



Second NACEE Conference of Young Researchers

PROCEEDINGS

**Research Institute for Fisheries, Aquaculture and Irrigation
Szarvas
2010**



Second NACEE Conference of Young Researchers

Szarvas, Hungary, 30-31 August 2010

**Research Institute for Fisheries, Aquaculture and Irrigation
Szarvas
2010**

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PROGRAMME

29 August 2010, Sunday

09.00-10.00 Arrival, transfer to Szarvas

19.00 Welcome dinner

30 August 2010, Monday

09.00-09.30 Registration

09.30-09.50 ***Opening speech***
Status and trends of European aquaculture
Dr. László Váradi, director general
Research Institute for Fisheries, Aquaculture and Irrigation

Keynote presentations

09.50-10.10 Determining factors of per capita seafood consumption in Central
and Eastern Europe
Gergő Gyalog, László Váradi

10.10-10.30 The Network of Aquaculture Centres in Central and Eastern
Europe (NACEE): the way forward
Péter Lengyel

Section I – Fish health, genetics, molecular biology

10.30-10.50 Immune response of genetically different common carp (*Cyprinus
carpio* L.) variants against *Aeromonas hydrophila*
**László Ardó, Galina Jeney, Alexandra Adams, Zsigmond
Jeney**

10.50-11.10 Distribution and residue depletion of some veterinary drugs in
common carp following different routes of administration
Zsuzsanna J. Sándor, Zsuzsanna Gy. Papp, István Csengeri

- 11.10-11.30 Development of a genetic certificate for Belarussian selection breeds of common carp
Aksana Koneva, Sergey Dromashko
- 11.30-11.50 Reintroduction of the Poljana and Nasice common carp strains to their farms of origin with the support of molecular biological methods
István Lehoczky, Tea Tomljanović, Duška Ivelić, Ines Jablan, Tomislav Treer, János Bakos, Zsigmond Jeney

11.50-12.05 Coffee break

Section II – Reproductive biology

- 12.05-12.25 Reproductive biology of the channel catfish (*Ictalurus punctatus* Raf.) reared in net cages in the cooling waters of a thermoelectric power plant
Ivan Iliev, Tania Hubenova, Angel Zaikov
- 12.25-12.45 Morphometric characteristics of oocytes and ultrastructure of their envelopes in Sakhalin sturgeon *Acipenser mikadoi*
Alexandra Novosadova, Ekaterina Mikodina, Andrey Presnyakov, V.E. Khrisanfov
- 12.45-13.05 Dependence of *Coregonus* fish sperm motility duration on temperature
Natalya Smeshlivaya, Sergey Semenchenko

13.05-14.00 Buffet lunch

Section III – Technology

- 14.00-14.20 Possibilities of rearing paddlefish (*Polyodon spathula*) fry in protected conditions – Hungarian experiences
Balázs Kucska, Tibor Feledi, András Rónyai
- 14.20-14.40 Effect of spawning practice and water temperature on the mass production and quality of larvae in perch (*Perca fluviatilis*)
Tomáš Policar, Jiří Křišťan, Vlastimil Stejskal, Jan Kouřil
- 14.40-15.00 The peculiarities of large European perch cultivation in the South of Russia
Fedorovykh, Yuliya
- 15.00-15.15 Coffee break

Section IV – Ecology and fisheries

- 15.15-15.35 Nutrient reusing capacity of a combined pond aquaculture system
Dénes Gál, Éva Kerepeczki, Tünde J. Kosáros, Ferenc Pekár
- 15.35-15.55 Aquaculture effluent treatment in constructed wetlands
Éva Kerepeczki, Dénes Gál, Tünde J. Kosáros, Réka Hegedűs, Gergő Gyalog, Ferenc Pekár
- 15.55-16.15 Periphyton utilisation in aquatic ecosystems – improvement of fish production and water treatment
Tünde J. Kosáros, Ferenc Pekár, Dénes Gál, Gyula Lakatos
- 16.15-16.35 The status of mollusc catches in the last decade over the Black Sea area
Iulian Oancea, Stefan Mihai Petrea, Isabelle Metaxa
- 16.35-16.45 Coffee break

Section V – Poster section

- 16.45-17.45 Application of vegetal feed supplements in intensive common carp production
Tibor Feledi, András Rónyai, Dénes Gál, Tünde J. Kosáros, Ferenc Pekár, István Csengeri
- Toxicity study of selected phthalate esters in *Sander lucioperca* L.
Sylwia Jarmołowicz, Krystyna Demska-Zakęs, Zdzisław Zakęs, Konrad Partyka
- Intensive rearing of pike (*Esox lucius*) in illuminated cages
Balázs Kucska, Tibor Feledi, András Rónyai
- Development of a multifunctional fish culture system
Balázs Kucska, Tibor Feledi, András Rónyai, Gyula Borbély
- Biological features of isolates of spring viraemia of carp virus (SVCV) isolated in different regions of Ukraine
Nataliya Kharkavliuk, Yuriy Rud, Nataliya Matvienko
- The zooplankton food resources in the fish farming in the Republic of Moldova
Liubovi Lebedenco

Selective breeding of common carp (*Cyprinus carpio*) in Serbia
**Zoran Marković, Kari Kolstad, Vesna Poleksić, Milan Spasić,
Marko Stanković, Ivana Živić, Zorka Dulić, Božidar
Rašković**

19.00- Dinner

31 August 2010, Tuesday

09.00-12.00 Visit to HAKI facilities (laboratories, intensive African catfish farm)

12.00-13.00 Lunch

13.00 Sight-seeing in Szarvas (visit to the Arboretum, boat trip on River Körös)

19.00 Farewell dinner

1 September 2010, Wednesday

Departure

ABSTRACTS

Status and trends of European aquaculture

László Váradi

Research Institute for Fisheries, Aquaculture and Irrigation, Hungary

Abstract

Although the contribution of Europe to the global aquaculture production is only about 4 percent, the European aquaculture is considered a world leader in the production of some high value species (e.g. salmonids, sea bass, sea bream, turbot) and contributes significantly to the global aquaculture development through knowledge and technology transfer. The European market is increasingly dependent upon imports, some 1.65 million tonnes of farmed seafood products were imported into Europe in 2008. Harvest and post-harvest services are significant components of the aquaculture industry,.

The first European Union strategy for sustainable aquaculture development was developed in 2002, while in 2009, the European Commission launched a new initiative “Building a sustainable future for aquaculture. A new impetus for the Strategy for the Sustainable Development of European Aquaculture”, aiming to make EU aquaculture more competitive, ensure sustainable growth and improve the sector's image and governance.

EU legislation covers all stages of the production, processing, distribution and placing on the market of food. Special attention is devoted to labelling of fishery and aquaculture products, traceability, fair trade, animal welfare and environmental impacts such as protection of overexploited fish stocks, food miles and more locally sourced products. The markets have responded to this with voluntary certification and labelling schemes operated on a trans-national basis.

While Europe as a whole enjoys a rich aquaculture research environment, it is very diversified and fragmented between public and private institutes, higher education establishments and private companies. There is a considerable overlap in research programmes and dissemination and the diversity of language is a barrier to communication and cooperation, as well as to the uptake and application of research results. In 2000, the EU created the European Research Area (ERA), creating a unified area across Europe, to address these issues. European organisations have considerably increased their cooperation in recent years to address some of the above issues. The European Fisheries and Aquaculture Research Organisation (EFARO) brings together 23 research institutes in 19 European countries and the Network of Aquaculture Centres in Central and Eastern Europe (NACEE) now consists of 45 institutes from 15 countries.

Inter-regional cooperation is generally less advanced, although new initiatives are addressing cooperation between Europe and other aquaculture regions. A pan-European education network that is a good model for other regions is AQUA-

TNET, the European thematic network in the aquaculture, fisheries and aquatic resource management sector, bringing together more than 100 partners from almost every EU member country, as well as associated partners from other countries outside of Europe. Dissemination has also developed, including consumer organisations in Europe, and the criteria for evaluating research proposals made in EU calls put significant weight on technology transfer and training issues.

An important recent development has been the establishment of the European Aquaculture Technology and Innovation Platform (EATIP) for a better dialogue – based on best governance practices – between the aquaculture industry, the research community, consumers and policy makers, focusing on exploiting the potential for innovation and technological development in the European aquaculture value chain.

Increasing competition for resources with other economic activities (urbanization, agriculture, industry, tourism, nature conservation, environmental protection, etc.) is probably the major challenge for the further development of European aquaculture.

The future success of the modern, professional European aquaculture sector may increasingly be dependent on the availability of high-quality services in seed and feed supply and also in veterinary support and services. There will also be an increasing need for special services such as financing, insurance, public relations and skill management. Significant variability may also be expected in terms of the scope and rate of future aquaculture development in individual countries or sub-regions of Europe, which will depend mainly on socio-economic realities in those countries or sub-regions. New aquaculture technologies, such as offshore systems, recirculating aquaculture systems or integrated production systems provide opportunities for development, although these are unlikely to represent the majority of European production systems in the next decade.

The efforts made by the production sector are indeed significant, but the average European citizen, keen to consume healthier seafood, is not aware of these and has a somewhat a priori negative image of aquaculture, often due to ignorance of whether the product bought is actually farmed or lack of access to balanced information about how it is produced. The frequent opposition to the development of new production sites will probably not decrease and efforts to explain the significance of a local production of certified high-quality aquatic products in close proximity to European markets will be of the highest importance.

The last decade has seen significant advances in the interrelations between European organisations involved in aquaculture and most notably in initiatives that have sought to involve consumer organisations, both at European and national level. Aquaculture products face stiff competition with beef, pork and poultry, although the sector has grown the most over the last decade. Better communication within the value chain and towards consumers will be of high importance in a seafood sector where European products fill a relatively high-priced niche position compared to other aquaculture products and other animal protein sources.

Determining factors of per capita seafood consumption in Central and Eastern Europe

Gergő Gyalog and László Váradi

Research Institute for Fisheries, Aquaculture and Irrigation, Hungary

Abstract

Although it is commonly thought that income, degree of urbanization and share of coastal population positively affects, while terrestrial meat consumption negatively affects fish consumption, it is not true in Central and Eastern Europe as per capita seafood consumption in the post-Soviet states is markedly higher than in new EU members of the region and in Balkan countries.

Statistical analysis of fish consumption in the members of the European Union and other Western European states shows that share of coastal population and per capita GDP are in positive correlation with per capita seafood consumption. At the same time, analysis of the fish consumption in Central and Eastern Europe (including Caucasian states) reveals that per capita GDP, share of urban population, share of coastal population are not significant in explaining variables for the differences found in per capita seafood consumption in different states of the region. On the other hand, in spite of the presuppositions, meat consumption positively correlates with fish consumption both along countries and years. Contrary to per capita fish consumption, per capita meat consumption in the region is significantly affected by income (GDP per capita).

