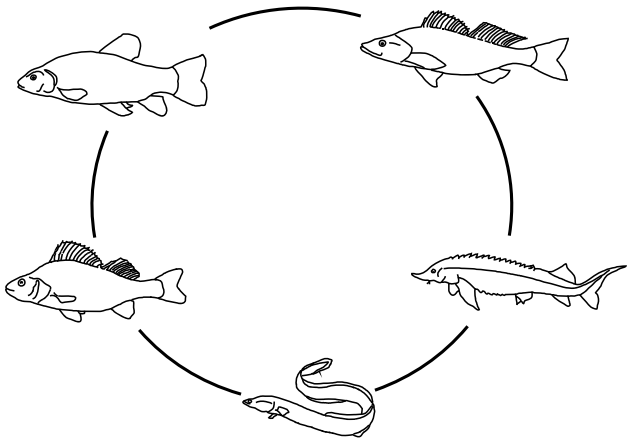
Fakulta rybářství
a ochrany vod
Faculty of Fisheries
and Protection
of Waters

Jihočeská univerzita
v Českých Budějovicích
University of South Bohemia
in České Budějovice

Diversification in Inland Finfish Aquaculture II (DIFA II)

September 24–26, 2013
Vodňany, Czech Republic



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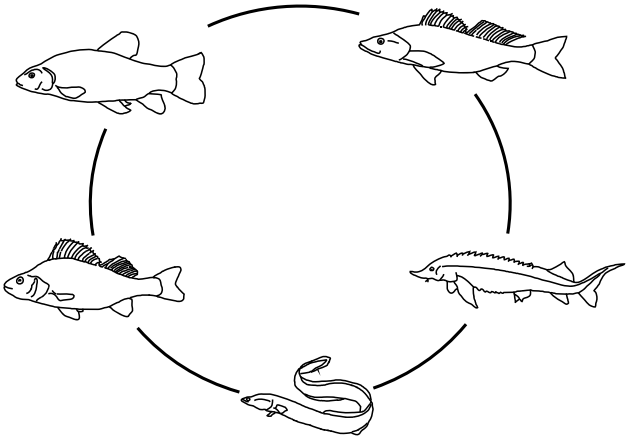

Diversification in Inland Finfish Aquaculture II (DIFA II)
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WELCOME AT DIFA II

Dear friends and colleagues,

we would like to heartily welcome you at the second run of the scientific workshop with the title „Diversification in Inland Finfish Aquaculture"(DIFA II) taking place in Vodňany, Czech Republic, on the occasion of the opening of the new facilities of the Faculty of Fisheries and Protection of Waters, University of South Bohemia in České Budějovice (FFPW USB) – South Bohemian Research Center of Aquaculture and Biodiversity of Hydrocenoses (CENAKVA).

The DIFA II follows the previous successful DIFA workshop, which was held in Písek, Czech Republic, in May 2011 on the occasion of launching the CENAKVA project with celebration in the Hluboká castle.

The workshop topics follow the main tasks of both the Research Institute of Fish Culture and Hydrobiology of FFPW USB and the CENAKVA project, addressing both traditional and modern methods of production of many freshwater aquatic species, exploring farming technologies and products. Main topics of the workshop will be focused on: quality of fish flesh and nutrition; reproduction and biotechnology; detection, exposure, effect, risk and elimination of pollutants in the aquatic environment; and freshwater aquaculture of commercially interesting fish.

Thanks are due to organizing and scientific committees of the DIFA II workshop for enthusiastic preparation of this event and to all sponsors (CENAKVA, FSBI – An International Society for Fish Biology, and OLYMPUS) for their financial and promoting support.

We wish you a nice time spent in Vodňany and we hope that the workshop will be a real opportunity for an in-depth exchange of experience, for meeting old friends, as well as to establish new professional contacts.

Martin Pšenička and Ivana Němcová

WORKSHOP PROGRAM

TUESDAY, THE 24TH OF SEPTEMBER, 2013

08.00–09.00 Registration

09.00–09.30 Welcome words (Otomar Linhart, the Dean of the Faculty of Fisheries and Protection of Waters and Director of CENAKVA)

SESSION 1: QUALITY OF FISH FLESH AND NUTRITION

Chairmen: Sachi Kaushik, Jana Pickova, Jan Mráz

09.30–09.50 Flesh quality of farmed fish fed diets with low levels of fishmeal and fish oil. (***Sachi Kaushik***) pp. 16

09.50–10.10 New strategies when thinking sustainable feeds to fish. (***Jana Pickova***, *Anders Kiessling, Volkmar Passoth*) pp. 17

10.10–10.30 Lipids in common carp and effects on human health. (***Jan Mráz***, *Pavel Kozák, Tomáš Zajíc, Věra Adámková, Jana Pickova*) pp. 18

10.30–10.50 Preliminary study of optimal feed dosage determination of *Cyprinus carpio* L. x *Carassius auratus* L. hybrid reared in cages in cooling water. (**Jacek Sadowski**, *Magdalena Wielopolska, Marek Bartłomiejczyk, Mirosław Półgęsek, Ewelina Giedziun*) pp. 19

10.50–11.20 COFFEE BREAK + POSTERS (session "Quality of Fish Flesh and Nutrition")

11.20–11.40 Effects of feeding different vegetable foods on output and product quality of marketable Carps (*Cyprinus carpio*). (***Gert Füllner***, *Matthias Pfeifer*) pp. 20

11.40–12.00 Different susceptibility to body deformities in juvenile cyprinid fish intensively fed dry diet under controlled conditions. (***Justyna Sikorska***) pp. 21

12.00–12.20 The role of zooplankton as food in carp pond management. (***Maria Anton-Pardo***, *David Hlaváč, Zdeněk Adámek*) pp. 22

12.20–12.40 Purging the „off-flavour" from barramundi (*Lates calcarifer*) after artificial contamination with geosmin. (***Zsuzsanna Gy. Papp***, *J. Biró, T. Feledi, A. Adorjan, V. Pencze, Zsuzsanna J. Sándor*) pp. 23

12.40–13.40 LUNCH

SESSION 2: REPRODUCTION AND BIOTECHNOLOGY

Chairmen: Katsutoshi Arai, Steven D. Mims, William L. Shelton, Martin Flajšhans

13.40–14.10 **Invited lecture –** Dimorphic expression pattern of sex differentiation-related genes in morphologically undifferentiated gonads of Amur sturgeon and Russian sturgeon. (***Shigeho Ijiri***, *Seishi Hagihara, Ryohei Yamashita, Hatsumi Suzuki, Shinji Adachi*) pp. 24

