low and uniform, less than 1%, and SD values ranged from 0.2 to 1.5.

DISCUSSION

The results from Sava Lake suggest that fyke nets represent a highly selective fishing tool for black bullhead. During the four-year research, it was observed that in stable conditions (2009, 2010, the first half of 2011, and the second half of 2012), the percentage of black bullhead in the total catch ranged from 79.1% to 100%. Only from May 2011 (when the mass mortality event occurred) until the first half of 2012 (when the population stabilized), the catch of the black bullhead was variable. The percentage of other non-native invasive species that are considered undesirable, such as pumpkinseed and gobies, were higher than for native species, except for the Eurasian perch. The catch of the commercially most important fish (northern pike, common carp, and pikeperch) was at the level of statistical error. It is important that the caught individuals of these species stay alive, so that they can be returned unharmed into the water.

Other studies have also confirmed the efficiency and selectivity of fyke nets for black bullhead. In 28 lakes of South Dakota these nets proved to be very effective, especially in the catch of smaller black bullhead individuals (Hanchin et al., 2002), and this was confirmed by Pedicillo et al. (2008) for Corbara Lake (Italy). Hanchin et al. (2002) also observed that, for black bullhead population monitoring in waterbodies where black bullhead abundance is high, fyke nets provide a better estimate of the relative abundance, expressed as the mean catch-per-unit effort of stock-length (≥ 15 cm total length) bullheads, than gill-nets, probably due to faster saturation of gill-nets. Krueger et al. (1998) showed that fyke nets represent a particularly effective tool for sampling of benthic fishes, such as black bullhead, in lakes and reservoirs. A study in the De Maten Nature Reserve (Belgium), which consists of a series of interconnected small and shallow ponds, suggests that double fyke nets, when combined with the mark-recapture technique, are a very useful tool for the efficient and reliable assessment of brown bullhead populations; the recapture efficiency of fyke nets for brown bullhead was 66% and for pumpkinseed, Prussian carp, rudd (*Scardinius erythrophthalmus*), and tench (*Tinca tinca*) it ranged between 23% and 47% (Louette & Declerck, 2006). The results of the same study also suggest that double fyke nets may potentially be a cost-effective tool for the mass removal of non-native brown bullhead (*Ameiurus nebulosus*) populations from small to medium-sized shallow water bodies. It proved that double fyke nets do not damage other fish species, they are easy to use, and therefore they could help managers to reduce the number of reproductive individuals within one year. If the selective removal continues during following years, young-of-the-year individuals could be prevented from reaching sexual maturity. This could consequently lead to a reduction of abundance or even to eradication of these species from the ecosystem (Louette & Declerck, 2006). Considering the great similarity of these two species, the results of this research can be fully compared with the results from Sava Lake.

The results of fyke nets selectivity assessment for black bullhead in Sava Lake suggest that the mass removal would be most effective if conducted during the summer and autumn period (when the population density is at its peak), in the zone of the macrophyte vegetation. Juvenile individuals should be removed along with adult (reproductive) individuals. This model, with some modifications, could be applied to most lake ecosystems in Serbia. However, long-term monitoring of these ecosystems would be necessary, in order to keep track of the recruitment and possible rapid renewal of the black bullhead population. In addition to these management measures, it would be desirable to occasionally restock these ecosystems with native fish species as well as to evaluate possible habitat alterations.

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MAJOR HISTOCOMPATIBILITY COMPLEX (MHC) POLYMORPHISM IN TWO MAJOR SOCKEYE SALMON (*ONCORHYNCHUS NERKA*) POPULATIONS IN ASIA

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POLIMORFIZAM GLAVNOG HISTOKOMPATIBILNOG KOMPLEKSA (MHC) KOD DVE ZNAČAJNE POPULACIJE ALJASKOG CRVENOG LOSOSA (*ONCORHYNCHUS NERKA*) U AZIJI

Apstrakt

U studiji je praćena varijabilnost Onne-DAB gena, koji enkodira β lanac glavnog histokompatibilnog kompleksa klase II (MHC II) koristeći jednonukleotidni polimorfizam One_MHC2_109, One_MHC2_190v2 i One_MHC2_251v2 u dve najveće populacije Aljaskog crvenog lososa (*Oncorhynchus nerka*) sa Pacifičke obale koje se razmnožavaju u rekama Ozernaja i Kamčatka. Razlike u nasleđivanju i stepenu polimorfizma One_MHC2_190v2 i One_MHC2_251v2 su uočene u uzorcima riba iz oba rečno-jezerska sistema, dok to nije bio slučaj sa One_MHC2_109. Uzorci sakupljeni iz Kurilskog jezera su bili karakteristični po visokom stepenu intrapopulacijskog diverziteta, dok interpopulacijske razlike u učestalosti zajedničkog MHC2 lokusa nisu pronađene. Ovakvi rezultati mogu biti rezultat balansirane prirodne selekcije u sekvencama koje kodiraju MHCII peptidno-vezujući region u ovoj populaciji lososa. Ovo je kasnije potvrđeno Evans-Vatersonovim testom. Sa druge strane, u uzorcima lososa iz reke Kamčatke, primećen je nizak genetički diverzitet i značajna heterogenost haplotipskih učestalosti na lokusu MHC2, očigledno zbog selekcije uzrokovane patogenima u Onne-DAB genu nađene na nekim lokalitetima ovog jezersko-rečnog sistema. Analize rezultata kod lososa iz reke Kamčatke su pokazale da su razlike u intrapopulacionoj varijabilnosti i interpopulacionoj diferencijaciji sa različitih delova sliva bile uzrokovane selekcijom na patogene na pojedinim lokalitetima. Znaci balansirane selekcije su otkriveni na subpopulacijama nastanjenim u srednjem delu toka, gde je direkciona selekcija bila izuzetno uspešna, za razliku od subpopulacija koje žive u gornjem i donjem toku reke. Rezultati multijavarijatne PCA analize su pokazali da su dve principijelne komponente (PC) objasnile 72% varijabilnosti genetičkih karakteristika u uzorcima. Značajna korelacija je ustanovljena između druge PC ose i geografske razdaljine od svake pritoke do potoka Azabačija (Jezero Azabačija je izabrano kao kontrolna tačka, jer ovo jezero služi kao gajilište mlađi lososa iz različitih pritoka i nalazi se u blizini ušća reke Kamčatke). Pored toga, nađena je visoko značajna korelacija između druge PC i učestalosti pojave plerocerkoida (*Diphyllobothrium* sp.) u ribama iz pritoka.

Ključne reči: *losos, MHC, SNP, Ozernaja reka, Kamčatska reka*
Keywords: *sockeye salmon, MHC, SNP, Ozernaya River, Kamchatka River*

INTRODUCTION

Sockeye salmon, *Oncorhynchus nerka* Walbaum, is highly commercially important species in the Russian Far East, where it is most abundant in the basins of the Ozernaya River (the size of the Kuril'skoe Lake population constitutes more than 70% of the total number of Asian sockeye salmon) and Kamchatka River (where in certain years almost 70 to 80% of the total number of Eastern Kamchatka sockeye salmon is reproduced) (Bugaev, 1995). Both metapopulations are characterized by extremely complex structure and are represented by different spawning ecotypes, subpopulations and seasonal races.

In order to study genetic structure of the populations we chose a DNA-marker with adaptive load - *Onne-DAB* gene, encoding β-chain of class II histocompatibility complex molecule (MHCII). Its variability was estimated by single-nucleotide substitutions (SNP) analysis in tree loci: *One_MHC2_109, One_MHC2_190v2* and *One_MHC2_251v2*.

The first two are located in 109 and 190 positions of the exon β1 encoding the most variable N-terminal domain of *β* chain. *One_MHC2_251v2* is located in the intron, 61 bp away from the second locus. The *β1* exon of the MHC class II genes is translated into the peptide-binding region (PBR) on the membranes of B cells and macrophages, thereby participating in the formation of the immune response to the action of extracellular pathogens. MHC class II genes are highly polymorphic in vertebrates; moreover, the highest number of non-synonymous substitutions is concentrated in the peptide-binding region (PBR). A high level of polymorphism in the MHCII genes is maintained by balancing selection because distinct alleles of them are associated with the organism's resistance to a specific infection or a parasite, as well as the directional selection influence in favor of some alleles is possible in some localities.

MATERIALS AND METHODS

Five samples of early and late running sockeye salmon from Kamchatka River lower course, as well as a sample from Azabachye Lake tributary (lower part of the Kamchatka River drainage), and early running sockeye salmon from Ozernaya River were analyzed by allele-specific PCR. In addition, open data of Habicht et al. (2010) (http://www.tandfonline.com/doi/suppl/ 10.1577/T09-149.1?scroll=top) on the loci variability in sockeye salmon ecotypes from distinct spawning sites (littoral and riverine) in Kurilskoye Lake watershed (Ozernaya R.) and upper, middle and lower course tributaries of Kamchatka River were involved (Fig. 1.).

Tissue samples were fixed in 96% ethanol. Genomic DNA was extracted from liver and fin fragments by standard methods. The genotyping method is described in detail in (Khrustaleva et al., 2010).

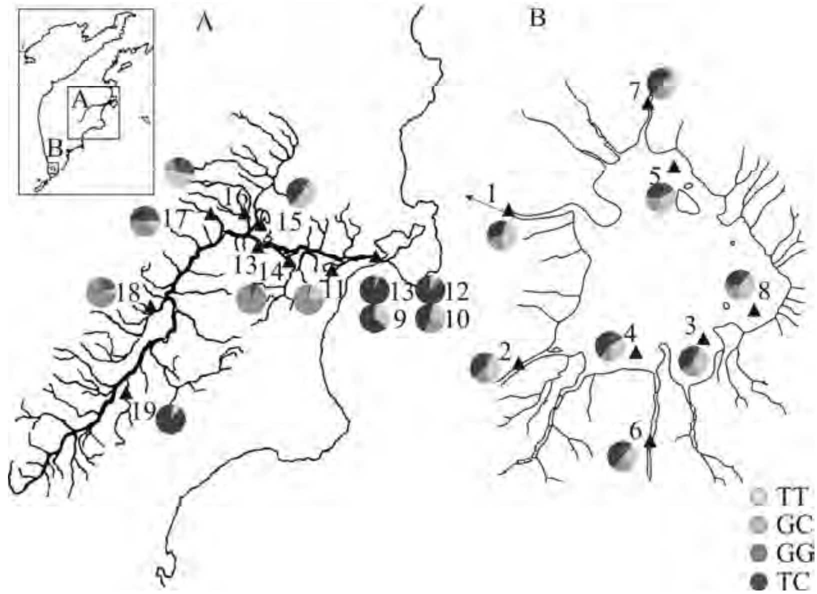

Figure 1. Schematic map of the study area showing sampling locations and TT, GC, GG, TC haplotypes frequencies distributions of MHC2 locus in Kamchatka River (A) and Kuril’skoye Lake (B) watersheds. The sample numbers are: 1 – Ozernaya R. (2003), 2 - Etamink R. (2002), 3 - Gavruschka Bay (2002), 4 - Khakizun Bay (2002), 5 - North-Far Bay (2002), 6 - Kirushutk R. (2000), 7 - Vichenkiya R. (2000), 8 - Olada Bay (2000), 9, 10, 12, 13 – Kamchatka R. mouth and lower course, early and late running sockeye salmon (1998, 2004, 2005), 11 - Azabachye Lake, KKa (2004), 14 - Hapiza River, KKhap (1998), 15 – Elovka R., KKel (1994, 1995), 16 - Dvu’yurta R., KKdv (1994, 1995), 17 - Belaia R., KKbel (1994, 1995), 18 – Kozireuka R., KKkoz (1994), 19 - Kitilgina R., KKkit (1998).

RESULTS AND DISCUSSION

Differences in inheritance and degree of polymorphism of *One_MHC2_190v2* and *One_MHC2_251v2* were observed in samples from both lake-river systems, whereas *One_MHC2_109* was poorly informative (monomorphic in one location or linked to *One_MHC2_251v2* in the other). Tests for linkage disequilibrium for *One_MHC2_190v2* and *One_MHC2_251v2* were significant in six samples from Kamchatka River and only in two samples of early stream-spawning sockeye salmon from Kurilskoe Lake. Because both loci are located in immediate proximity to each other there is sufficient reasons to combine them and consider as four multi-SNP haplotypes (allelic variants) of one joint locus *MHC2*.

Samples collected from the lake and river spawning grounds of Kurilskoe Lake (Ozernaya River) were characterized by high estimates of intrapopulation genetic diversity, whereas no inter-sample differences in the frequencies of haplotypes of the joint *MHC2* locus were revealed (Fig. 1B). That can be interpreted as the result of balancing selection in sequences encoding PBR in Kurilskoe Lake sockeye salmon population. It’s obviously due to the fact that pathogenic conditions in the watershed are homogenous. Our conclusions were confirmed by Ewens-Watterson neutrality tests.

On the contrary, in the samples of early (spring) sockeye salmon from the Kamchatka River basin low genetic diversity estimates and significant heterogeneity of haplotypic frequencies at the MHC2 locus were observed, apparently due to the action of directional pathogen-induced selection in *Onne-DAB* gene in some localities of the lake-river system (Fig. 1A). A possible cause for the diversity is a difference in pathogenic loads in sockeye salmon subpopulations in this river.

The more detailed study of Kamchatka River sockeye salmon showed that the differences in intra-population variability indices and inter-population differentiation estimates in the samples from different parts of the watershed were caused by action of different types of pathogen-induced selection in certain localities of the lake-river system. Signs of balancing selection were revealed in the middle course subpopulations, whereas directional selection was highly feasible by the results of the neutrality tests in subpopulations both from the upper river course and the lower course.

By the results of factor analysis (PCA) two principal components (PC) were extracted (Fig. 2a). In sum they explained 72% of general variability of genetic characteristics in the samples. A significant correlation between the second PC (33.4%) values and geographical distances from the specified tributary to Azabachya creek (Azabachye Lake was selected as a reference point because it is the nursing lake for young sockeye from several tributaries and it is located near the Kamchatka River mouth) was revealed (Fig. 2b). Moreover a highly significant correlation between the second PC and plerocercoid (*Diphyllobothrium* sp.) prevalence in fish from the tributaries was derived (Fig. 2c).

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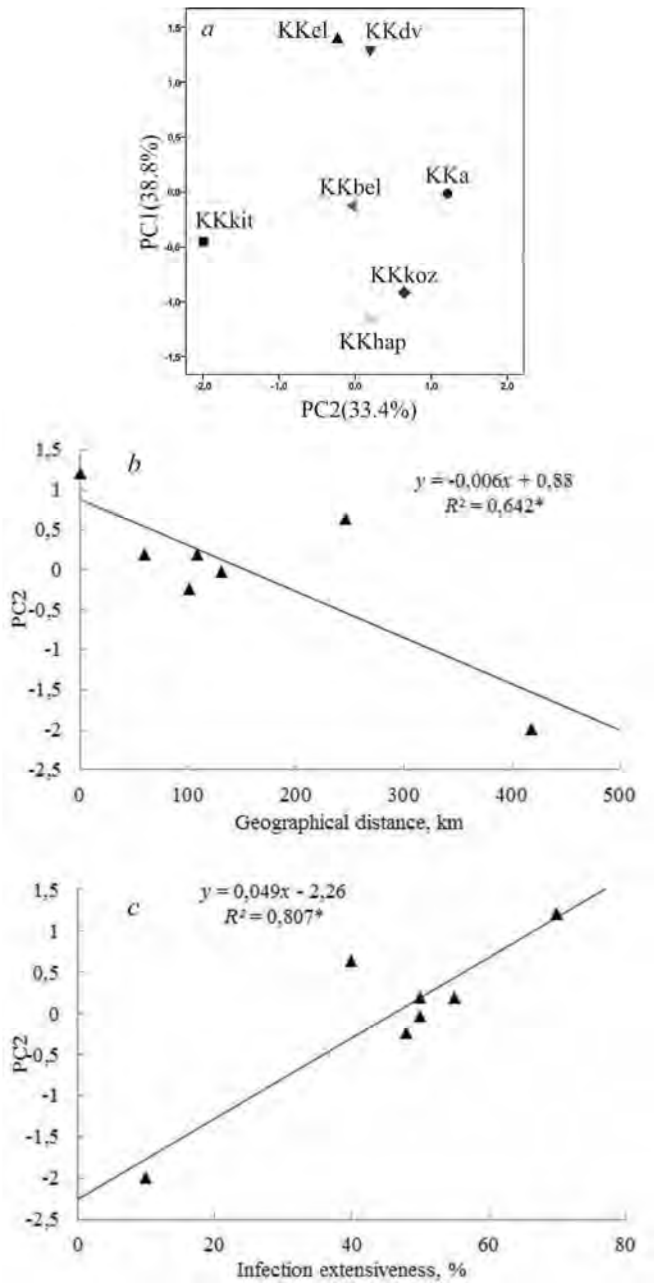

Figure 2. PCA plot (a) and dependencies of PC2 on geographical distance (b) and Diphyllobothrium sp. infection extensiveness (c) for sockeye salmon from different tributaries and spawning sites of Kamchatka River. Sample key as in Fig. 1.

LENGTH-WEIGHT RELATIONSHIP AND CONDITION FACTOR OF THE AXILLARY SEABREAM (*PAGELLUS ACARNE*) IN COASTAL WATERS OF LIBYA

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DUŽINSKO-TEŽINSKI ODNOS I FAKTOR KONDICIJE KOD BATOGLAVCA (*PAGELLUS ACARNE*) U PRIOBALNIM VODAMA LIBIJE

Apstrakt

Batoglavac (*Pagellus acarne*) je komercijalno značajna vrsta ribe koja se lovi duž istočne obale Atlantika i u Sredozemnom moru. Ova vrsta je protandrični hermafrodit (većina jedinki su prvo mužjaci koji postaju ženke pri dužinama od oko 17 do 29 cm, pri uzrastu od 2 do 7 godina). Dužinsko-težinski odnos i faktor kondicije analizirani su na uzorku od 100 jedinki batoglavca iz komercijalnog ulova. Ribe su ulovljene mrežama poponicama u septembru 2015. godine, u priobalnim vodama zapadne Libije, u području Sabrate. Za svaku jedinku određeni su totalna dužina tela (TL) u mm, težina tela (W) u g i pol. Dužina tela uzorkovanih jedinki obuhvatila je raspon od 150 mm do 215 mm. Sve jedinke su podeljene u šest dužinskih klasa od 10 mm, a najveći procenat jedinki spadao je u dužinsku klasu 160-169 mm (33%). Više od 80% jedinki bilo je duže od 160 mm. Težina tela analiziranih jedinki obuhvatila je raspon od 40 do 125 g. Parametri regresije dužinsko-težinskog odnosa iznosili su: $a = 0.00004$, $b = 2.76$, $r^2 = 0.80$, što ukazuje na negativni alometrijski rast. Srednje vrednosti Fultonovog faktora kondicije bile su slične za mužjake (1.32) i ženke (1.36). Uočeno je da ženke imaju veću vrednost faktora kondicije od mužjaka iste dužine.

Ključne reči: *batoglavac, dužinsko-težinski odnos, Fultonov faktor kondicije, priobalne vode, Sredozemno more*

Abstract

Axillary seabream *Pagellus acarne* is an important commercial species, fished along the eastern Atlantic coasts and the Mediterranean Sea. It is a protandric hermaphrodite (most individuals are first males, becoming females at sizes of about 17 to 29 cm, at age of two to seven years). Length-weight relationship and condition factor were analyzed on a sample of 100 specimens from a commercial catch. Fish were caught with trammel nets in September 2015, in coastal waters of western Libya offshore Sabratha. For each individual, the total body length (TL) in mm, body weight (W) in g, and sex were determined. Total body length of the sampled fish ranged from 150 mm to 215 mm, and all individuals were arranged into six 10-mm length classes. More than 80% of individuals were larger than 160 mm. The highest percentage of individuals were in the 160-169 mm length class (33%). Weight range of analyzed specimens ranged from 40 to 125 g. The length-weight regression parameters were: a = 0.00004, b = 2.76, r^2 = 0.80, which indicates a negative allometric growth. The mean value of the Fulton's condition factor was similar for males (1.32) and females (1.36). However, it was observed that females of the same length have better condition than males.

Keywords: *axillary seabream, length-weight relationship, Fulton's condition factor, coastal waters, Mediterranean Sea*

LENGTH-WEIGHT RELATIONSHIP AND CONDITION FACTOR OF THE COMMON BREAM (*ABRAMIS BRAMA*) IN THE DANUBE RIVER NEAR BELGRADE (1168-1170 RKM)

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DUŽINSKO-TEŽINSKI ODNOSI I FAKTOR KONDICIJE KOD DEVERIKE (*ABRAMIS BRAMA*) U DUNAVU KOD BEOGRADA (1168-1170 RKM)

Apstrakt

Deverika (*Abramis brama*) je značajna vrsta u privrednom i sportskom ribolovu i lovi se mrežama tokom cele godine. Koristi se i kao mamac pri ribolovu na grabljive ribe. Analizirani uzorci deverike potiču iz komercijalnog ulova obavljenog u periodu od decembra 2014. do februara 2015. godine na dva lokaliteta u Dunavu kod Beograda, Jojkića Dunavac (lokalitet 1) i ušće Save u Dunav (lokalitet 2). Uzorkovanim jedinkama izmerene su totalna dužina tela (TL) u mm i težina tela (W) u g, i određena im je starost očitavanjem naraštajnih zona na krljuštima. Korelaciona i regresiona analiza odnosa totalne dužine i težine tela urađena je kako za ceo uzorak, tako i za svaku uzrasnu kategoriju posebno. Za uzorke sa oba lokaliteta određeni su i Fultonov faktor kondicije ($K = W/L^3$ x 100) i alometrijski faktor kondicije ($C = W/L^b$ x 100). Srednja dužina tela (± SD) analiziranih jedinki iznosila je 242 ± 19 mm, a srednja težina (± SD) 151 ± 45 g. Starost jedinki kretala se od 1+ do 5+ godina. Najviše jedinki pripadalo je dužinskoj klasi od 231-260 mm i uzrasnim klasama 2+ i 3+, što znači da su u ulovu najviše zastupljene jedinke koje još nisu polno sazrele. Na taj način, onemogućeno je razmnožavanje ove ekonomski važne vrste ribe, a remeti se i dinamika brojnosti populacije, čime se smanjuje i ulov. Jedinke sa lokaliteta 1 pokazuju negativan alometrijski rast (a = 0.017, b = 2.85, r^2 = 0.86), dok jedinke sa lokaliteta 2 pokazuju pozitivan alometrijski rast (a = 0.004, b = 3.26, r^2 = 0.90). Prosečne vrednosti Fultonovog faktora kondicije bile su slične kod jedinki sa lokaliteta 1 (K = 1.04) i sa lokaliteta 2 (K = 1.05), dok su prosečne vrednosti alometrijskog faktora kondicije bile veće kod jedinki sa lokaliteta 1 (C = 1.70) nego kod jedinki sa lokaliteta 2 (C = 0.45). Rezultati su pokazali da kod uzoraka deverike sa oba lokaliteta najbolju kondiciju imaju jedinke koje pripadaju uzrasnoj klasi 4+ (sr. vr. K = 1.08 i C = 1.77 na lokalitetu 1; sr. vr. K = 1.11 i C = 0.47 na lokalitetu 2).

Ključne reči: *deverika, dužinsko-težinski odnos, Fultonov faktor kondicije, alometrijski faktor kondicije, Dunav*
Keywords: *common bream, length-weight relationship, Fulton's condition factor, allometric condition factor, Danube*

INTRODUCTION

The study of the length-weight relationship is an approach that is widely applied in fisheries management as it provides important information on stock condition (Bagenal and Tesch, 1978). These data, combined with data on age, are valuable in assessment of growth, condition, yield, and biomass (Kohler et al., 1995). A number of factors are known to influence the length–weight relationship in fish, including growth phase, degree of stomach fullness, gonad maturity, sex, health and general fish condition, size range, season, as well as preservation techniques (Bagenal and Tesch, 1978). The common bream (*Abramis brama*) is a widespread, commercially important species in Serbian waterbodies. The aim of the present study is to provide baseline data on length-weight relationship and condition of the common bream from two locations in the Danube River near Belgrade (Serbia).

MATERIALS AND METHODS

The analysed specimens were obtained from a commercial catch in the Danube at two sampling sites from December 2014 until February 2015. Site 1 is the small channel Jojkića Dunavac (44° 50' 4.90" N, 20° 28' 11.59" E), located upstream the Pančevo Bridge, and site 2 is the rivermouth of the Sava River into the Danube (44° 50' 1.94" N, 20° 26' 50.74" E). The total body length (TL, in mm) and body weight (W, in g) were measured for 137 sampled individuals of the common bream. Age was estimated from scales for 110 individuals.

The length-weight relationship (LWR) was calculated using the equation $W = aL$b. The values of constants, a and b, were estimated using the least-square method applied to the log transformed data $log\ W = log\ a + b\ log\ L$ (Ricker, 1975), where W (g) is the body weight of the fish, L (mm) is the total length, a is the intercept of the regression curve, and b is the regression coefficient. Value of the exponent b provides information on fish growth. When $b = 3$, the increase in weight is isometric, otherwise it is allometric (positive allometric if $b > 3$, negative allometric if $b < 3$). The Fulton's condition factor (K) was estimated from the equation $K = W/L$3 x 100, and the allometric condition factor from the equation $C = W/L^b$ x 100. Condition factors are used for comparing condition or well-being of fish, based on the assumption that heavier fish of a given length are in better condition. Fishes with Fulton's condition factor values greater than one (≥ 1) are considered in very good condition, while those with values less than one (< 1) are in poor condition.

RESULTS AND DISCUSSION

The total length of the sampled common bream individuals ranged from 204 to 330 mm, and the total weight from 100 to 414 g. Mean total body length (± SD) was 242 ± 19 mm, and mean weight (± SD) 151 ± 45 g. Total length-at-age and weight-at-age for each sampled site are shown in Table 1. The majority of individuals were in the lenght class of 231-260 mm. Age ranged from 1+ to 5+ years, with a predominance of ages 2+ to 3+, which represent individuals that have not yet reached sexual maturity (Table 2). Female bream attain maturity in their sixth year of life, whereas males mature a year later (Cowx, 1983). The predominance of sexually immature individuals in the commercial catch can impede spawning of this commercially important species and disturb its population dynamics, which will further result in a decline of the catch itself.

The values of the length-weight regression parameters for the site 1 were: a = 0.017, b = 2.85, r^2 = 0.86, and for the site 2: a = 0.004, b = 3.26, r^2 = 0.90. Growth in a fish stocks is isometric when b = 3, and it depends on species, sex, age, seasons, and feeding (Le Cren, 1951; Bagenal and Tesch, 1978). Individuals in the sample from site 1 have a negative allometric growth, while those from the site 2 have a positive allometric growth.

Table 1. Total length-at-age and weight-at-age of the common bream in the Danube near Belgrade.

		TL (mm)				W (g)			
Age	n	Mean	SD	Min	Max	Mean	SD	Min	Max
Site 1									
1+	3	229	13	220	244	123	8	115	130
2+	35	237	13	217	273	137	22	100	205
3+	31	241	12	223	270	149	25	115	225
4+	4	276	44	229	330	244	129	130	414
Site 2									
2+	9	238	11	229	264	144	24	125	205
3+	19	248	17	227	278	160	42	110	245
4+	6	280	5	273	289	244	51	205	340
5+	3	286	7	280	294	238	25	210	255

Table 2. Number of individuals of specific age in each length class.

SITE 1							
TL (mm)	0+	1+	2+	3+	4+	5+	Sum
201-230	0	2	10	10	1	0	23
231-260	0	1	24	20	1	0	46
261-290	0	0	1	1	1	0	3
291-320	0	0	0	0	0	0	0
321-350	0	0	0	0	1	0	1
Sum	0	3	35	31	4	0	73
%	0%	4%	48%	42%	5%	0%	100%
SITE 2							
TL (mm)	0+	1+	2+	3+	4+	5+	Sum
201-230	0	0	2	4	0	0	6
231-260	0	0	6	11	0	0	17
261-290	0	0	1	4	6	2	13
291-320	0	0	0	0	0	1	1
321-350	0	0	0	0	0	0	0
Sum	0	0	9	19	6	3	37
%	0%	0%	24%	51%	16%	8%	100%

The Fulton’s condition factor at site 1 ranged from 0.83 to 1.35, with the mean value (± SD) of 1.04 ± 0.08, and at site 2 it ranged from 0.90 to 1.41, with the mean value (± SD) of 1.05 ± 0.10 (Figure 1). The Fulton’s condition factor decreased with length at site 1 and increased at site 2. Allometric condition factor at site 1 ranged from 1.36 to 2.16, with the mean value (± SD) of 1.70 ± 0.13, and at site 2 it ranged from 0.37 to 0.58, with the mean value (± SD) of 0.45 ± 0.04 (Figure 2).

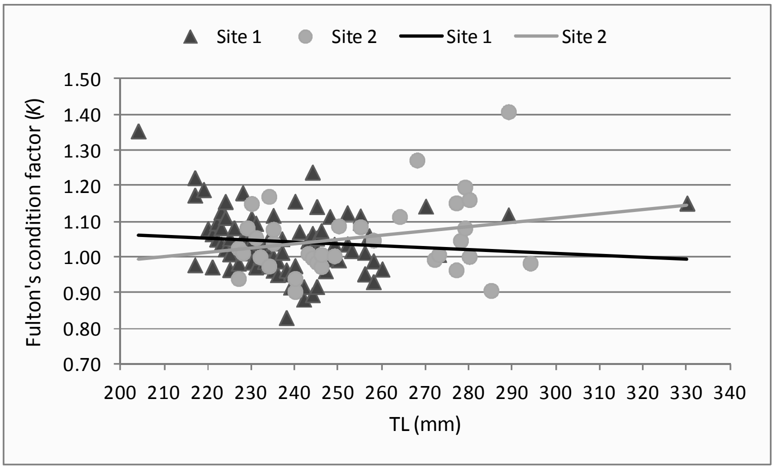

Figure 1. Simple linear regression between total length (TL) and Fulton’s condition factor (K) for the common bream in the Danube near Belgrade.

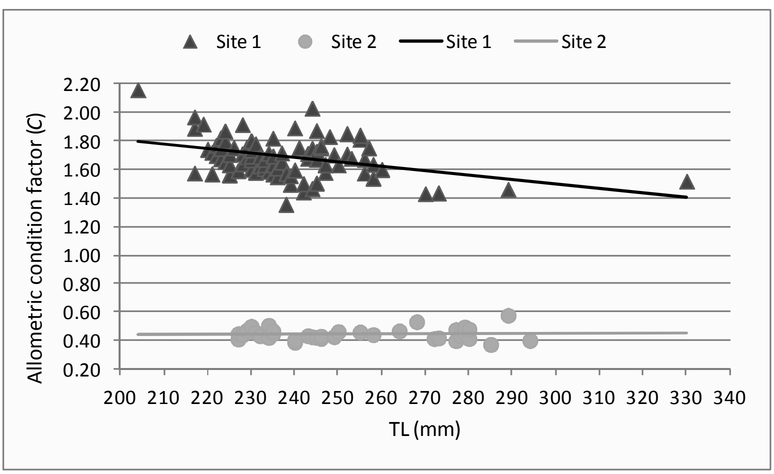

Figure 2. Simple linear regression between total length (TL) and allometric condition factor (C) for the common bream in the Danube near Belgrade.

According to Treer et al. (2003), the common bream from the Danube in Croatia (near the Drava rivermouth) have an isometric growth (b = 3.01) and very good and stable condition factor throughout its life (*K* = 2.44). Marčeta (2015) observed that the sampled individuals of the common bream in Slovenia had a positive allometric growth (*b* = 3.14). Individuals sampled from the Marmara region in Turkey also had a positive allometric growth (*b* = 3.25) (Tarkan et al. 2006), the same as individuals from Poland (*b* = 3.26), which also had a good condition (1.97< *K* < 3.15, mean 2.44) (Kakareko, 2001). However, Kleanthidis et al. (1999) observed a negative allometric growth of the common bream in Lake Volvi in Greece (*b* = 2.97).

The results of this study show that the growth in length and weight of the common bream from the Danube near Belgrade is somewhat slower compared to other countries in the region, but it is still near the average for the species. The lower values of the Fulton's condition factor than in other studies might be due to the fact that the sampling was performed during winter, when the food is scarce and feeding activity reduced. This species has a commercial value and, as such, its populations are under high fishing pressure. In Serbia, it is protected from April 15 to May 31, during the spawning period. The results of this study may provide insight into growth and population dynamics of the common bream in this region.

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THE DEVELOPMENT OF AN INNOVATIVE TECHNOLOGY FOR CARNIVOROUS FISH PRODUCTION THAT FITS WELL IN THE TRADITIONAL PRODUCTION ENVIRONMENT

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RAZVOJ INOVATIVNE TEHNOLOGIJE ZA PROIZVODNJU KARNIVORNIH VRSTA RIBA U USLOVIMA TRADICIONALNE PROIZVODNJE

Apstrakt

Osnovni cilj ovog istraživanja bilo je povećanje udela značajnih grablljlivih vrsta riba kao što su štuka (*Esox lucius*), som (*Silurus glanis*) i smuđ (*Sander lucioperca*) u tradicionalnoj proizvodnji ribe u polikulturi. Eksperimenti su obuhvatali razvoj akvakulture i povećanje efikasnosti tehnologije veštačkog mresta u ribnjacima.

Saradnja dva preduzeća sa Szent István Univerzitetom je bila fokusirna na eksperimente vezane za ribnjačku akvakulturu, jedno preduzeće je Tógazda Plc koje poseduje ribnjački sistem nastao pregrađivanjem rečnog toka, a drugo Szegedfish Ltd. sa ribnjačkim sistemom okruženim nasipima i sa mrestilištem. Ishrana prirodnom hranom je bila dopunjena kompletnom hranom koja je ili bila direktno iskorišćena od strane grabljivih vrsta ili usvajana kroz ekonomski značajne vrste (kao što su *Abramis brama*, *Scardinius erythrophthalmus*, *Rutilus rutilus* etc.).

U okviru projekta jednogodišnje i dvogodišnje grabljivice su gajene u polikulturi na jezeru Tógazda kompanije. Eksperimenti su sprovedeni na 20 ribnjačkih jezera sa sedam različitih ribnjačkih sistema vodene površine od 937 ha. Projekat je bio fokusiran na unapređenje prinosa predatroskih vrsta riba primenom kompletne hrane za ribe.

Na osnovu rezultata nakon prve godine projekta, primena kompetne hrane za ribe je rezultirala većim prinosom jednogodišnjeg soma uz prosečan prinos od 109 kg/ha, a u slučaju dvogodišnje smuđa i soma prinos je bio 142 kg/ha i 124 kg/ha. Tokom druge godine projekta planirani su odabir i ponavljanje eksperimenata sa najboljim rezultatima iz prethodne godine u cilju standardizacije sistema gajenja i ishrane.

Infrastruktura i oprema su bili renovirani pre rada u mrestilištu kako bi se unapredila efikasnost razmnožavanja obzirom da većina infrastrukture potiče iz 60-ih godina prošlog

veka. Eksperimenti u mrestlištu su bili fokusirani na povećanje efikasnosti reprodukcije štuke kroz povećanje kvliteta jaja i razvoj metode krioprezervacije sperme. Krajnji cilj je bilo unapređenje efikasnosti mresta soma kroz primenu krioprezervacije sperme u industrijskim razmerama i testiranje upotrebe krioprezervirane sperme u mrestilištu.

Iako ovulacija štuke može biti indukovana injekcijama rastvora hipofize riba, nepotpuna ovulacija i nizak stepen oplodnje predstavljaju ograničavajuće faktore za masovnu produkciju mlađi štuke. Proces hipofizacije je modifikovan primenom preparata sa produženim delovanjem, hidrofilnim biorazgradivim matiksom Na - karbksimetilceluloze (CMC-Na) i Carbopol smole. Primena hipofize šarana u 8.0% rastvoru CMC-Na ili u 2.0% vodenoj disperziji Carbopol-a je rezultirala višim prosečnim indeksom fekunditeta i povećanim stepenom oplodnje u poređenju sa tretmanom hipofizom.

U prvoj godini ovog dvogodišnjeg projekta testirani su različiti imobilizaconi rastvori, mogućnosti skladištenja sveže sperme u period od 6 sati, kvalitet sveže uzorkovane sperme i sperme uzorkovane iz izolovanog testis, efekat dva različita rastvora za kriooprezervaciju na bazi glukoze, kao i kvalitet sperme hormonski tretiranih i netretiranih riba, kao i kvalitet sperme nakon skladištenja od 48 sati etc.

Eksperimenti o krioprezervaciji sperme soma u prvoj godini projekta su bili fokusirani na testiranje kvaliteta sperme tokom skladištenja u trajanju od 96 časova, poređenje različitih načina i brzine zamrzavanja (kompjuterski kontrolisano zamrzavanje ili tradicionalno zamrzavanje u stiropornoj kutiji).

Planirani rezultati ovog projekta su razvijanje tehnologije proizvodnje za ribnjačku akvakulturu, a na kraju projekta i objavljivanje i predstavjanje tehnologije razvijene za masovni komercijalni mrest smuđa i soma uključujući i tehnologiju krioprezervacije sperme kao modela za praktičnu primenu.

Ključne reči: *grabljivice, ribnjak, mrest, krioprezervacija sperme*

Abstract

The basic aim of this work is increasing the ratio of significant carnivorous species, such as pike (*Esox lucius*), catfish (*Silurus glanis*) and pikperch (*Sander lucioperca*) in traditional polyculture fish production. The experiments include both the development of pond aquaculture and increasing the effectiveness of induced breeding technology in hatcheries.

The cooperation of two enterprises and Szent István Universities focus on the experiments for pond aquaculture, one is Tógazda Plc with barrage pond system and at Szegedfish Ltd. with paddy pond system and hatchery building. The natural feed is complemented by full-fledget fish feed that is either utilised directly in the carnivorous fish, or through economically important bream species (such as *Abramis brama*, *Scardinius erythrophthalmus*, *Rutilus rutilus* etc.)

Within a framework of the research project one and two years old carnivore fish species are bred in polyculture on the lakes of Tógazda company. Experiments are running on 20 ponds of 7 different pond systems, covering 937 ha of water surface. The project focuses on the improvement of yield of predator fish species by providing complete fish feed.

Based on the results of the first year, complete fish feed gave higher yields in the case of one-year old wels catfish with an average yield of 109 kg/ha and in the case of two-year old pike-perch and wels catfish, resulting in 142 kg/ha and 124 kg/ha yields, respectively. During

the second year of the project selection and repetition of the best-resulting experiments are planned in order to standardize the stocking and feeding system.

All facilities and equipment had been renovated before the hatchery work in order to improve the efficiency of the reproduction as the infrastructure was built in the 1960s. The hatchery experiments focus on increasing the effectiveness of pike reproduction through increasing egg quality and the development of the methodology of the cryopreservation of testicular sperm. We aim to improve the efficiency of hatchery wels catfish breeding through the application of sperm cryopreservation on industrial scale and the testing of cryopreserved sperm in hatchery environment.

Although ovulation in pike can be induced by injections of dried fish pituitary in saline solutions, incomplete ovulation and low fertilization rates limit large-scale production of pike fry. The pituitary treatment was modified by using sustained release vehicles, a hydrophilic biodegradable matrix of carboxymethylcellulose sodium (CMC-Na) and a Carbopol resin. Administration of carp pituitary in an 8.0% solution of CMC-Na or in a 2.0% aqueous dispersion of Carbopol resin resulted in a higher mean fecundity index and improved fertilization rate compared to pituitary injected in saline.

In the first year of this two-year project we have tested possible immobilisation solutions and the storability of native sperm in a 6-hour period, the quality differences of stripped sperm and sperm from removed testicles, the effect of two different glucose-based diluents on cryopreservation, sperm quality of hormonally treated and non-treated fish, the quality of sperm stored for 48 hours etc.

Our research for the cryopreservation of wels catfish sperm focused on testing the quality of sperm during a 96-hour storage period, the comparison of cooling rates and methods (controlled-rate freezer or traditional polystyrene box), and fertilization tests in the first year of the project.

Planned results are the development of pond aquaculture technology and at the end of the project a technology description for factory-sized hatchery breeding technology for pikeperch and catfish including sperm cryopreservation technology description and a model for practical application.

Keywords: *carnivorous fish, pond culture, hatchery practice, sperm cryopreservation*

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THE IMPACT OF NANOPARTICLES ON FERTILIZATION PROSESS OF RAINBOW TROUT (*ONCORHYNCHUS MYKISS* WALBAUM) AND EMBRYONIC DEVELOPMENT STAGES

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UTICAJ NANOČESTICA NA PROCES OPLOĐENJA I STADIJUME EMBRIONALNOG RAZVIĆA KALIFORNIJSKE PASTRMKE (*ONCORHYNCHUS MYKISS* WALBAUM)

Apstrakt

U naučnoj literaturi nema dovoljno informacija o brzom razvoju nanotehnologije i njenoj primeni u različitim oblastima, kao i o efektima koje imaju nanočestice na određene faze embrionalnog razvića riba, a koji su posebno osjetljiviji na spoljašnje sredinske faktore. Stoga je osnovna ideja ovog istraživanja ustanoviti efekte koje izazivaju nanočestice Fe3O4 (20-30nm) i Al (18nm) na polne ćelije (spermatozoide i jajne ćelije) kalifornijske pastrmke (*Oncorhynchus mykiss*, Walbaum 1792) pre sazrevanja polnih ćelija u akvakulturi, efekte koje izaziva kod razvojnih faza embriona nakon sazrevanja, kao i apsorpcija nanočestica kroz hranu i ulazak u lanac ishrane i razmatranje moguće bioakumulacije.

Material je prikupljen tokom perioda od januara do marta tokom 2017. i 2018. godine na salmonidnom (pastrmskom) ribnjaku Gabala u Azerbejdžanu. Nanočestice (Skyspring Nanomaterials Inc, USA, Houston TX) su dodavane u polne ćelije (spermatozoide i jajne ćelije) i ikru u različitim količinama (0,0001 g, 0.001 g, 0.005 g i 0.05 g), kao i u sazrelu ikru pre oplodjenja. Korišćena je binokularna lupa «Karl Zeiss», Stemi 2000C i praćen je efekat koji nanočestice imaju na faze embrionalnog razvića. Svaki dan, u procesu inkubacije, su uklanjani uginuli embrioni u svakoj od faza i to je beleženo, a nakon toga su prebrojavani preostali živi embrioni.

Na osnovu rezultata, utvrđeno je da je dodavanjem nanočestica $Fe3O_4$ (20-30nm) i Al (18nm) u ikru kalifornijske pastrmke pre sazrevanja procenat oplođene ikre bio veći, odnosno ostajalo je više dobrih živih embriona. Nanočestice Fe3O_4 (20-30nm) su imale stimulišući efekat kada su dodate u količini od 0.05 g (2018), dok je kod dodavanja Al (18 nm) stimulišući efekat bio u količini od 0.005 g (2018). Možemo zaključiti da nanočestice imaju katalitički efekat na akrozome spermatozoida, da ubrzavaju aktivnost i na taj način dovode do povećavanja stope fertilizacije, odnosno do većeg oplodnje ikre.

Kalifornijska pastrmka preneta je u Azerbejdžan još 1981. Godine, njen reproduktivni fond je formiran i već se godinama uspešno eksploatiše. Međutim, usled takvih okolnosti prisutna je visoka stopa inbridinga. Visoka je stopa mortaliteta kod mlađi i u mnogim slučajevima kvalitet reproduktivnog materijala je loš, a njihovo potomstvo je osetljivo na različite sredinske faktore, o čemu svedoče statistički podaci iz određenih ribnjaka koji su dugi niz godina aktivni. Stoga se dobijeni rezultati mogu koristiti za smanjivanje gubitaka nastalih tokom embrionalnog razvoja u akvakulturi salmonidnih riba, naročito kod kalifornijske pastrmke, te se može uticati i na povećanje ukupne produktivnosti.

Ključne reči: *kalifornijska pastrmka, nanočestice, polne ćelije, fertilizacija, faze embrionalnog razvića.*

Abstract

There is insufficient information in the scientific literature on rapid development of nanotechnology and its impact on fish reproduction, particularly to embryonic development stages, which are more sensitive to external environmental factors in connection with its application to various fields. Therefore, the main purposes of the current research were to investigate: the effect of nanoparticles of Fe3O_4 (20-30nm) and Al (18nm) on sexual cells (sperm, egg cell) of rainbow trout (*Oncorhynchus mykiss* Walbaum 1792) before maturation in the aquaculture process; the effects on embryonic developmental stages after maturation; as well as absorption of nanoparticles by feedstuffs and entry into the food chain; possible bioaccumulation.

The material for the research was carried out in January-March of 2017-2018 at Gabala salmonid fish hatchery named after G.Yusifov of the Republic of Azerbaijan. Fe3O_4 (20-30nm) and Al (18nm) nanoparticles purchased from Skyspring Nanomaterials Inc, USA, Houston TX were used in experiments. These nanoparticles were added to the sexual cells of rainbow trout (sperm, egg cell) and roe in various amounts (0,0001 g, 0.001 g, 0.005 g and 0.05 g), as well as to the roe before fertilizing and their effect on embryonic development stages was studied using “Karl Zeiss”, Stemi 2000C binocular. Every day in the incubation process, dead embryos were removed and the free embryos are then counted.

According to the results of the research, when Fe_3O_4 (20-30nm) and Al (18nm) nanoparticles are added in roe of rainbow trout prior to maturation, the percentage of fertilization of roe was higher, and the release of free embryos was higher than in other variants. Fe_3O_4 (20-30nm) nanoparticles have a stimulating effect at amount of 0.05 g (2018) and Al (18 nm) nanoparticles at the amount of 0.005 g (2018). It can be assumed that these nanoparticles have a catalytic effect on spermatozoa acrosomes, accelerate energy activity, and eventually increase the activity of spermatozoids, which leads to an increase in the rate of fertilization of the roe.

Rainbow trout (*Oncorhynchus mykiss*) was brought to Azerbaijan in 1981, its reproductive fund has been formed and has been exploited for many years by Azerbaijan’s salmonids fish hatcheries. In such a situation, the high rate of relationships between individuals leads to the occurrence of inbreeding. That is why the percentage of deaths among small fries at the fish growing process is high, in many cases the quality of the producers' sexual products is low and the new generation emerging from them is intolerant to the various environmental effects. There are statistical data on this in connection with hatchery's activity for many years.

Thus, the obtained results can be used to reduce the losses occurring during the embryonic development stage in the aquaculture process of salmonids, especially in the rainbow trout, and to increase overall productivity.

Keywords: *Rainbow trout, nanoparticles, sexual cells, fertilization, embryonic stages.*

EUROPEAN GRAYLING D-LOOP MTDNA HAPLOTYPES DIVERSITY AND POSTGLACIAL COLONIZATION OF THE RUSSIAN PART OF AREAL

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DIVERZITET EVROPSKOG LIPLJENA NA OSNOVU HAPLOTIPOVA D-PETLJE MTDNK I POSTGLACIJALNA KOLONIZACIJA RUSKOG DELA AREALA

Apstrakt

Evropski lipljen (*Thymallus thymallus* L.) je važan element zajednice riba u Severnom ledenom okeanu. Biološke karakteristike ove vrste: slatkovodna, stenohalina, ne migrira - omogućavaju da se odrede mogući pravci kolonizacije nakon prestanka glacijalnog perioda. Postojeća filogeografska slika mtDNK haplotipova baziranih na D-petlji najverovatnije reflektuje pravce kolonizacije iz postojećih refugijuma. Fragment mtDNK koji je uključivao kompletnu D-petlju je analiziran u studiji. Analizirane su ukupno 224 ribe sa 23 lokacije, od kojih su 198 bile evropski lipljeni, a 26 arktički lipljeni (*T. arcticus*). ukupno je opisano 32 haplotipa, od kojih 29 po prvi put. Haplotipovi evropskog lipljena pripadaju “Skandinavskoj” filogenetskoj grupi i formiraju dva haplotipa. Ovo može da ukaže na postojanje dva refugijuma tokom poslednjeg glacijalnog perioda, od kojih je kolonizacija evropskih lipljena severoistočnog dela oformila postojeći areal. Iz jednog od refugijuma, verovatno “zapadnog” su kolonizovani Fenoskandija, poluostrvo Kola i brojne pritoke Severne Dvine. Sledeći refugijum može biti “istočni”, koji je u poslednjoj glacijaciji postojao u regionu proglacijalnog jezera Pečora, i odakle je krenulo verovatno širenje lipljena na istok i severoistok. U uzorcima riba iz reke Umba i jezera Sejdozero na poluostrvu Kola, od svih 47 analiziranih evropskih lipljena, nađeni su haplotipovi D-petlje arktičkog lipljena. Otkriveni haplotipovi arktičkog lipljena u uzorcima evropskog lipljena sa poluostrva Kola mogu značiti postojanje paleoareala arktičkog lipljena na teritoriji evropskog dela Severnog

ledenog okeana, kao i postglacijalnu introgresiju evropskog lipljena na teritoriju koja je ranije bila naseljena arktičkim lipljenom.

Ključne reči: *Thymallus thymallus, Thymallus arcticus, haplotipovi D-petlje mtDNK, postglacijalna kolonizacija, introgresivna hibridizacija*
Keywords: *Thymallus thymallus, Thymallus arcticus, D-loop, mtDNA haplotypes, postglacial colonization, introgressive hybridization*

INTRODUCTION

European grayling (*T. thymallus* L.) is an important part of the Arctic Ocean basins aquatic communities. In recent years the scientific interest in this species is rather high, but in the Russian part of the areal most of the populations remain still unexplored, the data are fragmentary or relate only to some rivers (Koskinen et al., 2000; Marić et al., 2014; Weiss et al., 2002). The advantage for genetic studies of grayling in Russian European North is the natural environment of populations: in relatively sparsely populated regions, with low anthropogenic influence, and unaffected by translocations. In addition, the features of this species – freshwater, stenohaline, not making long migrations – make the grayling a good object for phylogeographical studies and allow to reconstruct ways of colonization from existed refugia after glacial retreat.

Accordingly, the objective of our study was to clarify the phylogenetic position of the European grayling on the territory of Russian European North. To define the ways of forming the phylogeographic picture, we chose the D-loop as a marker. In addition, the objective was to clarify the relatedness of the Arctic grayling haplotype revealed by Koskinen et al., 2000 in the sample of European grayling from the Syamzhenga River (the Northern Dvina basin). In this connection, samples of Arctic grayling from European/Arctic grayling border zone – waterbodies of the Ob' River basin from the Subpolar Urals – were analyzed.

MATERIALS AND METHODS

A total of 224 fish from 23 locations (Fig. 1) were analyzed, 198 of them were European graylings and 26 were Arctic graylings. The DNA extraction and D-loop amplification were performed by standard methods. To confirm species identification, nuclear markers were used: *tumor necrosis factor α* (exon 2-3) *TNFα*, *follicle-stimulating hormone β* (*FSH β*). Multiple alignment of sequences and phylogenetic analyzes were performed in the Geneious® 6.0.5 program (Biomatters Ltd.). Deposited in the GenBank® (NCBI) D-loop mtDNA nucleotide sequences were used for dendrograms construction.

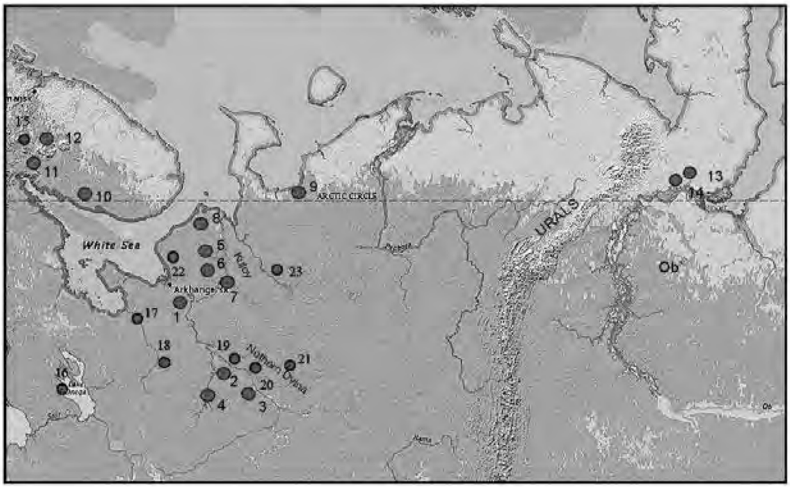

Figure 1. Samples location map. The Northern Dvina tributaries: 1 – Oboksha, 2 – Lemen’ga, 3 – Kestvazh, 4 – Ustya, 19 – Nizhnjaya Toima, 20 – Iorga, 21 – Vyya; the Kuloy tributaries: 5 – Soyana, 6 – Laka, 7 – Kelda; 8 – Megra; 9 – Oma; 10 – Varzuga, 11 – Umba, 12 – Seydozero Lake 13 – Bol’shoe Schuchye Lake, 14 – Bol’shaya Hodata Lake, 15 – Pecha, 16 – Onega Lake, 17 – Onega River (mouth), 18 – Shondoma, 22 – Zolotica, 23 – Irasa

RESULTS AND DISCUSSION

1046 bp mtDNA fragment including complete D-loop sequence, was analyzed. 32 haplotypes were identified, 29 haplotypes were described for the first time, taking into account indels 4 more haplotypes. The haplotype, designated by us as “Main”, was found in most samples of European grayling, with the exception of samples from the rivers of the Kola Peninsula and the Onega Lake. Additionally, it was found in more than half of the studied fishes. 6 haplotypes were identified in the Kuloy, 3 of which were variants of the “Main” with single nucleotide substitutions (Fig. 3), and 2 other haplotypes with a large number of substitutions, one of which corresponds to the haplotype AF522430 (At6) (Fig. 3) founded in sampling from the Baltic Sea basin (Weiss et al., 2002). In the N. Dvina, 5 haplotypes were identified. The haplotype ND_Kestvazh_98 belongs to the group of the mass haplotype “Main” with 1 substitution, and the haplotype ND_Obok_71 differs from the “Main” by 5 substitutions and is similar to the haplotype AF522426 (At2) revealed in the Tana in Norway (Weiss et al., 2002) (Fig.3). 2 haplotypes of Arctic grayling were identified in the sample from the Vyya, eastern tributary of the N. Dvina, one of them corresponded to the previously revealed AY779014 (Sja05) (Weiss et al., 2002). In other rivers of the White Sea coast, most fishes had “Main” or its variants differing by 1-2 substitutions. In the Varzuga, only 2 haplotype Var_152,159 were found, characteristic only for fish from this river, differing from “Main” by 7 substitutions and belonging to the same group as ND_Obok_71 from the N. Dvina (Fig. 3). In the Pecha in the Kola Peninsula, all grayling haplotypes were ND_Obok_71 (AF522430).

In 26 Arctic graylings from the lakes of the Ob' basin, there were revealed 5 new haplotypes. Haplotypes of Arctic grayling form 2 haplogroups, differing by 5-6 substitutions. 4 haplotypes from the lakes form an independent clade, whereas the haplotype of the European grayling from the Syamzhenga (NDvina_1) clustered with 1 of the haplotypes of the Arctic grayling from the Subpolar Urals (Ob'_29), differing only in 2 substitutions. In the Umba and Lake Seydozero, all analyzed haplotypes belonged to the Arctic grayling (Fig. 2). A total of 10 haplotypes and 13 with indels were detected. All fishes from these samples (47 in total) were examined, in none of them the haplotypes of European grayling were revealed. To confirm their identification as European grayling, these fishes were analyzed using nuclear markers *TNF α* and *FSH β*. All studied fishes were European graylings.

The haplotypes belong to the "Scandinavian" phylogenetic line and form 3 clusters (2 with strong support), presumably reflecting the refugia from which European grayling postglacial migration occurred. Geographical distribution of haplotypes allows to suppose the existence of a western refugium, from where European grayling could spread over Fennoscandia, the Kola Peninsula and to some tributaries of the N. Dvina. Another supposed refugium, the eastern one, could have been the Pechora Proglacial Lake, from where the European grayling could spread to the eastern and northeastern rivers. This is supported by the fact that "Main" was spread with a high frequency in samples from waterbodies situated to the east of the N. Dvina. "Main" is in a central position in the group of haplotypes differing from it by 1-2 substitutions, and the star-like cluster topology indicates a relatively recent population reduction and rapid expansion of graylings in this territory.

Thus, we can divide European grayling of the European North into 2 lineages – the western and the eastern branches. The haplotypes of the European grayling from the Vyya, the Umba and Lake Seydozero clustered with haplotypes of Arctic grayling from the Ob' basin, differing by 2-3 substitutions from one of the identified haplotypes from the Ob' basin. It indicates existence of the Arctic grayling paleoareal on the territory of the European Arctic basin. As a result of climatic changes, the fragmentation of areal took place, Arctic grayling could survive only in certain refugia, and because of lack of spawners of its species hybridization with European grayling occurred. The diversity of Arctic grayling haplotypes (9 plus 3 with indels) in European grayling samples, and the presence of Arctic grayling haplotypes in fishes from 2 waterbodies on the Kola Peninsula suggest the existence of a refugium in the Khibiny Mountains during glacial periods. At the same time, it is surprising that in all studied graylings from the Umba and Lake Seydozero no haplotypes of European grayling were revealed, although they belonged to European grayling by nuclear markers.

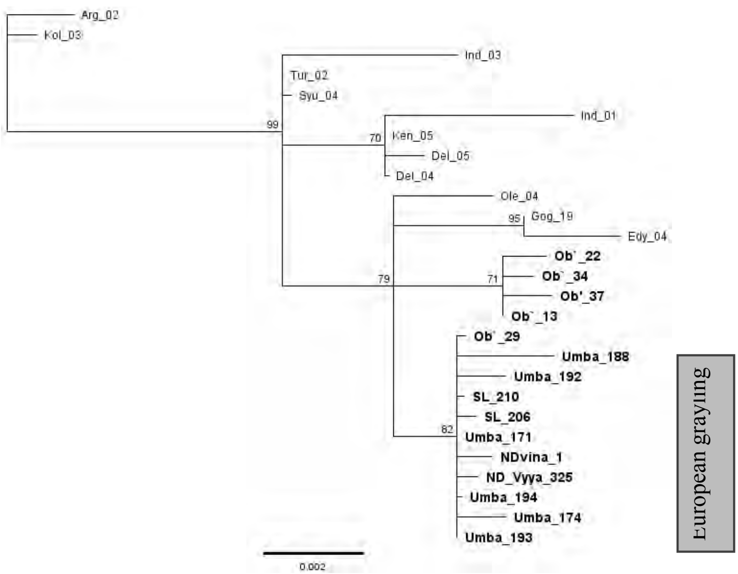

Figure 2. Neighbour-joining consensus tree (HKY model) of D-loop haplotypes of Arctic grayling. The bootstrap values are indicated in the tree nodes. Haplotypes obtained in our work are in bold.

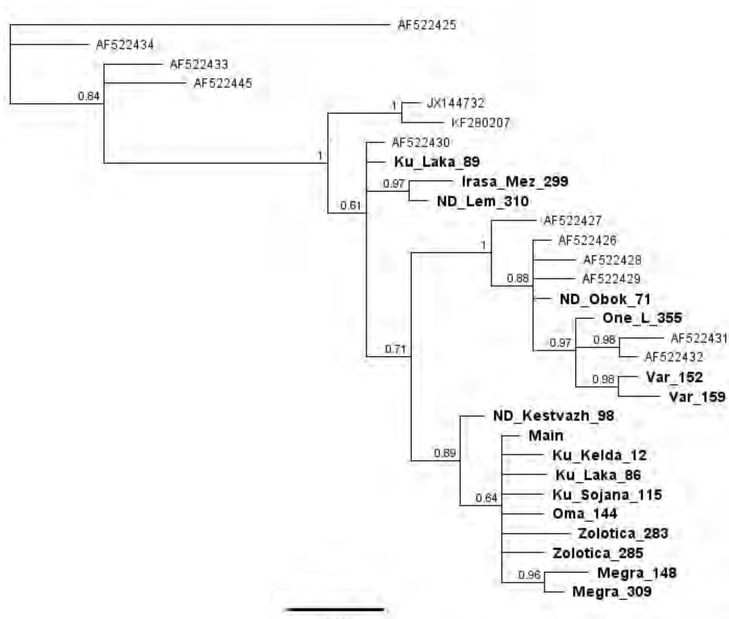

Figure 3. Tree of D-loop haplotypes (HKY85) of European graylings constructed in MrBayes 3.2.6. (Huelsenbeck, Ronquist, 2001). The posterior probabilities are in tree nodes. Haplotypes obtained in our work are in bold.

CONCLUSIONS

Thus, our findings supplement the picture of distribution and diversity of European grayling and Arctic grayling haplotypes. Nuclear markers (partial sequence of *TNFα* and *FSH β* genes) were developed and applied to confirm the species of grayling.

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ASSESSMENT OF FISH SPECIES DIVERSITY AND WATER QUALITY IN FIVE RESERVOIRS IN SERBIA BASED ON THE SHANNON'S DIVERSITY INDEX

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PROCENA DIVERZITETA VRSTA RIBA I KVALITETA VODE U PET AKUMULACIJA U SRBIJI ZASNOVANA NA ŠENONOVOM INDEKSU DIVERZITETA

Apstrakt

Značaj riba kao bioindikatora kvaliteta vode potvrđen je brojnim istraživanjima, a zasniva se na njihovoj dugovečnosti, mobilnosti, pripadnosti različitim funkcionalnim trofičkim grupama i osetljivosti na širok spektar antropogenih stresora. Cilj ovog istraživanja bilo je poređenje diverziteta vrsta riba u akumulacijama Garaši, Perućac, Zaovine, Ovčar i Međuvršje na osnovu Šenonovog indeksa diverziteta (H), kao i procena kvaliteta vode na osnovu poređenja vrednosti ovog indeksa sa rezultatima merenja fizičkih i hemijskih parametara vode. Uzorkovanje riba je vršeno mrežama (20-50 m dužine, 1.5-2 m visine i 28-100 mm promera okca) tokom leta 2017. godine. Ukupno je uzorkovano 795 jedinki riba koje pripadaju 21 vrsti. Multiparametarska sonda YSI 6600 V2-2 korišćena je za prikupljanje podataka o fizičkim, hemijskim i biološkim karakteristikama ispitivanih akumulacija. Praćene su vrednosti pH, rastvorenog kiseonika (DO) i hlorofila *a*. Prema vrednostima Šenonovog indeksa diverziteta za pomenute akumulacije, diverzitet naselja riba pokazao je sledeći trend: Perućac (H = 1.81) > Ovčar (H = 1.69) > Garaši (H = 1.52) > Zaovine (H = 1.45) > Međuvršje (H = 1.27). Akumulacije Garaši, Perućac i Ovčar imale su kvalitet vode koji spada u II-III klasu, dok je lošiji kvalitet (III-IV klasa) zabeležen za akumulacije Zaovine i Međuvršje. Procena kvaliteta vode na osnovu Šenonovog indeksa diverziteta poklapa se sa procenom kvaliteta vode baziranom na merenju pH vrednosti, rastvorenog kiseonika (DO) i hlorofila *a*, osim za akumulaciju Zaovine. Rezultati istraživanja sugerišu da kombinovanje Šenonovog indeksa diverziteta i merenja fizičkih i hemijskih parametara može pružiti pouzdanu procenu kvaliteta vode u istraživanim akumulacijama.

Ključne reči: *diverzitet vrsta, riba, kvalitet vode, akumulacije, Šenonov indeks diverziteta*
Keywords: *species diversity, fish, water quality, reservoirs, Shannon's diversity index*

INTRODUCTION

Species diversity is related to the function of ecological systems and it helps to understand the effects and mechanisms of environmental disturbances (Connel, 1978; Wootton, 1998). Rise of concern regarding the water quality and water availability have lead to a development of effective ecological tools capable of detecting the extent of anthropogenic impact. Necessity to monitor water quality arise from the obligation of each European country to protect and restore aquatic ecosystems and preserve environmental conditions (EC, 2000). According to the European Water Framework Directive, fish fauna is considered one of the key biological quality elements for the classification of ecological status of surface waters. The significance of fish as biological indicators of water quality has already been well documented, and it relies on their longevity, mobility, diversity of functional guilds, and sensitivity to a wide spectrum of anthropogenic stressors, including physical habitat degradation, organic enrichment and acidification (Karr, 1981; Wootton, 1992). The aim of this study was to compare fish diversity and water quality in five reservoirs in Serbia using the Shannon's diversity index (*H*) (Shannon and Weaver, 1949).

MATERIAL AND METHODS

A total of 795 individuals, belonging to 21 fish species, were caught during the summer of 2017 in the following reservoirs in western Serbia: Garaši, Perućac, Zaovine, Ovčar, and Međuvršje (Fig. 1). Sampling was performed by gill nets (20-50 m length, 1,5-2 m height, and 28-100 mm mesh size).

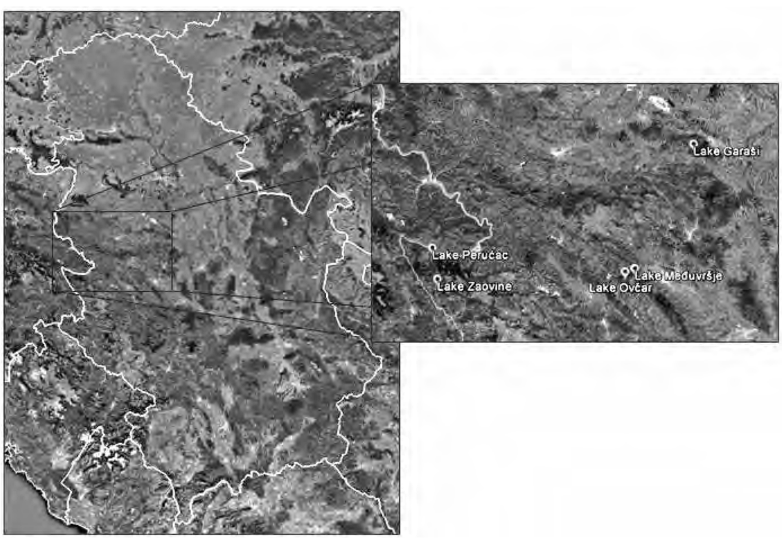

Figure 1. Map of the sampling sites.

Multiparameter water quality probe YSI 6600 V2-2 was used for physicochemical and biological water analysis. Measured parameters included pH value, dissolved oxygen (DO), and chlorophyll *a*.

$$H = -\sum_{i=1}^{S_t} p_i \ln p_i \qquad H_{\max} = \ln S \qquad p_i = \frac{n_i}{N}$$

The Shannon’s diversity index (*H*) was used for fish species diversity analysis:

where *pi* is the proportion of individuals of a particular species (*ni*) divided by the total number of individuals of all species (N), and *S* is the total number of species.

The classification of physicochemical, and biological parameters and associated water quality class (WQC) in Serbia is shown in Table 1 (Official Gazette of RS, 74/2011).

Table 1. Physicochemical and biological parameters and associated water quality class (Official Gazette of RS, 74/2011).

Parameter	Unit	Water quality class (WQC)		
		II-III	III-IV	IV-V
PHYSICOCHEMICAL PARAMETERS[1]				
pH value		6.5–8.5	6.5–8.5	< 6.5–8.5 <
Dissolved oxygen (DO)	mg l^{-1}	7	5	4
BIOLOGICAL PARAMETERS				
Chlorophyll a	μg λ^{-1}	50	100	250
Shannon’s diversity index (H)		1.5	1.2	0.5

[1]The value of physicochemical parameters is determined as the average value at three points along the water column: at 0.5 m from the surface, at central depth of the thermocline, and at 10% depth from the bottom.

RESULTS AND DISCUSSION

The list of fish species and the number of individuals sampled in each reservoir are presented in Table 2.

According to measured pH values, all reservoirs were between II-III and III-IV WQC (Table 3). Based on DO, Garaši, Zaovine, and Ovčar pertain to II-III WQC, while Perućac and Međuvršje pertain to III-IV WQC. No difference in WQC was observed as relates the chlorophyll *a* (II-III WQC).

Table 2. The list of fish species sampled in each reservoir in western Serbia.

Species	Garaši	Perućac	Zaovine	Ovčar	Međuvršje
Oncorhynchus mykiss			+		
Esox lucius					+
Abramis brama	+				+
Alburnus alburnus			+	+	
Barbus barbus		+	+	+	+
B. meridionalis			+	+	
Carassius auratus					+
Chondrostoma nasus		+	+	+	+
Cyprinus carpio	+				+
Gobio gobio				+	
Leuciscus cephalus	+	+	+	+	+
Rhodeus sericeus				+	
Rutilus pigus		+			
Rutilus rutilus	+	+	+	+	+
Gymnocephalus cernuus					+
Perca fluviatilis	+	+	+		+
Sander lucioperca	+				
S. volgensis		+			
Lepomis gibbosus		+			
Silurus glanis		+	+		+
Ictalurus nebulosus					+
Number of individuals	71	163	175	167	219

Table 3. Water quality class (WQC) of the studied reservoirs based on values of pH, dissolved oxygen (DO), and chlorophyll a (Chl).

	pH		DO		Chl	
	Values	WQC	Values (mg/l)	WQC	Values (µg/l)	WQC
Garaši	7.86	II-III / III-IV	8.10	II-III	4.93	II-III
Perućac	6.77	II-III / III-IV	7.12	II-III	2.30	II-III
Zaovine	7.29	II-III / III-IV	7.50	II-III	2.31	II-III
Ovčar	7.46	II-III / III-IV	7.08	II-III	3.06	II-III
Međuvršje	7.74	II-III / III-IV	5.45	III-IV	3.20	II-III

As indicated by the values of the Shannon’s diversity index (*H*), the diversity of the fish assemblages in the reservoirs had the following trend: Perućac > Ovčar > Garaši > Zaovine > Međuvršje (Fig. 2). Based on this values three reservoirs – Perućac, Ovčar, and Garaši pertain to II-III WQC, whereas Zaovine and Međuvršje pertain to III-IV WQC.

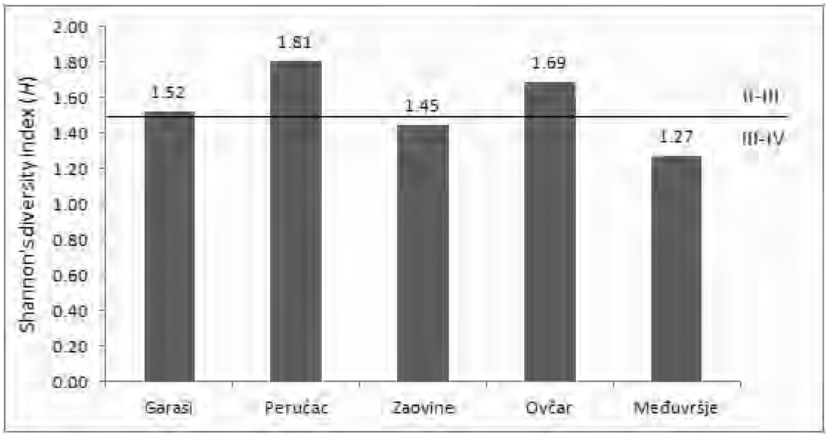

Figure 2. Values of the Shannon’s diversity index (H) for studied reservoirs. Horizontal line represents the boundary line between II-III and III-IV water quality class.

There was no difference in WQC in relation to the values of the Shannon’s diversity index (*H*) and values of pH, DO, and chlorophyll *a* for Garaši, Perućac, and Ovčar. For the reservoir Međuvršje, the values of the Shannon’s diversity index matched pH and DO, but not chlorophyll *a*. Finally, in the reservoir Zaovine there was a mismatch between the values of the Shannon’s diversity index and values of pH, DO, and chlorophyll *a*.

CONCLUSIONS

The Shannon’s diversity index (*H*) accounts for both abundance and evenness of the species present. Based on its values, the highest diversity of fish species was observed in the reservoir Perućac, and the lowest in the reservoir Međuvršje. Furthermore, the same values indicated that the reservoirs Garaši, Perućac, and Ovčar pertained to II-III WQC, whereas Zaovine and Međuvršje pertained to III-IV WQC. Water quality assessment obtained from the Shannon’s diversity index coincide with the assessment based on values of pH, DO, and chlorophyll *a*, except for the reservoir Zaovine. The results suggest that the combination of the Shannon’s diversity index and physicochemical parameters could provide an overall and reliable assessment of water quality in the studied reservoirs.

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THE EFFECT OF GENDER ON RED BLOOD PICTURE VALUES IN *ABRAMIS BRAMA* L. FROM THE ARTIFICIAL MODRAC LAKE

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UTICAJ SPOLA NA VRIJEDNOSTI ERITROCITNE LOZE *ABRAMIS BRAMA* L. IZ HIDROAKUMULACIJE MODRAC

Apstrakt

Hematološki parametri su dobri pokazatelji kondicionog i zdravstvenog stanja riba. Zbog velikog uticaja spoljašnjih faktora, navedeni parametri riba se karakterišu širokim rasponom variranja, kao velikim individualnim varijacijama. Njihovom analizom moguće je utvrditi nivo uticajavanjskih faktora na fiziološki status riba. Tokom istraživanja prikupljeno je ukupno 200 jedinki deverike (*Abramis brama*, Linnaeus, 1758), iz akumulacije Modrac, od čega je 152 mužjaka i 48 ženki. Krv uzeta punkcijom srca korištena je za dalju analizu, bez dodavanja antikoagulansa. Svim jedinkama je određen ukupan broj eritrocita, vrijednost hematokrita, koncentracija hemoglobina. Na osnovu vrijednosti navedenih parametara računski su određene vrijednosti hematoloških indeksa. Rezultati dobijeni analizom istraživanih parametara komparirani su u odnosu na polnu pripadnost. Najveće prosječne vrijednosti broja eritrocita kod mužjaka (1,728 x 10^{12}/L) i kod ženki (1,732 x 10^{12}/L) utvrđene su tokom ljeta. U periodu ljeta jedinke deverike oba pola imale su prosječno najveći hematokrit, dok je najmanja srednja vrijednost ovo gparametra zabilježena tokom zime. Koncentracija hemoglobina najveću prosječnu vrijednost dostiže u ljeto, a najnižu u periodu proljeća, za jedinke oba pola. Kod ženki deverike konstatovane su najmanje i najveće prosječne vrijednosti MCV – 184,848 fl (zima) i 284,345 fl (proljeće). Najveću prosječnu vrijednost srednje zapremine eritrocita (MCH) mužjaci su imali u ljetnjoj sezoni (70,302 pg), dok su najmanju vrijednost ovog parametra imale ženke, u periodu proljeća (60,688 pg). Kod ženki deverike utvrđena je najmanja i najveća srednja vrijednost srednje koncentracije hemoglobina u eritrocitima (MCHC) (221,051 g/l u periodu proljeća i 347,289 g/l u periodu zime). Između mužjaka i ženki zabilježena je signifikantna razlika ($p< 0,05$) za

vrijednosti hematokrita i koncentraciju hemoglobina. U ispitivanom uzorku kod mužjaka deverike, registrovane su više prosječne vrijednost i za većinu analiziranih hematoloških parametara u odnosu na ženke.

Ključne riječi: *eritrociti, hematokrit, hemoglobin, hematološki parametri, deverika*
Keywords: *erythrocytes, hematocrit, hemoglobin, hematologicala parameters, common bream*

INTRODUCTION

The physiological status of fish body is directly related to inner and outer factors, biotic and abiotic influences which acting to the organism (Stosik et al., 2002). Fish blood cells (RBC, WBC counts) are good indicators of systemic response to external stimulus and any changes are therefore reflected in their morphology and distribution in the blood (Srivastava and Choudhary, 2010). A wide range of variation of hematological parameters and large individual variations due to the pronounced environmental impact are the characteristics of ectothermic vertebrates.Their homeostatic system and control mechanisms are much less specialized in relation to mammals, which is the reason why it is very difficult to maintain certain parameters in a narrow interval (Homątowska et al., 2002).Species, gender, age and season, fish condition, oxygen concentration, and pH values of water affect the number of erythrocytes in fish (Bogut et al., 2006). Individuals of *Abramis brama* live in tranquil rivers and lakes.They have a high and lateral flattened body, covered with tiny scales. The artificial Modraclake was built in 1964. The reservoir is recharged by the river Spreča and the river Turija with its tributaries as well as small tributaries.

MATERIALS AND METHODS

The research was carried out during 2011. For research purposes, a total of 200 samples of common bream individuals (*Abramis brama*, Linnaeus, 1758) were collected from the accumulation Modrac: 50 individuals in each of four seasons.In the total sample, the presence of 152 males and 48 females was determined. Samples of blood were taken by heart puncture, using a sterile 1.1 mm needles, from place that was previously disinfected. For further analysis, native blood was used without adding anticoagulant. The total count of erythrocytes was determined in 20 µl of blood and added to a test tube with 4 ml Natt Herrick’s solution.Content was homogenized and poured into Bürcker-Türck’s chamber for erythrocytecounting. Erythrocyteswere counted at magnification at the 40x. The hemoglobin concentration was measured by Drabkin’s method. The hematocrit values were determined by microhematocrit method, using capillarytubes (Hasković and Ivanc, 2007). The fish blood was centrifuged for 5 minutes at 15,000 rpm. The following hematological indexes were determined: mean corpuscular volume (MCV) which represents relation between hematocrit and count of erythrocytes, mean corpuscular hemoglobin (MCH) which represents relation between hemoglobin concentration and count of erythrocytes, mean corpuscular hemoglobin concentration (MCHC) which represents relation between hemoglobin concentration and hematocrit. The gender was determinedby dissection of sexually mature individuals and by reviewingtheir gonads. The determination of analyzed individuals was carried out using the key for fish determination – Kottelat and Freyhof, 2007.

RESULTS AND DISCUSSION

The mean values of analyzed hematological parameters were measured during the different seasonsof 2011, in relation to the gender, and they are presented in the table1. The highest mean count of erythrocytes was measured during the summer, in both gender.In individuals of bream in both gender, the highest average hematocrit was measured in the summer, 0.409% (♂♂) and 0.439% (♀♀). The lowest mean values of hematocrit was detected during the winter, in females 0.288 and males 0.328. The average values of hematocrit for males *Abramisbrama* are higher than for females in all seasons, except in the summer. The values of this parameter differed significantly according to sex in all fish species under study (*Salmo trutta m. fario, Thymallus thymallus, Chondrostoma nasus* and *Leuciscus cephalus*), with higher mean values in males (Lusková, 1997). The highest value of hemoglobin concentration (Hb) of females and males of bream were measured in the summer. It occurred due to an increase in water temperature of accumulation Modrac. This caused a decrease in oxygen concentration. The increase of hemoglobin concentration is adaptive reaction of organisms to those conditions. The lowest value of this parameter was found in the spring, for both gender.

In bream females, the lowest and highest average MCV values were observed, 184.848 fl (winter) and 284.345 fl (spring). The highest average value of MCH was measured in males in the summer (70.302 pg), while the lowest value of this parameter was measured in females in the spring (60.688 pg). The lowest and highest mean values of MCHC (221.051 g/l in spring and 347.289 g/l in winter) were determined in females.

Table 1. Average values of hematological parameters in the bream during different seasons in relation to the gender

Parameters	Winter (N = 33)	Spring (N = 40)	Summer (N = 45)	Autumn (N = 34)	Winter (N = 17)	Spring (N = 10)	Summer (N = 5)	Autumn (N = 16)
	Male				Female			
The count of erythrocytes (10^{12})	1.702	1.456	1.728	1.628	1.613	1.325	1.732	1.613
Hematocrit (l/l)	0.328	0.375	0.409	0.400	0.288	0.369	0.439	0.392
Hemoglobin (g/l)	108.132	93.110	118.951	104.983	98.139	79.855	110.144	100.595
MCV (fl)	200.815	263.838	241.624	248.191	184.848	284.345	252.864	246.676
MCH (pg)	65.894	65.311	70.302	65.047	63.479	60.688	63.988	63.404
MCHC (g/l)	335.299	250.678	295.022	267.306	347.289	221.051	257. 146	258.903

In the total sample, significant differences between males and females ($p<0.05$) in values of hematocrit and hemoglobin concentration were determined (Table 2). By comparing the count of erythrocytes of males and females *Abramis brama* from the artificial Modrac lake, it was determined that there was no statistically significant difference ($p = 0.321$). The values of count of erythrocytes in *Salmo trutta m. fario* and *Chondrostoma nasus*, did not differ in terms to sexes, while in *Thymallus thymallus* the values of count of erythrocytes differed in terms to sex (Lusková, 1997). There was no statistically significant difference in

MCH values between *Abramis brama* males and females($p = 0.128$). The values of MCH of *Chondrostoma nasus* did not differe to sex (Lusková, 1997).

Table 2. Test results of intergenderdifferences in the analyzed bream population

Test Statistics[a]

♂♂/♀♀	Erc	Hct	Hb	MCV	MCH	MCHC
Mann-Whitney U	3301	2946.5	2666	3386.5	3115.5	3379
Wilcoxon W	4477.000	4122.5	3842	4562.5	4291.5	4555
Z	-0.993	-2.007	-2.809	-0.748	-1.523	-0.769
Asymp. Sig. (2-tailed)	0.321	*0.045*	*0.005*	0.454	0.128	0.442

a. Grouping Variable: Gender

The results of correlation and statistical significance (p) for the investigated parameters of male bream in the total sample are presented in Table 3. In male individuals of bream from the artificial Modrac lake, a significant correlation between Hb concentration and Hct ($r = 0.5655$), as well as in females ($r = 0.5136$) was determined. Also, in *Leuciscus cephalus*, there was found a significant correlation between Hb and Hct (males, $r = 0.61$, females, $r = 0.62$) (Lusková, 1997).

Table 3. The coefficients of correlations (r) between analysed parameters inmales

Gender: Male/ Correlation	Hct	Hb	Erc	MCV	MCH	MCHC
Hct	1.0000	.5655**	.3975**	.5129**	.1267	-.4955**
Hb		1.0000	.6019**	-.0471	.3876**	.4116**
Erc			1.0000	-.5571**	-.4803**	.1785*
MCV				1.0000	.5840**	-.5935**
MCH					1.0000	.2715**
MCHC						1.0000

*Significant $p < 0.05$; ** Significant $p < 0.01$

The coefficient of correlation and statistical significance (p) of the investigated parameters of females are presented in Table 4. In bream males and females from Modrac accumulation, the correlation of MCV with Hct, count of erythrocytes, MCH and MCHC was determined (Tables 3, 4). In *Chondrostoma nasus* significant correlations of MCV with count of erythrocytes ($r = - 0.74$), Hct ($r = 0.36$) and MCH ($r = 0.74$) were found (Lusková, 1997).

Table 4. The coefficients of correlations (r) between analysed parameters in female

Gender: Female/ Correlation	Hct	Hb	Erc	MCV	MCH	MCHC
Hct	1.0000	.5136**	.2967*	.6698**	.1445	-.6613**
Hb		1.0000	.5498**	.0031	.4222**	.2589
Erc			1.0000	-.4689**	-.5048**	.0872
MCV				1.0000	.4682**	-.7075**
MCH					1.0000	.2352
MCHC						1.0000

*Significant $p < 0.05$; ** Significant $p < 0.01$

CONCLUSIONS

In the total sample,the males of *Abramis brama* have higher mean hematocrit, hemoglobin concentration, count of erythrocytes, MCH, MCHC, while females have a higher mean value of mean corpuscular volume. Males and females of bream significantly differ ($p<0.05$) in values of hematocrit and hemoglobin concentrations. Bream males show a higher average values of most haematological parameters in comparison to females.