The above mentioned possible explanatory variables cannot explain strongly the per capita seafood consumption in Central and Eastern Europe, as considerable variance remains above these variables (income, percentage of coastal population, coastal (1) or landlocked (0) country, per capita meat consumption, percentage of urban population). However, a clear trend from this variance can be uncovered, that is, fish consumption is determined to be higher in post-Soviet states. Putting an extra explanatory variable (a dummy variable) into the statistical model connecting with being a post-Soviet state or not, a strong correlation can be found.

It means that in post-Soviet states (especially Russia, the Ukraine, Rep. of Moldova, Belarus, the Baltic countries) per capita seafood consumption is significantly higher than it would have been predicted solely based on the other explanatory factors of income, urbanization, coastal population and meat consumption. This “Soviet-effect” in consumption makes a huge

impact on the fishery sector, especially on the demand of marine fish. Although CEE states are not so dependent on marine fish consumption than Western European states (including landlocked states), freshwater fish consumption accounts for a larger share in total fish consumption compared to Western countries. Probably that fact is reflected in the low explanatory power of the share of coastal and urban population on per capita seafood consumption in Central and Eastern Europe.

The Network of Aquaculture Centres in Central and Eastern Europe (NACEE): the way forward

Peter Lengyel

Research Institute for Fisheries, Aquaculture and Irrigation, Hungary

Abstract

The Network of Aquaculture Centres in Central and Eastern Europe (NACEE) was officially established in Szarvas, Hungary, in 2004 by the directors and representatives of 23 research and educational institutions and producer associations from 13 countries of Central and Eastern Europe (CEE), in the common understanding that closer cooperation and information exchange was needed between the region's aquaculture-related organizations in order to re-establish the previously existed ties lost during the political and economic transition, overcome the language barrier and avoid redundant research, thus helping to bring the gap between CEE and Western Europe.

In the six years passed since its establishment, NACEE has made a notable progress. It currently consists of 45 member institutions and organizations from 15 countries of the region. It has become a recognized player in the European and global aquaculture scene, maintaining ties with numerous international organizations, e.g. Aqua-TT, the European Aquaculture Society (EAS), the European Aquaculture Technology and Innovation Platform (EATIP), the European Fisheries and Aquaculture Research Organization (EFARO), EUROFISH and the Network of Aquaculture Centres in Asia-Pacific (NACA). It obtained legal status with FAO, as FAO's official partner organization. NACEE has taken part in the preparation of several information materials on the fisheries and aquaculture of the region and has effectively represented the interests of the region in different international fora.

However, as the initial „information hunger” of the members is subsiding, they are beginning to have new expectations towards NACEE. Currently, the network is facing the challenge of redefining itself and its member services along the lines of these new demands.

The most pressing tasks for NACEE for the near future are the following:

- **Financial stabilization.** FAO provided invaluable support for the establishment and startup of NACEE and continues to finance specific programmes of the network, but it has gradually withdrawn the support provided to the operation and the annual directors' meetings. At the moment, NACEE mostly exists on the membership fees and a significant contribution by the Coordinating Institution. Project funds are badly needed in order to make NACEE self-sustaining.

- ***Transformation into an INGO.*** Currently, NACEE lacks legal personality, which does not allow it to apply for project funds as an independent entity, it can only take part in dissemination. Transformation into an international non-governmental organization (INGO) is a crucial step on the way to financial independence.
- ***Smaller and more efficient organization.*** NACEE has a relatively high percentage of passive members, who do not take any part in NACEE's activities and pay the membership fees irregularly. This practically blocks the efficient work of NACEE. Under the current rules of procedure, the exclusion of any member is a very difficult and lengthy process. However, the transformation offers NACEE an opportunity to both ask all members for renewal of their membership and adopting new, stricter rules of procedure.
- ***Higher efficiency of the directors' meetings.*** In order to avoid that NACEE meetings become formal and meaningless, accents should be changed during NACEE meetings, placing higher emphasis on the working groups, whose work should constitute the principal part of the directors' meetings. Working group members should work together in the inter-sessional period as well and come to the meeting with their own agenda and specific proposals.
- ***Better involvement of the member institutions' staff in NACEE activities.*** In many member institutions, the staff is poorly informed of NACEE's activities and the available opportunities. They need to be better involved in NACEE's work, especially thematic information exchange, managing the NACEE webpage, initiating joint projects, etc.

Especially in the latter point, young researchers of NACEE member institutions are expected to play a much more significant role than before. NACEE's framework offers them an excellent opportunity not only to meet their peers from other institutions, but to be aware of each other's research topics, identify partners for research projects, find training courses or practice opportunities, etc. A better involvement of young researchers is expected in the NACEE working groups, as well as a decentralized management of the NACEE website. All young researchers are encouraged to consult the NACEE website or directly contact the NACEE Coordinating Institution whenever they need information or contact from other countries or when they search for partners for a project. NACEE can, without doubt, offer much for young researchers, and likewise, young researchers can give a new stimulus for NACEE.

Immune response of genetically different common carp (*Cyprinus carpio* L.) variants against *Aeromonas hydrophila*

László Ardó¹, Galina Jeney¹, Alexandra Adams² and Zsigmond Jeney¹

¹*Research Institute for Fisheries, Aquaculture and Irrigation, Hungary*

²*Institute of Aquaculture, University of Stirling, UK*

Abstract

Motile Aeromonad Septicaemia (MAS) caused by the bacterium *Aeromonas hydrophila* is a worldwide problem for fish farmers. Vaccines are being developed against this pathogen; however, they are not yet commercially available. Antibiotics and chemotherapeutic agents used to control the disease can accumulate in the fish meat or in the environment, and there is a risk of developing antibiotic resistant bacteria.

An alternative approach is to develop strains of fish that are resistant to the disease by selective breeding for increased disease resistance and to identify secondary markers that can be used for the selection. In our experiment, 96 genetically different common carp (*Cyprinus carpio* L.) families were established using four different strains as parents. Twenty fish from each family were challenged with *A. hydrophila*, and the ten most resistant and ten most sensitive families were selected. Fish belonging to these families were selected and challenged again with *A. hydrophila*. Phagocytic and respiratory burst activities of phagocytic cells, lysozyme activity of the blood plasma were determined. Level of specific antibodies against *A. hydrophila* and level of natural antibodies were measured in the samples taken at the end of the experiment. Non-challenged fish were used as controls. Phagocytic and lysozyme activities as well as the level of specific antibodies were significantly higher in the challenged resistant families than in the sensitive ones. There were no significant differences of these parameters between the control groups. We can conclude that phagocytic, lysozyme activity and specific antibody titre are higher in the resistant families than in the sensitive ones following an infection with *A. hydrophila*.

Distribution and residue depletion of some veterinary drugs in common carp following different routes of administration

Zsuzsanna J. Sándor, Zsuzsanna Gy. Papp and István Csengeri

Research Institute for Fisheries, Aquaculture and Irrigation, Hungary

Abstract

Oxytetracycline (OTC) is a broad-spectrum antibiotic widely used for treatment of systemic bacterial infections. During the last decades it was the most important antibacterial agent used in fish farming. Recently, the substance has been increasingly replaced by other drugs, such as quinolones, which have more favourable pharmacokinetic properties. Flumequine (FLU) belongs to the group of fluorinated “second generation” quinolones which have a strong and rapid antibacterial activity against Gram-negative bacteria. Several studies have shown that OTC persist for a long time in fish tissues and long withdrawal times are necessary, moreover the OTC residues have immunosuppressive effects and cause liver damage. Flumequine is known to have a short distribution and elimination period. Information about toxicology of its metabolites and their accumulation capacities in common carp are scarce.

The aim of our studies was to study the uptake and depletion of OTC and FLU in the tissues of common carp as well as to screen the active compound plus metabolite total levels. In the first part of our experiments two groups of fish were fed during 7 consecutive days with OTC and FLU medicated feeds (doses: 120 and 24 mg/kg body weight/day) followed by a period of feeding without antibiotic for another 8 days. Samples of muscle + skin and liver were taken from three medicated animals 21 times. The samples have been taken usually before daily feeding except on the first, seventh and eighth days, when two and three more samples have been collected. The samples were analysed for OTC and FLU content by HPLC, for total tetracycline and fluoroquinolone quantities by enzyme immunoassay method (ELISA). In the second experiment 133 mg/kg body weight/day of OTC single dose was applied to fish with intraperitoneal injection method. Blood was collected from three different fish in each sampling time during 9 days and serum samples were analysed with HPLC method. The results of the feeding experiments showed a continuous increase of OTC level in skin + muscle during the treatment period and a very long elimination period. The maximum detected OTC concentration in muscle was 2810 $\mu\text{g kg}^{-1}$, the withdrawal period determined for MRL (100 $\mu\text{g kg}^{-1}$) was 59 day post-treatment time. The concentration data in liver reflect a fast absorption and elimination during the day, but accumulation of OTC can be seen during the medication and a very long elimination period could be estimated.

For the FLU a bimodal absorption could be observed in the liver and muscle also, which is followed by a fast elimination. The maximum measured concentrations in muscle were $2120 \mu\text{g kg}^{-1}$, in the liver $5327 \mu\text{g kg}^{-1}$. In the elimination period the FLU level has reached a mean value of $107 \pm 16 \mu\text{g kg}^{-1}$ in muscle, which was below the MRL = $600 \mu\text{g kg}^{-1}$ concentration, but total elimination of FLU was not observed during the experiment. The pharmacokinetic evaluation of OTC in serum samples in the second experiment has shown a bimodal absorption from peritoneal cavity of fish. The bimodal character of curve could be due to the metabolic intensity in connection with the circadian rhythm of fish or with the extreme high doses of drugs. The pharmacokinetic parameters determined were compared with some of literature data.

The concentration values and elimination half-lives measured in tissues and blood confirm the accumulation of persistent OTC and its metabolites in drug-treated animals. After some months of the treatments, residues of OTC can be detected in the liver of common carp and active OTC molecules in the muscle plus skin. Our experimental data suggest that the recommended 25 days ($500\text{day}^\circ\text{C}$) for withdrawal time on 20°C for common carp is not enough according to the food safeties regulations. According to this observation administration of OTC antibacterial drug is not recommended for common carp farming. The experimental data from flumequine administration in feed of fish suggest that FLU might use securely in common carp farming.

Development of a genetic certificate for Belarussian selection breeds of common carp

Aksana Koneva and Sergey Dromashko

Institute of Genetics and Cytology, Belarus

Abstract

The development of DNA technologies today enables us to define the taxonomic status of animals and plants with a high accuracy. Such a progress in biology opens up new possibilities in the field of the genetic identification of valuable agricultural breeds for the purpose of using their potential, optimizing the conservation of their gene pools, as well as preventing economic damage from manipulations when valuable agricultural animal breeds are sold to farms.

The aim of our work is to develop a genetic certificate technology for Belarussian selection breeds of common carp (“Tremlyanskaya” scaly and mirror varieties, “Lahvinskaya” scaly and mirror varieties, “Isobelenskaya” (“Triprim”, “Stolin XVIII”, “mirror mix” and “scaly mix” crosses) as a methodological basis for the genomic registration of the tested breeds for the purpose of their identification, using their potential and optimizing the conservation of their gene pools.