14.10–14.30 Development of sturgeon primordial germ cells: differences of developmental patterns among Xenopus, sturgeon, and zebrafishes. (***Taiju Saito***, *Martin Pšenička, Rie Goto-Kazeto, Kunio Inoue, Shinji Adachi, Katsutoshi Arai, Etsuro Yamaha*) pp. 25

14.30–14.50 Acipenseriform gynogenesis: progress & re-examination. (***William L. Shelton***, *Steven D. Mims*, *Rafael Cuevas*) **pp. 26**

14.50–15.10 Sex determination in sturgeons (Acipenseriformes): a short overview and future perspectives. (***Massimiliano Rampin***) **pp. 27**

15.10–15.30 Yolk formation in fish: multiple vitellogenins and their receptors. *(**Naoshi Hiramatsu**, Hiroko Mizuta, Wenshu Luo, Osamu Nishimiya, Meiqin Wu, Yuji Mushirobira, Benjamin J. Reading, Craig V. Sullivan, Takashi Todo, Akihiko Hara)* **pp. 28**

15:30–15:50 Recent progress in studies on androgenesis of fish. *(**Katsutoshi Arai**)* **pp. 29**

15:50–16:20 COFFEE BREAK + POSTERS (session "Reproduction and Biotechnology")

16:20–16:40 Attempts to simulate possible autotriploidization mechanisms in two sturgeon species, the sterlet, *Acipenser ruthenus* and the Siberian sturgeon, *A. baerii*. *(**Martin Flajšhans**, Marek Rodina, Miloš Havelka, David Gela, Dmytro Bytyutskyy)* **pp. 30**

16:40–17:00 Sperm maturation in sturgeon: verification of existence and possibilities for application. *(**Viktoriya Dzyuba**, Borys Dzyuba, Jacky Cosson, Sergei Boryshpolets, Marek Rodina, Pavlo Fedorov, Taiju Saito, Martin Pšenička, Olga Bondarenko, Otomar Linhart*) **pp. 31**

17:00–17:20 Embryonic stages and primordial germ cells development in pikeperch *Sander lucioperca* (Teleostei: Percidae). (***Hilal Guralp***, *Kseniia Pocherniaieva, Miroslav Blecha, Tomáš Policar, Martin Pšenička, Taiju Saito*) **pp. 32**

17:20–17:40 Androgenic development of the brook charr (*Salvelinus fontinalis*, Mitchill 1814) *in the eggs originated from the interspecific charr hybrids*. (***Konrad Ocalewicz***, *Oliwia Michalik, Stefan Dobosz*) **pp. 33**

17:40–18:00 Dynamics of changes in cytological characteristics of bester oocytes under controlled conditions. (***Svetlana Piyanova***, *Olga Filippova, Sergey Zuevskiy*) **pp. 34**

18:00–18:20 Comparison of the quality of pikeperch (*Sander lucioperca*) sperm in different spawning seasons. (***Miroslav Blecha***, *Jiří Křištan, Azin Mohagheghy Samarin, Marek Rodina, Tomáš Policar*) **pp. 35**

WEDNESDAY, THE 25TH OF SEPTEMBER 2013

SESSION 3: POLLUTANTS IN THE AQUATIC ENVIRONMENT

Chairmen: Ingvar Brandt, Werner Kloas, Tomáš Randák

09:00–09:20 Impact of seasonal soil fertilizers on crayfish cardiac and behavioural activity. (***Iryna Kuklina***, *Antonín Kouba, Aliaksandr Pautsina, Petr Císař, Pavel Kozák*) **pp. 36**

09:20–09:40 Environmental pollutants alter both regulation and catalytic activity of the CYP1 system in fish. (***Ingvar Brandt***, *Maria Jonsson, Kristina Beijer, Kai Gao, Bjorn Brunstrom*) **pp. 37**

09:40–10:00 Complex approach to monitoring of aquatic environment contamination in Czech Republic. (***Tomáš Randák***, *Roman Grabic, Ganna Fedorova, Kateřina Grabicová, Viktoriia Burkina, Oksana Golovko, Christoph Steinbach, Vladimír Žlábek*) **pp. 38**

10:00–10:20 High resolution mass spectrometry as a bridge between environmental chemistry and toxicology. (***Roman Grabic***, *Tomáš Randák, Ganna Fedorova, Kateřina Grabicová, Viktoriia Burkina, Oksana Golovko, Christoph Steinbach, Vladimír Žlábek*)

10:20–10:50 COFFEE BREAK + POSTERS (session "Pollutants in the Aquatic Environment")

10:50–11:10 Contaminant bioaccumulation in paddlefish and hybrid striped bass fingerlings cultured in reclaimed effluent water at a decommissioned wastewater treatment facility. (***Steven D. Mims***, *Rafael Uribe-Cuevas*) **pp. 39**

11:10–11:30 Recent approaches to assess endocrine disruptors in aquatic environments. (***Werner Kloas***, *Frauke Hoffmann, Josefin Garmshausen, Andrea Zikova, Ilka Lutz*) **pp. 40**

11:30–11:50 Analgesics, psycholeptics, antidepressants and illicit drugs in aquatic environment of Czech Republic. (***Ganna Fedorova***, *Tomáš Randák, Oksana Golovko, Vít Kodeš, Kateřina Grabicová, Roman Grabic*) **pp. 41**

11:50–12:10 Photodegradation characteristics of pharmaceuticals in water under impact of UV and sunlight. (***Oksana Golovko***, *Roman Grabic, Jerker Fick, Richard Lindberg, Marcus Östman*) **pp. 42**

12:10–12:30 Occurrence, toxicity and fate of natural progesterone and synthetic progestin in aquatic environment: a review. (***Vimal Kumar***, *Roman Grabic, Hana Kocour Kroupová*) **pp. 43**

12:30–12:50 Screening of emerging pollutants by passive sampling in middle and lower reaches of Yangtze river. (***Vladimír Žlábek***, *Tomáš Randák, Ganna Fedorova, Viktoriia Burkina, Huijun Ru, Yunfeng Li, Zhaohui Ni, Daqing Chen, Roman Grabic*) **pp. 44**