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THE METHODICAL IMPROVEMENT AND LARGE-SCALE CRYOPRESERVATION OF NORTHERN PIKE (*ESOX LUCIUS*) SPERM

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UNAPREĐENJE METODE I MASOVNA KRIOPREZRVACIJA SPERME ŠTUKE (*ESOX LUCIUS*)

Apstrakt

Štuka (*Esox lucius*) je ekonomski značajna vrsta slatkovodn ribe sa značajnim potencijalnom za ribolov. Iako je veštački mrest štuke već razvijen, postoji prostor za unapređnjenje efikasnosti te metode. U periodu mresta, dobar kvalitet i velike zpremine sperme se mogu obezbediti disekcijom testisa (Lahnsteiner et al 1998). Krioprezervacija je pomoćna metoda u reproduktivnoj tehnici koja omogućava dugotrajno skladišteje sperme za dalje korišćenje u akvakulturi ili konzervaciji (Cabrita et al 2010). Cilj ovog istraživanja je bilo unpaređenje efikasnosti masovne krioprezervacije sperme štuke pomoću kompjuterski kontrolisanog zamrzavanja u kriotubicama zapremine 10 mL. Matične jedinke štuke (*N*=16) korišćene ovom prilikom su bile gajene u Szegedfish Ltd. Pre eksperimenata, jedinke su bile hormonski indukovane rastvorom isušene hipofize šarana (3.5 mg/telesne mase kg^{-1}). Nakon 48 časova, ribe su bile anestezirane pomoću 2-fenoksietanola rastvorenog 0.4 ml L^{-1} u sistemskoj vodi. Izolovani testisi (12±2 g) svih jedinki su bili proceđeni kroz mrežu promera 200 μm. Uzorci sperme su čuvani na 4 °C. Parametri pokretljivosti sveže i odmrznute sperme (progresivna pokretljivost – pMOT%, krivolinijska brzina – VCL μm/s i linearnost -STR %) su određivani pomoću CASA sistema (Computer Assisted Sperm Analisys, Sperm VisionTM v. 3.7.4., Minitube of America, Venture Court Verona, USA). Sperma je bila aktivirana aktivacionim rastvorom (100 mM NaCl, 10 mM Tris, pH: 8, Lahnsteiner i sar., 1998) uz dodatak 0.01g mL^{-1} BSA (albumin iz seruma govečeta). Sva merenja su izvršena u duplikatu. KRioprezervacija je sprovedena na dva različita načina prema eksperimentalnom dizajnu. Sperma je bila razblažena u ekstenderu koji se sastojao od 150 mM glukoze, 75 mM NaCl, 30 mM KCl, 1 mM $Na_2HPO_4x12\ H_2O$, 1 mM $MgCl_2x6H_2O$, 1 mM $CaCl_2x2H_2O$, 20 mM Tris, and 0.5% BSA (pH: 8) u odnosu 1:3 (Bernáth i sar., 2017).

Kao intracelularni krioprotektant korišćen je 10 % metanol. Za krioprezervaciju su bile korišćene cevčice zapremine 0.5mL i 10 mL kriotubice. Uzorci sperme su bili odmrzavani u vodenom kupailu na 40 °C.

Eksperiment 1: Poređenje dve metode krioprezervacije
Cevčice zapremine 0.5 mL napunjene su spermom sedam mužjaka. Uzorci su bili zamrzavani na stiropornom ramu na visini od 3 cm od površine tečnog azota u trajanju od 3 minuta (Horváth i sar., 2003) ili pomoću kompjuterski kontrolisanog zamrzavanja (CRF, IceCube 14s, IceCube Series v. 2.24, Sy-Lab, Neupurkersdorf, Austria) prema programu dizaniranom za grgeča (od 7.5°C do -160°C, stopa hlađenja: 56°C/min, Bernáth i sar., 2016).

Eksperiment 2: Primenljivost kriotbica zapremine 10 mL za krioprzervaciju sperme štuke
Kriotubice zapremine 10 mL napunjene su supermom devet mužjaka (ukupna zapremina: 8 mL) i zamrzavane pomoću CRF. Program hlađenja (od 4 °C do -160 °C, stepen lađenja: 15 °C/min) je bio meren tokom 7 minuta na 3 cm iznad nivoa tečnog azota pomoću termokapilare (Digi-Sense DualLogR®**, Eutech Instruments Pte Ltd., Singapore**) (Bokor i sar., 2010).

U prvom eksperimentu pMOT se značajno smanjio nakon odmrzavanja obe krioprezervrane grupe (CRF: 42±17%, Frame: 45±11%) u poređenju sa svežom kontrolom (69±4%). Nije bilo zabeleženo statistički značajnih razlika u tri merena parametra pokretljivosti između uzoraka zamrzavanih na stiropornom ramu i pomoću kontrolisanog zarzavanja. U drugom eksperimentu

vrednosti za pMOT (25±10%) i VCL (77±9µm/s) su značajno opale nakon topljenja 10 mL kriotubica u poređenju sa svežom kontrolom (pMOT: 68±10, VCL: 146±15µm/s), dok STR nije pokazao značajan pad. Naša metoda krioprezrevacije dizajnirana za spermu štuke je uspešno prilagođena za CRF metodu. Prema dosadašnjim informacijama, kriotubice zapremine 10 mL su prvi put testirane ovom prilikom za krioprezervaciju sperme štuke. Ove kriotubice su se pokazale korisnim za masovnu krioprezervaciju koja može biti podrška tokom veštačkog mresta štuke.

Ključne reči: *štuka, sperma, krioprezervacija, masovna proizvodnja, kriotubice*

Abstract

The northern pike (*Esox lucius*) is an economically important freshwater fish species with a notable angling potential. Its artificial propagation was already developed, however its efficiency and frugality can be improved. During the hatchery process, good quality and large volume of sperm can be obtained with the dissection of the testis (Lahnsteiner et al 1998). Cryopreservation is an assisted reproduction technique which allows the long-term storage of sperm for both aquaculture and conservation purpose as well (Cabrita et al 2010). The aim of our study was to improve the efficiency of large-scale cryopreservation of northern pike sperm using a controlled-rate freezer and 10 mL cryotube. A broodstock of pike males (*N*=16) was purchased from the Szegedfish Ltd. Prior to the experiments, spermiation was hormonally induced by carp pituitary(3.5 mg/body weight kg^{-1}). Following 48 hours, fish were anaesthetized using 2-phenoxyethanol in a dose of 0.4 ml L^{-1} hatchery water. Testis (12±2g) of all individual were dissected and squeezed using a 200µm net.

Samples were stored at 4 °C. Fresh and thawed sperm motility parameters (progressive motility-pMOT %, curvilinear velocity-VCL μm/s and straightness-STR %) were recorded using a CASA system (Computer Assisted Sperm Analisys, Sperm VisionTM v. 3.7.4., Minitube of America, Venture Court Verona, USA). Sperm was activated using a simple saline solution (100 mM NaCl, 10 mM Tris, pH: 8, Lahnsteiner et al. 1998) in a mixture with approximately 0.01g mL^{-1} BSA (bovine serum albumin). Measurements were carried out in duplicates. Cryopreservation was performed using 2 different methods according to the experimental design. Sperm was diluted in an extender composed of 150 mM glucose, 75 mM NaCl, 30 mM KCl, 1 mM Na_2HPO_4x12 H_2O, 1 mM MgCl2x6H_2O, 1 mM $CaCl_2$x2H_2O, 20 mM Tris, and 0.5% BSA (pH: 8) in a ratio 1:3 (Bernáth et al. 2017). As intracellular cryoprotectant, 10% of methanol was used. Diluted samples were loaded into 0.5mL straws or 10 mL cryotubes according to the experimental design. Freezed sperm was thawed using a waterbath at 40 °C.

Experiment 1. The comparison of two cryopreservation method

Sperm from 7 males was loaded into 0.5 mL straws. Samples were freezed using a Styrofoam frame 3 cm above the liquid nitrogen for 3 minutes (Horváth et al. 2003) or were cryopreserved with a controlled-rate freezer (CRF, IceCube 14s, IceCube Series v. 2.24, Sy-Lab, Neupurkersdorf, Austria) using a cooling program designed for Eurasian perch (from 7.5°C to −160°C, cooling rate: 56°C/min, Bernáth et al. 2016).

Experiment 2. The applicability of 10 mL cryotube for pike sperm cryopreservation

Samples from 9 males were loaded into 10 mL cryotubes (total volume: 8 mL). Sperm was freezed using CRF. The cooling program (from 4 °C to -160 °C, cooling rate: 15 °C/min) was measured at 3 cm above the liquid nitrogen using a thermo couple (Digi-Sense DualLogR®**, Eutech Instruments Pte Ltd., Singapore**) for 7 minutes (Bokor et al. 2010).

In *Experiment 1.*, pMOT reduced significantly following thawing in both cryopreserved groups (CRF: 42±17%, Frame: 45±11%) compare to the fresh control (69±4%). No significant differences were observed in the 3 motility parameters between the CRF and frame groups. In *Experiment 2.*, pMOT (25±10%) and VCL (77±9μm/s) decreased significantly after thawing using the 10 mL cryotube in comparison with the fresh control (pMOT: 68±10, VCL: 146±15μm/s) where STR did not show a significant decrement.

Our cryopreservation method designed for northern pike sperm was successfully adapted for a standard device (CRF). According to our knowledge, the 10 mL cryotube was tested first time in pike sperm cryopreservation. The 10 mL cryotube can be beneficial in large-scale cryopreservation which can support the artificial propagation practice in northern pike.

Keywords: *pike, sperm, cryopreservation, large-scale, cryotube*

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A COMPARISON OF FISH DIVERSITY AND ABUNDANCE BETWEEN THE MAIN COURSE AND AN ARMLET OF THE DANUBE RIVER NEAR BELGRADE (1168-1170 RKM)

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POREĐENJE DIVERZITETA I BROJNOSTI NASELJA RIBA U GLAVNOM TOKU I RUKAVCU DUNAVA KOD BEOGRADA (1168-1170 RKM)

Apstrakt

Diverzitet i struktura naselja riba predstavljaju važne karakteristike dinamike akvatičnih ekosistema. Kako nastanjuju različita staništa i pripadaju različitim trofičkim nivoima, ribe predstavljaju jedan od najznačajnih elemenata za procenu ekološkog statusa akvatičnih ekosistema. Dunav u toku kroz Srbiju nastanjuje 68 vrsta iz 50 rodova i 16 familija (Simonović & Nikolić, 1997). Sastav faune beogradskog sektora Dunava karakteriše prisustvo oko 55 vrsta riba, od kojih oko 25 ima i ribolovni značaj (Hegediš et al., 2013). Važnu ulogu među njima imaju i alohtone vrste. Izgradnjom HE "Đerdap" dolazi do kritične ugroženosti ili nestanka anadromnih vrsta jesetri (fam. Acipenseridae). Što se biomase tiče, javlja se dominacija tzv. «bele ribe». Pored deverike (*Abramis brama*), značajan udeo imaju i rečna mrena (*Barbus barbus*), šaran (*Cyprinus carpio*) i jaz (*Leuciscus idus*) (ibid.). Cilj ovog rada jeste poređenje primenljivosti četiri indeksa diverziteta (Shannonov indeks, indeks ekvitabilnosti, Simpsonov i Margalefov indeks) u analizi raznovrsnosti naselja riba na beogradskom sektoru Dunava, između 1168 – 1170 rkm, u dve sezone (zima 2014. god. i proleće 2015. god.) i dva različita tipa staništa (otvorena voda na ušću Save u Dunav kod Velikog ratnog ostrva i rukavac Jojkića Dunavac). Indeksi diverziteta se koriste za kvantifikaciju raznovrsnosti zajednice, a njihove različite vrednosti mogu ukazati i na različit intenzitet antropogenog pritiska. Uzorci riba analizirani u ovom radu potiču iz komercijalnog ulova. Izvršeno je 10 uzorkovanja (po 5 u zimskom i prolećnom periodu). Uzorkovanje je vršeno mrežama (dužine 20-50 m, dubine 1,4 m i promera okaca 4, 4,5, i 5 cm). U uzorku koji je obuhvatio 747 jedinki identifikovano je 20 različitih vrsta riba iz pet porodica. Familija Cyprinidae, tzv. "bela" riba, ima najveću zastupljenost (preko 95%). Uočeno je postojanje razlike u sastavu naselja riba između ispitivanih lokaliteta. U mirnijim vodama Jojkića Dunavca prisutne su stagnofilne vrste, štuka (*Esox lucius*), bodorka (*Rutilus rutilus*) i grgeč (*Perca fluviatilis*). S druge strane, u otvorenoj i bržoj vodi oko Velikog ratnog ostrva zabeleženo je prisustvo reofilnih vrsta, kesege (*Abramis ballerus*), bucova (*Aspius aspius*), rečne mrene (*B. barbus*), skobalja (*Chondrostoma nasus*) i smuđa (*Sander lucioperca*). Vrednosti analiziranih indeksa pokazale su da je diverzitet vrsta ujednačen na oba lokaliteta. Na osnovu vrednosti Shannonovog indeksa, kvalitet vode na oba ispitivana lokaliteta varira između II-III i III-IV klase (umereno zagađene vode) u zavisnosti od sezone.

Ključne reči: *indeksi diverziteta, ribe, reofilne vrste, stagnofilne vrste, Dunav*
Keywords: *diversity indices, fish, rheophilic species, stagnophilic species, Danube*

INTRODUCTION

Diversity and structure of fish assemblages are important features of the dynamics of aquatic ecosystems, because changes in diversity reflect changes in various ecosystem processes, such as productivity, energy pathways and material flow, disturbance regime, abiotic stress, and biological interactions (Brown et al., 2001). As they live in different habitats and belong to different trophic levels, fishes have a potential to be used as bioindicators of the condition of aquatic ecosystems (Chovanec et al., 2003).

The Danube is an important river for commercial exploitation. This trend, however, brings along an increasing number of water management problems, as well as pollution, which has become a limiting factor in the rational utilization of rivers (Gavrilović and Dukić, 2002). Sixty-eight fish species from 50 genera and 16 families inhabit the Danube River in Serbia (Simonović and Nikolić, 1997). Fish fauna in the Danube in Belgrade consists of about 55 species of fish, of which about 25 species are commercially important (Hegediš et al., 2013). Allochthonous species play an important role among them (from 12% to 20%). After the construction of the hydroelectric power stations Iron Gate I and II, anadromous sturgeons became critically endangered or extirpated from the upper and middle Danube (Lenhardt et al., 2005). On the other hand, the dominance of the cyprinid species is recorded, first of all, of the white bream (*Blicca bjoerkna*), common bream (*Abramis brama*), and roach (*Rutilus rutilus*). As regards the biomass, besides the common bream, species like the common barbel (*Barbus barbus*), common carp (*Cyprinus carpio*), and ide (*Leuciscus idus*) play a significant role.

Diversity indices are used for quantification of the community diversity. There are many different non-parametric indices. In this study, the following indices were applied: the Shannon's index (H'), evenness index (E), Simpson's index (D), and Margalef's index (D_{Mg}), with the aim to compare their applicability in fish diversity analysis in the investigated section of the Danube in two different habitats (the main channel and an armlet) and two seasons (winter and spring). Differences in values of these indices can also indicate the intensity of anthropogenic pressure at studied sites.

MATERIAL AND METHODS

All samples of fish were caught at two sites in the Danube River in Belgrade (between 1168 and 1170 rkm) by professional fishermen, between December 2014 and May 2015. The first site (44° 50' 1.94" N, 20° 26' 50.74" E) is located at the river mouth of the Sava River into the Danube – Veliko ratno ostrvo (VRO), and the second site (44° 50' 4.90" N, 20° 28'

11.59" E) is located upstream the Pančevo Bridge – Jojkića Dunavac (JD). VRO represents the main channel of the Danube, with fast water flow, and JD is a left armlet of the Danube, with slow water flow. Both sites are located in the vicinity of the urban part of the city of Belgrade and in the vicinity of agricultural areas. Samplings (5 samplings in winter and 5 in spring at each site) were performed by gill nets (20-50 m length, 1.4 m height, and 4, 4.5, and 5 cm mesh size). A total of 747 fish, belonging to 20 different species and five families, were sampled. The following indices of diversity were used for the analysis of the fish assemblage:

1) Shannon's index (H') $H' = -\sum_{i=1}^{S_t} p_i \ln p_i \qquad H_{\max} = \ln S \qquad p_i = \frac{n_i}{N},$

where *pi* is the abundance of the *i*th species in each individual sample (*ni*) divided by total abundance (*N*), and *S* is the total number of species.

2) Evenness index $E = \frac{H}{H_{\max}}$

3) Simpson's index (*D*) $D = 1 - C$

4) Margalef's index (D_{MG}) $C = \sum_{i=1}^{S_t} p_i^2 \qquad D_{Mg} = \frac{S-1}{\ln N}$

The values of *H*' and the associated water quality class (Official Gazette of RS, 2011) is presented in Table 1.

Table 1. The values of the Shannon's diversity index and the associated water quality class (Official Gazette of the Republic of Serbia, 74/2011).

Shannon's index (*H*')	Water quality class
>2,20	I-II
2,19-1,50	II-III
1,49-1,20	III-IV
1,19-0,50	IV-V

RESULTS AND DISCUSSION

A total of 747 individuals were caught, 322 in winter, and 425 in spring (Table 2). VRO and JD are sites with different habitat configurations – JD is a side channel with slower flow, and VRO represents the main channel with fast open water. These characteristics led to different composition of fish assemblages. Stagnophilic species, such as northern pike (*Esox lucius*), roach (*R. rutilus*), and European perch (*Perca fluviatilis*), were caught in higher numbers at JD. On the other hand, rheophilic species, such as blue bream (*Abramis ballerus*), asp (*Aspius aspius*), barbel (*B. barbus*), nase (*Chondrostoma nasus*), and pike-perch (*Sander lucioperca*) were caught in higher numbers at VRO (*cf.* Hirzinger et al., 2004).

The values of the estimated diversity indices (Shannon’s, evenness, Simpson’s, and Margalef’s) are shown in Table 3. The values of all diversity indices, except for D_{Mg}, were somewhat lower in the spring than in winter at VRO, probably due to the dominance of the white bream (*B. bjoerkna*) in the spring sample ($p_i = 0.63$). At JD, the values of *H’*, *E*, and *D* were higher in spring, again probably because of the dominance of the common bream (*A. brama*) ($p_i = 0.43$) and the white bream ($p_i = 0.37$) in the winter sample.

Based on the values of the Shannon’s index, the section of the Danube River in Belgrade can be classified as moderately polluted, in both seasons and at both sampling sites (Table 3).

Table 2. A list of species and the number of caught individuals at two sampling sites in the Danube – Veliko ratno ostrvo (VRO) and Jojkića Dunavac (JD).

Taxon	No. of individuals		Taxon	No. of individuals	
	VRO	JD		VRO	JD
Esocidae	**0**	**4**	*Rutilus pigus*	1	0
Esox lucius	0	4	*Rutilus rutilus*	11	31
Cyprinidae	**486**	**235**	*Vimba vimba*	11	3
Abramis ballerus	71	8	**Lotidae**	**2**	**0**
Abramis brama	57	95	*Lota lota*	2	0
Abramis sapa	25	4	**Percidae**	**11**	**7**
Aspius aspius	1	0	*Gymnocephalus cernuus*	1	0
Barbus barbus	4	0	*Perca fluviatilis*	1	4
Blicca bjoerkna	288	89	*Sander lucioperca*	7	2
Carassius gibelio	6	0	*Sander volgensis*	2	1
Chondrostoma nasus	8	3	**Centrarchidae**	**2**	**0**
Leuciscus cephalus	1	0	*Lepomis gibbosus*	2	0
Leuciscus idus	2	2			

Table 3. Estimated values of diversity indices and water quality classes (associated with the Shannon’s index) of the fish assemblage at two sampling sites in the Danube – Veliko ratno ostrvo (VRO) and Jojkića Dunavac (JD).

	Winter 2014/ 2015		Spring 2015		TOTAL	
	VRO	JD	VRO	JD	VRO	JD
Shannon’s index (*H'*)	1.56	1.36	1.37	1.61	1.53	1.52
Water quality class	II-III	III-IV	III-IV	II-III	II-III	II-III
Evenness index (*E*)	0.68	0.59	0.48	0.90	0.52	0.61
Simpson’s index (*D*)	0.71	0.66	0.57	0.78	0.63	0.70
Margalef’s index (D_{Mg})	1.95	1.67	2.67	1.53	2.90	2.00

CONCLUSIONS

The results indicate that the composition of fish assemblages at two studied sites were different, because of the differences in water flow and habitat preferences of rheophilic and stagnophilic fish species. The limitation of the Shannon’s, evenness, and Simpson’s indices is that their values are impacted by the dominance of one or two species. The Shannon’s index is the only diversity index that is included in the national legislation of the Republic of Serbia, as it indirectly indicates the level of pollution and the quality of water. Both sampling sites, in both seasons, can be classified as moderately polluted, between the II-III and III-IV water quality class.

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EFFECT OF PROBIOTIC ON SPERMATOLOGICAL PARAMETERS OF RAINBOW TROUT (*ONCORHYNCHUS MYKISS*) BROODSTOCK

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UTICAJ PROBIOTIKA NA SPERMATOLOŠKE PARAMETRE MATIČNOG JATA KALIFORNIJSKE PASTRMKE (*ONCORHYNCHUS MYKISS*)

Apstrakt

Probiotici imaju širok spektar pozitivnih uticaja na vodene životinje. Nedavna istraživanja jasno ukazuju na prednosti ovih suplemenata u vidu modulacije imunog sistema, tolerancije na stres i stope rasta gajenih riba (Carnevali et al., 2004; Dimitroglou et al., 2011; Merrifield and Ringo, 2014), ali manji broj studija bio je usmeren u pogledu efikasnosti ovih suplemenata za ishranu matičnog jata. Pored toga, u istraživanjima je ustanovljeno da se poboljšavanjem kvaliteta hrane i ishrane matičnog jata znatno utiče na kvalitet ikre i mleča (Izquierdo et al., 2001). Studije takođe ukazuju da je problem kvaliteta semene tečnosti u kontrolisanoj reprodukciji riba podjednako važan kao i kvalitet oocita (Targońska et al., 2008; Bobe and Labbé, 2010). Shodno tome, ovo istraživanje je imalo za cilj da utvrdi uticaj probiotika na spermatološke parametre matičnog jata kalifornijske pastrmke.

U ovom eksperimentu, 44 mužjaka kalifornijske pastrmke prosečne inicijalne težine od 1365,7 ± 33,4 g hranjeno je tokom 12 nedelja, hranom koja je sadržala 0; 0,5; 1 i 2 $g.kg^{-1}$ Bio-Aqua probiotika. Ribe su podeljene u četiri grupe (n= 4 po svakom replikatu) u okviru pregrađenih betonskih bazena. Po svakom tretmanu, razmatrana su 3 replikata. Tokom perioda istraživanja, prosečna temperature vode bila je 9.2 ± 3.7°C. Na kraju eksperimenta, mleč je sakupljen od tri mužjaka po svakom replikatu u cilju određivanja spermatoloških parametra. Određeni su: zapremina spermatozoida, spermatokrit mleča, pokretljivost spermatozoida, kao i koncentracija spermatozoida (Fauvel et al., 2010). U cilju utvrđivanja statistički značajnih razlika između srednjih vrednosti tretmana, korišćeni su jednofaktorska analiza varijanse (*one-way ANOVA*) i Dankenov MR test (*Ducam multy range test*) upotrebom SPSS softvera.

U ovom istraživanju, najniža srednja vrednost zapremine, najviše srednje vrednosti pokretljivosti spermatozoida, spermatokrita mleča i koncentracije spermatozoida utvrđene su kod tretmana sa 1 $g.kg^{-1}$ probiotika, koje su se statistički značajno razlikovale u odnosu na vrednosti kontrolne grupe. Kod većine slatkovodnih vrsta, pokretljivost spermatozoida obično traje kraće od dva minuta, a u mnogim slučajevima spermatozoidi mogu biti vrlo aktivni u vremenskom interval kraćem od 30 sekundi (Rurangwa et al., 2004). U tom pogledu, istraživanja su pokazala da duža održivost spermatozoida utiče na veću mogućnost fertilizacije (Duplinsky, 1982). Ranije studije su potvrdile direktnu korelaciju između koncentracije spermatozoida i spermatokrita mleča, kao i direktnu korelaciju između koncentracije spermatozoida i oplođenja (Ciereszko and Dabrowski., 1993; Tvedt et al., 2001). Pored toga, kako navode Alavi i saradnici (2008), smanjenje zapremine spermatozoida kod zdravih riba nadoknađuje se povećanjem koncentracije spermatozoida, što se veoma dobro može uočiti na osnovu rezultata ove studije.

Ovo istraživanje ukazuje da odgovarajući nivo probiotika može uticati na poboljšanje kvaliteta mleča, povećavajući uspešnost veštačkog mresta. Količina Bio-Aqua probiotika od 1 $g.kg^{-1}$ preporučuje se kao najoptimalnija za poboljšanje kvaliteta mleča matičnog jata kalifornijske pastrmske.

Ključne reči: *kalifornijska pastrmka, kontrolisana reprodukcija, probiotici, parametri kvaliteta sperme*
Keywords: *rainbow trout, controlled reproduction, probiotics, sperm quality parameters*

INTRODUCTION

Probiotics are causing a wide range of beneficial effects on aquatic animals. Recent studies have clearly represented the benefits of this feed additive on immune system modulation, stress tolerance and growth rate of farmed fish (Carnevali et al., 2004; Dimitroglou et al., 2011; Merrifield and Ringo, 2014), but limited studies have been done about the effectiveness of these feed additives on broodstock nutrition. Furthermore, according to the studies, improvement the broodstock nutrition and feeding has been shown to greatly improve egg and sperm quality (Izquierdo et al., 2001). Studies also indicated that the problem of semen quality in controlled reproduction of fish is equally important as the quality of oocytes (Targońska et al., 2008; Bobe and Labbé, 2010). In this regard, this study aimed to investigate the influence of probiotics on spermatological parameters of rainbow trout broodstock.

MATERIAL AND METHODS

In this experiment, 44 rainbow trout males with an average initial weight of 1365.7 ± 33.4 g were fed for 12 weeks with diets containing 0, 0.5, 1 and 2 $g.kg^{-1}$ Bio-Aqua probiotic. Fish were divided into four groups (n= 4 in each replicate) in a partitioned raceway pond. For each treatment, 3 replicates were considered. During the period, the average of water temperature was 9.2 ± 3.7°C. At the end of the experiment, fresh semen was collected from three males in each replicate for measurement of spermatological parameters. Sperm volume, spermatocrit, motility time and sperm concentration were determined (Fauvel et al., 2010). One-way ANOVA and Duncan's multiple range tests were used to analyze the significance of the difference among the means of treatments by SPSS software.

RESULTS AND DISCUSSION

The results of the present study are shown in Table 1.

Table 1. Effects of Bio-Aqua probiotic complex on spermatological parameters of rainbow trout (Oncorhynchus mykiss) broodstock after 12 weeks (mean ± SD)

Spermatological parameters	Probiotic levels (g/kg diet)			
	0	0.5	1	2
Volume (ml)	16.6 ± 0.8^{a}	13.8 ± 0.7^{ab}	9.4 ± 1.9^{c}	11.56 ± 2.7^{bc}
Spermatocrit (%)	14.3 ± 0.9^{c}	17.0 ± 1.4^{b}	20.2 ± 1.1^{a}	16.6 ± 1.3^{b}
Motility time (Second)	65.2 ± 8.6^{b}	75.6 ± 12.2^{b}	114.2 ± 7.4^{a}	96.1 ± 12.1^{a}
Concentration ($\times 10^9$/ml)	13.3 ± 1.3^{c}	16.9 ± 1.4^{b}	22.0 ± 1.6^{a}	18.7 ± 1.6^{b}

Groups with different alphabetic superscripts in the same row differ significantly at $P < 0.05$.

In this study, the lowest volume of sperm, highest spermatozoa motility time, highest spermatocrit and spermatozoa concentration was obtained at 1 g.kg^{-1} treatment and were significantly different with the control group. In most freshwater species, spermatozoa usually moves for less than 2 min and in many cases is highly active for less than 30 s (Rurangwa et al., 2004). In this regard, studies demonstrated that higher viability of spermatozoa can caused increases the chances of fertilization (Duplinsky, 1982). Earlier studies showed a direct correlation between sperm concentration and spermatocrit and also a direct correlation between spermatozoa concentration and fertilization (Ciereszko and Dabrowski., 1993; Tvedt et al., 2001). In addition, according to Alavi et al. (2008) reducing the volume of sperm in healthy fishes will be compensated by increasing the sperm concentration and this can be observed very well in the current study.

This study shows that an appropriate level of probiotics can increase sperm quality and as a result, increases the chances of success in artificial reproduction. Accordingly, Bio-Aqua probiotic at the level of 1 g.kg^{-1} is the best-suggested level for improving sperm quality of rainbow trout broodstock.

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THE EFFECTS OF ANTIDEPRESSANTS ON NON-TARGET ORGANISMS IN SURFACE WATER

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EFEKTI ANTIDEPTESIVA NA NECILJNE ORGANIZME U POVRŠINSKIM VODAMA

Apstrakt

Zbog pritisaka modernog sveta, stresa i brzog tempa života, ljudi su u današnje vreme često primorani da posegnu za depresivima kao efektivnoj i lakoj pomoći. Ova činjenica rezultuje u porastu njihovih koncentracija u površinskim vodama; farmaceutici se generalno mogu naći u koncentracijama između ng i µg po litru površinske vode. Štaviše, farmaceutici su poznati po visokoj biološkoj aktivnosti (Baker and Kasprzyk-Hordern, 2013) i od skoro se smatra da predstavljaju problem ne samo za ljude, već i za životnu sredinu zbog njihove široke i rastuće rasprostranjenosti širom sveta (Santos et al., 2010).

Glavni izvori farmaceutika u životnoj sredini su izlučivanje iz tela, oslobađanje iz otpadnih voda iz domaćinstava i bolnica itd. (Frédéric and Yves, 2014). Takođe, nepotpuno uklanjanje farmaceutika tokom prečišćavanja u postrojenjima za prečišćavanje otpadnih voda rezultuje prisustvom ovih aktivnih sastojaka u životnoj sredini (Santos et al., 2010). U današnje vreme raste zabrinutost o efektima antidepresiva na vodene organizme.

Prema dosadašnjim istraživanjima, antidepresivi koji se pojavljuju u površinskim vodama mogu uticati na ponašanje, razmnožavanje, razvoj i preživljavanje vodenih beskičmenjaka i kičmenjaka. Naša studija se fokusira na tri antidepresiva iz različitih klasa. Amitriptilin pripada grupi tricikličnih antidepresiva i može indukovati inhibiciju usvajanja serotonina i noradrenalina u krajevima presinaptičkih nerava. Ova klasa antidepresiva se

uobičajeno koristi pri tretmanu depresije, anksioznog poremećaja, poremećaja u ishrani i sindroma bola (Maubach et al., 1999). Nasuprot tome, ova klasa takođe ima širok spektar štetnog dejstva, kao što su toksičnost za kardio-vaskularni (Mannesström et al., 2006) i nervni sistem (Mannesström et al., 2006). Drugi odabrani antidepresiv, sertralin, pripada grupi antidepresiva koji selektivno inhibiraju usvajanje serotonina (SSRI) blokirajući presinaptičke transportere za usvajanje serotonina (Munari et al., 2014). Nekoliko sporednih efekata SSRI-ja u ponašanju uključuju anksioznost, napade panike, insomniju, razdražljivost, agresivnost, impulsivnost, psihomotorički nemir itd. (Murphy et al., 2008). Treći antidepresiv koji smo testirali je venlafaksin, koji pripada grupi selektivnih inhibitora za usvajanje serotonina-noradrenalina (SNRI). Ovaj farmaceutik je naširoko prepisivan za tretiranje depresije i anksioznosti (Vaswani et al., 2003). Venlafaksin sprečava usvajanje serotonina, i u manjoj meri noradrenalina (Asnis et al., 2004). Među čestim sporednim efektima su antiholinergijski efekat, hipertenzija, astenija, vrtoglavica, glavobolja, pospanost, nesanica, seksualna disfunkcija i suvoća usta (Tarleton et al., 2016).

Svi antidepresivi su korišćeni u koncentracijama relevantnim za životnu sredinu (prema izvorima iz literature) i u 100 puta višim koncentracijama radi evaluacije dozno-zavisnih efekata. Organizmi korišćeni u ovoj studiji su bili embrioni *Danio rerio* i *Xenopus tropicalis*. Embrioni *Danio rerio* su bili izloženi antidepresivima tokom 6 dana, embrioni *Xenopus tropicalis* su bili izloženi tokom 2 dana.

Želeli smo da verifikujemo da li ovi antidepresivi mogu uticati na gensku ekspresiju i količinu mitohondrijalne RNK odabranih gena - BMP4, NKX2.5, OTX2 i PAX6 gena. Ovi geni su odabrani prema funkcijama koje obavljaju u organizmu, a koje bi potencijalno mogle biti pod uticajem istraživanih antidepresanata.

Ključne reči: *beskičmenjaci, Xenopus tropicalis, Danio rerio*

Abstract

Due to the pressure of modern world, stress and rush life, people are nowadays more often forced to look up the antidepressants as an effective and easy way of help. This fact results in arise of their concentrations in surface water; pharmaceuticals are, in general, found in amount between ng to μg per litre of surface water. Moreover, the pharmaceuticals are known for their high biological activity (Baker and Kasprzyk-Hordern, 2013) and they are recently considered to be an issue not only for humans, but also for the environment due to their worldwide and ubiquitous increasing occurrence (Santos et al., 2010).

The main sources of pharmaceuticals in the environment are excretion from the body, discharge of domestic sewage, hospital wastewater, etc. (Frédéric and Yves, 2014). Also, an incomplete removal of pharmaceutical during wastewater treatment plant processes result in findings of these active compounds in the environment (Santos et al., 2010). Nowadays, the concerns about effects of antidepressants on aquatic biota grow.

According to current knowledge, antidepressants occurring in surface water are able to affect the behaviour, reproduction, development, and survival of aquatic invertebrates and vertebrates. Our study focused on three antidepressants from different classes. Amitriptyline, belonging to tricyclic antidepressants, can induce the inhibition of serotonin and noradrenaline uptake in presynaptic nerve endings. This class of antidepressants is commonly used for treatment of depression, anxiety disorders, eating disorders, and pain syndromes (Maubach et al., 1999). In contrast, this class also have a wide range of adverse effects,

such as cardiotoxicity (Callaham et al., 1988), or neurotoxicity (Mannerström et al., 2006). The second chosen antidepressant, sertraline, belongs to the selective serotonin reuptake inhibitor (SSRI) antidepressants, which block presynaptic serotonin reuptake transporters (Munari et al., 2014). Several behavioural side-effects of SSRIs include anxiety, panic attacks, insomnia, irritability, aggressiveness, impulsivity, psychomotor restlessness, etc. (Murphy et al., 2008). The third tested antidepressant was venlafaxine belonging to the group of selective serotonin-noradrenalin reuptake inhibitors (SNRI). It is a pharmaceutical widely prescribed to manage depression and anxiety (Vaswani et al., 2003). Venlafaxine prevents the re-uptake of serotonin, and to a lesser extent noradrenalin (Asnis et al., 2004). Among common side effects are anticholinergic effects, hypertension, asthenia, dizziness, headache, somnolence, insomnia, sexual dysfunction, and dry mouth (Tarleton et al., 2016).

All antidepressants were used in environmentally relevant concentrations (according to literature sources) and in 100x higher concentration for evaluation of dose-dependent effect. The organisms, used for this study, were *Danio rerio* and *Xenopus tropicalis* embryos. The *Danio rerio* embryos were exposed to the antidepressants for six days; the *Xenopus tropicalis* embryos were exposed for two days.

We wanted to verify, whether these antidepressants can affect the gene expression and mRNA amount, of selected genes – BMP4, NKX2.5, OTX2 and PAX6 gene. These genes were chosen according to their function in the body system, which could be possibly affected by these selected antidepressants.

Keywords: *invertebrates, Xenopus tropicalis, Danio rerio.*

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DIET OF EUROPEAN MUDMINNOW (*UMBRA KRAMERI*) IN BAČKA, MAČVA, AND SEMBERIJA

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ISHRANA CRNKE (*UMBRA KRAMERI*) U BAČKOJ, MAČVI I SEMBERIJI

Apstrakt

Uzorak od 61 jedinke crnke (Umbra krameri) prikupljen je sa tri lokaliteta, dva iz Srbije – Lugomir u Bačkoj (45° 46′ 33″ N; 19° 00′ 04″ E; sliv Dunava) i Bakreni Batar u Mačvi (44° 55′ 45″ N; 19° 28′ 34″ E; sliv Save), i jedan iz Bosne i Hercegovine – Gromiželj u Semberiji (44° 51′ 58″ N; 19° 18′ 29″ E; sliv Save), tokom juna i jula 2011. godine. Izlovljavanje je obavljeno uređajima za elektroribolov, kao i pletenom korpom od pruća. Na osnovu želudačno-crevnog sadržaja uzoraka analizirana je ishrana, primenom sledećih pokazatelja: indeks vakuiteta %*VI* (procenat praznih stomaka), procentualna učestalost %*F* (proporcija stomaka sa određenom kategorijom plena), procentualni udeo %*N* (proporcija jedinki određene kategorije plena u odnosu na ukupan broj jedinki svih kategorija plena), prosečan broj komada plena po stomaku predatora, kao i širina trofičke niše (kao Shannonov indeks diveziteta *H*). Najznačajnije kategorije plena određene su korišćenjem indeksa značajnosti ($PV=CN\sqrt{F}$). Takođe, obavljeno je i uzorkovanje beskičmenjaka iz mulja i submerzne vegetacije u cilju utvrđivanja selektivnosti ishrane crnke (kao indeks elektivnosti po Ivlevu E_i).

Indeks vakuiteta bio je najniži u uzorku sa lokaliteta Gromiželj (*VI* = 11.1%), nešto veći sa lokaliteta Lugomir (*VI* = 13.0%), i najveći na lokalitetu Bakreni Batar (*VI* = 33.3%). Najzastupljenija kategorija plena na lokalitetu Lugomir bili su predstavnici porodice Chironomidae, dok je račić *Asellus aquaticus* (Isopoda) bio najzastupljeniji plen u uzorcima sa lokaliteta Gromiželj i Bakreni Batar. Spektar ishrane na lokalitetu Lugomir obuhvatio je šest kategorija plena iz tri grupe (Crustacea, Insecta,vodene makrofite). Kod 95% ana-

liziranih jedinki u stomacima su nađeni samo ostaci Arthropoda (%*PV* = 99.8), od čega kod 65% samo Chironomidae (%*PV* = 93.5), i kod 5% samo *A. aquaticus* (%*PV* = 3.4). Na lokalitetu Bakreni Batar, ishrana je obuhvatila pet kategorija plena iz tri grupe (Mollusca, Crustacea, Insecta). Kod 86% jedinki nađeni su samo Arthropoda (%*PV* = 96.6), od čega kod 43% samo *A. aquaticus* (%*PV* = 73.8). Na lokalitetu Gromiželj, ishrana je obuhvatila šest kategorija plena iz tri grupe (Mollusca, Crustacea, Insecta). Kod 92% jedinki nađeni su samo Arthropoda (%*PV* = 98.8), od čega kod 63% samo *A. aquaticus* (%*PV* = 83.4). Širina trofičke niše, izražena kao Shannonov indeks diverziteta, bila je najmanja na lokalitetu Lugomir (H = 0.31), zbog dominacije samo jedne kategorije plena (larve hironomida), nešto veća na lokalitetu Gromželj (H = 0.57), i najveća na lokalitetu Bakreni Batar (H = 0.91).

Indeks selektivnosti po Ivlevu (E_i) ukazuje na postojanje preferencije prema određenoj kategoriji plena. Od ukupno devet potencijalnih kategorija plena detektovanih na tri ispitivana lokaliteta, crnka je na lokalitetu Lugomir pokazala pozitivnu selektivnost samo prema hironomidama, (E_i = 0.76), na lokalitetu Bakreni Batar prema amfipodama (E_i = 0.22) i izopodama (E_i = 0.66), i na lokalitetu Gromiželj samo prema izopodama (E_i = 0.4).

Najveća vrednost prosečnog broja komada plena po stomaku predatora zabeležena je u uzorku sa lokaliteta Lugomir (7.3), dok su znatno niže i ujednačenije vrednosti zabeležene kod uzoraka sa lokaliteta Gromiželj (2.8) i Bakreni Batar (2.6). Ove vrednosti su u korelaciji sa dobijenim vrednostima za širinu trofičke niše.

Rezultati istraživanja pokazuju da je crnka oportunistički predator. Iako je jedna kategorija plena (larve hironomida) izrazito dominirala u uzoku sa lokaliteta Lugomir, prisustvo ovog plena bilo je znatno manje na lokalitetu Bakreni Batar, dok je potpuno odsustvovao na lokalitetu Gromiželj, što ukazuje na to da ova vrsta eksploatiše širok spektar dostupnih trofičkih resursa.

Istraživanje je podržano od strane Ministarstva prosvete, nauke i tehnološkog razvoja, projekat OI173045.

Ključne reči: *crnka, analiza stomačnog sadržaja, širina niše, sliv Dunava, sliv Save*

Abstract

A total of 61 specimens of the European mudminnow (Umbra krameri) were sampled from three locations, two in Serbia – Lugomir (45° 46′ 33″ N; 19° 00′ 04″ E; the Danube River basin), in the region of Bačka, and Bakreni Batar (44° 55′ 45″ N; 19° 28′ 34″ E; the Sava River system), in Mačva, and one in Bosnia and Herzegovina – Gromiželj (44° 51′ 58″ N; 19° 18′ 29″ E; the Sava River system), in the region of Semberija, in June and July 2011, using electrofishing and wattle baskets. The diet of the sampled individuals was analysed from gastrointestinal contents, using the following indices: vacuity index %*VI* (percentage of empty stomachs), frequency of occurrence %*F* (proportion of stomachs containing a specific prey category), numerical abundance %*N* (number of individuals of a specific prey category divided by the total number of individuals of all prey categories), average number of prey items per predator stomach, and trophic niche breadth (as Shannon' diversity index H). The most important prey categories were defined using the prominence value (PV=CN$\sqrt{\text{F}}$). Invertebrates from mud and submersed vegetation were sampled to estimate diet selectivity (as Ivlev's index of electivity E_i) of the European mudminnow.

Vacuity index in the sample from Gromiželj was the lowest (VI = 11.1%), followed by Lugomir (VI = 13.0%), and Bakreni Batar (VI = 33.3%). The family Chironomidae was the

most frequent prey of specimens from Lugomir, while waterlouse *Asellus aquaticus* (Isopoda) was the most frequent prey of specimens from Gromiželj and Bakreni Batar. The diet at Lugomir consisted of six prey categories from three groups (crustaceans, insects, water macrophytes). In 95% of analyzed stomachs only arthropod remains were found (%PV = 99.8), of which Chironomids in 65% of stomachs (%PV = 93.5) and waterlouse in 5% (%PV = 3.4). At Bakreni Batar, the diet consisted of five prey categories from three groups (molluscs, crustaceans, insects). Only arthropods were found in 86% stomachs (%PV = 96.6), of which waterlouse in 43% (%PV = 73.8). The diet at Gromiželj consisted of six prey categories from three groups (molluscs, crustaceans, insects). Only arthropods were found in 92% stomachs (%PV = 98.8), of which waterlouse in 63% (%PV = 83.4). The values of the trophic niche breadth was lowest at Lugomir (H = 0.31), due to a marked dominance of one prey category (chironomid larvae), somewhat higher at Gromželj (H = 0.57), and the highest at Bakreni Batar (H = 0.91).

Ivlev’s index of electivity (E_i) illustrates the preference for a particular prey category. Of nine potential prey categories observed at three studied sites, mudminnows positively selected only chironomids (E_i = 0.76) at Lugomir, amphipods (E_i = 0.22) and isopods (E_i = 0.66) at Bakreni Batar, and only isopods (E_i = 0.4) at Gromiželj.

The largest average number of prey items per predator stomach was observed at Lugomir (7.3), whereas lower and more uniform values were observed at Gromiželj (2.8) and Bakreni Batar (2.6). These values are correlated with estimated trophic niche breadths.

The results suggest that the European mudminnow is an opportunistic predator. Although one prey category (chironomid larvae) distinctly dominated at Lugomir, it was less present at Bakreni Batar, and even absent in Gromiželj, which indicates that this species exploits a wide spectrum of available feeding resources.

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Keywords: *mudminnow, stomach content analysis, niche breadth, Danube River system, Sava River system*

PRELIMINARY RESULTS REGARDING THE GILL ANTIOXIDANT ENZYMATIC RESPONSES OF STELLATE STURGEON (*ACIPENSER STELLATUS* PALLAS, 1771) DURING METAL EXPOSURES

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PRELIMINARNI REZULTATI ODGOVORA ENZIMA OKSIDATIVNOG STRESA ŠKRGA KOD PASTRUGE (*ACIPENSER STELLATUS* PALLAS, 1771) TOKOM IZLOŽENOSTI TEŠKIM METALIMA

Apstrakt

Mnogo je informcija o efektima teških metala, naročito bakra i cinka na ribe. Ipak, kada je u pitanju značajna grupa Acipenserida, pastruga, informacije o toksičnosti pomenutih metala su oskudne. Cilj ispitivanja bio je evaluacija efekata različitih koncentracija bakra, cinka i njihovih mešavina na enzime oksidativnog stresa škrga i bioakumulaciju kod *Acipenser stellatus.* Mlađ ove vrste bila je izložena tokom 7 i 14 dana koncentracijama 10% i 25% od LC_{50} bakra i cinka, ranije određenih kao 0.547 mg/l za bakar i 34.22 mg/l za cink. Studija je takođe nameravala da evaluira efekte oba metala na aktivnost antioksidativnih enzima u eksperimentu interakcije metal-metal, sa 10% i 25% LC_{50} svakog metala, tokom 7 dana. Odgovor antioksidativnih enzima škrga (superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase, glutathione-s-transferase, glucose-6-phosphate dehydrogenase) i ne-antioksidativnih enzima (redukovani glutation) su određivani. Enzimska aktivnost je značajno varirala zavisno od vrste metala, koncentracije i trajanja izloženosti. Rezultati istraživanja ukazuju da su škrge organ osetljiv na teške metale. Aktivnost antioksidativnih enzima može biti potencijalni biomarker kontaminacije teškim metalima za pastrugu, čak i u tvrdoj vodi.

Ključne reči: *Acipenser stellatus, škrge, enzimi oksidativnog stresa*

Abstract

There is a lot of information on the effects of heavy metals, especially copper and zinc on fishes, however information on the toxicity of these two metals individually and combined on a valuable group as sturgeons (Acipenseridae) is very poor. The aim of this study was to evaluate the effects of different concentrations of copper, zinc and their mixtures on *Acipenser stellatus* gill oxidative stress enzymes and their bioaccumulation. *Acipenser stellatus* juveniles were exposed for 7 and 14 days to 10% and 25% of LC_{50} of copper and zinc, previously determined as 0.547 mg/l and 34.22 mg/l respectively. Also, the study tried to evaluate both metals effects on the antioxidant enzymes activity in a metal-metal interaction experiment, with 10% and 25% LC_{50} of each metal, for 7 days. The responses of gill antioxidant enzymes (superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase, glutathione-s-transferase, glucose-6-phosphate dehydrogenase), non-antioxidant enzyme (reduced glutathione) were determined. The enzymes activity varied considerably according to metal type, concentration and duration of metal exposure. The results of the present investigation suggest that gill is a sensitive organ to the toxicity of heavy metals. The activity of antioxidant enzymes can be used as a potential biomarker of metal contamination for stellate sturgeon, even in hard water.

Keywords: *Acipenser stellatus, gill, oxidative stress enzymes*

METHOD DETERMINATION OF POLYCIKLIC AROMATIC HYDROCARBONS IN SMOKE FISH PRODUCTS

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METODA ODREĐIVANJA POLICIKLIČNIH AROMATIČNIH UGLJOVODONIKA U DIMLJENIM RIBLJIM PROIZVODIMA

Apstrakt

Policiklični aromatični ugljovodonici (PAH) predstavljaju grupu organskih jedinjenja koja se sastoje od dva ili više spojena aromatična prstena. PAH-ovi imaju nisku rastvorljivost u vodi a dobro su rastvorni u organskim rastvaračima. Ova jedinjenja nastaju kao nusprodukti nepotpunog sagorevanja, pri čemu su najznačajniji izvori emisije PAH-ova u atmosferu termo-energetska postrojenja, izduvni gasovi automobila sa benzinskim i dizel motorima kao i posledice intenzivne aktivnosti hemijske industrije. U poslednje vreme vlada veliko interesovanje za ispitivanje policikličnih aromatičnih ugljovodonika pošto je dokazano da neka jedinjenja iz ove grupe imaju kancerogena, mutagena i teratogena svojstva. Većina primenjenih metoda za određivanje PAH-ova u uzorcima hrane zasnovane su na tečno- tečnoj ekstrakcija (LLE) ili tečno –čvrstoj ekstrakciji dok se kvantifikacija radi uz pomoć gasne hromatografijom sa masenim detektorom

Cilj ovog rada bio je da se validuje metoda za određivanja nivoa kontaminacije kod različitih vrsta komercijalnog dimljenog mesa i ribljih proizvoda sa policikličnim aromatičnim ugljovodonicima (PAHs) za koje su utvrđene granične vrednosti u Uredbom evropske komisiej (EU) 2015/1125. Ovi PAH su benzo (a) piren (BaP) i zbir sledećih četiri PAH: benzo (a) piren, benzo (a) antracen (BaA), benzo (b) fluoranten (BbFA) i krisen (Chr).. Metoda uključuju proces ekstrakcije frakcije policikličnih aromatičnih ugljovodonika, a potom kvalitativno i kvantitativno određivanje PAH-ova gasnom hromatografijom sa masovnim detektorom (GCMS). Gasni hromatograf sa masenom detekcijom omogućava i potvrdne testove sa bazom analita sadržanih u NIST-ovoj biblioteci , podržanoj sa softverom MassHunter, gde se poređenjem spektara ispitivanog jedinjenja sa spektrom iz biblioteke dobija i procenat pouzdanosti rezultata. Da bi nestandardna metoda bila prihvatljiva urađena je validacija metode. Validacioni plan je uključivao proveru linearnosti u celom opsegu ispitivanja, proveru ponovljivosti, reproduktivnosti i tačnosti metode preko matriksa obogaćenog sa PAH-ovima u poznatoj koncentraciji, kao i određivanje granice detekcije i kvantifikacije. Linearnost je testirana u rasponu od 5 do 500 mg kg^{-1} i bila je zadovoljavajuća u celom opsegu (R^2> 0,999). Granica detekcije bila je u rasponu od 0,33 do 0,87 mg kg^{-1} i granična kvantifikacija u rasponu od 1,05 do 2,91 mg kg^{-1}. Prinos metode se kretao u opsegu od 81,53 do 100,31% dok su preciznost i reprodktivnost bile manje od 20%.

Ključne reči: *PAH, kontaminacija, GCMS, dimljena riba*
Keywords: *PAH, contaminants, GCMS, smoked fish*

INTRODUCTION

Smoking is one on the oldest food preservation methods. This process involves saturation of brined or salted food with components of the smoke followed by its desiccation (Zachara and Juszczak, 2016; Šimko, 2002; Zachara, 2017). Chemical compounds of the smoke act as preservatives with antioxidant and bacteriostatic properties as well as flavouring, dying and impregnating agents (Kubiak, 2014; Tokarczyket al., 2011; Škaljac et al., 2014). In addition to these desired compounds the smoke can also contain substances which are undesirable for health, such as polycyclic aromatic hydrocarbons (PAHs) (Phillips & Phillips, 1999). Polycyclic aromatic hydrocarbons (PAHs), also known as polyaromatic hydrocarbons, are organic compounds composed of multiple aromatic rings, the number of which determines their physicochemical properties and toxicity (Zachara, 2017). Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous environmental contaminants that are produced by incomplete combustion of organic matter (WHO, 2006). Over 100 PAHs have been identified, and they occur as complex mixtures throughout air, water, and soil (Wisciano *et al*., 2009; Novakov, 2017). Those contaminants can be found in different foods, including raw and unprocessed products as well as processed and cooked foods (Phillips & Phillips, 1999). The PAHs usually come from environmental pollution and thermal treatment (smoking, roasting, grilling, and cooking) during food manufacturing (Guillén *et al*., 2004; Yurchenko & Mölder, 2005). High molecular PAHs are considered to be carcinogenic, while benzo(a)pyrene represents the most potent PAH carcinogen (Novakov, 2017). The European Commission recently established maximum levels permitted in food (EC, 2014a) and set benzo (a) pyrene and sum of benzo (a) pyrene, benzo (a) anthracene, benzo (b) fluoranthene, and chrysene as priority PAH pollutants.

MATERIJAL AND METHOD

In this study we validate method for four PAHs. PAH analyzed components included benzo (a) anthracene (BaA), chrysene (CHr), benzo (a) pyrene (BaP), benzo (b) fluoranthene (BbF). For sample preparation we used multiresidue preparation that insure quick, easy, cheap, effective, rugged and safe preparation (QuEChERS), adapted from the Association of Analytical Communities (AOAC) Official method 2007.01 for extraction and clean up.

Reagents and Chemicals

All chemicals and reagents used were of analytical grade with high purity.

Standard Solutions

Standard solution were prepared using a PAH mix of 16 polycyclic aromatic hydrocarbons, manufacturer Ultra Scientific. lot. CL-6064, USA, concentration of 500 ± 0.2µg ml-1.

For internal control we used PAH mixture, manufacturer Ultra Scientific. lot CH-0209,USA. In order to eliminate the influence of the matrix, calibration through matrix blank sample was performed as well (SANCO, 2014).

Sample Preparation

Kartalovic *et al*. (2016) used adapted method of sample preparation for organochloric pesticides (OCP), polychlorinated biphenyl’s (PCB) for PAHs determination. That method was based on the extraction with acetonitrile (ACN, Sigma-Aldrich) in the presence of anhydrous magnesium sulfate ($MgSO_{4;}$ *Merck*, Darmstadt, *Germany*) and anhydrous sodium acetate (CH_3COONa; *Merck*, Darmstadt, *Germany*). Sample (3 g) was measured and transferred into centrifuge tube, 3 ml of water and 3 ml of Acetonitrile were added. After intensive stirring on a vortex, 3 g of anhydrous magnesium sulfate and 1 g of anhydrous sodium acetate were added. Exothermic reaction occurred within 1 min after the intense stirring on vortex. The sample was then centrifuged until 5 min at 3000 rpm. 1 ml of upper acetonitrile extract is transferred into the 5 ml tube, which contained 150 mg of anhydrous magnesium sulfate, 100 mg of Primary and Secondary Amine (PSA), Merck manufacturer (Darmstadt, Germany), and 50 mg of C18, Merck manufacturer (Darmstadt, Germany), (Anastassiades *et al*., 2003). The tube content was centrifuged for 5 min at 3000 rpm. After centrifuging, purified and clear extract was obtained. Then, 0.5 ml of the extract was evaporated in nitrogen and reconstituted with hexane. A sample prepared in this way was ready for the analysis on GCMS (Agilent 7890B/5977A, USA).

GCMS Analysis

The PAHs identification were based on comparison of the retention times of the peaks and target ions, with those obtained from standard mixture of PAH (standards supplied by instrument manufacturer).

Quantification was based on matrix calibrations curves prepared from the standard solution of PAH mix. The coefficients of determination (r2) for the PAHs standard calibration plots were more than 0.99.

Instrumentation

Agilent 7890B/5977A MSD, gas - mass chromatography was used for analyzing. The GC operating conditions were as follow: fused silica column [30m*0.25µm film of HP-5M (thickness)]; injection temperature was set at 280 °C using splitless mode and volume injected was 4 µL. The column temperature was programmed as following: hold at 50°C for 0.4min; 50-195 °C at 25 °C/min, hold 1.5 min; 195-265 at 8 °C/min and maintained at 315°C for 1.25 minutes on 20 °C/min, MSD temperature was 280 °C. Verification of peaks was carried out based on retention times and target ions, compared to those of external PAH. Procedural blank and solvent blanks were analyzed and quantified, but no PAHs was found in these blanks.

Data handling

Statistic 12 software and Excel (Microsoft Excel, 2007), data analysis was using to determine the descriptive statistic parameters.

RESULTS AND DISCUSSION

The determination of trace analytes in complex matrices is a serious challenge, and needs a comprehensive set of quality assurance tools. The reliability of analysis results can be supported by external quality control tools such as proficiency tests (PTs) and certified reference materials (Zelinkova and Wenzl, 2015). The validation of analytical methods is thus of extreme importance to demonstrate that they are fit for purpose. Fundamental guidance on the management of, among others, analytical chemistry laboratories and the demonstration of their competence is provided by ENISO/IEC17025:2005. Currently, great emphasis is placed on food control laboratories to follow ISO 17025 practices and to obtain accreditation according to this standard for their analytical methods in order to ascertain a high level of reliability of the generated data (Gowik, 2009; Wenzl, 2006).

In this study validation plan included determination of precision, reproducibility, accuracy, linearity, LOQ, LOD and uncertainty.

The method precision was evaluated by repeatability using the smoke fish meat without PAHs fortified with PAH concentrations injected in triplicate (50.0 μg kg^{-1}, n=20). Accuracy was calculated by recovery.

Linearity of detector was tested in range of 5 to500 μg kg^{-1}, and was satisfactory in all range.

Limit of detection (LOD—standard deviation equal to 3) and limit of quantification (LOQ— standard deviation equal to 10) were calculated using the excel program. The LOD values ranged from 0.3 to 1.6μg kg^{-1}, and the LOQ varied from 1.0 to 5.2μg kg^{-1} for PAH.

For calculating measurement uncertainty was use the contributions of PT (FAPAS-PAH in smoked fiash product, November 2017), the contribution of reproducibility and contribution of bias are taken into account (Kartalovic, 2016).

Table 1. The average values of precision, reproducibility, accuracy, linearity, LOQ and LOD for PAH

PAH	Precision (%)	Reproducibility (%)	Recovery (%)	Linearity (r^2)[a]	LOQ (μg kg^{-1})	LOD (μg kg^{-1})
benzo(a) anthracene	9.44	8.6	89.72	0.997	1.32	0.39
chrysene	5.33	8.2	92.52	0.998	1.12	0.33
benzo(a)pyrene	3.23	3.81	96.81	0.998	2.13	0.63
benzo(b) fluoranthene	8.52	14.25	86.43	0.994	1.30	0.39
min	3.23	3.81	89.72	0.994	1.12	0.33
max	9.44	14.25	96.81	0.998	2.13	0.63

Note: [a]r^2 – correlation coefficient

CONCLUSIONS

The determination of PAHs in foodstuffs has already a long history. In the past, only benzo[a]pyrene was targeted but now to see each toxicological effect of PAHs is need to determinate PAH4.

The obtained results may provide useful information for PAH method determination wich application can give us useful information for the exposure assessment in risk assessment of PAHs from smoked fish product.

GCMS method is simply, fast and certain for PAH determination in foodstuff.

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DIET COMPOSITION OF BROWN TROUT (*SALMO TRUTTA* L., 1756) FROM THREE RIVERS WITHIN SPECIAL NATURAL RESERVE «UVAC»

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SASTAV ISHRANE POTOČNE PASTRMKE *(SALMO TRUTTA* L., 1756*)* IZ TRI REKE NA PODRUČJU SPECIJALNOG REZERVATA PRIRODE „UVAC"

Apstrakt

Ispitivana je ishrana potočne pastrmke iz tri vodotoka na području Specijalnog rezervata prirode „Uvac": Mali Uvac (gornji deo toka Uvca); Tisovica i Zlošnica. Aparatom za elektroribolov ulovljeno je ukupno 29 primeraka pastrmke (14 iz Uvca, 11 iz Tisovice i 4 iz Zlošnice). Pregled crevnog sadržaja obuhvatio je identifikaciju konzumiranih kategorija plena i njihovu brojnu zastupljenost. Na osnovu dobijenih podataka izračunati su indeksi učestalosti, abundantnosti i značajnosti za svaku od utvrđenih kategorija plena. Utvrđeno je da pastrmke iz istraživanih voda, u pogledu diverziteta plena, odlikuje relativno širok spektar ishrane. Generalno, pastrmke iz ovih voda pretežno se hrane akvatičnim beskičmenjacima, pri čemu larve Trichoptera predstavljaju najznačajniji element ishrane. Kao vizuelni predator, pastrmka ispoljava jasan afinitet ka krupnijem i mobilnijem plenu. Dobijeni rezultati, budući da se radi o relativno malim uzorcima prikupljenim tokom jednog mesečnog izlaska (septembar), mogu se smatrati preliminarnim i iziskuju dalja istraživanja.

Ključne reči: *pastrmka, ishrana, sastav ishrane, akvatični beskičmenjaci*
Keywords: *brown trout, feeding, diet composition, aquatic invertebrates*

INTRODUCTION

The brown trout (*Salmo trutta* Linnaeus 1756) inhabits almost all rivers and streams running through the area of Special Natural Reserve «Uvac» (southwest of Serbia). In some of them it represents the only present fish species, and studies of its diet are important issue to understand its biology. Analysis of its diet provides information on its trophic requirements, interactions with other species, and habitat use in the investigated ecosystem (Oscoz et al., 2005; Ruginis, 2008). There are numerous studies concerning feeding of brown trout in European waters and elsewhere (Kara and Alp, 2005; Osoz et al., 2005; Montori et al., 2006; Ruginis, 2008; and references therein). On the contrary, such studies are scarce in Serbia and, to the best of our knowledge, in last 25 years only one paper concerning this matter was published (Skorić et al., 2012). The aim of this study is to describe diet composition of brown trout in the three rivers within the area of the Special Natural Reserve «Uvac».

MATERIAL AND METHODS

Fish were collected in September 2016 in the three rivers within Special Nature Reserve «Uvac»: Mali Uvac (upper course of the Uvac River), Tisovica River (upper course), and Zlošnica River (middle course). General information on the sampling sites for each stream is given in Table 1.

Table 1. Positions of the sampling sites and their basic characteristics: A. - altitude; A. W. – average width; A. D. – average depth; S. – substratum.

River	Latitude N	Longitude E	A.(m)	A. W.(m)	A. D.(cm)	S.
Mali Uvac	43° 17.521'	19° 55.184'	1058	4	25	gravel
Tisovica	43° 29.027'	19° 57.955'	1023	3	50	gravel
Zlošnica	43° 27.495'	19° 50.350'	944	3	40	gravel

An area of approximately 100 m^2 has been fished at each sampling site (profile length 25-35 m). The studied areas in Mali Uvac and Zlošnica were lotic, while in Tisovica were combined lotic and lentic conditions (pool presence). Fish sampling was conducted by electrofishing device (Honda, 1.2 kW; 6 A). In total 29 brown trout were caught, of which 14, 11, and 4 in Mali Uvac, Tisovica and Zlošnica, respectively. Immediately after capture, they were frozen for later study. In the laboratory, after melting, for each fish total and standard length in millimeters (± 0.1 mm), weight (± 0.1 g) and sex were recorded. For dietary analyses a complete digestive tract was examined. Dietary analyses were performed using stereomicroscope for the determination (mainly order level was observed) and counting of ingested organisms. The percentage of occurrence (F) and percentage of abundance (A) were calculated as: $\%F=(N_i/N_t)x100$; $\%A=(\Sigma S_i / \Sigma S_t)x\ 100$; where N_i is the number of fish with food item *i*, N_t is the total number of fish in the sample with stomach content; S_i is the number of food item *i*, and S_t the total number of food items in the entire sample (Amundsen et al., 1996).The percentages of occurrence and abundance were used to calculate the index of prominence (PV) (Hickley et al., 1994; Lorenzoni et al., 2002): $PV = A \sqrt{F}$; $\%PV = (PV / \Sigma PV)\ x\ 100$. Basic water quality indicators (temperature, dissolved oxygen, pH, conductivity) were measured using portable WTW instruments.

RESULTS AND DISCUSSION

Table 2 contains data on water quality indicators for three investigated rivers based on physical and chemical parameters. Recorded values indicate favorable conditions for salmonids.

Table 2. Water quality parameters recorded at sampling sites.

Parameter	Mali Uvac	Tisovica	Zlošnica
T (ºC)	12.4	11.3	11.7
C (µS/cm)	271	251	350
pH	7.1	6.7	7.1
O_2 (mg/l)	10.94	10.47	10.53
O_2 (%)	116.1	108	108.9

The only found fish species in investigated stretches of Mali Uvac and Zlošnica was the brown trout (*Salmo trutta*). In investigated stretch of Tisovica, the brown trout and bullhead (*Cottus gobio*) were found. According to their morphological, abiotic and biotic characteristics, studied waters represent typical upper salmonid region. The ranges of total length and mean length of examined fish were: 80.5–151.6 mm, 101.2 ± 22.18 mm; 65–153 mm, 115.2 ± 31.24 mm; 168–254 mm, 217 ± 40.8 mm; for Mali Uvac, Tisovica and Zlošnica, respectively. Regarding weight data the corresponding values were: 5–31.5 g, 10.5 ± 8.05 g; 2.6-42.8 g, 19.1 ± 13.25 g; 43.9–212.8 g, 135.9 ± 77.64 g; for Mali Uvac, Tisovica and Zlošnica, respectively. For all examined fish, there were no empty guts found. Figure 1 depicts diet composition for each individual trout from three investigated rivers.

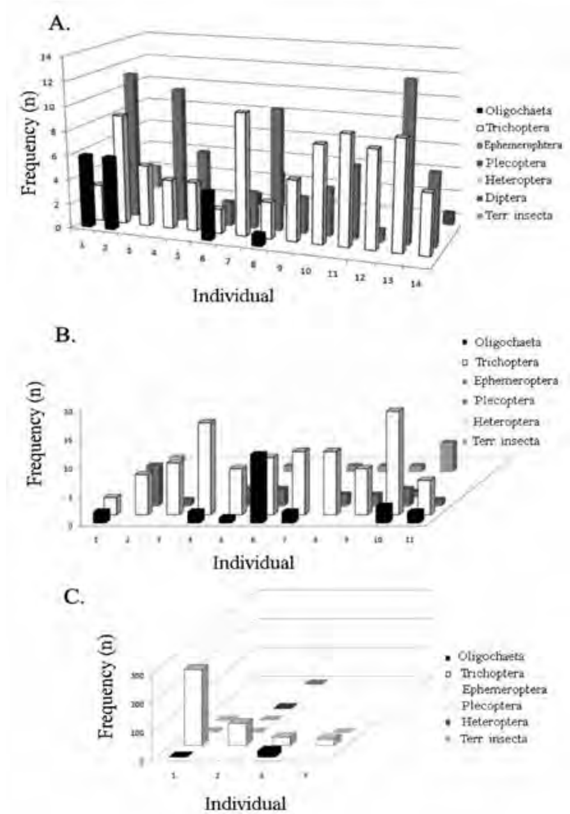

Figure 1. Gut contents of examined brown trout from three investigated rivers: A-Mali Uvac; B-Tisovica; C-Zlošnica.

A total of 198 aquatic and terrestrial prey items were found in the diets of the trout from Mali Uvac River (Fig. 1, inset A; Tab. 3). The number of ingested items varied in 8-27 range within examined fish. The most frequent prey were Trichoptera larvae (100%), followed by Ephemeroptera larvae (92.9%), also both were the most abundant prey constituting 46.6% and 43.5% of diet composition, respectively. According to %PV they represent 90% of the diet, with almost equal participation. In total, 98% of prey items were benthic aquatic organisms, while participation of terrestrial organisms was very low.

In guts of trout from Tisovica River a total of 155 aquatic and terrestrial prey items were detected, and the number of consumed prey varied in 5-31 range per individual (Fig. 1, inset B). Trichoptera larvae were detected in gut content of all examined fish and represented the most frequently consumed prey (Tab. 3). Moreover, high values of %F were noted for Ephemeroptera (72.7%) and Oligochaeta (63.6%). By abundance, Trichoptera represented the largest proportion of the diet, while the contribution of Oligochaeta and Ephemeroptera reached significantly high proportion. The index of prominence value reveals that previously mentioned three food categories represent about 95% of the diet, with Trichoptera being the most important (69.8%). The contributions of remainder food items in the diet of trout from Tisovica River were of low rate.

Table 3. Diet composition of trout from three investigated streams: %F-occurrence; %A-abundance; %PV-index of prominence.

Prey category	**Mali Uvac**			**Tisovica**			**Zlošnica**		
Aquatic organisms	%F	%A	%PV	%F	%A	%PV	%F	%A	%PV
Oligochaeta	28.6	8.6	5.08	63.6	14.4	12.5	50	6.1	4.42
Ephemeroptera larvae	92.9	40.9	43.5	72.7	13.2	12.3	75	1.7	1.51
Plecoptera larvae				9.1	0.6	0.2	25	0.2	0.1
Heteroptera	28.6	2.5	1.48	18.2	1.8	0.84			
Diptera	35.7	3.5	2.31						
Trichoptera larvae	100	42.4	46.6	100	64.1	69.8	100	91.7	93.9
Terrestrial organisms									
Insecta (unidentified)	21.4	2	1.02	45.5	6	4.41	25	0.2	0.1

A total of 424 food items were detected in the diets of trout from river Zlošnica (Fig. 1, inset C). The number of consumed prey varied greatly among examined fish, ranging in 22-272 prey items. In terms of all assessed indices the most important food item were species from order Trichoptera (Tab. 3).

Stream dwelling brown trout is known as generalist and opportunistic species with potential to utilize all kinds of habitat and food resources in a river (Kara and Alp, 2005; Osoz et al., 2005; Montori et al., 2006; Ruginis, 2008). Within the 29 specimens in which gut contents were analyzed, 8 individuals were identified as fed on aquatic and terrestrial organisms and 5 of them were caught in the river Tisovica. However, the low contributions of terrestrial organisms in diets indicate that trout from studied rivers feed mainly on aquatic invertebrates, which is in concordance with reported data from studies elsewhere (Osoz et al., 2005; 2005; Montori et al., 2006; Ruginis, 2008). Diet composition was characterized by relatively wide food spectrum, which was similar to reported findings, especially in comparison with literature data concerning trout diet from different European rivers (Osoz et al., 2005; Montori et al., 2006; Ruginis, 2008; Skorić et al., 2013). The diet of brown trout in studied rivers principally consists of caddis fly larvae, while relative importance of other food items varies among the investigated rivers. Observed differences most probably reflect differences in prey availability, types of habitat and fish size among the rivers. In terms of relative prominence of consumed prey, it is obvious that examined fish showed affinity towards larger and mobile prey, what was expected as a trout is a visual predator (Osoz et al., 2005). Finally, we have to point out that our results are based on relatively small number of examined fish collected in one month and further studies are needed.

CONCLUSION

Results on diet composition showed that brown trout in the investigated rivers feed mainly on aquatic invertebrates with Trichoptera larvae as the most important food item.

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THE COMPARISON OF TWO HORMONAL INDUCTION METHOD IN THE HEVIZ CARP (*CYPRINUS CARPIO CARPIO MORPHA HUNGARICUS*) MALES

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POREĐENJE DVE METODE HORMONSKE INDUKCIJE KOD MUŽIJAKA HEVIZ ŠARANA (*CYPRINUS CARPIO CARPIO MORPHA HUNGARICUS*)

Apstrakt

Divlja populacija šarana *Cyprinus carpio carpio* (Evropska podvrsta, Pintér, 1989) se stalno smanjuje usled intenzivne hibridizacije sa domaćim rasama. Od 2008. godine divlji šaran se nalazi na crvenoj listi IUCN. Ribnjački šaran se nalazi u kategoriji „ranjivi takson"; nasuprot njemu Dunavska subpopulacija (*Cyprinus carpio carpio morpha hungaricus*, Heckel 1836) je u kategoriji „krajnje ugrožen takson" (Freyhof and Kottelat, 2008). Jezero Hévíz je najveće, biološki aktivno, prirodno, termalno, lekovito jezero na svetu. Visoka temperatura (srednja vrednost: 30,5°C) i poseban hemijski sastav jezera zajedno doveli su do biološki jedinstvene zajednice u jezeru (Ponyi, 2002). Prema našim saznanjima, u jezeru se može naći izolovana jedna od poslednjih genetski čistih populacija *Cyprinus carpio carpio morpha hungaricus.* Ipak, posebni termalni uslovi doveli su do jedinstvenih ihtioloških, fizioloških i reproduktivnih osobina ove izolovane populacije: relativno niska standardna dužina i težina, stalna proizvodnja gameta, niska tolerancija na promene temperature i stres itd..). Unapređenje veštačkog razmnožavanja bi moglo da podrži konzervaciju ove važne, genetički čiste subpopulacije šarana. Cilj studije je bio razvoj dve različite metode indukcije oslobađanja spermatozoida, spermijacije, kod mužijaka iz jezera Hévíz.

Za oba eksperimenta ribe su lovljene odgovarajućim mrežama 4 x 36 m ili pecanjem iz jezera Hévíz. Spermijacija je hormonski indukovana na osnovu eksperimentalnog plana. Parametri pokretljivosti [progresivna pokretljivost-pMOT (%), kurvilinearna brzina-VCL-(µm/sec) pravolinijska brzina–VSL (µm/sec), linearnost-LIN (%), amplituda lateralnog po-

meranja glave-ALH (µm), frekvencija otkucaja-BCF (Hz)] su zabeleženi posle aktivacije upotrebom rastvora za ciprinide (45 mM NaCl, 5 mM KCl, 30 mM Tris, pH 8±0.2, Saad et al., 1988) u mešavini sa oko 0.01g BSA (Bovine Serum Albumin). Motilitet je analiziran pomoću CASA sistema (Computer-assisted Sperm Analysis, Sperm VisionTM v. 3.7.4., Minitube of America, Venture Court Verona, USA). Merenja su rađena najmanje u duplikatu.

Plan eksperimenta:

Eksperiment 1. Hormonalna indukcija na terenu

Obrazovane su dve eksperimentalne grupe (*N*=8-8). Jedna grupa mužijaka je indukovana odmah na Jezeru Hévíz (4 mg ekstrakta hipofize šarana na kg^{-1}), dok druga grupa nije indukovana, služila je kao kontrola. Pokretljivost je beležena u obe grupe 12 sati posle injekcije.

Eksperiment 2. Hormonalna indukcija mužijaka u recirkulacionom sistemu RAS

Mužijaci (*N*=9) su držani u RAS (od oko 2-4 nedelje) su hormonski indukovani postepeno tokom 1 nedelje (1, 2, 2 i 4 mg ekstrakta hipofize šarana na kg^{-1} u intervalu od dva dana). Parametri pokretljivosti sperme su beleženi posle 1 nedelje.

Značajno viši pMOT (43±25%) je zabeležen kod indukovane grupe u odnosu na kontrolu (15±18%). Nije bilo značajnih razlika u VCL (injektovano: 68±16µm/sec, ne injektovano: 54±13µm/sec), VSL (injektovano: 51±13µm/sec, ne injektovano: 40±15µm/sec), LIN (injektovano:1±0.1%, ne injektovano: 1±0.1%), ALH (injektovano:1±0.3µm, ne injektovano:2±1µm) i BCF (injektovano: 23±3 Hz, ne injektovano: 20±5Hz).

Postepeno indukovanje spermijacije tokom jedne nedelje indukovalo je spermijaciju mužijaka u RAS (Table 1).

Tabela 1: Parametri pokretljivosti dobijeni sekvencijalnom indukcijom (N=9). Tabela predstavlja srednje vrednosti i SD.

Progressive motility (%)	**VCL (µm/s)**	**VSL (µm/s)**	**LIN (%)**	**ALH (µm)**	**BCF (Hz)**
94±3	**139±21**	**102±14**	**73±5**	**2±0.4**	**30±1**

Može se zaključiti da su obe metode hormonske indukcije bile uspešno primenjene na populaciju *Cyprinus carpio carpio morpha hungaricus* osetljivu na stres. Naši rezultati podržavaju veštačko razmnožavanje ove genetski čiste populacije šarana u budućnosti.

Ključne reči: *Hévíz šaran, sperma, pokretljivost, krioprezervacija, CASA*

Abstract

The wild populations of *Cyprinus carpio carpio* (European subspecies; (Pintér, 1989)) are continuously decreasing because of the intensive hybridization with domestic carp landraces. Since 2008, the wild common carp is listed on IUCN Red List. The common carp is in "vulnerable" category; in contrast the Danube subpopulation (*Cyprinus carpio carpio morpha hungaricus*, Heckel 1836) is "critically endangered" fish (Freyhof and Kottelat, 2008). Lake Hévíz is the biggest, biologically active natural thermal spa lake in the world. The high temperature (average: 30.5°C) and the especial chemical composition of the Lake

Hévíz together eventuated a unique biological community in the pond (Ponyi, 2002). According to our knowledge, one of the last genetically pure *Cyprinus carpio carpio morpha hungaricus* population can be found isolated in the lake. However, the special thermal conditions resulted an unique ichthyologic, physiological and reproductive attributions to this isolated carp population (relatively low average standard length and body weight, continuous gamete production, low water temperature and stress tolerance etc.). The improvement of the artificial propagation can support the conservation of this important genetically pure carp subpopulation. The aim of our study was to develop two different methods for the induction of spermiation in the males caught from Lake Hévíz.

For both experiments, fish were caught with a 4 x 36 m benthic gill net or by angling from Lake Hévíz. Spermiation was hormonally induced according to the experimental design. Motility parameters [progressive motility-pMOT (%), curvilinear velocity-VCL-(µm/sec) straight line velocity–VSL (µm/sec), linearity-LIN (%), amplitude lateral head displacement-ALH (µm), andbeat cross frequency-BCF (Hz)] were recorded following activation using a saline solution designed for cyprinids (45 mM NaCl, 5 mM KCl, 30 mM Tris, pH 8±0.2, Saad et al., 1988) in a mixture with approximately 0.01g BSA (Bovine Serum Albumin). Motility was analysed with a CASA system (Computer-assisted Sperm Analysis, Sperm VisionTM v. 3.7.4., Minitube of America, Venture Court Verona, USA). Measurements were carried out at least in duplicates.

Experimental design:

Experiment 1. Hormonal induction in field condition

Two experimental groups (*N*=8-8) were established following. One group of males was hormonally induced immediately on field at Lake Hévíz (4 mg carp pituitary body weight kg^{-1}) where the other group was not injected (as control). Motility was recorded in both groups 12 hours post injection.

Experiment 2. Hormonal induction of males kept in recirculating system

Males (*N*=9) kept in the recirculating system (since approximately 2-4 weeks) was hormonally induced sequentially for 1 week (1, 2, 2 and 4 mg carp pituitary body weight kg^{-1} in two days interval). Sperm motility parameters were recorded after 1 week.

A significantly higher pMOT (43±25%) was recorded with the injected group compare with the not injected (15±18%). No significant difference was measured in VCL (injected: 68±16µm/sec, not injected: 54±13µm/sec), VSL (injected: 51±13µm/sec, not injected: 40±15µm/sec), LIN (injected:1±0.1%, not injected: 1±0.1%), ALH (injected:1±0.3µm, not injected:2±1µm) and BCF (injected: 23±3 Hz, not injected: 20±5Hz).

The one week sequential injection induced the spermiation in males kept in a recirculating system (Table 1).

Table 1. The motility parameters obtained from one week sequential injection (N=9). The table represents Mean and SD.

Progressive motility (%)	VCL (µm/s)	VSL (µm/s)	LIN (%)	ALH (µm)	BCF (Hz)
94±3	**139±21**	**102±14**	**73±5**	**2±0.4**	**30±1**

In conclusion, both methods of hormonal induction were successfully applied for this stress sensitive population of *Cyprinus carpio carpio morpha hungaricus*. Our results can support the effective artificial propagation of this genetically pure carp population in the future.

Keywords: *Hévíz carp, sperm, motility, cryopreservation, CASA*

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ECOLOGICAL STUDY ON HOLOTHURIA (HOLOTHUROIDEA, ECHINODERMATA) IN THE ROCKY SHORE IN THE NORTH OF PERSIAN GULF

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EKOLOŠKA STUDIJA MORSKIH KRASTAVACA (HOLOTHUROIDEA, ECHINODERMATA) NA STENOVITOJ OBALI NA SEVERU PERSIJSKOG ZALIVA

Apstrakt

Tokom 2013 godine, sva četiri godišnja doba, ispitivana je ekologija roda Holothuria, na 6 lokaliteta izabranih na stenovitoj obali Iranskih voda u persijskom zalivu. Identifikovano je 2 vrste morskih krastavaca i određena je njihova abundanca. Na dva lokaliteta morski krastavci nisu utvrđeni.nije bilo značajne razlike u ekološkim indeksima. Indeks diverziteta (H’) i indeks dominantnosti (D) klasifikovani su u klasu « nisko ».

Ključne reči: *Holothuria, eologija, stenovita obala, Persijski zaliv*
Key words: *Holothuria, ecology, rocky shore, Persian Gulf.*

INTRODUCTION

Holothuroidea which is known as sea cucumbers, are marine animals with leathery skin contained small calcareous spicules and elongated body (Barnes *et al.*, 2009). They mostly live in the intertidal area (Smirn*ov et a*l., 2000). Sea cucumbers are dominant in shallow water and litoral ecosystems (Dar and Ahmad, 2006). Distribution of Holothuroidea are mostly in tropical and subtropical shallows, but the greatest abundance and diversity are reported from Indo-pacific tropical (Al-Rashdi *et al.*, 2007). Also, they play an important role in coral reef and food chains as detritus feeders and suspension feeders (Bruckner *et al.*, 2003), and help to increase coral reef production (Wheeling *et al.*, 2007).

Sea cucumbers can affect structure and chemical characters of sediment, decrease microalgae productions, and bacterial activity by consumption and decomposing detritus and other organic matter in the sea bottom sediment (Slater and Carton, 2009). Sea cucumbers serve a useful role in the marine ecosystem as they help recycle nutrients, breake down detritus and other organic matter after which bacteria can continue the degradation process.

Whereas, there is not much publication and information about the distribution of the sea cucumbers in the Persian Gulf and Gulf of Oman (Pourvali *et al.* 2014 a,b), this study was carried out in the Iranian waters in the Persian Gulf. Our objectives were to determine the diversity and abundance related to the physic-chemical conditions.

MATERIALS AND METHODS

During April 2013 to December 2013 the diversity, density and distribution of Holothuroidea species were studied in 6 stations in intertidal zone of Iranian water in the Persian Gulf (Figure 1, Table 1). The present study was carried out in four seasons namely spring, summer, autumn and winter. At each station, three transect line were selected (English *et al.,* 1997) which the abundance of sea cucumber was counted using 10*20 m^2 quadrat with three replications in each transect. Sampling was conducted in maximum low tide. All sea cucumber found in each transect were counted and some individuals were collected for identification. Physico-chemical parameters were recorded by portable CTD (Digital SP701) in each station.

Table 1. Position of site sampling

stations	longitude	latitude
St. 1	50º 19' 28/32" E	29º 44' 10/80" N
St. 2	50º 51' 12/88" E	28º 51' 25/58" N
St. 3	51º 54' 11/46" E	27º 49' 56/88" N
St. 4	52º 37' 25/72" E	27º 24' 6/16" N
St. 5	54º 64' 87/43" E	26º 52' 95/65" N
St.6	54º 90' 14/28" E	26º 57' 50/18" N

Figure. 1. Map of site sampling in the Persian Gulf.

Data were processed by PAST software and SPSS 16 (Hammer *et al.,* 2001). Comparison of Holothuroidea diversity and distribution were analyses using Margalef species richness (d) index, Dominance (D) index, Shannon-Wenear (*H’*) index, Pielou’s evenness (J’) index and similarity (B) index. Also, the density of individuals (m2) per species were calculated for each site.

RESULTS AND DISCUSSION

The mean of salinity, temperature, DO and pH are shown in the Table 2. Results of one way ANOVA for salinity, temperature and DO showed a significant difference between seasons (0.05>P‹ Kruskal-Wallis H). There was no significant difference for pH.

Table 2. mean of salinity, temprature, DO and pH

Factor	Spring	Summer	Autumn	Winter
Temperature	32.60±2.32	37.13±1.98	27.45±2.00	25.76±2.41
DO	10.73±2.09	9.57±2.93	11.30±1.69	11.51±1.77
Salinity	42.68±0.10	44.48±0.17	41.32±0.15	42.03±0.09
pH	8.25±0.18	8.44±0.24	8.68±0.17	8.60±0.29

Annual temperature changes in study area show a decrease in autumn and winter followed by an increase of the temperature in spring and summer. Statistical analyses indicated a significant difference between sampling sites. Regarding changes in physico-chemical parameters such as: DO and CO_2 (Lawson, 1995), it is possible that salinity affected sea cucumber reproduction. Sea cucumber body osmotic pressure changes due to changes in sea water parameters (Dong *et al.*, 2008). Temprature has limited and direct effect on animal activity, while salinity has indirect effect - metabolism changes, growth, feeding and life history (Kinne, 1971).

The maximum temperature was in summer by 39.40±0.17 and the minimum temperature was 21.60±0.19 in winter. The maximum salinity was 45.72±0.16 in summer and the minimum salinity was 39.82±0.16 in autumn. The maximum DO was in winter by 13.78±0.16 and the minimum was 5.88±0.16 in summer. The maximum pH was recorded in summer by 8.99 and the minimum was 8.19 in winter.

During four seasons sampling, two species of Holothuria were observed throughout all the sites sampled (Table 2). Both of these two species have been previously recorded in Iranian water: *Holothuria parva* and *Holothuria arenicola*. But there is no any survey on sea cucumber ecology in this area. Moreover, no Holothuria species were sampled in stations 1 and 6 (Table 3).

Table 3. Total sea cucumber sampled

Station	Species	Spring	Summer	Autumn	Winter
St. 1	*H. parva*	0	0	0	0
	H. arenicola	0	0	0	0
St. 2	*H. parva*	12	15	22	0
	H. arenicola	1	2	2	1
St. 3	*H. parva*	54	47	38	41
	H. arenicola	4	3	7	4
St. 4	*H. parva*	17	13	15	0
	H. arenicola	5	2	4	3
St. 5	*H. parva*	37	55	47	32
	H. arenicola	6	2	4	5
St. 6	*H. parva*	0	0	0	0
	H. arenicola	0	0	0	0
Sum		136	139	139	86

Since during the survey, in stations 1 and 6 no Holothuria species were found; therefore, these sites was omitted from the Biodiversity analyses. The mean of ecological indices are given in the Table 4. There is no significant difference for the ecological indices (Margalef species richness index, Dominance index, Shannon-Wenear index, Pielou's evenness index), in P>0.05.