For developing the genetic certificate of the Belarussian common carp, we have chosen RAPD-markers as molecular markers permitting to reveal a high level of DNA-polymorphism and to analyse a great part of the carp genome. This technology is not expensive and is suitable for mass analysis. The method does not require preliminary cloning and fragment sequencing for selecting the primers. It is especially important as data on the genome sequence of common carp are not available for us. By today, only the full primary sequence of the mitochondrial genome has been established in common carp and polymorphism of its separate sites has been investigated in some Asian and European populations.

According to the literature, foreign studies also use mainly RAPD-analysis and microsatellites as DNA-typing methods. By means of these methods, the high genome variability of some European (Hungary) and Asian (China) common carp breeds have been revealed.

For the preliminary analysis, 7 primers (OpB-01, OpE-06, OpF-05, OpE-16, 21, 45, 15/19) have been selected in a random way in order to reveal the effective ones: those that give amplicons, suitable for identification and differentiation of the breeds studied by us. Some of the primers have shown positive results, allowing to use them for the search of a molecular marker of the breed.

We suggest to include the following information into the genetic passport of the breed: 1) the method used for genetic typing; 2) primer (name, sequence), giving effective amplicons for breed identification; 3) molecular weight of the PCR-fragment(-s) found to be a genetic marker for the breed (in base pairs); 4) the image of the gel with the amplification products using a given primer, showing the sites containing molecular markers of the breed (for facilitating the comparison of results while testing other individuals).

If the aforementioned information is presented in the form of a mathematical formula, the result will be the following:

RAPD-1517_{N21} or **RAPD-N21**₁₅₁₇, where

RAPD – the method with the help of which the genetic typing was made in this case (it can be also ISSR, SSR, AFLP, etc.);

N21 – the short name of the primer (or the locus) which was used in genetic typing and appeared effective for identification of the breed, cross, etc.;

1517 – molecular weight (in base pairs) of the PCR-fragment(-s) (amplicon), found to be a genetic marker of breed.

It is also possible to add information on the frequency differences on some other amplicons in the declared breed into the genetic passport.

The research has been conducted in the frame of Task 38 of the government program „Innovative biotechnologies”.

Reintroduction of the Poljana and Nasice common carp strains to their farms of origin with the support of molecular biological methods

Lehoczky István¹, Tomljanović Tea², Ivelić Duška², Jablan Ines², Treer Tomislav², Bakos János¹, Jeney Zsigmond¹

¹*Research Institute for Fisheries, Aquaculture and Irrigation, Hungary*

²*Faculty of Agriculture, University of Zagreb, Croatia*

Abstract

The probability of mixing and loss of broodfish of the cultivated common carp strains of ex-Yugoslavia was high during the hard years of the Croatian war of independence (1991-1995). The carp breeders of Croatia believed that the pure Croatian common carp strains, such as the Nasice and Poljana strains, were lost. In this unfortunate situation, the scientists of the UZFA (University of Zagreb, Faculty of Agriculture) and HAKI (Research Institute for Fisheries, Aquaculture and Irrigation) recognised the significance of the ex-situ common carp gene bank of HAKI, where the ex-Yugoslavian strains were still maintained.

The ex-situ gene bank of common carp strains is more than 40 years old and the number of maintained common carp strains exceeds thirty. Together with the fifteen Hungarian strains, more than fifteen strains from foreign countries are maintained (including the Nasice and Poljana strains). In the gene bank, the original gene pool of the three strains still exists, while it was probably lost in their farms of origin.

The two institutions carried out the genetic characterisation and repatriation of the two strains together. The samples for the genetic study were collected from the gene bank of HAKI and from the original farms.

The results of the genetic study showed that the gene bank strains, as well as the Croatian local strains, deviated significantly from the Hardy-Weinberg equilibrium. This suggests a high level of inbreeding in all examined populations. The gene bank populations originating from the ex-situ gene bank at Szarvas showed a higher level of genetic variability compared to the Croatian local populations. The mean allelic richness (A_r) values of the gene bank populations were higher than those of the local populations, suggesting that the local populations had lost some part of their original genetic variability, probably due to inbreeding or non-designed breeding. The mean number of alleles per locus and the number of individual alleles of the gene bank strains always exceeded the same parameters of the two Croatian strains. The inbreeding coefficient (F_{is}) was found to be always higher in case of Croatian strains. The reason for the higher level of inbreeding can be probably explained with the low number of broodfish for propagation after the war.

The results of the population genetic study confirm that the repatriation of gene bank populations into their original farm environment can improve the genetic variability of the local strains and can guarantee a good genetic basis for the breeding of these strains.

Based on the molecular genetic results in the frame of an intergovernmental bilateral science and technology development project (CRO-27/2006 OMFB-01007/2007) the broodfish from the three strains were propagated and 100.000 larvae were transported to the original farms. Unfortunately, due to logistic errors, the first attempt was not successful. The next spring, the propagation of the broodfish was repeated and this time the transportation was successful. The repatriation of the carp strains gave good results in case of the Nasice mirror strain (Donji Miholjac fish farm), while we have no reliable information from the Poljana fish farm. The repatriated fish were controlled several times and they were healthy and showed good performance. At the Donji Miholjac fish farm, the staff of the two institutions took part in sorting out the best future spawner individuals to establish a good quality and stable broodfish stock.

Reproductive biology of the channel catfish (*Ictalurus punctatus* Raf.) reared in net cages in the cooling waters of a thermoelectric power plant

Ivan Iliev, Tania Hubenova and Angel Zaikov

Institute of Fisheries and Aquaculture, Bulgaria

Abstract

The aim of the study was to investigate the size and age at which the channel catfish reared under the conditions of a thermal power station cooling lake in Bulgaria reach sexual maturity. The individuals were reared in net cages situated in the cooling lake where the water temperature during the winter months does not fall under 10°C, which allows the fish to feed actively during the autumn-winter period and generate growth. In total, 39 channel catfish of one-summer-old age, 26 of one-year-old age, 20 of two-summer-old age and 15 of two-year-old age were analyzed. For one-summer-old catfish, body weights of 100.4 g for males and 81.2 g for females were established. Their length was 17.4 cm and 16.4 cm, respectively. For one-year-old channel catfish, the registered body weight was 163.4 g for males and 133.2 g for females. Their length was 20.3 cm and 19.1 cm, respectively. Differentiation of the gonads was clearly pronounced as early as one-summer-old age. The ovary had a weight of 0.12 g and the testis 0.03 g. GSI was 0.19% in the females and 0.04% in the males. By one-year-old age, the ovaries doubled in size with an average weight of 0.21g, and the testes, of 0.04 g. The GSI had values of 0.23% in the females and 0.02% in the males. The maturity stage of the ovary and the testis was determined as II. In the ovary, follicles in the phase of previtellogenesis (B1 and B2), characterized by the progressive growth of the oocytes, were predominant, and in one-year-old fish, B3 oocytes were rarely found as well. In the testis, primary spermatocytes showing large nuclei and secondary spermatocytes were predominant.

For two-summer-old catfish (16-17 months), an average body weight of 897.8 g for the males and 958.9 g for the females was established. Their length was 43.7 cm and 45.45 cm, respectively. The ovary had an average weight of 12.7 g, and the testis, of 2.6 g. The GSI was 1.68% for females and 0.35% for males. The ovary was in the III-IV stage of maturity and their predominant fraction consisted of early vitellogenic follicles containing oocytes in phase D3-D4, i.e. oocytes in the phase of vacuolization of the ooplasm. Initially, in some follicles, small yolk globules were evident in the cortical region of the oocytes; then they filled the ooplasm in the perinuclear region. At an age of 18-19 months, the ovary was in the IV stage of maturity, and the vitellogenic follicles were the main fraction in the ovary. In the terminal stage of the spermatogenesis, spermatozoa were present in the lumen of the spermatocyst. The spawning takes

place during late May-mid June, and the fishes that are already two-year-old with an average body weight of 1007.8 g for the males and 985.2 g for the females, and length of 49.2 cm and 48.6 cm, respectively, can participate successfully in the reproduction process. Postovulatory, atretic follicles, characterized by an absence of zona radiata and hypertrophy of the follicle cells were observed in the ovary during July-August, i.e. the ovary was in VI-II maturity stage. The values of the absolute (7 583 eggs) and relative (8 702 eggs/kg) fecundity for the two-year-old matured channel catfish were lower than those typical for the older sexually mature fishes. The weight and diameter of the eggs were 0.0071 g and 2.12 mm, respectively.

Morphometric characteristics of oocytes and ultrastructure of their envelopes in Sakhalin sturgeon *Acipenser mikadoi*

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Abstract

The Sakhalin sturgeon *Acipenser mikadoi* Hilgendorf, 1892 is a rare species of the Far East sturgeons of Russia included in five Red Data Lists – IUCN, Russian Federation and three Far East RF entities (Primorye and Khabarovsk Territories and Sakhalin Region). The base for its artificial reproduction is shown to be biology of its natural spawning; however, the available information is limited and the morphology of eggs has been hardly studied.

The material was sampled in May-June 2005 and 2009. Oocytes from the biopsy samples from three wild (the Tumnin River, Khabarovsk Territory) and one hatchery-reared Sakhalin sturgeon female (Okhotsk Fish Farm, Sakhalin Island) were measured, weighed and fixed. The follicular layer was removed from 100 fixed oocytes (Podushka, 1992) and examined using light microscope (LEICA DMLS), and 30 of them were examined with the use of an electron scanning (raster) microscope (JSM-6380LA).

Native oocytes were obtained from the ovaries at stage IV from mature Sakhalin sturgeon females of different sizes, the weight of which varied from 18.3 to 33.0 kg, total length (TL) was between 148 and 170 cm. The oocytes of wild females were black, while those of hatchery-reared females were of grey color. All of them had an oval shape. Their average length varied from 3.2 to 4.4 mm, and the length was between 3.4 and 4.6 mm. One of the wild females of 26 kg in weight and 156.1 cm of TL had oocytes 3.4±0.04 mm wide and 3.9±0.04 mm long (electron chip D154), while another one of 33 kg and 170 cm had oocytes of 4.0±0.04 and 4.3±0.03 mm, respectively. The average egg diameter of these females was 3.7±0.02 and 4.14±0.02 mm, respectively. These data demonstrate a high size variability of Sakhalin sturgeon eggs. The oocytes' weight averages between 23.4±0.38 mg (19.2 - 29.3) at CV equal to 13.3. A direct positive relationship was found between the weight of females and their eggs in the Sakhalin sturgeon, which corresponds to the available information on other fish species, including sturgeons (Slutsky, 1980; Labenets, Novosadova, 2010). The Sakhalin sturgeon oocytes are shown to be the largest ones among other species of the genus *Acipenser* inhabiting Russian water bodies, except the

great sturgeon (beluga) that has been recently assigned to this genus (Vasil'ev et al., 2008). At the same time, on the average, they are smaller than in the green sturgeon *A. medirostris* (4.33 mm) inhabiting the North America Pacific coast (Deng, 2000).