12:50–13:50 LUNCH

13:50–14:10 Bioconcentration of antidepressant in fish exposed to wastewater treatment plant´s effluent. (***Kateřina Grabicová***, *Richard H. Lindberg, Marcus Ostman, Roman Grabic, Tomáš Randák, Jerker Fick*) **pp. 45**

14:10–14:30 A histology-based health assessment of selected fish species from two rivers in the Kruger national park, South Africa. (***Ina Wagenaar***, *Warren Smith, Nico Smit*) **pp. 46**

14:30–14:50 How does vinclozolin modulate reproductive genes in male goldfish? (***Mahdi Golshan***, *Azadeh Hatef, Magdale-na Socha, Mirosław Sokolowski-Mikolajczyk, Hamid Reza Habibi, Sayyed Mohammad Hadi Alavi*) **pp. 47**

14:50–15:10 *In vitro* inhibition of hepatic cytochrome P450-mediated reactions by human pharmaceuticals in rainbow trout microsomes. (***Viktoriia Burkina***, *Vladimír Žlábek, Galia Zamaratskaia*) **pp. 48**

15:10–15:30 Distribution of some pharmaceutical residues in the aquatic ecosystem of rivers. (***Zsuzsanna J. Sándor***, *Percze V., Biró, J., Fodor A., Györe K., Józsa V., Zsuzsanna Gy. Papp)* **pp. 49**

15:30–15:50 Crayfish monitoring system. (***Aliaksandr Pautsina***, *Dalibor Štys, Petr Císař*) **pp. 50**

SESSION 4: FRESHWATER AQUACULTURE OF COMMERCIALLY INTERESTING FISH – 1ST PART
Chairmen: Patrick Kestemont, Dariusz Kucharczyk, Tomáš Policar

15:50–16:20 COFFEE BREAK + POSTERS (session "Freshwater Aquaculture of Commercially Interesting Fish")

16:20–16:40 Improvement of larval rearing in percid fish through dietary enrichment with nutrients, immunostimulants and probiotics. (***Patrick Kestemont***, *Robert Mandiki)* **pp. 51**

16:40–17:00 Effect of age, size and digestive tract development on weaning effectiveness in crucian carp, *Carassius carassius* (L.), under laboratory condition. (***Dariusz Kucharczyk***, *Beata Łączyńska, Daniel Żarski, Joanna Nowosad, Katarzyna Palińska-Żarska, Maria Biłas, Sławomir Krejszeff, Tamas Müller*) **pp. 52**

17:00–17:20 Processing traits of European catfish (*Silurus glanis*) from outdoor flow-through and indoor recycling aquaculture units. (***Zdeněk Adámek***, *Iulia Grecu, Isabelle Metaxa, Laurent Sabarich, Jean-Paul Blancheton*) **pp. 53**

17:20–17:40 Sturgeon and caviar production and the misleading market of caviar imitations. (***Paolo Bronzi***) **pp. 54**

THURSDAY, THE 26TH OF SEPTEMBER 2013

SESSION 4: FRESHWATER AQUACULTURE OF COMMERCIALLY INTERESTING FISH – 2ND PART
Chairmen: Patrick Kestemont, Dariusz Kucharczyk, Tomáš Policar

09:00–09:20 Differences in antioxidant status among important representatives of Cyprinidae, Acipenseridae and Siluridae reared in cage culture. (*Radosław Drozd*, ***Remigiusz Panicz***, *Dorota Jankowiak, Arleta Drozd, Arkadiusz Nędzarek*) **pp. 55**

09:20–09:40 First detection of Herpesvirus anguillae (*HVA*) in Poland from various eel (*Anguillidae* sp.) samples. (*Jolanta Kempter,* ***Paulina Hofsoe****, Remigiusz Panicz*) **pp. 56**

09:40–10:00 Nursing of pikeperch (*Sander lucioperca* L.) fry in shallow fishponds. *(Balázs Csorbai,* ***Tamás Szabó****, Éva Kovács, Ádám Németh, Beatrix Beres, László Horváth*) **pp. 57**

10:00–10:20 Preliminary results of on-growing during hatching of burbot (*Lota lota*) using chironomidae larvae and chicken liver. (***Mirosław Półgęsek****, Jacek Sadowski*) **pp. 58**

10:20–10:50 COFFEE BREAK

10:50–11:10 Effect of temperature and stocking density on food utilization and growth of European whitefish (*Coregonus lavaretus* L.) from lake constance. (***Susanne E. Göbel****, Jan Baer*) **pp. 59**

11:10–11:30 Efficiency of two spawning techniques evaluated by reproductive performance in pikeperch (*Sander lucioperca* L.). (***Tomáš Policar****, Miroslav Blecha, Jiří Křišťan*) **pp. 60**

11:30–12:00 Awards for best student presentation and best student poster

Closing words (Martin Pšenička, DIFA II workshop convenor)

12:00–13:00 LUNCH

13:30 Ceremonial opening of the new CENAKVA facilities

POSTER SESSION

SESSION 1: QUALITY OF FISH FLESH AND NUTRITION

SESSION 2: REPRODUCTION AND BIOTECHNOLOGY

SESSION 3: POLLUTANTS IN THE AQUATIC ENVIRONMENT

ORAL SESSION

FLESH QUALITY OF FARMED FISH FED DIETS WITH LOW LEVELS OF FISHMEAL AND FISH OIL

Sadasivam J. Kaushik*

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The sustainable development of aquaculture implies the optimal use of feed ingredient resources. In this context, much emphasis has been put on the research for alternatives to fish meals and fish oils, but still meeting the macro and micro nutrient requirements of fish and shrimp. Such efforts have also led to the development of feeds by the industrial sector with low levels of fish meal and fish oil. One major question which arises often is that on whether such changes in feeds have or can have adverse effects on the "quality" of farmed fish and shrimp. Based on a number of studies undertaken with different species of fish, the general conclusion is the absence of distinct differences in objective flesh quality attributes or nutritional profiles such as free amino acids or fatty acids caused by dietary protein sources. The effects of replacement of fish oil by alternative sources such as vegetable oils do have significant effects on the fatty acid profiles of flesh as well as moderate effects on some sensory attributes. Studies on replacement of both fish meal and fish oil have also been undertaken with several species of fish, not only at an experimental level but also at full scale farm levels. In all such studies, objective quality evaluation criteria have been used. Models of changes in fatty acid profiles have been developed. Different feeding strategies have been developed in order to maintain and ensure the nutritional value of farmed fish. Studies have also taken into account changes in quality due to possible contaminants of dietary origin. The presentation will try to bring out some of the major findings with different species under aquaculture and summarise the issues.