Table 4. Mean of ecological indices

Ecological index	Spring	Summer	Autumn	Winter
Margalef (d) index	0.203	0.202	0.224	0.160
Shannon-Wenear (*H'*) index	0.497	0.313	0.513	0.579
Dominance (D) index	0.198	0.109	0.205	0.242
Pielou's evenness (J') index	0.522	0.346	0.535	0.612

The maximum Margalef species richness was recorded in summer. The maximum and minimum Shannon-Wenear index were recorded in winter and summer, respectively. The maximum and minimum Pielou's evenness and Dominance index were recorded in winter and summer, respectively.

According to Mason (1981), the diversity index (H') could be classified into three categories, namely low ((H' < 1), moderate (1 ≤ H' ≤ 3) and high (H' > 3). Therefore, it could be suggested that species diversity of Holothuroidea in Persian Gulf was low. Shannon diversity index (H') in study area is less than H' index of Echinoderms community in Tanjung Tiram (Tuapattinaja, *et al.,* 2014), Kema waters, North Sulawesi (Supono & Arbi 2010), and too much lower than lower than Diversity indexes of Echinoderms at Kairatu, West Seram (Rumahlatu et al 2008) and at Tanjung Merah, North Sulawesi (Yusron & Susetiono 2005).

Legendre and Legendre (1993) stated that the dominance index (D) could be divided into three categories: low (D < 0.4), moderate (0.4 < D <0.6) and high (D > 0.6). So, the Holothuroidea community in the Persian Gulf belonged to low value. Based on Magurran (1991), evenness index ranging from 0 to 1, in which E = 1 indicating equal number of individual for every species in the community. Furthermore, Odum (1975) stated that a community is in stable condition if the value of E ≥ 0.6. Based on this criterion, Holothuria community in winter in the study area is the only one which is in steady condition.

Results show that *H. parva* with 89% abundance was a dominant species in all sites. Heding (1940) find out *H. parva* had high resource and relative abundance in the study area. Distribution of sea cucumber will be affected by bottom characters, light intensity, food availability, salinity fluctuations (Mercier *et al.*, 2000, Pourvali et al., 2014 a)

H. parva and *H. arenicola* were found in Stations 2, 3, 4 and 5, which shows better condition for species presence, due to bottom heterogeneity or preferring of larval settling. The Holothuroidea prefer gravel, sand and muddy substrate. The Holothuroidea was also dominant in Hormuz Island and preferred mud and gravel, and rock with mud and gravel substrates (Pourvali et al. 2014 a). Dissanayke and Stefansson found high abundance of sea cucumber in rocky shore while mud and sand had low density of sea cucumber. It is reported that rocky shore saved holothuroidea against tidal current and predators (Dissanayake and Stefansson, 2010). It is suggested that the occurrence of echinoderms depend on environmental and biological factors (Uneputty *et al.*, 2017). Settlement is the initial processes determining the structure of population (Rodriguez et al. 1993)

Results show density changes between seasons has no significant difference. In the similar survey on Holothuria genus in Brazil no significant difference for density was reported (Mendes et al., 2006).

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INVESTIGATION OF EMBRYOGENESIS FOLLOWED FERTILIZATION WITH SPERM EXPOSED BY HEAVY METALS, IN ZEBRAFISH (*DANIO RERIO*)

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ISTRAŽIVANJE EMBRIOGENEZE KOD ZEBRICE (*DANIO RERIO*) POSLE OPLOĐENJA SPERMOM KOJA JE IZLOŽENA TEŠKIM METALIMA

Apstrakt

Cilj studije je bio ispitivanje efekata teških metala na fertilizacionu sposobnost, na preživljavanje tokom embriogeneze i na razvoj deformiteta posle izlaganja mleča zebrice teškim metalima. Mleč riba ima mnogih prednosti (neinvazivno dobijanje, merljive vrednosti, reakcija doza-odgovor kod toksične izloženosti) što je pogodno za *in vitro* toksikološke test sisteme. Zbog toga su poslednjih godina publikovane brojne studije u kojima je mleč riba korišćena kao indikator toksične izloženosti. Uglavnom su ispitivani parametri mortaliteta i odgovori antioksidanata kod izložene sperme. Istraživanje oplođenja posle izlaganja mleči različitim zagađujućim materijama nije uopšte široko rasprostranjeno u ovakvim studijama. Mleč zebrice, uprkos svojim prednostima (lako održavanje u recirkulatornim sistemima, mrest tokom cele godine, prikupljanje mleča konstantnog kvaliteta bez hormonske indukcije) je korišćena u *in vitro* toksikološkim ispitivanjima samo u tri navrata (Reinardy et al. 2013, Acosta et al. 2016, Kollár et al. 2018) i nema publikacija koje se odnose na fertilizacionu sposobnost izloženih spermatozoida zebrice.

Mleč zebrice dobijena ceđenjem i izložena različitim koncentracijama testiranih teških metala (Cr, Ni, Cu, Hg, As, Cd, Zn), tokom 30 ili 120 minuta. Ženke su takođe bile isceđene i jaja oplođena spermom koja je bila izložena teškim metalima. Ispitivana je stopa fertilizacije/oplođenja, 48h preživljavanje embriona i stopa embrionalnih deformiteta.

Primećen je sličan obrazac u slučajevima izloženosti Cr, Ni, Cu i Hg: gde dužina trajanja izloženosti nije uticala na stopu oplođenja, ali je efekat koncentracije bio značajan. Posle izlaganja Cr, stopa oplođenja je značajno opala na 100, 150 i 200 mg/L posle 120 minuta. U slučaju Ni već je 600 mg/L snizilo značajno stopu oplođenja posle 30 minuta, efekat se paralelno povećavao sa porastom koncentracije i dužine izlaganja. Posle izlaganja Cu, stopa oplođenja je značajno opala na 25 mg/L posle 120 min i na 50 mg/L posle 30 min, kao i posle 120 min. U slučaju Hg, stopa oplođenja se na 2,5 mg/L smanjila posle 30 min, a delovanje još više izraženo porastom koncentracije i vremena izlaganja. Samo u slučaju As i koncentracija i dužina izlaganja su delovale na stopu oplođenja: na 30 min, samo 200 mg/L je dovelo do redukcije stope oplođenja, dok je 50 mg/L snizilo stopu posle 120 min. U slučaju Cd i Zn ni dužina izloženosti mleča, kao ni koncentracije testiranih metala nisu značajno uticali na stopu oplođenja. 48 časovno preživljavanje embriona i stopa deformiteta embriona (edem žumancetne kese, perikardijalni edem, malformacije repa) nisu se značajno razlikovali u odnosu na kontrolu.

Poređenjem naših rezultata sa prethodno objavljenim radovima, može se tvrditi da je u nekim slučajevima fertilizaciona sposobnost osetljivija nego motilitet sperme, dok je u drugim slučajevima manje osetljiva. Povrh toga, bilo je slučajeva da su oba parametra izloženosti ista. Posledično, stopa fertilizacije može takođe da ukaže na toksičnu izloženost sperme, pa je upotreba ovog metoda takođe prihvatljiva za toksikološka *in vitro* ispitivanja. Teški metali snižavaju stopu oplođenja iako se oštećenja DNK koja utiču na preživljavanje embriona i embrionalno razviće nisu manifestovala u ispitivanim spermatozoidima koji su preživeli izloženost teškim metalima i zadržali sposobnost oplođenja.

Ključne reči: *zebrica, sposobnost oplođenja, preživljavanje embriona, embrionalni deformiteti, teški metali*

Abstract

The objective of our study was to investigate the effects of heavy metals on fertilizing ability of exposed zebrafish (*Danio rerio*) sperm, on embryonic survival and on development of embryonic deformities followed fertilization with exposed sperm. Fish sperm has many advantages (non-invasive gaining of sperm, measurable endpoints, dose-response reaction for the toxic exposure) which make it for a suitable *in vitro* toxicological test system. Due to this, numerous studies have been published in the last years in that fish sperm was used as indicator of toxic exposure. Mostly the motility parameters and antioxidant response of exposed sperm were examined in these works. The investigation of fertilization followed exposure of fish sperm to different toxicants is not a widespread endpoint in these studies at all. Zebrafish sperm, despite of its advantageous features (easy maintenance in recirculation systems, year-round spawning, sperm collection with constant quality without hormonal induction) has been used to *in vitro* toxicological-aimed studies only in three cases (Reinardy et al. 2013, Acosta et al. 2016, Kollár et al. 2018), however, there are not any publications regarding the fertilizing ability of exposed zebrafish sperm.

Zebrafish sperm was stripped and exposed to different concentrations of the tested heavy metals (Cr, Ni, Cu, Hg, As, Cd, Zn), the exposure duration was 30 or 120 minutes. Females were also stripped and the eggs were fertilized with the exposed sperm. The examined endpoints were the fertilization rate, the 48-hour survival of embryos and the rate of embyonic deformities fertilized with exposed sperm.

The similar pattern was observed in cases of Cr, Ni, Cu and Hg, where the exposure duration of sperm did not affect the fertilization rate, however, the effect of concentration was significant. Following exposure to Cr, the fertilization rate decreased at 100, 150 and 200 mg/L after 120 min significantly. In case of Ni, already 600 mg/L decreased the fertilization rate significantly after 30 min which effect increased in parallel with increasing of concentration and exposure duration. Following exposure to Cu, the fertilization rate de-

creased significantly at 25 mg/L after 120 min and at 50 mg/L after 30 as well as 120 min. In case of Hg, the fertilization rate reduced at 2.5 mg/L after 30 min which became more affected with increasing of concentration and exposure duration. Only in case of As, the concentration as well as the exposure duration affected the fertilization rate of exposed sperm. At 30 min, only 200 mg/L caused the reduction of fertilization rate, however, already 50 mg/L reduced this parameter after 120 min. In cases of Cd and Zn, neither the exposure duration of sperm, nor the concentration of the tested metals affected the fertilization rate significantly. The 48-hour survival of embryos and the rate of embryonic deformities (yolk oedeme, pericardial oedeme, tail malformation) did not differ from the control in case of any tested heavy metals significantly.

Comparing our results with the previously published articles, it can be stated that in some cases the fertilizing ability proved to be more sensitive than the motility of sperm, while in other cases it was less affected than the motility. Furthermore, there were cases in which the sensitivity of the two endpoints of sperm exposure were the same. Consequently, the fertilization rate can also indicate the toxic exposure of sperm, due to this, its application as *in vitro* toxicological endpoint is reasonable. Heavy metals can reduce the fertilization rate, however, impairments in DNA which would affect to the survival of embryos and to the embryonic development have not been manifested in those spermatozoa, which survive the heavy metal exposure and are able to fertilize.

Keywords: *zebrafish, fertilizing ability, embryonic survival, embryonic deformities, heavy metals*

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DEVELOPMENT OF SPERM VITRIFICATION METHODS FOR EIGHT FISH SPECIES

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RAZVOJ METODE VITRIFIKACIJE SPERME KOD OSAM VRSTA RIBA

Apstrakt

Iako su postupci za krioprezervaciju sperme razvijeni za brojne vrste i dalje se publiku-ju protokoli za vitrifikaciju sperme za pojedine vrste. Cilj ovog rada je bio razvoj species-specifičnih protokola za vitrifikaciju sperme sledećih vrsta: lipljen (*Thymallus thymallus*), pastrmka glavatica (*Salmo marmoratus*), potočna pastrmka (*Salmo trutta*), zebrica (*Danio rerio*), šaran (*Cyprinus carpio*), linjak (*Tinca tinca*), grgeč (*Perca fluviatilis*) i evropska jegulja (*Anguilla anguilla*). Vitrifikacija predstavlja promenu agreganog stanja tečnosti u amorfno ili staklasto stanje koje jedino može biti postigunto veoma brzim hlađenjem (10^6-10^{10} °C/s). Vitirfikacija sperme može da se sprovede bez formiranja kristala leda primenom malih zapremina tečnosti (u mikrolitrima). Za evaluaciju testiranih protokola praćena je progresivna pokretljivost vitrifikovane/zagrejane sperme pomoću CASA programa (Computer Assisted Sperm Analysis). Pored toga, u slučaju tri vrste (šaran, grgeč i zebrica) izvršen je i test oplodnje vitrifikovanom spermom. Tokom razvoja protokola testirani su različiti kriomedijumi, ekstenderi i razblaženja korišćenih supstanci. Za sve eksperimente, kako pribor za vitrifikaciju korišćeni su Cryotop nosači (Kitazato-Dibimed, za 2 µl rastvora).

Za testirane predstavnike porodice Salmonidae (lipljen, potočna i pastrmka glavatica) optimalan protokol je bio: razblaženje 1:1 (sperma:kriomedijum sa krioprotektantom), 15% metanola (MeOH) i 15% propilen glikola (PG). Progresivna pokretljivost uzoraka nakon zagrevanja za salmonide je varirala od 8.6±0.7% (lipljen) do 13.2±5.8% (potočna pastrmka). Među šaranskim vrstama (šaran, linjak i zebrica) optimalani protokoli su se znatno razlikovali po pitanju kriomedijuma i razblaženja. Uzrok za ove razlike može biti diverzitet u okviru porodice Cyprinidae. Među razvijenim protokolima, optimalna razblaženja su bila 1:4 (zebrica i linjak) i 1:100 (šaran), a krioprotektanti: 15% MeOH + 15% PG (zebrice) i 10% MeOH + 10% metil glikol +10% PG (šaran i linjak). Progresivna pokretljivost nakon zagrevanja sperme je varirala između 3.1±0.1 (linjak) i 10.8±5.2% (zebrica). U slučaju grgeča,

optimalan protokol za vitrifikaciju je: razblaženje 1:5, Tanaka ekstender, 15% MeOH + 15% PG. Progresivna pokretljivost sperme nakon zagrevanja je bila 14±1.6%, a test oplodnje je rezultirao izlegnutim larvama (4.9±4.8%). Optimalan protokol za jegulju je bio: razblaženje 1:1, Tanaka ekstender, 15% MeOH + 15% PG.

Ključne reči: *krioprezervacija, specifično za vrstu, protocol za vitrifikaciju, mleč*

Abstract

Fish sperm cryopreservation protocols had been carried out in numerous species, beside this in the recent years sperm vitrification protocols were published in some species. The aim of our studies was to develop species-specific sperm vitrification protocols in the following species: grayling (*Thymallus thymallus*), marble trout (*Salmo marmoratus*), brown trout (*Salmo trutta*), zebrafish (*Danio rerio*), common carp (*Cyprinus carpio*), tench (*Tinca tinca*), Eurasian perch (*Perca fluviatilis*) and European eel (*Anguilla anguilla*). 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). Sperm vitrification methods can be carried out without creation of ice crystals, using cooling volumes in the range of microliters. For the evaluation of the tested protocols, progressive motility of fresh and vitrified-warmed sperm was evaluated with computer assisted sperm analysis (CASA). Beside this, in case of three species (common carp, Eurasian perch, zebrafish) fertilization trials had been also carried out. Numerous cryomedia, extender and dilution rate had been tested. For all vitrification experiments Cryotop was used as cooling device (Kitazato-Dibimed, for 2 µl of solution).

In the Salmonidae family (grayling, brown trout, marble trout) the optimal protocol was as follows: dilution ratio of 1:1 (sperm:cryomedia including cryoprotectants), 15% methanol (MeOH) and 15% propylene-glycol (PG). The post-thaw progressive motility values in this family varied between 8.6±0.7% (grayling) and 13.2±5.8% (brown trout). In the Cyprinidae family (common carp, tench, zebrafish) the optimal protocols were considerably different regarding the optimal cryomedia and dilution rate. The reason of this can be the diversity of carp species. In the optimal protocols for the three species dilution rates varied between 1:4 (zebrafish, tench) and 1:100 (common carp), and different cryoprotectants had been used: 15% MeOH + 15% PG (zebrafish), and 10% MeOH + 10% methyl-glycol +10% PG (common carp, tench). The post-thaw progressive motility values in this family varied between 3.1±0.1 (tench) and 10.8±5.2% (zebrafish). In case of Eurasian perch the optimal vitrification protocol was as follows: 1:5 dilution ratio, Tanaka extender, 15% MeOH + 15% PG. The post-thaw progressive motility was 14±1.6%, and fertilization test with vitrified sperm resulted in hatching larvae (4.9±4.8%). In case of European eel the optimal vitrification protocol was as follows: 1:1 dilution ratio, Tanaka extender, 15% MeOH + 15% PG.

Keywords: *cryopreservation, species specific, vitrification protocol, sperm*

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HISTOPATHOLOGICAL CHANGES INDUCED BY COPPER IN THE LIVER OF STELLATE STURGEON (*ACIPENSER STELLATUS* PALLAS, 1771)

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HISTOPATOLOŠKE PROMENE IZAZVANE BAKROM U JETRI PASTRUGE (*ACIPENSER STELLATUS* PALLAS, 1771)

Apstrakt

Iako se smatra da je zagađenje Dunava teškim metalima veoma visoko, samo je nekoliko studija o histopatološkim efektima na jesetarske vrste. Zbog visokog sadržaja masti u telu, dužine života, dugog juvenilnog stadijuma i ishrane bentosom, jesetre su pod velikim potencijalnim rizikom akumulacije teških metala u tkivima. Cilj istraživanja je bio određivanje efekata bakra, jednog od najprisutnijih metala u Dunavu, na histologiju jetre pastruge (*Acipenser stellatus).* Mlađ pastruge je bila izložena 7 I 14 dana koncentraciji od 10% i 25% od vrednosti LC_{50} bakra, koja je prethodno određena kao 0.547 mg/l. u jetri kontrolnih riba, usled sadržaja masti, ćelije su postale hipertrofisane, aspekti povezani sa masnom jetrom (steatoza), što je promena vidljiva kod svih veštački hranjenih riba. Posle izlaganja bakru jetra pastruge je imala histopatološke promene koje su bile ozbiljnije sa rastom koncentracije i vremenom izlaganja. Bakar je uticao na oba tipa promena jetrinog parenhima : nekroza i proširenje prostora između hepatocita, kao i cirkulatorne promene kao što je proširenje sinusoidalnih prostora, kongestiju krvnih sudova i hemoragije. Takođe, povećan broj limfocita, melanomakrofagnih agregata i mast ćelija su uočeni. Istraživanje je pokazalo da bakar izaziva ozbiljne histološke promene i potvrdilo njegovo imunotoksično delovanje.

Ključne reči: *Acipenser stellatus, bakar, cink, histopatologija*

Abstract

Although water pollution by heavy metals in the Danube is considered to be very high, there are a few studies on their histopathological effects on sturgeons. Due to the high lipid content of their body, long lives, long juvenile stage and benthivorous diet, sturgeons are at a high potential risk for accumulating metals in their tissues.

The aim of this study was to determine the effects of copper, one of the most encountered metal in the Danube, on the liver histology of *Acipenser stellatus*. Stellate sturgeon (*Acipenser stellatus)* juveniles were exposed for 7 and 14 days to 10% and 25% of LC_{50} of copper, previously determined as 0.547 mg/l. In control liver, due to lipids loading, the cells became hypertrophied, aspects correlated with fatty liver (steatosis), as seen in all artificially fed fish. After copper exposure, stellate sturgeon liver presented histopathological changes that increased in severity with increasing concentration and exposure time. Copper determined both changes of hepatic parenchyma like necrosis and dilatation of the interhepatocyte space and circulatory changes as dilation of sinusoidal spaces, congestion of blood vessels, and hemorrhage respectively. Also increased number of lymphocyte, melanomacrophage aggregates and mast cell were seen. The study showed that copper is able to induce severe histological changes and confirms its immunotoxic action.

Keywords: *Acipenser stellatus, copper, zinc, liver, histopathology*

EFFECT OF NUTRITION LEVEL ON FEED CONVERSION, GROWTH AND VARIATION OF BODY LENGTH AND BODY WEIGHT OF BROWN TROUT (*SALMO TRUTTA* M. *FARIO*) FRY

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EFEKAT NIVOA ISHRANE NA KONVERZIJU HRANE, PRIRAST I VARIJACIJE DUŽINE I TEŽINE TELA MLAĐI POTOČNE PASTRMKE (*SALMO TRUTTA* M. *FARIO*)

Apstrakt

Eksperiment analize varijacija dužine i težine tijela potočne pastrmke realizovan je tokom 48 dana u laboratoriji za akvakulturu Poljoprivrednog fakulteta Univerziteta u Banjoj Luci. Ukupno je naseljeno 135 potočnih pastrmki u 9 protočnih akvarijuma iste zapremine (55 l), podijeljene u tri eksperimentalne grupe (n = 45/grupi) sa tri ponavljanja (n = 15 /ponavljanju). Prva grupa je L (velika) sa najvećom prosječnom individualnom masom (IBW) i dužinom tijela (IBL), druga grupa je M (srednja) sa srednjom IBW i IBL i treća grupa je S (najmanja) sa najmanjom IBW i IBL. Prosječna IBW, totalna (TL) i dužina tijela do račve repnog peraja (FL) iznosila je kod grupe "L" IBW = 1,05 g, TL = 4,63 cm and FL = 4,52 cm; kod grupe "M" IBW = 0,85 g, TL = 4,30 cm and FL = 4,20 cm i kod grupe "S" IBW = 0,76 g, TL = 4,21 cm and FL = 4,10 cm. Ukupna masa mlađi potočne pastrmke na početku je iznosila u L = 47,28 g, M = 38,04 g i S = 34,26 g. Masa i dužina tijela na početku i do 24 dana eksperimenta, statistički su se značajno razlikovale ($p<0,05$) između između L i M grupe, te L i S grupe. U zadnjem periodu, od 24 do 48 dana eksperimenta, utvrđena je statistički značajna razlika sredina ($p<0,05$) težine i dužine tijela između grupa L i S. U grupama L i M prisutan je trend rasta, a u grupi S pad koeficijenta varijacije težine tijela. Faktor kondicije sličan je u svim grupama (1,33-1,34). Diferencijacija težine tijela između jedinki izražena je u grupama L i M, kod kojih su utvrđene vrijednosti iznad 1, a u grupi S je ispod 1 što ukazuje na pad varijacije težine tijela. Diferencijacija totalne dužine tijela u svim slučajevima ima vrijednosti iznad 1. U grupi L zabilježen je najniži FCR i rast koeficijenta varijacije težine tijela. Povećanje dnevne količine hrane u grupama M i S manifestovalo se višom stopom rasta, višim relativnim prirastom težine i povećanjem FCR.

Ključne riječi: *varijacija težine i dužine, potočna pastrmka.*
Keywords: *weight and length variation, brown trout*

INTRODUCTION

Brown trout (*Salmo trutta* m. *fario*) is a species of Eurasian origin, but at present it is naturalized in many other areas all over the world (Sánchez-Hernández et al, 2011). Brown trout is a important species for fishing management in mountain rivers (Fisher & Burroughes, 2003; Augustyn et al., 2006), and represents a high-value species for recreational and commercial purposes, as well as for high quality cuisine as an alternative to rainbow trout (*Oncorhynchus mykiss*) (Turchini et al, 2005). During fish rearing, body weight and length variation can occur as a consequence of many different factors. Rauva et al. (1998) reported that in higher vertebrates, most of the heritable variation in BW is associated with differences in feed intake. For higher feeding levels, the variability decreased with the level of feed intake (Mambrini et al., 2004). The goal of this experiment was to establish effect of higher feeding levels on conversion coefficient, growth and body weight and length variation of the brown trout (*Salmo trutta* m. *fario*) fry with different starting, average and total body weight with high body weight variation coefficients.

MATERIAL AND METHODS

Lenght and weigh variation analysis experiment for brown trout was realised within 48 days in laboratory of aquaculture in Faculty of Agriculture of Banja Luka. 135 brown trout in total were inhabited in 9 flow system aquariums with same volume (55 l), divided in 3 experimental groups (n=45/exp. group) with 3 repeats (n=15/repeat). First group is L (large) with biggest average individual weight (IBW) and body length (IBL), the second group is M (medium) with medium IBW and IBL and the third group is S (small) with the lowest IBW and IBL. Average IBW, total body length (TL) and fork length (FL) amount was: "L" IBW = 1.05 g, TL = 4.63 cm and FL = 4.52 cm; group "M" IBW = 0.85 g, TL = 4.30 cm and FL = 4.20 cm and group "S" IBW = 0.76 g, TL = 4.21 cm and FL = 4.10 cm. Total mass of brown trout fry in the beginning was L = 47.28 g, M = 38.04 g and S = 34.26 g. The feed used was factory manufactured Nutra mixture. Fish were fed 2 times a day, and total feed spent was: L = 118.7 g; M = 114,0 g i S = 111.9 g. Basic water quality indicators (temperature, O_2 and pH) during experiment were measured with an oxi-meter (Oxi 330i/SET 2B20-0011, WTW Germany) and a pH-meter (pH 330i/SET 2A20-1011, WTW Germany), before first fish feeding. Body weight and length was measured with at the beginning of the experiment, after 24 days and after 48 days. Body weight was measured with a Acculab scale (±0.01 g), and body length was measured with a ihtiometer (±0.01 cm). Before measuring the individuals were anesthetisized. For calculations the following formulas were used: Weight gain: WG (%) = $((Wt-W_o)/W_o)*100$, where: W_t i W_0 final and initial body weight (g); Condition factor: CF = $(BW/L^3)*100$, where: BW-body weight (g), L-fork length (cm); Specific growth ratio: SGR = ((lnFBW-lnIBW)/D)*100, where: FBW-final body weight (g), IBW-initial body weight (g), ln- natural logarithm, D- days; Body weight coefficient of variation, CV_{BW} (%) = (100× body weight standard deviation (g))/mean body weight (g); Body length coefficient of variation, CV_{BL} (%) = (100× body length standard deviation (cm))/mean body length (cm); Body weight differentiation = $CV_{BWf} \times CV_{BWi}^{-1}$ Body length diferentiation = $CV_{BLf} \times CV_{BLi}^{-1}$ where: CV_{BWf}-coefficient of variation final body mass, CV_{BWi}- coefficient of variation initial body mass, CV_{BLf}- coefficient of variation final body length, CV_{BLi}-coefficient of variation initial body length; Feed conversion ratio: FCR = F/G, where: F-feed consumption, G-weight gain; Survival (%) = (N_t/N_0)x100 N_0-number of fish at the beginning; N_t-number of fish at the end.

For statistical analysis of the data (mean value, standard deviation and coefficient of variation, simple analysis of variance and LSD test) used computer program SPSS Statistics 17.0.

RESULTS AND DISCUSSION

By analysing the basic water characteristics (water temperature, dissolved oxygen, water oxygen saturation, and water pH) during the experiment, and statistical processing of the obtained results it was concluded that there were no significant differences of measuring aspects between experimental groups (Tab. 1.).

Table 1. The average value (Mean-SE) of measured water quality indicators

Parameter	Experimental groups		
	L	M	S
Water temperature (^oC)	14.98 ± 0.09^a	14.93 ± 0.10^a	15.11 ± 0.10^a
Dissolved oxygen (mg/l)	8.03 ± 0.07^a	8.04 ± 0.03^a	7.89 ± 0.05^a
Water oxygen Saturation (%)	80.39 ± 0.68^a	80.28 ± 0.83^a	79.19 ± 0.48^a
pH	7.215 ± 0.04^a	7.274 ± 0.04^a	7.173 ± 0.03^a

Values in the same row with the same letter index do not differ significantly statistically ($p>0.05$).

Distribution frequencies of total body length and body weight were shown on Figures 1. and 2., by experimental groups from the begging untill the end of experiment.

Weight and body length at the beginning and untill 24 days of experiment were significantly different ($p<0.05$) between L and M groups, and L and S groups. In the final period, from 24th to 48th day of experiment, a statistically significant average body weight and length difference ($p<0.05$) was present between groups L and S, and in the other combinations there were no significant average differences (Fig. 3.).

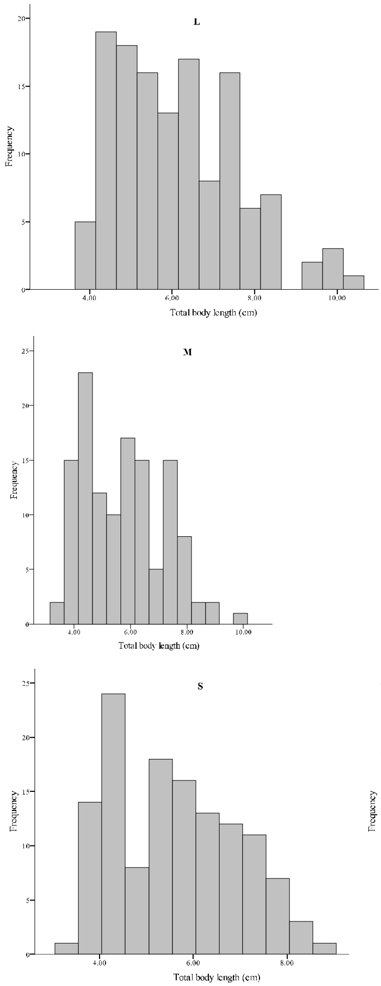

Figure 1: Frequency distribution of total body length (cm)

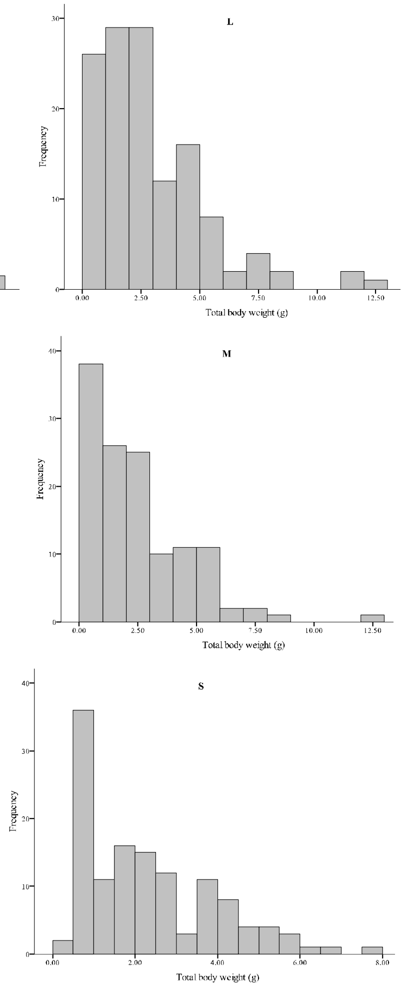

Figure 2: Frequency distribution of body weight (g)

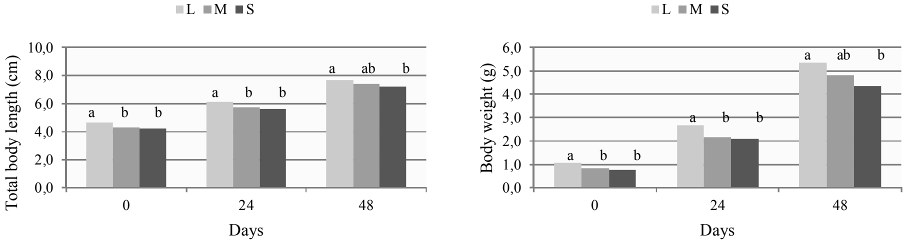

Figure 3: Total body length and weight of brown trout fry

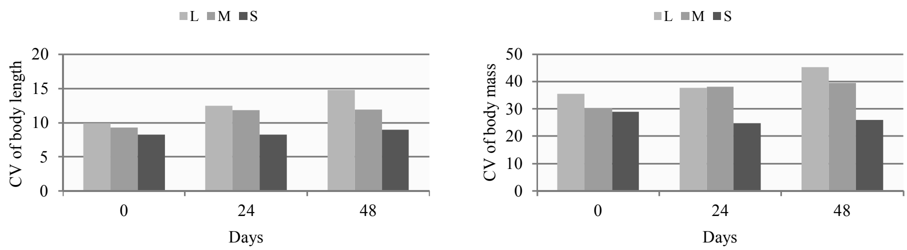

Figure 4: Coefficients of variation of the total body length and weight of brown trout fry

Body weight variation coefficient at the beginning was significantly manifested in all groups (Fig.. 4). In groups L and M a growth trend is present, and in group S body weight variation coefficient drop and in the end it was lower than the starting value, which was in compliance with allegations Rauv et al (1998) and Mambrini et al (2004). Body length variations of brown trouts were less expressed than body weight variations and have similar tendencies as with body weight.

Table 2: Final results of rearing brown trout fry with different nutrition levels (mean±SE)

Parameter		Experimental group		
		L	M	S
Condition factor (CF)		1.34±0.04[a]	1.34±0.01[a]	1.33±0.01[a]
Specific growth ratio (SGR)		3.39±0.03[a]	3.63±0.12[a]	3.62±0.02[a]
Feed conversion ratio (FCR)		0.67±0.05[a]	0.74±0.09[a]	0.83±0.03[a]
The levels of nutrition (%)	0-24 days	100	124	138
	25-48 days	100	130	129
Weight gain (%)		409.2	470.7	469.2
Initial body mass coefficient of variation (CV_{BWi}, %)		35.59	30.38	28.99
Final body mass coefficient of variation (CV_{BWf}, %)		45.23	39.45	26.05
Body weight diferentiation ($CV_{BWf} \times CV_{BWi}^{-1}$)		1.27	1.30	0.90
Initial body length coefficient of variation (CV_{BLi}, %)		9.93	9.30	8.26
Final body length coefficient of variation (CV_{BLf}, %)		14.77	11.90	8.93
Body length diferentiation ($CV_{BLf} \times CV_{BLi}^{-1}$)		1.49	1.28	1.08
Survival (%)		93.3	88.9	86.7

Values in the same row with the same letter index do not differ significantly statistically ($p>0.05$).

Condition factor was similar in all experimental groups (1.33-1.34) and it was introduced with a good body weight and length ratio, in accordance with the allegations Barnham & Baxter (1998) that the fish with condition factor arround 1.4 are proportional, in good condition that is. Individuals in groups M and S were given more feed then their suggested table values, but the FCR in those groups was higher, which points to lower usage feed. Relative weight growth and SGR were highest in groups with higher feeding norms (M and S), according to allegations of Savić & Mikavica (2017). Body weight differentiation between individuals was expressed in groups L and M, with values above 1, which points that there was a increase in variation of body weight compared to the beginning. In group S, body weight differentiation between individuals was below 1 which points to decrease of body weight variation of individuals fed with highest amount of feed compared to their body weight. Coefficient of variation of body weight and length are lower in S, which can be explained by less aggression in fighting for feed (Kindschi, 1988) because individuals in these groups had access to larger amount of feed. Total body length differentiation in all cases has values above 1, which is a sign of more expressed variability in the end compared to the beginning. Survival rate is highest in group L (93.3%), and the lowest in group S (86.7%).

In group L is the lowest FCR and body weight variation coefficient growth was recorded. Significantly higher daily feeding norms (compared to table values) in groups M and S have manifested in higher growth rate, higher relative weight growth, FCR increase, and in group S with body weight variation coefficient decrease.

CONCLUSION

By increasing daily feeding norms (higher feeding levels) for the brown trout fry, body weight variability decreases and growth rate increases, but at the same time there’s an increase in FCR.

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QUALITY ENHANCEMENT OF SUNFLOWER MEAL BY GRINDING AND AIR CLASSIFICATION FOR PRODUCING PROSPECTIVE PLANT PROTEIN SOURCE FOR FISH FEED

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OPLEMENJIVANJE SUNCOKRETOVE SAČME PRIMENOM USITNJAVANJA I VAZDUŠNE KLASIFIKACIJE U CILJU PROIZVODNJE POTENCIJALNOG IZVORA PROTEINA BILJNOG POREKLA U HRANI ZA RIBE

Apstrakt

Riblje brašno (RB) predstavlja glavni izvor proteina u ishrani uzgajanih riba. Visoka potražnja za RB i ograničena dostupnost sirovina za njegovu proizvodnju uzrok su smanjenje dostupnosti i visoke cene RB. U cilju održanja akvakulture alternativne visokoproteinske sirovine biljnog, mikrobiološkog i životinjskog porekla koriste se kao zamena za RB.

Zbog široke dostupnosti, visokog sadržaj proteina i relatvno niske cene, biljne proteinske sirovine se najviše koriste kao alternativa RB u industrijskoj proizvodnji hrane za ribe. Suncokretova sačma (SS) obično ima sadržaj proteina u rasponu 29–34% i primarno se koristi kao proteinsko hranivo za sve kategorije životinja. U poređenju sa drugim sačmama, prvenstveno sojinom, SS ima značajno manje antinutrijenata, ali i manju nutritivnu vrednost i nivo lizina. Osnovni konstituenti SS su ostaci proteinom bogatog suncokretovog jezgra i celulozom bogate ljuske koja je glavni uzrok visok sadržaj sirove celuloze (18–23%) zbog čega je i količina SS u ishrani riba ograničena. Uklanjanje ljuske smanjuje sadržaj celuloze i istovremeno povećava sadržaj proteina SS time i njenu nutritivnu i komercijalnu vrednost.

U cilju dobijanja visokoproteinskih frakcija SS, predložen je relativno jednostavan i povoljan tehnološki postupak. SS je najpre samlevena primenom čekićara, nakon čega su ljuska i ostaci jezgra razdvojeni na grubu i finu frakciju primenom cik-cak gravitacionog vazdušnog klasifikatora. Korišćena su tri sita čekićara različitog prečnika otvora 3, 2 i 1 mm (POS), dok su protok vazduha (5, 8,7 i 12,5 m^3/h) i protok materijala (30, 60 i 90% masksimuma) varirani tokom klasifikacije. Smanjenje POS uticalo je na povećanje sadržaja proteina grube frakcije. Povećanje protoka vazduha uticalo je na povećanje sadržaja pro-

teina grube frackije za sva tri korišćena sita, dok se istovremeno prinos ove frakcije smanjivao. Usitnjavanje SS polaznog sadržaja proteina 32,75% pri POS 1 mm, a zatim klasifikacije ovako usitnjene sačme pri 12,5 m^3/h i protoku 60% od maksimalnog dalo je grubu frakciju u prinosu od 10,88% sadržaja proteina 46,61% (VPSS). Ovaj rezultat predstavlja maksimalno relativno povećanje sadržaja proteina postignuto predloženim postupkom, ali ujedno i najveće relativno povećanje sadržaja proteina SS u poređenju sa bilo kojim drugim postupkom za proizvodnju visokoproteinske SS iz dostupne literature. Povećanje sadržaja proteina u grubim frakcijama praćeno je povećanjem koncentracije aminokiselina, naročito lizina (relativno povećanje od 38,8% u VPSS).

Ispitivanje tehnološkog kvaliteta proizvedene frakcije VPSS kao zamene za RB u hrani za ribe urađeno je proizvodnjom kontrolne i dve eksperimentalne grupe hrane za pastrmku primenom dvopužnog ekstrudera. Delimična zamena RB sa 10% i 20% VPSS uticala je na povećanje specifične mehaničke energije (SME) ekstrudiranja, što je imalo pozitivan efekat na važne parametre fizičkog kvaliteta granule za patrmku − ekspanzije proizvoda, kapacitet upijanja ulja, nivo otpuštanja ulja i stabilnost granule u vodi.

Ključne reči: *suncokretova sačma, vazdušna klasifikacija, hrana za ribe, ekstrudiranje*

Abstract

Aquaculture relies on fish meal (FM) as a main protein source in fish nutrition. High demand for FM and finite resources available for wild harvesting cause limited availability of the FM and high price of it. Alternative feed ingredients of plant, microbiological and other animal origin are used as a FM substitute in order to increase sustainability of aquaculture. It is important to evaluate the value of each novel high protein ingredient in fish feed in terms of nutritional and technological quality.

High availability, high protein content and relatively low cost of plant protein feedstuffs makes them most used alternative ingredients to FM in production of fish feed. Sunflower meal (SFM), with protein content ranging 29−34%, is a relatively inexpensive protein and energy source, primarily used as feed for all classes of animals. Comparing it with other oilseed meals, primarily soybean, SFM contains less antinutritional factors but with lower nutritional value and it is low in lysine. SFM consists of protein rich sunflower kernels and fiber rich hulls that is cause of high crude fiber content (18−23%) which limits the amount of SFM in fish diets. Removal of the fiber rich hulls decreases the fiber and increases the protein content of SFM, which improves the nutritive and economic value of it.

In order to obtain high protein SFM fractions novel technological process, that is relatively simple and low-cost, was proposed. SFM was ground by a hammer mill after which air zigzag gravitational air classifier was used for separating sunflower hulls from the kernels resulting in coarse and fine fraction. Three hammer mill sieves with sieve openings diameter (SOD) of 3, 2 and 1 mm were used, while air flow (5, 8.7 and 12.5 m^3/h) and feed rate (30, 60 and 90% of maximum) were varied during classification. Decrease in SOD lead to the increase in protein content of coarse fraction. The increase in airflow increased protein content in the coarse fraction for all three used SODs of hammer mill sieve, while fraction yield was decreased. Grinding of starting SFM with protein content of 32.75% by a hammer mill with the lowest SOD (1 mm) and later classification at 12.5 m^3/h and 60% feed rate resulted in coarse fraction (yield 10.88%) with 46.61% of protein (HPSFM), which was the highest relative protein enrichment of SFM obtained by proposed process, as well as by any other technological process for high protein SFM production to the best of our knowledge. Increase in protein content of coarse fractions was followed by increase in amino acid content, especially lysine (relative enrichment of 38.8% in HPSFM).

Investigation of HPSFM technological quality for replacing FM in fish feed was done by production of one experimental and two control groups of trout feed by twin-screw extruder. Partially replacement of 10% and 20% FM with HPSFM influenced higher SME values during extrusion that had positive effect on important physical quality parameters of trout feed − product expansion, oil absorption capacity, oil leakage and water stability.

Keywords: *sunflower meal, air classification, fish feed, extrusion*

MORPHOMETRIC AND SLAUGHTER CHARACTERISTICS OF SELECTED LOCAL PREDATORY FISH SPECIES IN BULGARIA

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MORFOMETRIJSKE I KLANIČNE KARAKTERISTIKE ODABRANIH LOKALNIH PREDATORSKIH VRSTA RIBA U BUGARSKOJ

Apstrakt

Akvakultura u Bugarskoj zasniva se uglavnom na gajenju šarana i kalifornijske pastrmke. Međutim, u posljednjih nekoliko godina zabeleženo je sve veće interesovanje za predatorske vrste, kako egzotičnih tako i domaćih, sa ciljem diverzifikacije vrsta. Cilj ovog istraživanja bio je ocenjivanje osnovnih morfometrijskih parametara, klaničnih karakteristika i približan sastav mesa četiri autohtone predatorske vrste. Uzorci konzumne veličine evropskog soma (*Silurus glanis* L.), smuđa (*Sander lucioperca* L.) i grgeča (*Perca fluviatilis* L.) ulovljeni su iz rezervoara u region Plovdiva (42^{o} 24' N geografske širine i 24^{o} 32' E geografske dužine), dok je štuku (*Esox lucius* L.) obezbedio ribnjak u oblasti Stara Zagora. Svi uzorci su sakupljeni u periodu oktobar-novembar 2016. godine. Istraživanje je pokazalo značajne razlike u morfometrijskim i kláničkim karakteristikama četiri vrste. Grgeč je imao najkompaktnije telo, najniže IHB i najviše IBB vrednosti. Nasuprot tome, som je posedovao najviše vrednosti IHB i najniže IBB indeksa među svim proučavanim vrstama. Najmanji relativni udeo očišćenog tela utvrđen je kod grgeča (66,9%), dok su najveći 70,88 i 70,74% pronađeni, redom, za grgeča i smuđa. Međutim, poslednji je imao najmanji relativni udeo fileta u težini očišćenog trupa i najveći relativni udeo glave (19,32%). Nasuprot tome, štuka je pokazala najniži relativni udeo glave (11,77%) i najveći relativni udeo fileta u odnosu na težinu očišćenog trupa (79,87%). Ovu ribu je takođe karakterisala visoka vrednost viscerosomatskog (15,.21), gonadosomatskog (6,24) i hepatosomatskog (2,49) indeksa. Najmanje razlike između ispitivanih vrsta utvrđene su za srčanosomatski indeks, a najveća vrednost (0,18) utvrđena je kod smuđa. Najveća vrednost za indeks slezine (0,14) utvrđena je za grgeča, dok su relativno manje vrednosti bile za smuđa (0,08) i štuku (0,05). Vrednosti Fultonovog i Klarkovog koeficijenta bile su najviše kod grgeča (1,86 i 1,70), dok su najniže vrednosti utvrđene za soma (0,7 i 0,6). Najveća količina lipida (6,06%) procenjena je kod soma, a zatim kod štuke (4,74%) i grgeča (4,14%). Na osnovu sadžaja lipida u svežem mesu, som i grgeč mogu da se svrstaju kao srednje masna riba. Najniži sadžaj lipida (3,91%) utvrđen je kod smuđa. Som je takođe sadržavao najveći nivo proteina (76,67%). Kao što je očekivano, meso soma bilo je najkaloričniji proizvod (2072,50 kJ/100 g), a zatim meso štuke (1956,78 kJ/100 g). Najviši sadržaj mineral utvrđen je u mesu grgeča (1,49%) i štuke (1,47%). Dobijeni podaci mogu olakšati razvoj akvakulture sa raznovrsnim ribljim vrstama.

Ključne reči: *ribe predatori, morfometrijski indeksi, klanične karakteristike, kvalitet mesa*

Abstract

Aquaculture in Bulgaria is based mainly on cultivation of carp and rainbow trout. However, growing interest in predatory species, both exotic and indigenous, aiming at species diversification was observed in recent years. The goal of current study was to evaluate basic morphometric parameters, slaughter characteristics and meat proximate composition of four autochthonous predatory species. Edible size samples of European catfish (*Silurus glanis* L.), pike-perch (*Sander lucioperca* L.) and perch (*Perca fluviatilis* L.) were caught from a reservoir in the Plovdiv region (42° 24' N latitude and 24° 32' E longitude), while pike (*Esox lucius* L.) was provided by a fish farm in the province of Stara Zagora. All samples were collected in October – November, 2016. The study demonstrated significant differences in morphometric and or slaughter characteristics of the four species. The perch had the most compact body, lowest high-backed and highest broad-backed indices. In contrast, the catfish possessed the highest high-backed index and lowest broad-backed index among all species studied. The lowest relative share of cleaned body was observed at perch (66.9%), while the highest ones, 70.88 and 70.74%, were found for perch and pike-perch respectively. However, the later had lowest relative share of the fillet in the cleaned carcass weight and highest relative share of the head (19.32%). In contrast, the pike exhibited the lowest relative share of the head (11.77%) and the highest relative share of the fillet in the cleaned carcass weight (79.87%). This fish was also characterized with high viscerosomatic (15.21), gonadosomatic (6.24) and hepatosomatic (2.49) indices. The smallest differences among studied species were established in by heartsomatic index, as the highest value (0.18) was established for pike-perch. The highest spleensomatic index (0.14) was established for perch, while it was relatively smaller for pike-perch (0.08) and pike (0.05). Fulton's and Clarc's coefficients for the perch (1.86 and 1.70) were the highest, while the lowest were established for the catfish (0.7 and 0.6). The highest amount of lipids (6.06%) was evaluated in catfish, followed by pike (4.74%) and perch (4.14%). Based on lipid content in fresh meat, catfish and pike can be categorized as medium fatty fish. The lowest lipid content (3.91%) was observed in pike-perch. Catfish contained the highest level of proteins (76.67%) as well. As expected, catfish meat was the most caloric product (2072.50 kJ / 100 g) followed by pike meat (1956.78 kJ / 100 g). Highest mineral contents were established in perch (1.49%) and pike (1.47%) meat. Acquired data would facilitate development of aquaculture with diversified fish species.

Keywords: *predatory fishes; morphometric indices; slaughter characteristics; meat quality*

DYNAMICS OF OXYGEN AND TEMPERATURE OF WATER IN CAGE SYSTEM FOR STURGEON GROWING IN SOUTHERN BULGARIA

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DINAMIKA KISEONIKA I TEMPERATURE VODE U KAVEZNOM SISTEMU ZA GAJENJE JESETRE U JUŽNOJ BUGARSKOJ

Apstrakt

Istraživanje je sprovedeno na farmi jesetri sa kaveznim sistemom koja se nalazi u branom stvorenom veštačkom jezeru tople vode u južnoj Bugarskoj. U periodu istraživanja (od početka aprila do kraja decembra) prosečne vrednosti temperature vode (TW) iznosile su od 10,6 do 26°C, rastvorenog kiseonika (DO) od 4,67 do 9,.86 mg.dm^{-3} i zasićenje rastvorenim kiseonikom (DOS) od 53,6% do 90,6%. Period sa optimalnom temperaturom za rast (OTR) za ovu vrstu (14-24°C) iznosio je 144 dana. Ako se kao maksimalna granica smatra 25°C, ovaj period se povećava za 21 dan. Od sredine jula do sredine septembra (62 dana), porast TW bio je viši od optimalnog - iznad 25°C (maksimalno 26,7°C). U toku perioda istraživanja vrednosti DO bile su u okviru prihvatljivog nivoa, sa vrednostima ispod 5 mg.dm^{-3} koje su zabeležene između kraja jula i kraja septembra (50 dana). DOS nije pao ispod 48% i period iznad 60% trajao je 140 dana. Najpovoljniji za rast riba bio je period od 1. maja do 10. jula, kada su svi ispitivani parametric bili u granicama optimalnih.

Ključne reči: *akvakultura, jesetre, kvalitet vode*
Keywords: *aquaculture, sturgeons, water quality.*

INTRODUCTION

The water quality is a subject of studies in different aquaculture ecosystems (Dulic et al., 2010; Jawecki et al., 2013). Oxygen content changes as a result of complicated interactions between biotic and abiotic factors (Radwan et al., 2003; Manasrah et al., 2006), and it is known that increasing of TW decreases the gases solubility. However, aquaculture ecosystems are diverse and their processes can vary considerably. For the precision of fish farming it is necessary to know dynamics of the main water parameters in different types of water basins and the optimal fish growth period. Therefore we set our goal to study the daily dynamics of TW, DO and DOS in a sturgeon cage farm located in a large reservoir in southern Bulgaria.

MATERIAL AND METHODS

The study was conducted during the period 1 April - 31 December 2017 on a cage sturgeon farm located in the Kardzhali reservoir. By type, it refers to the large, deep reservoirs - its area is 16.07 km^2, the volume 532.9 x 10^6 m^3. It is located in southeastern Bulgaria at 41° 37 ‘N latitude and 25° 20’ E longitude. It belongs to the South Bulgarian climate zone, the East Rhodopean climatic region. The average altitude is about 280 m. The Sturgeon Species grown on the farm are: Russian Sturgeon (*A. gueldenstaedtii*), Beluga (*H. huso*), Siberian Sturgeon (*A. baerii*), Sterlet (*A. ruthenus*), Stellate Sturgeon (*A. stellatus*), and hybrids. The fish are fed with specialized sturgeon granulated feeds. For the purpose of the study, each morning, at the same time, we performed an *in-situ* monitoring of water oxygen (DO, mg dm^{-3}), oxygen saturation (DOS, %) and temperature (TW, °C) by using the oximeter Elke Sensor MJ2000 Marvet junior. For statistical processing and graphic layout, Microsoft Excel (2010) and IBM SPSS Statistics 21 were used.

RESULTS AND DISCUSSION

Table 1 presents the average monthly values of the surveyed indicators and Figure 1 and 2 show their daily dynamics, respectively during the spring - summer and autumn - winter period.

Table 1. Average monthly values of the indicators surveyed

Month	TW, °C		DO, mg.dm^{-3}		DOS, %	
	ıl	SD	ıl	SD	ıl	SD
IV	12.47	0.67	9.86	0.39	90.5	2.84
V	17.10	1.17	8.99	0.45	90.6	2.89
VI	22.04	1.58	7.03	0.77	77.4	6.50
VII	25.15	0.43	5.26	0.26	61.1	2.70
VIII	26.07	0.36	4.77	0.30	56.3	3.40
IX	24.49	0.79	4.67	0.28	53.6	2.70
X	20.49	1.26	5.29	0.15	56.8	0.70
XI	16.08	1.89	5.72	0.15	56.5	0.80
XII	10.60	1.20	7.05	0.60	62.11	3.74
Average	19.35	5.45	6.51	1.81	67.23	14.5

During the study period, the water temperature varied from 10.6 to 26°C, DO from 4.67 to 9.86 mg.dm^{-3}, DOS from 90.6 to 53.6%. During the first month of observation the temperature raised, starting from 11.3°C at the beginning, increasing to 14.4°C at the end of the month. With increasing of temperature, DO decreased from 11 to 9.5 mg.dm^{-3}, and DOS from 110% to 91%. In the early days of May, the water got warmer to the favorable for the hydrobionts (15°C) and by the end of the first decade it was two degrees higher than at the end of the previous month. At the end of May the temperature raised by 4 degrees to 18.7°C. The oxygen content of 9.8 decreased to 8.1 mg.dm^{-3} while DOS levels were in optimal limits.

Kardzhali is an eutrophic to a hyper-eutrophic monomictic reservoir (Traykov, 2005), with relatively rich biodiversity and a great amount of algae (Dochin, 2015). Iliev and Hadjinikolova (2013) indicate that the surface layers in the reservoir are rapidly heated with warming the air temperature. During our study in the first month of summer the temperature raised every day. For the first decade, the increase was almost 3 degrees and about 1 degree each subsequent decade until the end of June when the temperature reached 24.2°C. The dissolved oxygen level at the beginning of June was 8.2 mgdm^{-3}, and at the end 5.8 mg.dm^{-3}, with the DOS level dropping below 70%. In July, the temperature stabilized and raised slower - in the first decade it was in the range 24.3 - 24.9°C; in the second one 25.1 - 25.3°C and the third 25.4-25.8°C. In the second decade the oxygen level dropped to 5 mg.dm^{-3}, as these levels were maintained by the end of the month. At the end of July DOS was about 60%. At the beginning of the third decade of the month the highest temperature for the monitored period was registered 26.7°C. At the end of August, the temperature dropped to 25.7°C. DO for most of the month was about 5 mg.dm^{-3}, at the end of which the minimum value for the study period was registered - 4.1 mg.dm^{-3}. Then the minimum DOS level (48%) was also recorded.

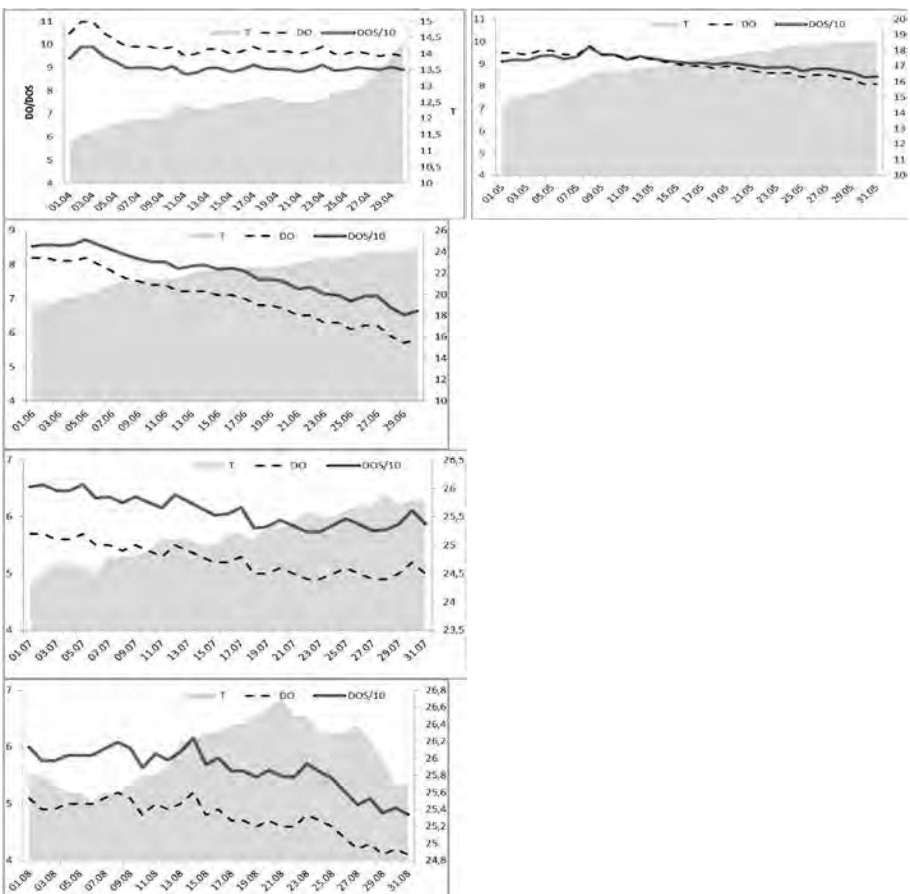

Figure 1. Dynamics of spring-summer period.

In September, the temperature began to decrease and from 25.7°C at the beginning of the month it reached 23.2°C at the end (Figure 2). DO gradually increased, stabilizing at 4.9-5.0 mg.dm^{-3} after the middle of the month. At the end of August, DOS was stabilized at around 50%, and since the beginning of September the indicator value started to increase. At the beginning of October the water temperature was 22.8°C and at the end - 18.9°C. DO did not vary significantly during the month, with reported values ranging from 5.0-5.5 mg.dm^{-3}.

In November the TW continued to decrease, with a decrease of 3 degrees at the end of the second decade of the month, and at the end of the month - by about 6°C reaching 12.5°C. DO throughout the month increased slowly to 6.0 mg.dm^{-3} at the end of the month. There was a tendency to increase DO, early in the winter (up to 8 mg.dm^{-3}) by lowering the water temperature (up to 9°C). DOS, however, remained below 70% by the end of the year, with saturation bringing the optimal values in the last decade of the month. The throughout the observation period there was a clear trend to reduce DO with an increase in TW.

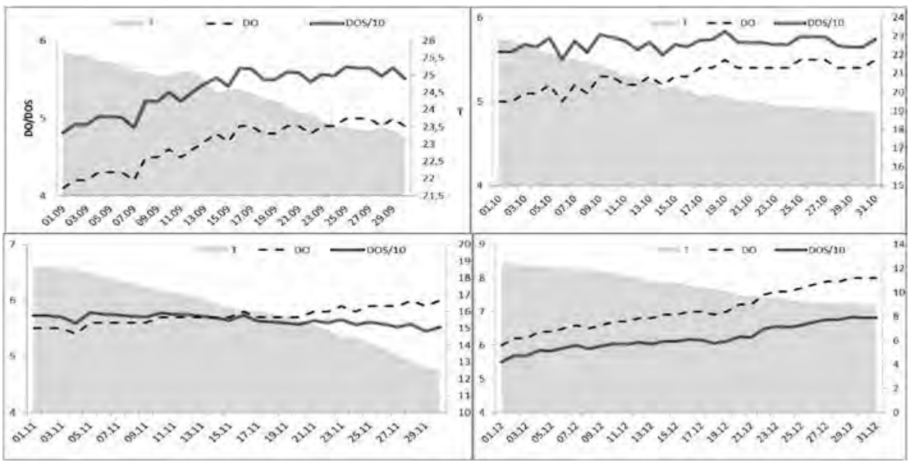

Figure 2. Dynamics of autumn-winter period.

With regard to the OTR for sturgeon, there are different views. Golovanov and Golovanova (2015) indicate that for sturgeon OTR is lower than the thermophilic eurythermal carp species, but is higher than the cold-loving stenothermal trout fish. According to the authors, the area of ecological and physiological optimum for adolescent sturgeons is in the range of 18-26°C, while in the older ones, the optimal limits are lower, compared to the younger (below 25-26°C). Vasilieva et al. (2006) indicate optimum for sturgeon growed in cages TW ranges of 14 to 24°C. In our water reservoir studies, the TW period within this range was 123 days. If assuming a maximum limit for OTR as 25°C, the optimal growth period increases by 21 days. A TW above the upper optimum limit of 25°C was maintained for a period of 62 days (mid-July to mid-September). For optimal DO when growing sturgeon in cages, Vasilieva et al. (2006) indicate values of 8-9 mg.dm^{-3}, with a minimum range of 5 mg.dm^{-3}. According to Chebanov and Galich (2013) the lower limit is 4 mg.l^{-1}. In our study, DO was within acceptable technological limits, with period below 8 mg.dm^{-3} being 202 days and less than 5 mg.dm^{-3}- 50 days (from the end of July to the end of September). Todorov and Ivancheva (1992) indicate for optimal fish growth waters with DOS over 70% and for life over 35-40%. According to Privezencev (1991), the minimum optimal DOS level for sturgeons is 60%. In the surveyed reservoir, the DOS period over 60% was 140 days. Since the second decade in July, the levels had fallen below this limit, but the saturation hadn`t dropped below 48% over the entire period.

CONCLUSIONS

In super-intensive farm for growing sturgeon in cages located in a large warm-water dam reservoir in southern Bulgaria, the average monthly water temperature during the period 1 April-31 December ranged from 10.6 to 26.07°C, DO from 4.67 to 9.86 $mg.dm^{-3}$, DOS from 53.6 to 90.6%. The period with OTR of sturgeon (14-24°C) was 123 days. If maximum 25°C is considered as a maximum limit the optimal growth period increases by 21 days. Temperature above 25°C (at max 26.7°C) had been held for 62 days (mid-July to mid-September). DO was in tolerable limits within the whole observation period, and the period below 5 $mg.dm^{-3}$ had been held for 50 days (from the end of July to the end of September). DOS did not fall below 48% and the period above 60% was 140 days. The most favorable for fish growth was the period from May 1 to July 10, when all the researched parameters were in optimal limits.

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USE OF ECHOGRAPHIC METHODS FOR SEX DETERMINATION OF THREE-YEAR OLD SIBERIAN STURGEON, REARED IN A CAGE FARM

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UPOTREBA EHOGRAFSKIH METODA ZA ODREĐIVANJE POLA KOD TROGODIŠNJE SIBIRSKE JESETRE, GAJENE U KAVEZNOM SISTEMU

Apstrakt

Istraživanje je sprovedeno na jatu trogodišnje sibirske jesetre (*Acipenser baerii).* Jednogodišnja riba gajena je na farmi sa kaveznim sistemom za vrlo intenzivnu proizvodnju u veštačkom jezeru sa toplom vodom, koje se nalazi u Južnoj Bogarskoj. Utvrđeno je da u ovim uslovima gajenja razvoj gonada sibirske jesetre omogućava razdvajanje po polu u uzrastu od tri godine primenom ultrazvučne dijagnostike. Na ehografskim snimcima vrlo dobro su prikazana različita tkiva. Kod ženskih jedinki heterogena struktura generativnog dela jajnika je jasno vidljiva, a kod mužjaka - homogeni karakter testisa.

Ključne reči: *akvakultura, jesetre, ultrazvučni metod, određivanje pola*
Key words: *aquaculture, sturgeons, ultrasound method, sex determination*

INTRODUCTION

Fish consumption is constantly increasing world-wide to the point of 20 kg a person per year nowadays (FAO, 2016). Meeting those ever-growing demands requires development of aquaculture given the fact that an increase of the catch in wild populations is impossible. Development of Sturgeon aquaculture has grown particularly important over the past decades which, on one hand, provides quality delicious products, and on the other – reduces the anthropogenic pressure on the endangered natural populations. According to Kuderskiy (2015) introduction of sturgeon fish in new environments with the intention of cultivating them in aquaculture farms is the most realistic solution of saving those species from extinction. Chebanov and Galich (2009) point out that up until recently, the main limiting factor for creating maternal Sturgeon schools was the absence of early sexing methods. This problem was alleviated after the introduction of methods for ultrasonic diagnostics in Sturgeon farming, which apply to multiple species (Chebanov and Galich, 2013; Masoudifard et al., 2011; Memis et al., 2016; Merkulov and Markov, 2017).

The interest in Siberian Sturgeon in Bulgaria has increased over the past several years and the annual production is 50-100 t (MZH, 2017). In this regard we set a goal to study the possibilities of applying methods for ultrasonic sex determination of Siberian Sturgeon at the age of three years, reared in a cage farm in Southern Bulgaria.

MATERIALS AND METHODS

The study was conducted at the end of February, 2018 with a part (n=445) of the three-year-old Siberian Sturgeon (*Acipenser baerii)* school. The average weight of the fish included in the study is 2.87±0.09 kg. Since their first summer of age fishes have been reared in a cage farm for super intensive production located in the Kardjali dam reservoir. By type, the reservoir refers to large, deep dam reservoirs - its area is 16.07 km^2, the volume 532.9 x 106. m^3. Kardzhali Dam reservoir is located in southeastern Bulgaria at 41° 37 'N latitude and 25°20' E longitude. It falls into the south-Bulgarian climate zone, the East Rhodopean climatic region. The average altitude is about 280 m. The fish are reared in separate 8x8 m cages with water depth 6 m from the surface. The net of the cages is double, of polyamide. Feeding is with specialized for sturgeon fish granulated feed. The non-invasive herd monitoring was carried out using a Mindray DP 50 echograph with a 75L38EA linear transducer (5-10 MHz). During the scans we strictly followed the methodical instructions of Chebanov and Galich (2009; 2013). The scannings were performed at the area around 3-4th abdominal bony scutes counting from the abdominal fin towards the head, on the left side of each fish. As a result, a number of ultrasound images were collected from which, for the purpose of the current study, we chose the most characteristic ones of the male and female individuals.

RESULTS AND DISCUSSION

Working with the fish, we started the examination with a frontal scan by touching the body of the fish with the transducer right next to the abdominal bony scutes line (Figure 1).

Figure 1. Positioning of the transducer on the fish in frontal scans

In transversal scans (Figure 2) the transducer is turned 90 degrees so that it is perpendicularly situated to the abdominal bony scutes line.

Figure 2. Positioning of the transducer on the fish in transversal scans

Chebanov and Galich (2009) point out that in order to successfully apply the methods of ultrasonic sex determination, it is essential to know very well the anatomic structure of the observed zone. The visual structure of the scanned object can be homogeneous or heterogeneous depending on the echogenicity of the different elements comprising the respective organ or tissue. For example, the structure of the mature testes is homogeneous while that of the ovaries is heterogeneous (Chebanov and Galich, 2013; Merkulov and Markov, 2017).

On Figure 3 there is an echographic image (frontal view) of a female Siberian Sturgeon and on Figure 4 – a transversal view of the same fish. The heterogeneous structure of the generative part and the pronounced hipoechogenicity (marked with red) can be easily seen; The ovarian fat with a well pronounced anechogenicity, which is visualized in dark, black areas on the screen (marked with white); a serous membrane with well-expressed hyperechogenicity (marked with green); musculature with hypoechogenic structure in different nuances (marked with blue).

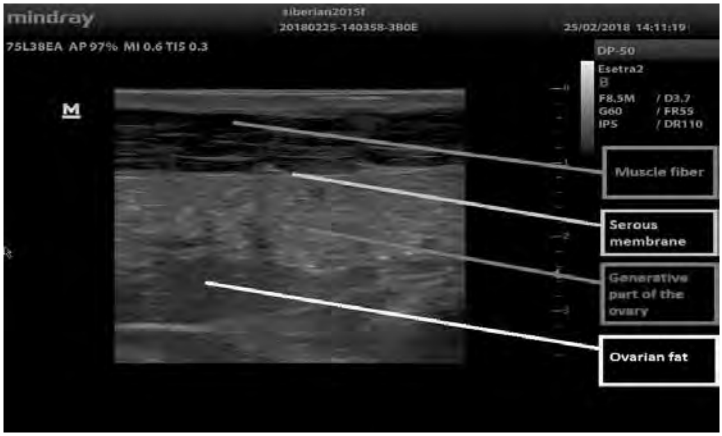

Figure 3. Echographic image (frontal view) of a 3-year-old female Siberian Sturgeon

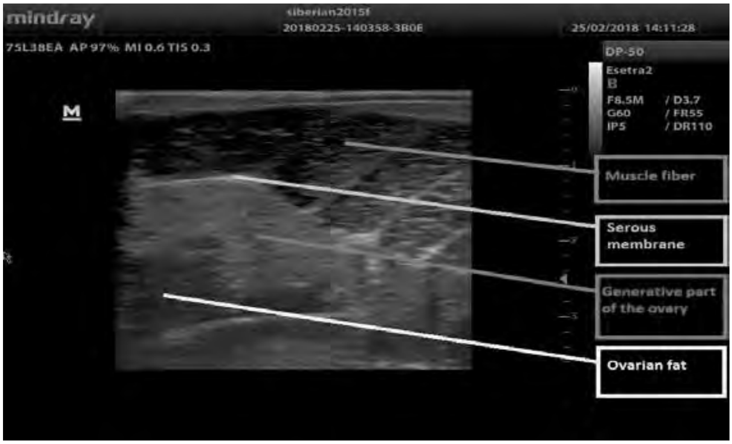

Figure 4. Echographic image (transversal view) of a 3-year-old female Siberian Sturgeon

On the frontal (Figure 5) and the transversal (Figure 6) echographic images of a male 3-year-old Siberian Sturgeon the tissues and the organs stand out clearly.

Figure 5. Frontal echographic image of a 3-year-old male Siberian Sturgeon.

Figure 6. Transversal echographic image of a 3-year-old male Siberian Sturgeon.

The testis is with a homogeneous structure and hyperechoic characteristics (marked with red). The serous membrane is with pronounced hyperechoic characteristics (marked with green). At that age the testicular fat is very low in quantity and with pronounced anaechoic characteristics (marked with white). The musculature is marked with blue and demonstrates mixed echogenicity with places of brighter nuances that are clearly visible.

The natural habitats of the Siberian Sturgeon are the rivers of Siberia (Ruban, 1999). Fish grow slowly there and the females mature after 20 years of age. The biological indicators rapidly change when the fish is reared in higher temperatures and with good feed base and they begin developing much faster (Kuderskiy, 2015). Konchits et al. (2011) point out that in fresh-water aquacultures, Siberian Sturgeon grows 7-9 times faster compared to the natural habitat.

In the living conditions studied by us, Siberian Sturgeon develops well which allows for an ultrasonic sex determination at a relatively early age.

CONCLUSIONS

Rearing in a cage farm for super intensive production located in a warm-water dam reservoir in Southern Bulgaria allows a completely successful sex determination of Siberian Sturgeon at three years of age using ultrasonic diagnostics. The different tissues and organs are visualized very well on the images. Among female individuals, the heterogeneous echostructure of the generative part of the ovaries stands out clearly and among males – the homogeneous characteristics of the testes.

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PARASITIC INFESTATIONS OF THE COMMON CARP (*CYPRINUS CARPIO*) IN FISH FARMS OF VOJVODINA REGION, SERBIA

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PARAZITSKE INFESTACIJE ŠARANA (*CYPRINUS CARPIO*) NA RIBNJACIMA U VOJVODINI, SRBIJA

Apstrakt

Ciprinidne ribe predstavljaju najbrojniju familiju slatkovodnih riba. Šaran (*Cyprinus carpio* Linnaeus, 1758) je jedna od najčešće gajenih ribljih vrsta u svetu, uključujući i Srbiju koja tradicionalno proizvodi šarana. Zbog svog širokog areala rasprostranjenosti i adaptacije na različite klimatske i geografske uslove brojni paraziti predstavljaju pretnju po zdravlje i produktivnost ove vrste. Prilikom procene da li je neki parazit samo prisutan na ribi ili izaziva bolest treba biti veoma obazriv. Paraziti mogu izazivati različite simptome od smanjenja telesne mase i slabljenja kondicije do slepila, respiratornih smetnji, intestinalnih simptoma, anemije, lezija kože, peraja i dr. Dovode i do pada imuniteta riba i stvaranja lezija koje predstavjaju ulazna vrata za delovanje bakterija, virusa i gljivica. Neki paraziti mogu biti i vektori i mehanički prenosioci određenih bolesti. Cilj ovog rada je da se da pregled najznačajnijih i najčešćih parazitskih infestacija prisutnih poslednjih godina na šaranskim ribnjacima u Vojvodini. Istraživanja su sprovedena na 18 šaranskih ribnjaka u Srbiji, od 2010-2016. godine a u sklopu sistemskog monitoringa najznačajnijih parazitoza šarana. Ukupno je pregledano 450 jedinki. Ribe su pregledane tokom čitavog vegetacionog perioda. Praćene su kliničke promene i uzimani su uzorci za nativnu mikroskopiju koja je rađena pomoću svetlosnog mikroskopa. Uzorci su najšćešće uzimani sa kože i škrga za preglede na protozoe, ali su pregledani i svi unutrašnji organi. Spore su pregledane takođe nativnom mikroskolijom. Od inficiranih jedinki uzimana su i različita tkiva u zavisnosti od patoloških promena 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. Nađene su različite vrste parazita od kojih su najdominantniji bili sledeći paraziti: *Ichthyophthirius multifiliis*, *Trichodina* i *Chilodonella piscicola* kao predstavnici protozoa trepljaša, te *Ichthyobodo necator*, jedan od najsitnijih bičara. Među detektovanim miksosporidijama treba istaći *Telohanellus nikolski* koji je bio prisutan tokom svih godina istraživanja u formi telohaneloze na perajima i telohaneloze na krljušti. Metilj *Dactylogyrus vastator* često je izolovan sa škrga, dok je *Diplostomum spathaceum* detektovan sporadično. Pantljičara *Bothriocephalus acheilognathi* i artropode *Lernaea cyprinacea* i *Argulus foliaceus* takođe su često dijagnostikovani kod šarana na našim ribnjacima. S obzirom na prisustvo velikog broja parazita i značajnih patoloških promena do kojih dovode kod šaranskih mladunaca treba stalno raditi na njihovom smanjenju i eradikaciji. Ključne mere za kontrolu parazitskih infestacija kod šarana su pravilno gajenje, isušivanje objekata, izmrzavanje, mehanička obrada tla i dezinfekcija krečom koja ima za cilj smanjivanje broja različitih prelaznih domaćina za parazite.

Ključne reči: *paraziti, šaran, Srbija*
Keywords: *parasites, common carp, Serbia*

INTRODUCTION

Carps of the family Cyprinidae, are the largest family of freshwater fishes in the world, that have been introduced beyond their native ranges (Nelson, 1994§). Cyprinids are also among the most frequently farmed fishes in the world, as well as in Central Europe. Serbia is country with a traditional production of common carp. Due to common carp’s adaptation to a wide range of climatic and geographical condition, many of parasites have been found in wild and domestic carp (Tekin-Özan et al., 2008). When defining the relationship between parasites and the fish host it is often difficult to decide whether the present parasite caused a disease or they did not damage to the health of the host. Parasites are among the important causative agents of diseases in fish responsible for manifestation of various symptoms of the disease as weight loss, gill damages, blindness, abnormal behavior, epithelial lesions, as well as increased sensitivity to other causes of the disease leading to the secondary fungal, bacterial and viral infections. Some external parasites may act as carriers of other pathogens being significant vectors of many diseases (Ćirković and Novakov, 2013).

The present paper is the part of a large study concerning parasites infected *Cyprinus carpio* from fish ponds in Vojvodina region, Serbia and give an overview of the most common and most important parasitic species and diseases present in recent years on our fish farms.

MATERIALS AND METHODS

Investigation was carried out in the northern Serbia (Vojvodina province) in 18 fish ponds, until 2010 to 2016 during a systematic survey of parasites of carp. 450 fishes were included in study. Common carps *Cyprinus carpio* L. 1758 were examined during the whole growing season. After clinical observations, fish were examined externally and internally for parasites. Skin, fins, gills and abdominal cavity smear were prepared by slight scraping and examined under a light compound microscope. Lens was dissected and removed from each eye then inspected under dissecting microscope for parasites. For the study of the internal parasites, the fishes were dissected from the ventral side. The body cavity, stomach, intestine, spleen, liver, kidneys, heart, muscles, swim bladder and gonads were separated and examined carefully under a dissecting microscope for the presence of parasites or cysts. Most of parasites were identified in a direct way by preparing smears of mucus which was scraped from the skin, fins and gills, onto a slide covered by slip and examined. Spores were studied fresh. Samples for pathohistology were fixed in 10% neutral formalin, were processed, sliced to 5-µm-thick paraffin-wax embedded tissue sections, mounted and stained in haematoxylin and eozine. The detected parasites were identified according to their morphology using the reference keys of Bykhovskaya-Pavlovskaya et al. (1964), Gussev (1985), Bauer (1987), Hoffman (1999) and Pugachev et al. (2010).

RESULTS AND DISCUSION

The present study showed the existence of numerous parasites. The most dominant protozoans including ciliats and flagellats were *Ichthyophthirius multifiliis* (Figure 1), *Trichodina* sp. (Figure 2), *Chilodonella piscicola* and *Ichthyobodo necator* (Figure 3).

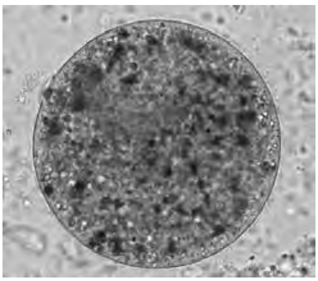

Figure 1. Ichthyophthirius multifiliis

Figure 2. Trichodina sp.

Figure 3. Ichthyobodo necator

Myxoxporidians were also present in carp fingerlings where the most abundant species was *Thelohanellus nikolskii.* Trematode *Dactylogyrus vastator* was a frequent parasite of the common carp gills, while the *Diplostomum spathaceum* were detected occasionally. Cestoda *Bothriocephalus acheilognathi*, and crustaceans *Lernaea cyprinacea* (Figure 4) and *Argulus foliaceus* (Figure 5) represented important pathogens often diagnosed.

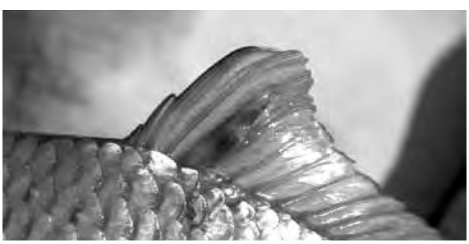

Figure 4. Lernea cyprinacea on common carp dorsal fin.

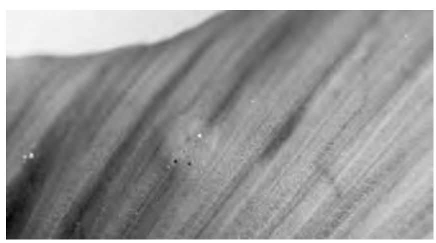

Figure 5. Argulus foliaceus on common carp fin.

Ciliated protozoan *Ichthyophthirius multifiliis* Fauquet, 1876, was found on the skin, fins and gills of *C. carpio* usually during the spring, causing necroses in the form of white prominent spots 0.1-1 mm in diameter. *Chilodonella piscicola* a ciliat, infected one-year-old fishes and provocated whitish opacity on the skin and gills what resulted in respiratory difficulties. *Trichodina*, also ciliated protozoan was isolated from the skin and fins and in small numbers did not pose a important problem to fish. Only in big number of isolated parasites, especially in fry led to skin and gill lesions. *Ichthyobodo necator* a flagellate, known as one of the tinest parisites of fish, caused excesive mucus production in young categories of carps. Myxosporidia *Thelohanellus nikolskii* caused disease called thelohanellosis which appeared in two forms fin and scale. The fin thellohanelosis occurred during July and September in one-year-old carp fingerlings in the form of cysts on the fins and being present on our fish ponds from the eighties (Ćirković, 1986). The scale thelohanellosis was present during the April and May in two-years-old common carps in the form of cysts on the scales. The disease was described for the first time in our ponds 10 years ago (Novakov, 2013). Monogenean trematoda *Dactylogyrus vastator* led to swollen gills covered with increased amount of mucus, haemorrhages and the fusion of the gill arches and consequently breathing difficulties. *Diplostomum spathaceum* was isolated from eye lens and manifested as blindness and mortalities in young fish infested with metacercaria. *Bothriocephalus acheilognathi,* the most detected common carp cestoda usually was found in one-year fry in intestines. The symptoms included body-weight loss, anemia and mortalities. Artropodes *Lernaea cyprinacea* and *Argulus foliaceus* are the ectoparasites very often present in common carp. Parasites are located in the skin and partly in the muscles, causing inflammation and ulcers at affected tissue. *A. foliaceus* penetrate the skin and suck blood after injecting anticoagulant toxins (Novakov et al., 2015). Those crustaceans are especially important in viral and bacterial diseases transmission.

CONCLUSIONS

Results of this study of parasitic infections in common carps support that infestations of parasites including protozoa, metazoan, trematodes, cestodes and arthropods are widespread treat causing losses in fish farms in Serbia. It is therefore necessary to continuously work on reducing their prevalence. This is possible by improving of fish rearing conditions and usage of prophylactic measures such as drying of objects, freezing, mechanical cleaning and disinfection with lime.

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CYANOBACTERIAL EFFECTS ON FISH – SERBIAN EXPERIENCE

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UTICAJ CIJANOBAKTERIJA NA RIBE – ISKUSTVO IZ SRBIJE

Apstrakt

Cijanobakterije su uobičajeni članovi fitoplantnonske zajednice akvatičnih ekosistema. Tokom leta i jeseni one često mog da se prenamnože i formiraju masovne populacije poznate kao cvetanje vode. Cijanobakterije mogu proizvoditi različite metabolite od kojih neki mogu biti ekstermno štetni (npr. cijanotoksini). Najčešće ispitvani cijanotoksini su mikrocistini (MCs), nodularin (NOD) i saksitootksini (STXs). Akvatični organizmi mogu biti izloženi cijanotoksinima direktno preko vode i mogu ih akumulirati u svom organizmu i/ili podnositi njihove negativne efekte.

Obimno istražvanje je sprovedeno u Srbiji na Ludoškom jezeru i 13 ribnjačkih jezera. Taksonomskim analizama vode sa svih ispitivanihi lokaliteta utvrđeno je prisustvocijanobakterija koje potencijalno mogu proizvoditii toksine (najbrojniji su bili *Microcystis aeruginosa, Planktothrix agardhii*, *Limnothrix redekei* and *Pseudanabaena limnetica*). Većina uzorka vode je sadžala mikrocisti i/ili nodularin, a nekoliko njih i saksitoksin. Osim toga, mikrocistini su bili detektovani u tkivima biljakak i životinja: u rizomima makrofita (*Phragmites communis, Typha latifolia* i *Nymphaea elegans*) i mišićima, crevima, bubrezima, gonadama i škrgama srebrnog karaša *Carassius gibelio* sa Ludoškog jezera, kao i u mišićima šarana *Cyprinus carpio* iz ribnjaka. Štaviše, histopatološke promene su registrovane na tjivima jetre, bubbrega, škrga i creva obe analizirane vrste riba. Oštećenja su takođe zabeležena na mišićnom tkivu šarana iz ribnjaka.

Rezultati ovog opsežnog istraživanja ističu značaj monitoring cijanobakterija i cijanotoksina , posebno u ribnjacima, s ciljem detekcije i smanjenja cijanotoksina i njihvoh potencijalno onegativnih efekata na ribe pa i na zdravllje ljudi koji koriste te ribe u ishrani.

Ključne reči: *cijanotoksini, jezero, ribnjak, patohistologija, akumulacija*

Abstract

Cyanobacteria are common members of phytoplankton community in aquatic ecosystems. During the summer and autumn they frequently overgrow and form mass populations known as cyanobacterial blooms. Cyanobacteria can produce different metabolites and some of them can be extremely harmful (e.g. cyanotoxins). The most frequently investigated cyanotoxins are microcystins (MCs), nodularin (NOD) and saxitoxins (STXs). Aquatic organisms can be directly exposed to dissolved cyanotoxins in water and can accumulate them in their organism and/or suffer from their negative effects.

An extensive study was performed in Serbia on Ludoš Lake and 13 fishpond lakes. Taxonomical analyses of water from all sampling sites indicated the presence of potential toxin-producing cyanobacteria (the most abundant *Microcystis aeruginosa, Planktothrix agardhii*, *Limnothrix redekei* and *Pseudanabaena limnetica*). Most samples contained microcystin, and/or nodularin, and in several saxitoxin was indicated Furthermore, microcystins were detected in tissues of plants and animals: in macrophyte rhizomes (*Phragmites communis, Typha latifolia* and *Nymphaea elegans*) and in the muscle, intestines, kidneys, gonads and gills of *Carassius gibelio* from the Ludoš Lake, as well as the muscle tissues of *Cyprinus carpio* from the fishpond lakes. Moreover, histopathological deleterious effects were observed in the liver, kidney, gills and intestines in both analyzed fish. Damage was also recorded in muscle tissue of *Cyprinus carpio* from fishpond lakes.

Findings from this survey emphasize the importance of cyanobacterial and cyanotoxin monitoring, especially in fishponds, in order to recognize and reduce cyanotoxins and their potential negative effects on fish used for human consumption and, further, on human health.