After removing the oocyte follicular layer, its surface is revealed and the chorion can be seen (Fig. 1). It is slightly plicated and penetrated by radial channels. Some follicular cells or their fragments may be observed on the chorion surface. The structure of the chorion surface of Sakhalin sturgeon provides a means for assigning it to the first (plane) structure type observed in anadromous sturgeons and beluga (Vorobyova, Markov, 1999).

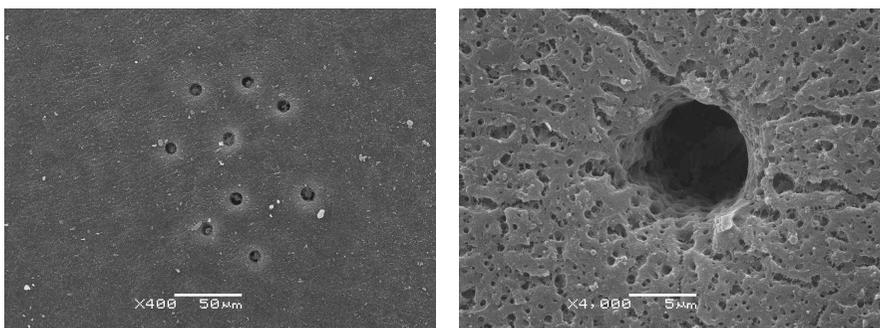


Fig. 1. Animal oocyte pole of Sakhalin sturgeon. Left - 9 micropyles, right - micropyle channel.

On average, micropyles of 7.7 ± 0.98 (5.6-11.3) μm in diameter are settled by 7.8 ± 0.27 (4-17) specimens at the animal oocyte pole positioned at 48.1 ± 1.11 (7.4-105.8) μm intervals. They have no funnels, while some of their inlets are covered with a closing cell of the follicular epithelium or its destroyed fragments.

Dependence of *Coregonus* sperm motility duration on temperature

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Abstract

Fish sperm motility duration is one of the major biotechnical factors indicating the quality of reproductive products of fish males. Temperature is the most significant environmental factor determining the rate of biological processes, including the motility of fish sperm (Kazakov, 1978). Previously, *Coregonus* sperm motility duration was studied by A.N. Kuzmin and A.M. Chuvatova (1970), G.M. Manenkova (1974), R.V. Kazakov and B.B. Voloshenko (1979). However, none of the above authors have ever studied the specific connection between the sperm motility and temperature.

The purpose of this study is to assess the influence of temperature on the *Coregonus* fish sperm motility duration in the Ob-Irtysh basin.

The study is based on the sperm of the following *Coregonus* taxa: *Coregonus tugun*, river and lake forms of *C. peled*, *C. lavaretus pidschian* and *C. nasus*. River forms of *Coregonus* were studied in October – November 2008 and 2009 at the river Lyapin in the basin of the river Severnaya Sosva (Khanty-Mansiysk Autonomous District). Male fish were caught during the spawning migration. Information about the lake form of *C. peled* was gathered in November – December 2008 and 2009 at Volkovskoe fish farm (Tobolsk area of Tyumen region) where a spawning stock of this ecological species is located.

Sperm was collected by means of stripping males, using anesthesia by clove oil. When measuring the sperm motility duration, we tried to ensure that the temperature of air, microscope, glass and water was identical. For each set of tests for determining the dependence of sperm motility duration on temperature we took sperm from the same male. Each test consisted in selection and further examination of an individual sperm portion at a particular temperature. The sample was examined immediately after being stripped. Approximately 0,05 ml of sperm were put on a cover-glass, then 0.2 ml of water were added to activate the sperm. The moment when the sperm contacted the water was taken as the start of motility. With the help of a microscope and a second-counter we recorded three consecutive moments: when 50% of sperm turned from forward motion to oscillatory motion, the start of oscillatory motion by all sperm and complete absence of any motion. The two last parameters were recorded during several sets of tests.

We have conducted two sets of tests in total with the river form of *C. peled* and *C. nasus* broadfish, and three sets of tests in total with *C. lavaretus pidschian*.

We studied the sperm motility duration in the river form of *C. peled* within the temperature range from 3,5°C to 13.0°C. When we increased the temperature during a test, the sperm motility duration gradually decreased. In particular, the average total sperm motility duration decreased from 385 s to 130 s; and the average duration of the forward movement of sperm decreased from 178 s to 67 s. The average total duration of sperm motility of *C. lavaretus pidschian* within the above temperature range (3,0 – 7.3°C) decreased from 435 s to 131 s, and the average duration of the sperm forward movement decreased from 229 s to 59 s. With the temperature increase from 4,8°C to 7,3°C, the duration of the transition period from forward to oscillatory movement for 50% of sperm decreased from 99 s to 31 s. As far as *C. nasus* is concerned, no dependence of the motility duration on temperature within the temperature range in question (1,4 – 4.1°C) has been observed. The average total *C. nasus* sperm motility duration within this temperature range amounted to 273 s, the average duration of the forward movement of the sperm, to 126 s. Taking into account a relatively narrow temperature range and a small number of tests conducted, it would be premature to draw any conclusion on the dependence of *C. nasus* sperm motility on the temperature specifically for that fish species.

The most prolonged sperm motility period of the *Coregonus* under study within the temperature range of 2-4°C was observed with *C. lavaretus pidschian* (tables 1, 2). Thus, the duration of the forward movement of sperm in *C. lavaretus pidschian* amounted to 229 s on average, and the total duration of motility amounted to 435 s. The least prolonged sperm motility period was observed with *C. tugun*. A more correct conclusion on the species-specific *Coregonus* sperm motility duration would require additional studies and tests.

Based on our data, the relation between the duration of forward movement of *Coregonus* sperm and total motility does not depend on temperature. The first factor constitutes 44 - 50% of the value of the second factor. The transition period of 50% of *Coregonus* sperm from forward to oscillatory movement amounts to 26-32% of the total motility time.

Species	Temperature, °C			
	0 – 2	2 – 4	4 – 6	Above 6
<i>C. tugun</i>	–	110	99	–
<i>C. peled</i> (river form)	–	163	126	74
<i>C. lavaretus pidschian</i>	–	229	124	59
<i>C. nasus</i>	129	162	118	–
<i>C. peled</i> (lake form)	121	–	–	–
<i>Coregonus</i> fish	125	163	116	67

Table 1. Duration of forward movement in *Coregonus* sperm at different temperatures, s

Species	Temperature, °C			
	0 – 2	2 – 4	4 – 6	Above 6
<i>C. tugun</i>	–	243	162	–
<i>C. peled</i> (river form)	–	340	216	158
<i>C. lavaretus pidschian</i>	–	435	211	131
<i>C. nasus</i>	226	293	262	–
<i>C. peled</i> (lake form)	310	–	–	–
<i>Coregonus</i> fish	288	327	237	145

Table 2. Total duration of *Coregonus* sperm motility at different temperatures, s

According to our records, the total average motility and forward movement duration within the spawning temperature range of 0,1 – 6.0°C of *Coregonus* in the Ob-Irtysh basin (Venglinskiy et al., 1979) amounted to 284 s and 138 s, respectively. The duration of sperm motility decreases very quickly at temperatures exceeding the spawning one.

According to A.N. Kuzmin and A.M. Chuvatova (1970), the total motility and forward movement duration of the sperm of *C. nasus* and *C. peled* from the Ob introduced in the Northern-Western Russia at the temperature of 2,5 – 3.5°C is 2.5 times less than in our study.

Thus, the total sperm motility duration of *Coregonus* in the Ob-Irtysh basin at the spawning temperature range has an interval of 4-5 minutes, and duration of forward movement amounts to 2-3 minutes. These data shall be considered during the adjustment of the artificial fertilization technique of fish eggs.

Possibilities of rearing paddlefish (*Polyodon spathula*) fry in protected conditions – Hungarian experiences

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Abstract

Paddlefish (*Polyodon spathula* Walbaum) is an excellent candidate for extensive pond culture, where it can partly substitute the lower-valued zooplankton-feeder species (*Cyprinus carpio* and *Aristichthys nobilis*). In the presence of abundant zooplankton (mainly *Daphnia* sp.) it grows fast; however, the fry production is highly vulnerable mainly because of the bird predation and competition by various insects and vertebrates, water quality and disease problems. Our studies aimed to investigate the possibilities of fry rearing of paddlefish in protected conditions: i.e. in cages or in tanks (first and second trial, respectively).

In the first experiment, a total of 3.000 larvae were randomly selected 10 days post-hatch (dph). Fish were stocked into four illuminated net cages (0.5 m³ each) at two different stocking densities (1.2 fish.L⁻¹ and 0.6 fish.L⁻¹) and into two 0.5 m³ outflow tanks (1.2 fish.L⁻¹). The cages were placed in a 0.2 ha earthen pond, which was continuously aerated with a paddlewheel aerator. During the 23-day-long trial, the water temperature fluctuated between 17 – 24 °C. To attract zooplankton, each cage was illuminated with a 12V / 60W bulb put into cages at a depth of 0.25 m. The water of the tanks was supplied from the same pond by pump with a turnover rate of twice per hour. Fish in the tanks were daily supplied in excess with collected zooplankton, but in cages, they fed exclusively on the prey organisms attracted by light. Fish in tanks had better growth and survival rates than fish in cages at the same stocking density (Table 1).

In the second experiment, 15 dph, 300 larvae were randomly selected and distributed into 6 circular tanks (each with a volume of 40 L). Two feeding treatments were applied in triplicate during 21 days. The first group was fed exclusively with the collected zooplankton at “ad libitum” level. The second group was gradually weaned during the first week from zooplankton to dry diet (Perla plus 2.0, Hendrix S.p.a Italy; crude protein: 55 %, crude fat: 11 %, crude ash: 18 %, particle size: 0.7 -1.1 mm), and thereafter, was fed exclusively with it in excess. Fish were exposed to a 16 h light and 8 h dark photoperiod and the water temperature fluctuated between 17 – 20 °C. The water flow in the tanks was regulated to ensure a 1.5 hour⁻¹ turnover rate. Fish fed on zooplankton grew better than fish fed dry feed but the differences were not significant (P>0.05).

However, the survival rates were significantly higher ($P<0.05$) in the pellet-feeding treatment (Table 2.).

Treatment	w_t (g)	l_t (mm)	SGR (%.day ⁻¹)	K (g.cm ⁻³)	S (%) **
Tank (1.2 fish.L ⁻¹)	2.44 ± 0.24	78.1 ± 4.0	16.82 ± 0.43	0.51 ± 0.03	38.9 ± 1.5 ^a
Cage (1.2 fish.L ⁻¹)	1.70 ± 0.25	66.3 ± 7.7	15.23 ± 0.65	0.59 ± 0.12	29.0 ± 0.5 ^b
Cage (0.6 fish.L ⁻¹)	2.21 ± 0.10	75.3 ± 1.6	16.39 ± 0.20	0.52 ± 0.01	39.2 ± 0.7 ^a

Table 1. Results of paddlefish fry rearing in tanks and cages^{*}.