Keywords: *farmed fish, nutrition, flesh quality, fish meal, fish oil*

NEW STRATEGIES WHEN THINKING SUSTAINABLE FEEDS TO FISH

Jana Pickova[1,2], Anders Kiessling[2], Volkmar Passoth[2]

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Microbes such as yeast and algae can become the future producers of essential nutrients if suitable species and growth conditions are applied to produce ´marine´ fatty acids and proteins. Marine algae have for long been recognized as the large producers of the long chain polyunsaturated fatty acids, especially DHA and EPA, in the aquatic food chain. Farmed fish contains high levels of such fatty acids since marine fishmeal is used in the feed formulation. It is necessary to use raw materials of other then from wild-caught fish and instead include alternate feed materials rich in long chain omega-3 fatty acids. Yeasts and algae are novel and sustainable ingredients possible to tailor for future fish feed formulations replacing fishmeal. Our goal is to produce lipids and proteins of favorable composition through large-scale cultivation of yeasts and algae in various organic wastes from pulp- and food industries. Our own preliminary small-scale results show that yeasts can grow on these types of organic wastes and that we can influence protein and fatty acid composition in yeasts and algae by modifying the growth conditions or by the addition of certain bioactive substances. The initiative will generate new knowledge on technologies and product prototypes that will enable ecologically sustainable fish farming while closing the loop for several organic waste streams. Thus, it can be expected that this project will generate results, which are of strategic importance for future economy of food production and for food security on global scale.

Keywords: *lipids, microorganisms, fish feeds, sustainable*

LIPIDS IN COMMON CARP AND EFFECTS ON HUMAN HEALTH

Jan Mráz[1], Pavel Kozák[1], Tomáš Zajíc[1], Věra Adámková[2], Jana Pickova[1,3]

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Fish is the major source of n-3 fatty acids n-3 (FA), especially eicosapentaenoic (EPA) and docosahexaenoic acid (DHA) which are known to be beneficial for human health, especially for cardiovascular system. Intake of fish as well as other n-3 FA sources is generally low in the Czech Republic. Common carp (*Cyprinus carpio*) is one of the most cultured fish species in Central Europe and therefore to increase the content of n-3 FA in carp flesh would be beneficial. We aim to develop a sustainable way to produce common carp with high content of n-3 FA without the use of fish oil and fish meal and test the effects of such fish in prevention and treatment of cardiovascular diseases. We have developed a novel supplemental feed based on rapeseed cake and extruded linseed as lipid source to increase n-3 FA in carp flesh. Flesh of carp supplemented by the feed contains in one serving (200 g) 300 mg of EPA+DHA and 1 g of n-3 FA. Such carp products are already present at the Czech market labelled with a trademark. Further, in a clinical study, we tested effects of full meals based on these carp products on human subjects after cardiovascular surgery during their follow up spa treatment. The subjects consumed for four weeks either standard spa diet or diet including two servings (200 g) of carp per week (carp feed supplemented either by cereals or by the novel rapeseed/linseed feed). Plasma lipid parameters were analysed at the beginning and after four weeks of intervention. The subjects consuming carp flesh had a significantly better improvement of their plasma LDL and HDL cholesterol, triglycerides and C-reactive protein compared to the control group receiving the standard spa diet. Meals from carp supplemented by the novel rapeseed/linseed feed had significantly higher effects compared to the carp supplemented by cereals. We conclude that carp with increased content of n-3 FA is a valuable healthy local product and its consumption should be increased by the Czech population for improvement of cardiovascular health.

Keywords: *cardiovascular diseases, DHA, EPA, feeding, sustainable*

Acknowledgements: The study was supported by projects CENAKVA CZ.1.05/2.1.00/01.0024, GAJU 074/2013/Z and NAZV QH92307.

PRELIMINARY STUDY OF OPTIMAL FEED DOSAGE DETERMINATION OF *CYPRINUS CARPIO* L. X *CARASSIUS AURATUS* L. HYBRID REARED IN CAGES IN COOLING WATER

Jacek Sadowski*, Magdalena Wielopolska, Marek Bartłomiejczyk, Mirosław Półgęsek, Ewelina Giedziun

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The intention of this study was to compare growth effect of hybrid fish reared in cages in the cooling water nourished with different doses of feed. The study took place at the Fisheries Experimental Station of the Department Aquaculture of West Pomeranian University of Technology in Szczecin, located at the power plant, "Dolna Odra" in Nowe Czarnowo, in cooling water channel about 250 m from the cooling water inlet. The experiment was conducted during 140 days (07/05 – 24/09). The studies used the feed Classic of Aller-Aqua (30.6 % of protein, 8.8 % of lipid, 47.5 % of carbohydrates and 18.1 $MJ.kg^{-1}$ of gross energy) with the following rations: 0.8 %, 1.2 %, 1.6 %, 2.0 %, 2.4 % of metabolic weight of fish. Each of the five variants were tested in triplicate, with the density of fish: 150 $pcs.cage^{-1}$. Every seven days all fish were weighted to determine the growth rate and food conversion ratio. During the experiment the cooling water temperature ranged in 17.1–34.3 °C, pH 7.5 – pH 9.0, while the oxygen content of 2.8–12.6 $mg.dm^{-3}$. Hybrid after the experience has achieved an average unit weight of individual variations in the rate of 0.8 % (150 g pcs^{-1}), 1.2 % (236 g pcs^{-1}), 1.6 % (345 g pcs^{-1}), 2.0 % (413 g pcs^{-1}), 2.4 % (430 g pcs^{-1}) with FCR coefficients: 1.44, 1.55, 1.61, 1.93 and 2.32. The results indicate a significant impact on the size of the feed dose increments hybrid weight. The optimal piecewise regression model preferred rations were 1.4 % of metabolic body weight.influences crucial points of somatotropic axis. Results will be enriched with other factors to use them in tench culture.

***Keywords**: cooling water, optimal feeding ration, hybrid carp x goldfish*

EFFECTS OF FEEDING DIFFERENT VEGETABLE FEEDS ON OUTPUT AND PRODUCT QUALITY OF MARKETABLE CARPS (*CYPRINUS CARPIO* L.)