Keywords: *cyanotoxins, lake, fishpond, pathohistology, accumulation*

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SPERM SUBPOPULATION OF COMMON CARP (*CYPRINUS CARPIO* L.)

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SUBPOPULACIJE SPERMATOZOIDA ŠARANA (*CYPRINUS CARPIO* L.)

Apstrakt

Cilj ovog istraživanja je bila analiza sperme šarana i identifikacja različitih subpopulacija spermatozoida u odnosu na njihove kinetičke osobine. Dodatni cilj rada je bilo testiranje efekta krioprezervacije i skladištenja sperme na sobnoj temperaturi i na 4 °C na pokretljivost spermatozooida i strukturu subpopulacija. Za eksperimente je korišćena sperma od tri mužjaka šarana ukupne pokretljivosti > 95% i progresivne pokretljivosti > 75%. Za identifikaciju subpopulacija spermatozoioda korišćena je dvostepena klaster analiza (k means klaster analiza i hijerarhijski klastering). Navedene analize ukazale su na postojanje tri glavna klastera koji predstavljaju subpopulacije (SP1 – SP3) koje se odlikuju specifičnim kinetičkim osobinama: SP1 čine relativno spori spermatozoidi sa niskim ili umerenim vrednostima za LIN (linearnost); SP2 čine brzi spermatozoidi sa visokim vrednostima za LIN i niskim ALH i opisana je kao sobpopulacija za koju je karakteristično brzo linearno kretnje; SP3 čine brzi spermatozoidi ali sa znatno nižim VSL (brzina po pravoj liniji) relatively to the VCL (brzina po zakrivljenoj liniji) i umerenim vredostima za LIN, tako da SP3 čine brzi spermatozoidi sa nelinearnim kretanjem.

Krioprzervacija i skladištenje u trajanju od šet dana su imali značajan uticaj na parametre kretanja spermatozoida šarana. Generalno, vrednosti za ukupne i progresivne pokretljivost, kao i VCL su bili za 50% manji nakon krioprezervacije u pređenju sa svežom spermom, dok su frekvencije subpopulacija sa visokim vrednostima parametara kretanja bile manje. Tokom skladištenja na sobnoj temperaturi i 4 °C, vrednosti kinetičkih osobina krioprezervirane sperme skladištene u nepromenjenom kriomediumu su značajno opale tokom prva dva sata skladištenja. Što se tiče sveže sperme i krioprezervirana sperma kod koje je zamenjen kriomedijum, motilitet je značajno opao tek posle 24 h skladištenja na

sobnoj temperaturi i nakon 72 h skladištenja na 4 °C. Slično je portvđeno i analizama subpopulacija (frekvencija SP2 i SP3 smanjena, dok je frekvencija SP1 i SP0 povećana).

Ovi rezultati pokazuju da populacije spermatozoida nisu homogene i da postoje subpopulacije spermatozoida sa različitim kinetičkim osobinama unutar istog ejakulata. Krioprezervacija i skladištenje utiču na parametre sperme i dinamiku subpopulacija. Tokom skaldištenja nakon krioprezervacije, zamena kriomedijuma za svež medijum moze značajno da utiče na kinetičke parametre spermatozoida nakon zamrzavanja.

Ključne reči: *krioprezervacija, klaster analiza, skladištenje*

Abstract

The main objective of this study was to identify the presence of different spermatozoa subpopulations according to their kinematic characteristics in the sperm of common carp. An additional aim was to test the effects of cryopreservation and prolonged storage at room temperature and 4 °C on spermatozoa motility and subsequently on sperm subpopulation dynamics. Sperm of three common carp males with total motility of > 95% and progressive motility of > 75% was used. Firstly, sperm subpopulations were identified by using two-step clustering analysis (kmeans clustering followed by hierarchical clustering). The analysis identified three main clusters each representing sperm subpopulations characterized by specific kinematic properties: SP1 contained spermatozoa with relatively low velocity and low/moderate STR and LIN values; SP2 was comprised of spermatozoa with high velocities, high STR and LIN values and low ALH and was described as the fast linear subpopulation while SP3 was characterized with high VCL, but considerably lower VSL relatively to the VCL and moderate LIN and STR. Therefore, SP3 was determined as fast non-linear subpopulation.

Both cryopreservation and the 6-day storage had a significant effect on sperm motility parameters. Generally, total motility, progressive motility and curvilinear velocity decreased after cryopreservation to approximately 50% of their value in fresh sperm, while the frequency of subpopulations characterized by high values of motility parameters declined in favor of those with low motility values and SP0 (immobile spermatozoa). During the storage at both room temperature and 4 °C, motility values of cryopreserved spermatozoa which were stored in the original cryomedium decreased significantly during the first two hours. As for the fresh spermatozoa and cryopreserved spermatozoa for which we changed the cryomedium for the fresh extender, motility values decreased significantly 24 h of storage at room temperature and after 72 h of storage at 4 °C. Similar was corroborated by subpopulation dynamics (decrease in the frequency of SP2 and SP3 and the increase in frequency of SP1 and SP0).

These results indicate that spermatozoa populations on common carp are not homogenous and that subpopulations of spermatozoa with different kinematic properties are found. Cryopreservation and prolonged storage influence sperm motility parameters and subpopulation dynamics. During the post-thaw storage, cryomedium in which the sperm was frozen should be replaced with the fresh medium in order to obtain favorable sperm kinematic properties after freezing.

Keywords: *cryopreservation, clustering analysis, storage*

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COMPENSATORY GROWTH AND HEMATOLOGICAL PARAMETERS IN SIBERIAN STURGEON AT DIFFERENT SIZES FOLLOWING RE-FEEDING

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KOMPENZATORNI RAST I HEMATOLOŠKI PARAMETRI RAZLIČITIH VELIČINSKIH KATEGORIJA SIBIRSKE JESETRE NAKON DODATNOG HRANJENJA

Apstrakt

Kompenzatorni rast dve veličinske kategorije juvenilne Sibirske jesetre, *Acipenser baerii*, je praćen tokom šestonedeljnog izgladnjivanja i smanjenja količine hrane (50% manje od zadovoljenja). Ribe svrstane u dve veličinske kategorije, veće i manje, su ispitivane u tri tretmana: ishrana *ad libitum*, smanjena količina hrane i izgladnjivanje. Ponovno hranjenje *ad libitum* je primenjeno tokom narednih 6 nedelja. Na kraju eksperimenta sve ribe su izmerene i uzeti su uzorci krvi radi analize hematoloških parametara. Izgladnjivanje tokom 6 nedelja je dovelo do smanjenja finalne mase ove dve veličinske kategorije riba. Nakon ponovnog hranjenja, kategorije riba koje su bile prethodno hranjene sa 50% manje hrane od *ad libitum* tretmana su skoro dostigle masu riba koje su bile u tretmanu *ad libitum*, dok su obe kategorije riba koje su izgladnjivane pokazale delimičan kompenzatorni rast. Hematološki parametri (broj crvenih krvnih zrnaca, hemoglobin i hematokrit) se nisu značajno promenili, dok je ponovno hranjenje pokazalo značajan porast broja belih krvnih zrnaca kod većih riba u tretmanu sa izgladnjivanjem u odnosu na veće ribe iz *ad libitum* tretmana. Rezultati pokazuju da Sibirska jesetra može da podnese ovakve nepovoljne promene u ishrani.

Ključne reči: *kompenzatorni rast, redukovana ishrana, Sibirska jesetra, veličinska klasa, izgladnjivanje, hematološki parametri*
Keywords: *compensatory growth, restricted feeding, Siberian sturgeon, size, starvation, hematological parameters,*

INTRODUCTION

Fish may experience starvation under natural and controlled cultured systems. Existence of stress conditions, aestivation, overwintering and also management strategy of fish farmers can lead to starvation (Abdel-Tawwab et al., 2006; Caruso et al., 2011; Xiao et al., 2011). During starvation period, fish consume energy stored in the body during the period when feed is available (Rios et al., 2002). Consequently, fish size (body weight) can affect energy stored and following the physiological condition (Wong et al., 2012) because fish size has an important role on metabolic rate (Heim et al., 2016). It seems that with reduced activities of fish, blood oxygen carrying capacity changes (Rios et al., 2002) and can affect the hematological parameters. Re-feeding may compensate the reduced energy.

Compensatory growth is the instinctive response and adaptive mechanism of an animal to variations in the nutritional status which occurs after re-feeding that is linked to increase of the appetite (Ali et al., 2003; Xiao et al., 2011). The period of starvation and re-feeding that provoke the compensatory growth differ among fishes (Tian and Qin, 2004). Therefore, the recognition of the suitable feeding strategies to achieve compensatory growth in fish are necessary. The purpose of this experimental study was to assess the effect of fish size during different nutritional status on hematological parameters and examine the ability of Siberian sturgeon to compensate growth following re-feeding.

MATERIALS AND METHODS

At first, fish were classified into large (average weight: 465.75 ± 11.18 g) and small (average weight: 250.40 ± 12 g) size classes. Large and small fish in control treatments were fed up to satiation to twelve weeks (LA and SA, respectively). Large and small fish in restricted treatments were subjected to restricted feeding (50% satiation feeding; LR and SR, respectively) and large and small fish in deprived treatments were experiencing food deprivation (LS and SS, respectively) for six weeks, then re-fed up to six weeks. At the end of experiment, 400 mg L^{-1} clove powder solution were used for anaesthetizing (Ghiasi et al., 2014). Fish were weighted and then blood was withdrawn from the caudal vein. Number of red blood cells, white blood cells and hemoglobin concentration were evaluated spectrophotometrically utilizing cyanmethemoglobin procedure (Blaxhall and Daisley 1973). Hematocrit (Hct) was quantified in microcapillary tubes centrifuged at 3500 g for 7 min.

Data were analyzed by SPSS 16.0 (Chicago, IL). Normality and and homogeneity of variances were assessed by the Kolmogorov–Smirnov test and Levene’s test, respectively. Body weight was analyzed by one-way ANOVA. Hematological parameters were analyzed by two-way ANOVA. All results are reported as mean ± standard error of the mean (SEM).

RESULTS

After re-feeding, starved fish showed significant difference compared to satiated fish ($P < .05$) but restricted treatment showed no significant difference (Fig. 1; $P > .05$). RBCs, HB and HCT showed no significant differences by size and feeding strategies ($P > .05$) but WBCs changed significantly in LS treatment (Table 1; $P < .05$).

Table 1. Mean values of hematological parameters of Siberian sturgeon (Acipenser baerii) after re-feeding following starvation and feed restriction at two sizes (mean ± SE).

Treatments		RBC ($\times 10^3$ cell mm^{-3})	HB (g dL^{-1})	HCT (%)	WBC ($\times 10^3$ cell mm^{-3})
LA		691.80 ± 31.80	6.36 ± 0.29	37.83 ± 1.74	4.51 ± 0.12^{b}
LR		732.3 ± 28.7	6.53 ± 0.25	39.16 ± 1.40	5.71. ± 0.96^{ab}
LS		710 ± 18.2	6.31 ± 0.17	37.83 ± 1.07	6.65 ± 0.96^{a}
SA		741.10 ±14.8	6.60 ± 0.13	39.88 ± 0.77	6.58 ± 0.54^{a}
SR		715.6 ± 19.3	6.38 ± 0.17	33.83 ± 1.13	4.55 ± 0.36^{b}
SS		682.80 ± 19.52	6.06 ± 0.17	36.22 ± 1.06	5.34 ± 0.73^{ab}
Two-way ANOVA	Size	$P > .05$	$P > .05$	$P > .05$	$P > .05$
	strategy	$P > .05$	$P > .05$	$P > .05$	$P > .05$
	S×S	$P > .05$	$P > .05$	$P > .05$	$P < .05$

Small letters indicate significant difference ($P < .05$) identified between treatments.
RBC: red blood cells; HB: hemoglobin; HCT: hematocrit; WBC: white blood cells
LA, LR, LS: large fish under satiation feeding, restriction feeding and starvation, respectively.
SA, SR, SS: small fish under satiation feeding, restriction feeding and starvation, respectively.

Small letters indicate significant difference ($P < .05$) identified between treatments.
RBC: red blood cells; HB: hemoglobin; HCT: hematocrit; WBC: white blood cells
LA, LR, LS: large fish under satiation feeding, restriction feeding and starvation, respectively.
SA, SR, SS: small fish under satiation feeding, restriction feeding and starvation, respectively.

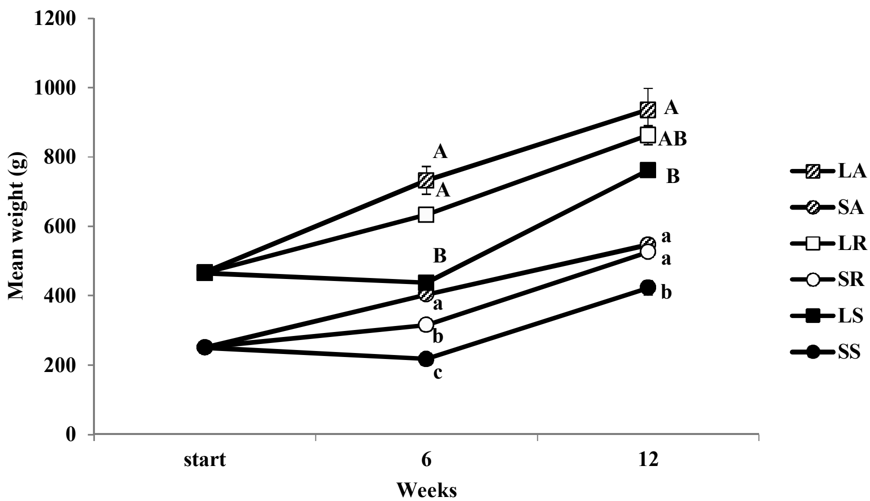

Figure 1. Mean values of body weight of Siberian sturgeon (Acipenser baerii) recorded in the six experimental treatments at two sizes (mean ± SE).
Large letters indicate significant difference (P < .05) identified between large fish.
Small letters indicate significant difference (P < .05) identified between small fish.

DISCUSSION

Weight loss of Siberian sturgeon after starving for six weeks was evident at both sizes. Previous observation performed by Hung et al. (1997) in lake sturgeon (*Acipenser transmontaus*) are in line with the current study. Interestingly, large fish in restricted treatment did not show significant difference compared to satiated treatment; however small fish under feed restriction showed significant decrease than the SA treatment. This result is correlated to fish size, because the relative resistance and growth under restriction feeding is correlated with energy reserves stored in the body (Navarro and Gutiérrez, 1995; Falahatkar, 2012). One of the aims of starvation period is to stimulate compensatory growth (Abdel-Tawwab et al., 2006). After re-feeding, recovered weight loss during starvation period for fish at both sizes was partial, while restricted group reached the level of control treatment. Incidence of compensatory growth is widely associated with the intensity and duration of starvation and feed restriction (Abdel-Tawwab et al., 2006). Complete compensation in small restricted fish (which was significant reduced for weight with 50% of feeding) suggests the potential of small fish to recover the weight when adequate food is provided.

Feeding strategies can affect the blood cells and these data can be used to monitor the physiological condition (Řehulka et al, 2004; Abdel-Tawwab et al., 2006). The Siberian sturgeon did not show adverse effects on hematological parameters when allowed to satiation feed following feed restriction and starvation which were observed in Nile tilapia *Oreochromis niloticus* (Abdel-Tawwab et al., 2006). Starvation can depress the erythropoiesis and hence reduce the hematocrit and hemoglobin levels that are linked to change in body water content (McCue, 2010), but in the current study no disruption in hematopoiesis were found after re-feeding. WBCs showed significant differences. In general, the WBC reflects immune function and can change with the nutritional condition (Blazer, 1992). Ushima et al. (2010) reported that immune function increases after re-feeding in rat, that is in agreement with the present study,. It seems that the increase in WBC can relate to increase in metabolic rate and accelerated growth. Generally, in addition to re-feeding following starvation, fish size did not adversely affect the physiological functions of Siberian sturgeon and confirm that this species can adapt to starvation period.

The present study did not find any significant damage for health of Siberian sturgeon at two sizes after a long-term starvation and feed restriction and subsequent re-feeding. The results showed that Siberian sturgeon will exhibit accelerated growth when resume the satiation feeding, although re-feeding period should be longer for starved fish. Additionally, feeding strategies and fish size in this species did not affect significantly hematological parameters except the number of white blood cells that suggest Siberian sturgeon can tolerate this adverse nutritional condition.

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DIFFERENTIAL BLOOD CELLS AND PLASMA BIOCHEMICAL COMPOSITION OF THE STERLET STURGEON, FOLLOWING THYROXINE ADMINISTRATION

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BIOHEMIJSKI SASTAV KRVNIH ĆELIJA I PLAZME DUGONOSE KEČIGE NAKON TRETMANA SA TIROKSINOM

Apstrakt

Ova studija istražuje uticaj tiroksina (T4) na biohemijski sastav različitih krvnih ćelija i plazmu dugonose kečige (*Acipenser ruthenus*). Za ovo istraživanje po 10 ženki je korišćeno u tri tretmana. Tretmani su se sastojali od kontrole (ulje kokosa), niske doze T4 (T1; 1 mg T4/kg telesne mase sa dodatkom kokosovog ulja) i visoke doze T4 (T10; 10 mg T4/kg telesne mase sa dodatkom kokosovog ulja). Ribe su injektirane intraperitonealno 4 puta tokom 170 dana. Na kraju eksperimenta, oko 1mL krvi je uzeto uz pomoć šprica sa heparinom za dalje analize. Ukupni proteini su ispitani korišćenjem Biuret metode, a albumin kolorimetrijskom metodom korišćenjem komercijalnih kitova. Takođe, analiziran je broj belih krvnih zrnaca, procenat limfocita, neutrofila, eozinofila i monocita. Rezultati su pokazali da su bela krvna zrnca, limfociti i neutrofili značajno brojniji u T_{10} tretmanu nego u drugim grupama ($p < 0.05$). Najviši nivo ukupnih proteina je utvrđen u T_{10} tretmanu ($p < 0.05$). Takođe, najviša koncentracija albumina je utvrđena u T_{10} tretmanu ($p < 0.05$). Rezultati ove studije pokazuju da injektiranje dugonose kečige sa T4 može uticati na neke imunološke parametre. Usled toga je korišćenje T4, posebno u dozama od 10 mg T4 po kilogramu mase preporučljivo, jer deluje kao imunološki stimulant za dugonosu kečigu.

Ključne reči: *tiroksin, injektiranje, Acipenser ruthenus, bela krvna zrnca, imunološki parametar*
Keywords: *thyroxine, injection, Acipenser ruthenus, WBC, immunological parameter.*

INTRODUCTION

The roles of thyroid hormones (THs) in fish have been evaluated generally on teleost fish species, but there is a small number of studies on sturgeon thyroid function. Furthermore, thyroid function of sturgeon and teleost may vary. Detlaf and Davydova (1974) indicated that THs are required for egg maturation in the stellate sturgeon (*Acipenser stellatus*). McEnroe and Cech (1994) reported that in the white sturgeon (*Acipenser transmontanus*) plasma concentrations of thyroxine (T4) and triiodothyronine (T3) are low and seasonally variable. Abdollahpour and Falahatkar (2017) showed that T4 improved larval growth and survival rate in Sterlet sturgeon (*Acipenser ruthenus*). Moreover, plasma THs concentration in sturgeon seems lesser than those of most teleosts. In the current study we used Sterlet sturgeon that is present in wild populations in rivers draining to Black, Azov and Caspian Seas (Billard and Lecointre 2000). Our aim was to determine the impact of T4 on differential blood cells and plasma biochemical composition of female Sterlet sturgeon.

MATERIALS AND METHODS

Thirty individuals of cultured Sterlet sturgeon 4 years old (body weight 707.97 ± 37.15 g were held in six circular concrete tanks (185 cm diameter, 35 cm depth, 940.7 ± 0.2 L volume), supplied with river water and the flow rate was set at 13 ± 0.3 L min^{-1} for each tank. The fish were fed for 170 days thrice a day (at 10:00h, 15:00h and 20:00h) with commercial sturgeon pelleted diet (Fara Daneh, Shahrekord, Iran) to satiation. During the trial, average water temperature was 12.0 ± 0.3 ºC. To determine the effects of T4 on differential blood cells and plasma biochemical composition of Sterlet, fish were divided into three groups each consisting of ten females. The trial treatments comprised of a control (coconut oil), a low dose of thyroxine (T1; 1 mg thyroxine/kg body weight plus coconut oil) and a high dose of thyroxine (T10; 10 mg thyroxine/kg body weight plus coconut oil). Fish were injected intraperitoneally four times over a period of 170 days. Fish were sampled at 170^{th} trial day. Fish were anesthetized with clove powder extract (400 ppm), and blood was withdrawn from the caudal vein with a heparinized syringe. Plasma was extracted from blood by centrifugation at 1600 g for 10 min and stored at –20 ºC until analyzing. Total protein was assayed by a colorimetric test based on Biuret method, and plasma albumin levels were determined with colorimetric method utilizing a commercial kit (Pars Azmoon, Karaj, Iran) (Falahatkar 2012). We also calculated the number of white blood cells (WBCs), percent of lymphocytes, neutrophils, eosinophils and monocytes as differential WBC count. One-way analysis of variance (ANOVA), followed by Duncan’s multiple tests, was used to analyzed significant differences among means ($p < 0.05$).

RESULTS

The number of WBC in different treatments showed a significant difference between trial treatments. The highest WBC was in high dose of T4 and the lowest were noted in control treatment (Table 1; $p < 0.05$). The highest and lowest neutrophil levels were observed in T_{10} and control groups, respectively (Table 1; $p < 0.05$). Also, the greatest blood lymphocytes levels were indicated in the T_{10} treatment ($p < 0.05$).

Table 1. Number of white blood cells and differential blood cells of Sterlet sturgeon (Acipenser ruthenus) after 170 days trial following injection with different doses of thyroxine including 0 (control), 1 (T1) and 10 (T10) mg thyroxine / kg BW.

	Thyroxine injection level (mg kg BW^{-1})		
Parameters	**Control**	**T_1**	**T_{10}**
WBC (× 10^3 cell mm^{-3})	3.97 ± 0.2^b	4.11 ± 0.1^b	4.96 ± 0.2^a
Lymphocytes (%)	71.00 ± 0.1^b	71.00 ± 1.5^b	73.00 ± 0.3^a
Neutrophils (%)	19.57 ± 0.7^b	20.00 ± 0.0^b	21.30 ± 0.7^a
Monocytes (%)	8.73 ± 0.4	7.55 ± 0.4	5.00 ± 0.6
Eosinophils (%)	0.70 ± 0.0	1.45 ± 0.2	0.70 ± 0.2

Values are mean ± S.E. derived from 10 fish for each group. Values in each row with various letters noted significant different ($p < 0.05$).

The results of monocyte and eosinophil levels in various treatments did not show any significant difference between the groups ($p > 0.05$). There were significant differences between groups on blood plasma total protein values. The highest total protein concentration were recorded in the high dose of T4 treatment and the lowest total protein level were noted in the low dose T4 treatment (Fig 1; $p < 0.05$). Plasma albumin levels showed significant differences between the various treatments. The highest amount of albumin was in T_{10} group and the lowest amounts of albumin were showed in control treatment (Fig 2; $p < 0.05$).

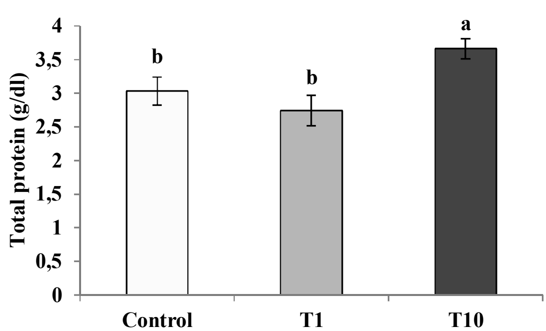

Figure 1. Changes in plasma total protein concentrations (mean ± S.E.) at the end of the experimental period compared among different doses of T4. Values in each column with various letters noted significant different (p < 0.05).

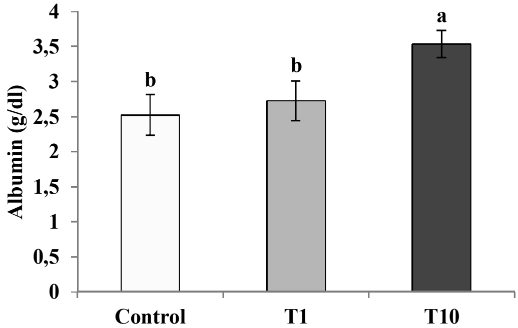

Figure 2. Changes in plasma albumin concentrations (mean ± S.E.) at the end of the experimental period compared among different doses of T4. Values in each column with various letters noted significant different ($p < 0.05$).

DISCUSSION

So far, there has been no study on the effect of T4 injection on the WBC and differential white blood cell percentage in sturgeon fish. Different studies have been conducted on the effects of THs on immunity and the improvement in survival rates at different stages of fish growth (Brown et al., 1989; Rubio et al., 1995). Sahoo (2003) studied the effect of oral administration of the T3 (0, 1, 5, 10 mg / kg of food) on the rohu (*Labeo rohita*). The results indicated that fish which received 5 and 10 mg/kg feed improved the growth and increased production of neutrophils and the fish showed more resistance to *Aeromonas hydrophila*. Due to the lack of studies on the effects of THs on fish immune system, further studies on the effects of T4 on IgM, C3 and C4, as well as the expression of genes involved in the immune system in Sterlet sturgeon should be conducted to determine the exact mechanism of the function of this hormone. According to the results of this study, the highest amount of total protein was obtained in T_{10} treatment and its lowest value in T_1. Moreover, plasma albumin levels indicated that the highest amount of albumin was showed in T_{10} treatment and the lowest amounts of albumin were displayed in the control treatment. In general, stimulation of protein metabolism was evident in the present study due to the increase in total protein and albumin levels compared to other treatments, which can be attributed to increased glutamic activity of liver transaminases. In addition, there are studies that show an enhancement of the contribution of amino acids to proteins after administrating THs that are needed for protein anabolism (Jackim and Laroche, 1973; Narayansingh and Eales, 1975).

CONCLUSIONS

The outcomes of the present study revealed that T4 injection of Sterlet sturgeon can influence WBC, neutrophils, lymphocytes, total protein and albumin of female broodstock, which are associated with thyroid function. Moreover, it was found that WBC and differential white blood cell in T_{10} treatment had a better enhancement than those in other groups and fish in T_{10} group where in a better health condition than the other treatment. These results can be used in developing new methods to positively improve the process of immunological status of Sterlet sturgeon.

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DETERMINATION OF ORGANOCHLORINE PESTICIDES RESIDUES IN FISH USING GAS CHROMATOGRAPHY – MASS SPECTROMETRY

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ODREĐIVANJE REZIDUA ORGANOHLORNIH PESTICIDA U RIBI KORIŠĆENJEM GASNE HROMATOGRAFIJE – MASENE SPEKTROMETRJE

Apstrakt

Razvijena je nova analitička metoda za istovremeno određivanje ostataka organohlornih pesticida (uključujući izomere) u hrani životinjskog porekla. U ovom radu opisana je upotreba brze, jednostavne, jeftine, efikasne, robusne i sigurne (QuEChERS) metode za ekstrakciju i prečišćavanje 17 ostataka pesticida koji su zanimljivi za dve vrste riba: skuša (*Merluccius merluccius*) i oslić (*Scomber scombrus*). Za ekstrakciju, glavni faktori (sadržaj uzorka i vode) su ispitivani i optimizovani u eksperimentima kako bi se postigle maksimalne vrednosti recovery pesticida. Acetonitril je odabran za ubrzanu ekstrakciju rastvarača (ASE) za efikasnu ekstrakciju pesticida iz masnih uzoraka. U metodi se koristi acetonitril (MeCN) za ekstrakciju praćenu dodavanjem anhidrovanog $MgSO_4$ i NaCl, što indukuje raspodelu MeCN ekstrakta iz vode u uzorku. Početni ekstrakt se zatim pomeša sa sorbentom primarnog sekundarnog amina (PSA), C18 i anhidrovanog $MgSO_4$ u jednostavnom pristupu koji se naziva disperzivnim čišćenjem čvrste faze (disperzivna-SPE). Pripremljeni uzorci su analizirani metodom gasne hromatografije - masena spektrometrija (GC/MS) u odabranom režimu za skeniranje jona (SIM) koristeći jedan target i dva kvalitativna jona za svaki analit. PCB 52 je korišćen kao interni standard. Prosečana ponovljivost većine pesticida bila je od 80,64% do 107,5%. Dobra linearnost ($r \geq 0,99$) je primećena između 0,05 i 1,0 mg mL-1. Primena razvijene procedure na realne uzorke ribe otkrila je prisustvo heptahlora, DDT-a i endosulfan sulfata na nivoima µg/kg.

Ključne reči: *QuEChERS, GC/MS,organohlorni pesticide, disperzivni SPE, riba*
Key words: *QuEChERS, GC/MS, organochlorine pesticides, dispersive-SPE, fish*

INTRODUCTION

Pesticide research has been an important part of agriculture (Mahugija et al, 2018). The contamination routes can lead to bioaccumulation of persistent pesticides in food products of animal origin such as meat, fat, fish, eggs and milk. OCPs have been identified as one of the major classes of environmental contaminants because of their persistence, long-range transport ability and human and animal toxic effects. OCPs are ubiquitous environmental contaminant which have spread globally and have been detected in food stuffs, meat, drinking water and sediments as well as wide range of biota including fish (Shinggu et al, 2015). Fishes are suitable indicators for environmental pollution monitoring because they concentrate pollutants in their tissues directly from water and also through their diet. The amount of research on ways to reduce solvent usage and laboratory time has increased since the 1990s, and several alternative extraction and clean-up approaches including matrix solid-phase dispersion (MSPD), (Barker, 2000), super critical fluid extraction (Herrero et al, 2006), and solid-phase microextraction (Seisonen et al., 2015) have been introduced. A range of methods and techniques used to determine the levels of these metabolites has been addressed in a number of papers (Nuapia et al, 2016; Bajwa and Sandhu, 2014).

The aim of this research was to adapt the QuEChERS method for routine pesticide multiresidue analysis in fish samples using gas chromatography - mass spectrometry. Current developments involve the use of extraction methods based on modifications to the QuEChERS procedure. These products contain a high lipid content, which can adversely affect pesticide recoveries and harm traditional chromatographic systems. The final method was validated for fishes in terms of recoveries and repeatabilities using GC/MS detection techniques.

MATERIALS AND METHODS

A method using gas chromatography with mass spectrometric detection (GC-MS) was developed and validated for simultaneously determining organochlorine pesticides residues in fish. A GC-MS analysis was carried out using a GC Clarus 680 PerkinElmer system comprising an autosampler and a gas chromatograph interfaced with an MS Clarus SQ8T instrument under the following conditions: capillary column Elite-5MS (30 x 0.25 mm ID x 0.25 μm df, composed of 95% dimethyl polysiloxane and 5% Phenyl), operating in the electron impact mode at 70 eV. Helium (99.999%) was used as the carrier gas at the constant pressure of 22.5 psi and an injection volume of 2 μL was employed (a split ratio of 50:1) at the injector temperature of 250 ºC; the temperature of the ion-source was 280 ºC. Mass spectra were taken at 70 eV; a scan interval of 0.2 seconds and fragments from 50 to 400 Da. The oven temperature program was initially set at 70 °C for 3 minutes and then ramped up to 150 °C at 25 °C/min, then to 200 °C at 3 °C/min, and finally to 280 °C at 8 °C/min for 10 min. Total GC running time was 41.87 min. The software was adapted to handle mass spectra and the chromatograms were Turbo Mass Ver 6.1.0.

Stock standard solutions (10 μg/ml) were supplied by Accustandard (New Haven, USA). Working solutions were obtained by appropriate dilutions with hexane. Matrix-matched solutions were also prepared by serially diluting the intermediate solution with blank fish sample extracts containing none of the tested analytes to perform matrix-matched calibration with the same concentrations as in the solvent. Twenty fish samples of mackerel and hake were scanned for the examination.

Organochlorine pesticides residues were extracted using the modified QuEChERS technique with acetonitrile and then purified using the dispersive solid phase extraction (d-SPE) cleanup. For the fish, 10 g of sample, 5 ml of water, 10 mL of acetonitrile, 1.6 g of $MgSO_4$, and 0.4 g of NaCl were used in the liquid-liquid partition, while 80 mg of C18, 80 mg of primary secondary amine and 150 mg of $MgSO_4$ were employed in the dispersive solid-phase extraction. Final extracts of acetonitrile were concentrated using a gentle stream of nitrogen and reconstituted into hexane. To achieve the best possible extraction results, extracts were re-purified using the C18 column before the analysis. Final extracts were evaluated by fast GC/MS analysis using the SIR (selected ion recording) mode with ion ratios for confirmation. Under the optimized conditions, the method was validated according to linearity, recovery, and precision.

RESULTS AND DISCUSSION

PCB 52 was used as an internal standard and its retention time was 17.85 min. The linearity of the analytical response across the studied range of concentrations (5 – 100 μg/kg) was excellent, obtaining correlation coefficients higher than 0.99 (Table 1). The LOD and LOQ of this method are also outlined in Table 1 I determinated using standard concentartion 0.025 μg/ml . The average recoveries of the pesticides from all matrices ranged from 80.64% to 107.5%, for fortification level of 20 μg/kg. The precision values, expressed as RSD values, were less than 10% for the pesticide in all matrixes (Table 2).

Matrix components, such as proteins and lipids, can induce ion suppression or enhancement effects. Clean-up method was evaluated in terms of fat removal from the extracts, recoveries and extraction precision. Thus, matrix effects were calculated to avoid any inaccurate quantifications of the analytes in different samples. The matrix effects under the QuEChERS conditions for various fish types at one spiking level are summarized in Table 2.

Calibration curves were performed by using matrix-matched because the fat samples contain many compounds that are co-extracted in the extraction organic solvent. The use of C18-SPE tries to avoid matrix effect using a clean-up step, but this not eliminates completely the problem. A matrix effect on the analytical signal due to the matrix was noticed for most pesticides (Table 2).

Linear calibration was checked in the range from 5 μg/kg up to 100 μg/kg. Fish samples were spiked at different analyte concentrations in quintuplicate then, extractions were performed and weighted linear regressions (1/x) were calculated for each pesticide. Residuals were calculated and showed a deviation by less than ± 20% from the calibration curve for each calibration level. Good linearity was achieved in all cases with correlation coefficients better than 0.990 (Table 1). The lowest calibrated level always had a qualifying transition with (single to noise) S/N ≥15. In all studied matrices, detector saturation was not a problem due to the very effective cleaning procedure proposed.

For recovery studies, one fortification level was selected: 20 μg/kg. All recovery experiments were performed five times at that level, as suggested by SANTE/11813/2017.

Table 1. Linear regression parameters of the calibration curve of organochlorine pesticides residues

Compound	Regression equation	R^2	LOD (μg/kg)	LOQ (μg/kg)
Aldrin	y=1.08677x + (-44.9351)	0.994454	1.8	6.1
α-HCH	y=1.07604x + (-45.2940)	0.994873	0.8	2.5
β-HCH	y=0.675262x + (-41.2580)	0.992612	0.7	2.3
γ-HCH	y=1.10742x + (-60.2206)	0.997591	2.0	6.7
δ-HCH	y=0.825525x + (-34.7934)	0.994498	0.9	2.9
4,4' - DDD	y=2.83435x + (-127.385)	0.993733	1.7	5.6
4,4' - DDE	y=1.90671x + (-82.0592)	0.994457	1.0	3.3
4,4' - DDT	y=2.16509x + (-105.751)	0.996042	1.0	3.3
Dieldrin	y=1.72220x + (-69.3284)	0.994395	2.0	6.7
Endosulfan I	y=0.277833x + (-16.6105)	0.998058	2.4	7.9
Endosulfan II	y=0.227093x + (-9.26686)	0.995752	2.0	6.7
Endosulfan sulfate	y=0.396962x + (-14.4923)	0.993422	1.1	3.6
Endrin	y=0.274841x + (-13.8913)	0.995432	0.8	2.8
Endrin aldehyde	y=0.800986x + (-30.7287)	0.994615	0.6	2.0
Heptachlor	y=1.23478x + (-54.3179)	0.994144	1.6	5.4
Heptachor epoxide	y=0.427601x + (-18.8694)	0.994398	1.0	3.2
Metoxychlor	y=4.25909x + (-194.094)	0.993330	0.3	1.0

Table 2. The effect of the matrix effect, recovery (Q) and relative standard deviation (RSD)

Compound	Matrix- mackerel			Matrix- hake		
	Q, %	RSD, %	Matrix effect, %	Q, %	RSD, %	Matrix effect, %
Aldrin	84.51	5.6	102.84	91.20	4.6	-90.76
α-HCH	93.50	7.8	101.12	89.50	5.2	-97.00
β-HCH	97.46	3.7	100.78	89.06	8.2	-97.80
γ-HCH	91.24	8.9	101.60	89.00	9.0	-84.30
δ-HCH	91.38	6.7	-99.22	84.38	8.3	-92.44
4,4' - DDD	93.40	4.5	-87.80	84.60	8.5	-98.70
4,4' - DDE	83.04	3.8	-82.64	99.74	8.5	-96.78
4,4' - DDT	93.46	6.1	-94.08	97.50	8.3	-96.80
Dieldrin	80.64	6.2	-88.08	98.00	6.8	-94.64
Endosulfan I	99.74	8.4	-89.06	97.80	3.6	-94.60
Endosulfan II	108.64	9.3	-92.60	90.72	4.4	-92.46
Endosulfan sulfate	92.70	6.4	-85.10	92.70	4.8	-92.00
Endrin	90.64	5.5	100.14	81.96	4.0	102.84
Endrin aldehyde	104.20	4.7	100.00	81.40	3.7	101.12
Heptachlor	107.50	4.8	101.80	92.74	4.9	100.78
Heptachor epoxide	93.46	6.7	100.22	95.52	5.0	101.60
Metoxychlor	99.99	3.4	100.05	95.80	3.0	100.90

The most pesticides detected in all determinated fishs were 4,4' DDD; 4,4' DDT 4,4' DDE, heptachlor and endosulfan sulfate. These results are in accordance with other author that found HCHs and DDTs the most compounds detected in fish samples. In general, it was observed that the one of p,p' isomers of DDE, DDT and DDD was detected in samples. All detected pesticides in fish samples did not exceed the MRLs established by the European Union for each compounds. Endosulfan sulfate was detected in one fish samples type of mackerel with a concentration of 10.2 μg/kg.

The results of the detected pesticides are shown in Table 3. The results showed that the incidence and levels of pesticides were higher in mackerel than in hake.

It should be noted that five samples of hake fish did not contain organohloric pesticides. Most of the concentrations were above the maximum residue limits (MRLs) indicating risks and concerns. All the concentrations of organochlorine pesticides residues detected in two type od fish were above the MRL of 0.01 mg/kg. The localization of pesticides in foods varies with the nature of pesticide molecule, type and portion of food material and environmental factors. Low volatility and high stability, to gether with lipophilic behaviour, are responsible critical factor for their persistence in the in fatty tissues through the food chain. Animal feed as well as animal fat are considered a very complex matrices with large number of components especially lipids. Triglycerides and sterol esters are the major components in fish fats. Sample clean-up was necessary for the removal of polar coextracted substances. C 18 cartridges have been employed for that purpose since that adsorbent has proved to be very efficient for the clean-up of all fish samples. The developed procedure represents an improvement over current methodologies for the analysis of organochlorine compounds (OCs) in fatty samples as regards to solvent consumption and automation using GC/MS.

Table 3. Mean values of results obtained using GC/MS

Compound	Mackerel, (μg/kg)	Hake, (μg/kg)
Aldrin	ND*	ND
α-HCH	ND	ND
β-HCH	ND	ND
γ-HCH	ND	ND
δ-HCH	ND	ND
4,4' - DDD	8.9 ± 1.9	7.9 ± 1.5
4,4' - DDE	ND	4.8 ± 0.8
4,4' - DDT	7.9 ± 1.8	7.2 ± 1.6
Dieldrin	ND	ND
Endosulfan I	ND	ND
Endosulfan II	ND	ND
Endosulfan sulfate	4.6 ± 0.8	6.7 ± 0.9
Endrin	ND	ND
Endrin aldehyde	ND	ND
Heptachlor	6.4 ± 1.5	ND
Heptachor epoxide	ND	ND
Metoxychlor	ND	ND

ND*- not detected

CONCLUSIONS

Finally, the proposed method was successfully applied to monitoring organochlorine pesticides residues in real samples, specifically fish imported from EU member states and used further in the process of manufacturing products for our market. Good linearities were obtained and the quantification confirmed a minor matrix effect. Successful application of this method is relevant to monitoring the impact of organochlorine pesticides residues contamination on the environment, primarily through monitoring the presence of OCPs in food, which will provide better insights into the present situation of the environment. This method, with certain modifications, may be employed in the future to test matrices such as soil and water.

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HISTOLOGICAL ANALYSIS OF GILLS OF BROWN TROUT (*SALMO TRUTTA MORPHA FARIO*) FROM THE RIVER PLIVA AND TROUT FISH FARM

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HISTOLOŠKA ANALIZA ŠKRGA POTOČNIH PASTRMKI (*SALMO TRUTTA MORPHA FARIO*) IZ REKE PLIVE I PASTRMSKOG RIBNJAKA

Apstrakt

Kvalitet i sadržaj rastvorenih materija u vodama reka direktno utiče na sve organizme koji žive u njima, a indirektno i na ljude. Škrge riba su organi koji su u direktnom kontaktu sa vodom u kojoj ribe žive. One su veoma osetljive na promene fizičkog, hemijskog i biološkog statava rečnih voda. Danas, kvalitet rečnih voda ima značaj zbog intenzivnog uzgoja riba u ribnjačkim sistemima širom Republike Srpske, jer se riba iz ribnjaka koristi u ishrani ljudi.

Cilj studije bio je da se ispita uticaj kvaliteta vode reke Plive na histološku građu škrga potočnih pastrmki (*Salmo trutta morpha fario* L. 1758). Uzorak se činio 30 jedinki potočnih pastrmki koje su uzete sa dva lokaliteta: prvi lokalitet bio je izvor reke Plive, gdje su one živele u prirodnim uslovima. Drugi lokalitet bio je ribnjak na reci Plivi gde su potočne pastrmke uzgajane. Histološka analiza svih potočnih pastrmki iz naše studije pokazala je srednje i intenzivne promjene anatomije škrga. Promijenila se volumenska gustina epitelnih, mišićnih i hrskavičavih ćelija škrga potočne pastrmke, koje su uzgajane u ribnjacima u odnosu na prirodne uslove. Škrge potočnih pastrmki koje su uzgajana u ribnjacima imale su i hiperplaziju i hipertrofiju epitela.

Prikazani rezultati studije jasno pokazuju da su histološke promene škrga potočnih pastrmki uzrokovane različitim kvalitetom vode u ribnjacima u odnosu na prirodne uslove. Kvalitet vode u ribnjacima na reci Plivi menja se u odnosu na kvalitet vode sa njenog izvora pod uticajem komercijalne hrane kojom se hrane potočne pastrmke, slabijim protokom

rečne vode kroz ribnjake, manjom količinom kiseonika i promenjenim sadržajem makrozoobentosa.

Ključne reči: *potočna pastrmka; Pliva; histologija; škrge; epitelne ćelije*
Keywords: *brown trout; Pliva; histology;gills; epithelial cells*

INTRODUCTION

In the gills cavity, there are four pairs of gills' arches on which are the gills lamellae situated. Lamellae are rich in blood vessels, whose outer surface is covered by a respiratory epithelium through which gas exchange between the water and the blood of the fish is carried out (Hristić and Bunjevac, 2011). By organization of the gill's epithelium a considerable increase in the respiratory surface is achieved, since it is located in a relatively small volume. Hence, fish have approximately 100 times bigger respiratory surface than the skin surface (Kalezić, 2001). In addition to adequate fish nutrition, oxygen and chemical elements of the environment, are the most important factors for successful fish production in fishponds. Soluble in water, oxygen is essential for the metabolism of most hydrobionts. The concentration of oxygen in river waters can change very much during the different periods of the year, even during the twenty-four hours of the day, while in other aquatic ecosystems the change is smaller (Hristić and Bunjevac, 2011). River Pliva has very high water flow, high concentration of oxygen and constant low temperature of water. For this reason, this river is often a place for breeding of brown trouts which lives in very clean waters. In addition to being very clean for its best growth and development, the brown trout require water with a high concentration of oxygen. In disrupted aquatic ecosystems as well as in ponds, the lack of oxygen is the main limiting factor of life (Filipović et al., 2005).

Histological analysis of tissues and cells of gills are an adequate indication of the water conditions in which they live (Lujić et al., 2013). Epithelial gill cells show great sensitivity to chemical and physical changes of water, as well as changes in osmotic pressure, salinity and possible pollutants. This study shows a medium to intense histological change in gills' epithelium, that is caused by the concentration of certain contaminants. Hyperplasia, aneurysms, lamellar fusion, loosening of the lamellas from the connective tissue, neoplasia, blood infiltrates or infections can occur on epithelial gill cells due to various water contamination. Connective and muscle cells have recently become interesting for histological research. These are also indicators of the change in water quality in which fish live. Connective and muscle tissue are very sensitive tissues because their blood flow and development depend on the state of the surface epithelium (Dolonec et al, 2009). The great influence on the growth and development of brown trout from the river Pliva also has the composition of macrozoobenthos that feed them. The trouts hunt their prey actively and the diversity of macrozoobenthos in the rivers allows them good condition and quality nutrition, which together affect the quality of brown trout meat. The quality of macrozoobenthos is richer in natural conditions than in fishpond systems, where it is reduced to a few types of organisms (Peuranen et al. 1994).

MATERIAL AND METHODS

The river Pliva is an extremely clean and fast river, which flows into river Vrbas and flows through the Republic of Srpska, Bosnia and Herzegovina. Brown trouts for our research were collected from two different sites on the river Pliva. The first site was a natural environment, three hundred meters from the spring of the river Pliva. The catchments that were used are sport fishing techniques conducted in the early morning and late evening hours. For our examination, 15 brown trouts were collected from first site of approximately same length and weight. The second site was a semi-intensive fishpond for growing trouts on the river Pliva, five kilometers away from its source. For our research 15 brown trouts were selected and caught from the fishpond by the drift net in the early hours of the morning.

At the same time as the brown trouts were picked, water from the Pliva river was taken from the same location for chemical analysis. Chemical analysis of water from the river Pliva was carried out in the licensed chemical laboratory, Department of Chemistry at the Faculty of Natural Sciences and Mathematics, Banja Luka, Republic of Srpska, Bosnia and Herzegovina by professional staff.

All unique brown trouts selected for our research were transferred to the Faculty of Natural Sciences and Mathematics in Banja Luka where their dissection and removal of gills were done. The gills were removed and fixed in Bouin solution and were processed using a standard procedure for paraffin embedding. Histological analysis by light microscopy was done at the Veterinary Institute “Dr. Vaso Butozan”, Banja Luka, at the Department of Pathology. Samples were cut in a frontal plane on a Leica rotary Microtome RM 2165, Leica Microsystems, Wetzlar, Germany, in 4 µm thick serial sections. For the histological analysis slices of gills were stained with hematoxylin-eosin (H&E) (Merck, Darmstadt, Germany). Morphometric and stereological analysis of fish gills was done using the following stained methods: Malory Azan and Masson’s trichrome staining, in order to visualize blood vessels i. e. endothelial cells. Victoria blue 8GX-floxin light green method was used in order to visualize connective tissue.

Stereological analysis of gill tissue was performed on the Leica 8000D microscope with MEGA VIEW camera and software for digital transmission and image analysis. Within the set goal of the work, measurements of the quantitative analysis’ parameters of the cross-section of the gills of all 30 brown trouts were performed. The parameters were: the height of the respiratory epithelium and the ratio of the volumetric density of the epithelial, connective and muscular cells of the gills.

The linear measurement was conducted by using the micrometer ocular scale and the software package for measuring the structures on the Leica DM 750 microscope with the camera determined. Stereological analysis of the cross-section of the gills' tissue was done with a point-counting technique using the Weibel multipurpose test system with 42 test points and 21 test lines. The volumetric density of epithelial, cartilaginous and muscular tissue was determined according to the following formula:

$V_{VF} = P_f / P_t$,

were parameters are:

V_{VF} – volume density of the gill’s cells

Pf - the number of points that affect the element

P_t - total number of points in the test system

Photographs of the best sections were made by Leica 8000D microscope with MEGA VIEW camera and software for digital transmission and image analysis. All numerical results are displayed as the mean ± standard deviation. The existence and level of differences between the median values of the accompanying stereological parameters among the observed groups were analyzed by F-test and t-test with the uncertainty of $p < 0.05$. Statistical tests were conducted by SSPS 2007 using Excel 2007 software. The cross-sections of brown trouts' gills tissue from both sites were additionally used for pathological analysis that included the classification of altered gill tissue in hyperplasia, hypertrophy, epithelial lifting and leukocyte infiltration (Table 1).

Table 1. Classification of pathological changes of gills of trout (Lujić et al., 2012)

reaction pattern	alteration
progressive change	epithelial hypertrophy
	epithelial hyperplasia
regressive change	epithelial lifting
	leukocytes infiltration

RESULTS AND DISCUSSION

After a comparative stereological analysis of the cross-sections of tissue of fish's gills from both sites of the river Pliva, data are summarized in Table 2. The analysis of the gill tissue of the brown trouts that were in the fishpond shows medium to intensive histological, cytological and pathological changes compared to fish gills from the natural conditions of the river Pliva.

The average height of the respiratory epithelium of brown trouts' gills (Table 2) decreased in brown trout in fishpond conditions (19.95 ± 1.73) compared to natural conditions (25.34 ± 2.59). This decrease is statistically significant, $p = 0.0124$. Reduction of the height of the epithelial gill cells is an indication of the changed water conditions in which the brown trouts lived. The height of the epithelial cells decreases in the event of water pollution, reduction in the concentration of oxygen or elevated water temperatures (Wu et al. 2012).

Table 2. Change in the values of histological and stereological parameters of brown trouts' gills from both sites

parameters	natural conditions	fishpond conditions
height of epithelium of gills (μm)	25.34±2.59	19.95±1.73
volume density of the epithelium (mm^0)	0.196 ±0.0262	0.109±0.0133
volume density of cartilage(mm^0)	0.145±0.0165	0.113±0.0091
volume muscle density(mm^0)	0.164±0.0112	0.127±0.011

The change in the quality of water in two observed places on the Pliva river, although not large, is evident (Table 3). The chemical composition of the water was changed due to the increased number of fish in the fishpond, as well as by addition of artificial commercial food into the pond, which is not existent in natural conditions and reduced at the speed of water flow in a time interval, which is smaller in the fishpond than in the natural conditions. Similar data are given by authors who analyzed the relationship of histopathological changes in fish’ gills in relation to the quality of the water in which the fish lived (Peuranen et al. 1994, Lujić et al. 2012).

Table 3. The chemical and physical parameters of the water from two sites on the river Plia from which brown trout were sampled

parameters	natural conditions	fishpond conditions
temperature of water (^{0}C)	8.3	12.9
pH	7.2	6.9
dissolved oxygen (mg/l)	11.7	7.5
biological oxigen demand (mg/l)	1.5	3.28

Stereological parameters of volumetric density: epithelium, connective and muscle of brown trouts' gills from both sites of the Pliva river show their decrease in brown trout from the fishpond in relation to natural conditions (Table. 1). The decrease in the volume density of the epithelium (0.196 ± 0.0262 to 0.109 ± 0.0133), muscle (0.164 ± 0.0112 to 0.127 ± 0.011) and connective tissue (0.145 ± 0.0165 to 0.113 ± 0.0091) was statistically significant for the trend of $p < 0.05$. Reducing the volume of tissue that builds brown trouts' gills is a consequence of their way of life and the environment in which they live (Figure 1). Namely, the brown trout that lives in fishpond is less likely to move, swim and actively hunt its food; it also has less dissolved oxygen in the water and more dissolved organic and inorganic matter. For these reasons, there is also a change in the tissue of the gills, which leads to changes in their functioning.

a.  b. 

Figure 1. Histological change in brown trout’s gills from both sites, a. natural conditions, b. fishpond conditions, HE, x10

Pathological changes in gills of trouts that lived in fishpond systems (Figure 2. a. b.) compared to natural conditions (Figure 1. a.) was hypertrophy of the respiratory epithelium. Different levels of expression of epithelial hyperplasia from a small proliferation of epithelial cells to complete fusion of secondary lamellas due to hyperplasia were observed (Figure 2). The intensity of hyperplasia differed among lamellas of the same gills of brown trouts that lived in the fishpond systems, while it was not existent in brown trouts from natural conditions (Figure 1.a.). Similar data are also provided by authors who analyzed the histological changes of gills of fish due to the pollution of water in which they lived (Dalzell and Macfarlane 1999, Pandey et al. 2008).

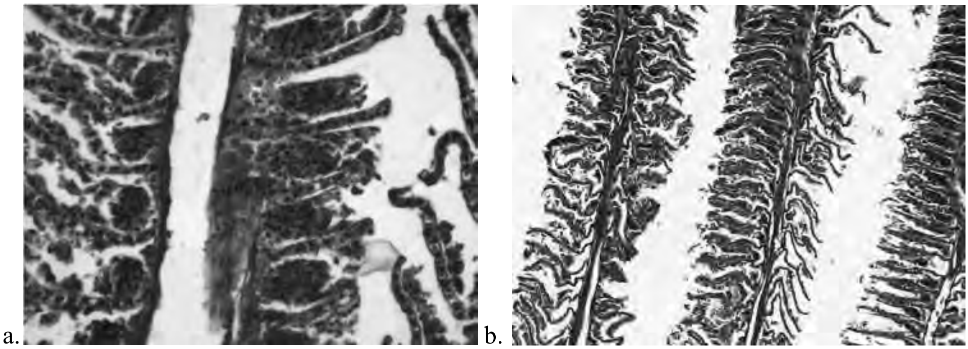

Figure 2. Display of hyperplasia of respiratory epithelium on gills of brown trouts (a. and b.) from fishpond conditions, HE, a.x40, b.x10

The hyperplasia of the respiratory epithelial cells of the gills of brown trouts that lived in the fishpond system is also evident (Figure 3). Hyperplasia occurs in a large percentage of gills' blades in response to a poorer environment in which trouts live. Hyperplasia of gills' fish is found in the papers of Lujić et al. 2012 where the pollution of the river they lived in is the incitement.

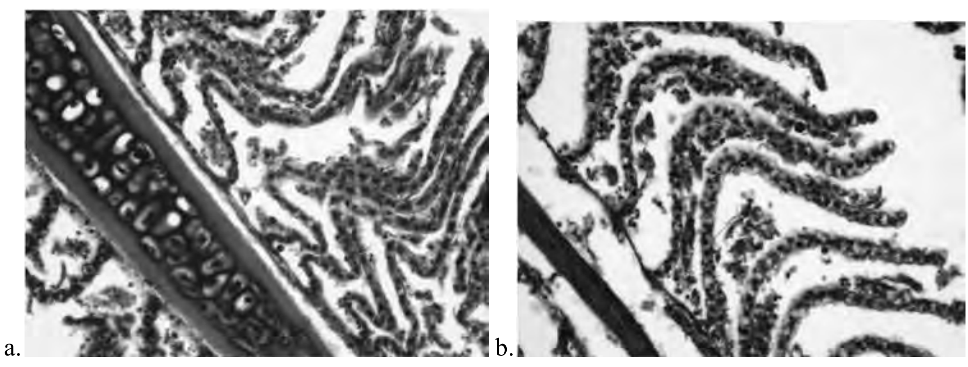

Figure 3. Display of epithelial hyperthrophy on gills of brown trouts (a. and b.) from the fishpond conditions, HE, x40

a. b.

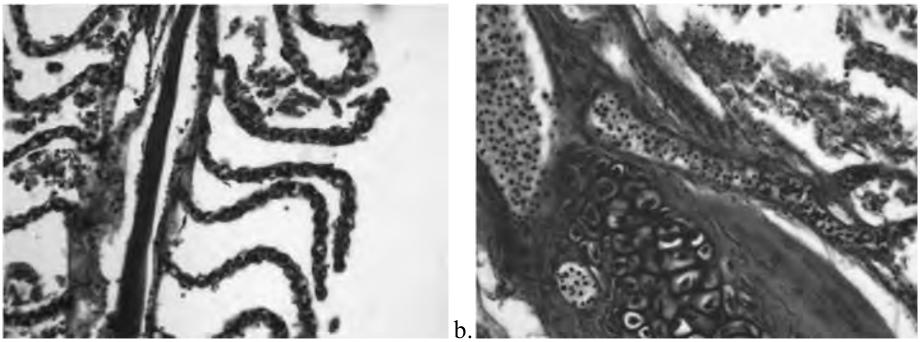

Figure 4. Display of: a. epithelial lifting, x40 and b. infiltration of leukocytes on gills of brown trout from fishery pond system, HE, x100

In our study lifting of respiratory epithelial cells (Figure 4a.) and infiltration of leukocytes of fish’ gills that were in the fishpond system (Figure 4b.) monitored as a response to the lifestyle and conditions of water of the brown trouts’ life. These pathologycal changes of gills are reversible and can go into a healthy state if the environmental conditions for brown trouts change (Arellano et al., 1999, Karan and Poleksić, 1999). On the other hand, they can be fatal if the conditions of water do not change, often leading to necrosis and infection of gills (Middauggh et al., 1980, Liu et al., 2010).

CONCLUSION

Histological analysis of brown trouts'gills that live in the river Pliva at two sites showed the change in their histological structure and the decrease in the quality of their tissues. Change of epithelial cells; volume density of epithelial, muscle and connective tissue; and pathological changes in the gills affect the quality of brown trouts’ body composition. The environment in which brown trouts live, quality of water, type of artificial commercial food, speed of water's flow through fishponds and the number of brown trouts in fish ponds directly affect the health of their gills. Healthy gills allow their functioning and therefore a healthy brown trout that is used in the fishpond system conditions for consumption.

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THE POTENTIAL EFFECTS OF PHARMACEUTICALS ON THE GROWTH OF EARLY JUVENILES OF *OREOCHROMIS MOSSAMBICUS* (PETERS, 1852)

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POTENCIJALNI EFEKTI LEKOVA NA RAST MLAĐI *OREOCHROMIS MOSSAMBICUS* (PETERS, 1852)

Apstrakt

Antiretroviralni (ARV) nevirapin (NVP) i antibiotici sulfametoksazol (SMX) i trimetoprim (TMP) su veoma česti u otpadnim vodama postrojenja za prečišćavanje i površinskim vodama Južne Afrike. Informacije o potencijalnim efektima ovih lekova na ribe su ograničene. Ovo istraživanje ispituje efekte NVP kao samostalne zagađujuće materije, ali i u mešavini sa SMX i TMP na rast mlađi *Oreochromis mossambicus* tokom hronične izloženosti, korišćenjem faktora kondicije (CF) kao biomarkera. Izloženost je počela sa embrionima 24 h po oplođenju i nastavljena do uzrasta od 60 dana. Korišćene su koncentracije tri leka relevantne za životnu sredinu u statičkom ogledu sa zamenom rastvora i kontrolom uslova sredine (27±1^{0}C; 14:10 sati dan/noć fotoperioda). Kondicioni faktor je izračunat za 10 individua uzorkovanih iz svakog tretmana na dane: 1, 30 i 60 po izvaljivanju. Nije bilo statistički značajne razlike (p > 0,05) u vrednostima faktora kondicije kod 30 i 60 dana stare mlađi između kontrolne i grupa izloženih lekovima.

Ključne reči: *antiretroviralno, antibiotici, površinske vode, mlađ riba, toksikologija*
Keywords: *antiretroviral; antibiotics; surface waters; fish juveniles; toxicology*

INTRODUCTION

Surface waters around the world are mixtures of diverse pollutants including human pharmaceuticals, and their unintended effects on aquatic animals including fish is still largely unknown (Brausch et al., 2012). There is a gap of information on the chronic effects of many single pharmaceuticals and their mixtures on fish. Recent studies in South Africa have shown that anti-retroviral drugs (ARVs) and a wide range of antibiotics are present at ng/L and μg/L levels in waste water treatment plants’ (WWTP) effluents, and in surface water (Swanepoel et al., 2015;). Among those pharmaceuticals, the ARV nevirapine (NVP) and the antibiotics sulfamethoxazole (SMX) and trimethoprim (TMP) were recurrently detected in most of the studied water resources (Wood et al., 2016;).

Pharmaceuticals exposure can disturb fish early life stages growth and development (Schoenfuss et al., 2016). Fish growth is dependent on the water quality; therefore, the presence of pharmaceuticals may affect fish growth directly by disturbing the body metabolism and physiology resulting in reduced growth (Schoenfuss et al., 2016). The condition factor (CF) of fish reflects the “degree of well-being” or “robustness” of a fish and is dependent on the food availability and water quality; it is a useful tool in fisheries to compare different fish species growth performances (Froese, 2006). A CF value of 1 indicates a healthy adult fish grown in good conditions while a low CF (< 1) is an indication of poor health state due to mediocre environmental conditions (Carlander, 1969). Healthy early life stages are characterised with a high CF > 1; as they grew and mature, the CF will decrease to get closer to the unity (Froese, 2006).

MATERIAL AND METHODS

Exposure chemicals

Environmental relevant concentration of pharmaceuticals in South African surface waters (NVP: 1.48 μg/L; SMX: 3.68 μg/L; TMP: 0.87 μg/L) and dimethyl sulfoxide (DMSO) were used as a solvent (Triebskorn et al., 2007; Wood et al., 2015). The first set of exposure experiments included three groups: control with dilution water only, DMSO and NVP (1,48 μg/L) and this was repeated four times. The second set of exposures included four groups: control, NVP (3,74 μg/L), and two mixtures ([NVP: 1.48 μg/L + SMX: 3.68 μg/L + TMP: 0.87 μg/L] and [SMX: 3.68 μg/L + TMP: 0.87 μg/L]). This experiment was done in two repeats.

Fish exposure

The experiment was conducted in a controlled environment: water temperature kept at 27 ± 1^{0}C; 14:10 day/night photoperiod maintained, and oxygen supplied. Exposure jars/tanks were checked twice daily. All the exposures started with embryos, ± 24 hours after fertilization and continued till the fish were 60 days old. At day 1, 30 and 60 post-hatching, at least 10 larvae/juveniles from each group were killed; the total length (TL) and wet weight were measured. Weight and length measurements were then used to calculate the condition factor (CF) of each fish (Carlander, 1969):

$$\mathrm{CF} = \frac{\text{Total body mass (g)} \times 10^5}{\text{Total body length (mm)}^3}$$

Water chemistry

The temperature, pH, electrical conductivity (EC), total dissolved substances (TDS) and the dissolved oxygen (DO) were measured daily. A half of the exposure media was renewed every 96 hours to maintain nevirapine concentration and water quality in recommended guidelines. Bole hole water was used for all the exposer and was analysed for inorganic and organic chemicals at an ISO 17025 accredited laboratory using respectively inductively coupled mass spectrometry (ICP – MS) and gas chromatography tandem mass spectrometry

(GC-MS). Nevirapine analysis was done using ultra-high-pressure liquid chromatography (UPLC) coupled to quadrupole time-of-flight mass spectrometry (QTOF/MS) (Ferrer and Thurman 2012).

Statistical analysis

The IBM SPSS Statistics (version 25) was used for statistical data analysis with $p < 0{,}05$. Data was checked for normality of distribution using Kolmogorov-Smirnov and Shapiro-Wilk Tests. To test the significance of differences between the exposed groups and the control group, the non-parametric Kruskal–Wallis and Mann-Whitney U test were used.

RESULTS AND DISCUSSION

One day old larvae showed a high mean CF in all groups (> 3.5), which is higher than 1, the normal CF for healthy adult fish from a healthy environment. However, this is not abnormal, as studies have shown that only adult healthy fish will have a CF closer to the unity, while early life stages, their CF will be > 1 as they increase more in length than they do in other dimensions (Froese, 2006). From day 1 to day 60 post-hatching, the CF in all groups, was coming down closer to 1 as shown in Figure 1 above. There was a statistical significant difference ($p = 0.022$) in the CF of day 1 old larvae between the control, NVP: 3.74 µg/L and the mixtures. Post-hoc analysis tests revealed significant differences between the control and NVP (3.74 µg/L) at $p = 0.043$; the control and the antibiotic mixture ($p = 0.017$); and between the two mixtures ($p = 0.034$). However, 30 and 60 days old juveniles showed no significant differences in CF ($p > 0.05$) in all the groups. This suggests that NVP at 3.74 µg/L and the mixtures of the tested pharmaceuticals may have slowed the growth of early life stages of *O. mossambicus* in their early larval stages, but the fish retrieved.

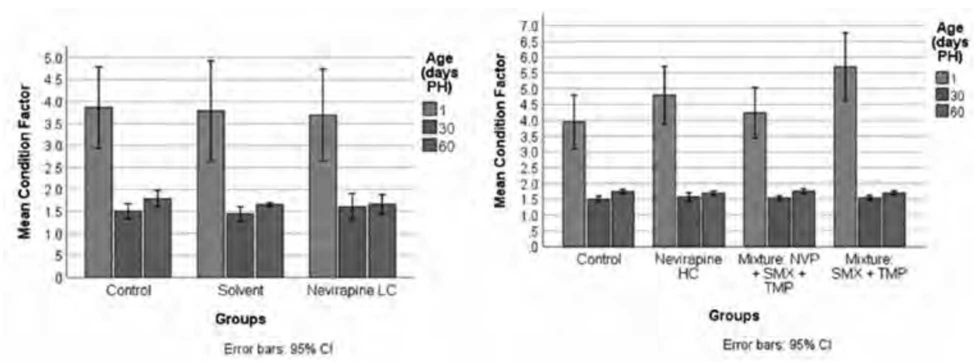

Figure 1: Mean CF of O. mossambicus larvae and juveniles in the different exposure groups at day 1, 30 and 60 post-hatching

CONCLUSION

Nevirapine as a single toxicant and its mixture with antibiotics SMX and TMP at their relevant concentrations in South African surface waters had no long-term adverse effects on *O. mossambicus* early juveniles’ growth.

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FATTY ACID COMPOSITION OF HOT SMOKED COMMON CARP MEAT

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MASNOKISELINSKI SASTAV TOPLO DIMLJENOG ŠARANA

Apstrakt

Proizvodnja dimljene ribe je jedan od najvažnijih sektora u ribarstvu Evrope. U našoj zemlji su zastupljeni dimljeni šaran (*Cyprinus carpio*) i dimljena pastrmka (*Oncorhynchus mykiss*) koji su u poslednje vreme sve prisutniji na tržištu. Dimljenje riba je jedan od najprihvatljivijih načina prerade mesa riba, ne zahteva skupu opremu, proizvodnja kratko traje, a prihvatljivost proizvoda na našem tržištu je, s obzirom na navike potrošača (kao što je dimljeno svinjsko meso), veoma dobra. Dimljena riba predstavlja značajan deo ishrane ljudi u svetu pre svega zbog poželjnih senzornih svojstava, kao i zbog visoke nutritivne vrednosti, visokog sadržaja polinezasićenih masnih kiselina, liposolubilnih vitamina, esencijalnih minerala i esencijalnih aminokiselina. Pored optimalnih količina esencijalnih masnih kiselina, veoma je bitan i njihov odnos. Prema preporukama Svetske zdravstvene organizacije odnos polinezasićenih i zasićenih masnih kiselina treba da bude iznad 0,4, a unutar polinezasićenih masnih kiselina, odnos ω6 i ω3 polinezasićenih masnih kiselina manji od 4. Ovaj odnos je u mesu riba uglavnom adekvatan.

U ovom radu je analiziran masnokiselinski sastav toplo dimljenog šarana prethodno upakovanog u vakuum i u modifikovanu atmosferu (MAP) sa argonom. Za određivanje sastava masnih kiselina je korišćen gasni hromatograf Agilent 7890A sa plameno-jonizujućim detektorom (FID) i kolonom Supelco SP-2560 (100 m x 0,25 mm; debljina stacionarne faze 0,20 µm).

Odnos polinezasićenih i zasićenih masnih kiselina u ovoj studiji je u skladu sa preporukama i iznosi čak i preko 1. Odnos ω6 i ω3 toplodimljenog šarana je u skladu sa preporukama, odnosno manji je od 4. Rezultati ove studije potvrđuju da bi toplodimljeni šaran s aspekta masnokiselinskog sastava trebalo da bude značajan deo balansirane ishrane.

Ključne reči: *riba, vakuum, MAP, polinezasićene masne kiseline*

Abstract

Production of smoked fish is one of the most important sector in European aquaculture. Smoked common carp (*Cyprinus carpio*) and smoked trout (*Oncorhynchus mykiss*) are present in our country and they are more present on the market in the recent years. Smoking fish is one of the most acceptable method of fish meat processing which is relatively inexpensive, has shortime of production, and is very acceptable on our market by the consumers due to their preference to smoked products (smoked pork). Smoked fish is significant part of human nutrition worldwide, due to the desirable sensory properties, high nutritional value, high content of polyunsaturated fatty acids, liposoluble vitamins, essential minerals and essential amino acids.

Apart from optimal amounts of essential fatty acids, the ratio of essential fatty acids is very important. The World Health Organization has recommended that the ratio of polyunsaturated and saturated fatty acids should be above 0.4, and within polyunsaturated fatty acids, the ratio of ω6 and ω3 polyunsaturated fatty acids should be less than 4. This ratio is adequate in the most type of fish and fishery products.

In the present study, fatty acid composition of hot smoked common carp meat packed in vacuum and modified atmosphere (MAP) with argon was analysed. For determination of fatty acid composition, gas chromatograph Agilent 7890A with flame ionization detector (FID) and column Supelco SP-2560 (100 m x 0,25 mm; width stationary phase 0,20 μm) was used. The ratio of polyunsaturated and saturated fatty acids was according to recommendation of the World Health Organization and it was above 1. Within polyunsaturated fatty acids, the ratio of ω6 and ω3 polyunsaturated fatty acids also was according to recommendation, less than 4.

The results obtained from present study confirm that hot smoked common carp meat should be significant part of well-balanced diet.

Keywords: *fish, vacuum, MAP, polyunsaturated fatty acids*

THE EFFECT OF DIFFERENT COMMERCIAL FEEDS ON THE GROWTH AND SURVIVAL OF WEATHERFISH LARVAE (*MISGURNUS FOSSILIS*) REARED IN CONTROLLED CONDITIONS

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UTICAJ RAZLIČITIH KOMERCIJALNIH HRANA NA PRIRAST I PREŽIVLJAVANJE LARVI ČIKOVA (*MISGURNUS FOSSILIS*) GAJENIH U KONTROLISANIM USLOVIMA

Apstrakt

Shodno izgrađenim rečnim regulacijama i posledičnom degradacijom plavnih područja, čikov (*Misgurnus fossilis*) je postao ugrožena vrsta zaštićena zakonom u velikom delu Evrope. Od skoro je na Crvenoj listi IUCN-a u kategoriji LC — takson za koji postoji mali rizik od izumiranja, u dodatku III Bernske konvencije i u dodatku II Direktive o pticama i staništu (Natura 2000 vrste). Veštački mrest i uzgoj larvi u kontrolisanim uslovima su metode koje bi mogle pomoći unapređenju populacija ove vrste. Ova studija je proučavala uticaje različitih živih i koncentrovanih hrana na rast i preživljavanje čikova u uzgoju u kontrolisanim uslovima. Larve korišćene u ovoj studiji dobijene su veštačkim mrestom divljih matica. Dan nakon inicijacije egzogene ishrane naupliusima Artmije, sedmog dana po valjenju (DPV) larve (početna dužina 7.3 ± 0.4 mm) su raspoređene u devet tankova zapremine 1.5 L, gde je u svaki tank nasađeno po 50 larvi. Tri tretmana su postavljena u tri replikacije, gde je svakom tretmanu nasumično dodeljeno tri tanka: Grupa A: hranjena isključivo naupliusima artemije; Grupa PL: hranjena starterom za morske vrste Perla Larvae Proactive 6.0 (62% protein, 11 % mast); i grupa SDS: hranjena starterom za zebrice

SDS 200 (60% protein, 14.5% mast). Ribe su hranjene ručno, šest puta na dan, *ad libitum*, od 6 do 20 časova. Od 7 do 16 DPV, tretmani PL i SDS hranjeni su isključivo koncentrovanom hranom, dok su od 17 do 22 DPV sve tri grupe hranjene isključivo naupliusima artemije. Nakon 15 dana hranjenja, sve preostale larve su prebrojane u svakom tanku. Telesna masa je određena nakon zbirnog merenja svih riba po tanku, dok je ukupna dužina merena nakon fotografisanja svih riba pomoću ImageJ programa. Mortalitet i telesne deformacije (distorzije kičme i glave) su povećane u velikoj razmeri tokom perioda hranjenja koncentrovanom hranom u obe grupe (PL i SDS). Nakon promene ishrane, dnevni mortalitet je opao, dok je udeo larvi sa deformitetom ostao visok, konačno ostavši značajno viši u grupama PL i SDS (74,03 ± 2,83%, odnosno 79,87 ± 10,15%), u odnosu na grupu A gde deformacije nisu zapažene. Grupa hranjena sa živom hranom isključivo je ispoljila značajno više parametre rasta ($p < 0.05$) u odnosu na obe grupe hranjene inicijalno koncentrovanom hranom. Konacna srednja ukupna dužina riba iznosila je 25,4 ± 2,2 mm, 14,9 ± 1,5 mm i 14,7 ± 1,5 mm; konačna srednja telesna masa bila je 116,2 ± 8,0 mg, 26,2 ± 1,7 mg i 24,8 ± 0,3 mg; dok je preživljavanje bilo 96,0 ± 3,5 %, 87,3 ± 1,2 % i 88,0 ± 6,0 %, u A, PL, odnosno SDS grupi. Shodno rezultatima ove studije, preporučeno je zalučiti larve na suvu hranu u starijem uzrastu, nakon inicijalnog perioda hranjenja isključivo živom hranom. Ova pionirska studija je prikazala prilično visoko preživljavanje i prirast kod larvi čikova, ukazujući na izvodljivost intenzivne proizvodnje mlađi za potrebe poribljavanja.

Ključne reči: *nativne, zaštićene vrste, poribljavanje, uzgoj larvi*

Abstract

With respect to the builded river regulations and consequently degradation of the flooding areas, the weatherfish (*Misgurnus fossilis*) became an endangered species protected by law in most of Europe. Recently, it is on the IUCN Red List in Least Concern category, in the appendix III of the Bern Convention and in the appendix II of the Birds and Habitat Directives (Natura 2000 species). Artificial propagation and larval rearing in controlled conditions could be an applicable methods to enhance the populations of this species. In the present study the effects of different live and dry diets were examined on growth and survival in weatherfish under the intensive rearing conditions. Larvae used in this study were obtained by artificial reproduction of wild breeders. One day after the start of exogenous feeding with newly hatched *Artemia* nauplii, 7 days post-hatch (DPH) larvae (initial mean body length 7.3 ± 0.4 mm) were distributed in nine 1.5 L tanks, with 50 larvae stocked in each tank. Three treatments were set in three replicates, each treatment randomly attributed to three different tanks: Group A: fed with *Artemia* nauplii exclusively*;* Group PL: fed with Perla Larvae Proactive 6.0 (protein 62%, fat 11%) marine larvae starter; and Group SDS: fed with SDS 200 (protein 60%, fat 14.5%) zebrafish larvae starter. Feeding was done manually six times per day in an *ad libitum* manner, from 6 AM until 8 PM. From 7 to 16 DPH, PL and SDS treatments were fed with dry feed exclusively and from 17 to 22 DPH all three groups received *Artemia* nauplii, exclusively. After 15 days of feeding all remained larvae were counted in each tank. Body mass was assessed by one sum measurement of all larvae per replication, while the total lengths of all fish were measured on photographs by ImageJ software. Mortality and body deformities (spine and head distortions) increased in large portion during the dry feed period in both group (PL and SDS). After the diet change daily mortality decreased, while the ratio of body deformities remained high, finally being significantly higher in PL and SDS groups (74.03±2.83% and 79.87±10.15%, respectively), compared to group A where no deformities were observed. Group fed with live food exclusively showed significantly ($p < 0.05$) higher growth parameters, than the other two groups fed with dry feeds initially. The mean final total length was 25.4 ± 2.2 mm, 14.9 ± 1.5 mm and 14.7 ± 1.5 mm; final mean body weight was 116.2 ± 8.0 mg, 26.2 ± 1.7 mg and 24.8 ± 0.3 mg; while the survival rate was 96.0 ± 3.5 %, 87.3 ± 1.2 % and 88.0 ± 6.0 %, in A, PL and SDS group, respectively. With respect to the results of the present study, it could be recommended to wean larvae onto dry diets at the later age, after the initial period of exclusive feeding with live food. This pioneer study showed that rather high survival and growth can be achieved in weatherfish larvae pointing to the feasibility of intensive juvenile production for restocking purposes.

Keywords: *native, protected, stock enchancement, larval rearing*

INTESTINAL PARASITES AND DIET OF COMMERCIALLY IMPORTANT FISH SPECIES IN THE BELGRADE STRETCH OF THE DANUBE RIVER (SERBIA)

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CREVNI PARAZITI I ISHRANA KOMERCIJALNO ZNAČAJNIH VRSTA RIBA U BEOGRADSKOM SEKTORU DUNAVA

Apstrakt

U radu su prikazani rezultati istraživanja crevnih parazita i crevnog sadržaja (ishrane) komercijalno značajnih vrsta riba Dunava u beogradskom sektoru. U tom cilju je urađena identifikacija i prikazan je spisak crevnih parazita, kao i identifikacija organizama makrobeskičmenjaka iz crevnog sadržaja. Tokom istraživanja pregledeno je ukupno 39 jedinki sedam vrsta riba (*Silurus glanis, Sander lucioperca, Cyprinus carpio, Esox lucius, Lota lota, Zingel zingel, Hypophthalmichthys molitrix).* Pregledom crevnog trakta riba, utvrđeno je da je parazitima zaražena 21 jedinka, odnosno 53,85%. Paraziti nisu nađeni u jedinki šarana. U crevnom sadržaju sakupljenih jedinki riba identifikovani su predstavnici faune dna, pripadnici različitih grupa, u različitom broju i odnosu (Bivalvia, Gastropoda, Amphipoda, Gammarida, Oligochaeta, Insecta). Mnogi od predstavnika faune dna su prelazni domaćini u životnom ciklusu unutrašnjih parazita.

Ključne reči: *paraziti; ishrana, komercijalne vrste riba, Beograd, reka Dunav.*
Keywords: *parasitic species; feeding; commercially fish; Belgrade, River Danube.*

INTRODUCTION

Infection of fish with parasites depends on the way of fish life, physiological tolerance to individual parasites, the ability of the host to avoid them, the distribution of hosts, fish feeding habitats, etc. Generally speaking, the degree of infestation depends on the density of the population (directly proportional), and the diversity of parasites from the size of the host’s body (directly proportional) (Simonović, 2010). The study of the parasite species and their abundance, intensity and prevalence of infection is of importance for the analysis of the fish population and also for ichthyofauna monitoring as a very important resource of aquatic ecosystems.

Composition of fish fauna in the Belgrade stretch of the Danube River still has autochthonous character. The construction of a hydro-energetic system „Djerdap“ caused changes in ichthyocoenosis and exstinction of anadromous sturgeon fish species (Acipenseridae) in this river section (Lenhardt et al., 2006). In the Danube near Belgrade, based on the analysis of commercial catch fish of K1 category (*Silurus glanis*, *Sander lucioperca)* constitute 16.7% per abundance and 35.7% by mass, while K2 fish (*Esox lucius*, *Cyprinus carpio, Lota lota, Hypophthalmichthys nobilis, Zingel zingel*) are not registered in catches (Hegediš et al., 2013).

The aim of this study is establishment of intestinal parasitic species in economically significant fish species from Belgrade catchment of the Danube River, as well as analyses of their intestinal content and identification of found macrozoobentic individuals.

MATERIAL AND METHODS

This study comprehends the data derived from two-year long investigations (2007-2009) within the Danube River in Belgrade section, on localities Zemun (1.173 rkm) and Višnjica (1.162 rkm). The work covers the investigations on endoparasites and the diet of commercially freshwater fishes. Fish specimens have been sampled by multimesh nets (32 – 50 mm). Fish were transported to the laboratory, where the analyses of their intestines for endoparasites and diet were conducted. The material was analyzed using standard parasitological procedure. Appropriate identification keys were used for determination of parasite fauna representatives to the lowest taxonomic level (Bykhovskaya-Pavlovskaya et al., 1962; Bauer 1987; Moravec, 1994). Determination of the macrozoobenthic organisms recorded in the fish intestines was conducted to the lowest taxonomic level using the appropriate identification keys.

RESULTS AND DISCUSSION

A total of 39 individulas of seven economically significant fish species (*Silurus glanis*, *Sander lucioperca*, *Esox lucius*, *Cyprinus carpio, Lota lota, Zingel zingel, Hypothalmihthis molitrix*) have been collected and examined during this study. . By examination of their intestinum, parasitic species have been identified in 21 fish specimens (Table 1).

Table 1. List of collected and examined fish species and prevalence of infection (number of infected fish and % of infestation)

Fish species	Number of examined fish specimens	Number of infected fish specimens	Percentage of infection (%)
Esox lucius (Linnaeus, 1758)	3	1	33.33
Sander lucioperca (Linnaeus, 1758)	5	2	40.00
Lota lota (Linnaeus, 1758)	4	4	100.00
Silurus glanis (Linnaeus, 1758)	1	1	100.00
Cyprinus carpio (Linnaeus, 1758)	1	0	0
Zingel zingel (Linnaeus, 1758)	11	10	90.91
*Hypophthalmichthys molitrix** (Valenciennes 1844)	14	3	21.43
Σ	**39**	**21**	**53.85**

*allochthonous fish species

Results of examination of fish intestine for identification of endoparasites and diet are presented in the Table 2. The presented findings of intestinal parasites in commercially important fish species of the Belgrade part of the Danube are significant from the aspect of consumption of selected fish species. None of the identified endoparasites has caused damage to the abdominal cavity, nor is the cause of zoonosis in humans. No parasites were found in the carp, while in the previous studies, a total of 17 were identified (Babić, 1935; Andrić, 1984, Cakić and Hristić, 1987; Kiškarolj and Tafro, 1988**;** Cakić, 1992). **O**nly one single pike, out of the three examined, was infected with acanthocephalus, while in previous years there were representatives among Cestoda (5), Trematoda (3), Nematoda (4) and Acanthocephala (5) (Babić, 1935; Andrić, 1984; Cakić, 1992; Cakić and Fišter, 1997). Two individuals of pike perch were infected, and in 2013 was the first observations of *Eustrongylides* larvae (Nematoda) (Bjelić-Čabrilo et al., 2013).

Table 2. The results of determined intestinal parasites and diet of seven commercially fish species

Fish species	**Intestinal parasite**				**Diet**
	Cestoda	Trematoda	Nematoda	Acanthocephala	
Esox lucius				*Pomphorhynchus laevis*	Bivalvia (*Sphaerium* sp.) Insecta.
Sander lucioperca	*Proteocephalus* sp.	*Rhipidocotyle campanula*	*Camallanus lacustris, Philometra rischta*		Annelida (fragmented, impossible to determine to a lower level), Bivalvia (*Dreissena polymorpha*), some small fish specimens.
Lota lota			*Contracaecum siniperca, Contracaecum* sp., *Camallanus truncates, Hysterothylacium bidentatum*	*Pomphorhynchus laevis, Metechinorchynchus truttae, Acanthocephalus anguillae,*	Crustacea (*Asellus* sp., Gammaridae), Bivalvia, Gastropoda (*Lithoglyphus naticoides*), Oligochaeta, some fish specimens
Silurus glanis			*Camallanus lacustris* *Camallanus* sp.		none
Cyprinus carpio					Bivalvia
Zingel zingel		*Bucephalus polymoprhus, Bunodera luciperca, Nicolla skrjabini, Bucephalus polymorphus*	*Hysterothylacium bidentatum,*	*Pomphorhynchus laevis Acanthocephalus lucii, Acanthocephalus* sp.	Crustacea (*Asellus* sp., Gammaridae), Gastropoda (*Lithoglyphus naticoides*), Oligochaeta, Insecta, specimens of small fish.
Hypophthalmichthys molitrix		*Allocreadium* sp.	*Camallanus* sp.		Oligochaeta algae

The individuals of common zingel, in previous years, were not subject to ihtioparasitological research. In our study, it is the fish with the highest percentage of infection (90.91%). All collected burbot specimens were infected with parasites, the presence of which was confirmed by previous studies (Babić, 1935; Kiškarolj and Tafro, 1988; Cakić, 1992). One caught wels catfish individual was infected with nematodes, while a much larger number of parasites were identified in the previous studies by Babić (1935); Andrić (1984) and Kiskarolj and Tafro (1988). With introduction of non-native fish species *Hypophthalmichthys molitrix* spreading of parasites to native taxa have been reported , supplemented by fish parasite fauna: *Trichodina nobilis (*Chen 1963)*, Lernaea cyprinacea* (Linnaeus 1758) and *Sinergasilus polycolpus* (Markevich 1940) (Cakić and Hristić, 1987; Cakić et al., 1996; Nikolić and Simonović 1998).