^{*} Initial weight and length: 0.051 ± 0.020 g and 22.6 ± 3.14 mm, respectively. Values are means ± SD of two replicates.

^{**} Values by columns with different superscripts are significantly different ($P<0.05$).

Feed	w_t (g)	l_t (mm)	SGR (%.day ⁻¹)	K (g.cm ⁻³)	S (%) ^{**}
Zooplankton	2.70 ± 0.26	84.9 ± 0.7	15.37 ± 0.46	0.44 ± 0.05	48.7 ± 7.0 ^a
Perla plus 2.0	2.27 ± 0.13	79.1 ± 3.5	14.55 ± 0.26	0.46 ± 0.04	59.3 ± 4.2 ^b

Table 2. Results of paddlefish fry feeding on live preys and dry diets^{*}.

^{*} Initial weight and length: 0.11 ± 0.03 g and 24.4 ± 3.3 mm, respectively. Values are means ± SD of two replicates.

^{**} Values by columns with different superscripts are significantly different ($P<0.05$).

Effect of spawning practice and water temperature on the mass production and quality of larvae in perch (*Perca fluviatilis*)

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Abstract

Introduction

Intensive culture of perch requires a mechanism for controlling reproduction. Synchronization of egg ovulation is one possibility for controlled reproduction in perch. Hormonally induced ovulation using different hormones was studied in perch by Kucharczyk et al. (1998) and Kouril & Hamackova (1999).

Material and Methods

This study was carried out to investigate the effectiveness of two spawning techniques (semiartificial and artificial spawning) under two different temperatures (10 ± 0.2 °C and 15 ± 0.2 °C) during perch reproduction. The female's fecundity, latency, spawning synchronization, fertilization and hatching rates and broodstock mortality were evaluated in 4 groups. Each group was tested in triplicates (each triplicate: 10 females and 10 males). In total, 240 four years old broodstock (females: TL= 215.8 ± 57.0 mm; W= 190.2 ± 56.4 g and males: TL= 194.4 ± 37.2 mm; W= 91.4 ± 36.3 g) were used in this study. All females were injected intramuscularly by uniform hormonal treatment (50 µg GnRH_a.kg⁻¹). Males were not hormonally treated.

Results and conclusions

Fluctuating fecundity of females was found within each group. The average female's fecundity was the same in all groups without significant differences in minimum (22200 ± 15000 eggs) and maximum (32200 ± 18600 eggs) values. Longer latency was found in semiartificial spawning compared to the artificial one under both temperatures. Latency in semiartificial spawning under 10°C and 15°C was about 57.6 hours and 14.5 hours longer compared to artificial spawning, respectively. Higher temperature significantly shortened latency in both spawning techniques compared to lower temperature. In total, 80.0 – 87.5 % females spawned in all groups without differences. Good synchronization of

spawning was found in all groups, when females spawned during 3 or 4 days. Low broodstock mortality rates were found in males ($0\pm 0\%$ - $10\pm 2.5\%$) and females ($15\pm 2.5\%$ - $17\pm 4\%$) in all groups. However, very high mortality rates were evident in both sex (females: $95\pm 2.5\%$ - $99\pm 2.5\%$ and males: $85\pm 2.5\%$ - $92\pm 2.5\%$) 90 days after spawning period without differences among groups. The highest fertilization and hatching rates ($85.6\pm 8.7\%$ and $72.9\pm 12.3\%$) were found in semiartificial spawning under $15\text{ }^{\circ}\text{C}$. The lowest fertilization and hatching rates ($53.7\pm 7.9\%$ and $46.1\pm 9.8\%$) were recorded after artificial spawning under $10\text{ }^{\circ}\text{C}$. Generally, lower fertilization and hatching rates were found under lower temperature and after artificial spawning.

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The peculiarities of large European perch cultivation in the South of Russia

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Abstract

One of the ways to diversify the aquaculture sector in the conditions of South of Russia is breeding of European (river) perch (*Perca fluviatilis*). The high abundance of its population, its economic value and central status in the fish communities of many water basins motivate the intense interest in its research in different countries.

The river perch was not considered a food fish or a valuable object of fishing to present day in Russia. It can be explained by the low demand for this fish in Russia and its infestedness by eustrongylides. *Eustrongylides excises* is a pathogenic species of nematodes, widespread in the delta of Volga River. It can be found as a larva in the abdominal cavity of perch, in the walls of its intestines, liver, eggs and in the spinal muscular tissue. The number of these parasites can reach 30 and more per fish. These nematodes damage the marketable look, which leads to discarding the fish products. However, because of the sharp decrease in the main valuable fish: sturgeons, salmonids, some anadromous cyprinids and zander, the river perch gains importance in the catches of Russian Federation.

The river perch cultivation is characterized with low costs and fast return rates for the aquaculture enterprises. Perch fillet has a high dietary value, but the percentage of edible part of perch is near 37,5% of the undressed fish, while for zander, it is 47%. Thus, we should find methods to increase the economic efficiency of perch cultivation.

The body length of river perch in the net catches in the Volga-Caspian basin ranges from 10,5 cm to 38,0 cm, the most numerous size group is fish of 17-25 cm. In the western part of the Volga delta lowlands, a significant part of the catches (25-30%) consists of large fishes of 26-33 cm. These fishes are called "depth" perch – a large race or morph of this species. Perch of this race reach a larger size and have a higher growth rate in contrast to the small race – the "grass" perch. The "depth" perch live in the open water at bigger depths and have solitary habits. The females of large perch have higher fecundity than those of the smaller one.

The technology of large perch cultivation in industrial conditions is being developed by Aquabiotechnopark (Astrakhan State Technical University). The development of a large perch broodstock, obtaining and further breeding of the offspring, feeding on artificial diets allow us to avoid infection by eustrongylides and other parasites occurring in the natural environment, and also to reduce the rearing period to marketable size. It will make the perch fillet more attractive for consumers in Russia.

Nutrient reusing capacity of a combined pond aquaculture system

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Abstract

Nutrient retention into fish biomass varies only between 20 and 30% of the introduced fish feed in various aquaculture practices. The combination of intensive fish production with extensive pond culture affords a possible solution to increase the nutrient utilisation of aquaculture. In the frame of the SustainAqua project, pilot scale experiments were implemented in HAKI in order to investigate the potential of nutrient reusing capacity of integrated aquaculture. The investigated combined intensive-extensive aquaculture system (IES) operated in a close interaction of the intensive and extensive production units. The key element of the proper operation was the treatment capacity of the extensive unit; hence the investigations were focused on the periphyton application on the nutrient utilisation and water quality of the production system. The aim of this study was to estimate the potential of nutrient recovery by additional production activities in combined systems.

The experiments of IES were carried out in three ponds (area 310 m², depth 1 m) that served as extensive units, where to a cage was placed as an intensive unit (volume 10 m³) in each pond. Three different setups of extensive ponds were studied: additional area for periphyton development equalled about 0, 100 and 200% of the pond surface area. In the intensive units, African catfish were cultured and fed with pellet – initial stocking biomass was 200 kg –, while 200 kg common carp and Nile tilapia were stocked in each extensive unit (at the ratio of 1:1) and were raised without any artificial feeding. All ponds were subjected to the same regime of feeding and fish stocking. The average feed load was 0.9 g N m⁻² day⁻¹ (maximum 1.8 g N m⁻² day⁻¹). The growth rate (SGR 1.0%) and feed conversion (FCR 1.5%) were similar in all intensive units.

The nutrient utilisation of fish production in IES expressed in the percentage of the total introduced nutrients is summarised in Table I. There were only negligible differences in the nutrient accumulation between the intensive units. The nutrient reuse by the additional fish production in the extensive unit was the highest where the periphyton area was 100% of the pond surface. Between the treatments, there were no significant differences in the measured water quality parameters ($p > 0.05$).

Results of the study proved that combination of intensive aquaculture with extensive fishponds enhances the nutrient utilisation efficiency and fish production in IES. The combined fish production resulted in higher protein

utilisation by 26%; even this ratio can be increased by 40% with periphyton application. The operation of the constructed wetland system was characterized by effective nutrient removal and additional revenue possibilities. Besides the adequate treatment capacity, the constructed wetlands have a remarkable potential in energy crop production as well.

The experimental work resulted in higher nutrient utilisation efficiency and reduced environmental emissions of fish production with increased production capacity. The application of the combined intensive-extensive pond fish production system could contribute to the sustainable use of natural resources (i.e. higher nutrient utilisation efficiency and reduced environmental emissions) and to the economical sustainability as well (i.e. increased production capacity). The combined system is addressed to traditional pond farmers who intend to diversify their income with predatory fish production. It helps carp farmers to use their water more efficiently by producing valuable species in their large reservoir ponds or extensively used ponds and increases the economical performance of fish production.

Table I. Nutrient accumulation in fish biomass in the percentage of the total input (%)

	IES/1			IES/2			IES/3		
	N	P	C	N	P	C	N	P	C
Input (kg/ha)	1790	310	9700	1800	320	9700	1800	310	9700
Output (kg/ha)	760	130	3100	840	140	3900	720	130	3200
Retention (%)	58	60	67	53	55	59	60	60	67

Table 1. Nutrient accumulation in fish biomass in the percentage of the total input (%)

PA: Periphyton area

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Aquaculture effluent treatment in constructed wetlands

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Abstract

Several intensive flow-through fish production plants were established in Hungary in the last decades, representing a significant share of the total fish production. However, the full management of the discharged waste from such systems is still unsolved. Searching new sustainable methods for treatment and utilisation of output nutrients, a pilot-scale constructed wetland system was built in Szarvas (Hungary) in 2000. The aim of this study is to present the nutrient removal efficiency and the potential of the wetland system to transform discharged nutrients into valuable by-products in year 2008.

The pilot-scale treatment system consisted of two subsystems (“A” 1.1ha, “B” 0.4ha) of similar structure: one stabilisation pond and one fishpond were connected serially; the water was channelled from the fishpond into macrophyte ponds (surface-flow wetlands) and additionally spread on one irrigated field (B_SAI). The effluent from the intensive flow-through African catfish farm was pumped into the stabilisation pond through a pipeline, where a paddle-wheel aerator was operated and supplement river water was added. The fishpond was stocked in polyculture of silver carp (*Hypophthalmichthys molitrix*), common carp (*Cyprinus carpio*) and grass carp (*Ctenopharyngodon idella*) (900kg ha⁻¹, 60:35:5%) in April and raised without feeding. Four macrophyte species were tested: common reed (*Phragmites australis*) (A_PH), cattail (*Typha latifolia* and *T. angustifolia*) (A_TY), energy willow (*Salix viminalis*) (B_SA) and giant reed (*Arundo donax*) (B_AR). The fish was harvested in November, the plant biomass was estimated from the weight of three representative samples of 1 m² area. The dry weight, the total Kjeldahl nitrogen and the total phosphorus content of tissues were measured. The fuel value of plant samples was determined in a bomb calorimeter in O₂ and at a pressure of 30 bar. The concentration of main nutrient compounds (TN, TP, VSS) in water samples were analysed according to standard methods.