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Saxon State Institute for Environment, Agriculture and Geology – Fisheries Department, Gutsstraße 1, 02699 Königswartha, Germany

We reared marketable carps by feeding different vegetable supplementary feeds in ponds of the experimental fish farm Königswartha. With all used diets except corn we achieved good rearing results in average body mass gain as well as in gain per hectare and in Feed Conversion Efficiency. We realized best output by feeding rye, but there are no significant statistic differences to other feeds. Feeding with protein-rich vegetable feeds like lupine, pea or a cheap high protein vegetable mixed feed did not show better results on output than feeding with low-protein feeds like wheat, rye or barley.

Feeding with different vegetable feeds resulted in different slaughtering amounts and flesh qualities of carp, too. Highest gain and highest slaughtering result are not identic with high flesh quality. In our slaughtering examinations we identified differences between feeding groups, but first of all between sexes. Females had a better slaughtering result, because they had lower gonad mass during a three summer production time. By analyzing some physical-chemical parameters of carp filets, we detected that there was only a significant difference for luminance (parameter L in the CIE L*a*b* Color space) between the feeding groups barley and wheat, but not between the other groups or sexes. Color values a and b were different between sexes and only in part between trials.

Fat content of carp filet can oscillate partly within the group especially in groups that were fed with grain. Feeding with grain always resulted in the highest fat content. Carps that were fed by legumes achieved a low fat content only; however, a little more than carps that were fed exclusively with natural food. There was no dependency between flesh luminance and fat content.

In standardized tastings carps fed with peas and low fat content reached best results, better than fish that were fed with natural food only. On the other side, corn-fed carps were evaluated worst by all testers. Therefore, corn should never be used as supplementary food for marketable carps. In addition, carps fed with corn in spite of optic results received the worst evaluation not only for taste but also for yield in pond, food conversion and slaughtering parameters.

From an economical point of view, rye was the best feed for carp. However, best flesh quality of carps will be reached by feeding legumes which is caused by a low but not to low fat content of carp filets produced in that way. But feeding costs from high protein vegetable feeds like lupine, peas or cheap mixed feed from vegetable components double compare to grain without a similar increase of yields per hectare. The higher production costs result in a higher feed price only, but not in a worse feeding conversion. That is why use of high protein vegetable feeds is too expensive for the production of marketable carps.

Finally, our results show that the fat content is the most important parameter for the taste evaluation of carps. The higher the fat contents, the worse the product quality of Common carp. Therefore, a moderate fat content should be aspired. This can be reached by maximum yields per hectare and high protein vegetable feeds or by lower yields per hectare and a slight feeding with grain. The last way corresponds with better revenue; additionally, it meets the interests of ecology.

***Keywords**: supplementary feed, yield, product quality, fat, Common carp*

DIFFERENT SUSCEPTIBILITY TO BODY DEFORMITIES IN JUVENILE CYPRINID FISH INTENSIVELY FED DRY DIET UNDER CONTROLLED CONDITIONS

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In today's aquaculture, one of the most important problem of this branch are considered morphological abnormalities in fish, especially those affected by intensive feeding with commercial dry feeds. Particularly prone to dry-diet influenced abnormalities are Cyprinidae. Cyprinids, as stomachless fish with no acid secretion in the gut, are able to digest dry feeds much less effectively than fish with a functional stomach. In dry diet-fed young cyprinids, particularly in early juvenile period of their life, the most common morphological abnormalities are spinal curvatures. The share of individuals with such deformities was found to be positively dependent on the intensity of feeding, with the maxima recorded at or over the level of satiation.

The aim of the present work was to find out whether, under comparable conditions, the share of fish with deformities can be also species-dependent.

To answer the question posed, several series of 60-day feeding experiments at 25 °C were perfomed. They comprised 13 cyprinid fish species, of similar initial mean body weight of 0.5–0.8 g and the same density of 1.6 indiv. per dm^3. Fish were fed 5 times daily over 13 h a day with a commercial starter feed (Aller Futura, Denmark), at mean daily rate of approximately 2.5 % of fish biomass what is close to satiation.

The results of the experiments made it possible to distinguish three groups of cyprinid species of significantly ($P \leq 0.05$) different susceptiblity to deformities (mainly scoliosis) in early juvenile period of life. The group of low susceptibility to deformities consisted of four the following species: *Aspius aspius*, *Barbus barbus*, *Chondrostoma nasus* and *Cyprinus carpio*, for which the final share of juveniles with deformities was 0–3 %. Members of group of medium susceptibility were *Vimba vimba* and *Tinca tinca* with 11 and 24 % of fish with deformities, respectively. To group of high susceptibility belonged seven species: *Abramis brama*, *Carassius carassius*, *Leuciscus cephalus*, *L.* i*dus*, *L. leuciscus*, R*utilus rutilus* and *Scardinius erythrophthalmus*. In the particular cases, the share of juveniles with deformities ranged from 50 to 87 %.

The results of the work lead to the conclusion that it may not be feasible to work out formula of one universal dry diet, of comparable high usefulness, to be used for the controlled rearing of juveniles of all common Cyprinidae species of economic interest.

***Keywords**: cyprinid fish, deformities, dry diet, juveniles*

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THE ROLE OF ZOOPLANKTON AS FOOD IN CARP POND MANAGEMENT

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Commercial cyprinid fishes in Central Europe are usually reared in earthen ponds with the advantage that natural food will be easily found in the habitat. This work aims to present a review of the current knowledge of zooplankton as a natural food resource in carp ponds, including information about the management strategies to strengthen the link between zooplankton and fish in ponds.

Zooplankton is the main component of the diet for carp larvae, while, as the individuals grow, they change to benthivorous feeding, and then, chironomids are the dominant food. However, zooplankton remains in a variable proportion in its diet, depending on the availability of food in the environment, the season and other factors. Regarding the presence of different zooplanktonic organisms, they are selected in relation to fish size (and thus, the mouth size), increasing from small rotifers to large cladocerans.

In addition, zooplankton presents a high content of proteins (above 50 % for some groups) and lipids (approximately 10 % of dry weight, with a significant proportion of unsaturated fatty acids). Therefore, fish fed with natural food could have also a high nutritional content, better fitting the requirements of healthy human nutrition.