In the intestinal contents of the sampled individuals of commercial fish species representatives of the bottom fauna were identified (Bivalvia, Gastropoda, Amphipoda, Gammarida, Oligochaeta, Insecta). *Dreissena polymorpha* and *Sphaerium* sp. were identified within Bivalvia group ., while within Gastropod *Lithoglyphus naticoides.* Many of the benthic organisms are intermediate hosts for larval stages of certain groups of parasites. The feeding habits of fish and its diet are influenced by the available local invertebrate fauna, which is, in turn, determined by water quality and habitat composition.

Table 3. The review of fish intestinal parasites from the Belgrade strech of the Danube River

Intestinal parasites	**Class**
Proteocephalus sp. (Weinland, 1858)	Cestoda (1)
Bunodera lucioperca (O.F. Müller, 1776) *Bucephalus polymorphus* (von Baer, 1827) *Nicolla skrjabini* (Ivanitzky, 1928) *Allocreadium* sp. (Looss, 1900) *Rhipidocotyle campanula* (Dujardin, 1845) Trematoda-ciste Trematoda gr.spp.	Trematoda / Digenea (7)
Hysterothylacium bidentatum (Linstow, 1899) *Contracaecum siniperca* (Dogiel & Achmerov, 1946) *Contracaecum* sp. (Railliet & Henry, 1912) *Camallanus lacustris* (Zoega, 1776) *Camallanus truncatus* (Rudolphi, 1814) *Camallanus* sp. (Railliet & Henry, 1915) *Philometra rischta* (Skrjabin, 1923)	Nematoda (7)
Acanthocephalus anguillae (Müller, 1780) *Acanthocephalus lucii* (Müller, 1776) *Acanthocephalus* sp. (**Koelreuter, 1771)** *Pomphorhynchus laevis* (Müller, 1776) *Pomphorhynchus* sp. (Monticelli, 1905) *Metechinorhynchus truttae* (Schrank, 1788)	Acanthocephala (6)

Catch statistics in the period 1969-2010 indicates an increasing presence of Abramidae fish and allochthon species in total fish catch (Jarić et al., 2016). It was noticed that the most numerous species are bream, barbus and prussian carp (40–70% in catch), silver and bighead carp (4%), while economically valued species are less represented: sterlet (8–10%), pike perch (3–5%), and even more rarely, wels catfish, northern pike and common carp (each with a 1–2%) (Smederevac-Lalić, 2013).

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MACROZOOBENTHOS COMMUNITY OF THE SAVA LAKE – BIOINDICATORS OF WATER QUALITY

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ZAJEDNICA MAKROZOOBENTOSA SAVSKOG JEZERA - BIOINDIKATORI KVALITETA VODE

Apstrakt

Savsko jezero, nekadašnji desni rukavac reke Save§ kod Ade Ciganlije, formirano je 1967. godine izgradnjom nasipa sa gornje i donje strane rukavca. Jezero je fizički podeljeno na dva dela, u južnom delu nalazi se taložnik površine 181 000 m^2 (preko kojeg se jezero snabdeva vodom) i znatno veći sportsko-rekreativni deo jezera površine 785 000 m^2. Dno Savskog jezera je veoma heterogeno, priobalni delovi jezera su šljunkoviti§ dok je u ostalom delu ono muljevito§-peskovito§ i muljevito-glinovitog§ karaktera.

Procena kvaliteta vode Savskog jezera, na osnovu hemijskih i fizičkih parametara je višegodišnja i obavlja se na mesečnom nivou od strane nadležnih institucija u Beogradu, dok su zajednice zoobentosa, fito i zooplanktona do sada samo sporadično istraživane. Za potrebe realizacije međunarodnog projekta (O-15205), u jesen 2017. godine obavljena su preliminarna istraživanja faune dna Savskog jezera na ukupno 6 lokaliteta (LS1-LS6), po tri lokaliteta duž obe obale. Uzorci su sakupljeni na dubini od 0,5m *Kick and Sweep* metodom korišćenjem ručne bentosne mreže, kao i sa 2m dubine pomoću bagera tipa Van-Veen.

U uzorcima je zabeležena prilično uniformna, kvantitavno i kvalitativno slabo razvijena zajednica koju čine 12 taksonomskih grupa makrozoobentosa, sa ukupno 36 identifikovanih taksona (determinisanih do nivoa tribusa, familije, roda ili vrste). Dominatne grupe su: Oligochaeta (8 taksona), Gastropoda (9 taksona) i Chironomidae (6 taksona).

U uzorcima prikupljenih sa 2 m dubine dominantni su puževi (*Valvata piscinalis* - 981 ind/m^2; *Esperiana esperi* - 500 ind/m^2 i *Bithynia tentaculata* 461 ind/m^2 na LS1) i školjke (*Dreissena* sp. - 58 ind/m^2 na LS1), dok su na 0,5m dubine najučestalije jedinke familije Chironomidae (*Ablabesmyia* sp.- 28 ind/m^2 i tribus *Chironomini* 25 ind/m^2 na LS5). Nasu-

prot pomenutim, u fauni dna Savskog jezera zabeleženi su i retki nalazi vrsta *Mysis relicta* (Crustacea, Mysidae) na LS4 (0,5m), *Craspedacusta sowerbii* (Cnidaria, Hydrozoa) na LS4 (2m), statoblaste vrste *Pectinella magnifica* (Bryozoa, Lophopodidae), kao i larve vodenih insekata *Orthotrichia costalis* (Trichoptera, Hydroptilidae) na 0,5m dubine i *Donacia* sp. (Coleoptera, Chrysomelida) na 2 m dubine, obe sa LS1.

Na osnovu identifikovanih taksona makrozoobentosa izvršena je procena kvaliteta vode. Vrednosti saprobnog indeksa (Zelinka i Marvan, 1961) variraju od 1,84 (LS5) do 2,41 (LS3), ukazujući na vodu II ili III klase kvaliteta. Vrednosti BMWP indeksa (Chesters, 1980) variraju u rasponu od 19 (LS3) do 47 (LS5), što ukazuje na organski opterećenu ili vodu izmenjenog kvaliteta.

Ekološki status Savskog jezera procenjen je na osnovu sastava i strukture zajednice makrozoobentosa prema nacionalnoj legislativi (Službeni Glasnik Republike Srbije, 74/2011) za prirodna vodna tela. Dobijene vrednosti saprobnog indeksa ukazuju da je ekološki status Savskog jezera između I i II klase (visok-dobar) na većini istraživanih lokaliteta, dok je na LS1 i LS3 ekološki status između II i III klase (dobar-umeren). Vrednosti BMWP indeksa oslikavaju ekološki status između II i III klase (dobar-umeren) na skoro svim lokalitetima, izuzev na lokalitetu LS3 čije su vrednosti ovog indeksa ukazivale na ekološki status između III i IV klase kvaliteta (umeren-loš).

Međutim, ukoliko se dobijenim rezultatima pridoda veliki antropogeni pritisak (broj posetioca, kupališna sezona u trajanju od juna do septembra, različite sportske aktivnosti, ugostiteljski objekti), za kvalitet vode i ekološki status Savskog jezera moglo bi se reći da su na zadovoljavajućem nivou.

Ključne reči: *Savsko jezero, akvatični beskičmenjaci, biološki monitoring, indeks saprobnosti, biotički indeks, ekološki status*

Abstract

Sava Lake, as a former right arm of the Sava River near Ada Ciganlija, was formed in 1967 by constructing the dam on the opposite sides of the arm. The lake is divided into two parts, with settling pond of 181 000m^2 in the southern part of the lake (through which the lake is supplied with water) and significantly larger sports-recreational part of the lake of 785 000 m^2. The substrate of the Sava Lake is very heterogeneous; the costal part of the lake is dominant by pebble, while the rest of the bottom area is characterized by the presence of mud, sand and gravel.

Chemical and physical water quality assessment of the Sava Lake is perennial and carried out monthly by the competent institutions in Belgrade, while zoobenthos, phyto and zooplankton communities have been only sporadically researched so far. For the purpose of implementing the International project (O-15205), preliminary survey of bottom fauna was carried out in autumn 2017, on six localities (LS1-LS6) - three localities along both sides of the Sava Lake. The samples were collected at the depth of 0.5 m with D-shaped net (Kick and Sweep method) and at the depth of 2 m using Van-Veen grab.

We recorded quite uniform, qualitatively and quantitatively poorly developed community, consisting of 12 taxa groups with 36 identified taxa (determined at the tribe, family, genus or species level). The dominant groups were: Oligochaeta (eight taxa), Gastropoda (nine taxa) and Chironomidae (six taxa).

In samples taken at the depth of 2 m, the dominant taxa were: snails (*Valvata piscinalis* - 981 ind/m^2; *Esperiana esperi* - 500 ind/m^2 i *Bithynia tentaculata* 461 ind/m^2 na LS1), bivalves (*Dreissena* sp. - 58 ind/m^2 na LS1), while at the depth of 0.5 m the most common individuals were among Chironomidae family (*Ablabesmyia* sp.- 28 ind/m^2 i tribus *Chironomini* 25 ind/m^2 na LS5). Contrary, some rare taxa were also recorded in bottom fauna of the Sava Lake: *Mysis relicta* (Crustacea, Mysidae) on LS4 (0.5 m), *Craspedacusta sowerbii* (Cnidaria, Hydrozoa) on LS4 (2m), statoblasts of *Pectinella magnifica* (Bryozoa, Lophopodidae), and also the larvae of aquatic insects: *Orthotrichia costalis* (Trichoptera, Hydroptilidae) at 0,5m and *Donacia* sp. (Coleoptera, Chrysomelida) at 2 m, both on LS1 locality.

Water quality assessment was performed based on determined macrozoobenthos taxa. Saprobity index values (Zelinka and Marvan, 1961) varied between 1.84 (LS5) to 2.41 (LS3) and therefore indicate on II or III water quality class. The values of BMWP (Chesters, 1980) index were between 19 (LS3) to 47 (LS), presenting polluted or moderately impacted water.

Ecological status of the Sava Lake was assessed based on macrozoobenthos community composition and structure according to national legislation (Official Gazzete of the Republic of Serbia, 74/2011) for natural water bodies. Obtained saprobity index values indicate ecological status class between I and II (high-good) at most of investigated sampling localities, and between II and III ecological status class (good-moderate) at locality LS1 and LS3 respectively. Calculated values of BMWP Score indicate ecological status class between II and III (good-moderate) at most of the localities and ecological status class between III and IV (moderate-poor) only at the LS3 sampling locality.

However, by taking into consideration large anthropogenic pressure (number of visitors, bathing season from June to September, various sports activities, catering facilities), we can conclude that water quality and ecological status of the Sava Lake are at satisfactory level.

Keywords: *Sava Lake, aquatic macroinvertebrates, biological monitoring, saprobity index, biotic index, ecological status*

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RADIOACTIVITY AND ELEMENTAL ANALYSIS OF MOLLUSK SHELLS (MUSSEL *MYTILUS GALLOPROVINCIALIS* AND OYSTER *OSTERA EDULIS*) FROM THE BOKA KOTORSKA BAY

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RADIAKTIVNOST I ELEMENTARNA ANALIZA LJUŠTURA MEKUŠACA (DAGNJA *MYTILUS GALLOPROVINCIALIS* I KAMENICA *OSTERA EDULIS*) IZ BOKOKOTORSKOG ZALIVA

Apstrakt

Morski mekušci imaju sposobnost akumulacije velikog broja elemenata i sve češće se koriste kao indikatori zagađenja morske sredine. Vrste kao što su dagnje (*Mytilus galloprovincialis*) i kamenice (*Ostrea edulis*) intenzivno su ispitivane i korišćene kao bioindikatori velikog broja elemenata, a među njima i toksičnih metala, u morskoj sredini. S obzirom da je meko tkivo efikasniji akumulator elemenata u odnosu na ljušturu, naučna pažnja usmerena je upravo na upotrebu mekog tkiva kao bioindikatora. Ipak, akumulacija elemenata u ljušturama i njihovo određivanje mogu obezbediti zapis o nivou elemenata u životnoj sredini. Takođe, sa stanovišta monitoringa kontaminacije vodene sredine elementima, ljušture imaju i značajne praktične prednosti u odnosu na meko tkivo: 1) manja varijabilnost, 2) integracija elemenata tokom celog života jedinke, 3) obezbeđivanje uvida u koncentracije elemenata i posle smrti jedinke što omogućava i uvid u koncentracije iz prošlosti i 4) značajne prednosti u pogledu očuvanja i skladištenja uzoraka.

Sa druge strane, ljušture dagnji i kamenica predstavljaju značajan ekološki problem širom sveta jer se proizvode u velikim količinama za potrebe ljudske ishrane. U najvećem broju slučajeva otpadne ljušture se deponuju na kopnu u blizini uzgajališta ili deponijama ili se vraćaju u more. Ovaj otpad nagomilava se u priobalnim područjima i izaziva negativne efekte u životnoj sredini, uključujući zagađenje priobalnih voda, neprijatan miris

usled raspadanja ostataka mesa na ljušturama, zdravstvene i kanalizacione probleme, trajno oštećenje prirodnog pejzaža i ugrožavanje mnogih nativnih biljnih i životinjskih vrsta usled dugogodišnjeg i sve intezivnijeg deponovanja ljuštura na morskom dnu. Na području Bokokotorskog zaliva na godišnjem nivou generiše se velika količina otpada nastalog ovom vrstom proizvodnje, pa se potencijalno ozbiljni ekološki problem mora sprečiti.

Po našem saznanju, u dostupnoj literaturi ne postoje podaci o radioaktivnosti i/ili koncentraciji elemenata u bilo kojoj vrsti ljuštura iz Bokokotorskog zaliva. Shodno tome, istraživanja u ovoj studiji predstavljaju prvi pokušaj da se ljušture dagnji (*Mytilus galloprovincialis*) i kamenica (*Ostrea edulis*) koriste kao bioindikatori zagađenja elementima u Bokokotorskom zalivu. Takođe, rezultati ovog istraživanja pružiće uvid u bezbednost otpadnih ljuštura kao materijala i mogućnost njihove upotrebe u druge svrhe i namene.

Ključne reči: *radioaktivnost, elementarna analiza, ljušture dagnji i kamenica, Bokokotorski zaliv*
Keywords: *radioactivity, elemental analysis, mussel and oyster shells, Boka Kotorska bay*

INTRODUCTION

Marine molluscs are well known to accumulate a wide range of elements and are increasingly used as indicators of marine pollution (Stankovic et al., 2012). The species such as mussels *Mytilus galloprovincialis* and oysters *Ostrea edulis* have proved to be important tools for the biomonitoring of heavy metals pollution in coastal areas (Jović and Stanković, 2014; Rainbow, 1995). The soft tissues of molluscs have been recognized as more efficient accumulators of elements than the shells, and consequently, scientific attention has been directed to the use of soft tissues as indicators (Protasowicki et al., 2008; Stankovic and Jovic, 2012). Determination of element accumulation in shells is very useful since such investigations can provide a record of environmental element levels. Also, shells have important practical advantages over the use of the soft tissues in monitoring element contamination of the aquatic environment, such as: (1) reveal less variability, (2) integrate elements over the lifetime of the animal, (3) preserve the metals after death and provide information on the concentration levels in the past, and (4) offer considerable advantages with respect to both sample preservation and storage (Protasowicki et al., 2008).

On the other hand, waste mussel and oyster shells present a significant environmental problem around the globe, since they are produced in vast quantities. Waste shells are often just dumped in landfill or in the sea. This waste piles up at coastal areas and causes many environmental problems including pollution of coastal fisheries, management problem of public water surface, damage of natural landscape and health/sanitation problems (Jung et al., 2012). Finding solutions to the problem of such waste would be a step towards reducing the environmental problems.

To the best of our knowledge, there is no reported data in literature for radioactivity and elemental analysis for any kind of shellfish from the Boka Kotorska bay. Therefore, the work reported in this study is a first attempt to use the shells of mussels *M. galloprovincialis* and oysters *O. edulis* in a biomonitoring study investigating radioactivity and trace elements pollution in the Boka Kotorska bay. Also, this research will serve to determine the safety of the waste shells and to assess the possibility of its use for other purposes.

MATERIALS AND METHODS

Mussel and oyster samples were collected at two locations in the Boka Kotorska bay, Montenegro, on the Southeastern part of Adriatic coast, Table 1. At each sampling location about 5 kg of mussels and oysters were collected, placed in bags with seawater and transported to the laboratory. Shellfish were washed and cleaned out, opened raw and the flesh was scraped out of the shells. After that shells were oven-dried at 60°C, than pulverized using a planetary mono mill with agate milling balls (Pulverisette 6, Fritsch GmbH) and the resulting homogeneous fine powders stored for subsequent analysis.

Table 1. Information related to collected samples and sampling locations

Species	***Mytilus galloprovincialis***	***Ostrea edulis***
Sampling date	July 2016	June 2016
Sampling locations	COGImar, Orahovac	Sv Neđelja, Kamenari
Coordinates	42°29'08.2"N 18°44'44.8"E	42°27'31.1"N 18°40'21.2"E

The total content of investigated elements was determined following US EPA 3051A procedure for microwave assisted acid digestion (U.S. EPA 3051A, 2007). Element concentrations were measured by an inductively coupled plasma mass spectrometer (ICP-MS) model Thermo iCAP Q (Thermo Scientific, United Kingdom). Gamma spectrometry measurements were performed by low-level HPGe detectors (Canberra Industries, Meriden, Connecticut, USA) with relative efficiency 18% and resolution of 1.89 keV at 1332 keV. The samples were packed in Marinelli beaker geometry of 0.5 L. The counting time was 160000 s and data were statistically analyzed by Genie 2000 software.

RESULTS AND DISCUSSION

Concentrations of ^{210}Pb, ^{238}U, ^{235}U, ^{226}Ra, ^{232}Th, ^{40}K and ^{137}Cs (in Bq kg^{-1}) and Pb, Cr, Mn, Cu, Zn, V, Ni, As, Cd and Co (in mg kg^{-1}) measured in shells of *M. galloprovincialis* and *O. edulis* sampled from the Boka Kotorska bay are summarized in Table 2 and Table 3.

Table 2. Activity concentrations of 210Pb, 238U, 235U, 226Ra, 232Th, 40K and 137Cs in shells from the Boka Kotorska bay (Bq kg-1).

	^{210}Pb	**^{238}U**	**^{235}U**	**^{226}Ra**	**^{232}Th**	**^{40}K**	**^{137}Cs**	**Ra_{eq}**
M. galloprovincialis	18±2	<2	<0.1	2.5±0.3	<0.3	4.2±0.9	<0.05	3.0
O. edulis	12±1	<2	<0.1	0.9±0.1	1.1±0.2	17±2	<0.05	3.8

In the current study, the activity concentrations of ^{137}Cs, ^{238}U, ^{235}U, for both shell samples, and ^{232}Th for *M. galloprovincialis* were found to be below of instrument detection limits. The activity concentrations of ^{210}Pb and ^{226}Ra were found to be higher in *M. galloprovincialis* than in *O. edulis*, while in the case of ^{40}K activity concentration in *O. edulis* was significantly higher ($p<0.05$) than in *M. galloprovincialis.* The levels and patterns of accumulation of radionuclides vary from species to species and region to region, but also from body size. In general for seashells, concentrations of radionuclides decrease with increasing body size

(Alam et al., 2000). Obtained activity concentrations were found to be similar or lower than those obtained for seashells in previous studies (Alam et al., 2000; Kılıç and Çotuk, 2011) which suggest that there is no pollution caused by investigated radionuclides.

Radium equivalent activity (Ra_{eq}) in Table 2, calculated by using the equation: $Ra_{eq}=C_{Ra}+1.43C_{Th}+0.077C_K$, where C_{Ra}, C_{Th} and C_K are determined Ra, Th and K activities, respectively, show that for investigated shell samples Ra_{eq} were <370 Bq kg^{-1} which represents the maximum permissible value for raw building materials and products for safe use (Jelić et al., 2018). This indicates that bivalve shells, which are mainly made of $CaCO_3$, can be safely used in building materials.

Table 3. Elements concentrations in shells samples (mg kg−1).

	Pb	Cr	Mn	Cu	Zn
M. galloprovincialis	1.29	31.2	13.5	5.98	2.99
O. edulis	53.1	13.2	69.9	7.14	9.65
	V	**Ni**	**As**	**Cd**	**Co**
M. galloprovincialis	0.67	0.80	<0.1	<0.1	0.14
O. edulis	1.43	3.96	2.01	0.23	0.50

All investigated concentrations were found to be higher in *O. edulis* than in *M. galloprovincialis*, except for Cr (Table 3). Differences in elements concentrations between shells can be attributed to the species type, region, but also to the contamination degree of the investigated locations. Semi-enclosed systems, such as Boka Kotorska bay, are very sensitive to contamination which primarily comes from the mainland due to various human activities (Jovic et al., 2011). High concentration of Pb was detected in *O.edulis* shells from Sv Neđelja, Kamenari i.e. location with intensive marine traffic. In comparison to other literature reports (Çevik et al., 2008; Protasowicki et al., 2008; Szefer and Szefer, 1985) the Pb level found in Kamenari was higher, indicated that the elevated Pb concentration is primarily the consequence of human activities in the Bay. Also, elevated Cr and Mn concentrations in comparison to other literature data (Çevik et al., 2008; Protasowicki et al., 2008) were obtained. However, an analysis of the element content of the Boka Kotorska bay sediments prior to its Industrialization period indicated that the sea bed is rich in Mn and Cr (Jovic et al., 2011) and that their presence in the shells are rather of natural than of anthropogenic origin. Concentrations of the remaining elements were found to be similar to those reported in literature.

CONCLUSIONS

Activity concentrations of ^{210}Pb, ^{238}U, ^{235}U, ^{226}Ra, ^{232}Th, ^{40}K and ^{137}Cs in *M. galloprovincialis* and *O. edulis* shells were found to be similar or lower than those obtained in previous studies suggesting that there is no pollution caused by investigated radionuclides. Radium equivalent activities (Ra_{eq}) for investigated shell samples were <370 Bq kg^{-1} which indicates that bivalve shells can be safely used in the production of building materials. High concentration of Pb detected in *O.edulis* shells indicated anthropogenic impact as a result of marine traffic, while elevated Cr and Mn concentrations are the consequence of sediments composition in the Bay. The study demonstrates that shells of mussels *M. galloprovincialis* and oysters *O. edulis* are useful tool for biomonitoring and water quality assessment in the Boka Kotorska bay.

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USABILITY OF THE ZAPADNA MORAVA RIVER WATER FOR FISHING PURPOSE

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UPOTREBLJIVOST VODE REKE ZAPADNE MORAVE ZA RIBARSTVENE NAMENE

Apstrakt

Cilj rada bio je da se na osnovu analize standardnih fizičko-hemijskih i mikrobioloških (bakterijskih) parametara kvaliteta vode srednjeg toka Zapadne Morave izvrši procena upotrebljivosti vode za gajenje šaranskih riba. U ihtiološkom pogledu Zapadna Morava predstavlja mrensko-ciprinidni region. Ihtiofaunu ispitivanog sektora formiraju 23 vrste iz 8 porodica. Izrazita je dominacija šaranskih riba (fam. Cyprinidae). Mala brojnost predatorskih vrsta doprinosi nepovoljnoj strukturi riblje zajednice. Analiza vode na tri lokaliteta (**1**-**3**) urađena je u periodu 2016–2017 godine. Rezultati dobijeni za fizičko-hemijske (pH-vrednost, rastvoreni kiseonik, biohemijska potrošnja kiseonika, ukupni organski ugljenik, amonijum jon, nitrati, ukupni fosfor, ortofosfati i hloridi) i bakterijske (ukupne koliformne bakterije, fekalne koliformne bakterije i intestinalne enterokokne bakterije) parametre kvaliteta vode srednjeg toka reke Zapadne Morave ukazuju da ispitivana voda uglavnom zadovoljava osnovne uslove za opstanak riba. Vrednosti većine ispitivanih hemijskih parametara (rastvoreni kiseonik, ukupni organski ugljenik, amonijum jon, ukupni fosfor i hloridi) na svim ispitivanim lokacijama su bile veće u 2017. godini u poređenju sa rezultatima dobijenim u 2016. godini. Isti trend primećen je za ortofosfate i nitrate, sa izuzetkom na lokalitetima **1** odnosno **3** gde su veće vrednosti izmerene u 2016. godini. Rezultati bakterijske analize ukazuju na veći sadržaj ukupnih koliformnih i fekalnih koliformnih bakterija u 2017. godini na lokalitetima **1-3** i intestinalnih enterokoka~~ma~~ na lokalitetu **3**. Generalno, deo vodotoka nizvodno od Čačka karakteriše visok stepen organskih zagađenja i bakterijske kontaminacije, tako da je rečna voda ovog sektora neupotrebljiva za bilo koji oblik gajenja riba i/ili poljoprivrednih aktivnosti. Dobijeni rezultati ukazuju na

potrebu izgradnje sistema za prečišćavanje otpadnih voda iz Čačka koji bi doprineo poboljšanu kvaliteta vode ovog vodotoka.

Ključne reči: *reka Zapadna Morava, kvalitet vode, ihtiofauna*
Keywords: *the Zapadna Morava River, water quality, ichthyofauna*

INTRODUCTION

Intensive degradation processes exacerbate the conditions for survival of living organisms in water ecosystems. Degraded water quality characterizes most of our rivers including the Zapadna Morava River, central watercourse of Serbia. Length of the direct course of the Zapadna Morava River is 308 km. Hydrographic network of the basin includes over 1800 smaller and bigger watercourses (Ocokoljić, 1987). There are two accumulations on the river (Ovčar Banja and Međuvršje) which are in the most part with deposition and distinct process of eutrophication (Lenhardt et al. 2009). Watercourses of the Zapadna Morava basin are large recipients of communal, industrial and agricultural waste waters. Presence of phenols, toxic elements and other pollutants which degrade water quality and endanger survival of hydrobionats has been registered in the water of the direct course of the Zapadna Morava River (Lazić et al., 2003; Obradović and Filipović, 2009; Novaković, 2013; Mašković et al. 2018).

This work aimed to evaluate the usability of the middle course of the Zapadna Morava River water for carp fish culture and forming fish ponds of this type, based on the analysis of physico-chemical and microbiological (bacteriological) parameters for water quality obtained during 2016 and 2017.

MATERIALS AND METHODS

During 2016 and 2017 sampling and analysis of the river water from three localities (**1-3**) of the Zapadna Morava’s middle course (Moravica county area, Serbia) were performed, Figure 1. Locality **1** (N 43° 54’/E 20° 14’) is situated downstream from the dam of hydroelectric power plant (HE) «Međuvršje», at the 182nd km of the river course; Locality **2** (N 43° 54’/E 20° 20’) is situated by the sports center «Mladost» in the city of Čačak, at the 170th km of the river course; Locality **3** (N 43° 52’/ E 20° 26’) is situated in the village Stančići, at the 162nd km of the river course. This locality is placed downstream from the inflow of the Čačak sewage system.

Figure 1. Study area; localities 1, 2 and 3 on the middle course of the Zapadna Morava River

Selected physico-chemical parameters (pH, dissolved oxygen (DO), biochemical oxygen demand (BOD), total organic carbon (TOC), ammonium (NH_4^+) ion, nitrates, and chlorides) were determined using literature standard methods (Kostić et al., 2016), while bacteriological (total coliforms, fecal coliforms and intestinal enterococci) analyses, as basic water quality parameters, were performed according to National Standard Methods for the Hygienic Examination of Water (Pantelić et al., 2017). Also, determination of total phosphorous and orthophosphate ion was done according to colorimetric EPA 365.3 - method (EPA, 1978). Obtained results were compared to basic criteria that the water must fulfill for fish culture (Bhatnagar and Devi, 2013).

Interpolation based on Kriging methodology was used for presenting water quality data on Figures 2 and 3. Kriging is a statistical technique that posits a certain statistical model for the data, namely that the response at a given location is the sum of two components: an unknown underlying surface, which we are trying to estimate, plus some additional noise. ArcGIS uses a somewhat ad hoc method, estimating something called the variogram using weighted least squares, and then relies on some further ad hoc approaches to reduce the computational burden of producing an estimate of the underlying surface and estimates of uncertainty in the surface estimate. For a surface estimate based on a default approach to estimate the variogram, in this paper, we used the Spatial Analyst extension (https://www.hsph.harvard.edu/; https://www.hsph.harvard.edu/gis/arcgis-tips/kriging/).

RESULTS AND DICUSSION

According to basic physico-chemical and bacteriological characteristics quality of surface waters in Serbia is divided into five classes; from class I (the best) to class V (the most polluted and unusable for any purposes) (Anonymous 2011). It was noticed that the values of dissolved oxygen, total organic carbon, ammonium ion, total phosphorous and chlorides were higher in 2017 in all studied locations when compared with the results obtained in 2016. The same trend was observed for orthophosphates and nitrates with the exceptions on localities **1** and **3** respectively, where higher values were recorded in 2016. Results of bacteriological analysis indicated higher content of total coliforms and fecal coliforms in 2017 on localities **1-3**, and intestinal enterococci on the locality **3** (Table 1).

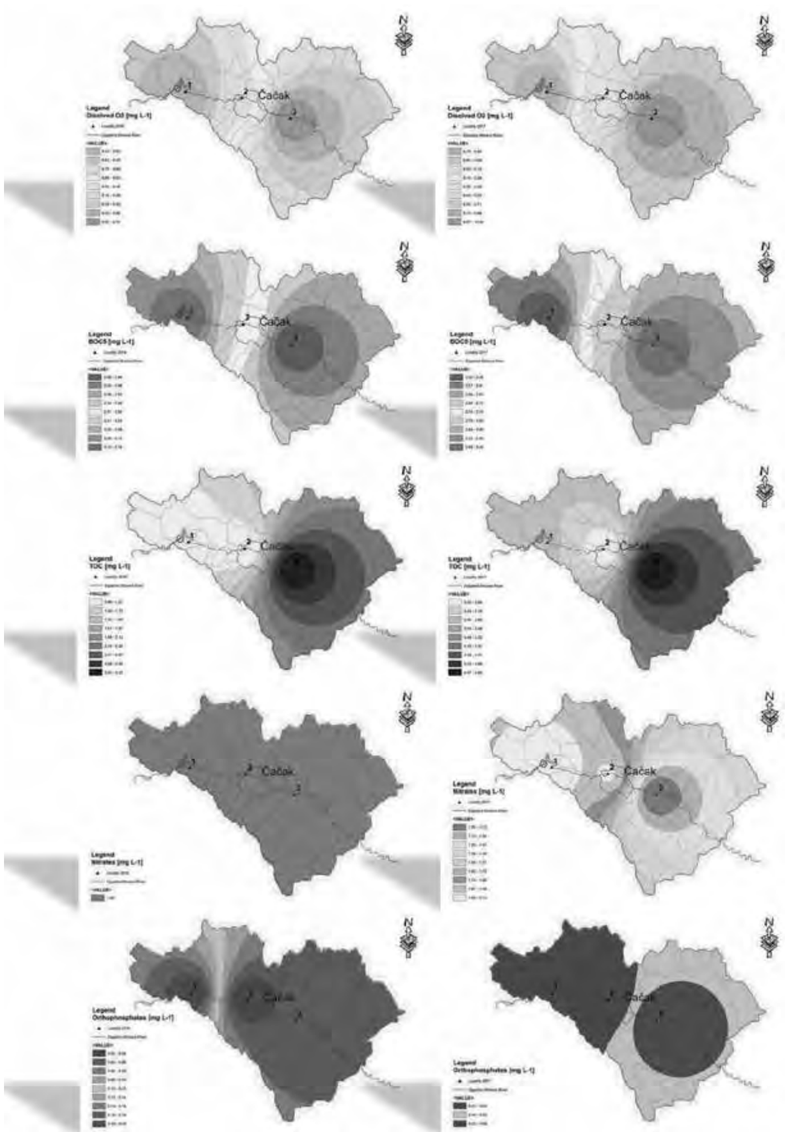
Table 1. Chemical and bacteriological analysis of the Zapadna Morava River water

Parameter*	unit	Locality 1		Locality 2		Locality 3	
		2016	2017	2016	2017	2016	2017
pH	mg L^{-1}	7.1	7.8	8.0	7.9	8.2	8.0
DO		8.5	8.7	8.8	9.4	9.7	10.0
BOD		3.16	2.5	3.0	2.8	2.8	3.0
TOC		< 1	3.8	< 1	3.5	3.2	4.8
NH_4^+ ion		0.25	0.3	0.2	0.2	0.2	0.5
Nitrates		1.9	2.1	1.9	2.0	1.9	1.0
Orthophosphates		0.19	0.03	0.02	0.03	0.04	0.06
Total phosphorous		0.03	0.04	0.03	0.04	0.05	0.07
Chlorides		8.85	9.1	7.74	9.3	9.94	11.7
Total coliforms	cfu 100 mL^{-1}	18 083	78 400	12 775	96 750	251 683	279 058
Fecal coliforms		2 016	37 040	1 692	28 225	67 141	69 400
Intestinal enterococci		485	170	348	157	3 234	11 297
Classes of surface waters (mean status)		**III**	**III**	**III**	**III**	**IV**	**IV**

* – Average values

Changes in values of chemical and bacteriological parameters on locations **1**, **2** and **3** during the study period (2016-2017) are presented in Figures 2 and 3, respectively. Obtained results of the studied water quality parameters of the Zapadna Morava River in 2016/2017 indicated its uneven and generally deteriorated quality.

Water quality of the locality **1** corresponded to moderate ecological status, i.e. class III of surface waters. Evident pollution, primarily microbiological, was a consequence of the inflow of communal and industrial waters from many populated areas located upstream from the profile, as well as eutrophication processes occurring at the accumulation Međuvršje. Water quality of the locality **2** (in the city of Čačak) also corresponded to moderate ecological status (class III of surface waters).



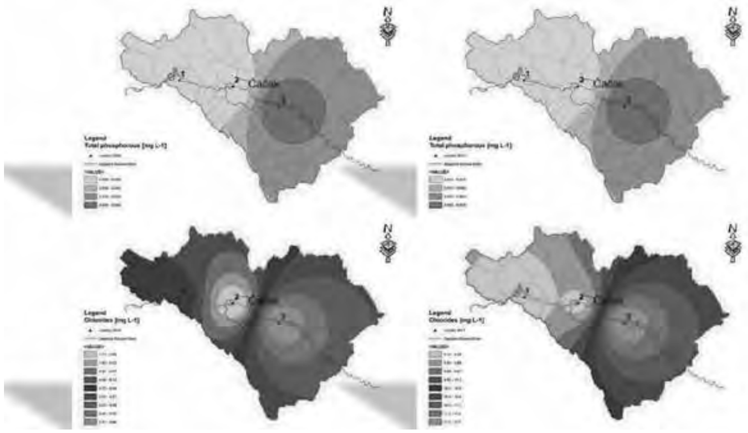


Figure 2. Changes of the chemical profile of the Zapadna Morava River on localities 1-3 during the period 2016/2017

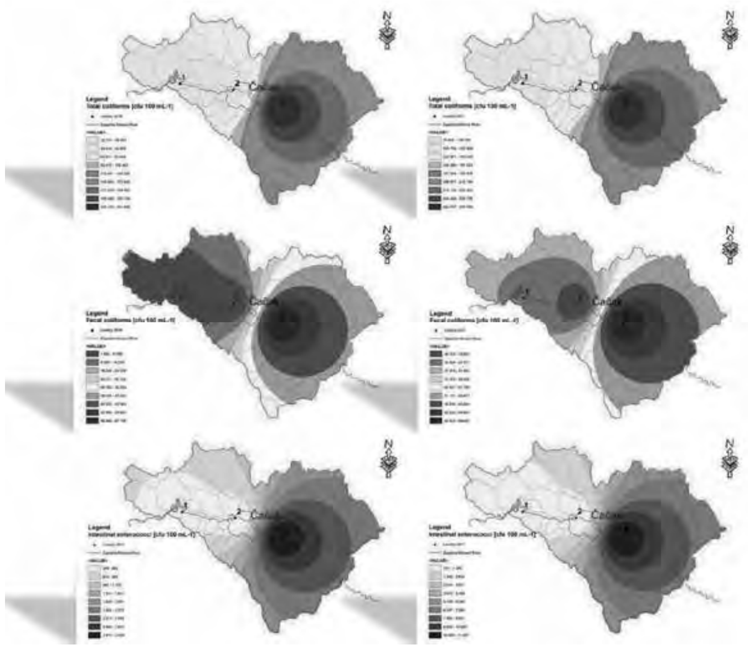


Figure 3. Changes of the bacterial profile of the Zapadna Morava River on localities 1-3 during the period 2016/2017

On the river course between profiles **1** and **2**, there are no big sources of pollution. During the summer this part of the river becomes Čačak bathing resort. Longer retention and elevated water temperature intensify the decomposition of organic pollutants and degrade the quality. Water quality of the locality **3** was significantly worse compared to the previous ones (**1** and **2**). The profile was under enormous influence of the waste waters from the Čačak sewage system which unpurified reach the watercourse. Based on the average values of NH_4^+ ions, total coliforms and fecal coliform bacteria the water quality of this profile corresponded to poor ecological status (class IV of surface waters). The degree of bacteriological contaminations was especially high (Table 1), which confirms high degree of organic pollution of this part of the watercourse.

From the ichthyological point of view, the Zapadna Morava River represents a barbel-cyprinid region. The ichthyofauna of the examined sector comprises 23 species from 8 families with distinct domination of carp fish (fam. Cyprinidae) (Table 2).

Table 2. Ichtyofauna of the Zapadna Morava river (profile Čačak) (Marković et al. 2013)

Fam. Cyprinidae		**Fam. Siluridae**	**Family Cobitidae**
Alburnus alburnus	*Gobio gobio*	*Silurus glanis*	*Cobitis taenia*
Alburnoides bipunctatus	*Leuciscus aspius*	**Fam. Esocidae**	**Fam. Centrarchidae**
Abramis brama	*Squalius cephalus*	*Esox lucius*	*Lepomis gibbosus**
Barbus balcanicus	*Pseudorasbora parva**	**Fam. Percidae**	**Family Icatulirade**
Barbus barbus	*Rhodeus sericeus*	*Perca fluviatilis*	*Ameiurus nebulosus**
*Carassius gibelio**	*Rutilus rutilus*	*Zingel zingel*	**Family Gobiidae**
Chondrostoma nasus	*Vimba vimba*	*Gymnocephalus cernuus*	*Neogobius fluviatilis**

* *allochthonus /alien/ species*

There is an especially big number of commercially less significant species – bleak (*Alburnus alburnus*), nase (*Chondrostoma nasus*) and Prussian carp (*Carassius gibelio*), with the reduced number of carp (*Cyprinus carpio*). Small number of predator species contributes to the unfavorable structure of the fish community.

By comparing the results of the physico-chemical water analyses of the middle course of the Zapadna Morava River with certain criteria of water quality for fish culture presented in Table 3, it can be concluded that the river fulfills the basic conditions for fish survival (Bhatnagar and Devi, 2013).

Table 3. The optimum range of various water quality parameters (Bhatnagar and Devi, 2013)

Parameter		Acceptable range	Desirable range	Stress
Temperature	(^{o}C)	15-35	20-30	<12, >35
Turbidity	(cm)		30 – 80	< 12, > 80
Water color		Pale to light green	Light green to light brown	Clear water, Dark green & brown
pH		7-9.5	6.5-9	< 4, > 11
Dissolved O_2	(mg L^{-1})	3-5	5	< 5, > 8
BOD	(mg L^{-1})	3-6	1-2	> 10
CO_2	(mg L^{-1})	0-10	< 5, 5-8	> 12
Alkalinity	(mg L^{-1})	50-200	25-100	< 20, > 300
Hardness	(mg L^{-1})	> 20	75-150	< 20, > 300
Calcium	(mg L^{-1})	4-160	25-100	< 10, > 250
Ammonia	(mg L^{-1})	0-0.05	0. < 0.025	> 0,3
Nitrite	(mg L^{-1})	0.02-2	< 0.02	> 0,2
Nitrate	(mg L^{-1})	0-100	01-4.5	> 100, < 0.01
Phosphorous	(mg L^{-1})	0.03-2	0.01-3	> 3
H_2S	(mg L^{-1})	0-0.02	0.002	Any detectable level
Primary productivity	($CL^{-1}D^{-1}$)	1-15	1.6-9.14	< 1.6, > 20.3
Plankton	(No. L^{-1})	2000-6000	3000-4500	< 3000, > 7000

Exceptions were elevated concentrations of NH_4^+ ions at the locality **3** (Stančići). Besides that, this profile was especially characterized by significant bacteriological contaminations which declare the river water of the profile as unusable for fish growing and/or any agricultural activities. Before it might be used, it is necessary to perform disinfection treatments which would significantly increase the expenses of processing and question the cost-effectiveness of the entire process. Obtained results pointed out the need for building a System for purification of waste waters coming from the Čačak city.

CONCLUSIONS

Results of the standard physico-chemical and microbiological (bacteriological) parameters for water quality of the middle course of the Zapadna Morava River obtained in the period 2016/2017, indicated its uneven and generally deteriorated quality. High degree of organic pollution and bacteriological contamination of the part of the watercourse downstream from Čačak, caused by the inflow of unpurified waste waters from the city, makes the river water unsuitable for fish culture, irrigation, and any agricultural activities.

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SUPEROXIDE DISMUTASE AND CATALASE ACTIVITIES IN HEPATOPANCREAS OF RAINBOW TROUT (*ONCORHYNCHUS MYKISS*) FROM DIFFERENT AQUACULTURE FACILITIES

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AKTIVNOSTI SUPEROKSID DISMUTAZE I KATALAZE U HEPATOPANKREASU KALIFORNIJSKE PASTRMKE (*ONCORHYNCHUS MYKISS*) IZ RAZLIČITIH RIBNJAKA

Apstrakt

Dve populacije juvenilnih jedinki kalifornijske pastrmke *Oncorhynchus mykiss*, uzete su iz Centra za ribarstvo i primenjenu hidrobiologiju «Mali Dunav» Poljoprivrednog fakulteta (*eng.* CEFAH) i pastrmskog ribnjaka Sisevac (*eng.* Trout fish farm Sisevac – TFS). Pastrmke su u svakom ribnjaku hranjene sa različitom komercijalnom hranom za ribe. Hrana za ribe u TFS je sadržala 2% više sirovih proteina, 5% više sirovih masti, 0.8% više pepela i 3% više svarljive energije nego hrana za ribe korišćena u CEFAH. Variranje izmerenih fizičkih i hemijskih parametara vode je bilo zanemarljivo između dva ribnjaka.

Hepatopankreas je glavni organ kod riba za skladištenje i metabolizam masti, samim tim antioksidativni status ovog organa utiče na nivo metabolizma masti. Cilj ispitivanja u našem radu je bio da se detektuju moguće promene u aktivnostima antioksidativnih enzima, superoksid dismutaze (SOD) i katalaze (CAT) u hepatopankreasu kalifornijskih pastrmki (n = 10 jedinki u svakoj populaciji) u odnosu na njihove ishrane sa različitim procentualnim sastavom hranljivih materija. U oba ribnjaka, ribe su na dnevnoj bazi dobijale hranu u količini od 1.3% njihove ukupne mase tela dok jedinke nisu dostigle starost oko 12 meseci i težinu tela od 125,7 ± 17,2 g. Kalifornijske pastrmke iz ribnjaka TFS su imale težinu tela u proseku za 10% veću u poređenju sa jedinkama iz ribnjaka CEFAH, ali nije dobijena statistički značajna razlika u kondicionom faktoru (CF) i hepatosomatskom indeksu (HSI) između dve populacije. Sa druge strane, dobijene su statistički značajno više aktivnosti enzima SOD (15%, $p < 0.01$) i CAT (9%, $p < 0.05$) u hepatopankreasu kod TFS populacije u odnosu na CEFAH populaciju. Primena Pearsonovih korelacija između merenih enzima je pokazala postojanje statistički značajne pozitivne međusobne povezanosti u hepatopankreasu TFS riba. Dobijeni rezultati ukazuju na to da hrana koja sadrži veći udeo masti i svarljive energije utiče na uvećan rast kalifornijskih pastrmki, praćen povećanjem aktivnosti antioksidativnih enzima SOD i CAT. Dalja istraživanja su neophodna da bi se bolje razjasnio mehanizam koji leži u osnovi promena antioksidativnih enzima koje prate promene u metabolizmu masti verovatno nastale zbog ishrane kalifornijskih pastrmki sa hranom koja ima različit kvantitativni sastav hranljivih materija.

Ključne reči: *kalifornijska pastrmka, ribnjak, komercijalna hrana, SOD, CAT*

Abstract

The two aquaculture populations of rainbow trout juveniles, *Oncorhynchus mykiss* (from Center for Fishery and Applied Hydrobiology "Little Danube" Faculty of Agriculture - CEFAH, and Trout fish farm Sisevac – TFS) were fed with two commercial feeds. Fish from TFS were fed with feed that contains 2% of crude protein, 5% of crude lipids, 0.8% of ash and 3% of digestible energy more than feed used for CEFAH population. The variation of most physico-chemical parameters of water in two aquacultures was in narrow range, with data showing no statistically significant difference. Knowing that the hepatopancreas is major lipid storage and metabolism organ in fish, antioxidant status of this tissue affects the metabolism rate of lipids. The aim of our study was to determine the possible variations of antioxidant defense enzymes, superoxide dismutase (SOD) and catalase (CAT) in hepatopancreas of rainbow trout ($n = 10$ per group) in relation to feeds with various proximate compositions. Fish were fed daily by hand at feeding rate of 1.3% of total fish body mass in both aquacultures, until specimens were about 12 months old and weighing 125.7 ± 17.2 g. Rainbow trouts from TFS had body weights cca 10% higher in comparison with fish from CEFAH, but there were no statistical differences in condition factor (CF) and hepatosomatic index (HSI) among the two groups. It was noticed that SOD and CAT activities were significantly higher, 15% ($p < 0.01$) and 9% ($p < 0.05$) respectively, in hepatopancreas of TFS fish group compared to that of CEFAH group. In addition, statistically significant positive Pearson's correlations between the SOD and the CAT activity in hepatopancreas of TFS fish were observed. The obtained results suggest that diet containing more lipids and digestible energy is effective in enhancing rainbow trout growth, followed by increasing enzymatic antioxidant capacity through increasing SOD and CAT activities. However, further investigation is necessary to fully understand the underlying mechanism of these diet-composition mediated antioxidant responses in the aquaculture fish species.

Keywords: *rainbow trout, aquaculture, commercial feed, SOD, CAT*

SELECTION OF PHOTOSYNTHETIC MICROORGANISMS CONSORTIA ABLE TO REMOVE NITRATE AND PHOSPHORUS, TO BE FUTHER USED IN RAS

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SELEKCIJA KONZORCIJUMA FOTOSINTETIČKIH MIKROORGANIZAMA SPOSOBNIH ZA UKLANJANJE NITRATA I FOSFORA, ZA KORIŠĆENJE U RAS SISTEMIMA

Apstrakt

Upotreba fotosintetičkih organizama u recirkulacionim sistemima u akvakulturi (RAS) je relativno nova tema, uprkos tome što su sposobnosti ovih organizama pre 60 godina privukle pažnju u oblasti prećiščavanja otpadnih voda iz domaćinstava. Cilj ovog rada je da predstavi rezultate selekcije populacija fotosintetičkih organizama koje su sposobne da koriste nitrate i neorganski fosfor kao hranjive materije, time snižavajući njihove koncentracije na vrednosti koje mogu biti od interesa za RAS sisteme i/ili tretman otpadnih voda iz sistema za njihovo prećiščavanje. Selektivno gajenje bogatih mešavina fotosintetičkih organizama u BG_{11} medijumu sa niskim koncentracijana nitrata (40mg nitrata/L), veštačkom medijumu koji služi kao model otpadnih voda, omogućava povećanje sposobnosti fotosintetičkih organizama da absorbuju nitrate i ortofosfate. Selektovani konzorcijumi, sačinjeni od cijanobakterija i mikroalgi, uklanjaju nitrate stopom od 9.8 – 59.0 mg dnevno za tri selektovana konzorcijuma, smanjujući njihovu koncentraciju sa 40 mg/L na 3mg//L. Odabrani mikrobijalni konzorcijumi koji su imobilizovani na hidrofobičnom supstratu smanjuju koncentracije ortofosfata sa 147 mg/L na 3.7 mg/L za 24 časa, stopom od 143 mg ortofosfata dnevno za 200 cm^2 imobilizovanih fotosintetičkih mikroorganizama. Tokom uklanjanja nitrata i ortofosfata, javlja se akumulacija polifosfatnih tela unutar nekih ćelija, kao i očigledan porast masti od 15%. Dalji rad na selektovanim konzorcijumima je neophodan kako bi se dostigao nivo efikasnosti rada potreban za otpadne vode iz RAS sistema, u laboratoriji i *in situ*.

Ključne reči: *fotosintetički mikroorganizmi, selektivno gajenje, nitrati, ortofosfati, recirkulacioni sistemi u akvakulturi.*
Keywords: *photosynthetic microorganisms, selective cultivation, nitrate, orthophosphate, recirculating aquaculture systems*

INTRODUCTION

The ability of photosynthetic microorganisms to use inorganic nitrogen (NO_3^-, NH_4^+ or ammonia) and orthophosphate as nutrients to sustain their growth has retained attention in the last six decades for the use of these cells as biocatalysts in waste water treatment (Oswald, and Gotaas, 1957; Oswald, 1988; Benemann, 1989; Arredondo-Figueroa et al., 2007; Ramli et al., 2017; Manea and Ardelean, 2016; Tiron et al., 2017; Chun et al., 2018). Recirculating aquaculture systems (RAS) are intensive, usually indoor tank-based systems, very useful where ideal sites are unavailable, which achieve high rates of water re-use by mechanical, biological chemical filtration and other treatment steps (Dalsgaard et al., 2013; Murray et al., 2014). The use of photosynthetic microorganisms in RAS, up to our best knowledge, is a rather new topic but with promising results (Burut-Archanai et al., 2013; Valeta and Verdegem, 2015; .Ramli et al., 2017, 2018; Chun et al. 2018). The aim of this paper is to present the results concerning the selection of photosynthetic microorganism populations able to use nitrate and inorganic phosphorous as nutrients thus strongly decreasing their concentration at values which can be of interest for RAS and/or treatment of outlet waters from domestic waste water plants.

MATERIALS AND METHODS

Photosynthetic microorganisms. The diversity of starting biological material for further selection was increased as much as possible by mixing different photosynthetic assemblages of populations already existing in the Microbiology Department of Institute of Biology Bucharest as well as mixtures of populations from the Plutonița fish farm and from domestic aquaria. The mixture of consortia was grown in BG_{11} medium at 20°C under continuous white fluorescent illumination. The selection of photosynthetic microorganisms for RAS was done by growing them in a modified BG_{11} medium, a medium with low nitrate concentration (40mg nitrate/L), model for synthetic waste water. The cells were grown either immobilized or free in solution. Immobilization was done by growing them in the presence of a solid support (hydrophilic (e.g. cotton) or hydrophobic). Selection of free photosynthetic microorganisms was focused predominantly on filamentous ones which can grow and form a network in which unicellular photosynthetic microorganisms can reside. Cell morphology was investigated microscopically in bright field without any added dye or treated with alkaline methylene blue which, in the presence of polyphosphate inclusions, becomes reddish. Nitrate and phosphorus determination were performed using the Spectroquant® Nitrate test kit 1.09713.0001 (Merck) and Spectroquant® Phosphate Test 1.14842.0001 (Merck). All the NO3 and PO4 concentrations were calculated using a standard curve prepared with Nitrate IC-STD solution 119811 (Merck) and Phosphate IC-STD solution 119898. The lipid content of algal cells was estimated, after Nile red addition, by quantification of fluorescence emission, as previously shown (Ardelean et al., 2017) in agreement with Anthony et al. (2014). The presence of both prokaryotes and eukaryotes photosynthetic microorganisms was done following Lee et al. (1995) by measuring the intensity of the fluorescence signal at 640nm and 680 nm.

RESULTS AND DISCUSSION

Table 1 presents the time evolution (9 weeks) of nitrate removal capacity of photosynthetic microorganisms either free (vessel 2) or immobilized on cotton (vessel 1) or on a hydrophobic substrate (vessel 3). The NO_3 removal rates are expressed in mg of NO_3/24 hours, photosynthetic microorganisms occupying a surface of 200cm^2.

Table 1. NO3 removal rates of the photosynthetic microbial populations (mg NO3/24 hours)

Week	1	2	3	4	5	6	7	8	9
Vessel 1	4.1	5.0	6.5	6.2	7.3	8.2	8.5	8.1	9.8
Vessel 2	10.2	11.2	18.2	16.0	21.6	18.0	25.4	28.7	33.6
Vessel 3	35.7	40.4	45.0	38.6	47.4	53.8	58.0	55.4	59.0

There was an increase in the rate of nitrate removal in time, with concentrations in 24 being decreased from 40mg/L to 3mg/L. The ability to remove phosphorus of the selected microbial consortium immobilized on hydrophobic substrate (vessel 2) was also checked, the decrease in orthophosphate being from 147mg/L to 3.7mg/L in 24 hours with a rate of 143mg orthophosphate /24 hours/200cm^2 of immobilized photosynthetic microorganisms. These results demonstrate the ability of photosynthetic microorganisms grown in modified BG_{11} medium to consume nitrate and orthophosphate, in agreement with results already reported in the literature using true outlet waters from functional RAS (Valeta and Verdegem, 2015; Ramli et al., 2017; Ramli et al., 2018, Chun et al., 2018) but, for the time being, it is difficult to compare the reported rates with ones reported here. During orthophosphate consumption process from this model of synthetic waste water, the accumulation of polyphosphate bodies inside some microbial cells can occur, as indicated by specific alkaline methylene blue labelling (results not shown). Microscopic investigations argue for the presence of photosynthetic microorganisms, both prokaryotes (cyanobacteria) and eukaryotes (microalgae) in the selected consortium (results not shown). This information was confirmed by measuring fluorescence emission at 640nm (excitation at 620nm) at 680nm (excitation at 440nm) (Lee et al., 1995). Table 2 shows the intensity of the fluorescence signal of the selected consortia of photosynthetic microorganisms as compared with the signal produced by pure cultures of *Synechocystis* PCC 6803 (cyanobacteria) and *Chlorella sorokiniana* UTEX 1230 (microalgae).

Table 2. Fluorescence emission of the selected consortium of photosynthetic microorganisms as compared with pure cultures of cyanobacteria and microalgae

	Fluorescence Ex620/em 645	Fluorescence Ex440/em 680	Ratio em 640/em680
Synechocystis PCC 6803	0.1589	0.0320	4.9937
Chlorella sorokiniana UTEX 1230	0.0780	1.8550	0.0420
Selected microbial consortium	0.280	0.100	2.8000

These results further argue that the selected photosynthetic microbial consortium contains both prokaryotes and eukaryotes, in agreement with microscopic observations. Fluorescence estimation of lipid content indicates a 15% increase in the lipid content of the consortium immobilized on hydrophobic substrate during nitrate and orthophosphate consumption, in agreement with the idea that the biomass of photosynthetic microorganisms could be a source of lipids (Benneman, 1989).

CONCLUSIONS

Selective cultivation of rich mixtures of photosynthetic microorganisms in BG_{11} with low nitrate concentration (40mg nitrate/L), model for synthetic waste water, allows the increase ability of photosynthetic microorganisms to take up nitrate and orthophosphate. The selected consortia, composed of both cyanobacteria and microalgae, eliminate nitrate at rates of 9.8-59.0 mg nitrate/ 24 hour, for three different selected consortia, lowering the concentration from 40mg/L to 3mg/L. The selected microbial consortium immobilized on hydrophobic substrate decreases orthophosphate concentration from 147mg/L to 3.7mg/L in 24 hours with a rate of 143 mg orthophosphate/24 hours/200cm^2 of immobilized photosynthetic micro-organisms. During the removal of nitrate and orthophosphate, the accumulation of polyphosphate bodies occurs inside some cells, as well as an apparently 15% increase in the lipid content. More work needs to be done in order to reach the efficiency level needed for true waste waters from RAS, at laboratory level and *in situ*.

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EFFICIENCY OF NITROGEN AND PHOSPHORUS REMOVAL OF SELECTED BACTERIAL CONSORTIA FOR THE BIOTREATMENT OF WASTEWATER IN RAS

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EFIKASNOST UKLANJANJA AZOTA I FOSFORA OD STRANE SELEKTOVANIH BAKTERIJSKIH KONZORCIJUMA ZA BIOLOŠKO TRETIRANJE OTPADNIH VODA U RECIRKULACIONIM SISTEMIMA

Apstrakt

Selektovani bakterijski konzorcijumi efikasno uklanjaju 88% NH_4 iz veštačkog medijuma i 100% NO_3 i time predstavljaju interesnu temu za recirkulacione sisteme gajenja u akvakulturi (RAS).

Uklanjanje azota iz otpadnih voda trenutno je zasnovano na aktivnosti nitrifikujućih i denitrifikujućih organizama. U ovom radu proučavali smo sposobnost nitrifikacije, aerobne denitrifikacije i uklanjanja fosfata od strane selektovanog bakterijskog konzorcijuma uzgojenog u veštačkom medijumu. Bakterijski konzorcijumi su izolovani iz biofiltera iz akvarijuma, obogaćeni aerobnim denitrifikujućim bakterijama i gajeni u osnovnom medijumu sa acetatom kao jedinim izvorom ugljenika, $KMnO_4$ kao izvorom fosfora i NH_4 ili NO_3 kao izvorom azota. Koncentracije NH_4, NO_3 i PO_4 merene su kolorimetrijski, pomoću Spectroquant® test kita (Merck). Neorganski ugljenik (IC), ukupni organski ugljenik (TOC) i ukupni azot (TN) mereni su pomoću Multi N/C 3100 analizatora (Analitik Jena).

Bakterijski konzorcijum, održavan na 30°C, postigao je smanjenje koncentracija NH_4 za 88%, NO_3 za 100%, TOC za 18% i TN za 96% uz retenciju od 5 dana, dok smanjenje PO_4 nije postignuta. Stope smanjenja su bile 3.03 mg NO_3/L dnevno i 0.1 mg NH_4/L dnevno, počevši od inicijalnih koncentracija od 160.43 mg/L za NO_3 i 22.28 mg/L za NH_4.

Rezultati ukazuju na to da testirani bakterijski konzorcijum može efikasno da prečisti otpadne vode u laboratorijskim uslovima. Potrebno je dalje unapređivanje obogaćene populacije, zajedno sa čistim laboratorijskim linijama, kako bi se povećao kapacitet prečišćavanja prilagođenih bakterijskih konzorcijuma za upotrebu u uzgajalištima riba i u postrojenjima za prečišćavanje komunalnih voda.

Ključne reči: *RAS, nitrifikacija, denitrifikacija, uklanjanje fosfora, bakterijski konzorcijum*

Abstract

The selected bacterial consortia efficiently removed 88% of the NH_4 from the synthetic medium, 100% of the NO_3, and being of real interest for RAS.

Currently, N removal from wastewaters is essentially based on the activity of nitrifying and denitrifying microorganisms. In this paper we studied the nitrifying, aerobic denitrifying and phosphate removal performances of a selected bacterial consortium, grown on synthetic media.

The bacterial consortia was isolated from an aquaria biofilter, enriched in aerobic denitrifying bacteria and cultivated into basal media with acetate as sole C source, KH_2PO_4 as P source, and NH_4 or NO_3 as the N source. The NH_4, NO_3 and PO_4 concentrations were measured colorimetrically with Spectroquant® test kits (Merck). The inorganic carbon (IC), total organic carbon (TOC), and total nitrogen (TN) were measured with a Multi N/C 3100 analyzer (Analytik Jena).

The bacterial consortium maintained at 30˚C showed an 88% of NH_4 reduction, 100% of NO_3, 18% in TOC, and 96% in TN, while no PO_4 reduction was obtained after 5 days retention time. The reduction rates were 3.03 mg NO_3/L/day and 0.1 mg NH_4/L/day with 160.43 mg/L and 22.28 mg/L of initial NO_3 and NH_4 concentrations respectively.

The results argue that the tested bacterial consortium can efficiently clean wastewaters at laboratory level. The enriched population together with expected purified strains should be further improved to increase the cleaning capacities of a tailored bacterial consortium in fish farms and municipal waste water plants, as well.

Keywords: *RAS, nitrification, denitrification, phosphate removal, bacterial consortium*

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LITERATURE SURVEY ON FISH TISSUES CONTAMINATION BY HEAVY METALS AND ELEMENTS IN THE DANUBE RIVER, FROM 1433-845 RKM

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PREGLED LITERATURE O KONTAMINACIJI TKIVA RIBA TEŠKIM METALIMA I ELEMENTIMA U RECI DUNAV, OD 1433-845 RKM

Apstrakt

Teški metali i elementi mogu prodreti i akumulirati se u akvatičnim organizmima putem lanaca ishrane, te se smatraju kritičnim kontaminantima vodenih ekosistema. S obzirom da su ribe nalaze na vrhu lanca ishrane, mogu akumulirati velike količine teških metala i elemenata u tkivima. Dunav je druga najduža reka u Evropi i prolazi kroz Srbiju dužinom od 587,4 km. Najveći industrijski gradovi nalaze se na obalama Dunava (Beograd, Novi Sad, Pančevo i Smederevo) od kojih ni jedan grad nema sisteme za preradu komunalnih i industrijskih otpadnih voda. U okviru ove studije analizirani su objavljeni radovi o akumulaciji teških metala u različitim tkivima riba na Dunavu od 1433 rkm do 845 rkm toka, kako bi se procenilo koje su vrste, tkiva i elementi najzastupljeniji u analizama. Konačan uzorak obuhvatao je 16 članaka objavljenih od 1996. do 2017. godine. Rezultati pokazuju da je najčešće proučavano tkivo mišić (26,67%), a slede jetra (21,67%) i škrge (18,33%). Tkiva koja su takođe proučavana bile su gonade (6,67%), creva (8,33%) i slezina (3,33%). Najčešće ispitivane vrste su som (*Silurus glanis*) (15,52%), šaran (*Cyprinus carpio*) (12,07%) i smuđ (*Sander lucioperca*) (10,35%). Manje zastupljene u analiziranim radovima su deverika (*Abramis brama*) (8,62%) i mrena (*Barbus barbus*) (6,9%). Najviše analizirani teški metali i elementi bili su As, Pb, Cd, Cu, Zn, Fe, Ni, Mn i Co. Elementi koji su takođe bili zastupljeni u analizama bili su Cr, Hg, Sr, Al, Ba i Se. Na osnovu istraživanja literature, dunavski deo koji nije bio proučavan, predstavlja rečni tok od 1132 do 863 rkm koji velikim delom pripada Nacionalni Park “Đerdap” gde treba usmeriti buduće istraživanje.

Ključne reči: *Dunav, teški metali, elementi, ribe, tkiva*
Keywords: *Danube River; heavy metals; trace elements, fish; tissues*

INTRODUCTION

Heavy metals have high potential to enter and accumulate in food chains and therefore are considered as critical contaminants of aquatic ecosystems (Erdoğrul and Erbilir 2007). Given that fish are situated at the top of the food chain they can accumulate large amount of heavy metals (Yilmaz et al. 2007). Heavy metals are taken up through different organs of the fish because of the affinity between them, and then are concentrated at different levels in different organs of the body (Bervoets *et al.* 2001). Accumulation of heavy metals in muscle tissues was the most studied, given that muscle tissue is the main fish part that is consumed by humans (Begum et al. 2013; Storelli et al. 2006). However, it is important to analyze other tissues since muscles are not always a good indicator of the whole fish body contamination (Has-Schön et al. 2006).

The Danube River is the second longest river in Europe and runs through Serbia at a length of 587.4 km. The biggest industrial capacities are largely located on the Danube river banks - Belgrade, Novi Sad, Pančevo and Smederevo (Petrović 2015). The main problem is that none of them has any kind of system for treating municipal wastewaters (Veljković 2005).

Within this study, published articles regarding heavy metal accumulation in different tissues of fish in the Danube in Serbia were analyzed in order to evaluate which species, tissues and elements were the most represented in the analyses. The main aim was to assess present practices in research of heavy metal pollution in this region, as well as to identify key gaps regarding the particular river sections, types of pollutants, tissues, and trophic and functional groups of fish communities that were inadequately represented in research

Figure 1. Map of the study area on the Danube River (river km): 1) Maletin et al. 1996 2) Poleksić et al. 2010; 3) Jarić et al. 2011; 4) Višnjić-Jeftić et al. 2010; 5)Lenhardt et al. 2012; 6) Sunjog et al. 2012; 7) Zrnčić et al. 2012; 8) Rašković et al. 2014; 9) Subotić et al. 2013; 10) Jovanović et al. 2017; 11) Jovičić et al. 2014; 12) Ivanović et al. 2016; 13) Milošković et al. 2016; 14) Morina et al. 2016; 15) Kostić et al. 2017; 16) Subotić et al. 2013

MATERIAL AND METHODS

We conducted a literature survey on current studies dealing with the heavy metal uptake in the Danube fish species inhabiting different trophic levels. The survey included previous research in the Danube Basin, between 852 river km and 1432 river km. Danube enters Serbia at 1433rkm and up to 1296 rkm represents boarder area between Serbia and Croatia (137km). Boarder area represents 58 % of the Croatia's overall area used for commercial freshwater fishing (Zrnčić et al. 2012). Serbia boards with Romania from 1075 to 845rkm (230km). The localities that are shown on the Figure 1and represents studies included in our literature survey are (rkm): 1. Danube-Tisa-Danube channel and Danube River near Novi Sad (1250); 2. Bačko Novo Selo, Zemun, downstream Đerdap II dam (1319, 1173, 861); 3. Bačka Palanka, Beograd, downstream Đerdap II dam (1299, 1169, 863); 4. Prahovo, downstream Đerdap II dam (863); 5. Zemun (1173); 6. Zemun (1173); 7. Batina, Aljmas, Dalj (1432, 1354, 1381); 8. Zemun (1173); 9., 10., 11. Beograd (1169); 12. Grocka (1132); 13. Novi Sad, Zemun, Radujevac (1257, 1173, 852); 14. Zemun (1173); 15. Beograd (1169); 16. Zemun, Grocka (1173, 1132).

Articles were obtained from personal records of relevant publications collected previously by authors. Final sample comprised 16 articles published during 1996-2017.

RESULTS AND DISCUSSION

The results of the literature survey are presented in the Table 1. The results indicated that the most studied fish tissue was muscle (26.67%), followed by liver (21.67%) and gills (18.33%). Tissues that were also studied with the smaller percentage were gonads (6.67%), intestines (8.33%) and spleen (3.33%). Only a single author reported concentration of heavy metals in less studied tissues such as operculum, swimming bladder, gallbladder, heart, brain, vertebrae (1.67%). As for the analyzed species, species that were most studied included wels catfish (*Silurus glanis*) (15.52%), carp (*Cyprinus carpio*) (12.07%), and pike-pearch (Sander lucioperca) (10.35%). Bream (*Abramis brama*) and barbell (*Barbus barbus*) were also studied, but less frequently (8.62%, 6.90%). Prussian carp *(*Carassius gibelio), sterlet (*Acipenser ruthenus*) and silver carp (Hypophthalmichthys molitrix) were represented with 5,17%, while bleak (Alburnus alburnus), pike (*Esox lucius*), burbot (*Lota lota*) accounted for 3.45%. Other studied species were represented with 1.72%. The most analyzed elements were As, Pb, Cd, Cu, Zn, Fe, Ni, Mn and Co. The elements that were also represented in the analyzes were Cr, Hg, Sr, Al, Ba and Se.

Based on the literature survey, the Danube section that was not studied, included river stretch from 1132 to 863 rkm. Given that Danube flows through Serbia at a length of 587.4 km, unstudied section includes 269 rkm, which represents nearly a half of the total Danube flow through Serbia. From 1039 rkm (Golubac) to 933rkm (Kladovo) extends National Park Đerdap. According to Milenković et al. (2005) the Iron Gate (Đerdap Gorge-117 km long) as a biggest hydropower dam and reservoir system has not been investigated. Iron Gates I and II along with the hydrotechnical regulation works along the Danube tributaries have changed the sediment discharge of the Danube (Panin & Jipa 2002). It has been documented that the Iron Gate reservoirs, are significant sinks for nutrients, as well as pollutants (Panin & Jipa 2002). Based on our study, the territory of the National Park Đerdap represents the least studied part of the Danube.

Muscle was the most frequently studied tissue, which is probably not surprising as it represents the main fish for human consumption (Storelli *et al.* 2006). Liver is the

Table 1. Data regarding studied research, locality, species, elements and tissues (M-muscle, G-gills, L-liver, SP-spleen, GO-gonads, I-intestine, WB-whole body, K-kidney, H-heart, BR-brain, V-vertebrae, SB-swimming bladder, GI-gizzard, GB-gallbladder, OP-operculum)

Authors	**Locality (rkm)**	**Species**	**Elements**	**Tissues**
Maletin *et al.,* 1996	around 1250	*Alburnus alburnus,Carassius auratus,Cyprinus carpio, Tinca tinca, Anguila anguila, Esox lucius, Micropterus salmoides,Silurus glanis,Stizostedion lucioperca*	Cd, Co, Cu, Fe, Mn, Ni, Pb,Zn	G,M,L,SP,GO
Poleksić *et al.*,2010	1319; 1173; 861	*Acipenser ruthenus*	Cd, As, Pb, Cr, Hg, Cu, Ni, Fe, Mn, Zn	M, G, L, I
Jarić *et al.,* 2011	1299; 1169; 863	*Acipenser ruthenus*	Ag, Al, As, B, Ba, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, Se, Sr, Zn, Li	M, G, L, I
Višnjić-Jeftić *et al.,* 2010	863	*Alosa immaculata*	Al, As, Cd, Cu, B, Ba, Fe, Mg, Sr, Zn, Li, Co, Cr, Mn, Mo, Ni, Pb	M, G, L
Lenhardt *et al.,*. 2012	1173	*Hypophthalmichthys molitrix,Abramis brama,Blicca bjoerkna, Cyprinus carpio, Silurus glanis*	Al, As, B, Ba, Cd, Co, Cr, Cu, Fe, Li, Mn, Mo, Ni, Pb, Se, Sr i Zn	M, G, L, GO
Sunjog *et al.*,2012	1173	*Barbus barbus*	Al, As, B, Ba, Cd, Co, Cr, Cu, Fe, Li, Mn, Mo, Ni, Pb, Sr, Zn	M, G, L, GO
Zrnčić *et al.,* 2012	1432; 1354; 1381	*Chondrostoma nasus, Ctenopharingodon idella, Leuciscus idus, Alburnus alburnus, Rutilus rutilus, Carassius gibelius, Cyprinus carpio, Abramis brama, Aspius aspius, Esox lucius, Sander lucioperca, Silurus glanis, Lota lota, Hypophthalmichthys molitrix, Lota lota*	As, Cd, Hg, Pb	M
Rašković *et al.,* 2014	1173	*Barbus barbus, Acipenser ruthenus*	As, B, Ba, Cd, Co, Cu, Fe, Hg, Li, Mn, Mo, Ni, Pb, Se, Sr i Zn.	M, G, L
Subotić *et al.,* 2013	1168-1170	*Sander lucioperca, Silurus glanis, Lota lota, Cyprinus carpio*	Al, As, B, Ba, Cd, Co, Cr, Cu, Fe, Hg, Li, Mn, Mo, Ni, Pb, Se, Sr, i Zn	M, G, L
Subotić *et al.,* 2013	1170	*Sander lucioperca, Silurus glanis, Cyprinus carpio, Neogobius gymnotrachelus, Neogobius melanostomus*	As, Cu, Fe, Hg, Mn i Zn	M,L,WB
Jovičić *et al.,* 2014	1169	*Silurus glanis*	As, Cd, Co, Cr, Cu, Fe, Hg, Mn, Ni, Pb, Se i Zn	M,G,L,SP,K, I, GO, H, BR, V, SB, GI, GB, OP
Ivanović *et al.*,2016	1132	*Hypophthalmichthys molitrix, Cyprinus carpio, Silurus glanis*	As, Cd, Hg, Pb	M,L,I
Milošković *et al.*,2016	1257;1173;852	*Sander lucioperca,Silurus glanis, Abramis brama*	Al, As, Cd, Co, Cr, Cu, Fe, Hg, Mn, Ni, Pb I Zn	M
Morina *et al.*,2016	1173;	*Barbus barbus*	Al, As, B, Ba, Co, Cr, Cu, Hg, Mn, Mo, Ni, Pb, Se, Sr i Zn	M,G, L,I
Kostić *et al.*,2017	1170	*Abramis brama*	Al, As, B, Ba, Cd, Co, Cr, Cu, Fe, Li, Mn, Mo, Ni, Pb, Sr i Zn	M,G,L
Jovanović *et al.,* 2017	1173; 1132	*Carassius auratus gibelio,Barbus barbus,Abramis brama,Cyprinus carpio, Stizostedion lucioperca, Silurus glanis*	As, Cd, Cu, Fe, Hg, Pb, Zn	M

second most frequently analyzed tissue. It is metabolically active tissue and has higher accumulation potential for metal accumulation than muscle tissue (Storelli *et al.* 2006). The gills are considered to be the main site of the metal uptake from the water, and high metal concentrations in gills can indicate that the main route of contamination is the water (Bervoets and Blust 2003; Storelli *et al.* 2006). The spleen is considered as important metal storage tissue as the kidneys and the liver (Yancheva *et al.* 2014). Other fish organs and tissues have been poorly studied so far.

The most frequently analyzed species was the wels catfish. It is economically important and highly prized fish species and well represented in fishermen's catch (Hegedis *et al.* 2013). Carp was the second most frequently studied fish species, which is understandable given that it is the most popular species in the diet of the population in Serbia (Hegedis *et al.* 2013). Pike-perch is an economically important fish species for commercial and recreational fishing (Hegedis *et al.* 2013). Bream and barbell were also studied. Bream is among higher quality fish while the barbell is a common species in fishermen's catch and an extremely attractive species for recreational fishing (Hegedis *et al.* 2013).

In analyzed studies As, Pb, Cd, Cu, Zn, Fe, Ni, Mn and Co were the most frequently studied elements. These elements represent some of the major pollutants regarding environmental impact and human health (Schenone *et al.* 2014). With the exception of Mn and Ni, maximum allowed concentrations (MAC) in fish meat were established for the above mentioned elements for the utilization in human diet. Nevertheless, other elements should also be included in analyses in order to evaluate their potential impact on fish.

CONCLUSIONS

To conclude, the most frequently studied fish tissue was muscle, while the most frequently studied species was wels catfish. As, Pb, Cd, Cu, Zn, Fe, Ni, Mn and Co were the most frequently analyzed elements. Future studies should include more fish species and include those tissues that have been neglected so far. Also, future research efforts should also cover the section of the Danube River in Serbia that has been neglected so far (1132 rkm-863 rkm).

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PRELIMINARY ASSESSMENT OF THE DEGREE OF VULNERABILITY AND HEALTH RISK IN SOME FISHING WATERS BASED ON CYANOBACTERIA IN 2017

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PRELIMINARNA PROCENA STEPENA RANJIVOSTI I ZDRAVSTVENI RIZIK NEKOLIKO RIBOLOVNIH VODA NA OSNOVU ANALIZE CIJANOBAKTERIJA U TOKU 2017. GODINE

Apstrakt

U uslovima klimatskih promena, sve učestalije opterećenosti nutrijentima u vodama je sve prisutnija pojava cvetanja cijanobakterija koja može da bude praćena produkcijom toksina opasnih po sve vodene organizme. U takvim uslovima širom sveta zabeležena su akcidentna masovna uginuća riba. Toksini, takođe, mogu da se akumuliraju u različitim organima riba i da predstavljaju zdravsteni rizik po ljude koji ih konzumiraju.

Analizom zajednice fitoplanktona u 15 akumulacija u Srbiji u periodu od aprila do septembra 2017. godine, zabeleženo je prisustvo 27 taksona Cyanobacteria. iz rodova Aphanizomenon, Aphanocapsa, Aphanothece, Chroococcus, Chrysosporum, Cuspidothrix, Cylindrospermopsis, Dolichospermum, Gloeocapsa, Leptolyngbya, Microcystis, Oscillatoria, Phormidium, Planktolyngbya, Planktothrix, Pseudanabaena, Snowella i Woronichinia.

Prema preporukama WHO, u odnosu na brojnost cijanobakterija, praćene ribolovne vode u periodu istraživanja su kategorisane kao vode niskog (Barje, Divljana, Kamenica, Marića bara, Potpeć, Prvonek, Savsko i Zvorničko jezero), srednjeg (Bojnik, Garaši i Zaova) i visokog stepena (Vučkovica, Smoljinac, Srebrno jezero) opasnosti i zdravstvene ugroženosti. U Srebrnom jezeru zabeležena je invazivna vrsta Cylindrospermopsis raciborskii, čije širenje areala u Srbiji i često masovno umnožavanje nosi visok rizik po zajednice vodenih organizama, a pre svega riba.

Prilikom pripreme Programa upravljanja ribolovnim vodama i predviđanja daljih aktivnosti na njima, neophodno je uzeti u obzir rezultate dobijene monitoringom koji se odnose na prisustvo i brojnost cijanobakterija, obzirom da direktni negativni efekti i akumulacija cijanotoksina predstavljaju rizik za ribe, njihovo zdravlje, kvalitet, ali i direktno i indirek-

tno za zdravlje čoveka. Ukoliko je moguće, uz različite metode prevencije i sanacije, predvideti i biomanipulaciju adekvatnim ribljim vrstama.

Takođe, neophodna je edukacija upravljača ribolovnih voda, kao i korisnika (pre svega ribolovaca) u cilju prepoznavanja potencijalnih rizika i preduzimanja mera kontinuiranog monitoringa, i privremene ili trajne zabrane korišćenja voda, ukoliko rezultati pokažu visok stepen rizika i opasnost po zdravlje.

Ključne reči: *Cyanobacteria, ribolovne vode, zdravstveni rizik*
Keywords: *Cyanobacteria, fishing waters, health risk*

INTRODUCTION

Due to increasingly present eutrofication of the aquatic ecosystems comes to frequent occurrence of Cyanobacterial blooms, often followed by the production of cyanotoxins in their cells. Cyanotoxins are very poisonous substances, which when released into the water represent a threat to both aquatic and terrestrial organisms (Sedmak & Svirčev, 2011). The massive accidentally fish kill caused by blooming of cyanobacteria, in Serbia are already known (Đorđević & Simić, 2014; Đorđević et al., 2015).

Fish comes into contact with cyanobacteria and their toxins in various ways, which can affect their growth, development, reproduction, survival (Palikova et al., 2007; Drobac et al., 2016). Fish exposure to cyanotoxins can occur in two ways: by active introduction through drinking and consuming of cyanobacterial cells and other organisms that have accumulated cyanotoxins in their tissues, or by direct contact of the epithelial cells with the surrounding water containing toxins (Palikova et al., 2007). Cyanotoxins can accumulate in fish and other hydrobionts organs, and their consuming represent a potential risk to human health.

Accumulation of cyanotoxins in fish may leads to desorders of the antioxidant defense system, as well as histopathological changes in various fish organs, including the liver, intestines, kidneys and muscles (Drobac et al., 2016).

The health effects that cyanobacteria and their toxins have, both on fish and potentially on human which in their diet use cyanotoxin-infected fish, have led to an increase in interest in cyanobacteria research, especially in waters used for fishing purposes.

By the Decision on the Determination of Fishing Areas (Official Gazette of the Republic of Serbia, 90/2015) it has been established 17 fishing areas on the territory of the Republic of Serbia. The research of fishing waters carries out with the goal to get an Fishing Water Management Program, according to the Law on the Protection and Sustainable Use of Fish Stock (Official Gazette of the Republic of Serbia, 128/2014). Within this research, the nekton community (qualitative analysis, biomass, real and potential production of fish) is primarily enquired, while a lot less attention is dedicate to the benthos and plankton communities.

The aim of this paper is to provide a preliminary assessment of the degree of vulnerability and health risk in some fishing waters in Serbia, based on the presence of potentially toxic Cyanobacteria and the total number of this group of organisms during the research period.

MATERIAL AND METHODS

Field research of 15 reservoirs of different purposes in the territory of the Republic of Serbia was conducted in the period from April to September 2017 (Table 1).

The samples for the phytoplankton qualitative analysis were collected by sweeping a plankton net (netframe 25 cm ø, mesh net e.g. 22 µm), while the samples for the quantitative analysis were collected from a depth of one meter by Rutner's bottle (2 l).

The qualitative analysis was done by the light microscope Motic BA310 with digital camera Bresser (9MP) and MicroCamLab software. Taxonomic identification of Cyanobacteria was made using appropriate identification keys. The quantitative analysis of phytoplankton was conducted using the Sedgewick-Rafter counting chamber, with a Motic BA310 microscope.

Assessment of the degree of hazard and health risk (for the research period) was done according to the number of Cyanobacteria and on the basis to the recommendation of the World Health Organization (WHO) (Chorus & Bartram, 1999).

RESULTS AND DISCUSSION

Qualitative analysis of phytoplankton community during the 2017 years in 15 Serbian reservoirs resulted with a total of 27 identified taxa, recorded in 14 reservoirs (Table 1).

Some of the potentially toxic cyanobacteria (Chorus & Bartran, 1999), such as *Aphanizomenon flos-aquae, Dolichospermum flos-aquae* (as *Anabaena flos-aquae*) *Microcystis aeruginosa, M. viridis*, *Cuspidothrix issatschenkoi, Cylindrospermopsis raciborskii* and *Snowella lacustris* were recorded (Table 1).

Table 1. Qualitative and quantitative analysis of Cyanobacteria in the investigated fishing waters

Reservoir/ month 2017	Taxa	Abundance of Cyanobacteria (cell/ml)
	Fishing area „Južna Morava 1“	
Barje June	*Planktolyngbya* sp. Anagn. & Komárek *Snowella lacustris* (Chodat) Komárek & Hindák *Woronichinia compacta* (Lemmerm.) Komárek & Hindák	2 118
Divljana July	-	0
Prvonek June	*Aphanocapsa inserta* (Lemmerm.) Cronberg & Komárek *Dolichospermum planctonicum* (Brunnth.) Wacklin et al. *Pseudanabaena* sp. Lauterborn	2 307
	Fishing area "Južna Morava 2"	
Bojnik June	*Chroococcus* sp. Nägeli *Cuspidothrix issatschenkoi* (Usachev) Rajan., Komárek, Willame, Hrouzek, Kastovská, Hoffm. & Sivonen *Dolichospermum planctonicum* (Brunnth.) Wacklin et al. *Dolichospermum* sp. (Ralfs ex Bornet & Flahault) Wacklin, Hoffman & Komárek *Planktolyngbya limnetica* (Lemmerm.) Legn. & Cronberg *Snowella lacustris* (Chodat) Komárek & Hindák	23 898

Fishing area "Zapadna Morava"		
Vučkovica September	*Microcystis viridis* (A. Braun in Rabenhorst) Lemmenrm. *Phormidium* sp. Kütz. ex Gomont *Planktolyngbya* sp. Anagn. & Komárek	967 259
Marića swamp July	*Chroococcus* sp. Nägeli *Leptolyngbya notocorum* (Bornet ex Gomont) Anagn. & Komárek *Pseudanabaena* sp. Lauterborn *Phormidium* sp. Kütz.ex Gomont	1 998
Potpeć May	*Phormidium irriguum* (Kütz. ex Gomont) Anagn. & Komárek *Planktolyngbya* sp. Anagn. & Komárek *Pseudanabaena* sp. Lauterborn	2 627
Fishing area "Kolubara"		
Garaši July	*Chroococcus dispersus* (Keiss.) Lemmerm. *Dolichospermum* sp. (Ralfs ex Bornet & Flahault) Wacklin, Hoffman & Komárek *Gloeocapsa* sp. Kütz. *Planktolyngbya* sp. Anagn. & Komárek *Planktothrix agardhii (Gomont)* Anagn. & Komárek *Snowella lacustris* (Chodat) Komárek & Hindák	21 615
Kamenica July	*Aphanocapsa inserta* (Lemmerm.) Cronberg & Komárek *Gloeocapsa* sp. Kütz. *Leptolyngbya* sp. Anagn. & Komárek	6 965
Savsko August	*Leptolyngbya* sp. Anagn. & Komárek *Phormidium* sp. Kütz. ex Gomont	5 087
Zvorničko July	*Leptolyngbya* sp. Anagn. & Komárek *Planktolyngbya limnetica* (Lemmerm.) Legn. & Cronberg	7 214
Fishing area "Velika Morava 1"		
Šumarice April	*Aphanizomenon flos-aquae* Ralfs ex Bornet & Flahault *Chroococcus limneticus* Lemmerm. *Chroococcus minutus* (Kütz.) Nägeli Chrysosporum minor (Kiselev) Komárek *Cuspidothrix issatschenkoi* (Usachev) Rajan., Komárek, Willame, Hrouzek, Kastovská, Hoffm. & Sivonen *Microcystis aeruginosa* (Kütz.) Kütz. *Oscillatoria tenuis* Agardh ex Gomont *Snowella lacustris* (Chodat) Komárek & Hindák	2 349

Fishing area "Mlava"		
Smoljinac July	*Aphanocapsa inserta* (Lemmerm.) Cronberg & Komárek *Cuspidothrix issatschenkoi* (Usachev) Rajan., Komárek, Willame, Hrouzek, Kastovská, Hoffm. & Sivonen *Dolichospermum flos-aquae* (Bréb. ex Bornet & Flahault) Wacklin et al. *Microcystis aeruginosa* (Kütz.) Kütz. *Microcystis viridis* (A. Braun in Rabenhorst) Lemmenrm. *Pseudanabaena catenata* Lauterborn	377 672
Srebrno July	*Aphanizomenon flos-aquae* Ralfs ex Bornet & Flahault *Cylindrospermopsis raciborskii* (Woloszyńska) Seenayya & Subba Raju *Leptolyngbya* sp. Anagn. & Komárek *Planktolyngbya limnetica* (Lemmerm.) Legn. & Cronberg *Pseudanabaena* sp. Lauterborn	277 057
Zaova July	*Aphanizomenon flos-aquae* Ralfs ex Bornet & Flahault *Aphanothece* sp. Nägeli *Cuspidothrix issatschenkoi* (Usachev) Rajan., Komárek, Willame, Hrouzek, Kastovská, Hoffm. & Sivonen *Microcystis aeruginosa* (Kütz.) Kütz. *Pseudoanabaena catenata* Lauterborn *Planktolyngbya* sp. Anagn. & Komárek	39 776

According to the WHO recommendations (Chorus & Bartran, 1999), in relation to the abundance of cyanobacteria, fishing waters are classified as low probabilities of adverse health effects (Barje, Divljana, Kamenica, Marića swamp, Potpeć, Prvonek, Savsko and Zvorničko), as waters of moderate probability of adverse health effects (Bojnik, Garaši and Zaova), and as water of high risk of adverse health effects (Vučkovica, Smoljinac and Srebrno), based on preliminary results in the research period.