The nutrient removal of the wetland systems was most efficient in the case of organic carbon in the “A” subsystem; for all nutrients the removal efficiency reached 90%, except for the phosphorus in the “A” subsystem, where the lowest efficiency was found (80%). From the input nutrient amounts, 20.4 kg N (1.5%), 3.98 kg P (2.6%) and 93.5 kg OC (3%) were transformed into fish biomass. The highest biomass in dry weight was observed for the cattail and the giant reed with

nearly 20 000 kg ha⁻¹. The lowest biomass growth of 5 400 kg ha⁻¹ was found in the willow plantation in the surface flow wetland unit (B_SA). Cattail, giant reed and willow had higher average fuel values near to 18 000 J g⁻¹, while a comparatively low fuel value of 13 840 J g⁻¹ was found for common reed. The highest total fuel value was produced in the cattail pond.

The total fuel value of the plant biomass amounted to more than 180 000 MJ. For the operation of the treatment system, 62 946 MJ energy was consumed, resulting in a positive energy budget. The common carp was the most valuable by-product showing a considerable net yield of 1 147 kg ha⁻¹ and representing a market value of €2 kg⁻¹. The silver carp and grass carp showed low net yields and their market prices were also lower (€0.6 kg⁻¹ and €1.6 kg⁻¹, respectively). The total market value of by-products provided a supplementary income of 1 787 € annually.

The operation of the constructed wetland system was characterized by effective nutrient removal, positive energy budget and additional revenue possibilities. The ecological sustainability was enhanced by the production of renewable energy source; the substitutions of fossil gas by plant biomass produced in the treatment system can diminish the CO₂ emission by 11 250 kg annually. The surface-flow ponds assured optimal conditions for common reed and cattail; however, to increase the growth potential of willow and giant reed more efforts and management changes are required.

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Periphyton utilisation in aquatic ecosystems – improvement of fish production and water treatment

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Abstract

Introduction

In our study, the periphyton appearing on natural and artificial substrates in different types of experimental ponds was examined. Since traditional periphyton-based aquaculture does not exist yet in Hungary, detailed knowledge on the quantitative and qualitative changes of the periphyton may give possibilities to increase fish yield and improve water quality in fish ponds, even under temperate climate.

Materials and methods

The experiment was carried out in three different treatments at the Research Institute for Fisheries, Aquaculture and Irrigation (HAKI), Szarvas, Hungary:

- (i) wetland-type pond system for wastewater treatment (W1, W2, W macro: W3 and W4) – 2001-2009 –,
- (ii) two fish ponds in combined intensive-extensive fish farming systems (IE1 and IE2) – 2007-2009 – and
- (iii) three traditional polyculture fish ponds (FP1, FP2 and FP3) in 2007.

The periphyton samples were collected from epiphytic (from underwater surface of macrophytes) and epyphalotical habitats (plastic pipe substrates – diameter 1.8 cm – placed vertically in the ponds).

Results and discussion

The dry matter (DM) of periphyton was significantly higher in the samples that developed in the fish ponds (IE: $42.2 \pm 2.96 \text{ g m}^{-2}$ $n=40$, FP: $39.0 \pm 6.31 \text{ g m}^{-2}$ $n=22$, W2: $37.6 \pm 3.45 \text{ g m}^{-2}$ $n=48$) than samples taken from W1 ($13.4 \pm 1.28 \text{ g m}^{-2}$ $n=58$) and W macro ($3.81 \pm 0.900 \text{ g m}^{-2}$ $n=106$), where wastewater treatment was the main function of ponds.

Comparing the annual averages of dry matter in the wetland-type wastewater pond systems (W1: $13.4 \pm 1.28 \text{ g m}^{-2}$ $n=58$, W2: $37.6 \pm 3.45 \text{ g m}^{-2}$ $n=48$, W macro:

3.81±0.900 g m⁻² n=106), the highest values were found in the fish pond units. The amount of periphyton in the last two units (W macro) was low, which was explained by the nutrient removal efficiencies in the system, as well as the shading effect of the aquatic plants. The largest quantity of periphyton in the W2 fish pond can be used as a reference for expanding the surface of artificial substrates. A potential average of up to 94 kg of periphyton was estimated on the surface of 2500 m². Beside its effect on water quality, this quantity of periphyton would provide a significant mass of natural food source for aquatic organisms and would also contribute to the growth of fish.

The mean DM observed on our substrates reached 42.2 g m⁻² on plastic pipes in the IE. This is relatively high, since 8 studies out of 10 found values under 50 g m⁻². Dry matter changes were primarily determined by the sampling date and the type of treatments ($R^2=0.996$).

The maximal mean organic periphyton (AFDM) observed in the fish ponds (21.6 – 30.1 g m⁻²) was greater than means observed by other authors, which generally did not exceed 10 g m⁻². The lowest AFDM was found in the W macro ponds, which was explained by the nutrient removal efficiency in the system.

Periphyton can provide special, often protein-rich natural food for fish and invertebrates. The range of crude protein content of the periphyton biomass varied between 8.82±0.086% and 32.9±0.324% in our experiments. In our study, the nitrogen and phosphorus uptake by periphyton were as follows: TN: 6.69±0.097–32.5±0.219 mg m⁻² day⁻¹, TP: 1.92±0.036–39.8±0.593 mg m⁻² day⁻¹.

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The status of mollusc catches in the last decade over the Black Sea area

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Abstract

Living conditions offered by the Black Sea

- Located between Europe and Asia, bordered by Russia, Ukraine, Romania, Bulgaria, Turkey and Georgia .
- Opens to the Sea of Marmara (Bosphorus), Azov (Kerch), Aegean (Dardanelles);
- It covers an area of 423,488 km² (the deepest point is 2245 m below sea level);
- Main collected rivers are : Danube and Dnieper in Europe; Scaria and Enige in Asia;
- Water temperature varies from 29°C at surface in summer to 0°C in winter ;
- Light penetrates into the open sea to a depth of 150-200 m;
- Oxygen is absent in depth, only hydrogen sulphide gas exists;
- Currents have low intensity, vertical and horizontal waves may occur in winter, reaching 5-10 m;
- Salinity of 17-18 ‰ is low compared to 24-34 ‰ in other seas and oceans;
- The bottom consists of rocks, sand, shores, organic or mineral deposits;
- The Black Sea biotope comprises four floors:
 - Over-seaboard (coastal areas occasionally covered or sprinkled by waves) biocoenosis (angiosperms, aerobic bacteria, crustaceans, insects, worms, etc.);
 - Average seaboard (wave breaking zone, between 0 and - 0.5 m); biocoenosis (algae, molluscs, etc.) ;
 - Beneath-seaboard (located at depths of 0.5 to 12 to 18 m) biocoenosis (multicellular plants and biomass) .

The main species of molluscs in the Black Sea area are: bivalves (oysters, mussels, scallops, clams) and gastropodes (*Rapana venosa*, *Hesaplex trunculus*, *Murex brandaris*).

Ukraine and Turkey are the primary countries that record the most important catches in this area.

The representative species from each class that we had studied are *Rapana venosa* (a gastropode) and *Mytilus galloprovincialis* (a bivalve). The evolution of the catches is structured in the following table:

Year	Mytilus galloprovincialis						Rapana venosa					
	BUL	GEO	ROM	RUS	TUR	UCR	BUL	GEO	ROM	RUS	TUR	UCR
1990	0	0	0	9	2544	2189	0	0	0	156	6094	0
1991	0	0	0	88	26	399	0	0	0	11	3730	0
1992	0	0	0	0	5678	449	0	0	0	192	3439	14
1993	0	0	0	0	5914	210	0	0	0	29	3668	3
1994	0	0	0	0	6038	226	3000	0	0	2	2599	5
1995	0	0	0	0	5741	578	3120	700	0	54	1198	303
1996	5	0	0	0	1400	74	3260	711	0	1	2447	376
1997	57	0	0	0	2952	159	4900	118	0	440	2020	476
1998	92	0	0	0	2435	82	4300	0	0	46	3997	369
1999	100	0	0	4	1584	155	3800	0	0	45	3588	619
2000	0	0	0	0	178	111	3800	184	0	182	2140	913
2001	7	0	0	0	17	61	3353	517	0	224	2614	395
2002	55	0	0	0	2500	71	698	503	0	56	6241	91
2003	15	0	0	1	4050	68	325	295	0	62	5500	149
2004	34	0	0	9	2867	78	2428	65	0	62	14834	159

The status of molluscs catches in the last decade (tonnes)

As a conclusion, we can see that the catches recorded show a significant decline caused by the negative impact of human activity over the environment, leading to the decrease of oxygen supply and, consequently, the mollusc populations.

The actual legislation pleads for the reduction of molluscs catches, favouring the regeneration of the biomass.

Application of vegetal feed supplements in intensive common carp production

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Abstract

According to the medical and biochemical studies on the sanitary effects of fish meat consumption in the last decades, it has been stated that the essential fatty acids in fish have a big importance in the healthy functioning of the human organism and prevention of cardiovascular diseases. During our work, feeding and pond manuring technologies were investigated in intensive pond conditions, which could be suitable for producing fish meat with a higher essential fatty acid content than in the case of the traditionally mainly extensive cereal feeding technologies, applying vegetal-oil-supplemented artificial feeds with high protein content.

In pond experiments related to the Aquamax (EU FP 6) international project, common carp was reared in polyculture. Natural food was supplemented with high-protein-content artificial diets. The production parameters of carp stocks and the fatty acid composition of the fish meat were examined. The trials of Experiment 1 were carried out in 12 experimental ponds of the Research Institute for Fisheries, Aquaculture and Irrigation with surface areas of 0.15 ha from April to the middle of November 2007. In the beginning, the whole stock was fed exclusively with wheat and, from June, five experimental trials were established in duplicates. The applied feeds (without fish meal and fish oil) contained 26% protein and lupine (*Lupinus albus*) in two different proportions (6.5% - LL, and 10.5% - HL). The feeding trials were combined with two different manuring methods: 5 t/ha in single dose (SD) and 3+2 t/ha in partitioned dose (PD). The control stock was fed exclusively with wheat besides a single 5 t/ha manuring dose. In Experiment 2, fish were reared in 0.15-ha-surface ponds and fed with wheat until the middle of June. Thereafter, they were fed with a complete (protein: 34.3%, fat: 11%) feed (SKR) supplemented with *Camelina sativa* oil. The aim of Experiment 2 was a comparison between the results of the experimental ponds and the data of a barrage pond located in Transdanubia, where the fish were fed mainly with triticale.