Proven the benefits of zooplankton for carp pond farming, some strategies to ensure and increase the production of this community could be used in the management of fish ponds. These practices include the monitoring of zooplankton organisms to stock the fish in the appropriate moment, the control of the environmental variables which favor the development of zooplankton, or the use of sustainable fish densities. An appropriate feeding regime, based on the application of supplementary feeds (mainly cereals), adapted according to the results of zooplankton monitoring, remains a big challenge for further studies.

Keywords: *carp larvae, cladocerans, rotifers, fish pond management*

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PURGING THE „*OFF-FLAVOUR*" FROM BARRAMUNDI (*LATES CALCARIFER*) AFTER ARTIFICAL CONTAMINATION WITH GEOSMIN

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Artificially breaded barramundi (*Lates calcarifer*) usually has unfavourable „*off-flavours*" without purging. Odorous compounds responsible for preharvest off-flavours may be accumulated from the water or diet. The most common natural causes of off-flavour in cultured fish are odorous metabolites of different aquatic microorganisms. Muddy-earthy odours and test in fresh water fish are the most common off-flavours, caused mainly by geosmin and 2-methyl-isoborneol which are metabolites of different microorganisms. These microorganisms might settle to the wall of the culture systems and they might cause unfavourable test of fish flesh, so they must be removed before harvesting. According to this our aim was to study a model of removing treatment with artificial pollution of geosmin.

A feeding experiment was carried out to study the model of tainting with "off-flavour". Four groups of 300 g average weight barramundi were fed during a week with different diets: "absolute" control: fish were fed with diet without geosmin and in an independent recycling system; "0" control: fish were fed without geosmin, but in the same system with the tainted groups; "low" geosmin: 50 $\mu g.kg^{-1}$ in the diet; "high" geosmin: 400 $\mu g.kg^{-1}$ in the diet. Purging was carried out for 7 days without feeding. Fish were sampled at the beginning of experiment, after the geosmin contamination, at the 4th and at the 7th days of purging. Off-flavour was detected by trained testers ("panel") with triangle test and geosmin was instrumentally analysed by GS-MS.

Our sensory and instrumentally measured results are shown that only small amount of the geosmin remained in the fish flesh already after 4–5 days purging and weight loss was negligible. Fish might be enjoyable after purging even if it was heavily contaminated by geosmin.

Keywords: *barramundi, off-flavour, purging, sensory analylsis, geosmin*

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DIMORPHIC EXPRESSION PATTERN OF SEX DIFFERENTIATION-RELATED GENES IN MORPHOLOGICALLY UNDIFFERENTIATED GONADS OF AMUR STURGEON AND RUSSIAN STURGEON

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Sex control is of interest in the aquaculture industry, specifically for sturgeons. To identify genetic sex, identification of sex-linked DNA markers is of particular importance. It may allow production of super females (WW), leading to a genetically all-female population. However, previous attempts to isolate sex-linked markers from genomic DNA have not been successful, probably because of large genomic size and complex sequences in sturgeons. On the other hand, RNA sequence analysis based on high-throughput sequencing technology has enabled the isolation of a sex-determining gene located on the Y chromosome in rainbow trout. The present study aimed to isolate the sex-determining gene from sturgeons by RNA sequence analysis using morphologically undifferentiated sturgeon gonads.

The expression levels of sex differentiation-related genes are considered useful markers for identification of potential ovary or testis at a morphologically undifferentiated stage. In previously studied fish, foxl2 and cyp19a1a (P450arom) or dmrt1 were characteristically detected in female or male undifferentiated gonads, respectively. In the Amur sturgeon, full-length cDNAs encoding foxl2 and 2 types of dmrt1 (dmrt1a and dmrt1b) were isolated. RT-PCR analysis revealed that foxl2 was expressed in the ovary and not in the testis. In contrast, dmrt1b was distinctly expressed in the testis and not in the ovary. Dmrt1a was expressed in both gonads. At 9 months of age, qPCR analysis showed high foxl2 level in three differentiating ovaries. The expression level of foxl2 was very low in one differentiating testis and two undifferentiated gonads. Other three undifferentiated gonads showed high foxl2 expression level. Before 9 months, such dimorphic expression pattern was not evident in undifferentiated gonads. Dmrt1a and 1b did not show such dimorphic expression pattern. A similar expression pattern was observed in the Russian sturgeon. In addition, cyp19a1a cDNA was isolated from the Russian sturgeon. The expression pattern of cyp19a1a showed a pattern similar to that of foxl2. These results probably indicate that undifferentiated gonads expressing high foxl2 and cyp19a1a would differentiate into an ovary (potential ovary).

To identify other gonadal sex differentiation-related and sex-determining genes, RNA sequence analysis was carried out for undifferentiated gonads from 5 juvenile Russian sturgeons (age, 9 months). A total of 45,686,832 (8,498 Mb) and 79,743,269 (7,887 Mb) sequence reads were obtained using Ion PGM and HiSeq 2000 sequencers, respectively, from 3 potential ovaries and 2 potential testes; these were assembled into 338,648 contigs. tBLASTx analysis identified 26 potential sex differentiation-related genes. Within them, read mapping analysis found that gsdf and hsd17b1 were characteristically expressed in potential testes and ovaries, respectively. This clear contrast between the dimorphic expression patterns in gsdf and ovary-related genes (foxl2, cyp19a1a, and hsd17b1) may aid us to distinguish potential ovary and testis even before 9 months of age. Furthermore, read mapping analysis identified approximately 700 unique contigs (> 300 bp) that were found only in the potential ovaries. These contigs need to be examined by genomic PCR to identify sex-linked markers, which might help us identify the sex-determining gene.