The occurrence of the invasive tropical species *Cylindrospermopsis raciborskii* in Srebrno Lake is especially significant. Increasingly frequent finding of this species in Serbia (Cvijan & Fužinato, 2011; Karadžić et al., 2013; Đorđević & Simić, 2014; Đorđević et al., 2015) indicates the expansion of its area. *C. raciborskii* can produce very strong toxin cylindrospermopsin and in case of massive occurrence, could lead to fish kills (Đorđević et al., 2015).

CONCLUSIONS

During the preparation the Fishing Water Management Program and anticipating further activities on them, it is necessary to take into account the monitoring results that are related to the presence and the number of cyanobacteria, because of the cyanotoxins and its direct negative effects and the risk for fish, their health, quality, and therefore directly and indirectly for human health. It is also necessary to anticipate biomanipulation with adequate fish species, with different methods of prevention and rehabilitation, if it is possible.

The training of fishery water managers and users (primarily fishermen) is needed with the goal of identify potential risks, to take measures of continuous monitoring and temporary or permanent water use prohibition, if the results show a high risk of adverse health effects.

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RESERVOIRS AS FISHING WATERS IN SERBIA – THEIR SIGNIFICANCE AND FURTHER PERSPECTIVE

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AKUMULACIJE KAO RIBOLOVNE VODE U SRBIJI – NJIHOV ZNAČAJ I PERSPEKTIVA

Apstrakt

Cilj ovog istraživanja je analiza kvalitativnog i kvantitativnog sastava ihtiofaune sedam akumulacija od značaja za rekreativni ribolov i ribolovni turizam u Srbiji i to: Bovan, Barje, Gazivode, Vrutci, Gruža, Srebrno i Borsko jezero. Istraživanja su sprovedena od aprila do septembra 2017. godine, a za potrebe izrade Programa upravljanja ribarskim područjima za period 2017. - 2026. godine. Analiza ihtiofaune vršena je standardnom metodologijom, u zavisnosti od tipa ekosistema i objektivnih uslova.. Da bi se dobio što potpuniji uvid u stanje ribljeg fonda, procenjena je biomasa, realna i potencijalna produkcija. Analizom rezultata procene biomase i produkcije riba može se zaključiti da je stanje ribljeg fonda u većini istraživanih akumulacija više ili manje zadovoljavajuće. U akumulacijama kao što su : Gazivode, Bovan i Gruža, zapaženo je manje prisustvo grabljivica, pre svega smuđa. Nasuprot, u Borskom jezeru zabeležena je povećana produkcija grgeča, delom i smuđa što je uticalo na smanjenje biomase i produkcije “plena” pre svega uklije, krupatice i šarana. U Srebrnom jezeru uočava se disbalans biomase i produkcije alohtone vrste babuške u odnosu na druge ribolovno značajne vrste. U akumulaciji Vrutci zabeleženo je značajno prisutvo i produkcija soma kao glavne i dominantne grabljive vrste, ali se i u ovoj akumulaciji javlja disbalans, odnosno znatno veća produkcija plena (deverika, uklija, bodorka) u odnosu na predatore. Ovakvo stanje najverovatnije je posledica odsustva i drugih predatorskih vrsta u ovoj akumulaciji, kao što su smuđ i štuka. Rezultati ukazuju da je formiranje ribljeg fonda u akumulacijama osetljiv, složen i dugoročan proces i da se mora zasnivati na veoma preciznim naučnim podacima.

Ključne reči: *akumulacije, biomasa i produkcija riba, Srbija*
Keywords: *reservoirs, fishe biomass and production, Serbia*

INTRODUCTION

Serbia is a highly continental country with a very small number of natural lakes (Ostojić et al., 2007). Accordingly, Serbia has constructed artificial lakes to replace the lack of natural lakes (Čomić and Ostojić, 2005). The largest number of such lakes was built on rivers for the needs of electricity generation. Artificial lakes were also built for the purpose of water supply for settlements and economic activities. They are also used for irrigation, as fishponds while some others were built for tourism purposes (Bubalo-Živković et al., 2018). In this study we investigated a qualitative and quantitative composition of the seven reservoirs, and all of them were constructed for a range of water needs. In order to understand their significance and further perspective, it is necessary to have information on the current state of fish populations. Selected reservoirs are significant fishing waters, indicating a high level of recreational fishing. Because of a high socio-economic and socio-cultural benefits of recreational fishing, there is a need for permanent monitoring of attractive fishing species. Recreational fisheries through their practices (*e.g.* overharvest, habitat change, release mortality, stocking and dispersal of non-native fishes), however, have been implicated in negative effects on populations and ecosystems (Cooke et al., 2015).

MATERIALS AND METHODS

The field research was conducted from April to September 2017 for the needs of the Program for the Management of the Fisheries Areas for 2017-2026 (Simić et al., 2018). Seven reservoirs were investigated: Bovan, Barje, Gazivode, Vrutci, Gruža, Srebrno and Borsko. We analyzed ichthyofauna using a standard methodology. Data on the fish community and fish livestock were obtained using two methods: electro-fishing (Aquatech IG 4000) and netting tools (10 to 40 mm mesh size and total length 300 m). Also, we analyzed the catch of fishermen and anglers. Depending on the type of ecosystem and the objective conditions, the most appropriate method was used, or all methods were used together. Collected fish samples were processed on the field (measurement of weight and total body length of fish) and returned to their natural habitat. In addition to the qualitative analysis of the fish community, the biomass, real and potential production estimation was assessed in accordance with Chapman (1971) and Huet (1964).

RESULTS AND DISCUSSION

The results are presented in the form of a table. By analyzing the results of the biomass estimation and the production of fish from the Tables 1 and 2, it can be concluded that the state of the fish stock in most of the fishing waters is more or less satisfied.

In Srebrno reservoir is noticed disbalance between biomass and production of Prussian carp (*Carassius gibelio*) in relation to other fish (Table 1 and 2). The one study which was conducted in north-western Turkey provides results on the effect of Prussian carp on native fish species over a 6-year period in a mesotrophic drinking water reservoir. A dramatic decrease in relative density of native species correlated significantly with an increase in Prussian carp relative density (Tarkan et al., 2012). Of the total number of samples in the Srebrno reservoir, over 38% was a Prussian carp (Simić et al., 2018). The decrease in the number of piscivorous fish species was also observed in Gruža reservoir (Simić et al., 2018). The fishery management program recommends stocking with pikeperch for the stabilizing the population of this species in Gruža reservoir. There is also an ecological potential for their growth, primarily in terms of the existence of peaceful fish (Simić et al., 2018). For nearly 20 years, most Czech reservoirs for supplying drinking water have been under statutory protection which permitted reservoir managers to manipulate fish stocks in order to maintain a sustainable water quality. The most common biomanipulative measure adopted was stocking with piscivorous fish (mostly 5 cm long fry) using an annual stocking level of approx. 25 000 fish per reservoir (Seda et al., 2000). Piscivorous fish species are very important for maintaining the entire ecosystem. The first ichthyological research for Gruža reservoir carried out immediately after the formation (1987) indicates the presence of 16 species from 6 families. At this stage of the existence of the ecosystem, the fish unit was characterized by the presence of ritron species such as Barbus peloponnesius (L), Alburnoides bipunctatus (L), Barbatus barbatus (L) and others (Šorić, 1996). Currently situation is different and based on the results from Table 1, we notice that there are currently a large number of Prussian carp. Also, based on the obtained results in Table 1, it can be concluded that the situation in Vrutci reservoir is different, since the presence of number of catfish species has increased. It can be possible because there is no other predator species such as pikeperch and pike. Consequently, there is an imbalance in the production of prey and predators due to increased production of prey.

Table 1. Biomass of fish species in seven investigated reservoirs

Reservoirs	Biomass (kg/ha)											
	Bream (*Abramis brama*)	**Carp** (*Cyprinus carpio*)	**Bleak** (*Alburnus alburnus*)	**Eurasian perch** (*Perca fluviatilis*)	**Nase** (*Chondrostoma nasus*)	**European catfish** (*Silurus glanis*)	**Roach** (*Rutilus rutilus*)	**Prussian carp** (*Carassius gibelio*)	**Chub** (*Squalius cephalus*)	**Pikeperch** (*Sander lucioperca*)	**Silver bream** (*Blicca bjoerkna*)	**FINAL SCORE**	**FINAL SCORE FOR TOTAL AREA**
Bovan	136,54	92	109.8	9	0,34	109,33	55	226,83	-	38,13	-	671,82	282,16
Barje	135,7	-	117,86	68,68	-	-	187,53	50	20,5	52,05	-	632,32	82,2
Gazivode	202.76	6.34	127.34	65.78	57.77	-	-	127.50	24	9.9	-	621.39	161.56
Vrutci	348.8	7.66	722.5	206.2	225.7	725.1	14.83	2258.5	-	-	-	4509.2	2705.5
Srebrno	18	-	243	16.55	-	103.7	-	258	-	25.25	-	664,5	265,8
Gruža	112.33	93.53	149.56	-	-	52.26	22.83	195.03	-	48.72	-	715.41	643.8
Borsko	-	-	16.56	196.20	-	-	111.16	16.83	-	138.53	3.96	588.4	104.7

Table 2. Real and potential production of fish species in seven investigated reservoirs

Reservoirs	Real production and potential production (kg/ha)																							
	Bream (*Abramis brama*)		**Carp** (*Cyprinus carpio*)		**Bleak** (*Alburnus alburnus*)		**Eurasian perch** (*Perca fluviatilis*)		**Nase** (*Chondrostoma nasus*)		**European catfish** (*Silurus glanis*)		**Roach** (*Rutilus rutilus*)		**Prussian carp** (*Carassius gibelio*)		**Chub** (*Squalius cephalus*)		**Pikeperch** (*Sander lucioperca*)		**FINAL SCORE**		**FINAL SCORE FOR TOTAL AREA**	
	rp[1]	pp[2]	rp	pp	rp	pp	rp	pp	rp	pp	rp	pp	rp	pp	rp	pp	rp	pp	rp	pp	rp	pp	rp	pp
Bovan	98,92	118	-	-	76,44	91,21	-	-	-	-	95,7	115	63	75,17	136,23	120	-	-	32,54	95	502,83	600	211,1	252
Barje	89	1 137	-	-	73	74 119	72	469	-	-	-	-	165, 5	189,8	-	50,6	-	20,7	40	76 52	441,45	640	57,38	70,04
Gazivode	147,89	189,25	-	5,91	92,11	118,8	67,37	61,39	-	53,92	-	-	-	-	153,7	119	-	22,40	2,62	9,24	463,69	580	120,55	150,8
Vrutci	245.53	154.7	2.32	3.39	486.5	320.45	167.54	91.46	159.23	100.1	-	321,6	-	6,57	853.8	1001.72	-	-	-	-	1914.1	2000	1148.4	1200
Srebrno	-	12,46	-	-	148	168,22	8,55	11,45	-	-	-	71,78	-	-	224	178,6	-	-	-	17,47	380,55	460	152,2	184
Gruža	60.26	117.76	45.65	98.05	83.65	156.79	-	-	-	-	43	54.78	20.21	23.93	188.95	204.45	-	-	32.38	51.07	529.5	750	476.5	675.7
Borsko	-	-	-	-	9,2	7,59	120, 66	90,03	-	-	-	-	-	-	-	-	-	-	82,5	63,56	256,15	270	7,684	8,1

rp - real production

pp – potential production

Certain changes are observed in reservoirs of Gazivode, Bovan and Gruža. There is noticed presence of piscivore species, primarily of pikeperch. Moreover, in Borsko reservoir, increased production of Eurasian perch, partly and pikeperch, which affected the reduction of biomass and the production of prey (common bleak and common carp). In order to establish a balance between the number of „prey" and „predators", it is necessary to carefully stocking with species that are in deficit. „The concept of cascading trophic interactions predicts that an increase in piscivore biomass in lakes will result in decreased planktivorous fish biomass, increased herbivorous zooplankton biomass, and decreased phytoplankton biomass" (Drenner and Hambright, 2002).

CONCLUSION

Based on conducted research, the following can be stated: Compared to the previous results there is no significant difference between real and potential production of attractive fish species in the investigated reservoirs. By analyzing the results of the biomass estimation and the production of fish, it can be concluded that the state of the fish stock in most of the investigated fishing waters is more or less satisfied. However, there is need for a permanent monitoring. The significance and perspective of investigated reservoirs is reflected in sustainability assessment and management plans in the stability of the entire ecosystem.

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JOHN DORY, *ZEUS FABER*, AS A SUGGESTED FLAGSHIP SPECIES OF AQUARIUM BOKA

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ŠANPJER, *ZEUS FABER*, KAO PREDLOG ZA VODEĆU (FLAGSHIP) VRSTU AKVARIJUMA BOKA

Apstrakt

Univerzitet Crne Gore – Institut za biologiju mora, Kotor grantom Norveške vlade započeo je realizaciju projekta „Centar za očuvanje biodiverziteta Akvarijum Boka - MonteAqua» (http://www.aquariumboka.ucg.ac.me/) u saradnji sa Centrom za ribarstvo i zaštitu biodiverziteta kopnenih voda Instituta za biologiju i ekologiju Prirodno matematičkog fakulteta Univerziteta u Kragujevcu. Osnivanje morskog Akvarijuma Boka kao integralnog dijela Centra za zaštitu biodiverziteta mora je jedna od najvažnijih projektnih aktivnosti. Za podršku aktivnostima akvarijuma u zaštiti biodiverziteta, unapređivanju javne svijesti i obezbeđivanju sredstava za zaštitu biodiverziteta, projektom je predviđen izbor jedne morske vrste kao vodeće (eng, Flagship). U radu se opisuje razvijena jednostavna metodologija za izbor vodeće vrste. Metodologija ima tri koraka. U prvom koraku definisano je 6 kriterijuma za selekciju: 1. Status osetljivosti, 2. Vizuelna prepoznatljivost vrste, 3. Značaj za lokalnu zajednicu, 4. Atraktivnost vrste, 5. Ekonomski značaj, i 6. Gastronomska vrijednost. U drugom koraku definisani kriterijumi su primjenjeni na 8 dobro poznatih vrsta riba kao potencijalnih kandidata za vodeću vrstu. U trećem koraku distribuiran je upitnik relevantnim zainteresovanih stranama (ribari, predstavnici lokane zajednice, relevantni istraživači) u kome se svaki kriterijum ocenjuje na skali 1 do 4, gde 4 ukazuje da vrsta ispunjava sve kriterijume. Na osnovu analize 60 upitnika kao vodeća vrsta Akvarijuma Boka u formiranju odabrana je vrsta kovač, šanpjer, Zeus faber (Linnaeus, 1758). Razlozi za odabir ove vrste na prvom mjestu leže u njegovoj atraktivnosti, karakterističnim vizuelnim karakteristikama, interesantnom obliku tijela i upadljivoj crnoj mrlji na bokovima. Pored karakterističnog izgleda kojim ova vrsta treba da privuče pažnju javnosti, u obzir je uzet

i socio-ekonomski, odnosno privredni značaj koju ova vrsta ima za ribarstvo Crne Gore. Očekuje se da će korišćenje ove vrste podržati konzervatorske aktivnosti Akvarijuma.

Ključne reči: *Akvarijum Boka, vodeća (flagship) vrsta, šanpjer, Crna Gora*
Keywords: *Aquarium Boka, flagship species, John Dory, Montenegro*

INTRODUCTION

The Institute of Marine Biology of the University of Montenegro is granted by the Norwegian Ministry of Foreign Affairs to implement a project "Marine Biodiversity Conservation Center "Boka Aquarium" (MonteAqua)" (http://www.aquariumboka.ucg.ac.me/*)* in cooperation with the Center for Fisheries and Biodiversity Conservation of Inland Waters, Institute of Biology and Ecology, Faculty of Science, University of Kragujevac. One of the main project activities is setting-up marine Aquarium Boka, as integral part of the Marine Biodiversity Conservation Center. Aquarium Boka is planned as first Montenegrin public aquarium and conservation advocacy center which will promote implementation of marine protected areas in Montenegro and all means of marine biodiversity protection. Particular focus will be on sustainable seafood choices. Aquarium Boka is envisaged as main player in conservation and restoration of key threatened marine wildlife species in Montenegrin Adriatic, and beyond.

For supporting Aquarium Boka conservation activities, promote public awareness and raise funds for conservation, we have decided to select one Montenegrin marine species as flagship species. This paper describes criteria and selection process of Aquarium Boka flagship species.

MATERIAL AND METHODS

Several authors state that flagship species must fulfill different criteria for a desired conservation action. In selection process of flagship species for a desired conservation action various combinations of ecological, phenotypic, cultural and policy-related traits must be taken into consideration (Dietz *et al.,* 1994; Caro and O'Doherty 1999; Bowen-Jones and Entwistle 2002; Farjon *et al.,* 2004; Home *et al.,* 2009; Veríssimo *et al.,* 2009). Following Kalinkat *et al.,* (2016) recommendation, the main criteria for selecting flagship species should be based on sociocultural considerations.

As pre-requirement for selection of flagship species for Aquarium Boka we defined that it need to be well recognized regionally (in the South Adriatic) and in particular locally (in Montenegro). On the other hand, our wish was to avoid selection of species which are already exploited as flagship species by other Adriatic aquaria or environmental organizationis, like WWF, IUCN or UNEP.

In first step of the process of selection flagship species, following defined pre-requirements and literature recommendations, project team has composed list of six criteria for selection of the flagship species, as follows: 1. Vulnerability status; 2. Visual identity of species; 3. Importance for local community; 4. The attractiveness of the species; 5. Economic valuing, and 6. Gastronomic value (character).

In the second step of the process, we have applied defined criteria on 8 well known fish species as candidate flagship species for Aquarium Boka (Error: Reference source not found). We have selected only fish species, considering them as most known locally and with the highest sociocultural considerations among other species.

Table 1. List of candidate flagship fish species

Scientific name	***English name***	***Local name***
Mullus barbatus	Red mullet	Barbun, Trlja od blata
Pagellus erythrynus	Common Pandora	Arbun Rombun
Merluccius merlucius	Hake	Oslić, Luc
Zeus faber	John dory	Kovač, Šanpjer
Sardine pilchardus	Sardine	Srdela, Gavica
Engraulis encrasicolus	Anchovy	Inćun, Sardun
Argyrosomus regius	Meagre	Hama
Epinephelus marginatus	Dusky grouper	Kirnja

In the third step of the process, we distributed a questionnaire to relevant stakeholders including fishermen, representatives of local community, and relevant scientists from the region, in order to obtain their opinion/answers how well candidate species meets given criteria. For each candidate species and for each criterion we offered 4 level score: 1. Species doesn't meet criteria at all; 2. Species meet criteria in small extent; 3. Species meet criteria in high extent; 4. Species fully meets criteria. All answers were calculated and for each species and each criterion average value of answers was used to obtain final score for each species from all questionnaires.

RESULTS AND DISCUSSION

We have collected total of 60 questionnaires, 31 from relevant scientists (marine and conservation biologists from Adriatic region), 16 from representatives of local communities along Montenegrin coast and 13 from Montenegrin fishermen. All values from 60 questionnaires for each species and each criterion was calculated and averaged in order to obtain average value in each field of the table and to obtain final score for each candidate species (Table 3).

Table 3. Results of interviews and final score for each candidate species

Criteria / Species	*Vulnerability status*	*Visual identity*	*Importance for local community*	*Attractiveness of the species*	*Economic value*	*Gastronomic character*	*Final score*
Mullus barbatus	3.1	2.7	3.2	1.8	3.2	3.6	2.9
Pagellus erythrynus	2.5	2.9	2.6	2.2	2.8	3.0	2.6
Merluccius merlucius	3.2	1.9	3.2	1.8	3.4	3.4	2.8
Zeus faber	3.8	3.9	3.6	3.8	4.0	4.0	3.8
Sardine pilchardus	2.6	1.3	3.2	1.6	2.2	3.2	2.3
Engraulis encrasicolus	2.6	1.9	3.2	1.4	2.2	2.8	2.3
Argyrosomus regius	3.9	3.6	2.8	3.2	4.0	4.0	3.6
Epinephelus marginatus	3.9	3.7	2.6	3.3	4.0	4.0	3.6

Based on all questionnaires the highest value of the final score was obtained for species John Dory (*Zeus faber*) - 3.8, followed by final scores of 3.6 for Meagre (*Argyrosomus regius*) and Dusky grouper (*Epinephelus marginatus*), respectively. Other candidate species obtained lower final scores due to low values obtained for criteria of visual identity and attractiveness of species. These findings corresponds to definition of "flagship species" by different authors which defines them as "known charismatic species that serve as a symbol or focus point to raise environmental consciousness" (Samways *et al.,* 1995) or "popular charismatic species that serve as symbols and rallying points to stimulate conservation awareness and action" (Heywood, 1995). John Dory represents important species for coastal area of Montenegro, this species is categorized as a high quality fish (1st class fish) with high economic value (25 euros/kg at the market) and there is high demand for this species at restaurants. Species has very characteristic visual identity, body is laterally compressed olive-yellow color with a large dark spot, and long spines on the dorsal fin. The dark spot is used to flash an 'evil eye' if danger approaches. This eye spot on the side of its body also confuses prey, which are scooped up in its big mouth. Numerous legends about name of this fish exists, according to one the apostle Peter grabbed the fish with his hands. He caught the fish, and in the places where he touched her fingers, black spots remained. From there, this fish got the name of St. Peter's fish, and also the Italian version of the «šanpjer».

CONCLUSION

For selection of Montenegrin marine species as flagship species of Aquarium Boka under establishment authors developed simple methodology, based on valuing six criteria, as follows: 1. Vulnerability status; 2. Visual identity of species; 3. Importance for local community; 4. The attractiveness of the species; 5. Economic valuing, and 6. Gastronomic value (character). Based on 60 analysed questionnaires the highest value of the final score was obtained for species John Dory (*Zeus faber*) which is suggested as flagship species of Aquarium Boka under establishment. It is expected that using this species will support Aquarium Boka conservation activities, promote public awareness and raise funds for conservation.

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DIET OF GREAT CORMORANT (*PHALACROCORAX CARBO)* IN THE „CARSKA BARA" SPECIAL NATURE RESERVE, WITH A PARTICULAR REFERENCE TO THE CARP (*CYPRINUS CARPIO*) SHARE

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ISHRANA VELIKOG KORMORANA (*PHALACROCORAX CARBO*) U SPECIJALNOM REZERVATU PRIRODE „CARSKA BARA", SA POSEBNIM OSVRTOM NA UDEO ŠARANA (*CYPRINUS CARPIO*) U ISHRANI

Apstrakt

Veliki kormoran se u potpunosti hrani ribom. Dnevna količina hrane koju veliki kormoran pojede kreće se između 425-700 grama, odnosno u proseku od 15 do 17 % svoje telesne težine. Opsežnija istraživanja ishrane velikih kormorana u Srbiji nisu vršena. Jedini podaci o ishrani kormorana i drugih ihtiofagnih vrsta ptica kod nas odnose se na štete koje one nanose proizvodnji ribe na ribnjacima. Podaci o ishrani kormorana van ribnjaka odnose se na ishranu tokom sezone gnežđenja u koloniji u Kopačkom ritu i zimsku ishranu na akumulaciji Gruža. Analiza ishrane velikog kormorana i značaj šarana u njegovoj ishrani rađena je na području Specijalnog rezervata prirode „Carska bara". U periodu od 2007 do 2010 godine prikupljane su gvalice za analizu ishrane velikog kormorana. Gvalice su prikupljane u dve sezone: sezona gnežđenja (mart-jun) i jesenje-zimskom periodu (oktobar-februar). Prikupljane su na mestima gde se ptice odmaraju i u samom mestu gnežđenja. Analiza gvalica je najčešće korišćen metod analize ishrane kod kormorana. U periodu oktobar–februar, 2007 – 2010. godine prikupljeno je 507 gvalica iz kojih su identifikovani ostaci 2433 jedinke od 16 različitih vrsta riba, od kojih je 9 vrsta bilo iz familije Cyprinidae, zatim tri vrste iz familije Percidae i po jedna vrsta iz familija Gadidae, Esocidae, Ictaluridae i Gobiidae. Ostaci šarana identifikovani su u 184 gvalice (36,29%). Šaran je u ovom periodu bio zastupljen sa 18,10%, u pogledu brojnosti. Po masenom udelu u plenu najznačajnija je vrsta, sa učešćem od 36,24%.

Tokom sezona gnežđenja od 2007. do 2010. godine prikupljeno je 80 gvalica. U plenu, identifikovanom iz gvalica, po brojnosti su dominirale dve vrste, babuška i šaran, a registrovano je prisustvo 11 vrsta riba iz 5 familija (6 Ciprinidae, 1 Ictaluridae, 1 Esocidae, 2 Percidae,1 Gobiidae). Ostaci šarana su registrovani u 44 (55%) gvalica. Šaranske vrste riba su još značajnije zastupljene u ishrani kormorana tokom sezone gnežđenja nego u jesenjem i zimskom periodu. Tako, one čine 85% ukupnog broja ulovljenih jedinki, a udeo u biomasi plena je oko 90%. Rezultati istraživanja ishrane velikih kormorana na području Carske bare u skladu su sa mnogim studijama sprovedenim širom Evrope.

Ključne reči: *ishrane, veliki kormoran, šaran, ribnjak, Carska bara.*
Keywords: *nutrition, great cormorant, carp, fishpond, Carska bara.*

INTRODUCTION

The great cormorant is completely feed by the fish. The daily amount of food eaten by the great cormorant ranges between 425-700 grams, or an average of 15 to 17% of its body weight (Van Doben, 1952). The rapid increase in the number of cormorants across Europe has led to dissatisfaction among fish and fish farmers due to the losses caused by birds. This has led to many studies in many European countries over the last 20 years, with the aim of determining the daily intake of food, composition and size of prey, and so on (Sutter, 1997; Leopold et al., 1998; Keller and Wisser, 1999; Grémillet et al., 2003; Gwiazda, 2004; Santoul et al., 2004; Opačak et al., 2004).

Carp is the main species in fish production in Serbia with a share of over 85% in carp fishponds, accounting for around 80% of total production (Marković and Poleksić, 2011). Since these ponds are mainly located in Vojvodina, near the lowland rivers, they represent the ideal feeding base for ichthyophagous birds.

Extensive research of the great cormorants diet in Serbia has not been carried out. The only data on nutrition of cormorants and other ichthyophagous bird species in our country relate to the damage they cause on the production of fish on fish farms (Pihler et al., 2000). Also, in the former SFR Yugoslavia, data on nutrition of cormorans are mainly related to fishponds (Đorđević and Mikuška, 1986; Mikuška, 1986), while the data on nutrition of cormorants outside the fishpond is relate to feed during the breeding season in the colony in Kopački rit (Mikuška, 1983) and winter food on the accumulation of Gruža (Skorić, 2013/14).

The aim of this paper is point to the significance of the carp in the diet of great cormorant in Serbia.

MATERIALS AND METHODS

The analysis of the great cormorant’s diet and the importance of the carp in its diet was made in the area of the special nature reserve «Carska bara». Cormorants have wide activities area, and within it, along the water areas within the reserve (Stari Begej, Carska bara) there are the rivers Tisa and Begej, and above all, the lake of the Ečka fishing farm, which represent the largest aquatic areas in this area and are of particular importance to wading birds.

In the period from 2007 to 2010, pellets were collected for analyzing the nutrition of great cormorants. The pellets were collected in two seasons: breeding season (March-June) and autumn-winter period (October-February). They are collected at places where birds rest in the nest itself. Pellet analysis is the most commonly used method of nutrition analysis in cormorants.

Only fresh and complete pellets were collected, which were then placed individually in plastic bags and freezed at -20ºC. The bone structure of the collected pellets used to identify the carp is the otolith, the gallbladder and the keratinous structures on the basicocipital bone - the so-called «Chewing pads». These structures are separated and measured. The number of individuals present in the pellets is defined as the largest number of fish residues identified. To make a reference collection, a carp from aquatic habitats of the investigated area was sampled. Measure is the total length (TL: length from the tip of the mouth to the end of the tail fin) and weight, on a technical scale of precision of 0.1 g. The fish were then cooked for several minutes, and the length of bones separated for identification were measured. Regression equations have correlations between the total length of the reference fish body (TL) and the length of bitter teeth or otolith, and only those values with high degree of correlation (r> 0.95) were taken into account. The weight estimate was made according to the exponential equation of the mass and body length ratio: $W = a \times TL^b$

On the basis of the obtained values, the correlations between the length of the body and bitter teeth or otolites are the estimates of the length and weight of the fish whose residues are identified in pellets.

RESULTS AND DISCUSSION

In the period from October to February 2007 - 2010, 507 pellets were collected, from which 2433 specimens of 16 different fish species were identified, of which 9 species were from the Cyprinidae family (*Cyprinus carpio*, *Carassius gibelio*, *Abramis brama*, *Blicca bjoerkna*, *Scardinius erythrophthalmus*, *Rutilus rutilus*, *Leuciscus idus, Chondrostoma nasus*, *Aspius aspius*), then three species from the Percidae family (*Perca fluviatilis*, *Gymnocephalus* sp., *Sander lucioperca*) and one species from the families Gadidae (*Lota lota*), Esocidae (*Esox lucius*), Ictaluridae (*Ameiurus melas*) and Gobiidae (*Neogobius* sp.). The average length of prey was 17.12 cm and ranged from 3.7 cm (*Neogobius* sp.) to 37.3 cm (*Sander lucioperca*); the average weight was 86.17 g in the range of 0.46 g (*Neogobius* sp.) to 582 g (*Abramis brama*). The remains of carp were identified in 184 pellets (36.29%). The most numerous prey in the autumn-winter period is prussian carp (24.62%), followed by Eurasian ruffe and carp (18.50% and 18.10%). By weight in the prey, the most important species is carp (36.24%). The average length of the carp in the winter period was 223.22 mm and ranged from 89.96 mm to 338.89 mm; and the average weight was 179.29 g and ranged from 10.98 g to 578.92 g.

During the breeding season in the period 2007-2010, 80 pellets were collected. In the prey identified from pellets, two species were dominant - prusian carp and carp, and also 11 fish species from five families - 6 Ciprinidae (*Cyprinus carpio, Carassius gibelio, Abramis brama*, *Blicca bjoerkna*, *Rutilus rutilus*, *Scardinius erythrophthalmus*), 2 Percidae (*Perca fluviatils*, *Gymnocephalus* sp.), 1 Ictaluridae (*Ameiurus melas*), 1 Esocidae (*Esox lucius*), 1 Gobiidae (*Neogobius* sp.) were recorded. The average length of prey was 17.53 cm and ranged from 6.3 cm (*Carassius gibelio*) to 43.5 cm (*Esox lucius*); the average weight was 94.77 g and ranged from 1.45 g (*Carassius gibelio*) to 625 g (*Cyprinus carpio*). The remains of carp are registered in 44 (55%) pellets. Carp fish species are even more important in feeding cormorants during the breeding season than in autumn and winter periods. Thus, they account for 85% of the total number of caught individuals, while the share of prey biomass is about 90%. Prussian carp and carp are the most important species in the diet during the breeding season with a share of 40.26% and 37.26% in number and 29.41% and 55.33% in biomass, respectively. The length of the carp during the breeding season is 232.7 mm, and ranged from 105.92 mm to 347.72 mm, while the average weight was 208.12 g and ranged from 17.9 g to 625.19 g. Average length and weight of carp as a prey of great cormorant in different seasons in the period from 2007 to 2010 is presented in Table 1.

Table 1. Average length and weight of carp in different seasons in the period from 2007 to 2010

Year	Season	Number of ind.	Average prey length (mm)			Average prey weigth (g)		
			Mean value ± SD	Min	Max	Mean value ± SD	Min	Max
2007	breeding							
	winter	6	241.71±30.19	206.78	294.74	219.19±87.19	132.22	381.43
2008	breeding	56	218.67±47.03	105.92	347.72	177.32±111.74	17.9	625.19
	winter	99	208.75±49.51	86.96	338.89	158.25±103.04	10.98	578.92
2009	breeding	14	260.76±31.06	223.43	315.46	275.05±101.52	166.64	467.3
	winter	227	222.79±34.16	117.13	312.4	176.69±79	24.18	453.9
2010	breeding	21	251.4±28.17	209.16	318.86	245.65±84.44	136.82	482.5
	winter	107	236.5±20.62	187.77	297.46	202±54.72	99.09	392.04

Statistically significant differences were found between the length and weight of the carp as the prey of cormorants between different seasons (autumn-winter and breeding season) (t-test, $p < 0.05$) (Figure 1).

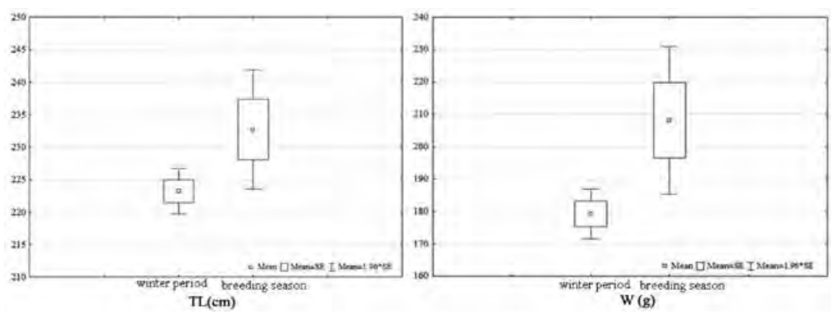

Figure 1. Differences between the length and weigth of the carp in different season

Many studies on the nutrition of great cormorants used pellet analysis. In a short time and with a minimal disturbance of birds, a large sample can be collected (Duffy and Jackson, 1986). Basically, this analysis provides good information on the qualitative composition of the diet content (percentage representation of species) (Warke and Day, 1995), and a number of authors (Dirksen et al., 1995; Keller, 1995, Engstrom, 2001; Gwiazda, 2004; Santoul et al., 2004) calculates the length of the fish (and therefore the weight) based on the residues from the pellets, and estimates the total fish weight per pellet considering as the daily intake of food. Cormorant diet depends on the type of aquatic ecosystems that birds inhabit, as well as the seasons (Kirby et al., 1996). Also, the probability of catching a larger prey is more frequent in winter, since the lower water temperature reduces the speed of fish swimming. Although many fish species are involved in the diet spectrum of cormorants, only a few of them are dominant in its diet. The carp dominates in the cormorant diet in areas where colonies were located near the pond, while the share of fish from the open waters is smaller. These examples are found in several areas of Poland (Mellin et al., 1997; Gwiazda, 2004), France (Trolliet, 2002), Croatia (Opačak et al., 2004; Đorđević and Mikuška, 1986; Mikuška, 1986).

The results of the great cormorants diet in the Carska bara region are in accordance with the mentioned studies, relative to Prussian carp and carp, making the most significant prey in both quantity and biomass. Cormoran is opportunistic and feeds on the most numerous and available prey (Grémillet et al., 2001), which are definitely Prussian carp and carp. This is especially the case during the breeding period when the share of these species in the diet is about 80%. Also, as in many studies conducted throughout Europe (Keller, 1995; Gwiazda, 2004; Santoul et al., 2004; Čech and Vejřik, 2011), the share of carp species in cormorant diet in our research was between 50 and 90%.

A great cormorant catches a prey of a wide range of body size. In studies carried out in many European countries, the results show that the prey length ranges from 2 to 3 cm, up to 70 cm and weight from less than 1 g to 900 g (Keller, 1995; Sutter, 1997). The most frequently hunted prey is in the range of 10 to 25 cm (Martyniak et al., 1997; Gwiazda, 2004; Opačak et al., 2004; Santoul et al., 2004). The range of the prey length, as well as the average values of the prey length, measured in our study are in compliance with the results of above research. The average size of carp in this study is larger than in the studies carried out by Adámek (1991) where the most frequently caught specimens were 13 to 17 cm, Mellin et al. (1997) from 8 to 10.9 cm and Opačak et al. (2004) from 10 to 15 cm. According to Schreckenbach et al. (1998), when carp are larger than 600 g it is «safe from cormorants», which was confirmed by this study. Only one individual had weight over this value (625 g).

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NEW RECORDS OF NATIVE CRAYFISH *AUSTROPOTAMOBIUS TORRENTIUM* (SCHRANK, 1803) IN SERBIA

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NOVI NALAZI AUTOHTONE VRSTE *AUSTROPOTAMOBIUS TORRENTIUM* (SCHRANK, 1803) ZA SRBIJU

Apstrakt

Potočni rak *Austropotamobius torrentium* (Schrank, 1803) je autohtona vrsta Evrope koja naseljava čiste brdsko-planinske potoke sa kamenito-šljunkovitom podlogom. Zbog opadajućeg trenda brojnosti populacija i ugroženosti staništa *A. torrentium* je zaštićen u većini zemalja. Za bolju ocenu statusa ugroženosti u Srbiji, neophodno je prikupiti što više podataka o ovoj autohtonoj vrsti, što je i cilj rada. Detaljna terenska istraživanja sprovedena su tokom 2017. godine od juna do oktobra i obuhvatila su 45 vodotokova u Srbiji. Rakovi su hvatani ručno ili uz pomoć zamki primenom standardizovanog protokola. Prisustvo vrste prvi put je zabeleženo na ukupno 8 vodotokova. Kao najinteresantniji nalaz može se izdvojiti populacija zabeležena na Fruškoj gori (Rakovački potok) koja predstavlja najseverniji nalaz vrste kod nas. Možemo izdvojitii nalaz sa Divčibara, gde je u reci Kamenici pored potočnog raka zabeleženo i prisutvo druge nativne vrste *Astacus astacus* Fabricius, 1775. Prema novijim istraživanjima, zagađenje vodenih staništa se ne smatra faktorom ugrožavanja ove vrste u Srbiji, ali je potrebno ukazati na rizik koji predstavljaju mini hidrocentrale, čija izgradnja je u ekspanziji u poslednjoj deceniji. Fragmentacija i degradacija staništa predstavljaju ozbiljan rizik za opstanak populacija ove zaštićene vrste, te je stoga neophodan nastavak istraživanja i sprovođenje standardizovanog monitoring radi bolje ocene statusa i nivoa ugroženosti.

Ključne reči: *Austropotamobius torrentium, Srbija, novi nalazi, faktori ugrožavanja.*
Keywords: *Austropotamobius torrentium, Serbia, new record, endangering factors*

INTRODUCTION

The stone crayfish *Austropotamobius torrentium* (Schrank, 1803) is one of a few native European crayfish species distributed throughout Central and Southeastern Europe (Laurent, 1988; Souty-Grosset et al., 2006; Longshaw & Stebbing, 2016). Due to decreasing populations trends caused by habitat destruction and crayfish plague this species is listed in the Red List of Threatened Species of the IUCN, categorized as «Data Deficient» according to IUCN criteria (Version 3.1) (Füreder et al., 2010).

Austropotamobiu storrentium preferably inhabits relatively unpolluted cold-water headwaters, springs, brooks and streams with stony substrate and often turbulent flow (Streissl & Hödl, 2002; Huber & Schubart, 2005; Füreder et al., 2006). In Serbia the species is found dominantly at higher altitudes of the western, southern and eastern part of the country, where it was recorded at 48 localities (Simić et al., 2008, 2015; Petrović et al., 2013; Markovic et al., 2017).

Having in mind that detailed and continuous study on distribution and population status of stone crayfish in Serbia have not been done since 1960's (Karaman 1961, 1962, 1963), arises a need to conduct more detailed survey, checking and compiling literature data, with recent field investigation, in order to update our knowledge regarding this species and to better assess its current status, which is necessary for conservation and protection.

Here we present new findings of stone crayfish in Serbia, as a result of recent extensive field survey.

MATERIAL AND METHODS

The field survey was conducted from June to October 2017 including 45 watercourses from all main drainages in Serbia. In order to efficiently cover as many habitats of stone crayfish as possible, a selection of watercourses was done based on literature data and our knowledge. Some of selected watercourses were uninvestigated before, while regarding others our intention was to check (confirm or deny) presence of *A. torrentium*. The period of survey (June-October) was chosen not only because of better hydrological conditions, but also in order to minimize disturbance of hatching females with fertilized eggs (which occurs in Winter-Spring period).

Stone crayfish specimens were collected by hand or by LiNi traps placed in a stream along transect of 100 m and left over night. The caught crayfish were sexed, measured and released back into the water unharmed. If present, damage, physical defects and visible symptoms of the disease and the presence of parasites were recorded.

RESULTS AND DISCUSSION

Based on extensive field investigation covering 45 watercourses in Serbia, the presence of *A. torrentium* was confirmed at total of eight watercourses with no previous findings of this species. The main data of these localities are provided in Table 1.

Majority of these newly recorded populations were found at typical habitats for *A. torrentium* (Streissl & Hödl, 2002; Huber & Schubart, 2005; Füreder et al., 2006) – i.e. small cold-water streams at a higher altitude, situated in a forest or surrounded by dense vegetation, with a strong water current and hard-bottom substrate. Of particular interest could be finding of presumably isolated stone crayfish population in the Pannonian (i.e. northern) part of country at the Fruška gora Mountain (Fruška Gora National Park).

Another remarks that should be noted was possibility of population overlapping between *A. torrentium* and another native crayfish species *Astacus astacus* Fabricius, 1775 in the river Kamenica on Divčibare mountain. The locality where *A. astacus* was found is located few kilometers downstream, in the populated area with anthropogenic influence.

Regarding sex-ratio in most populations approximately the same proportion of males and females was recorded. Found domination of males in populations from streams Županska reka and Rakovački potok may occurred due to seasonal differences in male and female activity (Aleknovich et al., 1999) as the investigation at this localities was done late (October).

The absence of *A. torrentium* in the tributaries of the Drina River (Trešnjica, Rača, Ljubovиđa and Rogačica) should be mentioned. All localities at these rivers meet the habitats requirements of *A. torrentium*. We could only speculate that devastating floods occurred in the Spring of 2014 might had some influence on crayfish populations in these watercourses, meaning that populations probably were not yet recovered from this event. The reason for rare findings of stone crayfish in numerous watercourses belonging to Aegean Sea drainage in south-eastern Serbia the Dragovištica (Božica, Lisina, Ljubata, Dukatska reka) and Pčinja (Tripušnica, Kozjedolska reka) catchments could be the same as above. Moreover, the presence of stone crayfish at all mentioned watercourses was confirmed relatively recently (Simić et al., 2008).

Table 1. New findings of Austropotamobius torrentium (Schrank, 1803).

river/stream (river basin)	coordinates	elevation (m)	number of catched female/male
Kamenica/Divčibare (Drina)	N 44.097891° E 20.022914°	799	8F/12M
Sovljak/Tara (Drina)	N 43.898973° E 19.523992°	940	3F/5M
Jarevac/Tara (Drina)	N 43.900670° E 19.528571°	944	6F/6M
Užički potok/Tara (Drina)	N 43.860123° E 19.585959°	750	9F/11M
Uvac (Drina)	N 43.292452° E 19.926834°	1059	8F/12M
Pesača/Đerdap (Danube)	N 44.573582° E 21.987418°	402	9F/11M
Rukjavica (Danube)	N 44.041467° E 22.147816°	269	8F/12M
Zupska reka/Stara planina (Timok)	N 43.406761° E 22.590512°	820	7M
Rakovački potok/Fruška gora (Danube)	N 45.176626° E 19.772833°	222	4M

Throughout *A. torrentium* areal the declining populations’ trend was noted (Füreder et al., 2010). Negative anthropogenic impact on its habitats, either as water pollution, habitat degradation, damming or introducing of invasive species and diseases (plague), were signed out as the main cause (Maguire et al., 2011). In Serbia, according to previous studies (Simić et al., 2008), water pollution was probably not being a limiting factor for this species (i.e. cause of populations decline) due to very good water quality status of hilly-mountainous streams as its preferred habitat. Construction of numerous small hydropower plants could play important role in presumably declining of *A. torrentium* populations. Importance of spatial continuity for natural dispersion of crayfish has already been proven and habitat fragmentation and isolation due to damming is recognized as one of key factors causing populations declines (Bohl, 1997). Without natural dispersion populations could become isolated from one another resulting in genetic decline (reduced heterogeneity) which linked with some other negative factors (such as low number of individuals, etc.) eventually could lead to extinction.

Having in mind sharp increase of other negative factors in last decade, a prolonged detailed study of *A. torrentium* in the region is necessarily in order to adequately assess its ecological status, diversity and vulnerability. The reports of new findings, as presented in this paper, should contribute to our knowledge by providing new data of its distribution as a foundation for more comprehensive research.

ACKNOWLEDGEMENTS

This paper was supported by the Rufford Foundation, project *Stone crayfish Austropotamobius torrentium (Schrank, 1803) in Serbia: distribution, population density, genetic diversity and conservation* and the Ministry of Education, Science and Technological Development of the Republic of Serbia, projects TR 37009 and III 43002. Authors wish to express their gratitude to dr Stefan Skorić (Institute for Multidisciplinary Research),Goran Žikić (Đerdap National Park), dr Dragiša Savić (Fruškagora National Park), Siniša Jeftić (Tara National Park), Ninoslav Kostadinović (Srbijašume) and Ninoslav Kostić (Association Veternica - Vlasina) for valuable help during fieldwork.

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ASSESSMENT OF INVASIBILITY OF THE DANUBE SECTION IN SERBIA

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OCENA INVAZIBILNOSTI PODRUČJA NA DELU TOKA DUNAVA KROZ SRBIJU

Apstrakt

Popis alohtonih vrsta određene teritorije i procena stepena njihove invazivnosti predstavljaju polazni korak na osnovu koga se može pratiti efikasnost sprovedenih mera borbe protiv invazivnih vrsta. Važan aspekt metodologije predstavlja i definisanje potencijalno osetljivih teritorija sa aspekta bioloških invazija, odnosno procena stepena invazibilnosti područja. U ovom radu data je procena invazibilnosti dela toka Dunava kroz Srbiju na osnovu parametara za koje pretpostavljamo da imaju najveći uticaj na osetljivost vodenih ekosistema prema bioinvazijama. To su: nadmorska visina, razuđenost reljefa, broj stanovnika, nivo hidroloških promena i intenzitet rečnog saobraćaja. Na osnovu ukupne ocene koja je dobijena kao srednja vrednost pojedinačne ocene za svaki od navedenih parametara, možemo zaključiti da je sektor od Beograda do brane „Đerdap I" najosetljiviji na biološko zagađenje, kao i deo toka nizvodno od Novog Sada. Na sektor od Beograda do brane „Đerdap I" najveći uticaj na visoku klasu invazibilnosti ima prvenstveno intezivan rečni saobraćaj, kao i nivo hidromorfoloških promena.

Ključne reči: *Dunav, Srbija, biološke invazije, invazibilnost područja.*
Keywords: *Danube river, Serbia, biological invasions, section invasibility*

INTRODUCTION

The ecological status of surface waters is one of the two main determinants of the Water Framework Directive (WFD, 2000). In addition to the ecological status, it is envisaged that all signatory countries identify significant hydromorphological changes on water bodies.

Although hydrological and morphological data in many countries are part of the standard procedure, the mutual dependence of hydromorphological changes and ecological status of the river is poorly known (Babić Mladenović and Kolarov, 2010). Only a few countries have developed a system to integrate hydromorphological changes into ecological assessments. For the Danube River Basin, this was done within the International Commission for the Protection of the Danube River (ICPDR).

In this paper, an estimation of the invasibility of the Danube in Serbia is given based on parameters which we presume to have the greatest impact on the sensitivity of aquatic ecosystems to bioinvasions.

MATERIAL AND METHODS

Assessment of the invasibility of the Danube was done on data collected during the International expedition Joint Danube Survey 3 supported by International Commission for the Protection of the Danube River (ICPDR). Macroinvertebrate samples were collected in August and September 2007. In total, 22 localities along the 588 km of main course of the Danube in Serbia were examined: Batina (JDS40), upstream from the Drava confluence (JDS41), Bogojevo (JDS43), Dalj (JDS44), Bačka Palanka (JDS45), Novi Sad (upstream, JDS46 and downstream, JDS47), Stari Slankamen (JDS48), Belegiš (JDS50), Pančevo (upstream, JDS52 and downstream, JDS53), Grocka (JDS54), Velika Morava confluence (upstream, JDS55 and downstream, JDS57), Stara Palanka (JDS58), Banatska Palanka (JDS59), Golubac (JDS60), Donji Milanovac (JDS61), Tekija (JDS62), Iron Gate II (JDS63), Vrbica (JDS64) and locality upstream from the Timok confluence (JDS65). Benthic samples were taken by benthological hand net (mesh size 500 μm and 1000 μm).

The assessment of the invasibility of the area was obtained by assessing the impact of the selected parameters that supposedly have the greatest impact on the sensitivity of aquatic ecosystems to bioinvasions. These are: elevation, relief characteristics, number of inhabitants, level of hydromorphological changes and intensity of river transport. Scales of rated classes by parameters are given in Table 1.

Parameters are divided into five classes, except for relief characterization and intensity of river transport. The final value of the impact is calculated as the mean value of the classes of all presented parameters. As the values of the two parameters are evaluated in three classes, final values are multiplied by the factor 5/3.

Classes for the assessment of the level of hydromorphological changes were given by Schwarz and Kraier (2008), and taken over Babić Mladenović and Kolarov (2010).

The estimation of the number of inhabitants was based on the number of inhabitants of the municipalities located along the Danube bank, in order to assess the water load of various types of pollution, urban and industrial wastewater, as well as water from the agricultural area. The number of inhabitants is given according to the 2011 census of the Republic of Serbia from Statistical Office of the Republic of Serbia (www.popis2011.stat.rs).

The impact of river transport intensity was estimated by traffic density in t/km according to the data of the Statistical Office of the Republic of Serbia 2007 (2009).

Table 1. Classes of selected parameters. 1 – very low risk, 2 – low risk, 3 – moderate risk, 4 – high risk, 5 – very high risk.

parameter/ class	**1**	**2**	**3**	**4**	**5**
elevation (m)	>801	501-800	201-500	101-200	1-100
relief characteristics	very diverse	moderately diverse	unique	/	/
number of inhabitants	below 10.000	10.000-49.999	50.000-99.999	100.000-999.999	over 1.000.000
hydro-morphological changes	nearly natural	sligthly modified	moderately modified	severely modified	totally modified
water transport (t/km)	below 5.000	5.000-10.000	over 10.000	/	/

RESULTS AND DISCUSSION

The results of the hydromorphological assessment show slightly to moderately modified channel in upper stretch of the river, upstream from Pančevo (JDS52). The backwater effect of power plants "Iron Gate I" and "Iron Gate II" is felt on downstream sections characterized by fluctuating flow regime. Twenty kilometres of flow downstream from the "Iron Gate II" (JDS63) is characterized as moderately altered. High level of hydromorphological changes along the Iron Gate reservoirs are also contributed by fully coated shore as a protection against high water levels.

The parameter estimation of the number of inhabitants was based on the municipalities located along the Danube bank. Belgrade (JDS53) is the only town with more than one million inhabitants (class 5). The largest number of cities along the Danube belongs to the second class with a population between 10,000 and 49,999. From this aspect, the most affected part of the Danube represents 150 km of river flow from Novi Sad to Smederevo (JDS47-JDS57). Downstream of the dam "Iron Gate I" (JDS60) the number of inhabitants is decreasing.

Regarding the intensity of river transport, it is noticeable that it is increasing downstream along the river. Sectors with the highest intensity of transport have a traffic density over 10,000 t/km (JDS52-JDS59), while the smallest density is only about 3,500 t/km (JDS40-JDS41).

In its major part, Danube is typical lowland river with a slope of 0.05-0.04 per thousand (Paunović et al., 2007). Sector from the state border (JDS40) to the site of Banatska Palanka (JDS59) has highly variable characteristics of the river channel (class 3). Due to its relatively uniform morphological characteristics, the next sector, namely the Iron Gate Gorge (JDS60-JDS62) has assigned to the class 1. Danube sector downstream of the "Iron Gate II" dam (JDS63-JDS66), has typical characteristics of the lowland river with higher river terraces (class 2).

Based on the overall analyses of selected parameters considered to be important for intensity of biological invasions, we concluded that part of the Danube stretch between Belgrade and Iron Gate I dam, as well as locality downstream Novi Sad are most affected (Figure 1). High class of invasibility was determined due to high intensity of water transport, as well as the level of hydromorphological changes.

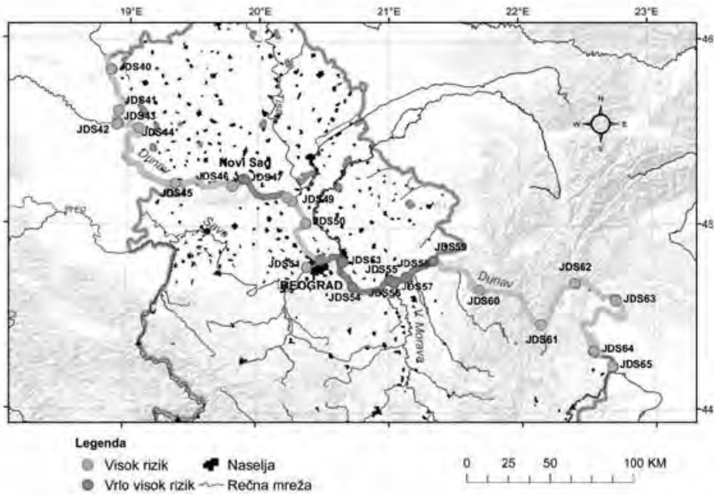

Figure 1. Assessed invasibility of the Danube River in Serbia

In future, research a larger number of environmental parameters should also be included in the analysis, primarily physico-chemical parameters of water and sediment.

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THE NITROGEN DYNAMICS IN INTEGRATED MULTI-TROPHIC AQUACULTURE SYSTEMS

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DINAMIKA AZOTA U INTEGRISANIM MULTI-TROFIČNIM SISTEMIMA AKVAKULTURE

Apstrakt

U centralnoj i istočnoj Evropi proizvodnja riba se obavlja uglavnom u tradicionalnim produkcionim sistemima. Imajući ovo u vidu, neophnodno je osmisliti strategije koje će obezbediti smanjenje uticaja nutrijenata na kvalitet recipijentnih voda. U cilju zadovoljavanja ovih potreba i obzirom da isplativa proizvodnja u akvakulturi zahteva efikasno iskorišćavanje unetih nutrijenata, primena integrisane multi-trofične akvakulture (IMTA) se može smatrati odgovarajućim izborom. Kruženje nutrijenata, posebno N, je značajno za IMTA produkcioni sistem. Dakle, radi evaluacije dinamike N u ribnjacima, neophodno je da se utvrde stope akumulacije ovog hemijskog elementa u nekoliko ključnih tačaka: voda ribnjaka, sediment i mišićno tkivo riba. Ova studija ima za cilj da identifikuje potencijal IMTA sistema za korišćenje azotnih jedinjenja koja potiču od gajenja Ciprinida, i doprinose održivosti ove grane privrede.

Ekperimentalna postavka: Koristili smo dva zemljana bazena veličine 0.45 ha sa prosečnom dubinom od 1.5 m (Slika. 1). PCP se koristi za gajenje u polikulturi 2500 jedinki šarana – CC, 100 jedinki belog amura – GC, 40 jedinki sivi tolstolobik – BC i 40 jedinki beli tolstolobik. CP-PP objekat je podeljen pomoću mreže: prvi deo površine od 0.15 ha CP (šaranski objekat – 2000 jedinki), i drugi deo površine 0.33 ha PP (objekat sa polikulturom, 500 šarana, 40 belog amura, 40 sivog tolstolobika i 100 belog tolstolobika). Riblja hrana (sa 28% proteina) je ručno distribuirana dva puta dnevno, samo u PCP (756.82 kg) i CP (285.41 kg) 5 dana u nedelji. Eksperiment je počeo 15.06.2016 a završio se 05.09.2016, sa intemedijarnim uzokovanjem nakon 52 dana.

Biohemijska analiza je urađena na svim uzorcima riba i sedimentu obuhvatila je: macro-Kjeldahl metoda za TKN (ukupni Kjeldahl azot), spektrofotometrijske metode, jedna za $N\text{-}NO_3$

bazirnoj na diazotization-coupling reakciji sulfanilamida sa N-(1-naphthyl) etilenediamine dihidrohlorida a druga za $N\text{-}NO_2$ određenoj redukcijom nitrata sa kadmijumum. Koncentracije $N\text{-}NO_3$, $N\text{-}NO_2$ i $N\text{-}NH_4$ u vodi su određene korišćenjem continuous flow analizatorom.

Tokom delimičnog izlova jedinke babuške – PrC su nađeni i u polikulturi i u IMTA objektima, i kategorisani su kao invazivne vrste. Obzirom da na početku eksperimenta objekti nisu nasađeni sa ovom vrstom, babuška je mogla ući preko ulivnih kanala, kroz otvore na mrežama. Međutim, i ovi ribe su podvrgnute analizi koncentracije N u mesu.

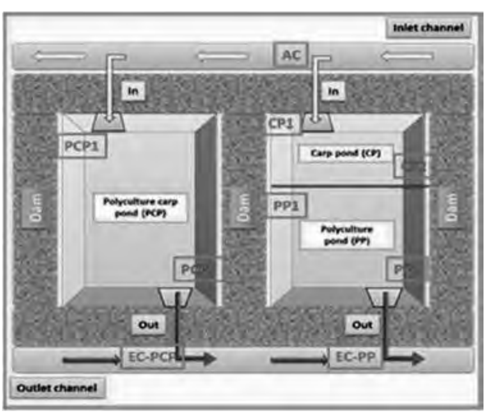
CP-PP menadžment ishrane, zajedno sa testiranim tehničkim rešenjima (podela objekta na dva dela) dovela je do većeg unosa azota od strane šarana, i boljeg stanja vodene sredine. Može se zaključiti da je primena IMTA tehničkog rešenja primenjenog u CP-PP dovela do smanjene akumulacije azota u životnoj sredini.

Ključne reči: IMTA, zemljani ribnjaci, ciprinide, azor, održiva akvakultura

Abstract

In central and eastern part of Europe, the national fish production is assured mainly by the traditional pond aquaculture production system. Considering this aspect, strategies targeting to reduce the impact of nutrients on water quality in receiving waters must be made. Therefore, in order to meet the needs and since economically feasible aquaculture activity requires efficient utilization of nutrients inputs, the application of integrated multitrophic aquaculture (IMTA) may be considered the proper solution. The cycling of nutrients, especially N, is critical for the sustenance of pond IMTA. Thus, for the evaluation of N dynamics in fish ponds effluents, it is necessary to identify the main accumulation rates of this chemical element in a series of key targets as follows: pond water, pond sediments and fish muscle tissues. The present study aims to identify the potential of IMTA pond systems to use the nitrogen compounds resulting from cyprinids culture and therefore, improve the sustainability of this economic activity.

Experimental design: For our research, we used two earth ponds with an area of 0.45 ha each one and an average water depth of 1.5 m (Fig. 1).



Sampling areas: AC – inlet channel, EC-PCP – outlet channel from polyculture carp pond, EC-PP – outlet channel from polyculture pond, PCP – polyculture carp pond,

CP – carp pond, PP – polyculture pond.

Površina za uzorkovanje: AC – ulivni kanal, EP-PCP – izlivni kanal iz šaranskog objekta sa polikulturom, EC-PP – izlivni kanal iz ribnjačkog objekta sa polikulturom, PCP – ribnjački objekat sa polikulturom šarana, CP – šaranski ribnjački objekat, PP – ribnjački objekat sa polikulturom.

Figure 1. Experimental design and sampling areas
Slika 1. Ekperimentalni dizajna i mesta uzorkovanja

Table 1. TKN, N-NO2 and N-NO3 concentrations in fish meat
Tabela 1. Koncentracija TKN, N-NO2 i N-NO3 u mesu ribe

Fish sp.	Pond	Sampling date	Individual average biomass (g/fish)	TKN (g % FW)	Nitrites (mg/kg FW)	Nitrates (mg/kg FW)
C. carpio (CC)	15.06.2016		61.4±10.0	2.6±0.1	1.2±0.2	21.1±1.4
	CP	5.08.2016	144.0±60.7	2.2±0.1	3.7±0.1	12.5±1.2
		5.09.2016	175.1±63.9	3.5±0.4	4.4±0.7	25.1±3.2
	PP	5.08.2016	98.0±14.8	2.1±0.1	4.1±0.2	14.8±1.6
		5.09.2016	184.9±65.5	1.5±0.1	6.4±0.3	31.3±1.9
	PCP	5.08.2016	196.6±42.8	3.1±0.1	8.1±0.5	25.6±2.9
		5.09.2016	213.5±57.3	2.1±0.1	4.6±0.3	13.7±1.3
A. nobilis (BC)	15.06.2016		1880.6±187.1	2.8±0.1	3.0±0.3	22.4±0.6
	PP	5.08.2016	3391.3±317.4	3.6±0.2	5.6±0.2	41.2±1.9
		5.09.2016	3248.3±406.1	2.2±0.1	3.6±0.3	25.0±3.4
	PCP	5.08.2016	3368.0±455.8	2.4±0.3	4.3±0.4	21.5±1.3
		5.09.2016	3187.2±412.7	2.2±0.2	2.2±0.1	11.3±0.6
H. molitrix (SC)	15.06.2016		2025.2±251.8	2.8±0.1	2.5±0.3	21.1±0.9
	PP	5.08.2016*	-	-	-	-
		5.09.2016	2801.0±250.1	2.3±0.2	4.7±0.3	21.7±0.7
	PCP	5.08.2016*	-	-	-	-
		5.09.2016	2958.8±417.5	2.6±0.2	4.4±0.2	18.2±1.9
C. idella (GC)	15.06.2016		199.9±20.3	2.9±0.1	3.2±0.3	21.4±0.5
	PP	5.08.2016*	-	-	-	-
		5.09.2016	1226.5±489.5	3.4±0.1	4.0±0.3	26.9±3.3
	PCP	5.08.2016	1528.0±386.6	3.4±0.2	6.0±0.3	23.8±3.0
		5.09.2016	1434.5±624.8	3.3±0.2	3.3±0.2	19.1±1.7
C. gibelio (PrC)	CP	5.08.2016	14.1±2.0	3.2±0.1	3.4±0.4	18.6±1.0
		5.09.2016	14.5±2.0	3.6±0.1	8.3±0.3	34.2±4.5
	PP	5.08.2016	10.7±1.6	2.5±0.2	2.6±0.5	10.8±1.3
		5.09.2016	8.7±0.9	2.3±0.1	2.1±0.3	21.1±1.8
	PCP	5.08.2016*	-	-	-	-
		5.09.2016	8.5±0.9	3.0±0.1	6.8±0.3	10.9±0.4

* No results are available during the experimental period, at intermediary stages, because not enough specimens were harvested in order to elaborate proper statistical analysis.

The PCP was used for rearing, in polyculture, 2500 common carp specimens – CC, 100 grass carp specimens - GC, 40 bighead carp specimens - BC and 40 silver carp specimens - SC. The CP-PP pond was divided by using a net, as follows: first part with an area of 0.15 ha CP (carp pond – 2000 specimens) and the second part with an area of 0.30 ha PP (polyculture pond - 500 common carp specimens, 40 silver carp specimens, 40 bighead specimens and 100 grass carp specimens). Feed (28% protein) was manually administered twice/day, only in PCP (756.82 kg) and CP (285.41 kg), for five days/week. The experiment started on 15/06/2016 and ended on 05/09/2016, with an intermediar sampling after 52 days.

The biochemical analysis were performed on all samples of fish and sediments, as follows: macro-Kjeldahl method for TKN (total Kjeldahl nitrogen), spectrophotometric methods, one for $N\text{-}NO_3$ based on the diazotization-coupling reaction of sulphanilamide with N-(1-naphthyl) ethylenediamine dihydrochloride and the other spectrophotometric method for $N\text{-}NO_2$ determined by reduction to nitrite with cadmium. The $N\text{-}NO_3$, $N\text{-}NO_2$ and $N\text{-}NH_4$ concentrations in water were determined by using a continuous flow analyser.

RESULTS

During the partial harvestings, prussian carp - PrC exemplars have been found in both polyculture and IMTA ponds and were categorized as invasive species. As at the beginning of the experiment it was not stocked in neither of the ponds, the prussian carp may have entered into both ponds through the water inlet screen holes. However, those invasive accidental exemplars were also analysed in terms of N concentration in meat.

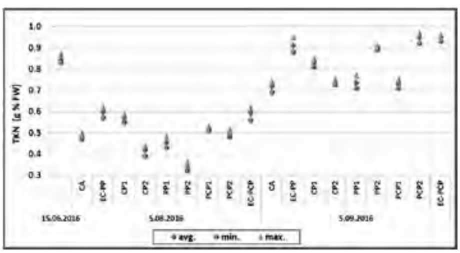


Figure 2. The dynamics of TKN in pond sediments
Slika 2. Dinamika TKN u sedimentu

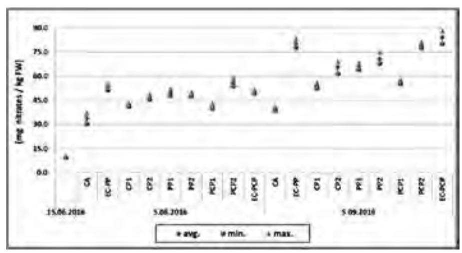


Figure 3. The dynamics of N-NO3 in pond sediments
Slika 3. Dinamika N-NO3 u sedimentu

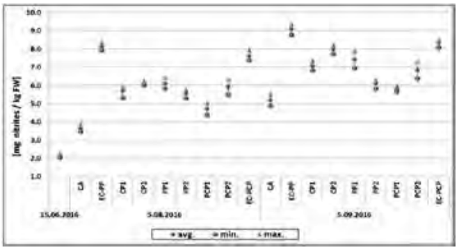


Figure 4. The dynamics of N-NO2 in pond sediments
Slika 4. Dinamika N-NO2 u sedimentu

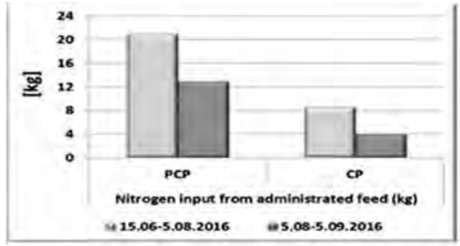


Figure 5. Nitrogen input from administrated feed
Slika 5. Unos azota od distribuirane hrane

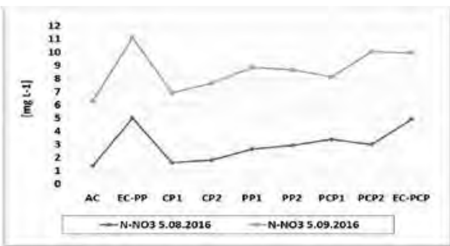


Figure 6. The dynamics of N-NO3 in pond water
Slika 6. Dinamika N-NO3 u vodi ribnjaka

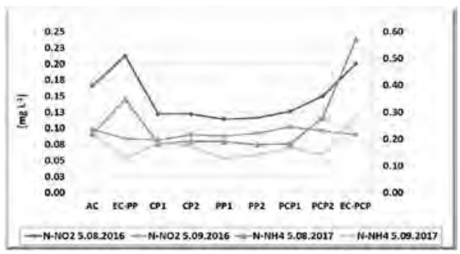


Figure 7. The dynamics of N-NO2 and N-NH4 in pond water
Slika 7. Dinamika N-NO2 i N-NH4 u vodi ribnjaka

The CP-PP feeding management, together with the tested technical solution (pond dividing) generated a higher common carp nitrogen intake and also, better water conditioning performances. Thus, implementing the IMTA technical solution applied in CP-PP generates a reduced loading of nitrogen on the water environment.

Keywords: *IMTA, ponds aquaculture, cyprinids, nitrogen, sustainable aquaculture*

ACKNOWLEDGEMENTS

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CYT-B SEQUENCE VARIATION OF CASPIAN ROACH, *RUTILUS CASPICUS* (YAKOVLEV, 1870) IN THE SOUTHERN CASPIAN SEA

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VARIJACIJA SEKVENCE CYT-B KASPIJSKE BODORKE (*RUTILUS CASPICUS*, YAKOVLEV, 1870) IZ JUŽNOG KASPISKOG MORA

Apstrakt

Kaspijska bodorka *Rutilus caspicus* (Yakovlev, 1870) je semi-anadromna komercijalna vrsta riba koja se pojavljuje u plitkim brakičnim vodama Kaspijskog mora. Ranije morfološke studije su ukazivale na postojanje dve udaljene linije u jugozapadnom i jugoistočnom delu obale Kaspijskog mora u Anzali laguni (*kurensis*) i u zalivu Gorgan (kinopowitschi) ili dve podvrste *Rutilus rutilus*. Nekoliko fragmenta mitohondrijalnog gena (Cyt-b,1140 bp) iz svake populacije je amplifikovano i sekvencirano za testiranje ovih pretpostavki. Genetička varijabilnost unutar populacija je bila 0.7% i 0.2% (bp) za Anzali lagunu odnosno zaliv Gorgan, dok je prosečna genetička udaljenost bila veoma niska, 0.5% (bp). U poređenju sa *Rutilus rutilus* haplotipovi su bili veoma udaljeni, kao kod različitih vrsta, sa preko 4.4 % bp prosečne genetičke razlike. Haplotipovi koje dele sa vrstom *Rutilus frisii* su takođe pokazali da postoji hibridizacija dve vrste u nekoliko slučajeva. Na osnovu sekvenci Cyt-b nije utvrđeno postojanje podvrsta ili linija *Rutilus caspicus* u južnom Kaspijskom moru, a morfološke razlike su uglavnom rezultat faktora okruženja. Takođe, svi haplotipovi pripadaju *Rutilus caspicus* u okviru monofiletske grupe.

Ključne reči: *bodorka, Kasijpijsko more, genetička udaljenost*
Keywords: *Roach, Caspian Sea, Genetic distance*

INTRODUCTION

Caspian Roach, *Rutilus caspicus* is regarded as important commercial fish species of Cyprinidae, from the southern coast of the Caspian Sea (Coad, 2016). Because of overfishing and deterioration of spawning grounds, this species is considered to be included in the list of threatened species of the region (Kiabi et al., 1999). It was believed that there are two subspecies of *Rutilus rutilus* at the Iranian coast of Caspian Sea with Gorgan Bay and Anzali Wetland which are about 400 km far from each other. Bogutskaya and Naseka (2004) and Kottelat and Freyhof (2007) recognised *Rutilus caspicus* (Yakovlev, 1870) as the semi-anadromous species in the Caspian Sea with *R. rutilus* in rivers and lakes. The Anzali Wetland population differs from the Gorgan Bay population by a greater body depth and smaller eyes (Abdoli, 1999). Interestingly, *R. caspicus* and *R. frisii* showed low genetic divergence in Ketmaier *et al.* (2008), a study using DNA with a low sample size. In this study we analyze the validity of these two populations of *R. caspicus* by comparing cyt-b sequence with *R. rutilus* and *R. fri*sii data beside genetic distance between them.

MATERIAL AND METHODS

Fourteen Caspian roach specimens collected from Anzali Lagoon in the southwestern Caspian sea and the Gorgan Bay in south eastern during spring 2016 (Fig. 1). After identification (Coad, 2016), left pectoral and pelvic fin were sampled and preserved in 96% ethanol.

Phenol-chloform Protocol was used for DNA extraction. Full sequence of the mitochondrial Cyt-b gene amplification was performed by Glu F: 5′AACCACCGTTGTATTCAACTACAA3′, and Thr R: 5′ACCTCCGATCTTCGGATTACAAGACCG3′(Machordomand Doadrio, 2001).The polymerase chain reaction product was sequenced (Source Macrogen, Seoul, SouthKorea) and aligned with some of previously submitted sequences from conger species; *Rutilus rutilus*, *Rutilus frissi* and *Garrarufa* (as out group). A phylogenetic tree of Cyt-b sequences with the Maximum likelihood (ML) method was constructed. The best fit models of DNA substitution (TN+I) was chosen based on the Akaike Information Criterion (AIC) approach by Modeltest 3.7 (Posada and Crandall 1998).

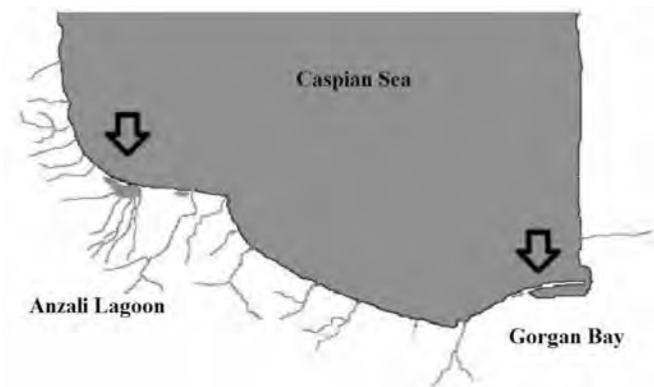

Figure 1. Two stations for collecting Caspian Roach (Rutilus rutilus) specimens along southern coast of the Caspian Sea

RESULTS AND DISCUSSION

Average within group mean distances were 0.47% and 0.16% for Anzali Lagoon and Gorgan Bay specimens respectively while this distance in comparison between population was 0.33% for 1140 bp sequence of Cyt-b gene. Results indicate no distinct population or subspecies in these areas with 400 km distances. Averages between group distances for both locations were about 4.5% vs. *R. rutilus* and 7% vs. *R. frissi* (Table 1).

Table 1. within group and between group mean distances base on base pair percent in 1140 bp fragment of Cyt-b gene.

		1	2	3	4
1	***R. caspicus* (Anzali Lagoon)**	**0.47%**			
2	***R. caspicus* (Gorgan Bay)**	0.33%	**0.16%**		
3	***R. rutilus***	4.56%	4.54%	**1.12%**	
4	***R. frisii***	7.03%	7.06%	6.86%	**0.13%**

Most of identified haplotypes from Anzali Lagoon and Gorgan Bay were located in a monophyletic sister group position with *R. rutilus* haplotypes (Figure 2).

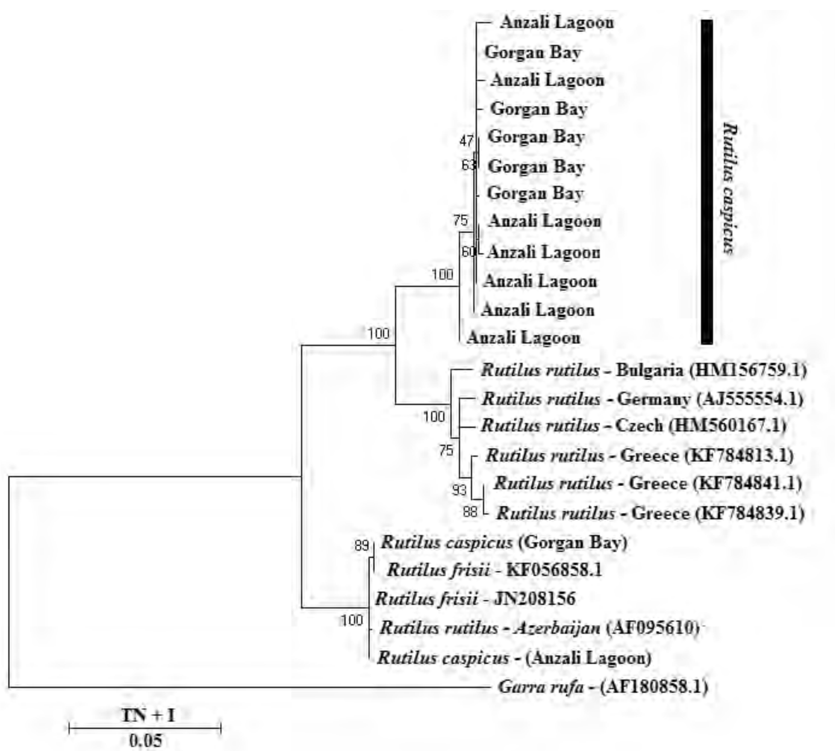

Figure 2. Maximum likelihood estimates of phylogenetic relationships of two population of Rutilus caspicus with some congeneric taxa (Rutilus frisii) using Cyt-b sequences (1140 bp). Nodes are labeled with bootstrap support.

DISCUSSION

Validity of specimens in the southern Caspian Sea as *R. caspicus* were well supported by Cyt-b sequence. Within analyzed haplotypes, one haplotype from Anzali lagoon, one from Gorgan Bay and one from gene bank (Samur River, Azerbaijan), were located in *R. frisii* group which indicated hybridization between these two species. This phenomena was previously reported by Ketmaier et al. (2008). There are also some reports of artificial hybrids with *Rutilus frisii*in in Iran (IFRTO, 1996). Introgressive hybridization is a common phenomenon in Cyprinidae (Freyhof *et al*., 2005).

CONCLUSION

These results indicate high genetic similarity of two populations and it seems that migration occurs between populations and morphological differences are more related to short term adaptation to environmental conditions. However this phenomenon may be attributed to artificial homogenization across watersheds, through breeding and restocking (Triantafyllidis et al., 2002; Salzburger et al., 2003).

The regions around the coasts of the Black Sea and the Caspian Sea are 'hot-spots' for the genetic diversity of *Rutilus* in Eurasia, and need a lot of attention. This species is cultured and released into the Caspian Sea for restocking. Regular monitoring of genetic variability among the progenies is essential to avoid the loss of current polymorphism due to inbreeding problems.

ACKNOWLEDGMENTS

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SOME BIOLOGICAL FEATURES OF EUROPEAN CATFISH (*SILURUS GLANIS)* IN THE UVAC RESERVOIR

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NEKE BIOLOŠKE ODLIKE SOMA (*SILURUS GLANIS*) IZ AKUMULACIJE „UVAC"

Apstrakt

Som je najveća primarno slatkovodna vrsta ihtiofaune Srbije i u staništima koja naseljava predstavlja vrhunskog predatora. Ova vrsta ne spada u nativnu ihtiofaunu u vodama Rezervata Uvac, a vreme introdukcije, količina i uzrasni sastav unetih riba su nepoznati. Generalno, podaci o biologiji prirodnih populacija soma u otvorenim vodama u Srbiji su siromašni i do sada ne postoje studije o populaciji u akumulaciji Uvac. Ciljevi ovog istraživanja su određivanje starosne i polne strukture populacije somova i njegove distribucije u akumulaciji. Definisane su tri zone akumulacije u kojima je vršeno uzorkovanje, i to: zona I (gornji kanjonski deo akumulacije), zona II (srednji deo akumulacije sa ulivima dve pritoke) i zona III (donji deo akumulacije u oblasti brane). Mrežarskim alatima ulovljeno je ukupno 20 somova. Među ulovljenim jedinkama 7 je bilo mužjaka, 9 ženki i kod 4 jedinke nije bilo moguće makroskopski odrediti pol. Dužina je varirala od 60-86 cm kod mužjaka; 66-113 cm kod ženki i 51-57.5 cm kod polno nezrelih jedinki. Težina ulovljenih primeraka kretala se u opsegu 993-4 000 g kod mužjaka; 1 520-9 000 g kod ženki; i 652-984 g kod polno nezrelih jedinki. Među mužjacima su dominirale jedinke starosti 3^+, dok su kod ženki sve starosne klase bile jednako zastupljene (2^+-7^+). Sve polno nezrele jedinke su bile starosti 2^+. Som u akumulaciji Uvac dominantno naseljava gornji (kanjonski) deo, a na osnovu starosne structure populacije pretpostavlja se da je som u akumulaciju introdukovan pre više od 10 godina.

Ključne reči: *som, distribucija, polna stuktura*
Keywords: *wells, distribution, sex composition*

INTRODUCTION

Due to intentional and unauthorized introductions, the fish fauna of the Uvac Reservoir has changed from the native state to current fish community representing combination of native cold-water and alien warm-water species. It is supposed that anglers conducted unauthorized introduction of European catfish (*Silurus glanis*), and origin, quantity and size composition of introduced fish are unknown. Since then, species has acclimatized and established self-sustaining population, becoming important fishery resource. The European catfish is the largest freshwater fish in Europe and in the waters that inhabits represents top predator species feeding on wide spectrum of food items (Ristić, 1977; Bora and Gül, 2004; Carol et al., 2009). Generally, information on biology of natural populations of European catfish in the Serbian waters is poor and, until now, the studies on population in the Uvac Reservoir do not exist. Therefore, having in view its strong predatory behavior, data on biological features of European catfish from the Uvac Reservoir are of vital importance to understand its ecological status and impacts on fish community, especially those concerning native species. The purpose of this paper is to present some results of first studies of an ongoing investigation on biology features of European catfish in the studied water body. The objectives are to record longitudinal distribution and age and sex structure of European catfish population in the Uvac Reservoir.

MATERIAL AND METHODS

The Uvac Reservoir occupies the highest position (985 m.a.s.l.) in the row of three river impoundments built on the middle course of the Uvac River. It is the youngest among three reservoirs (1979 year of formation) with following morphometric features: length up to 25 km, surface area 6.1 km^2, volume 212 x 10^6 m^3, maximal depth 108 m. The reservoir has a pronounced canyon-shaped morphometry with two small tributaries (Kladnica and Veljušnica). Fish sampling was preformed on four occasions during the middle summer-early autumn 2017 period (02-04. 08; 22-24. 08; 12-14. 09; 10-12. 10); using benthic and pelagic trammel nets (inner mesh sizes 45-80 mm). Three zones were recognized (Fig. 1): zone I (upper canyon zone), zone II (middle part of reservoir with two bays), and zone III (lower part of reservoir in the dam area). Samplings were performed on 26 sampling sites, of which 9, 12 and 5, were within zone I, zone II and zone III, respectively. Nets were set overnight and 20 specimens were caught; at field fish were measured (± 1 mm), weighed (individuals up to 3 kg ± 1 g; individuals > 3 kg ± 50 g) and dissected for sex determination and diet analyses. Pectoral spines were used for age determination. Pectoral spines were sectioned using a jeweler’s saw. Sections for each spine were ground with sandpaper to make the annuli more distinct for age reading, and read under dissecting microscope.

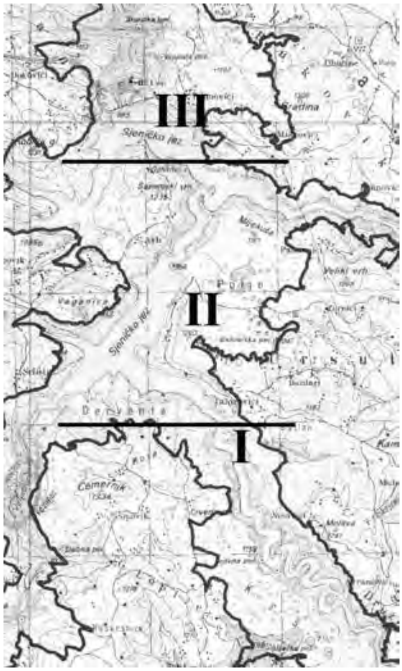

Figure 1. The Uvac Reservoir and its zonation: I- upper canyon zone; II- middle zone of reservoir; III- lower zone in the dam area.