There were significant differences between the production parameters of the exclusively cereal-fed carp groups and the co-fed groups (e.g. $\text{Yield}_{\text{cereal}} = 1057 \text{ kg/ha}$ vs. $\text{Yield}_{\text{artificial feed}} = 1798 \text{ kg/ha}$) especially in terms of food conversion ($\text{FCR}_{\text{cereal}} = 3.62$ vs. $\text{FCR}_{\text{artificial feed}} = 1.5$).

Toxicity study of selected phthalate esters in *Sander lucioperca* L.

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Abstract

Introduction

Phthalates are esters of phthalic acid and are mainly used as plasticizers (substances added to plastics to increase their flexibility). In the European Union, five of the most widely used phthalates have been reviewed by the European Chemicals Bureau: di-2-ethylhexyl phthalate (DEHP), dibutyl phthalate (DBP), diisononyl phthalate (DINP), diisodecyl phthalate (DIDP) and butyl benzyl phthalate (BBP). There is a wide range of different phthalates, but acute toxicity to aquatic organisms is demonstrated only in these esters with alkyl chain lengths lower than C6 (Staples et al., 1997) such as DBP and BBP. The physico-chemical properties of selected phthalate esters are shown in Table 1. DBP is used in such products as plastic (PVC) piping, varnishes and lacquers, nail polishes, paper coatings, pharmaceuticals and plastic food wrap. In turn, BBP is used mainly in vinyl floor tiles, vinyl foam and carpet backing, in cellulosic resins and as an organic intermediate. High phthalate concentrations in the environment are mostly found near production and processing sites in waste water and nearby surface water. They are also found in aquatic and soil-dwelling organisms near to sources and in sediment and soil. Raising concerns about possible environmental and health effects, the risks of human and animals exposure to phthalates are being kept under close review by national and international agencies.

The purpose of the presented work was to determine the acute lethal toxicity (LC₅₀) of DBP and BBP in concentrations near their solubility in water on pikeperch (*Sander lucioperca*). The percids, including pikeperch are of great interest to global aquaculture. This species is highly prized by consumers and it also plays an important role in the biomanipulation of aquatic ecosystems.

Material and methods

Acute toxicity (static) tests were conducted in the laboratory at the Department of Ichthyology, University of Warmia and Mazury, Olsztyn, Poland. Juvenile *S. lucioperca* selected for this study were obtained from the Dgal

Experimental Hatchery of the Inland Fisheries Institute in Olsztyn. They were brought to the laboratory and acclimatized to the appropriate temperature for two weeks before being tested. The initial body length and body weight for the juvenile fish tested with DBP were: 6.90 cm (± 0.15); 2.44 g (± 0.22) and with BBP: 6.63 cm (± 0.11); 2.60 g (± 0.10), respectively. There was no mortality in a 48 h period prior to test initiation. To start each experiment, all aquariums (8 L capacity) were filled with 6 L tap water. Chemically pure phthalate esters (Sigma Aldrich, Poznań, Poland) were dissolved in 99,8% ethanol for the preparation of the desired stock solutions. The following nominal concentrations in weight per volume of DBP were used in test: 0.0, 2.09, 4.18, 6.28, 8.37, 10.46 mg L⁻¹, and BBP: 0.0, 0.55, 0.99, 1.54, 2.09, 2.64 mg L⁻¹, respectively. Seven fish were stocked into each aquarium containing the above different concentrations of phthalates. During experiments constant air was supplied to all the test mediums with an air pump. The behaviour and mortality of the test fish were observed and recorded from 3 h and above, especially with higher concentrations of phthalate esters. Dead fish were immediately removed. The observations of water temperature, pH, dissolved oxygen concentration were made at 12 h intervals during the 96-h determination of LC₅₀ and lethal concentrations (100% mortality) for the fish. The LC₅₀ values for the juvenile *S. lucioperca* were estimated for 48 and 96 h of exposure time by using Probit analysis.

Results and Discussion

The quality analyses of the water before and during the experiments are shown in Table 2. No mortality was observed in the control groups. The acute toxicity values for *S. lucioperca* in our tests were 6,82 mg L⁻¹ (48 h LC₅₀) and 6,56 mg L⁻¹ (96 h LC₅₀) (Table 3). There were changes in the frequency of movement of the fish subjected to different concentrations of DBP. Behavioral changes such as uncoordinated movements, erratic swimming and increase in operculum ventilation, respiratory distress, and sudden quick movements were observed. In acute static toxicity tests with other species, the yellow perch (*Perca flavescens*) and the channel catfish (*Ictalurus punctatus*) were the most sensitive freshwater fish with 96 h LC₅₀ values of 0.35 and 0.46 mg DBP L⁻¹ respectively (Mayer and Ellersieck, 1986). The pikeperch was similarly sensitive to DBP as rainbow trout (*Oncorhynchus mykiss*) with LC₅₀ of 6.47 mg L⁻¹ (Mayer and Sanders, 1973). The most sensitive marine fish species identified was the sheepshead minnow (*Cyprinodon variegates*), for which a 96 h LC₅₀ of 0.6 mg DBP L⁻¹ has been reported (CMA, 1984). No mortality was observed in pikeperch after 96 h exposure to BBP even at the highest concentration (2,66 mg L⁻¹). LC₅₀ values appear to differ within reviewed test species. Very often, acute BBP toxicity occurred only at concentrations significantly above its water solubility, e.g. for rainbow trout, 96 h LC₅₀ was 3,30 mg L⁻¹ (Gledhill et al., 1980) and bluegill (*Lepomis macrochirus*) 96 h LC₅₀ was 43.0 mg L⁻¹ (Buccafusco et al., 1981). The most sensitive freshwater fish was fathead minnow (*Pimephales promelas*) with a 96 h LC₅₀ of 0.78 mg L⁻¹ (Adams et al., 1995). In studies with marine fish, LC₅₀ values range from 3.0 mg BBP L⁻¹ (Gledhill et al., 1980) to 440 mg BBP L⁻¹ (nominal concentrations) (Heitmuller et al., 1981) for sheepshead minnow. These data show that one of the tested lower phthalates, DBP, is acutely

toxic to pikeperch at concentrations below its solubility and pikeperch is less sensitive to this toxicant than most tested species.

Properties	DBP*	BBP**
Physical state	oily liquid	oily liquid
Molecular formula	C ₁₆ H ₂₂ O ₄	C ₁₉ H ₂₀ O ₄
Molecular weight	278.35	312.35
Melting point (°C)	-35	-40.5
Boiling point (°C)	340	370
Water solubility (mg L ⁻¹)	11.2	2.7
Density at 25°C (g mL ⁻¹)	1.04	1.12
Vapor pressure at 25°C (mm Hg)	2.7·10 ⁻⁵	6.0·10 ⁻⁶
Log K _{ow}	4.45	4.59

Table 1. Physicochemical properties of dibutyl phthalate (DBP) and butyl benzyl phthalate (BBP)

* Staples at al. (1997)

** CMA (1999)

Phthalate ester	Temperature (°C)	pH	Dissolved oxygen (mgO ₂ L ⁻¹)
Dibutyl phthalate DBP			
initial	24.1 ± 0.55	7.45-7.51	89.92 ± 0.57
24 h	24.0 ± 0.71	7.22-7.46	91.10 ± 0.98
48 h	24.2 ± 0.45	7.64-7.21	89.98 ± 1.55
72 h	24.0 ± 0.64	7.72-7.31	90.78 ± 1.13
96 h	24.3 ± 0.55	7.55-7.60	91.08 ± 1.44
Butyl benzyl phthalate BBP			
initial	24.1 ± 0.31	7.80-7.76	89.72 ± 1.53
24 h	24.0 ± 0.23	7.84-7.83	89.10 ± 1.51
48 h	24.2 ± 0.31	7.94-7.79	89.18 ± 1.46
72 h	24.1 ± 0.13	7.93-7.91	89.82 ± 0.90
96 h	24.0 ± 0.36	7.97	89.75 ± 0.63

Table 2. Mean (± SD) and range of the physico-chemical parameters of test mediums during the 96-h acute toxicity trails

Phthalate ester	Measured/ Nominal (M/N)	Test type	Test duration	Test endpoint	48h LC ₅₀ (mg L ⁻¹)	96h LC ₅₀ (mg L ⁻¹)	Lethal concentration (mg L ⁻¹)
Dibutyl phthalate DBP	N	static	96h	survival	6,82	6,56	8,37
Butyl benzyl phthalate BBP	N	static	96h	survival	-	-	-

Table 3. LC₅₀ and lethal concentration values for the acute aquatic toxicity of DBP and BBP on pikeperch

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Intensive rearing of pike (*Esox lucius*) in illuminated cages

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Abstract

The pike (*Esox lucius*) is one of the most important predatory fish species in Hungary. Its propagation and pond rearing has great traditions in the country. It is one of the most preferred fish by anglers worldwide, and contributes to the ecological balance of freshwater ecosystems. Nowadays, demand for the most popular pond species – common carp – is stable or is slowly decreasing. However, the demand for pike is growing all over Europe, thanks to the rapid development of sport fishing. The aim of our experiment was to investigate of illuminated cage rearing of pike larvae in Hungarian conditions, which is used worldwide in nursing of other fish species (Coregonidae, Percidae Esocidae). The basis of this method is the accumulation of food organisms by light exploiting the positive phototaxis of zooplankton. The advantage of this method is that the pre-nursing is cost-effective and needs minimal labour.

Fish were stocked into four illuminated net cages (0.5 m³ each) at two different stocking densities (1.0 fish.L⁻¹ and 2.0 fish. L⁻¹). The cages were placed in a 0.2-ha earthen pond, which was continuously aerated with a paddlewheel aerator. During the 12-day-long trial, the water temperature fluctuated between 14-19°C. To attract zooplankton, each cage was illuminated with a 12V / 60W bulb put into the cages at a depth of 0.25 m. The light was switched on at 19:30 and switched off at 06:30 automatically with a timer. In the cages, the fish fed exclusively on prey organisms.

The fish had a slower growth with higher standard deviations at high stocking densities (see Table 1).

	Stocking densities (fish/l)	Final length (mm)	Final weight (g)	CV%	SGR	Survival
1	1	46,0±2,49	0,59±0,09	15,8	27,8	431pc (86%)
2	1	45,9±1,66	0,61±0,07	11,3	27,7	425pc (85%)
3	2	39,9±5,47	0,43±0,16	37,8	24,8	750pc (75%)
4	2	42,3±4,98	0,49±0,15	30,1	26,3	770pc (77%)

Table 1. Experimental data

CV%=: $100 \times SD / w_t$

SGR= $100 \times (\ln w_t - \ln w_0) / t$ (%/day)

This project was supported by the Bolyai János Grant.

Development of a multifunctional fish culture system

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Abstract

This project aimed to develop a multifunctional recirculation fish rearing facility, which is suitable for a large-scale fry and fingerling production of different fish species with different ecological demands. During the planning, it was an important aspect that it has simple technical solutions and is easily installed. In the system, environmental parameters (e.g. temperature, salinity etc.) can be set according to the needs of the target species requiring either freshwater or saltwater in their optimal temperature range.