DEVELOPMENT OF STURGEON PRIMORDIAL GERM CELLS: DIFFERENCES OF DEVELOPMENTAL PATTERNS AMONG XENOPUS, STURGEON, AND ZEBRAFISHES

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Sturgeon primordial germ cells (PGCs) would provide us with valuable information to understand gametogenesis, sex differentiation, and evolution of PGCs development in vertebrates. However, the origin and migration of PGCs are unstudied, although location of PGCs after 6 days post fertilization (dpf) was previously reported with conventional and electron microscopy {Grandi:2007gx}{Grandi:2008bi}. Sturgeon eggs have holoblastic cleavage, as in anurans {Elinson:2009eq}, although many teleosts have meroblastic pattern. Development of sturgeon embryos is more similar to Xenopus than a teleost, not only in their cleavage pattern but also in many other aspects. Here, we raise several questions about PGCs development in sturgeon: the presence or absence of maternally supplied germplasm in the sturgeon egg, 2) the position where PGCs are specified, and 3) migratory pattern of PGCs. To study these questions, we tried to visualize germ plasm and PGCs by injecting chimeric RNA that contains GFP and PGCs specific gene sequences (*nanos3* or *bucky ball* from zebrafish) into fertilized starlet (*A. ruthenus*) eggs. Germ plasm and PGCs were visualized only when mRNAs were injected into the vegetal pole of eggs. The PGCs initially appeared at the surrounding region of the tailbud after the closed neural tube stage, and then, they migrated toward the gonadal ridge. Next, to investigate whether the migratory mechanisms of PGCs is conserved among Actinopterygii species, the visualized PGC was transplanted into goldfish, *Carassius auratus*, blastula embryo. Transplanted donor PGC migrated toward the gonadal region of host embryo. Our results clearly show that the specification pattern of the sturgeon PGCs closely resembles that of anurans, and the mechanisms governing migration of PGCs are conserved widely among Actinopterygian fishes.

Keywords: *primordial germ cells, PGCs, embryonic development, sturgeon*

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ACIPENSERIFORM GYNOGENESIS: PROGRESS & RE-EXAMINATION

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The American paddlefish is one of two extant species in the family Polyodontidae and is closely related to other economically important species of Acipenseriform fishes. The American paddlefish will become increasingly a valued foodfish and a source of caviar, therefore, continued improvements in artificial propagation are critical. Verification of sex determination through progeny testing of mature gynogenote females is being conducted in an attempt to identify the viability of purported WW-females. Ten 15–16 year-old gynogenote (PF♀gynF_1) females were spawned over the past two years and progeny are being grown for sexing. Also, modifications to the gynogenetic induction protocol is being re-evaluated. The primary induction modification being examined is the ultraviolet treatment of heterologous donor male spermatozoa as a requirement for ova activation.

Keywords: *paddlefish, artificial propagation, induced gynogenesis, sex determination, progeny testing*

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SEX DETERMINATION IN STURGEONS (ACIPENSERIFORMES): A SHORT OVERVIEW AND FUTURE PERSPECTIVES

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Sex determination in sturgeons (Acipenseriformes) is one of the most stimulating research topics not only because of the innate curiosity of the scientists but also because its knowledge will be helpful for breeding assisted programs aimed to the restoration of these endangered species and for aquaculture as well. Presently, sex determination in sturgeons is poorly understood. It is not currently possible to distinguish between males and females on the basis of morphological characters at early stages of development as well as in juvenile and adult specimens. In sturgeons' adult populations the sex ratio is 1 : 1. This observation suggests that sturgeons have gonochoristic reproduction system and the sex is genetically determined. To date, many studies have been carried out to analyze the sex determination systems in sturgeons. Sex identification by surgical biopsy is reliable, but it is necessary to have sexually differentiated gonads and this diagnostic method is invasive. Ultrasound diagnostic technique allows early sexing of juveniles, but at age of 1–2 years, not during the early stages of development. Plasma steroids examination could be used to identify the sex, but these steroid indicators are influenced by several environmental factors (age, water temperature, husbandry conditions). All the sturgeon's species don't exhibit any defined sex chromosomes: both sexes have homomorphic chromosomes. Gynogenesis is an efficient chromosomal manipulation technique to investigate sex-determining mechanisms in sturgeons and allowed to infer female heterogamety in three sturgeon species, *Acipenser transmontanus*, *Huso huso* and *Acipenser brevirostrum* as well as in the paddlefish *Polyodon spathula*. The results obtained in these experiments, however, do not exclude that other chromosomal or polygenic factors could affect the sex ratio. Moreover, sex determination could be controlled by multiple chromosomes involving many sex determining loci due to the extensive genome duplication events occurred during the evolutionary history of Acipenseriformes. Several approaches have been used for the isolation of sex-associated DNA markers in sturgeons (RAPD, AFLP, ISSR) but none of them gave any positive results. Recently, we tried to apply Comparative Genomic Hybridization (CGH) methodology in sterlet (*Acipenser ruthenus*) in order to try to identify if some differences are present between males and females chromosomes. The first experiments didn't give any positive results: if sex specific sequences are present, probably they are too small to be visualized by CGH. However, an optimized experimental protocol is needed to confirm these results. The most promising system to identify genetic sex markers is the massive parallel sequencing by means of Next Generation Sequencing platforms and the application of genotyping by sequencing (GBS) methods to these species using SNPs as non-conventional morphological markers. The ultimate goal is to develop a reliable diagnostic test that will allow to identify the sex at juvenile/sub-adult stages of development.

Keywords: *sex determination, sturgeons, DNA sex markers, CGH, Next Generation Sequencing*

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YOLK FORMATION IN FISH: MULTIPLE VITELLOGENINS AND THEIR RECEPTORS

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Recent investigations have revealed multiplicity of maternal yolk precursors (vitellogenins: Vtgs) and their corresponding ovarian lipoprotein receptors (Lrs) in diverse oviparous vertebrates, including fishes. This study briefly reviews evidence for a system of fish egg yolk formation mediated by multiple ovarian Lrs, including classical Vtg receptor (Vtgr, an orthologue of mammalian very-low-density lipoprotein receptor or Lr with 8 ligand binding repeats: Lr8), low-density lipoprotein receptor (Ldlr or Lr with 7 ligand repeats: Lr7), and recently described novel Lrs with multiple ligand binding repeats (LrX+1). Our findings have been obtained by a combination of conventional molecular and biochemical analyses, and modern proteome and transcriptome technologies. A hypothetical model of ''multiple Vtgs and ovarian Lrs'' is proposed based on our current and previous knowledge of fish yolk formation.