RESULTS AND DISCUSSION

During the studied period 31 trammel nets totaling 5 961 m^2 were set, and 20 specimens of European catfish were caught (Tab. 1). Obviously, there are big differences, both in terms of number and biomass of captured fish, in catches between the investigated zones of reservoir. In relation to total catch, catch from zone I (upper canyon part of reservoir) comprised 80% and 82.1% in number and biomass respectively, while in the zone III (dam area) none individual of European catfish was caught. Compared to data for zone II (middle part of reservoir), the catch of European catfish in zone I outnumber and overweigh by 4 and almost 5 times, respectively.

Table 1. Catch data on European catfish for the investigated zones of Uvac Reservoir: FE – fishing effort; N – number of caught individuals; ΣW – total biomass of caught fish; CPUE – catch per unit effort.

Parameter	Zone of reservoir		
	I	II	III
FE (m^2)	2580	2121	1260
N	16	4	0
ΣW (g)	51 152	11 123	0
CPUE (ind./m^2)	0.006	0.002	0
CPUE (g/m^2)	19.8	5.2	0

Despite a relatively high fishing effort the amount of caught fish could be considered as low, reflecting difficulties in sampling such a large species by conventional fishing techniques in large water ecosystems (Carol et al., 2007; Benejam et al., 2007). Obtained results clearly demonstrate longitudinal distribution pattern of population of European catfish in the Uvac Reservoir. Homogenous or random pattern of distribution of fishes within their environments is seldom found, and their distribution is usually determined by numerous factors including physical, chemical and historical constrains of environmental conditions, biotic interaction and the intensity of human use of a water body (Benson and Magnuson, 1992; Mehner et al., 2003; Prchalova et al., 2008). The European catfish pre-dominantly inhabits upper part of the reservoir what is most probably related to suitable environmental and forage conditions in this section of water body. Further studies are needed, in order to detect factors that influence recorded distribution pattern and to assess habitat use of catfish.

Table 2 contains data on age and sex structure of sampled fish. The age of captured fish ranged from 2^+ to 7^+ years. Obtained results indicate that introduction of this species in the reservoir occurred at least 10 years ago.

Table 2. Number of males, females and sex ratio of the European catfish in the Uvac Reservoir: TL – total length; W – biomass.

Sex	*n*	*TL (cm)*	*W (g)*	**Age (years)**				
		min-max	min-max	2^+	3^+	4^+	6^+	7^+
Males	9	60-86	993-4000	2	6	1		
Females	7	66-113	1520-9000	1	2	2	1	1
Juveniles	4	51-57.5	652-984	4				
Sex ratio	1♂ : 0.78♀							

Out of the 20 specimens of *S. glanis* sampled from the reservoir during present study, 9, 7 and 4 were males, females and specimens of unidentified sex, respectively (Tab. 2). Total length and biomass of collected fish varied from 60-86 cm and 993-4 000 g; 66-113 cm and 1 520-9 000 g; 51-57.5 cm and 652-984 g, for males, females and unsexed fish, respectively. Age class 3^+ dominated in males, while all recorded ages were equally distributed among females. All unsexed fish were 2^+ years old. Alp et al. (2004) reported, based on information from various waters that European catfish reach maturity at ages of 2-3 and 3-4 years, for males and females, respectively. Therefore, in accordance with this 55% of sampled fish were sexually mature. Males dominated in relation to females, but the overall sex ratio did not differ from 1 : 1 ratio statistically ($\chi^2 = 0.87$, df = 1, $P > 0.05$). The sex ratio of population from Menzelet Reservoir also was not different from the 1:1 ratio, but in this case, it was slightly in favor of females (Alp et al., 2004). Recorded sex ratio was expected, due to fact that most natural populations of fish have a sex ratio of 1:1 (Reay, 1984). The present study provides first information on European catfish population in the Uvac reservoir and can be useful for proper fishery management of this water body.

CONCLUSION

The European catfish pre-dominantly inhabits upper part of the Uvac Reservoir. According to the age structure of the population, it was introduced in the reservoir at least 10 years ago. Expected sex ratio of 1:1 was found, the ratio that is characteristic for most natural population of fishes.

ACKNOWLEDGMENT

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INFLUENCE OF SOME ENVIRONMENTAL FACTORS ON THE ABUNDANCE AND DISTRIBUTION OF *VIBRIO* SP. IN THE BAY OF MALI STON, EASTERN ADRIATIC, CROATIA

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UTJECAJ NEKIH ČIMBENIKA OKOLIŠA NA ABUNDANCIJU I DISTRIBUCIJU *VIBRIO* SP. U MALOSTONSKOM ZALJEVU, ISTOČNI JADRAN, HRVATSKA

Apstrakt

Suvremena akvakultura predstavlja učinkoviti način intenzivne proizvodnje akvatičnih organizama u uvjetima koje je moguće kontrolirati. Ova rapidno rastuća industrija iskusila je i značajne probleme kontrole širenja bolesti u uzgojnim sustavima, čije su posljedice bile visoke stope mortaliteta akvatičnih organizama. Mikroorganizmi roda *Vibrio*, koji obuhvaća više od 63 vrste, ubrajaju se u najzastupljenije bakterije u morskom okolišu. Većina vrsta su oportunistički patogeni. Određene vrste ovog roda uzrokuju vibrioze, skupinu najzastupljenijih bakterijskih bolesti uzgajanih riba na području Jadrana, dok druge (*V. cholerae, V. parahaemolyticus, V. vulnificus, V. mimicus, V. alginolyticus, V. hollisae*) predstavljaju humane patogene, te su česti uzročnici gastroenteritisa uzrokovanih konzumacijom zdravstveno neispravnih proizvoda iz mora. *Vibrio* sp. također ima značajnu ulogu u razlaganju organske tvari, a sudjeluje i u prijenosu otopljenog organskog ugljika na višu trofičku razinu u morskoj sredini. Cilj ovog istraživanja bio je procijeniti mogući štetni učinak kaveznog uzgoja ribe na mikrobnu kvalitetu okolnog mora te korelaciju između kaveznog uzgoja i abundancije *Vibrio* sp. u okolišu. U tu svrhu su istražene korelacije između abundacije mikroorganizama ovog roda, partikulrane tvari (ukupne, organske i anorganske) i temperature. Uzorkovanje je obavljano kvartalno od rujna 2016 do studenog 2017 na dvije postaje: kaveznom uzgajalištu ribe u uvali Kabli, smještenoj u vanjskom dijelu Malostonskog zaljeva (Istočni Jadran) te na kontrolnoj postaji u Malostonskom kanalu, udaljenoj dva kilometra od uzgajališta. Temperatura je mjerena ručnom sondom Mettler *in situ*, dok su uzorci mora za mikrobiološku i analizu partikularne tvari prikupljani Nisskin-ovim crpcem (vol. 8 L) na četiri dubine (0.5m, 5m, 10m i 0.5m iznad morskog dna). Odgovarajuća serijska razrjeđenja nanesena su na selektivni medij Difco ™ TCBS (BD) agar za brojanje*Vibrio* sp. kolonija i inkubirana na 35° C tijekom 24 sata te na 22° C kroz 3-5 dana.Bakterijski izolati su potom dalje potvrđeni pomoću MALDI TOF MS. Za statističku obradu podataka korištene su klaster analiza i metoda logaritamske linearne regresije. Rezultati su pokazali sezonske varijacije abundancije *Vibrio* sp. Broj kolonija inkubiranih na 22°C bio je uvijek veći za dubine do 10 m. Na obje postaje uzorkovanja, abundancija utvrđena za izolate s 22°C bila je u pozitivnoj korelaciji s dubinom i koncentracijom ukupne partikularne tvari, dok je negativno korelirala s portikularnom organskom tvari i temperaturom. Najveći broj kolonija *Vibrio* sp. utvrđen je u uzorcima izoliranim na 22°C koji su potjecali s kontrolne postajei 5m dubine u rujnu 2017 (1270 cfu/ml). Na obje postaje je broj kolonija *Vibrio* sp. inkubiranih na 35°C bio vrlo nizak i kretao se u rasponu 0-30 cfu/ml, osim u uzorcima prikupljenim 0.5m iznad dna na ribljoj farmi u studenom 2017 (140 cfu/ml).

Ključne riječi: *partikularna tvar, dubina, temperatura, akvakultura, kavezni uzgoj ribe*

Abstract

Modern aquaculture provides effective means for intensive seafood production under "controllable" conditions. This rapidly growing industry has experienced significant problems controlling the spread of disease in rearing systems, which as a result, has contributed to high mortality rates of aquatic organisms. Genus *Vibrio* is comprised of more than 63 species, are among the most abundant bacteria in the marine environment. Most of the strains are opportunistic pathogens. While some species cause Vibriosis, which is one of the most prevalent bacterial fish diseases in the Adriatic region, many *Vibrio* sp. (*V. cholerae, V. parahaemolyticus, V. vulnificus, V. mimicus, V. alginolyticus, V. hollisae*) are recognized as human pathogens and have been implicated in water and seafood-related outbreaks of gastrointestinal infections in humans. This genus also plays an important role in the degradation of organic matter, and acts as a link that transfers dissolved organic carbon to higher trophic levels of the marine food web. The purpose of this study was to investigate how fish farming could affect the microbial quality of the marine environment around aquaculture farms, and to assess possible correlation between marine cage fish farm operation and the abundance of *Vibrio* sp. For this purpose, correlations between the abundance of *Vibrio* sp., particulate matter (total, organic and inorganic), and temperature were examined. The sampling was carried out on the fish cage farm located in the Kabli, outer part of the Mali Ston Bay (Eastern Adriatic) and at the control site situated 2 km away from the farm in Mali Ston Channel. Sampling was performed quarterly from September 2016 through November 2017. Temperature was measured using the Mettler probe in situ, while water samples for microbiology and particulate matter analyses were collected using 8L Nisskin bottle at four depths (0.5m, 5m and 10m, and 0.5m above the sea bottom). Serial dilutions of the water were applied onto selective medium Difco™ TCBS (BD) agar for *Vibrio* enumeration and incubated at 35°C for 24h and at 22°C for 3-5 days. Bacterial isolates were further processed on MALDI TOF MS for identification. Statistical analyses included cluster analysis and log-linear regression. The results showed seasonal variations of *Vibrio* sp. abundance. Isolates at 22°C were always more abundant above depth of 10 m. At both sampling sites the abundance of isolates at 22°C was in positive correlation with depth and total particulate matter (TPM), while in negative with particular organic matter (POM) and temperature. The highest count of isolates on 22°C was recorded at the control site in September 2017 (1270 cfu/ml) at the depth of 5m. At both sampling sites, the counts of *Vibrio* sp. incubated at 35°C were negligibly low, in the range of 0-30 cfu/ml, except in the samples collected above the sea bottom of the fish farm in November 2017 (140 cfu/ml).

Keywords: *particulate matter, depth, temperature, aquaculture, fish cage farming*

THE BLOOD PHYSIOLOGICAL RESPONSE OF *ACIPENSER RUTHENUS* (L., 1758) JUVENILES IN BACTERIAL CHALLENGE AFTER PHYTOBIOTICS SUPPLEMENTED DIET

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FIZIOLOŠKI ODGOVOR KRVI MLAĐI *ACIPENSER RUTHENUS* (L., 1758) NA BAKTERISKU INFEKCIJU POSLE ISHRANE OBOGAĆENE FITOBIOTICIMA

Apstrakt

Upotreba biljnih ekstrakata u oblasti akvakulture je ograničena, uglavnom zbog specifičnog dejstva aktivnih supstanci. Smatra se da je dodavanjem ovih prirodnih proizvoda u hranu varenje poboljšano i, razumljivo, prirast optimizovan za dobijanje boljih vrednosti biotehnoloških parametara (specifična stopa rasta, faktor konverzije hrane, preživljavanje itd.). Pored toga, zbog njihovih antimikrobnih i antiparazitskih potencijala, neki fitobiotici su ispitivani u cilju kontrole bolesti životinja, ali način delovanja svakog od njih na domaćina je specifičan. Iz ove perspektive, prikazano istraživanje imalo je za cilj da se ispita efekat ulja pasjeg trna i majčine dušice kao imunostimulanata na organizam mlade kečige (*Acipenser ruthenus* L., 1758) primenom veštačke infekcije bakterijama posle eksperimentalnog davanja hrane sa ova dva fitobiotika.

Posle 60 dana izlaganja kečiga hrani sa imunomodulatornim dejstvom pasjeg trna i majčine dušice, izvedeno je izlaganje bakteriji *Pseudomonas fluorescens* in vivo u cilju otkrivanja otpornosti na bolest putem poboljšanja prirodne otpornosti riba tretiranih fitobiotikom. Iz svake hranidbene grupe (1-ribe hranjene 1% mešavinom pasjeg trna i majčine dušice/kg hrane, 2-ribe hranjene 1% majčine dušice/kg hrane, 3 – ribe hranjene 1% pasjeg trna/kg hrane i 4-kontrola, ribe hranjene netretiranom hranom), 7 riba su nasumično izlovljene i intramuskularno je svakoj ubrizgano po 0,2 ml Pseudomonas fluorescens 0,5 McFarland ćelijske suspenzije.

Infektivni proces se razvio subklinički sa periodom inkubacije neočekivano dužim nego što je bio slučaj kod drugih vrsta riba. U našem slučaju, kečige su postepeno ispoljavale karakteristične spoljašnje i unutrašnje znake sistemske bakterijske infekcije (površinske kožne nekrotične lezije, kongestija krvnih sudova na bazi peraja, petehijalnih krvarenja na telu i ventralnih štitova, promenjena žućkasta boja jetre, povećana zapremina bubrega i nakupljanje hemoragične tečnosti u abdomenu) tokom 21 dana. Posle prvog uginuća zabeleženog u kontrolnoj grupi, uzeti su uzorci krvi i analizirani rutinskim hematološkim metodama.

Podaci iz našeg eksperimenta pokazali su dinamiku fizioloških odgovora u krvi u ova 4 dijetetska tretmana, naglašenih izrazitim opadanjem, postinfektivno, vrednosti hematoloških pokazatelja (RBC, Ht, Hb), što ukazuje na to da je infekcija karakterisana anemijom do koje je došlo posle ekstravazacije krvi i uništavanja hematopoetskih organa, naročito bubrega. Izračunane vrednosti parametara eritrocita (MCV, MCH, MCHC) ukazuju na patofiziološki mehanizam hipohromne anemije koji je nastao kao rezultat *Pseudomonas fluorescens* infekcije. U pogledu imunoloških odgovora, naši preliminarni podaci svode se na argument da fitobiotici korišćeni u eksperimentu stimulišu humoralne komponente urođenog imunološkog sistema koji obezbeđuje zaštitu organizma od infekcije. Ovaj odgovor je održan uz nešto niže utvrđene vrednosti lizozima i ukupnih proteina kod riba u kontrolnoj grupi i relativno jednakim koncentracijama ukupnih imunoglobulina za sve četiri grupe, generalno ukazujući na pouzdan nespecifični imunološki sistem u slučaju grupa koje su tretirane fitobiotikom.

Ključne reči: *kečiga, otpornost na bolesti, hematološki parametric, imunološka reakcija*

Abstract

The use of plant extracts in the field of aquatic animals farming is limited, mostly due to the specific action of the active compounds. It is considered that by adding these natural products in feed, the digestion process is improved and, implicitly, the growth performance is optimized obtaining better values for biotechnological parameters (specific growth rate, feed conversion factor, survival etc.). In addition, due to their antimicrobial and antiparasitic potentials, some phytobiotics are studied for animals' diseases control but the action mode of each one on host is specific. From this perspective, the present study aimed to evaluate the effect of sea buckthorn and thyme oil as immunostimulants on the young sterlet's (Acipenser ruthenus L., 1758) organism by applying a bacterial challenge following an experimental feeding diet with those two phytobioyics.

After 60 days of the sterlets exposure to diet with immunomodulatory action of sea buckthorn and thyme, in vivo trial against Pseudomonas fluorescens was performed in order to reveal the disease resistance by the enhancement of the natural defense system in the fish treated with phytobiotics. From each feeding group (1-fish fed with 1% mix Sea buckthorn and thyme/kg feed, 2-fish fed with 1% thyme/kg feed, 3-fish fed with 1% Sea buckthorn/kg feed and 4-Control, fish fed with untreated feed), 7 fish were randomly extracted and injected intramuscularly with 0.2 ml/ fish of Pseudomonas fluorescens 0.5 McFarland cell suspension.

The infectious process evolved subclinical with an incubation period unexpectedly longer than it was encountered in other fish species. In our case, the sterlets have gradually revealed characteristic external and internal signs of systemic bacterial infection (superficial cutaneous necrotic lesions, congestion of the blood vessels at the base of the fin insertion, petechial haemorrhages on the body and ventral shields, modified yellowish liver color, enlarged volume kidney and hemorrhagic fluid accumulation in the abdomen) during 21 days. After the first death was recorded in the control group, blood samples were taken and analyzed by routine hematological methods.

Our experimental data showed the dynamics of the physiological responses in the blood in those 4 dietary treatments, highlighted by a pronounced decreasing, post-infection, of the values of the haematological indicators (RBC, Ht, Hb), suggesting that the infection was characterized by anemia resulted after blood extravasation and destruction of the hematopoietic organs, especially the kidney. The calculated values for the erythrocyte parameters (MCV, MCH, MCHC) indicate the pathophysiological mechanism of a hypochromic anemia installed as a result of Pseudomonas fluorescens infection. In terms of immunological responses, our preliminary data converge to the argument that the phytobiotics used in the experiment stimulate the humoral components of the innate immune system that ensure the protection of the fish organism against infection. This response was sustained by the slightly lower values recorded for lysozyme and total protein level in the control fish and by relatively equal concentrations of total immunoglobulins for all four groups, overall indicating a fortified non-specific immune system in the case of phytobiotic treated groups.

Keywords: *sterlet, disease resistance, hematological parameters, immunological reaction*

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BIODIVERSITY AND CONSERVATION STATUS OF THE FISH AND QUALITATIVE COMPOSITION OF THE MACROPHYTIC VEGETATION IN THE LAKE DOJRAN, REPUBLIC OFMACEDONIA

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BIODIVERZITET I KONZERVACIONI STATUS RIBA I KVALITATIVNI SASTAV MAKROFITSKE VEGETACIJE DOJRANSKOG JEZERA, REPUBLIKAMAKEDONIJA

Apstrakt

Vode Republike Makedonije pripadaju u trima slivovoma: egejskom, jadranskom i crnomorskom**.** Riblja fauna u R. Makedoniji zastupljena je sa oko 80 vrsta. U velikim prirodnim jezerima (Prespansko, Ohridsko i Dojransko jezero) opisana je 61 vrsta riba. U Prespanskom jezeru opisano je 11 nativnih i 12 introdukovanih ribljih vrsta, u Ohridskom su opisane 22 nativne i 7 introdukovanih vrsta riba, a u jezeru Dojran opisane su 15 nativnih i 7 introdukovanih vrsta riba.

U litoralnom regionu Dojranskog jezera distribuirane su različite populacije makrofitske vegetacije (emerzne, flotantne i submerzne makrofite).

Ključne riječi: *Egejsko slivno područje, ekoregion, riblja fauna, nativne vrste, uvedene vrste*
Keywords: *Aegean watershed, ecoregions, fish fauna, native species, introduced species*

INTRODUCTION

The waters from Republic of Macedonia belong to 3 watersheds: Aegean, Adriatic and Black Sea. At the territory of R.Macedonia are present total about 80 fish species from 15 families (Talevski, Talevska, 2010 c). Two species belong to family Petromyzonidae , Acipenseridae - 1 species, Anguillidae - 1 species, Clupeidae - 1 species, Cyprinidae - 46 species, Cobitidae - 5 species, Nemacheilidae - 2 species, Ictaluridae - 1 species, Siluridae - 1 species, Esocidae - 1 species, Salmonidae - 12 species, Poecilidae - 1 species, Cottidae - 1 species, Centrarhidae - 1 species, Percidae - 3 species and in Blennidae belongs 1 species (Talevski, Talevska, 2010 c).

According to the map of freshwater ecoregions of the world 426 ecoregions are delineated (Abell, et al. 2008). Fresh waters of the the R.Macedonia belong to the two ecoregions which are marked on the world map as ecoregion 420 (Southeast Adriatic watershed, which includes Lake Prespa and Lake Ohrid) and ecoregion 422 (Vardar watershed, which includes Lake Dojran).

The conservation status of the fish from R.Macedonia until now was researched on the watershed: Lake Prespa (Talevski et.al., 2010 b) and Lake Ohrid (Talevski et.al., 2010 a). Also were investigated and dangers of commercial fishing in the great natural lakes on the native species and the conservation status of native species in natural lakes of Drim system (Milošević, Talevski, 2015). The IUCN Red List is set upon precise criteria to evaluate the extinction risk of thousands of species and subspecies. These criteria are relevant to all fish species and all regions in the world. The aim is to convey the urgency of conservation issues to the public and policy makers, as well as to help the international community to try to reduce the species extinction. IUCN Red List is widely considered the most objective and authoritative system for classifying the species in terms of the risk of extinction.

The fish species are classified in nine groups, set through criteria such as rate of decline, population size, area of geographic distribution, and degree of population and distribution fragmentation.

In IUCN Red List are included the following categories: EX - Extinct, EW - Extinct in the Wild, CR - Critically Endangered, EN - Endangered, VU - Vulnerable, LR/cd - Lower Risk/conservation dependent, NT - Near Threatened (includes LR/nt - Lower Risk/near threatened), LC - Least Concern (includes LR/ LC - Lower Risk, least concern), DD - Data Deficient - Has not yet been evaluated against the criteria and NE - Not Evaluated - Has not yet been evaluated against the criteria.

MATERIAL AND METHODS

The materials from the Lake Dojran were collected by day and nighttime's experimental fishing with various fishing gill net. Conservation status of native fish fauna from Lake Dojran was determinated according to IUCN (2014), Kotellat & Freyhof (2007) and FishBase (Frose, Pauly 2014).

The saprobiological affiliation of present macrophyte species in Lake Dojran was determined using the list of indicators according to Hofrat and Ottendorfer (1983)

RESULTS AND DISCUSSION

The results of our research are presented in the lake Dojran, R. Macedonia Table 1.

Conservation status of autochthonous fish fauna in Lake Dojran is following: CR - Critically Endangered belong 2 species, *Alburnus macedonicus* and *Anguilla anguilla* and VU - Vulnerable, belongs 1 species - *Cyprinis carpio*. In group LC - Least Concern (includes LR/ LC - Lower Risk, least concern) belong 10 species: *Barbus balcanicus*, *Gobio bulgaricus*, *Rutilus rutilus*, *Scardinius erythrophthalmus*, *Squalius vardarensis*, *Tinca tinca*, *Silurus glanis*, *Cobitis vardarensis*, *Salaria fluviatilis* and *Perca fluviatilis. Pachychilon macedonicum* is DD - Data Deficient. In Lake Dojran seven species are introduced: *Carassius gibelio*, *Hypophthalmichthys molitrix*, *Hypophthalmichthys nobilis*, *Pseudorasbora parva*, *Rhodeus amarus*, *Gambusia holbrooki* and *Pygocentrus nattereri.*

The macrophyte vegetation is found thought the littoral zone of Lake Dojran and has significant role in the cycling of materials in it. Also, it represents habitat, shelter and food for a large number of fishes, and also their spawning ground.

In the littoral region of Lake Dojran are distributed different populations of macrophyte vegetation (emerged, floating and submerged macrophytes) that always present vertical zonal reparation. The qualitative composition of macrophyte vegetation is one of the indicators for the degree of water pollution in aquatic ecosystems (Hofrat, Ottendorfer, 1983) Macrophyte vegetation in Lake Dojran is presented by 27 different species (Table. 2.)

Table 1. List of native and introduced fish of Lake Dojran and its catchement area

	Species	Lake Dojran		Conservation Status/ Introduced
		Native species	Allien species	
	CYPRINIDAE			
1.	*Alburnus macedonicus* Karaman, 1928	+		CR
2.	*Barbus balcanicus* Kotlik Kotlik, Ráb i Berrebi, 2002	+		LC
3.	*Carassius gibelio* Bloch, 1782		+	In
4.	*Cyprinis carpio* Linnaeus, 1758	+		VU
5.	*Gobio bulgaricus,* Drenski 1926;	+		LC
6.	*Hypophthalmichthys molitrix* (Valenciennes, 1844)		+	In
7.	*Hypophthalmichthys nobilis* (J. Richardson, 1845)		+	In
8.	*Pachychilon macedonicum* Steindachner 1892	+		DD
9.	*Pseudorasbora parva* (Temminck & Schlegel, 1846)		+	In
10.	*Rhodeus amarus* (Bloch, 1782)		+	In
11.	*Rutilus rutilus* Linnaeus, 1758	+		LC
12.	*Scardinius erythrophthalmus (*Linnaeus, 1758)	+		LC
13.	Squalius vardarensis Karaman, 1928	+		LC
14.	*Tinca tinca* (Linnaeus, 1758)		+	LC
	ANGUILLIDAE			
15.	*Anguilla anguilla* (Linnaeus, 1758)	+		CR
	SILURIDAE			
16.	*Silurus glanis* Linnaeus, 1758	+		LC
	COBITIDAE			
17.	*Cobitis vardarensis* Karaman, 1928	+		LC
18.	*Sabanejewia doiranica* Economidis & Nalbant, 1996	+		
	POERCILLIDAE			
19.	*Gambusia holbrooki* Girard, 1859		+	In
	BLENIDAE			
20.	*Salaria fluviatilis* (Asso, 1801)	+		LC
	PERCIDAE			
21.	*Perca fluviatilis* Linnaeus, 1758	+		LC
	SERRASALMIDAE			
22.	*Pygocentrus nattereri* Kner, 1858		+	In
	TOTAL	14	8	

The species of *Chara* genus. are present in regions where water is I category. The species *Typha latifolia*, *Schoenoplectus lacustris*, *Potamogeton lucens*, and *Potamogeton gramineus* are present in regions where water is I-II category. The species *Sparganium ramosum, Persicaria amphibia, Hydrocharis morsus ranae*, *Lemna minor*, *Potamogeton perfoliatus*, *Myriophyllum spicatum*, *Myriophyllum verticillatum*, *Ceratophylllum demersum*, *Najas marina*, *Spirodela polyrrhiza* and *Lemna trisulca* are present in regions where water is II category. The species *Potamogeton crispus*, *Stuckenia pectinata* and *Zannichellia palustris* are present in regions where water is II-III category.

For the species *Phragmites australis*, *Typha angustifolia*, *Eleocharis palustris*, *Butomus umbellatus*, *Alisma plantago aquatica*, *Ceratophylllum submersum*, *Vallisneria spiralis* and *Najas minor* found in Lake Dojran, there are not data on their pollution indication according to Hofrat – Ottendorfer (1983).

Table 2. Qualitative composition of the macrophyte vegetation

N	Species	Saprobic index Hofrat – Ottendorfer
1.	*Phragmites australis* (Cav.) Trin ex Steud.	
2.	*Typha latifolia* L.	I, II
3.	*Typha angustifolia* L.	
4.	*Sparganium ramosum* Huds.	II
5.	*Schoenoplectus lacustris* (L.) Palla	I, II
6.	*Eleocharis palustris* (L.) Roem. & Schult	
7.	*Butomus umbellatus* L.	
8.	*Alisma plantago aquatica* L.	
9.	*Persicaria amphibia* (L.) Gray	II
10.	*Hydrocharis morsus ranae* L.	II
11.	*Lemna minor* L.	II
12	*Potamogeton perfoliatus* L.	II
13	*Potamogeton lucens* L.	I, II
14.	*Potamogeton crispus* L.	II, III
15.	*Potamogeton gramineus* L.	I, II
16.	*Stuckenia pectinata* (L.) Boerner	II, III
17.	*Zannichellia palustris* L.	II, III
18.	*Myriophyllum spicatum* L.	II
19.	*Myriophyllum verticillatum* L.	II
20.	*Ceratophylllum demersum* L.	II
21.	*Ceratophylllum submersum* L.	
22.	*Vallisneria spiralis* L.	
23.	*Najas marina* L.	II
24.	*Najas minor* All.	
25.	*Spirodela polyrrhiza* Schleid.	II
26.	*Lemna trisulca* L.	II
27.	*Chara* sp.	I

In the Lake Dojran were dominated macrophyte species bioindicators for the second category of water quality: *Sparganium ramosum, Persicaria amphibia, Hydrocharis morsus ranae*, *Lemna minor*, *Potamogeton perfoliatus*, *Myriophyllum spicatum*, *Myriophyllum verticillatum*, *Ceratophylllum demersum*, *Najas marina*, *Spirodela polyrrhiza* and *Lemna trisulca*.

The data indicate that water in the Lake Dojran is polluted, which means that anthropogenic pressure is very pronounced in them (the intake of various organic and inorganic materials is great), and there is present process of eutrophication.

CONCLUSION

1. The ichthyofauna in Lake Dojran is following: CR - Critically Endangered belongs 2 species, Vulnerable, 1 species LC - Least Concern 10 species and 1 species is DD - Data Deficient.

2. In Lake Dojran seven fish species are introduced.

3. From the macrophyte vegetation one genus is indicator of water for I category, 4 species are indicators for I-II category of water, 11 species are indicators for II category of water and 3 species are indicators for II-III category of water. For 8 macrophyte species that are found in Lake Dojran, there are not data on their indication of pollution according to Hofrat – Ottendorfer **.**

4. The data indicate that water in the Lake Dojran is polluted, which means that anthropogenic pressure is very pronounced.

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MACROPHYTE VEGETATION - HABITAT AND SPAWNING GROUND OF SOME CYPRINID FISH FROM LAKE OHRID

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MAKROFITSKA VEGETACIJA - STANIŠTE I MRESTILIŠTE NEKIH CIPRINIDNIH RIBA OHRIDSKOG JEZERA

Apstrakt

Osnovni cilj ovog rada bio je istraživanje makrofitske vegetacije i ciprinidnih riba Ohridskog jezera. Istraživanje je sprovedeno na 10 lokaliteta sa istočne obale Ohridskog jezera: Ajvan Plaža, Park, Ineks, Sveti Stefan, Granit, Metropol, Lagadin, Elesec, Kruša i Peštani. Dobijeni rezultati pokazuju da je na istraženim lokalitetima duž istočne obale Ohridskog jezera evidentirano ukupno 21 različita vrsta makrofita. Najzastupljenije makrofite su *Potamogeton perfoliatus* L., *Potamogeton crispus* L., *Potamogeton lucens* L., *Stuckenia pectinata* (L.) Börner, *Myriophyllum spicatum* L., *Zannichellia palustris* L., *Phragmites australis* i velike alge *Chara tomentosa* L. i *Cladophora* sp. Na istraživanim lokalitetima žive i mreste se *Alburnus scoranza*, *Barbus rebeli*, *Chondrostoma ohridanus*, *Cyprinis carpio*, *Gobio ohridanus*, *Pachychilon pictum*, *Rutilus ohridanus* i *Squalius squalus*. Takođe, konstatovano je da je početak mresta ciprinidnih riba istovremeno sa razvojem makrofitske vegetacije.

Ključne reči: *makrofitska vegetacija, istočna obala, ciprinidne ribe, mrest, Ohridsko jezero*
Keywords: *macrophyte vegetation, Eastern coastline, cyprinid fish, spawning ground, Lake Ohrid*

INTRODUCTION

Lake Ohrid is largest and deepest lake in Europe. It is a transboundary water body divided between R. Macedonia and R. Albania**.** At a sea level of 693.17 meters Lake Ohrid has a surface area of 358.18 km^2, volume of 58,64 km^3, maximal length of 30.5 km, maximal width of 15 km, and a shore line of 87.53 km. Lake has maximal depth of 288.7 meters, average depth of 163.71 meters and a great transparency of water - 21 meters. The Lake feeds primarily by underground springs and by rivers which inflow in Lake. From the Lake flows out river Crn Drim.

The macrophyte vegetation which is found throughout the littoral zone of Lake Ohrid is presented by vascular plants, a small number of aquatic mosses and some algae. In Lake Ohrid macrophyte vegetation is distributed *in* more or less continuous belts around the entire lake: belt of alga *Cladophora*, belt of reed - *Phragmites*, belt of pondweed - *Potamogeton* and belt of stonewort - *Chara* (Talevska 2002, 2003; Talevska, Trajanovska 2002, 2004; Talevska et al. 2009).

Macrophyte vegetation has great importance in the whole process of circulation of matter and energy in the lake ecosystem. Also represents food, habitat, shelter and place for spawning for many cyprinid fishes, and has a large role in protecting from theirs predators.

Cyprinid fish - carps from Lake Ohrid are represented by twelve species. The different cyprinid fishes are spawning in particular month in the spring-summer period in different regions of Lake Ohrid and on different substrates (Talevski 2001, 2003; Talevski et al. 2009).

In relation to the grounds where they lay their eggs, Ohrid cyprinids are mainly phytophyllic species, because they lay eggs upon plants, and their spawning period is in spring-summer (Talevska, Talevski 2003; Talevski, Talevska 2002, 2003, 2008).

MATERIALS AND METHODS

The researches of the macrophyte vegetation and the cyprinid fauna were performed in the course of 2015 and 2016, on 10 localities from Eastern coastline of Lake Ohrid: Ajvan plaza, Park, Inex, St.Stephan, Granit, Metropol, Lagadin, Eleshec, Krusha, and Peshtani.

The sampling of macrophyte species was performed during the spring and summer period by application of the standard lymnological method (Wetzel, Likens 1979).

Sampling of cyprinid fish was performed by daytime and the nighttime experimental fishing using nets with different mesh size (from 12 mm to 50 mm).

The collected materials (macrophytes and cyprinids) were determined in laboratories according to respective floras and keys for the determination of species.

RESULTS AND DISCUSSION

Macrophyte vegetation is distributed in littoral region of Lake Ohrid. Its development begining in spring period, and its maximal growth is in summer period.

In researched localities were evidenced total of 21 different macrophyte species, mainly emerged and submerged (Tab. 1.). Most present are submerged macrophytes *Potamogeton perfoliatus* L., *Potamogeton crispus* L., *Potamogeton lucens* L., *Stuckenia pectinata* (L.) Börner, *Myriophyllum spicatum* L., *Zannichellia palustris* L., and *Chara tomentosa* L. From emerged macrophytes was present *Phragmites australis.* In all researched localities was recorded and green alga *Cladophora* sp., which is widespread in the shallow lake water.

In researched localities there are differences in number of macrophytes species which varied from 5 (locality Inex) to 15 macrophyte species (locality Park).

Differences in qualitative composition of macrophytes are primarily due to different environmental conditions present in the researched localities (slope of the lake bottom, type of substrate, water movements, quantity of nutrients and other).Namely, in localities where are optimal ecological conditions, there are greater number of different macrophyte species which growing in dense populations (Park-15, St.Stephan-12, Peshtani-12, Metropol-10, and Ajvan plaza-9 species). In these areas the slope of the bottom is gradual, and the substrate is fine sand or mud.

In the rest of researched localities conditions for macrophytes growth and development are unfavourable and there is much lower number of macrophyte species which at the same time grow in rare populations (Inex-5, Eleshec-6, Lagadin-7, Krusha-7, and Granit-8 species).

In these regions of the littoral zone the conditions for growth and development of macrophytes are less favorable compared to other investigated localities: the slope of the lake bottom is steeper, and the substrate is stones and large sand.

Evidenced macrophyte species represent a food, habitat, shelter, and especially place for spawning for cyprinid fish.In researched localities were evidenced 8 species of cyprinid fish: *Alburnus scoranza* - Bleak, *Barbus rebeli* - Western Balkan barbell, *Chondrostoma ohridanus* - Ohrid undermouth, *Cyprinis carpio* - Carp, *Gobio ohridanus* - Ohrid gudgeon, *Pachychilon pictum* - Moranec, *Rutilus ohridanus* - Ohrid roach, and *Squalius squalus* - Chub (Tab. 2.).

The qualitative composition of cyprinid fish in researched localities from Lake Ohrid is different and primarily depends on used fishing networks, and qualitative and quantitative composition of present macrophytes.

Mainly, in the most researched localities macrophyte vegetation begins almost from shore, and is present up to 7 meters depth, up to 12.5 meters (locality St. Stephan), and even 14 meters depth (locality Eleshec).

Table 1. Evidenced macrophyte species in the researched localities from Lake Ohrid

Species		Locality									
		1	2	3	4	5	6	7	8	9	10
1.	*Phragmites australis* (Cav.) Trin. ex Steud.		+		+	+			+		
2.	*Potamogeton perfoliatus* L.	+	+	+	+	+	+	+	+	+	+
3.	*Potamogeton crispus* L.		+								+
4.	*Potamogeton lucens* L.	+	+		+	+			+		+
5.	*Stuckenia pectinata* (L.) Böerner.	+	+				+				+
6.	*Potamogeton pusillus* L.		+								+
7.	*Potamogeton nitens* Weber		+				+				
8.	*Zannichellia palustris* L.		+		+		+	+		+	+
9.	*Myriophyllum spicatum* L.	+	+		+		+			+	+
10.	*Elodea canadensis* Michx.	+	+		+						+
11.	*Vallisneria spiralis* L.										+
12.	*Najas marina* L.		+	+	+						
13.	*Chara tomentosa* L.	+	+	+	+	+	+	+	+	+	+
14.	*Chara aspera* (Deth.) Willd.	+	+		+	+	+	+			
15.	*Chara denudata* A.Braun							+			
16.	*Chara globularis* Thuiller				+	+		+			
17.	*Chara imperfecta* A.Braun		+				+			+	
18.	*Chara ohridana* Kostic	+		+			+		+	+	
19.	*Chara contraria* A.Braun ex Kutz.										+
20.	*Nitella opaca* (Bruzelius) C.Agardh				+	+					
21.	*Cladophora* sp. Kutz., 1843	+	+	+	+	+	+	+	+	+	+
	Total number of macrophyte species	9	15	5	12	8	10	7	6	7	12

Locality 1. Ajvan Plaza, 2. Park, 3. Inex, 4. St. Stephan, 5. Granit, 6. Metropol, 7. Lagadin, 8. Eleshec, 9. Krusha, 10. Peshtani

Favourable conditions where cyprinid fishes live and performed spawning mainly are evidenced at 2-6 meters depth. Cyprinid fishes are spawned at smaller depths (1-1.5 meters depth - locality Krusha, and 1.5-2.5 meters depth - locality Granit, while they are spawned at greater depths in localities Park (at 4-6.5 meters) and Inex (at 4-7 meters depth).

The mats of green algae *Cladophora* sp., are places where spawn many cyprinid fish species (mostly *Rutilus ohridanus*, *Pachychilon pictum*) and their cubs are staying there in the first weeks of his life. Also the populations of other submerged plants (*Potamogeton perfoliatus*, *Potamogeton crispus*, *Potamogeton lucens*, *Potamogeton pussilus, Potamogeton nitens*, *Stuckenia pectinata*, *Myriophyllum spicatum*, *Zannichellia palustris*, *Elodea Canadensis, Najas marina, Chara tomentosa, Chara aspera* and others) and *Phragmites australis* are places where live and performe spawning *Alburnus scoranza, Barbus rebeli, Chondrostoma ohridanus, Cyprinis carpio, Gobio ohridanus, Pachychilon pictum, Rutilus ohridanus,* and *Squalius squalus.* It has been notice that *Cyprinis carpio* mainly live and performed spawning in the reed belt, *Cladophora* sp. and *Chara* meadows. The populations of macrophytes in researched localities have large role in the protection of the cyprinid fishes from their predators.

Table 2. Cyprinid species present in researched localities from Lake Ohrid

Species		Locality									
		1	2	3	4	5	6	7	8	9	10
1.	*Alburnus scoranza* (Heckel et Kner)		+	+	+	+	+	+	+	+	+
2.	*Barbus rebeli* Koller					+				+	
3.	*Chondrostoma ohridanus* Karaman					+		+	+	+	+
4.	*Cyprinis carpio* Linnaeus	+	+	+	+	+	+		+	+	+
5.	*Gobio ohridanus* Karaman				+	+			+		
6.	*Pachychilon pictum* (Heckel et Kner)	+	+		+	+	+	+	+	+	+
7.	*Rutilus ohridanus* (Karaman)	+		+	+	+	+	+		+	+
8.	*Squalius squalus* (Bonaparte)							+	+	+	
	Total number of fish species	3	3	3	5	7	4	5	6	7	5

Locality 1. Ajvan Plaza, 2. Park, 3. Inex, 4. St. Stephan, 5. Granit, 6. Metropol, 7. Lagadin, 8. Eleshec, 9. Krusha, 10. Peshtani

Received results indicated that spawning grounds of cyprinid fish principally depend from qualitative composition of the macrophyte vegetation, and also from the slope of littoral, the type of substrate and other ecological conditions present in researched localities. Also, the results show that the beginning of the cyprinid fish spawning is at the same time with the development of macrophytes in the littoral region of the Lake.

It should be mentioned that in recent years anthropogenic influence in Lake Ohrid is increasingly expressed that causes changes in the quality of water, the composition of substrate, as well as in the qualitative and quantitative composition of macrophyte vegetation. Namely, in some localities the reed belt is destroyed, while in others submerged macrophytes are developing a lot. These unwanted changes of macrophyte vegetation in some localities from Lake Ohrid cause changes in the cyprinid fish qualitative composition, as well as on their spawning grounds. So, some cyprinids fish leave the natural habitats and natural spawning places.

CONCLUSION

Macrophyte vegetation primarily serves as a spawning place for cyprinid fishes, as well as food and shelter.

In the researched localities were evidenced 21 different macrophyte species and 8 species of cyprinid fish.

The period of beginning of cyprinid fishes spawning is at the same time with growth and development of macrophyte vegetation.

Any change in the qualitative and quantitative composition of macrophyte vegetation causes changes in spawning grounds of cyprinid fish.

Research indicate that localities Ajvan plaza, Park, Inex, St.Stephan, Granit, Metropol, Lagadin, Eleshec, Krusha, and Peshtani are important for spawning of some cyprinid fish species from Lake Ohrid.

Therefore, in the future it is necessary to take measures to protect macrophyte vegetation from Lake Ohrid from the negative anthropogenic impacts, especially in the places where the cyprinids performed spawning.

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EFFECTS OF DIFFERENT DIETARY OILS ON SOME BLOOD PARAMETERS IN COMMON CARP FINGERLINGS

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EFEKAT RAZLIČITIH DIJETETSKIH ULJA NA POJEDINE PARAMETRE KRVI MLAĐI ŠARANA

Apstrakt

Povećana količina masnoća u obroku može da utiče na funkcionalno stanje jetre riba i zastupljenost pojedinih supstanci u krvi u čijem metabolizmu jetra učestvuje. S tim u vezi, cilj ovog istraživanja bio je da se utvrdi da li dodavanje različitih biljnih ulja u komercijalnu ekstrudiranu smešu ima uticaja na vrednosti ukupnog holesterola (TC), triglicerida (TG), glukoze (GLU), aspartat-aminotransferaze (AST), alanin-aminotransferaze (ALT) i c-reaktivnog proteina (CRP) u krvi šarana. U protočnim tankovima sistema, u periodu od 90 dana, sproveden je ogled u 5 grupa sa po tri ponavljanja. Na početku ogleda je u svakom tanku nasađeno po 23 jedinke mlađi šarana (11,16±2,02 g). U grupi 1 (kontrola) riba je hranjena komercijalnom ekstrudiranom smešom sa 38% proteina i 7% masti. Ostale grupe dobijale su istu smešu (38/7) uz dodatak po 7% ribljeg, lanenog, sojinog ili repičinog ulja (redom: grupa 2, 3, 4 i 5). Krv riba je uzorkovana 60. i 90. dana ogleda. Rezultati su pokazali da su tretman i uzorkovanje, u interakciji ili zasebno, najveći uticaj ispoljili na vrednosti GLU, AST i ALT (p<0,05), dok se srednje vrednosti TC i CRP nisu značajno menjale (p>0,05). Dobijeni podaci pružaju korisne podatke o vrednostima odabranih biohemijskih parametara. Za precizniju procenu stanja jetre i nivoa stresa posle ishrane sa povećanim sadržajem lipida potrebni su dodatni podaci.

Ključne reči: *biljna ulja, jetra, stres, biohemija krvi, šaran*

Keywords: *vegetable oils, liver, stress, blood biochemistry, common carp*

INTRODUCTION

The use of commercial extruded mixtures for common carp (*Cyprinus carpio* L., 1758) has an increasing significance in Serbia. Considering great influence on fish growth, health and meat properties, finding the most suitable mixture composition for this omnivorous species is an important topic of research as is described by Poleksić et al. (2014) and Trbović et al. (2016). In this regards, increasing levels of lipids in the diet result in improved feed efficiency, providing biologically useful energy and thus preventing the use of proteins as a source of energy (Poleksić et al., 2014). The use of alternative lipid sources, especially of vegetable origin to replace fish oil has become a priority in the industry.

As in other vertebrates, high lipid content can influence liver function in fish (Huang et al, 2014; Yan et al., 2015). Nutritional status also can affect the fish health and the ways to deal with stress (Hemer et al., 1996). Liver is a principal site where the metabolism of carbohydrates, lipids and proteins takes place (Vikramjit and Metcalf, 2012). This organ is a glucose depot (in the form of glycogen), and it is involved in cholesterol and triglycerides synthesis (Jensen-Urstad and Semenkovich, 2012). Its function is also related to enzymes aspartate- and amino-transferase (Lui, 2018). Furthermore, several proteins of acute phase reactants are produced in the liver, for example C-reactive protein (Dorcas and Solomon, 2014). All these chemical compounds can be used for direct or indirect determination of liver functional condition. They also represent indicators of stress in fish which can be measured in blood (Shopinka et al., 2016).

Considering all facts mentioned above, the aim of this study was to determine whether feeding carp fingerlings with addition of different dietary oils in commercial feed has effect on blood biochemical parameters, related to liver functioning.

MATERIAL AND METHODS

The experiment was performed in flow-through aquaculture system of the laboratory for fish nutrition, University of Belgrade - Faculty of Agriculture, in feeding period of 90 days. In each of fifteen 120 L volume tanks, with a constant flow 0.34 l/min of dechlorinated tap water, 23 specimens of common carp yearlings were distributed. Individual average body mass was 11.16 ± 2.02 g and the initial fish density was 2.13 g/l/tank.

Water quality in tanks was regularly monitored and kept in optimal range for common carp. Electro-conductivity (µS/cm) and pH of the water were measured twice a week before feeding the fish using appropriate probes of the multifunctional device MULTI 340i/SET (WTW, Germany), while dissolved oxygen concentration (mg/l), saturation (%) and water temperature (°C) were measured daily at intervals of 10 min using the OxyGuard system (Denmark).

Fish were fed by average 3% feed in relation to the ichtiomass. On a precision scale (Radwag THB–600, Poland) daily rations were weighed out and continuously distributed during 12 hours light period by automatic belt feeders (AGK Kronavitter GmbH, Germany).

Based on the meal composition, five groups of tanks with three replicates were formed. Group 1 (control) was fed with an extruded commercial mixture for common carp fingerlings with 38% proteins and 7% fat (Soprofish 38/7 intensive effect, Veterinarski zavod Subotica, Serbia). Other groups were fed with the same 38/7 extruded mixture in addition of 7% fish oil (Group 2), 7% linseed oil (Group 3), 7% soybean oil (Group 4) or 7% rapeseed oil (Group 5).

For testing effects of different oils in feed on fish blood parameters, blood samples were taken on 60^{th} and 90^{th} day of the experiment (first and second sampling). In each sampling, total 45 fish (nine from each group) were first anaesthetized in the water with clove oil (Probotanic, Serbia). After measuring of body mass, their blood was taken from caudal vein and deposited in plastic 1.5 ml heparinised microtubes (Eppendorf Tubes® 3810X). Plasma obtained by centrifugation of blood samples (Eppenforf Centrifuge 5424 for 5 minutes on 4000 rpm) was transferred into the new microtubes for further analyses. The following five parameters were tested by Reflotron Sprint system (Roche diagnostics, Switzerland) and appropriate diagnostic kits: total cholesterol (TC, mmol/l), triglycerides (TG, mmol/l), glucose (GLU, mmol/l), aspartate aminotransferase (AST, IU/l) and alanine transaminase (ALT, IU/l). Also, C-reactive protein (CRP, mg/l) in samples was tested by Advia 1200 Clinical Chemistry System (Siemens Healthcare GmbH, Germany). Data were analyzed by STATISTICA 8.0 Software (StatSoft, Inc. 2007) and Microsoft Office EXCEL 2007. According to descriptive statistics and data character, the values were compared statistically using nonparametric tests, Kruskal-Wallis ANOVA and Mann-Whitney U-test at the 5% level of significance ($p < 0.05$).

RESULTS AND DISCUSSION

According to the results (Table 1), the applied treatments and samplings mostly influenced GLU, AST and ALT levels ($p<0.05$).

Table 1. Values of blood parameters in common carp fingerlings fed with different dietary oils addition

Group	**Total cholesterol** (mmol/l)			**Triglycerides** (mmol/l)			**Glucose** (mmol/l)		
	60. day*	90. day*	*p***	60. day*	90. day*	*p***	60. day*	90. day*	*p***
(1) commercial mixture	5.06±0.46	5.43±0.24	*0.453*	2.55±0.53	2.95±0.16	*0.233*	5.86±0.43	7.46±0.62	*0.145*
(2) commercial mixture +7% fish oil	6.06±0.78	5.15±0.31	*0.453*	3.59±0.94	3.77±0.18	*0.965*	4.85±0.28	6.82±0.57	***0.024***
(3) commercial mixture +7% linseed oil	5.33±0.35	6.39±0.30	*0.112*	2.88±0.20	3.71±0.24	***0.046***	6.96±0.57	7.24±0.33	*0.233*
(4) commercial mixture +7% soybean oil	5.24±0.69	5.65±0.27	*0.354*	4.79±0.46	2.93±0.28	*0.052*	7.62±1.06	4.25±0.57	***0.009***
(5) commercial mixture +7% rapeseed oil	5.42±0.54	5.33±0.31	*0.825*	3.60±0.25	2.83±0.28	*0.070*	5.55±0.15	6.30±0.60	*0.251*
*p****	*0.780*	*0.422*	-	*0.152*	*0.127*	-	***0.016***	***0.024***	-

Group	**Aspartate-aminotransferase** (IU/l)			**Alanine-transaminase** (IU/l)			**C-reactive protein** (mg/l)		
	60. day*	90. day*	*p***	60. day*	90. day*	*p***	60. day*	90. day*	*p***
(1) commercial mixture	34.70±5.07	21.20±1.91	***0.005***	16.00±1.22	8.00±2.02	***0.037***	0.42±0.03	0.45±0.03	*0.658*
(2) commercial mixture +7% fish oil	22.10±1.52	14.30±1.52	***0.003***	9.00±0.94	3.00±0.55	***0.002***	0.46±0.04	0.51±0.03	*0.250*
(3) commercial mixture +7% linseed oil	32.00±3.87	18.30±1.90	***0.009***	10.00±2.29	6.00±1.69	*0.076*	0.47±0.02	0.45±0.04	*0.756*
(4) commercial mixture +7% soybean oil	27.70±3.85	16.20±3.16	***0.038***	9.00±1.71	8.00±3.21	*0.929*	0.46±0.02	0.47±0.02	*0.658*
(5) commercial mixture +7% rapeseed oil	23.80±4.48	14.90±1.92	***0.001***	10.00±5.01	6.00±2.05	***0.032***	0.47±0.02	0.43±0.02	*0.092*
*p****	*0.134*	*0.282*	/	***0.020***	*0.117*	/	*0.715*	*0.587*	/

* Median±SE; ** p-values obtained by Mann Whitney U-test; *** p-values obtained by Kruskal–Wallis test

Physiological values of blood parameters in fish depend on many factors, including regime of nutrition and diet composition. In paper by Jezeh et al. (2014) the higher rate of glucose and cholesterol have been observed in carps fed with commercial dry feed comparing to others. Furthermore, data on normal glucose levels can vary in different study (Hertz et al., 1989; Hoseini and Ghelichpour, 2013; Jezeh et al., 2014). In our paper, differences between the control and other groups have been considered, as well as the difference related to the time of sampling what also refers to the length of the experimental feeding period.

Cholesterol is an important structural component of cell membranes, the outer layer of plasma lipoproteins, and the precursor of all steroid hormones. Decreased levels indicate a possible disease but increased levels point to physiological discomfort (stress) and/or dysfunction at lipid metabolism (Patriche et al., 2011). There were no significant changes in TC values in this experiment, regardless of the treatment or sampling time ($p>0.05$).

Triglycerides can be used as indicator of nutritional condition, as well as cholesterol. Their most important function is to store and provide cellular energy. They largely come from food and increased levels can be related to increased food intake or liver function damage. Reducing levels can be found in malnutrition, malabsorption and terminal conditions of liver diseases (Patriche et al., 2011). A significant increase in TG was found in group 3 fed with linseed oil addition ($p<0.05$) which may be related to feed intake and its digestion.

Glucose represents a permanent and immediate source of energy necessary for the function of heart and muscles. Serum glucose level is an efficient indicator of stress condition in fish. Reduced glucose concentration may be associated with lack of adrenaline antagonist hormones or disorders of glycogen metabolism and, sometimes, liver disease. Nutritional condition can have an important influence on glucose level, more than other blood parameters (Ologhobo, 1992; Patriche et al., 2011). In the second sampling, all GLU values (except in group 4) were higher than in the first. It is significant only in group 2 fed with fish oil addition ($p<0.05$). Elevated GLU levels can be related to stress during measuring and sampling. In study by Velisek et al. (2005) clove oil anaesthesia had no effect on other biochemical indices than significant increasing in glucose. In group 4 fed with soybean oil addition, value significantly decreased ($p<0.05$). This also may be also related to the stress reactions. Furukawa (2003) found that administration of soybean emulsion to the patients after surgical procedure amplified stress response in severely stressed patients.

Transaminases (transferases) are intracellular enzymes that participate in the metabolism of amino acids and carbohydrates. Aspartate-aminotransferase can be found mostly in liver and heart muscle. Elevated AST levels are present in myocardial infarction, muscular dystrophy and in liver disease. Elevated ALT levels indicate acute liver damage, most commonly caused by chemicals, and also in massive infarction. Serum ALT has diurnal variation, may vary day-today, and may be affected by exercise, as well as AST levels (Varga et al., 2013; Dorcas and Solomon, 2014). Significant differences ($p<0.05$) in AST and ALT values between two samplings could be the consequences of fish higher activity in the time of the first sampling.

The level of C-reactive protein in blood rises as a respond to trauma, injury or infection. It has been also released during cell death or exposure to exogenous harmful molecules, such as mercury (Falco et al., 2012). In our study, the mean CRP values match to those that have been determined by MacCarthya et al. (2008) in healthy, non infected carps. They are almost the same in all groups and in both measurements indicating that treatment or fish handling did not activate this protein.

CONCLUSIONS

This study most affected the levels of glucose and liver enzymes ($p<0.05$). Total cholesterol and C - reactive protein have been not influenced by extra content of different dietary oils, or by fish handling. The data provide useful information on the values of selected biochemical parameters. For a more accurate assessment of the liver condition and the level of stress after feeding with increased lipid content, additional data are needed.

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PERCENTAGE OF MORFOLOGICAL DEFORMITIES OF MOUTHPARTS IN *CHIRONOMUS PLUMOSUS* (DIPTERA: CHIRONOMIDAE) LARVAE FROM THE CARP PONDS

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PROCENAT MORFOLOŠKIH DEFORMITETA USNOG APARATA KOD LARVI *CHIRONOMUS PLUMOSUS* U ŠARANSKIM RIBNJACIMA

Apstrakt

Praćeni su efekti različitih tipova hrane kojima je hranjena šaranska mlađ, uticaj fizičkih i hemijskih parametara kvaliteta vode, kao i uticaj šarana kao predatora na pojavu deformiteta usnog aparata (mentum) kod larvi vrste *Chironomus plumosus* u šaranskim ribnjacima. Konstatovano je da u jezerima gde je korišćena hrana iz tretmana «KP» (sa većim procentom kukuruza i pšenice) i u kojima je konstatovana najmanja koncentracija kiseonika (7,29 mg/l), a najveća koncentracija ortofosfata (0.258 mg/l) i nitrata (1.253 mg/l), bio najveći procenat deformiteta mentuma (10,18%) *C. plumosus* u odnosu na jezera gde je za prihranu šarana korišćena hrana iz tretmana «SRB» (sa većim procentom sojinog proteina i ribljeg brašna). Najčešći tipovi deformiteta su kraći medijalni zub i asimetrični zubi. Takođe, praćen je efekat predatorstva na pojavu deformiteta. U jezerima gde je bio veći broj riba uočen je veći procenat deformiteta (10,18%) u odnosu na kontrolu, kada u jezerima nije bilo ribe, čime je pokazano da na pojavu deformiteta usnog aparata hironomida utiču i faktori koji nisu direktno povezani sa kvalitetom vode.

Ključne reči: *deformitet usnog aparata, Chironomus plumosus, šaranski ribnjak*
Keywords: *deformities of mouthparts, Chironomus plumosus, carp ponds*

INTRODUCTION

Chironomid larvae represent one of the most important groups of aquatic invertebrates in the macrozoobenthos community (Pery et al., 2004) because they live in virtually all types of aquatic habitats. Chironomids are also used successfully as bioindicators of pollution of aquatic ecosystems (Mebane et al., 2008; Michailova et al., 2009; Al-Shami et al., 2010). Their significance in food chains is great, and on carp farms they represent very important natural food for young carp and carp reared for human consumption (Broza et al., 2000; Marković, 2010).

Because of the need to achieve a multi-fold increase of ichthyomass per unit of area or volume of water, rearing of fish leads to deterioration of water quality on fish farms. During the process of production in rearing of fish, changes occur in the sediment and in the water, and in a very short time the environment can become unfavourable for the life of different organisms, causing various disturbances, including morphological deformities on the mouthparts of aquatic invertebrates. The occurrence of deformities in chironomid larvae, particularly deformities of the mentum, represent a response of the individual to changes in quality of the water and the sediment which sends an early warning signal of organic or inorganic pollution of the aquatic environment (Nazarova et al., 2004; Odume, 2011). Supplementary feeding of carp on fish farms with different commercial mixtures and cereals as an unavoidable technological operation in semi-intensive and intensive systems of rearing leads, among other things, to changes on the mentum of chironomids, since they feed on particles of undecomposed food falling on the bottom of the carp pond. In addition, the occurrence of deformities is also influenced changes in physical and chemical parameters of the water and by predation (Odume et al., 2012) which can affect the ability of chironomids to feed themselves (Odume et al., 2012).

The experiment whose results are presented in this paper was conducted in order to evaluate the effect of different types of feed given to young carp, physical and chemical parameters of the water quality and the influence of carp as predators on the occurrence of deformities of the mouthparts in the species *Chironomus plumosus* on carp farms.

MATERIAL AND METHODS

Chironomus plumosus larvae were collected at the "Little Danube" Centre of Fisheries and Applied Hydrobiology from six experimental ponds stocked with different densities of young carp. In three of the ponds, the fish were given feed of the "MW" type (30% soya grain, 30% maize, 29% wheat, 2% premix, and 3% soya protein concentrate). In the other three ponds, they were given feed of the "SFM" type (40% soya grain, 6% maize, 14% wheat, 12% fish meal, 3% premix, and 25% soya protein concentrate). Chironomid larvae were collected every 15 days for a period of two and a half months, from the end of July to the middle of October of 2015, using a Van Veen dredge with an area of 260 cm^2. The control consisted of chironomid larvae collected from the ponds before they were stocked with young fish.

Microscope slides of the chironomid mouthparts were prepared according to the method of Namiotko et al. (2011). A total of 278 specimens were analysed from the ponds with "MW" feed, 526 from the ponds with "SFM" feed, and 135 from the control ponds. These slides were examined under a Carl Zeiss microscope of the Axioskop 40 type to detect deformities. Throughout the experiment and before stocking of the ponds, water temperature, oxygen concentration, pH, and electroconductivity were measured with the aid of a MULTI 340i/SET (WTW, Germany). From each pond, water samples were taken at a depth of 30 cm below the surface with a 1-litre plastic bottle for chemical analysis, which was performed by standard methods in the Water Chemistry Laboratory of the Faculty of Agriculture in Zemun.

RESULTS AND DISCUSSION

Analysis of physical and chemical parameters of the water quality during realization of the experiment revealed decrease of oxygen concentration, pH, and electroconductivity in ponds with both types of feed, whereas the concentrations of orthophosphates, nitrates, sulphates, and HPK increased in relation to the control (Table 1). These changes of water quality were accompanied by changes on the mentum of chironomids. Thus, the ponds with "MW" feed where the lowest oxygen concentration (7.29 mg/l) and highest concentrations of orthophosphates (0.258 mg/l) and nitrates (1.253 mg/l) were recorded, showed the highest percentage of occurrence of deformities (10,18%, table 1), of which the most frequent were shorter median teeth and asymmetric teeth. As in the presented investigation in the study of Odume et al. (2012) was demonstrated a strong correlation between increase in the percentage of occurrence of deformities on the mentum of chironomids on the one hand and decrease in the concentration of dissolved oxygen and electroconductivity and increase in the concentration of orthophosphates and total inorganic nitrogen on the other.

It is known that the influence of poor water quality leads to developmental instability in larvae of the genus *Chironomus* and occurrence of deformities on their mentum, since asymmetry of the mentum in strong correlation with the concentration of HPK in the water was recorded in a previous study (Al-Shami et al., 2011). Moreover, seasonal changes of water temperature can bring about developmental instability in chironomid larvae (Servia et al., 2004). Contrary to the results of those investigators, temperature in our study did not significantly affect the occurrence of deformities on the mentum of *C. plumosus.*

Table 1. Percentage of larvae with deformities, number of fish, and physical and chemical characteristics of the water in carp ponds (mean values of the indicated parameters are given in the table).

	Control	"MW" type	"SFM" type
Deformities (%)	4.66	10.18	6.58
Number of fish	0	71	63
Temperature ^{o}C	28.16	21.11	21.16
pH	8.91	8.67	8.67
Oxygen – O_2(mg/l)	11.45	7.29	7.49
Electroconductivity (µS/cm)	1760	1735	1618
Orthophosphates PO_4-Pmg/L	0.113	0.258	0.250
Nitrates -NO_3mg/L	0.8	1.253	1.053
Sulphates -SO_4mg/L	35.02	35.67	38.76
HPK (from $KMNO_4$)mg O_2/L	17.93	23.16	23.25

The frequency and severity of morphological deformities in chironomid larvae are known to increase parallel with increase in the quantity of both inorganic and organic pollutants in aquatic ecosystems (Bhattacharyay et al., 2005; McDonald and Taylor, 2006). Meanwhile, Odume et al. (2012) state that it would be useful to investigate the influence of other factors not directly connected with water quality on the occurrence of deformities of the mouthparts in chironomids, factors such as predation and competition for example. In our study we therefore monitored the percentage of chironomid larvae with deformities of the mentum in relation to the number of fish in ponds with different types of feed. Here we found that with increase in the number of fish there was an increase in the percentage of larvae with deformities (shorter median teeth and asymmetric teeth), whereas the lowest percentage of larvae with deformities was in the control with no fish in the ponds (Table 1).

One of the responses to predation exhibited by larvae of the family Chironomidae is that they form tubes around their bodies in which they hide (Dudgeon, 1994; Baker and Ball, 1995) and bury these tubes deep in the sediment when predators are present (Hershey, 1987). However, these "tactics" of avoiding predators have certain consequences for chironomids (Holker and Steif, 2005). Such construction of tubes at great depths to avoid predators has as consequences the need to adapt to lower concentrations of oxygen in deeper parts of the sediment and reduced access to resources (Van der Bund et al., 1994). This leads to increase in the percentage of deformities of the mouthparts.

Moreover, the supplementary feed given to carp in fish ponds can cause the occurrence of deformities on the mentum of chironomids. Analysing changes on the mentum in ponds with the different types of feed used in our experiment, we found that in the case of ponds with feed having a high percentage of maize and wheat (the "MW" type) the number of deformed mouthparts in *C. plumosus* increased, whereas the percentage of larvae with deformities declined with increase in the percentage content of soya protein and fish meal in the "SFM" type of feed (Fig. 1).

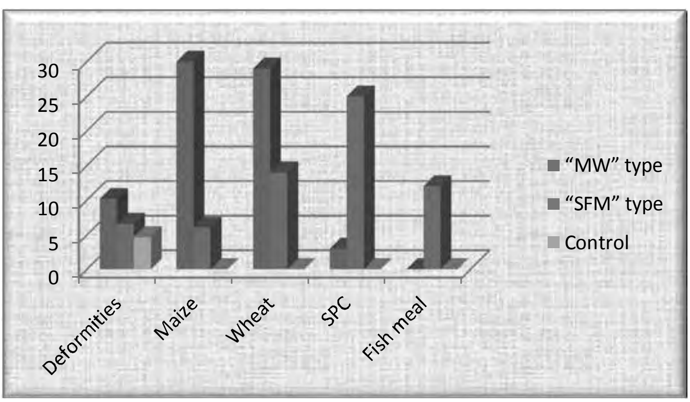

Figure 1. Percentage of larvae with deformities of the mentum and percentage content of different ingredients in two types of feed.

This can be attributed to the fact that soya protein concentrate of standard good quality with high protein content and easily digestible by young carp has a positive effect on maintenance of good quality of an aquatic environment (Stanković, 2013). On the other hand, feed containing cheaper and more readily available ingredients such as maize and wheat (De Silva, 2012) and having high content of dry matter causes significant deterioration of water quality (Diana et al., 2013), which affects the occurrence of deformities of the mentum in chironomids exposed to these organic pollutants (Kuhlmann et al., 2000).

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ALLOCHTHONOUS FISH SPECIES IN SOME WATER ACCUMULATIONS OF THE CENTRAL BALKANS

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ALOHTONE VRSTE RIBA NEKIH VODENIH AKUMULACIJA CENTRALNOG BALKANA

Apstrakt

Analizirane su alohtonie vrste u akumulacijama Centralnog Balkana, u radovima autora iz Srbije i Bosne i Hercegovine. Dati su podaci za Zlatarsko jezero, Međuvršje, Gružu, Ćelije i Zvorničko jezero,, koje nalazimo u radovima Kosorić (1975), Lenhardt i sar. (2009), Marković (2011) i Hegediš i sar. (2008). Iz Bosne i Hercegovine su analizirani radovi Adrovića i Skenderovića (2007), Adrovića (2012), Adrovića (2016), Škrijelja i sar. (2005), Škrijelja i sar. (2011), Korjenića i sar. (2011), Skenderovića, (2010) i Mikavice i Savića (1999). Zlatarsko jezero1975. godine, nije bilo naseljeno alohtonim vrstama riba. Mićković i sar. (2011) u ovom jezeru nalaze 14 vrsta riba, među kojima pet (35,17%) alohtonih i to: zlatovčica, babuška, šaran, sunčanica i smuđ. U Međuvršju (Lenhardt, 2008) 1955. godine nije bilo alohtonih vrsta, a kasnije su se pojavili babuška i bijeli amur, sunčanica, bezribica i američki somić, ali ne samo u Međuvršju. Marković i sar. (2013) su na lokaciji Kratovska Stena utvrdili 13 vrsta riba, među kojima je alohtona sunčanica; u Međuvršju je bilo 19 vrsta, od kojih su alohtone: babuška, **bijeli tolstolobik, bezribica,**sunčanica i američki somić. Preostale dvije lokacije naseljava 23 vrste riba, među kojima su i pomenute alohtone vrste. Na lokaciji Čačak se pojavljuje i Neogobius fluviatilis, koji se kasnije pojavio u akumulacijama Međuvršje i Gruža (Marković i sar. 2015). Među 24 vrste riba iz akumulacija Međuvršje, Gruža i Ćelije, Marković (2011) konstatuje, pomenute alohtonie vrstei bezribicu. Zvorničko jezero (Hegediš, 2008) naseljava 14 vrsta riba među njima i sunčanica (3,4%), babuška (5%), balavac (0,8%) i šaran (0,8%).

U Modracu (Zelinka, 1971), nalazi šarana, soma, balavaca, smuđa i sunčanicu, a Habeković i sar. (1984) šarana (4), soma (2), balavca (21) i smuđa (73 jedinke), čije prisustvo utvrđuje Adrović (2012). Skenderović (2010) konstatuje prisustvo babuške (11), šarana (24), soma (7), smuđa (63) i sunčanice (8 jedinki). Škrijelj i sar. (2011) potvrđuju babušku (2) i

smuđa (9). Sniježnicu naseljava 11 vrsta (Škrijelj i sar. 2005.), među kojima su alohtone šaran (1,17%), babuška (4,40%) i sunčanica (3,20%). Hazna (Korjenić i sar. 2011) je naseljena sa devet vrsta riba. Alohtone su šaran (2,13%), babuška (27,66%), smuđ (4,26%) i američki somić 12,75%. Vidaru naseljavaju alohtone vrste: šaran (2,04%), babuška (6,12%), smuđ (32,66%) i američki somić 28,57%). Akumulacije Bajina Bašta i Zvornik na Drini (Mikavica i Savić, 1999.) naseljava 16, odnosno 11 vrsta riba, kao i som i balavac, sa malim brojčanim učešćem.

Ključne riječi: *alohtone vrste, hidroakumulacije, Srbija, Bosna i Hercegovina*
Keywords: *allochthonous species, hydroaccumulations, Serbia, Bosnia and Herzegovina*

INTRODUCTION

The introduction of new fish species has been very frequent in the middle of the last century. There was no adequate knowledge about the consequences of introduction, which led to some types of species being lost due to the pressure of local predators or the impossibility of adapting to new living conditions. In case of a good adaptation of the introduced species, they have adversely affected the number of autochthonous species, and sometimes the introduced species are predatorsof their domestic speciesor their spawns. One particular problem related to the introduction of fish is the possibility of introducing ecto and endo parasites of fish and new fish diseases. Despite this knowledge, research studies ofallochthonousfishspecies in Serbia and Bosnia and Herzegovina has not been paid due attention, as evidenced by a relatively small number of publications on this issue. Although the area of north-eastern Bosnia is inhabitedby a number of allochthonous fish species, the condition of its populations was considered in just a few scientific papers that were published over the past decade. In Serbia, the situation is somewhat better, and in support of this are relatively numerous scientific papers of Serbian authors. In accordance with the above facts, the data on allochthonous fish species in hydroaccumulation in the Central Balkan area are provided.

MATERIAL AND METHODS

The previously published articles which contain the status of diversity and the number of allochthonous fish speciesas well as the consequences of their presence in some hydroaccumulation in the Central Balkan area were analyzed. For the area of Serbia,the articles of Kosorić (1975) for Zlatarsko lake, Lenhardt al., (2008) for Međuvršje, Marković (2011) for Gruža and Ćelije, Hegediš et al. (2008) for Zvornik Lake were analyzed. The data of allochthonous fish species in some accumulations from the territory of Bosnia and Herzegovina were found in articles by Adrović (2007, 2012, 2016), Škrijelj et al., (2005, 2011), Korjenićet al. (2011), Mikavica and Savić (1999), for the accumulation of BajinaBašta and Zvornik on the Drina River. The data cover the period from 1953, but they are not the result of continuous monitoring; they represent the synthesis of different results by different authors from different research periods.

RESULTS AND DISCCUSION

Zlatarsko Lake with a volume of about 250 million m3 was formed on the river Uvac (Mićković et al., 2011). The accumulation is inhabited by eight fish species (Kosorić et al., 1975), and the composition of ichthyocenosis has not changed after the accumulation formation. There were not any allochthonous species in ichthyocenosis. Later research studies (Mićković et al., 2011) indicate the presence of 14 different species of fishes, five of which (35.17%) were allochthonous. These are arctic char, giebel carp, common carp, pumpkinseed and zander. It is not known how most of these species entered the ichthyocenosis, while the zander were introduced with the aim of reducing the number of populations of bleak (Alburnus alburnus). The number of some populations of fish in the accumulation decreasing (arctic char, giebel carp, common carp), and the number of some populations oscillating (nase, chub, common barbel, Danubian roachRutilus virgo) which is likely to be related to hatching of zander. There is a disappearance of a schneider (Alburnoides bipunctatus), which was not recorded in the accumulation after the investigation by Kosorićet al. (1975). Water accumulation Međuvršje was formed in 1953 in the West Morava, with a volume of about 18.5 million m3, a maximum depth of about 20 m. Lenhardt et al. (2008) presented the data of fish populations from Međuvršje for the period from 1955 to 2000. According to these data, there were not any allochthonous species in Međuvršje in 1955, and later giebel carpand white grasscarp appeared, which were introduced by the recreational fishermen. Over the past decades, pumpkinseed, topmouth gudgeon and brown bullhead have spread rapidly, not only in Međuvršje, but also in other waters in Serbia. Markovićet al. (2013) investigated ichtyofauna of the Western Morava at the locations of Kratovska Stena, Međuvršje, Čačak and Kraljevo, in the period from 2010 to 2012. The authors emphasize that the area of Kratovska Stena is inhabitedwith 13 species of fish, including the allochthonous pumpkinseed. The accumulation of Međuvršje is inhabited by 19 species, but also by the following five allochthonous species: giebel carp, white grasscarp, topmouth gudgeon, pumpkinseed and brown bullhead. The remaining two locations are inhabited by 23 species of fishes, including the mentioned allochthonous species. In addition to the above, Neogobius fluviatilis appears in the area of Čačak. In ichtyofauna of Međuvršje and Gruža accumulations (Marković et al., 2015), during 2013-2014 the presence of Neogobiusfluviatilis was also registered. In Međuvršje, Gruža and Ćelije accumulations, Marković (2011) found 24 species of fishes, six of which were allochthonous. In all three accumulations, apart from the mentioned allochthonous species, the presence of the topmouth gudgeon was registered. Zvorničko jezero (Hegediš, 2008) is inhabited by 14 species of fishes, and there are the following allochthonous species: pumpkinseed (3.4%), giebel carp (5%), common ruffe (Gymnocephalus cernuus) (0.8%) and common carp(0.8 %).In ichtyofauna of Serbia (Marković, 2011), the presence of 23 introduced allochthonous species from 11 familieswas registered. The ways and reasons of the introduction are different, and the consequences of the introduction can generally be assessed as negative. Vuković and Kosorić (1978) indicated the problem of introducing allochthonous species into the open waters of Bosnia and Herzegovina. Since then, a number of papers that point to this problemhave been published (Adrović and Skenderović, 2007, Piria et al., 2017). The first data of the fish from Modrac are found in a fishing basics (Zelinka, 1971), where it is stated that common carp, wells and common ruffeare quite numerous, that there are few pumpkinseed and that zander is stocked but that during the research it is not caught. Habeković et al. (1984) registered common carp (4), wells (2), common ruffe (21) and zander (73 individuals) of allochthonous species in Modrac. Data from Adrović, (2012) indicate the presence of seven allochthonous species of fish in the Modrac reservoir. The abundance of these species is different. Thus, the abundance of giebel carp, common carp, wells and common ruffe is 0.09%, black bullhead (Ameiurus melas) 0.81%, zander (Sander lucioperca) 1.54%, and pumpkinseed 2.45%. Skenderović (2010) determines the presence of giebel carp(11), common carp (24), wells (7), zander (63) and pumpkinseed (8 individuals). Škrijelj et al. (2011) present the information on the presence of giebel carp (2) and zander (9). Fishing from Aprilof the current year (unpublished data) indicates an increase in the number of allochthonous species (71.56%) in comparison to autochthonous species (38.44%) and especially giebel carp (62.38%). The number of giebel carp populations is the result of uncontrolled and unplanned restocking, undertaken by local fishermen. Hydroaccumulation Sniježnica is inhabited by 11 species (Škrijeljet al., 2005), including one individual of common carp (1.17%), 22 giebel carp (4.40%) and 16 pumpkinseed (3.20%). Hydroaccumulation Hazna (Korjenić et al. 2011) is inhabited by nine species of fishes. With regard to allochthonous species, which accountfor 46% of the total number, common carp(2.13%), giebel carp(27.66%), zander (4.26%) and brown bullhead(12.75%) are present. The Vidara accumulation is inhabited by seven species of fishes, three autochthonous and four allochthonous species: common carp(2.04%), giebel carp (6.12%), zander (32.66%) and brown bullhead(28.57%). The composition of the Vidara ichtyopopulations has been altered for the benefit of allochthonous species in terms of the number of species (4: 3) and their abundance (69.39%: 30.61%). In the composition of ichtyofauna BajinaBašta and Zvornik on Drina accumulations, Mikavica and Savić (1999) found the presence of 16 and 11 species of fishrespectively. There were determinedtwo allochthonous species of fishes, wells and common ruffe, with a small number of participations.In the waters of Bosnia and Herzegovina, 25 allochthonous fish species were introduced (Piria et al., 2017), which originate from Africa (3), Asia (14), Eastern Europe (14), North Europe (5), North America (15), South America (3) and Western Europe (2).

CONCLUSIONS

Based on the presented data, a list of the following 14 species of allochthonous species occurring in the investigated accumulation was identified: *Salvelinus alpinus, Carassius gibelio, Ctenopharyngodon idella, Lepomis gibbosus, Pseudorasbora parva, Ameiurus nebulosus, Hypophthalmichthys molitrix, Pseudorasbora parva*, *Lepomis gibbosus, Neogobius fluviatilis, Gymnocephalus cernuus, Ameiurus melas* and *Sander lucioperca*. Their influence on autochthonous species is difficult to assess due to the lack of precise data on the structure and productivity of fish communities prior to their entry. It can be assumed that it is a key competitor for food and hatchery with native species, as most allochthonous specieshave low requirements for the environmental conditions.The current level of knowledge about the negative consequences and influences of allochthonous species on indigenous populations is not sufficient for total consideration of this problem, so additional systematic research on biology, ecology and invasive potential of allochthonous species will be useful as it will complement the knowledge in this insufficiently known area. The problem is the introduction and spread of parasites and parasitic diseases, whose effects are also relatively little known.

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DECISION MAKING OF STUDENTS IN AQUACULTURE: PROFESSIONALLY OR SOCIALLY DRIVEN?

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DA LI JE PROFESIONALNO DONOŠENJE ODLUKA STUDENATA VOĐENO SOCIJALNIM OKOLNOSTIMA?

Apstrakt

Na Poljoprivrednom fakultetu Univerziteta u Beogradu aktivno učenje/nastava (AUN) se praktikuju više od 15 godina, dok se uporedo obavljaju istraživanja ovih nastavnih metoda, njihova analiza i objavljivanje rezultata. U cilju ispitivanja sposobnosti donošenja profesionalnih odluka, koje nemaju često prilike da vežbaju tokom studija, studenti završne godine Osnovnih studija Zootehnike zamoljeni su da donesu individualno profesionalne odluke zasnovane na znanju i uverenjima koje su stekli tokom studija. Predstavljena su im dva slučaja iz oblasti akvakulture tj. trebalo je dva puta da donesu odluku: Slučaj 1 – pre serije interaktivnih časova; Slučaj 2 – posle učešća u dvodnevnom bloku interaktivnih časova namenjenom “osvežavanju” znanja o kvalitetu vode, zdravlju i dobrobiti riba i kvalitetu proizvoda, finalizirano studentskom debatom o zamišljenoj saradnji Srbije i Vijetnama. Ispitane su 2 generacije: školske 2015/16 (grupa A) i 2017/18 (grupa B). Ukupno je analizirano 115 upitnika (55 iz grupe A i 60 iz grupe B). U oba slučaja student su birali između 4 ponuđena odgovora, a izabrani odgovor je trebalo da obrazlože.

Slučaj 1

Po završenom fakultetu zapošljavate se kao glavni tehnolog na velikom ali neuspešnom ribnjaku. Vlasnik vam stavlja do znanja da traži vašu stručnu pomoć da podignete profit ribnjaka jer je zadužen kod banke koja će mu oduzeti ribnjak ukoliko ne bude podigao profit. Na tržištu se pojavila ekstrudirana hrana za ribe inostranog proizvođača, odličnog kvaliteta, koja je urađena po najboljoj tehnologiji i daje izvanredne rezultate prirasta ribe po istoj ceni kao i hrana domaćih proizvođača. Odlučujete se da isprobate tu novu hranu za ribe bez dodatnih analiza. Nakon kratkog vremena povećava se prirast riba i samim tim profit ribnjaka. Dobijate povišicu i nagradu od vlasnika. Posle nekog vremena, mediji otkrivaju i objavljuju da je sastav hrane za ribe ovog inostranog proizvođača i poreklo komponenti od genetski modifikovanih organizama (GMO). Otvara se ozbiljna afera o rizicima

od GMO u hrani a vaša firma je na listi korisnika ovog proizvoda. Naknadnim uvidom u dokumentaciju saznajete da se u pripremi te hrane zaista koriste komponente koje potiču od sirovina koje su genetski modifikovane. Kakvu odluku ćete doneti i kako ćete savetovati vlasnika? Izaberite neki od ponuđenih odgovora :

1. Nastavljamo sa uvozom i korišćenjem hrane, a u javnosti uporno branimo stav da je bezbedno i bez rizika za ljude
2. Nastavljamo sa uvozom, „pravimo se ludi“ do daljnjeg, ne uključujemo se u rasprave, izbegavamo novinare i odbijamo medijske nastupe o GMO
3. Prekida se uvoz ove hrane, nalazi se zamena, a u medijima se obrazlaže potencijalni rizik koji nosi GMO hrana
4. Nešto drugo (napišite na linijama)

Slučaj 2

Setite se prošlog upitnika:

Izgubili ste posao na ribnjaku, već neko vreme ste bez posla u struci. Iznenada Vam se javlja bivši gazda i nudi ponovo zaposlenje, ali da gajite organskog šarana. Ponovo biste bili glavni tehnolog na ribnjaku i potpuno odgovorni za proizvodnju i plasman organskog srpskog šarana. Investicija će početi da donosi dobit posle 4-5 godina, a dotle bi Vam bila zagarantovana plata od 50 000 RSD. Gotovo u isto vreme dobijate i ponudu od Vijetnamske firme da budete zastupnik u Srbiji i obezbeđujete uvoz i plasman pangasiusa u Srbiju i regionu. Početna plata je 1000 € + bonusi i procenat od prodaje. Kakvu odluku ćete doneti? Izaberite neki od ponuđenih odgovora:

1. Prihvatate posao na ribnjaku
2. Prihvatate posao kod Vijetnamske firme
3. Odlazite u inostranstvo da radite bilo šta, u struci ili van struke
4. Nešto drugo (napišite na linijama)

Dobijeni su sledeći rezultati :

Slučaj 1

U obe generacije preovlađuje profesionalna etika i uverenja struke: odlučuju se za odgovor da se prekida uvoz GMO hrane (oko 70 % u obe generacije), dok više od 20% (24% u grupi A i 22 u grupi B) bira nešto drugo. Kao razloge za izbor odgovora 3 navode najčešće brigu za zdravlje, sopstveno i celog “naroda” iako je još uvek nedovoljno dokaza o štetnosti GMO, pokazuju uverenja koja obrazlažu solidnim poznavanjem problema oko GMO. Ističu značaj proizvodnje domaće hrane bez GMO i sa potencijalno dobrim efektima na proizvodnju i prodaju. Pokazuju poverenje u znanje, nauku i Univerzitet. Smatraju da se primenom naučenog može proizvesti domaća hrana za ribe. Insistiraju na kontroli kvaliteta hrane i ribe, sledljivosti proizvoda i biosigurnosti.

Nastavak upotrebe hrane sa GMO (7 odnosno 4%) ili obmanjivanje javnosti – zanemarljivo (0 i 4%) (“GMO je neminovnost” i “Kontrola kvaliteta ribe i hrane za ribu mora da postoji”).

Slučaj 2

Posle senzibilizacije i podsećanja na uslove gajenja, dobrobit i kvalitet proizvoda, u obe generacije u proseku skoro 40% bira skromniju platu i opet profesionalna etika i uverenja da se gaji organski šaran preovlađuju. Kao razloge navode brigu za zdravlje, biosigurnost i kvalitet domaće ribe u odnosu na pangasiusa ("Panga će proći"). Obrazlažu kvalitetom domaćeg šarana, izazovima i velikim mogućnostima domaće proizvodnje, te primeni znanja stečenog na fakultetu u proizvodnji domaće ribe. Biraju i nižu platu, ali da ostanu u svojoj zemlji ("50000 je solidna plata" - odgovor iz grupe B).

I dok više od polovine (53,8% studenata) u grupi A, 27,3% u grupi B, bira primamljivu ponudu strane firme od 1000 €. Obrazlažu prevashodno većom platom, teškom situacijom u zemlji ("50000 je mala plata" - odgovor iz grupe B) i novim izazovima, tržištem i potrebom kontrole kvaliteta proizvoda i obezbeđivanja biosigurnosti.