The system contains a 1.1 m³ volume sedimentation tank and a biological filter with 400 litre “bioflow 9” filter media (870 m² surface/m³ volume). The water flow is provided with two pumps (each with a capacity of 6500 l/h), which are suitable for circulating fresh- and saltwater. The system also contains two 55-Watt UV lamps, and it is heated by electric heaters. The useful volume in the case of larval and fingerling rearing is 1.5 m³.

The biological process for purification of water was induced with urea, fish feed and inoculation of special bacterial strains. Urea provided the carbon and nitrogen source for the growth of bacteria and the fish feed provided the phosphorus and trace elements. For the rapid start of nitrification, 5 ml bacterial solution (produced by Aquaculture Supply, Geneva, Switzerland) was inoculated in the fourth week. During the test period, the chemical parameters of water were measured twice a week, and dissolved oxygen and temperature were checked 5 times a day. The activation of the biological filter needed about 1 month.

Based on our current experimental results, it can be stated that the system is suitable for nursing barramundi (*Lates calcarifer*) fingerlings, which reached an average weight of about 30 g (from 0.4 g initial weight) in two months. The total production was about 22 kg and the feed conversion ratio remained below 0.6 kg/kg.

This work was performed in the frame of the BARRA_09 project and was supported by the National Office for Research and Technology (NKTH) and, partly, by the Bolyai János Grant.

Biological features of isolates of spring viraemia of carp virus (SVCV) isolated in different regions of Ukraine

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Abstract

Spring viraemia of carp (SVC) is an important disease affecting cyprinids and is reported to be present in several European countries. In Ukraine SVCV causes mortality of carp (*Cyprinus carpio*) that can reach up to 70% during spring outbreaks and leads to considerable economic losses in fish industry. SVCV is currently classified as a tentative member of the genus Vesiculovirus of the family Rhabdoviridae.

Suitable cell lines are necessary tools for the virus proliferation, purification and further characterization. Therefore, in our investigation, we studied the morphology, physicochemical properties and growth characteristics of SVCV in several cell lines, such as BF-2, FHM and EPC.

The sensitivity of cell lines to SVCV was evaluated for two criteria: virus titer (TCID₅₀) and time for CPE appearing. The sensitivity of SVCV to chloroform, pH and heat treatment was also determined.

SVC is listed as a notifiable disease by the OIE. Therefore the virus propagation, including isolation, transportation and storage was based on OIE guidelines. The virus was obtained from the outbreak of SVC in 2009 from Lvivska, Rivnenska and Volynska regions. For the infection, BF-2, FHM and EPC cells grown in 25-cm² flasks were inoculated with the virus suspension diluted 10-fold and were maintained at 21,5°C. When the CPE was stable after serial infection, the virus titer was determined using the 50% tissue culture infective dose (TCID₅₀) method.

For electron-microscopic observation, the virions of SVCV were stained with 2% uranyl acetate and studied with an electron microscope EM-125 (SEO).

Susceptibility of the virus isolates to chloroform was tested by stirring a mixture (vol/vol) of infected cell culture fluid and chloroform at 4 0C for 4 h. For a temperature sensitivity test, the virus was heated at 56°C for 30 min. The pH sensitivity of the virus was determined by treating the virus with low pH (3.0) and high pH (12.0) at 4 0C for 20 min. After experiments, the infectivity of aliquots of serial 10-fold dilutions was assayed on EPC cells. Untreated infected cell culture fluid was also titrated and served as a control.

Cytopathic effects appeared in BF-2, FHM and EPC cell cultures 24 h after inoculation with supernatants of infected fish organ homogenates. Firstly, cell shrinkage and rounding were observed at 1–2 days post-infection (p.i.). Then the

number of rounding cells increased greatly at 3 days p.i. and reached a peak at 4 days p.i. Finally, numerous rounding cells began to detach from the flask and broke up after 5 days p.i. The monolayer of cell cultures was completely detached from the plastic surface 1 d later.

The results showed that the viral titers at 5–7 days were around $10^{6.4-7.5}$ TCID₅₀/0.1 ml, and no obvious difference was observed between the number of viral passages and cell lines. Transmission electronic microscopic assay showed numerous virus particles with the diameter of approximately 150–170×50–60 nm.

Following physico-chemical treatments including heat, exposure to low and high pH and organic solvents, the virus was titered in EPC cells. The virus was sensitive to both heat (56°C, 30 min) and pH (3.0 and 12.0). The virus was inactivated completely within 20 min after treatment with chloroform, suggesting that the virus is lipid enveloped.

Cell cultures, electron microscopy and physicochemical approaches yielded results consistent with the suggestion that SVCV, isolated in different regions of Ukraine was similar to reference strains of SVCV. For virus characterization, investigated cell lines may prove useful in SVCV propagation, but EPC cells, unlike BF-2 and FHM, are cheap to maintain.

The zooplankton food resources in the fish farming in the Republic of Moldova

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Abstract

The importance of zooplankton in the fish productivity of ponds is very difficult to overestimate. Numerous studies indicate that phytoplankton and zooplankton are often the only accessible and necessary natural food supply for most fish in the early stages of ontogeny. The creation of conditions for optimum development of zooplankton is a fundamental factor in shaping the size and productivity of most fish species. Cladocerans and *Daphnia* have great importance in the feeding of young fish, while for larvae, ciliates, rotifers and protococcus algae are the most important food items.

This work is based on the results of zooplankton studies in the fish ponds Gura Bicului and Ivancea in 2009. Sampling and processing of zooplankton samples was carried out by conventional hydrobiological methods (Guide to techniques of hydrobiological analysis of surface water and sediments, 1983). In calculating biomass and zooplankton production, published data were used on the individual weight and P/B ratio, respectively, of individual species and forms.

In 2009, 12 taxa were reported belonging to three groups (Rotatoria, Copepoda, Cladocera) in the studied fish ponds Gura Bicului and Ivancea. Of the 3 groups, the most diverse were Copepods, accounting for 50% of the total number of species. The share of rotifers and cladocerans accounted for 25% for each group. In general, the species composition of zooplankton in the experimental ponds was quite uneven. Some species evolved quite rapidly, others slowly. A common feature of the studied ponds is the dominating of *Acartocyclops viridis*. This species, including its naupliar and copepodite stages, had the main role in the formation of zooplankton biomass, which amounted to 2161.5 mg/m³ (97.7% of the total biomass) in Gura Bicului fish farms, and 21,268 mg/ m³ (99.3%) in Ivancea fish farms.

The total number and biomass of zooplankton was 264,000 ind./m³ (2.212.8 mg/m³) in Gura Bicului fish farms and 653,000 ind./m³ (21.406 mg/m³) in Ivancea fish farms (Table 1.2). Ivancea Fish Farm is 2.5 times more productive in terms of size and 9.7 times – in terms of biomass. The norm of zooplankton biomass as a natural food base for fish farm ponds in Moldova should be 6-10 mg/l.

Taxon	Ind./m ³	Biomass (mg/m ³)	Productivity (mg/m ³)
Rotifera	60500	12,3	2,82
Copepoda	203000	2186,5	128,81
Cladocera	5000	14,00	0,84
Total	264000	2212,8	132,47

Table 1. Indicators of zooplankton in Gura Bicului fish farms

Taxon	Ind./m ³	Biomass (mg/m ³)	Productivity (mg/m ³)
Rotifera	2500	8,5	2,19
Copepoda	643500	21374,5	375,1
Cladocera	7000	23,00	5,98
Total	653000	21406	383,27

Table 2. Indicators of zooplankton in fish farms Ivancea.

Judging from the above-mentioned data, the natural food supply of zooplankton is only 2.21 mg/l and is quite low. In Ivancea fish farms, on the contrary, it is developed sufficiently and is 21.406 mg/l.

The leading role in the shaping of zooplankton production in the studied ponds belongs to *Acantocyclops viridis* and its copepodite stages.

Selective breeding of common carp (*Cyprinus carpio*) in Serbia

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Abstract

In Serbia, the carp eggs and fingerlings are produced by a small number of common carp hatcheries (only three of them) and without any active selective breeding program. Hatcheries are genetically closed units producing their own stock of spawners. New spawners are selected according to their phenotypic performance. Often, only a small numbers of spawners are used in each generation. Also the frequency of replenishment of spawners is low. Consequently, although not documented, the rate of inbreeding is expected to be high and the seed quality low from such breeding systems. Such problems suggested an urgent need for better procedures for seed production. The aim of this selective breeding program was to assess the possibilities of improving production traits (weight, length and height) that are related to the growth of carp through selection in Serbia.

A collaborative research project between the Agricultural Faculty of Belgrade University, Serbia, and Nofima Marin (formerly AKVAFORSK), Norway, was initiated in 2005 with the aim of improving common carp through selective breeding. The approach was to form a base population by collecting breeders from hatcheries and rivers in Serbia.

The reproduction and culture of common carp was carried out at the Center for Fishery and Applied Hydrobiology (CFAH), Faculty of Agriculture, University of Belgrade, Serbia. Common carp broodfish were obtained from five local fish farms located at north part of Serbia and from the river Tisa. A partly factorial mating design was used to produce 48 full-sib families. In October 2007, 1812 fingerlings were randomly sampled and individually tagged with Passive Integrated Transponder (PIT) tags (11 x 2.1 mm), implanted into the abdominal cavity. The average number of tagged fingerlings per family were 38 and mean body weight was 13 g. Individual body weight, length and height of the fish were recorded twice, first at 5 months of age (at tagging in October 2007) and later at 18 months of age (in November 2008).

In our study, heritabilities and genetic correlations of growth-related traits in two-year-old carps were estimated. Based on a multitraits animals model, heritability estimates were high for all traits (0.39 ± 0.09 , 0.34 ± 0.09 and $0.45 \pm$

0.08 for weight, length and height, respectively). The genetic correlations between weight and the two other traits were high (0.81 ± 0.06 and 0.91 ± 0.03 for length and height, respectively), while height and length of the fish were moderately correlated (0.54 ± 0.12). Furthermore, residual correlations between the traits were also high, (between length and two other traits 0.89 ± 0.01 and 0.89 ± 0.02 , for weight and height respectively, and between weight and height 0.83 ± 0.02). Based on the current results, genetic improvement of growth rate is expected to be efficient. However, given the high residual correlation between the traits, the value of height and length as indirect traits for improvement of growth rate is of limited value when compared with direct recording of weight. Nevertheless, given the current results, improving growth rate in common carp through genetic selection is expected to be effective.

Based on the estimated genetic variation, one can expect significant genetic improvement in growth traits in common carp through selective breeding. This is in accordance with recently conducted studies (Gjerde et al., 2002, Vandeputte et al., 2004, Vandeputte et al., 2008) which showed that genetic improvement of growth and survival of carp can indeed be obtained through selective breeding. According to a review by Vandeputte (2003) about 20% increase in growth rate can be achieved per generation by means of simple mass selection of the best 3% of the population. This should be the basis for a successful selection breeding program for common carp in Serbia.