Svakako je očekivano da u obe generacije 90, odnosno 70 % studenata bira jedno od 2 ponuđena zaposlenja. Međutim, 2016. niko ne bira odlazak iz zemlje, ali 2018. ih ima 9%. Odgovor 4 – «nešto drugo» 2016. bira manje od 8%, a 2018. 3 puta više studenata. Ovi poslednji su ili kolebljivi ili žele da pokrenu sopstvenu poljoprivrednu proizvodnju.

Iz izloženog možemo zaključiti da studenti imaju profesionalna uverenja, da su osetljivi na kontroverzna pitanja kvaliteta hrane, ribe i proizvoda, da se zauzimaju za očuvanje zdravlja "naroda" i oslanjaju se na znanje stečeno na fakultetu, ali podležu i uticajima medija, osetljivi su i na biosigurnosne izazove.

Interesantno da često navode "patriotske" razloge, pokazuju brige za zdravlje, sopstveno i "celog naroda", navode razloge kao što su problemi u društvu i koriste često političko/medijski diskurs. Nastavak ispitivanja profesionalnih uverenja i sposobnosti donošenja odluka budućih generacija studenata će doprineti boljem razumevanju njihovih stavova i ukazati na mogućnosti unapređenja nastave/učenja u formiranja stavova zasnovanih na znanju koje stiču budući profesionalci u zootehnici i akvakulturi.

Ključne reči: *studije slučaja, upitnici, gajenje riba, kvalitet proizvoda, profesionalno odlučivanje*

Abstract

At the Faculty of Agriculture University of Belgrade active teaching/learning is practiced more than 15 years, together with the study of these teaching methodologies, analysis and results publication. In order to study students' professional decision making capacities, rather seldom tested during their studies, students of the final year of Bachelor studies in Animal Science were asked to make individual decisions based on their knowledge and beliefs acquired during 4 years of study. Two aquaculture cases were presented, students had to make individual decisions twice: Case 1 – prior to a set of interactive classis; Case 2 - after they participated in a two days block of interactive classes aimed to „refresh" knowledge on subjects related to water quality, fish health and welfare, and product quality, finalized by a debate about an imaginary possible cooperation in aquaculture of Serbia and Vietnam. Two generations were tested: in school years 2015/16 (group A) and 2017/18 (group B). In total 115 questionnaires (55 from A, 60 from B) were analyzed.

For both cases students had to decide between 4 offered answers (forth being offered as „Other answers"). They had also to explain/elaborate their decision.

Case 1

After you finished studies in Agriculture you get a job as a chief technologist at a big fish farm suffering economic losses. The owner asks for your professional competences to quickly raise profit since the bank may foreclosure the farm. A new imported extruded

fish feed has appeared on the market, made according to newest technology. Its quality is excellent, results of fish growth rate are superior and the price of this new feed is the same as the one already existent on the market locally produced. You decide to try this new feed without additional testing or analysis. Soon, fish growth rate increased farm profit as well. You are rewarded and the farm owner raises your salary. After a while, media find out and publish the composition of the imported feed with some components made from genetically modified organisms (GMO). A serious affair arises focused on GMO in food and feed and your company is listed among the users. The documentation checked confirms the GMO origin of several feed components. What is your decision? How are you going to advice the farm owner? Choose one of the options:

1. We continue to import and use the feed: in public we defend the opinion that consumption of GMO is safe for humans
2. We continue to import and use the feed but without comments and discussions, we avoid journalists and refuse appearing in media, especially when GMO is in question
3. We stop importing the fish feed, find replacement, in media we explain a potential risk from GMO in feed
4. Something else (please write on lines below)

Case 2

Remember the previous questionnaire:

You lost your job on a fish farm, you are without employment in your profession for some time. Your ex boss, the farm owner, offers you a job, but this time you should run an organic carp farm. You should, again, be a chief technologist and fully responsible for production and placement of the organic carp. The investment will bring profit after 4 to 5 years, until then, your salary will be fixed to 50.000 Serbian dinars (RSD). Almost at the same time you receive another offer from a Vietnamese company to become a representative in Serbia and secure import and placement of pangasius in Serbia and the region. Starting salary is 1000 € (app. 400 €), plus bonuses and percentage of sales. What is your decision? Choose one of the options:

1. You accept the offer to work on the organic fish farm
2. You accept the offer of the Vietnamese company
3. You decide to leave the country and get a job anywhere, in your or any other profession
4. Something else (please write on line below)

The results of questionnaires analysis:

Case 1

In both groups professional ethics predominates, as well as professional beliefs: termination of import of feed with GMO feed components (approx. 70 % in both groups), while more than 20% (24 and 22 in groups A and B, respectively) choose something else. Reasons for the choice of answer 3 are listed as: concern for health, their own and of the "people", although there is still not enough evidence about GMO adverse effects; students' beliefs are explained with good knowledge of problems around GMO. They emphasize the importance of domestic production without GMO and potentially beneficial effects on fish production and sale. Students demonstrated confidence in knowledge, science and the University. They consider that by knowledge application quality fish feed in Serbia could be produced, similar to high quality imported feed, they also insist on quality control, traceability and biosecurity. Answers concerning continuation of GMO use (for group A and B: 7% and 4% respectively), or cheating the public was negligible (0 and 4 %) ("GMO use is inevitable", "Quality control of feed and fish must be rigorous").

Case 2

After remembering rearing conditions, welfare and product quality in interactive classes, both generations, almost 40 %, on average, chooses the modest salary and, again professional ethics and beliefs to grow organic carp predominates. As main reasons student mention concern about health, biosecurity and domestically produced fish quality compared with the pangasius ("panga will pass"). They elaborate by the quality of "our" carp, challenges and great potential of development of carp production in Serbia, as well as applied knowledge acquired at the University. They choose lower salary, but decide to stay in the country ("50.000 RSD is a good salary" in 2018). While more than half of the students (53,8% in group A, 27,3% in the group B chooses the attractive offer of 1000 €. They explain this high salary choice primarily by the need of money, difficult situation in the country ("50.000 RSD is a low salary"), but also believe in new challenges, market, and necessity of quality control of products and biosecurity concern. It was expected that in both groups, a high percent, 90% and 70% respectively, chooses one of the two offered jobs (answers 1 and 2). However, in 2016 nobody chooses to leave the country, but in 2018, there is 9 % who would leave. Answer number 4 – "something else" in 2016 is chosen by less than 8 %, and in 2018, 3 times more students, being hesitant or wanting to start their own agricultural production.

It could be concluded that students demonstrated solid professional beliefs and that they are sensible to the controversial questions of feed, fish and product quality; they are concerned by the health of the population; they rely on knowledge acquired at the Faculty, but they are also under the influence of media, and sensible to biosecurity issues.

It is interesting that students often mention "patriotic" reasons, concern about their own and "nation" health status, problems in the Serbian society, and often use political and media discourse.

Further study on professional beliefs and decision making capacity of future generation of students will contribute to better understanding of their position and will show further possibilities of teaching/learning improvement for the development of knowledge based attitude of future professionals in animal sciences, including aquaculture.

Keywords: *case studies, questionnaires, fish culture, product quality, professional decision making*

GROWTH PERFORMANCE AND VARIABILITY OF TWO STURGEON HYBRIDS

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PARAMETRI PRIRASTA I VARIJAILNOST DVA HIBRIDA MORUNE

Apstrakt

Hibridi jesetarskih vrsta postaju sve popularniji u akvakulturi i to iz jednog od dva razloga: ili zbog boljih parametara prirasta u poređenju sa roditeljskim vrstama (heterozis), ili zbog drugih osobina, kao što je ranija polna zrelost u poređenju sa čistim linijama. Oba navedena razloga predstavljaju prednost koja potencijalno može povećati prinos mesa ili kavijara. Cilj ove studije je bio merenje parametara prirasta hibrida bester (Huso huso x A. ruthenus - B) i hibrida nastalog povratnim ukrštanjem bestera i morune Huso huso (BB) u ranim uzrasnim kategorijama odgajanim u protočnom sistemu. Sedam dana nakon izvaljivanja, oko 50% larvi su aktivno plivale i u tom momentu je počela ishrana. Sa ciljem adaptacije ribe na hranu sa visokim sadržajem proteina i malim gubicima, protocol progresivnog hranjenja: prvih 5 dana riba je hranjena isključivo zooplanktonom, sledeća 4 dana hranom koja je sadržala mešavinu zooplanktona i organizama bentosa - tubifeksa, dok je u periodu do 12. dana riba hranjena isključivo tubifeksom. Od 13. dana započeta je prihrana kompletnom hranom, dok je od 45. dana riba jela isključivo kompletnu hranu. Kada je riba dostigla 45 dana, oba hibrida su smeštena u devet 400 litarskih polipropilenskih tankova, u triplikatu (tri različite klase, po veličini: CM1, CM2 i CM3). U svaki tank je smešteno 50 jedinki prosečne mase: 0.06 ± 1.29 g; 22.58 ± 1.35g i 4.14 ± 0.77 g (B hibrid) i prosečne mase 43.4 ± 1.02 g, 19.72 ± 0.71 i 3.05 ± 0.77g za BB hibrid. Eksperiment je sproveden između 4. i 12.09.2017. godine, a riba je hranjena sa dve različite komercijalne hrane: sa 50% proteina (1-1.5 mm) is a 40% protein (2 mm). Na kraju eksperimenta riba je izmerena i određeni su parametri prirasta, varijabilnost i alometrijski rast. Zdravstveni status ribe je procenjen preko uzoraka krvi. nakon 70 dana trajanja eksperimenta, zaključili smo da je prirast BB hibrida bio malo viši u poređenju sa besterom, pogotovo za manje težinske kategorije. Finalna masa bester hibrida je iznosila: 272.00 ± 67.14 g; 201.25 ± 59.48 g i 150.70 ±

27.51 g za kategorije CM1, CM2 i CM3, dok je prosečna masa BB hibrida iznosila: 298.25 ± 67.72 g, 262.90 ± 49.87 g i 204.20 ± 38.29 g za CM1, CM2, i CM3, respektivno. Varijabilnost je bila malo viša kod BB hibrida. Koeficijent varijacije mase pojedinačnih riba kod BB hibrida je iznosio između 16 i 22% (više vrednosti su bile prisutne kod riba u grupi CM1), dok su kod bestera za grupu CM1 vrednosti koeficijenta išle do 19%. Što se tiče tehnoloških performansi dva hibrida, nije primećena značajna razlika SGR za hibride iz grupe CM1, međutim neznatno više vrednosti SGR-a su primećene kod hibrida BB. Vrednosti DGR za CM1 su iznosile 2.87 i 2.09 g/kg/dan za BB hibrid i bester respektivno, vrednosti za klasu CM2 su iznosile 3.47 za BB hibrid i 2.55 g/kg/dan za bester, dok su za CM3 klasu DGR vrednosti iznosile 2.87 g/ kg/dan za BB i 2.09 g/kg/dan za bester. Takođe je primećeno da je u slučaju BB hibrida vrednosti FCR i PER bile više nego kod bester hibrida (vrednost FCR se razlikovala za oko 15%). Zbog velike varijabilnosti BB hibrida, težinske kategorije moraju biti jasno uspostavljene. Brži prirast preporučuje BB hibrid za proizvodnju mesa. Hematološki indeks je pokazao bolji zdravstveni status BB hibrida u poređenju sa besterom u istom režimu gajenja.

Ključne reči: *akvakultura, hibridi jesetre, genetska varijabilnost.*

Abstract

Sturgeon hybrids are becoming more and more popular for aquaculture industry due to either better growth performance compared to parent species (hybrid vigour) or other traits such us earlier maturity comparing with pure line, both reasons representing a premise for their potential for meat and caviar production. The objective of the study was to determine the growth performance of the hybrid bester (B) and backcrossed hybrid between bester (Huso huso x A. ruthenus) and grate sturgeon - Huso huso (BB) in early life stages reared in the conditions of a flow-through open production system. At 7 days after hatching, about 50% of larvae began to swim actively, moment at which feeding was started. In order to adapt them to pre-starter feed with a high protein content with minimal losses, a progressive feeding protocol was applied in order to replace exclusive zooplancton diet (during the first 5 days) with mixed feed of zooplankton and benthic -tubifex organisms (in the next 4 days) and exclusively tubifex up to 12 days. On the 13th day the artificial feed was gradually introduced and in the 45th the fish were fed exclusively with artificial diet. Thus, at the age of 45 days post hatching, both hybrids were stocked in nine 400-liter PP tanks, in three triplicated experimental variants (3 size classes: CM1, CM2 and CM3). Each tank was populated with 50 ind. / tank with an average mass of 40.06 ± 1.29 g/ind; 22.58 ± 1.35g and 4.14 ± 0.77 g for the B hybrid and mean mass of 43.4 ± 1.02 g, 19.72 ± 0.71 g and 3.05 ± 0.77g for the BB hybrid. The experiment was conducted between 4.07-12.09.2017 during which 2 types of commercial feeds were administered with 50% protein (1-1.5mm) and 40% protein (2mm). At the end of the experiment fish were weighed and measured to quantify the growth performance, variability and allometric growth. In order to assess the health status blood samples were collected. After 70 experimental days it can be concluded that BB hybrids performed slightly better than those of bester, especially for small weight classes. Thus, the final average mass for the bester hybrid was 272.00 ± 67.14 g; 201.25 ± 59.48 g and 150.70 ± 27.51 g for CM1, CM2 and CM3, respectively, while for BB hybrid we recorded average masses of 298.25 ± 67.72 g, 262.90 ± 49.87 g and 204.20 ± 38.29 g for CM1, CM2, and CM3, respectively. Variability in batches was slightly higher for BB hybrid, with the individual mass variation coefficient (CVM) recording values between 16-22% (higher values being observed for the batches in CM1), while for bester, CVM registered values up to 19% for CM1. Regarding the technological performance of the two hybrids, in the conditions of the flow-through system, it can be said that no notable differences were observed between the SGR values of hybrids from CM1 groups, but slightly higher values of these indicators were registered for small and intermediate classes of BB hybrids. The DGR (daily growth rate) values for CM1 were 2.87 and 2.09 g/ kg/day for the BB hybrid and bester respectively, the values for CM2 were 3.47 for BB hybrid and 2.55 g/kg body weight per day for bester while for CM3 class DGR values were 2.87 g/ kg/ day for BB and 2.09 g/kg/day for bester. It is also noted that in the case of BB hybrids, nutrient efficiency indicators (feed conversion factor-FCR and protein-PER ratio) recorded better values than those of bester hybrids (FCR values of about 15 % smaller). Due to higher variability of backcrossed BB hybrid grading frequency must be established accordingly, with a higher frequency than bester. The faster growth rate recommends BB hybrid for meat production. The haematological indices revealed a stronger health status for BB hybrid comparing with bester held in the same rearing regime.

Keywords: *aquaculture, sturgeon hybrids, genetic variability.*

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COMPARISON OF TWO HISTOLOGICAL APPROACHES FOR ASSESSMENT OF FISH INTESTINAL HEALTH IN NUTRITION TRIAL

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POREĐENJE DVA PRISTUPA U PROCENI HISTOLOGIJE CREVA RIBA U EKSPERIMENTIMA ISHRANE

Apstrakt

U poslednjih 20 godina beleži se ekonomski rast u oblasti akvakulture zahvaljujući uspešnoj industriji hrane za ribe koja obezbeđuje održive izvore proteina kao zamenu ribljeg brašna u hranljivim smešama. Unapređenje formulacija hranljivih smeša i uvođenje novih hraniva su dva najvažnija pitanja na kojima počiva ishrana riba u akvakulturi. U procesu razvijanja novih hrana, ishranaški ogledi su najvažniji korak. Međutim, kada se u hranljive smeše uvedu neodgovarajući sastojci , javljaju se negativni efekti na fiziologiju riba. Poznata je inflamacija distalnog dela creva kod karnivornih riba hranjenih sastojcima biljnog porekla. Kod omnivornih ciprinida takođe se sreću znaci crevne inflamacije različitog intenziteta i trajanja. Stoga je praćenje stanja creva riba tokom ishranaških ogleda najvažniji preduslov i uglavnom se obavlja korišćenjem histoloških metoda. Ukoliko se efekti izazvani hranom ne mogu lako primetiti, evaluacija zdravlja creva radi se histomorfometrijom. Ovakav pristup obično uključuje određivanje dužine crevnih nabora, visine enterocita, visine supranuklearnog prostora, širine *lamina propria* i *tunica mucosa* ili prečnika creva. Dobijeni rezultati su validni, iako je zlatni standard za procenu histoloških preseka stereološko ispitivanje. U ovom istraživanju poredili smo dva pristupa: određivanje (merenje) dužine crevnih nabora (IFL), širine *tunica muscularis* (WTM) i prečnika creva (DI), i korišćenje testne mreže za brojanje (stereološki pristup) na istim presecima sa kojih su merene prethodno pomenute vrednosti. Strukture merene stereološkim pristupom bile su: *lamina epithelialis* (LE), *tunica mucosa* (TMUC), *tunica muscularis* (TMUS) i površina profila tkiva (SUR). U tu svrhu pregledano je 74 histološka preseka creva iz ogleda u kome je šaran (*Cyprinus carpio*) hranjen hranom koja je sadržala sojin proteinski koncentrat poreklom od različitih tipova soje. Za poređenja dve metode korišćen je Pirsonov koeficijent korelacije za svaki procenjeni parametar. Rezultati su pokazali visoke statistički značajne koeficijente korelacije za sve ispitivane parametre:

Koeficijenti korelacije između morfometrijskih i stereoloških parametara

	IFL	WTM	DI
SUR	NT	NT	0.86 (P<0.001)
LE	0.92 (P<0.001)	NT	NT
TMUC	0.92 (P<0.001)	NT	NT
TMUS	NT	0.64 (P<0.001)	NT

NT – nije testirano

Visok koeficijent korelacije je potvrdio validnost morfometrijskog merenja IFL za procenu površine epitela. Sa druge strane vrednosti DI i WTM merene na histološkim presecima treba uzeti sa rezervom budući da su vrednosti korelacije niže.

Ključne reči: *morfometrija, stereologija, hrana za šarana*

Abstract

Growth of aquaculture industry in the past 20 years is supported by feed production and its success in providing sustainable protein sources that could replace fishmeal in feed. Nutrition of fish in aquaculture strongly relies on feed, with improving feed formulations and introduction of novel feed ingredients being two of the most important issues. In the process of developing new fish feed, nutrition trials are the most essential step. Negative effects on fish physiology are often found when inappropriate ingredients are introduced to fish feed. A known example is inflammation of carnivorous fish distal intestine when fed plant-derived feed ingredients. In omnivorous cyprinids signs of inflammation of different intensity and duration are encountered as well. Therefore, monitoring of intestinal health in fish during nutritional trials is a crucial prerequisite and is usually done using histological methods. If the effects caused by fish feed are not easily observed, evaluation of intestinal health is done using histomorphometry. This typically include deriving length of intestinal folds, height of enterocytes, height of supranuclear area, width of *lamina propria*, width of *tunica mucosa* or diameter of intestine. Obtained results are valid, but the gold standard for assessing histological sections is the use of stereological probes. In the present study we compared two methodologies, by deriving length of intestinal folds (IFL), width of *tunica muscularis* (WTM) and diameter of intestine (DI), and from point counting test grid (stereological probe) superimposed over the same histological slides. Structural components quantified via point counting included: *lamina epithelialis* (LE), *tunica mucosa* (TMUC), *tunica muscularis* (TMUS) and complete surface area (SUR). For this purpose, 74 histological slides from nutritional trial where common carp (*Cyprinus carpio*) fed with feed included different origin of soy protein concentrate were used. For comparison of two methods, Pearson product-moment correlation coefficient was calculated for each structural component. Results revealed high and statistically significant correlation coefficients for all parameters that were tested:

Correlation coefficients between morphometrical and stereological parameters

	IFL	WTM	DI
SUR	NT	NT	0.86 (P<0.001)
LE	0.9w2 (P<0.001)	NT	NT
TMUC	0.92 (P<0.001)	NT	NT
TMUS	NT	0.64 (P<0.001)	NT

NT - Not tested

High correlation coefficients confirmed morphometrical measurements of IFL as an estimation of epithelial surface. On the other hand, caution has to be taken when DI and WTM measurements are done in histological sections, due to the lower values.

Keywords: *morphometry, stereological probe, carp feed*

OUT-OF-SEASON ARTIFICIAL REPRODUCTION OF COMMON DACE (*LEUCISCUS LEUCISCUS* L.) UNDER CONTROLLED CONDITIONS

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VEŠTAČKA REPRODUKCIJA KLENIĆA (*LEUCISCUS LEUCISCUS* L.) VAN SEZONE I U KONTROLISANIM USLOVIMA

Apstrakt

Klenić *Leuciscus leuciscus* (L.) je uz druge riblje vrste svrstan u grupu Evropskih rečnih ciprinida. *L. leuciscus* živi u mnogim evropskim rekama najčešće u gornjim i srednjim delovima toka. Poslednjih godina je značajno povećan interes za reofilne vrste (uključujući *L. leuciscus*) (Kupren et al., 2011; Nowosad et al., 2014; Targońska et al., 2015), kao posledica opasnosti od opadanja brojnosti ili čak potpune ekstinkcije u bliskoj budućnosti. Nestanak mesta za mrest izazvan zagađenjem vodene sredine ili regulacijom reka glavni su uzroci ove situacije. Poslednjih godina su zabeležene jake temperaturne fluktuacije u prolećnom periodu kada se ova riba mresti. Istraživanje koje su obavili Nowosad i sar., (2014) pokazalo je da fluktuacije temperature imaju nepovoljan uticaj na efikasnost mresta klenića, uključiv i uspeh ovulacije i stopu preživljavanja embriona. U radu su prikazani rezultati veštačke reprodukcije klenića *Leuciscus leuciscus* (L.) van reproduktivne sezone. Dobijeni rezultati su pokazali mogućnosti razmnožavanja jedinki ove vrste nekoliko nedelja pre sezone mresta. Stimulacija izmenjenim parametrima sredine bila je dovoljna da izazove spermijaciju. Kod ženki je hormonska injekcija bila neophodna da izazove završno sazrevanje ovocita (final oocytes maturation (FOM) i ovulaciju. Konstatovan je visok procenat spermijacije i ovulacije kao i nizak mortalitet među stimulisanim maticama. Rezultati dobijeni u ovom istraživanju mogu se primeniti za optimizaciju procesa proizvodnje nasadnog materijala, kao i ponuditi važnu alatku za očuvanje populacija ugrožene vrste *L. leuciscus*

Ključne reči: *reofilne vrste, temperaturne fluktuacije, stimulacija*

Abstract

Common dace *Leuciscus leuciscus* (L.) together with several other fish species is included in the group of European riverine cyprinids. *L. leuciscus* is present in large areas of Europe populating most frequently the upper and middle courses of rivers. During the recent years the interest in reophilic species (including *L. leuciscus*) has increased significantly (Kupren et al., 2011; Nowosad et al., 2014; Targońska et al., 2015), which was a consequence of the threat of a sure decrease, if not total extinction in the near future, of the local population or disappearance of such populations. The loss of spawning grounds caused by pollution of aquatic environment or regulation of rivers is the major causes of that situation. However, in last years, the strongly temperature fluctuations was noted during spring period when the dace spawning occurs. Study done by Nowosad et al., (2014) showed that temperature fluctuation have negative impact for *L. leuciscus* spawning efficiency, including ovulation success and embryo survival rate. The presents study the results of artificial reproduction of common dace *Leuciscus leuciscus* (L.) conducted outside the reproductive season. The obtained results showed the possibility of reproducing individuals of this species some weeks before the natural spawning season. Stimulation by environmental conditions was sufficient to obtain spermiation. In case of females, hormonal injections were necessary for involving final oocytes maturation (FOM) and ovulation. Generally, a high percentage of spermiation and ovulation as well as low mortality were observed among the stimulated spawners. The data obtained in present study could be applicable in optimization of stocking material production process and, as a consequence, will offer an important tool for conservation tools of *L. leuciscus* endangered populations.

Keywords: *reophilic species, temperature fluctuation, stimulation*

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THE POSSIBILITY OF APPLICATION OF AGGLOMERATE ELASTOMERS (EPP) AS MEDIA FOR BIOLOGICAL BED IN AQUACULTURE

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MOGUĆNOST KORIŠĆENJA SLEPLJUJUĆIH ELASTOMERA KAO MEDIJUMA ZA BIOLOŠKI FILTER U AKVAKULTURI

Apstrakt

U proteklih 50 godina, proizvodnja ribe se naglo uvećala. Do 1990. riba je najčešće lovljena iz okeana, mora, jezera i reka. Trenutno, oko 90 miliona tona ribe i vodenih beskičmenjaka se ulovi godišnji iz pomenutih vodenih tela (FAO, 2014; FAO, 2016). U poslednje vreme, ova cifra jako malo oscilira. Recirkulacioni sistemi za akvakulturu (RAS) su postrojenja za gajenje vodenih organizama, u kojima se otpadna voda ponovo koristi za gajenje, nakon određenih pre-tretmana ili tretmana. Kontrola životne sredine, zajedno sa efikasnim sistemima za prečišćavanje vode i korišćenjem kompletnih hraniva za ishranu ribe je omogućila povećanje proizvodnje, sa smanjivanjem potrebe za vodom. Korišćenjem RAS sistema za pojedine vrste košljoriba, omogućena je redukcija količine vode da bi se odgajio 1 kg ribe, i to sa 30 m^3, koliko je potrebno u konvencionalnim sistemima, do 0.3 m^3, koliko je potrebno u RAS-u. Pored toga, otpad (feces i nepojedena hrana) mogu biti bezbedno uklonjeni iz sistema i dalje korišćeni ili u poljoprivredi ili u proizvodnji bio gasa (Bregnballe 2015). Biološki filteri su obavezan deo RAS-a i njihovo pravilno korišćenje određuje kvalitet vode u sistemu, uključujući odgovarajući nivo azotnih jedinjenja. Amonijak je izuzetno toksičan za ribe (Wicks et al. 2002; Eding et al. 2006; Hurtado and Cancino-Madariaga 2014). Cilj ove studije je bio provera korišćenja eksperimentalnog medijuma (slepljujućih elastomera - EPP) u biološkom filteru, koji ima za cilja da pročisti vodu u RAS-u. RAS omogućava masovnu produciju ribe u maloj zapremini vode u okviru ograničenog prostora. Ova tehnika uključuje mogućnost mnogostrukog korišćenja vode tokom gajenja. Jedan od najjednostavnijih i najefektivnijih načina je kombinovanje dva tipa filtracije vode: mehaničkog i biološkog. Ovo je potrebno da bi se proučavao novi medijum za biološki filter da bi se uklonila azotna jedinjenja iz vode.

Ključne reči: *slepljujući elastomeri, RAS, biološki filter.*

Abstract

For the past five decades, the world production of fish has been rapidly increasing. Until the 1990s, fish were mainly sourced from oceans, seas, lakes and rivers. Currently, approx. 90 million tonnes of fish and aquatic invertebrates are caught in these bodies of water annually (FAO, 2014; FAO, 2016). In recent years, this value has only fluctuated slightly. Recirculating aquaculture systems (RAS) are facilities for farming aquatic organisms, in which the waste water after either pre-treatment or treatment is reused for production. Environmental controls, along with the use of efficient water purification systems and compound feeds for feeding fish enable the increase of production while reducing water demand. In RAS systems for some finfish species, it can be reduce the amount of water used to produce 1 kg of fish up to 0.3 m^3 while for conventional systems, up to 30 m^3 of water is used. Moreover, the emerging waste (faeces and uneaten feed) can be safely separated from the system and e.g. used either in agriculture or in the production of biogas (Bregnballe 2015). Biological filters are an essential element of the RAS and their proper operation determines the quality of water in the system, including the appropriate level of nitrogen compounds. Ammonia nitrogen is highly toxic to fish (Wicks et al. 2002; Eding et al. 2006; Hurtado and Cancino-Madariaga 2014). The aim of the study was to determine the possibility of experimental media (agglomerate elastomers EPP) application as biological media bed, which serves the purpose of water purification in recirculating aquaculture systems (RAS). RAS enables mass-production of fish in small volume of water in a limited area. This involves the possibility of multiple usage of water during culture. One of the simplest and most effective ways to achieve it is combining application of two types of water filtration: mechanical and biological. It is needed to study new media for biological bed with proper filling is able to purify water from toxic nitrogen compounds.

Keywords: *agglomerate elastomers, RAS, biological bed.*

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BACTERIAL INFECTION OF EUROPEAN EEL (*ANGUILLA ANGUILLA* L.) FEMALES UNDER FRESHWATER AND SALTWATER CONDITIONS

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BAKTERIJSKE INFEKCIJE ŽENKI EVROPSKE JEGULJE (*ANGUILLA ANGUILLA* L.) U USLOVIMA SLATKE I SLANE VODE

Apstrakt

Od 1980 se primećuje drastično smanjenje populacije Evropske jegulje (Dekker, 2003; Freyhof and Kottelat, 2010) koja je sada prevazišla sigurnosne granice za ovu vrstu (Amilhat et al., 2014). Od 2008 vrsta Evropska jegulja se smatra "kritično ugroženom" i uključena je na Crvenu listu Ugroženih vrsta međunarodne Unije za zaštitu prirode, Union for Conservation of Nature (IUCN). Jedan od problema tokom veštačke indukcije polne zrelosti ženki Evropske jegulje (*Anguilla anguilla* L.) u kontrolisanim uslovima je smrtnost uglavnom usled baterijske infekcije. Cilj ove studije je bio izolovanje bakterijskih sojeva iz 2 tipa nekrotičnih promena na koži ženki tokom veštačkog sazrevanja u dva različita okruženja/ životne sredine: slatkoj i slanoj void. Izolovano je dve stotine i četrdeset sojeva bakterija iz nekrotičnih promena iz obe vodene sredine: slatke i slane. Bakterije su izolovane na univerzalnim i selektivnim medijumima, bojenje, mikroskopsko posmatranje, testovi rezistencije na lekove, API test, 16S rDNA-PCR i sekvencioniranje. U slatkoj void je konstatovan veći broj i diverzitet bakterija nego u slanoj vodi. Psihrofilni bajterijski slojevi su bili dvostruko brojniji nego u slanoj vodi, naročito oni izolovani iz oblasti gde je injektovan čip (više od 10^{10} cfu/mL).

Sekvencioniranje ribozomalnih gena iz jedne cfu iz slatke vode ukazao je na prisustvo 20 osnovnih rodova bakterija, između ostalih: *Pseudomonas* sp., *Comamonas* sp., *Escherichia* sp., *Aeromonas* sp., *Citrobacter* sp., *Burkholderia* sp., *Pantoea* sp., *Enterobacter* sp., *Yersinia* sp., *Edwardsiella* sp., *Serratia* sp., *Bordetella* sp., *Morganella* sp. i *Chromobacterium* sp. U slanoj vodi je uočeno prisustvo 5 rodova bakterija među kojima: *Pseudomonas* sp., *Aeromonas* sp., *Yersinia* sp., *Vibrio* sp., *Edwardsiella* sp. U slatkoj vodi bakterije su bile rezistentne na 9 lekova. Veliki diverzitet bakterija tokom veštačkog sazrevanja Evropske jegulje u slatkoj vodi ukazuje da je potrebno više pažnje u održavanju aseptičnih uslova tokom svih stadijuma procesa reproducije kao i neophodnost povećanja saliniteta vode čim je pre moguće tokom sazrevanja. Poznavanje patogenih bakterija u ulcerativnim promenama ženki Evropske jegulje i njihove otpornosti na lekove može da pomogne boljem preživljavanju jegulja tokom sazrevanja u kontrolisanim uslovima.

Ključne reči: *veštačke indukcije, bakterijski sojevi, sekvencioniranje*

Abstract

Since 1980, a drastic decline in the population of European eel has been observed (Dekker, 2003; Freyhof and Kottelat, 2010) which has now exceeded the safety limits for this species (Amilhat et al., 2014). In 2008, the European eel species was considered "critically endangered" and was included on the International Union for Conservation of Nature (IUCN) Red List of Threatened Species. One of the problems during artificial induction of sexual maturation of European eel (*Anguilla anguilla* L.) females under controlled conditions is mortality mainly due to bacterial infection. The aim of this study was to isolate bacterial strains from two types of necrotic changes on the skin of females during artificial maturation under two different environment conditions: fresh and saltwater. Two hundred and forty bacterial strains were isolated from specimens originating from necrotic changes in both fresh and saltwater. Bacteria were identified by a plate culture on universal and selective media, staining, microscopic observation, drug resistance testing, API tests, 16S rDNA-PCR and sequencing. There was a greater bacterial number and biodiversity in fresh than in saltwater. Psychrophilic bacterial strains in freshwater were twice as abundant as in saltwater, especially isolated from the mucus from uncreative changes around the chip injection point (up to 10^{10} cfu/mL). Ribosomal gene sequencing of single cfu isolated from the freshwater revealed the presence of twenty main genera of bacteria, among others: *Pseudomonas* sp., *Comamonas* sp., *Escherichia* sp., *Aeromonas* sp., *Citrobacter* sp., *Burkholderia* sp., *Pantoea* sp., *Enterobacter* sp., *Yersinia* sp., *Edwardsiella* sp., *Serratia* sp., *Bordetella* sp., *Morganella* sp. and *Chromobacterium* sp. In saltwater, the presence of five genera of bacteria were found, including *Pseudomonas* sp., *Aeromonas* sp., *Yersinia* sp., *Vibrio* sp., *Edwardsiella* sp. In freshwater, the bacteria were resistant to 9 tested drugs. The high bacterial biodiversity during artificial maturation of European eel in freshwater suggest more attention to aseptic maintenance of all stages of reproduction operation and the necessity to increase the salinity of water as soon as possible during the maturation process. Knowledge of bacterial pathogens in ulcerative changes of European eel females and their resistance to drugs may help increase the survival of eel during the artificial maturation process under controlled conditions.

Keywords: *artificial induction, isolate bacterial, sequencing*

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THE OCCURRENCE OF LARVAL MALFORMATIONS, INCLUDING SIAMESE TWINS, FOLLOWING FERTILIZATION OF POST-OVULATORY AGEING OOCYTES IN IDE (*LEUCISCUS IDUS* L.)

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POJAVA DEFORMACIJA LARVI, UKLJUČUJUĆI SIJAMSKE BLIZANCE NAKON OPLOĐENJA POST-OVULATORNIH OVOCITA KOD JAZA (*LEUCISCUS IDUS* L.)

Apstrakt

Glavni cilj konverzacione akvakulture je restauracija ili poboljšanje populacija riba u prirodnim ekosistemima, najčešće kroz njihov nasad. Nasadni materijal treba da bude dobrog kvaliteta, kondicije i zdravlja. Visoko kvalitetni gameti su zajednički imenilac koji daje visoke stope fertilizacije košljoriba i razvića larvi bez deformacija (Nowosad i sar. 2014; Samarin i sar. 2017). Na embrionalno razviće nićih kičmenjaka utiče mnogo faktora, naročito ako se nalaze izvan majčinog tela i pod uticajem različitih abiotičkih i biotičkih faktora. U ovoj studiji, proučavani su efekti oplođenja post-ovulatornih ovocita jaza (Leuciscus idus L.) na pojavu deformisanih larvi pod kontrolisanim uslovima. Veštačko oplođenje je sprovedeno metodom koju su opisali Targońska i sar. (2012) sa modifikacijom procesa opisanom od strane Kucharczyk i sar. (2016). Ovociti su sakupljeni dva puta od istih ženki (n = 7): neposredno nakon ovulacije (T0) i osam časova nakon prvog prikupljanja jaja (T8). Između sakupljanja ovocita, temperature vode je bila konstantna i iznosila je 12.5°C. Oplođena jaja su inkubirana u Vajsovim inkubatorima na 12.5°C, što je optimalna temperature za razviće ove vrste (Kupren et al., 2011) do izvaljivanja. Analiza deformacija larvi je urađena koristeći Leica MZ16 A stereomikroskop i digitalnom kamerom sa rezolucijom od 5 Mpixel i softverom Leica DFC420 Image Analysis. Potvrđeni su negativni efekti kasnog oplođenja post-ovulatornih ovocita u jajniku na kvalitet potomstva, koji su uzrokovali smanjeno preživljavanje trećeg dana nakon izvaljivanja (68.3% prema 81.2% i 47.2% prema 79.7% za grupe T8 i T0, respektivno) i veća frekvencija brojnih defekta u razviću (19.3% prema 0.8% za grupe T8 i T0, respektivno), uključujući pojavu sijamskih blizanaca. Ovo je prvi put da je uočena pojava sijamskih blizanaca kod jaza. Sijamski

blizanci jaza su izgledali kao jaja sa dve glave i razlikovali su se od "tipičnih" sijamskih blizanaca kod drugih vrsta riba.

Ključne reči: *jaz, sijamski blizanci, deformiteti larvi.*

Abstract

The main goal of conservative aquaculture is to restore or enhance the naturally occurring fish population, most often through restocking. The material to be stocked should be of high quality, condition and health. High quality gametes are the common denominator resulting in high rates of finfish fertilization and larval development without deformation (Nowosad et al. 2014; Samarin et al. 2017). Embryonic development of lower vertebrates can be influenced by many factors, especially when outside of the mother's body and under the influence of variable abiotic and biotic factors. In this study, the effects of fertilization of post-ovulatory ageing (over-matured) oocytes of ide (Leuciscus idus L.) on the possibility of developing anomalies of ide larvae were assessed under controlled conditions. Artificial reproduction was carried out with the method described by Targońska et al. (2012) with the modification of the fertilization process as described by Kucharczyk et al. (2016).

The oocytes were collected from the same females (n = 7) two times: shortly after ovulation (T0) and eight hours after first stripping (T8). Between oocyte collections, the water temperature was a constant 12.5°C. The fertilized eggs were incubated in Weiss jars at 12.5°C, which was optimal for embryonic development for this species (Kupren et al., 2011) until hatching. Deformation analysis in larvae was performed using a Leica MZ16 A stereomicroscope and a digital colour camera with 5 Mpixel resolution for Leica DFC420 Image Analysis. There was a negative effect of delayed fertilization post-ovulatory ageing (over-matured) oocytes in the ovary on the quality of offspring, which caused reduced survival on 3rd DPF and on hatching stage (68.3% vs. 81.2% and 47.2% vs. 79.7% from over-matured and matured groups, respectively) and increased the occurrence of numerous developmental defects (19.3% vs. 0.8% from over-matured and matured groups, respectively), including the development of Siamese twins. This is the first reported case of the occurrence of Siamese twins in ide. The ide Siamese twins look like hen eggs with two heads and are different from "typical" Siamese twins in fish.

Keywords: *ide, Siamese twins, larval deformations.*

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HANDBOOK

PRIRUČNIK

STATE-OF-THE-ART IN ZANDER (*SANDER LUCIOPERCA*) ARTIFICIAL REPRODUCTION AT NAIK HAKI INSTITUTE

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SAVREMENA TEHNOLOGIJA VEŠTAČKOG MRESTA SMUĐA (*SANDER LUCIOPERCA*) NA INSTITUTU NAIK HAKI

UVOD

Veštački mrest smuđa (*Sander lucioperca*) je tema obimnog istraživanja u poslednjih par decenija (Zakęś i Szczepkowski, 2004; Rónyai, 2007; Křišt'an i sar, 2013). Kakogod, tek od skora je otvoreno novo poglavlje istraživanja – reprodukcija gajenih matica naviknutih na koncentrovanu hranu (Zakęś i sar., 2013; Ljubobratović i sar., 2017). Iako su rezultati sa divljim maticama bili prilično obećavajući, uskoro je postalo jasno da je gajenim maticama potrebna posebna pažnja. Zbog toga, skorašnje istraživanje na institutu NAIK HAKI bilo je upućeno ka prilagođavanju tehnologije u skladu sa osetljivošću gajenih matica i unapređenju praktičnog aspekta metodologije radi smanjenja fizičkog rada i rukovanja ribom. Cilj ovog članka je da opiše metodologiju veštačkog mresta matica smuđa gajenih u spoljašnjim uslovima.

Priprema matica za veštački mrest

Postoje dva osnovna tipa veštačkog mresta matica gajenih u spoljašnjim uslovima, sezonski i vansezonski. Sezonska reprodukcija podrazumeva hormonsku indukciju tokom perioda prirodnog mresta kada je većina ženki započela završno sazrevanje ovocita (FOM – eng. final oocyte maturation), dok mužjaci spermiraju. U ovom slučaju, matice su obično injektirane pri transportu u mrestilište i nadalje održavane na temperaturi mresta od 12 °C. Ženke se injektiraju ili sa 500 IU kg^{-1} (internacionalne jedinice – eng. international units) humanog horionog gonadotropina (hCG – eng. human chorionic gonadotrpin) ili sa 50 µg kg^{-1} lososovog gonadotropin-oslobađajućeg hormona (sGnRHa – eng. salmon gonadotropin releasing hormone analog), dok se mužjaci injektiraju sa 250 IU kg^{-1} humanog horionog gonadotropina.

Kakogod, u slučaju vansezonskog mresta, koji se obično obavlja u januaru i februaru, pri spoljašnjim temperaturama 0-6 °C, toplotna priprema je neophodna da bi se dobili gameti. Trenutno se izvode dve metode vansezonskog rukovanja maticama na institutu NAIK HAKI:

1. Po transportu, matice se smeštaju u mrestilište na temperaturu vode sličnu spoljašnjim uslovima te se vrši postepeno zagrevanje vode do 12 °C, dnevno po 1 °C. Kada temperatura vode dostigne 12 °C, matice se injektiraju sledećim hormonima:
   Ženke: sGnRHa u dozi 50 µg kg^{-1};
   Mužjaci: hCG u dozi 250 IU kg^{-1}.
   Po injekciji, temperatura se održava konstantno na 12 °C do poslednje ovulacije.
2. Neposredno po transportu, matice se injektiraju sa:
   Ženke: sGnRHa u dozi 5 µg kg^{-1} (početno injektiranje);
   Mužjaci: hCG u dozi 250 IU kg^{-1}.

Nakon injektiranja, matice se smeštaju u mrestilište na temperaturu vode sličnu spoljašnjim uslovima, voda se zatim postepeno zagreva do 10 °C, dnevno po 1 °C, i nadalje se temperatura vode održava konstantnom. Kada ovocite ženki dostignu stadijum pucanja germinalnih vezikula (GVBD – eng. germinal vesicle breakdown), sprovodi se završno injektiranje sa 25 µg kg^{-1} sGnRHa i ovulacija se očekuje nakon 6 sati.

Praćenje završnog sazrevanja ovocita

Prilikom injektiranja, te potom svakog dana, prati se stanje sazrevanja da bi se predvideo dan ovulacije. Ovocite se uzorkuju iz ženkinih gonada pomoću katetera. Cevčice za hranjenje odojčadi veličine CH06 su adekvatne za ovaj zahvat, obično povezane sa špricem 5-10 ml sa gumenim vrhom klipa (Slika 1.). Kraj cevčice se pažljivo postavlja u genitalnu papilu i dalje se polako uvlači u jajovod 4-5 cm. Pažljivo se povlači klip šprica uz istovremeni lagani pokret izvlačenja cevčice dok se ne zapazi prisustvo ovocita u cevčici. Kada je sakupljen uzorak od 20-30 ovocita u cevčici, klip šprica se oslobađa i cevčica se polako izvlači iz papile. Potrebno je posvetiti posebnu pažnju jačini i dužini trajanja povlačenja klipa šprica, da bi se sprečilo usisavanje ovocita u špric. Stoga, kada je uočeno prisustvo dovoljno ovocita u cevčici, pritisak klipa se otpušta. Nakon što se uzorkuju, ovocite se pažljivo rasporede u petri šolji. Odmah nakon stavljanja ovocita u petri šolju, prekrivaju se rastvorom za razbistravanje Serra. Tečnost Serra sadrži 60% alkohola (koncentracije 97%), 30% formalina i 10 % glacijalne sirćetne kiseline.

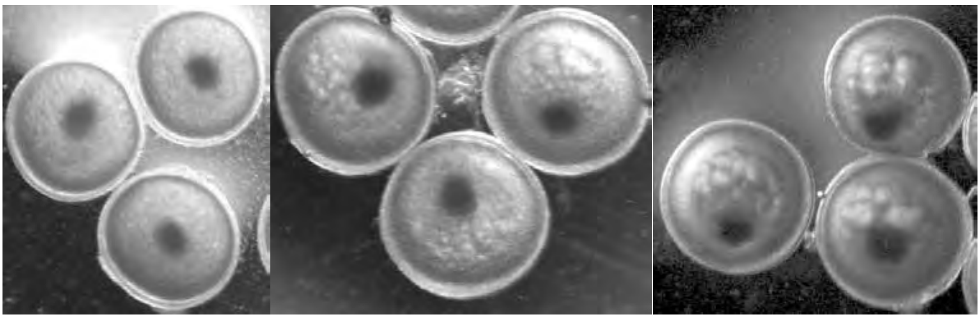
Slika 1. Kateter i špric odgovarajućih karakteristika za biopsiju ovocita smuđa.
Figure 1. Catether and syringe appropriate for oocyte biopsy in zander.

Nekoliko minuta nakon aplikacije sredstva za razbistravanje, ovocite su spremne za procenu statusa završnog sazrevanja ovocita. Za ovu proceduru se obično koriste stereo mikroskopi sa uvećanjem od 20 do 40 puta. Klasifikacija stadijuma konačnog sazrevanja ovocita se vrši po metodi koju su opisali Żarski i sar. (2012.a):

I – germinalna vezikula je centralno pozicionirana; bez vidljivog nakupljanja ulja (Slika 2.a);

II – GV je započela migraciju; opaža se nakupljanje ulja u vidu brojnih sitnih uljanih kapi (Slika 2.b);

III – GV očigledno počinje da migrira; opažaju se 3 do 4 veće uljane kapi (Slika 2.c);

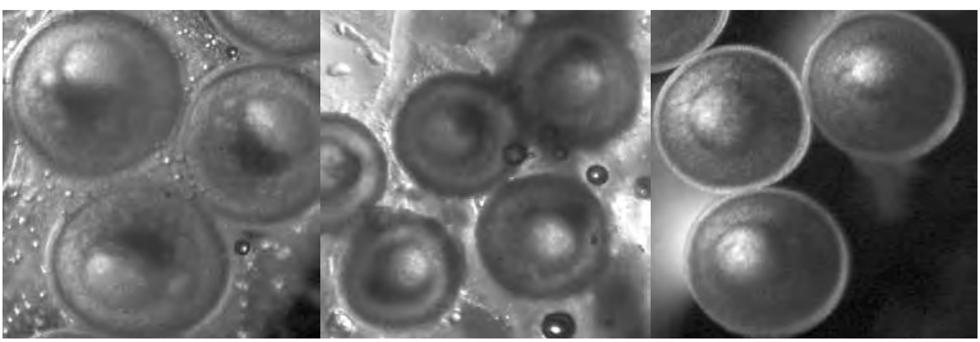


Slika 2. Ovocite u FOM stadijumu I (a), II (b) i III (c); klasifikacija po Żarski i sar. (2012.a);
Figure 2. Oocyte in FOM stadium I (a), II (b) i III (c); classification according to Żarski et al. (2012.a)

IV – GV je otprilike na polovini putanje do animalnog pola; opaža se jedna veća uljana kap, još uvek nepravilnog kružnog oblika, te nekoliko sitnijih uz nju (Slika 3.a);

V – GV je ili veoma blizu ili potpuno na animalnom polu ovocite; prisutna je samo jedna velika uljana kap kružnog oblika; ovocite su prilično bistre (Slika 3.b);

VI – pucanje germinalne vezikule - GVBD; ovocite su potpuno bistre (Slika 3.c).



Slika 3. Ovocite u FOM stadijumu IV (a), V (b) i VI (c); klasifikacija po Żarski i sar. (2012a);
Figure 3. Oocyte in FOM stadium IV (a), V (b) i VI (c); classification according to Żarski et al. (2012a)

Ispitivanje ovocita se obično obavlja u vreme injektiranja hormona. Naredno uzorkovanje zavisi od inicijalnog statusa konačne maturacije ovocita; tako u slučaju stadijuma I, drugo uzorkovanje se vrši 72 časa nakon injektiranja, u slučaju stadijuma II i III, 48 časova nakon injektiranja i u slučaju stadijuma IV i V, 24 časa nakon injektiranja. Pri temperaturama 10-12 °C, u većini slučajeva napredak nije brži od jednog stadijuma po danu. Ipak, dešavaju se slučajevi preklapanja stadijuma, tako u slučaju stadijuma V/VI potrebno je biti posebno oprezan, pogotovo pri temperaturi 12 °C ili višoj.

Sprečavanje spontane ovulacije

Spontana ovulacija i ispuštanje jaja u tank je redovna pojava u slučaju veštačkog mresta percida (Rónyai, 2007; Rónyai i Lengyel, 2010). Zbog toga, da bi se umanjio intenzitet rada i sprečio gubitak jaja, nakon utvrđivanja stadijuma VI u ovocita ženke, genitalna papila se zatvara šivenjem (Żarski i sar.., 2015). Za ovu proceduru, ženka se hirurški anestetizira i postavlja na sto za ceđenje jaja u poziciju trbuhom na gore. Adekvatni šivaći materijal za ovu proceduru sadrži sledeće karakteristike (Slika 4.):

Igla – lučnog oblika ¾ kruga; trougaonog ili kružnog poprečnog preseka; veličine oko 40 mm;

Konac – monofilament (jednonitni), prečnika 1 ili 2 USP (United States Pharmacopea) (4 ili 5 metrički).

Slika 4. Šivaći materijal odgovarajućih karakteristika za prevenciju spontane ovulacije smudja smuđa.

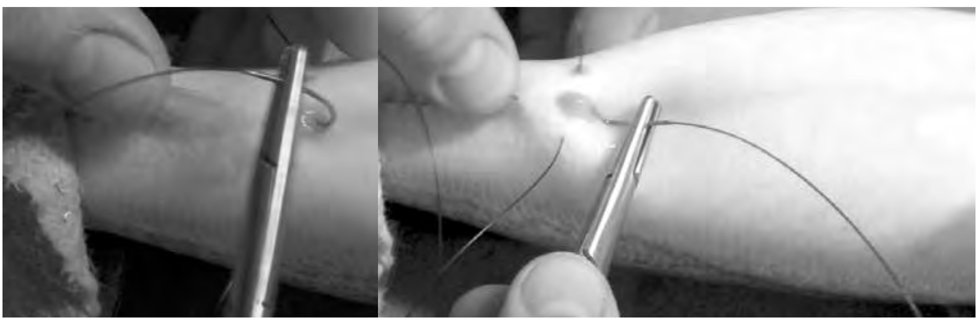
Figure 4. Suture of appropriate characteristics for prevention of spontaneous ovulation in zander.

Procedura šivenja:

1. Iglom ući u telo ribe 5-7 mm od genitalne papile i izaći 7-8 mm od genitalne papile sa druge strane, tako da mesto ulaska i izlaska igle zajedno sa genitalnom papilom formiraju pravu liniju koja je upravna na dužinsku osu ribe (Slika 5.);

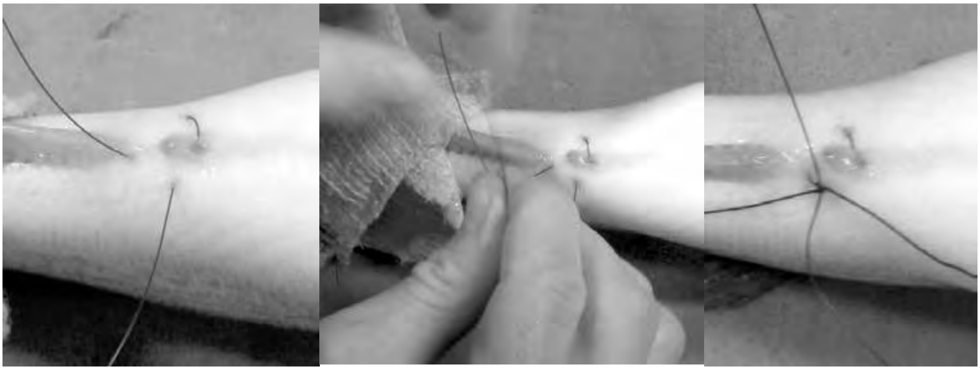
Slika 5. Prvi prolaz iglom pri zašivanju papile smuđa.
Figure 5. First needle penetration for the papilla suture in zander.

2. Povući konac dok ne preostane oko 10 cm ispred mesta ulaska igle;
3. Postaviti iglu direktno u anus ribe, usmerenu ka repu ribe. Nakon što je igla prodrla u telo ribe, postaviti je na gore i izvući između genitalne papile i analnog peraja (Slika 6.);



Slika 6. Drugi prolaz iglom pri zašivanju papile smuđa.
Figure 6. Second needle penetration for the papilla suture in zander.

4. Potpuno izvući iglu i konac i iseći ga na oko 10 cm od mesta izlaska igle (Slika 7.a);
5. Povući oba kraja konca da bi se zategla koža ribe (Slika 7.b);
6. Zavezati jednostavni čvor i držati pritisak na mestu čvora, tokom čega se postavlja parče konca dužine 10cm na čvor, preko koga se vezuje još jedan čvor. Parče konca između dva čvora služi da omogući višestruko otvaranje šava radi procene ovulacije (Slika 7.c).



Slika 7. Završena penetracija iglom i odsečen kraj konca (a); Zatezanje konca pred vezivanje čvora (b); Zavezan čvor sa sigurnosnim koncem između dva čvora (c).
Figure 7. Finished needle penetration and cutten end of the thread (a); Pull of the thread prior to tying the knot (b); Tied know with safety thread between the two knots (c).

Sakupljanje gameta

U slučaju metode indukcije mresta jednim injektiranjem ukupne količine hormona, nakon što je utvrđeno da su ovocite dostigle VI stadijum, ovulacija će se dogoditi u naredna 24 sata. Ipak, najčešće nije moguće predvideti tačan momenat ovulacije. Iako je gubitak jaja sprečen ušivanjem, bazirano na našim opažanjima, jaja smuđa su prilično sklona prezrevanju i gubitku vitalnosti. Zbog toga, nakon što se genitalna papila zatvori, provera se obavlja na svakih 6 sati dok se ne utvrdi ovulacija.

Da bi se proverilo stanje ovulacije, ušivena ženka se hirurški anestetizira i postavlja na sto za ceđenje jaja. Šav se otvori i olabavi, predeo oko papile izmasira da bi se opustilo tkivo i ovulacija se proverava pažljivom masažom stomaka u predelu gonada. Potpuna ovulacija se utvrđuje ispadanjem jaja iz papile u vrpci prečnika nekoliko milimetara. U tom slučaju, riba je spremna za ceđenje jaja (Slika 8.). Ipak, događaju se slučajevi kada se ovulacija utvrdi u ranom stadijumu i isticanje jaja se ne dešava u jasnoj i očigledno širokoj vrpci, nego u tankom sloju jaja pomešanih sa većom količinom ovarijalne tečnosti. U ovakvim slučajevima, papilu ženke je potrebno ponovo zatvoriti i proveriti nakon 3 sata. U slučaju da pri proveri ovulacije iz papile ne teku jaja, čvor se ponovo vezuje i procedura ponavlja nakon 6 sati.

Slika 8. Ceđenje jaja potpuno ovulirane ženke.
Figure 8. Eggs stripping in fully ovulated female.

Kada je utvrđena potpuna ovulacija, jaja se istiskuju u suvu posudu. Nakon završenog istiskivanja jaja, preporučuje se procena kvaliteta na malom uzorku od nekoliko stotina jaja postavljenih ispod mikroskopa (uvećanja 10-40 puta). Nakon aktivacije čistom vodom, dolazi do deformacije jaja dobrog kvaliteta (Slika 9.), kao posledica kortikalne reakcije koja je prilično intenzivna kod smuđa (Żarski i sar., 2012b). Jaja slabe vitalnosti se tokom aktivacije ne deformišu i trebalo bi ih isključiti iz dalje procedure.

Slika 9. Jaja smuđa visokog kvaliteta nekoliko minuta nakon activacije.
Figure 9. Zander eggs of high quality several minutes after the activation.

Nakon što su jaja isceđena, posuda se zatvara poklopcem i odlaže na hladnom mestu (10-15 °C) do oplodnje jaja. Preporučuje se da se oplođenje jaja izvrši u okviru 30 minuta nakon ceđenja.

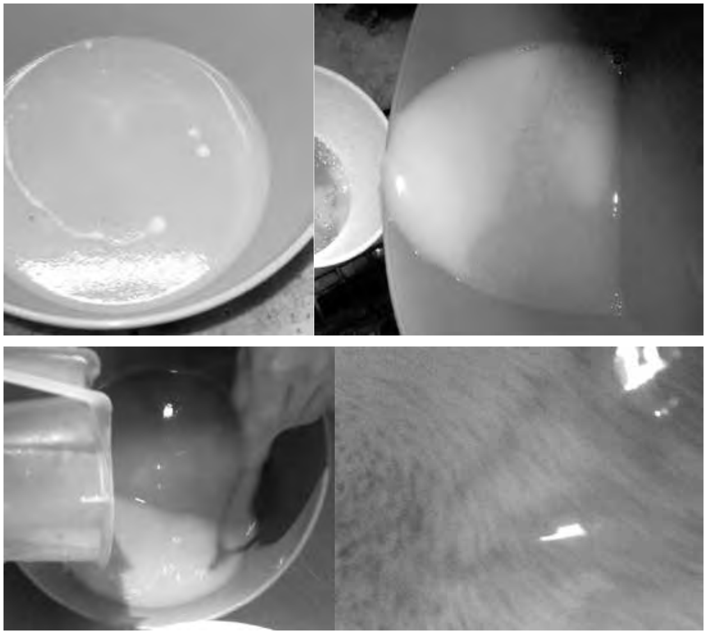
Kada su sakuljena jaja, pristupa se sakupljanju mleči mužjaka (Slika 10.). Mužjaci su blago anestetizirani za ovu proceduru i postavljaju se na sto za ceđenje jaja u položaju trbuhom na gore. Mleč se sakuplja pomoću katetera i u ovu svrhu se koriste cevčice za hranjenje odojčadi, veličine CH05 za mužjake mase 0,8-1,5 kg i CH06 za mužjake čija je masa iznad 1,5 kg. Cevčice se seku u komade dužine 6 cm i postavljaju unutar genitalne papile mužjaka oko 1-1,5 cm. Mleč se istiskuje pažljivom masažom stomaka, i potreban je poseban oprez da se stomak ne pritisne preterano snažno, što bi moglo da dovede do curenja mleča van katetera. Mleč se sakuplja u ependorf tube zapremine 2 ml. U zavisnosti od toga da li će mleč biti upotrebljen odmah nakon sakupljanja ili posle nekoliko sati, može se čuvati pored jaja ili na 4 °C do oplodnje.

Slika 10. Sakupljanje mleči smuđa upotrebom katetera.
Figure 10. Milt collection in zander using catether.

Oplođenje i otklanjanje lepljivosti jaja

Kada su sakupljeni gameti, oplođenje može da počne. Oplođenje kod smuđa se obavlja suvom metodom, gde se suv mleč postavlja na suva jaja (Slika 11.a), a zatim meša sa jajima i dalje aktivira običnom vodom iz mrestilišta. Mleč dvaju mužjaka se koriti za jaja svake ženke, u ukupnoj količini od 0,5-1 ml mleča na 100g jaja. Na početku, mala količina vode se sipa u posudu, toliko da prekrije sloj jaja u visini od nekoliko milimetara. Po sipanju vode, izuzetno je bitno podrobno izmešati jaja i vodu radi osiguranja potpune i efikasne aktivacije. Zatim, svakih pola minuta se u posude dodaje oko 100 ml sveže vode da bi se poboljšala pokretljivost spermatozoida (Boryshpolets i sar., 2009). Dva minuta nakon aktivacije, posuda se puni svežom vodom i jaja se ispiru od neiskorišćenog mleča.

Da bi se uklonila lepljivost, jaja se dalje mešaju u mlečnom rastvoru u trajanju od 30 minuta. Mleko korišćeno u ovu svrhu sadrži 3,5 % masnoće, UHT tipa i kao najefikasnije do sada za ovu proceduru se pokazao proizvod Mizo (Sole-mizo Zrt, Segedin, Mađarska). Proporcija mleka u odnosu na vodu iz mrestilišta u rastvoru je 1:5. Jaja nisu lepljiva u ovom rastvoru i lagano mešanje plastičnom kašikom po dnu posude ili samo konstatno pomeranje posude je dovoljno tokom ove procedure. Prilično široke posude, relativno velike površine ravnog dna se koriste za ovu proceduru. Treba napomenuti da je potrebno uzeti u obzir veličinu posude u odnosu na količinu jaja. Zbog toga, preporučene zapremine posuda su: do 150g ≈ 5l; 100-300g ≈ 10l; 300-1000g ≈ 15-20 l.



Slika 11. Suv mleč na suvim jajima pred mešanje i aktivaciju (a); odstranjivanje mleka od jaja pred konačnu kupku u kaolinu (b); položaj posude pri sipanju prve porcije suspenzije kaolina (c); izgled jaja nakon kupke u suspenziji kaolina i tokom ispiranja (d).
Figure 11. Dry milt on dry eggs prior to mixing and activation (a); milk removal from the eggs prior to final kaolin bath (b); bowl position at the time of pouring in the kaolin suspension (c); eggs appearance after the bath in kaolin suspension and during the wash (d).

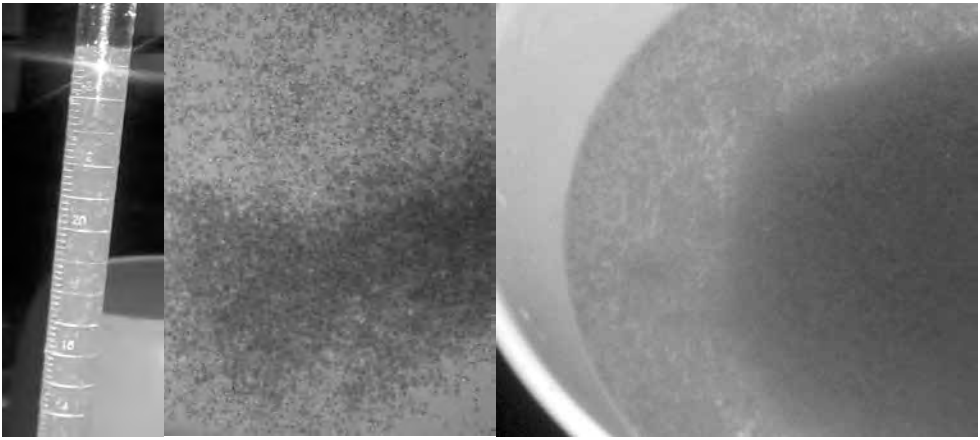
Pola sata nakon aktivacije, rastvor mleka se pažljivo izliva iz posude dok ne ostanu pretežno jaja u rastvoru, na ivici posude (Slika 11.b.). Posuda ostaje u nagnutom položaju sa svim jajima smeštenim uz ivicu posude i tako se spušta na sto. Tada se sipa rastvor gline u jaja. Proizvod korišćen za ovu proceduru je kaolin (Sipőcz Kft., Budimpešta, Mađarska), u koncentraciji 20 ml l^{-1} u svežoj vodi mrestilišta. Ukupno, 0,5 l rastvora se koristi na 100 g jaja. Momenat pre nego što će se dodati jajima, suspenziju kaolina treba dobro promešati zbog veoma brzog taloženja krupnijih čestica gline. Prva porcija suspenzije (30-50%) se sipa dok je posuda u nagnutom položaju sa jajima smeštenim na njenoj ivici i meša se nekoliko sekundi (Slika 11.c). Nakon toga, posuda je postavljena horizontalno na sto i dodaje se ostatak rastvora kaolina. Jaja se mešaju u rastvoru ukupno 2,5 minuta, nakon cega se dodaje čista voda u istoj količini kao rastvor kaolina i jaja se mešaju dodatnih 15-30 sekundi. Zatim, rastvor se ispušta iz posude, pazeći da jaja ostanu na dnu i dodatno ispiranje velikom količinom čiste vode iz mrestilišta se ponavlja (Slika 11.d) dok voda u posudi ne ostane skoro potpuno čista od suspenzije (Slika 12.).

Slika 12. Jaja u inkubatoru nakon odstranjivanja lepljivosti.
Figure 12. Eggs in the jar after the adhesiveness removal.

Inkubacija, valjenje i rano upravljanje larvama

Nakon tretmana odstranjivanja lepljivosti, jaja su spremna za smeštanje u inkubatore tipa "Zuger". Za inkubaciju se obično koriste inkubatori zapremine 2-8 L. Oko 50-100 g jaja se može smestiti po litru zapremine cugera, u zavisnosti od oblika inkubatora. Tokom prvih 24 časa inkubacije održava se prilično slab protok, taman dovoljan da pomera stub jaja ostavljajući liniju površine jaja ravnu, dok se, kako se embrioni razvijaju, protok pojačava da održava jaja adekvatno aerisana. Najveći deo mortaliteta embriona se dešava tokom prva tri dana inkubacije, a mrtva jaja su lako uočljiva zbog beličaste boje i manje težine, te se natalože na površini stuba jaja. Takva jaja se ili usisaju sa vrha stuba jaja ili čiste od ostatka jaja jačim protokom održavanim nekoliko minuta. Od drugog do petog dana posle fertilizacije se obavljaju profilaktičke kupke formalinom u koncentraciji 50 mg l^{-1}, u protoku, jednom ili dva puta dnevno radi sprečavanja gljivičnih infekcija. Temperatura tokom inkubacije iznosi 14-15 °C i presudno je da se održava stabilnom koliko god je moguće. Zasićenost vode kiseonikom se održava između 100 i 110% tokom prvih nekoliko dana inkubacije, a kako se valjenje približava, povećava se na 110 -130 % da bi se sprečilo spontano valjenje larvi.

Sedam do osam dana od početka inkubacije oči embriona postaju jasno pigmentisane i oni su spremni za valjenje. Prema tome, prvog ili narednog dana nakon utvrđivanja pigmentacije očiju, jaja se usisavaju iz inkubatora i izmeri se njihova ukupna zapremina (Slika 13.a). Zasnovano na našim merenjima, oko 400-600 jaja se nalazi u ml i prema tome, merenjem zapremine jaja, može se predvideti ukupan broj larvi. Prosejana jaja se smeštaju u lavore (Slika 13.b) i valjenje se javlja u kratkom roku kao posledica smanjenja koncentracije kiseonika i osvežavanja vode u posudama (Slika 13.c). U većini slučajeva, može se očekivati valjenje iznad 90 % pri korićenju objašnjene metode otklanjanja lepljivosti jaja.



Slika 13. Merenje zapremine jaja spremnih za valjenje (a); jaja sa pigmentisanim očima po smeštanju u lavor za valjenje (b); početak valjenja (c).
Figure 13. Volume measurement of eggs ready for hatching (a); eyed-eggs upon stocking into the hatching bowl (b); initiation of the hatching (c).

Novoizvaljene larve se čuvaju u tankovima sa uzlaznim tokom u maksimalnoj gustini od 1000 larvi po litru tokom endogene faze hranjenja. Osvežavanje vode u tankovima se održava na oko 25-30 % izmene po času, temperatura na 15-16 °C i zasićenost vode kiseonikom na 100-110 %. Održava se prilično nizak (2-5 lux) intenzitet difuzne svetlosti. Pet do šest dana nakon valjenja, larve su spremne za početak egzogenog hranjenja.

Pregled rezultata, perspektive, problema i mogućnosti unapređenja

Koristeći matice proizvedene na institutu NAIK HAKI i uzgajane konstantno u sistemu jezero-u-jezeru, opisana tehnologija omogućava preživljavanje od faze jaja do larvi u opsegu 50-80% u periodu od početka januara do sredine aprila. Proizvodnja po seriji varira od pola miliona do nekoliko miliona larvi. Najstarije matice su mrešćene tri puta, pri čemu je svaki naredni mrest bio uspešniji od prethodnog. Sa jedne tačke gledišta, razlog može biti unapređenje tehnologije, kakogod, zasnovano na dosadašnjim opažanjima, čini se isplativim ne mrestiti matice nakon prvog zimovanja jer njihova sposobnost reprodukcije ima tendenciju da varira unutar grupe.

Trenutno je najveći problem dosadašnje tehnologije mortalitet izmrešćenih ženki, koji varira od 10 do 40%. Obično, mortalitet se pojavljuje dve do osam nedelja nakon mresta i čini se uzrokovan oštećenim imunim sistemom, takođe, do sada nije bilo razlike kod različitih vrsta hormona koji su korišćeni. Najverovatnije će dalja domestikacija dovesti do ublažavanja ove pojave.

Uopšteno, naša metodologija je neprekidno poboljšavana da bi bila praktičnija. Iako se može predvideti dan ovulacije, nemogućnost da se predvidi preciznije dovodi do prekomernog rada, često u noćnim smenama, što je industrijski vrlo neprikladna karakteristika. Takođe, koristeći jednu injekciju gonadoliberina umesto gonadotropina poboljšava se učinak mresta, kakogod varijacija u vremenu latencije raste i obično je potrebno nekoliko dana dok sve ženke u grupi dostignu ovulaciju. Zbog toga, započet je rad na sinhronizaciji ovulacije unutar grupe pripremom riba sa sGnRHa na niskim temperaturama i dosadašnji rezultati nagoveštavaju da se na taj način može celoj grupi indukovati ovulacija u toku jednog dana. Treba dodati i da se zahvaljujući završnoj injekciji noćni rad može isključiti i vreme ovulacije predvideti. Ipak, ova metodologija je na samom početku svog razvoja, sa svega dve eksperimentalne grupe do sada, i zahteva dalja testiranja i poboljšanja pre konačnih zaključaka.

Ključne reči: *smuđ; veštački mrest; praktično uputstvo; biopsija ovocita; gameti.*

INTRODUCTION

Artificial reproduction in zander (*Sander lucioperca*) has been subject of extensive research in last couple of decades (Zakęś and Szczepkowski, 2004; Rónyai, 2007; Křišt'an et al., 2013). Nevertheless, just recently the new issue in this research topic has been opened – reproduction of cultured – feed-eating breeders (Zakęś et al., 2013; Ljubobratović et al., 2017). Although the results in case of wild breeders were rather promising, soon it became clear that cultured breeders need special concern. Therefore, recent research at NAIK HAKI was pointed towards refinement of the technology with respect to sensitivity of cultured breeders and to improvement of practical side of the methodology in order to reduce both labour and fish handling. The aim of this paper is to describe the methodology of artificial reproduction in intensively reared breeders cultured in outside conditions.

Preparation of breeders for artificial reproduction

There are two main types of artificial reproduction in breeders reared in outside conditions, seasonal and off-seasonal. Seasonal reproduction implies hormonal induction during the natural spawning period when most of the females entered the final oocyte maturation (FOM) and males are spermiating. In this case, breeders are usually injected at the time of transport to hatchery and further maintained on the spawning temperature of 12 °C. Females are injected with either 500 IU (international units) of human chorionic gonadotropin (hCG) or 50 μg kg^{-1} of salmon gonadotropin releasing hormone (sGnRHa), while the males are injected with 250 IU of hCG.

However, in case of off-season reproduction, which usually takes place in January and February, at outside temperatures 0-6 °C, thermal preparation is needed prior to obtain the gametes. Presently, two methods of off-season broodstock manipulation are being performed at NAIK HAKI:

1. Upon the transport, breeders are stocked to the water temperature similar to outside conditions and gradually heated up to 12 °C by 1 °C per day. Once the temperature reaches 12 °C, breeders are injected with following hormonal doses:
   Females: sGnRHa of 50 μg kg^{-1}
   Males: hCG 250 IU kg^{-1}
   Upon injection, temperature remains stable at 12 °C until the last ovulation.
2. Upon the transport, breeders are injected with:
   Females: sGnRHa of 5 μg kg^{-1} (priming injection)
   Males: hCG 250 IU kg^{-1}

Upon injections, water is gradually heated up to 10 °C by 1 °C per day, and further kept stable. Once the female's oocytes reach germinal vesicle breakdown (GVBD) stage it is resolved with 25 μg kg^{-1} sGnRHa and ovulation is expected in 6 hours.

Monitoring of FOM

At the time of injection and afterwards daily, status of maturation is being followed in order to predict the day of ovulation. Oocytes are collected from female's gonads by catheterisation. Infant feeding tubes of size CH06 are appropriate for this operation, usually attached on the 5-10 mL syringe with rubber piston (Figure 1.). End of the tube is gently placed into the genital papilla and further slowly inserted into oviduct for 4-5 cm. Gentle pull of the plunger followed with slow movement of the tube towards out is being performed until the oocytes are visible inside the tube. Once the sample of 20-30 oocytes is in the tube, plunger is released and tube is slowly pulled out of the papilla. Special caution needs to be given to the intensity and duration of plunger pull in order to prevent oocytes to be suctioned into the syringe. Therefore, once the sufficient amount of oocytes is visible in the tube, the pressure on the plunger is released. Once the oocytes are collected, they are gently disposed into the petri dish. Immediately upon placement of oocytes into the petri dish, they are being washed and covered with clarifying solution Serra. Serra solution is consisted of 60% alcohol (concentration 97%), 30 % formalin and 10% of glacial acetic acid.

Few minutes after the placement of clarifying solution, oocytes are cleared and ready for the evaluation of the FOM status. For this procedure, stereo microscopes with 20-40 times magnification are usually used. Classification of FOM stage is being evaluated according to Żarski et al. (2012a):

I – germinal vesicle (GV) is centrally positioned; no visible oil aggregation (Figure 2.a);

II – GV is started to migrate; visible oil aggregation in form of numerous small oil droplets (Figure 2.b);

III – GV clearly started to migrate; there are visible 3 to 4 bigger oil droplets (Figure 2.c);

IV – GV is around half way of migration to animal pole; one large oil droplet is visible of still not fully circle shape and several smaller droplets next to it (Figure 3.a);

V – GV is either close to or totally on the animal pole of the oocyte; only one large circle shape oil droplet is present; oocytes are being rather transparent (Figure 3.b);

VI – GVBD; oocytes are totally transparent (Figure 3.c).

Biopsy of oocytes is usually performed at the time of hormonal injection. Following time of sampling depends on the initial FOM status, thus in case of stage I, the oocytes are second time collected 72h post-injection, in case of stages II and III 48h post-injection and stage IV and V 24 hours post-injection. On temperature 10-12 °C, in most of the cases progress is not faster than one stage per day. Nevertheless, there are cases of overlapping stages, thus in case of stage V/VI special caution should be taken, especially on temperatures 12 °C or more.

Prevention of the spontaneous ovulation

Spontaneous ovulation and eggs disposal in the tank is a common feature in case of artificial reproduction in percid fish (Rónyai, 2007; Rónyai and Lengyel, 2010). Therefore, in order to reduce the labour intensity and prevent loss of eggs, once the female's oocytes are found to be in stage VI, genital papilla is being closed by suture (Żarski et al., 2015).

For this procedure, female is surgically anaesthetized and placed on the stripping table on the belly up position. The appropriate suture for this procedure is of the following characteristics (Figure 4.):

Needle – curved ¾; reverse cutting or round bodied; size around 40 mm;

Thread – monofilament; 1 or 2 USP (United States Pharmacopea) (4 or 5 metric).

Suture procedure:

1. Enter the body 5-7 mm from the genital papilla and exit on other side 7-8 mm from the genital papilla in such manner that the entering, exiting point and papilla are in the straight line which is making straight angle with the length axis of the fish (Figure 5.);
2. Take out the thread until there is around 10 cm left before the entrance point;
3. Place the needle straight into the anus of the fish, pointing towards the tail. Once the needle is in the body of the fish, reverse it up and exit between genital papilla and anal fin (Figure 6.);
4. Take out the needle and thread totally and cut it around 10 cm after the exit (Figure 7.a);
5. Pull the both ends of the thread in order to tight the skin (Figure 7.b);
6. Tie one simple knot and while keeping the pressure on the not, place the 10cm long piece of thread on the knot and tie additional knot. Piece of the thread between the two knots is to enable multiple opening of the suture to evaluate the ovulation (Figure 7.c).

Collection of gametes

In case of single injection, once the female's oocytes are found to be in stage VI, ovulation will occur in the following 24 hours. Nevertheless, usually it is not possible to predict the exact moment of the ovulation. Although the loss of the eggs is prevented by the suture, based on our observations zander eggs are rather prone to overripe and loose its viability. Therefore, once the genital papilla is closed, ovulation checks are being done in each 6 hours until the ovulation is recognized.

In order to examine the ovulation, sutured female is being surgically anaesthetized and placed on the stripping table. Suture is opened and loosen, area around papilla massaged in order to relax the tissue and ovulation is checked by gentle massage of the gonad area of fish belly. Full ovulation is recognized by the eggs flowing out of the papilla in a several millimetres thick string. In such case, fish is ready to be stripped (Figure 8.). Nevertheless, there are occasions when the ovulation is recognized at the rather early stage, and flow off the eggs is not in a clearly visible thick string, but rather flowing in a thin layer of eggs with lot of ovarian fluid. In such cases, the female should be closed back and checked again in 3 hours. In case there are no eggs flowing out of the papilla, knot is tied back and procedure is repeated in 6 hours.

Once the full ovulation is noticed, eggs are stripped in the dry container. Upon collection of eggs, it is advisable to assess its quality on a small sample of few hundred of eggs placed under microscope (10-40 magnification). Upon activation with pure water, good quality eggs are being deformed (Figure 9.), as a consequence of cortical reaction which is rather aggressive in case of zander (Żarski et al., 2012b). Eggs of low viability are not being deformed upon activation and should be excluded from the further procedure. Once the eggs are stripped, the container is closed with the lid and placed on the cool place (10-15 °C) until fertilisation. It is advisable to fertilise the eggs within 30 minutes after stripping.

Upon collection of eggs, milt is being collected from the males (Figure 10.). Males are slightly anaesthetized for this procedure and placed on the stripping table in belly up position. Sperm is collected by catheter and for this purpose the infant feeding tubes are used, size CH05 for males 0.8-1.5 kg and CH06 for males above 1.5 kg. The tubes are cut on the pieces 6 cm long and placed into male's genital papilla, 1-1.5 cm deep. Milt is being stripped by the gentle massage of the belly, and special caution is needed in order not to press the belly with excessive force which would lead to milt coming out aside from catheter. Sperm is collected into the 2ml eppendorf tubes. Depending whether the sperm will be used immediately upon collection or in several hours, it can be stored next to the eggs or at 4 °C until fertilisation.

Fertilisation and egg adhesiveness removal

Once the gametes are collected, fertilisation can be initiated. Fertilisation in zander is carried out on dry method, where the dry sperm is placed on the dry eggs first (Figure 11.a), then mixed around the eggs and further activated with plain hatchery water. Milt of two males is used for eggs of each female in total amount of 0.5-1 mL of sperm per 100g of eggs. Initially, the small amount of water is placed inside the bowl, to overlap the eggs layer by few millimetres. Once the water is poured in, it is very important to properly mix the eggs and water in order to secure the full and effective activation. Further on, every half minute around 100 mL of fresh water is added to the bowls in order to enhance the sperm motility (Boryshpolets et al., 2009). Two minutes after activation, bowl is filled with fresh water and eggs are washed of unused sperm.

In order to eliminate the adhesiveness, eggs are further being washed in milk solution for 30 minutes. Milk used for this purpose is 3.5% fat content, UHT type and the most effective type found so far for this procedure is the product of Mizo (Sole-mizo Zrt, Szeged, Hungary). Ratio of milk to hatchery water in the solution is 1:5. Eggs are not adhesive in this solution and either the gentle stirring the bottom of the container with spoon or just the slow constant shaking of the bowl is sufficient movement during this procedure. Rather wide, flat bottom bowls (washbowls) are used for this procedure with relatively large bottom surface. However, the size of the bowl compared to amount of the eggs should be taken into consideration for this procedure. Therefore, suggested volumes of bowls/washbowls are: up to 150 g ≈ 5 L; 100-300 g ≈ 10 L; 300-1000 g ≈ 20 L.

Half hour after activation milk solution is carefully poured out from the bowl until there are mainly eggs staying in the solution on the edge of the bowl (Figure 11.b). Bowl remains in banded position with all the eggs placed in the corner of the bowl and as such put on the table. Then the clay solution is poured into the eggs. Product used for this procedure is kaolin (Sipőcz Kft., Budapest, Hungary) in the concentration 20 mL L^{-1} in fresh hatchery water. In total, 0.5 L of solution is used per 100 g of eggs. Just prior to addition to the eggs, kaolin suspension should be properly mixed due to rather fast sedimentation of the larger clay particles. First batch of suspension (30-50%) is poured while the bowl is in the banded position with eggs placed in its corner and it is stirred for several seconds (Figure 11.c). Afterwards the bowl is placed horizontally on the table, and the rest of the kaolin solution is poured into the eggs. Eggs are stirred in the solution for in total 2.5 minutes, when the water is added in the same amount as the kaolin solution and mixed for additional 15-30 seconds. Further, solution is poured out of the eggs taking care that the eggs remain on the bottom and washing with lot of clean hatchery water is done several times (Figure 11.d) until the water remains by and large clear of the suspension (Figure 12.).

Incubation, hatching and early larvae management

Following the de-adhesion treatment, eggs are ready to be stocked in the incubation jar. Zug jars of 2-8 L volumes are usually used for the incubation. Around 50-100g of eggs can be stocked per litter of volume of the Zug jar, depending on the jar’s shape. During the first 24 hours of incubation very gentle flow is maintained, just enough to move the column of eggs leaving the eggs’ surface-line flat, while as the embryo develops, flow is being increased to keep the eggs properly aerated. Most of the embryonic mortality takes place during the first three days of incubation and dead eggs are rather visible by whitish colour and lower weight thus being sedimented on the top of the eggs column. Such eggs are either sieved of the top of the eggs column or are cleaned out of the rest of the eggs with stronger flow kept for several minutes. From second to fifth day post-fertilisation, prophylactic baths with 50 mg L^{-1} formalin in a flow-through mode are done once or twice per day in order to prevent the fungal infections. Temperature used for incubation is 14-15 °C, and it is crucial to keep it as stable as possible. Oxygen is maintained between 100-110% during the first several days of incubation and as the hatching approaches it is increased to 110-130% in order to prevent spontaneous hatching of the eggs.

After 7-8 days of incubation eyes of the embryos are getting visibly pigmented and ready to hatch. Thus either at the first day of eye pigmentation or the day after, eggs are being siphoned out of the incubator and their volume measured (Figure 13.a). Based on our measurements, there are around 400-600 eggs per mL and thus, measuring the volume of the eggs, the total number of larvae can be predicted. Siphoned eyed-eggs are placed in the bowl (Figure 13.b) and hatching occurs shortly upon as a consequence of reduction of oxygen koncentration and water refreshment in the bowls (Figure 13.c). In most of the cases more than 90 % hatching can be expected using the explained egg de-adhesion method.

Newly hatched larvae are kept in up-welling flow tanks with maximal density of 1000 larval L^{-1} during the endogenous feeding phase. Water exchange in the tank is kept at about 25-30 % exchange per hour, temperature at 15-16 °C and water oxygen saturation at 100-110%. Rather low (2-5 lux) light intensity in dispersed manner is maintained. Five to six days after hatching larvae are ready for start of exogenous feeding.

Overview on the results, prospects, concerns and improvement possibilities

Using the broodstock produced at the site of NAIK HAKI and reared constantly in pond-in-pond system described technology leads to survival from eggs to larvae in range 50-80% in period from early January to mid-April. Production per batch ranges from half to several millions of larvae. The oldest broodstock has been propagated for three times with each reproduction being more successful than previous. From one side it might be due to technology improvement, however, based on so far observations it seems worthwhile not to reproduce breeders after first wintering, as their reproduction performance tends to be variable inside the batch.

Presently main concern with so far technology is post-spawning mortality in females which ranges from 10 to 40%. Usually, mortality occurs from two to eight weeks after propagation and seems to be caused by impaired immune system and so far there were no differences between the applied hormones. Most likely, further domestication will lead to the reduction of this phenomenon.

In general, our methodology was constantly enhanced in order to be more practical. Although the day of ovulation can be predicted, inability to predict more precisely leads to excessive work often in night shifts which is rather repellent feature for the industry. Additionally, using the single injection of gonadoliberins instead of gonadotropins reproductive performance improves, however the variation in latency time expands and usually takes several days for whole batch of females to ovulate. Therefore, the work has been initiated to synchronize the ovulation inside the batch, with priming the fish with sGnRHa on low temperatures and so far results are suggesting that on such way the whole group can be induced to ovulate within one day. Additionally, due to resolving injection, the night work can be excluded and the ovulation time predicted. Nevertheless, this work is at the very beginning with so far two experimental batches and needs further tests and refinements prior to final conclusions.

Keywords: *zander; artificial reproduction; practical manual; oocyte biopsy; gamets.*

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