Keywords: *egg yolk, fish, low-density lipoprotein receptor super family, oogenesis, vitelogenin*

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RECENT PROGRESS IN STUDIES ON ANDROGENESIS OF FISHES

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Androgenesis (all-male inheritance) has been considered useful biotechnology for rapid fixation of important traits by cloning, recovery of endangered genotypes by using cryopreserved sperm, and sex-manipulation of aquaculture-oriented fishes. It has been artificially induced by fertilization of genetically inactivated eggs with normal sperm. The first step of induced androgenesis is genetic inactivation of egg nucleus and this has been achieved by gamma-, X-rays or ultraviolet (UV) irradiation. Gamma irradiation is necessary for inactivation of salmon and sturgeon with large eggs, but special facility is required for safety. UV-irradiation is easier to operate by commercially available germicidal lamp and has been widely applied in fish with relatively smaller egg sizes. Recently, we demonstrated in loach fish *Misgurnus anguilicaudatus* that cold-shock treatment (0–3 °C) of eggs for 30 to 60 min duration just after fertilization (within 10 s) can induce androgenetic development by eliminating the egg nucleus together along with the second polar body release and then development initiates by a decondensed sperm nucleus. By this cold-shock method without egg irradiation, viable diploid androgenetics were successfully produced in loach by using diploid sperm of a neo-tetraploid, which had been produced by inhibition of the second polar body release after the fertilization of eggs of wild-type diploid female with diploid sperm of naturally occurring tetraploid male. The mean yield rate of diploid androgenetics was about 13 % to total eggs. Doubled haploid androgenetics were also produced by the combination of cold-shock treatment just after fertilization with normal sperm and heat shock (42 °C, for 2 min, 65 min after fertilization). The yield of doubled haploids was about 11 % to the total number eggs and they were homozygous at 28 microsatellite loci examined. Applications of cold-shock androgenesis are now being examined in other fish species.

***Keywords**: clone, gene-banking, parthenogenesis, ploidy manipulation, sex-control*

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ATTEMPTS TO SIMULATE POSSIBLE AUTOTRIPLOIDIZATION MECHANISMS IN TWO STURGEON SPECIES, THE STERLET, *ACIPENSER RUTHENUS* AND THE SIBERIAN STURGEON, *A. BAERII*

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Several current studies within the last decade brought evidence about induced- or spontaneous triploidy in recent diploid, tetraploid and hexaploid sturgeons. Spontaneous triploidy in sturgeons could theoretically arise in consequence of meiotic dearrangements during gametogenesis, by spontaneous diploidization of the maternal chromosome set (SDM) in an oocyte due to its post-ovulatory ageing followed by fertilization with a haploid spermatozoon or, by dispermic fertilization of a haploid oocyte. Firstly, oocytes of recent diploid sterlet, Acipenser ruthenus were half-stripped out and stored for 0 (control batch), 2, 4 and 6 hours at 8.2 ± 0.17 °C *in vitro* prior to fertilization. Oocytes remaining *in vivo* in the fish kept at 14.9 ± 0.1 °C were collected and fertilized in the same time intervals. The second trial considered prolongated storage of oocytes for 0 (control batch), 2, 4, 6, 8 and 10 hours at 15.0 ± 1.15 °C also in aerated media, the Persian sturgeon artificial coelomic fluid (PSACF; Sohrabnezhad et al., 2006) and Leibovitz L-15 prior to fertilization. Hatching rate mostly decreased after 2–6 h storage of oocytes *in vitro* and after 8–10 h storage of oocytes *in vitro* in media. Analysis of relative DNA content by flow cytometry revealed only the diploid larvae in all control batches. Autotriploid larvae were found after 2–6 h *in vivo* storage (1.19–2.38 %), and after 8–10 h in vitro storage in L-15 medium (4.75–6.67 %). Both the *in vitro* and in *vivo* prolongated storage also generated haploids (2.08–8.33 %) and two discrete groups of aneuploids (hypodiploid, 33.33–78.27 % and hyperdiploid, 1.15–2.78 %) in different time intervals, while no haploids, nor aneuploids were found after the in vitro prolongated storage in media.

Oocytes of recent tetraploid Siberian sturgeon, *A. baerii* were fertilized with doses of 150 x 103 spermatozoa per oocyte (control batch) and with 3.3x, 6.6x, 10x and 13.3x more concentrated doses of spermatozoa per oocyte. Later, sterlet oocytes were fertilized with doses of 810 x 103 spermatozoa per oocyte (control batch) and with 5x, 10x and 50x more concentrated doses of spermatozoa per oocyte. Hatching rate did not significantly differ among the respective variants within each species. Analysis of relative DNA content did not reveal any autotriploid larvae but 14.29 % aneuploids (hypotetraploid) in one replicate of the 5fold variant for Siberian sturgeon.

These experiments might contribute to the evidence that SDM could be a more probable mechanism of autotriploidization in sturgeons.

Keywords: *flow cytometry, oocyte ageing, polyspermy, sterlet, Siberian sturgeon*

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SPERM MATURATION IN STURGEON: VERIFICATION OF EXISTENCE AND POSSIBILITIES FOR APPLICATION

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Sperm maturation, as a process of acquisition of potential for motility and fertilization in testicular spermatozoa, is quite well understood in several teleost species but is not described in sturgeon so far. Sturgeons possess specific excretory system anatomy, in which testicular efferent ducts are in close contact with kidneys, where testicular sperm is presumably mixed with urine. Using sterlet, *Acipenser ruthenus*, we recently discovered that sperm maturation phase exists, taking place in Wolffian ducts where spermatozoa combine with urine. This statement is based on the experiments conducted on testicular spermatozoa. Testicular spermatozoa collected from any part of testes or efferent ducts were unable to initiate any motility after dilution by different activating media (AM) differing in ionic composition, pH or osmolality. Pre-incubation of testicular sperm in seminal fluid, collected from normally spawn control sperm, led to acquisition of ability to motility activation (maturation) in AM. After in vitro maturation, sperm motility parameters (such as velocity, motility percentage and duration) did not differ significantly from those of ejaculated control sperm. We demonstrated for the first time the existence of sperm maturation phase in sturgeon spermiogenesis. Furthermore, we have shown that this process takes place outside of testes, implicating activity of seminal fluid components of high molecular weight and requires presence of external Ca^{2+} ions. We determined that sperm maturation process was followed by significant changes in parameters of seminal plasma prooxidant-antioxidant system. These changes quite probably arise from physiological mixing of testicular sperm with urine. We also demonstrated that proteolytic activity of seminal fluid quite probably is involved in this process. The components of seminal fluid, which are responsible for sperm maturation process, are not species specific for sturgeon species. Application of these substances is able to improve sperm motility in samples with low motility parameters. After *in vitro* maturation testicular spermatozoa are able to survive cryopreservation and after thawing they are able to fertilize eggs.

We discuss the possibility to use testicular sturgeon spermatozoa in aquaculture or conservation programs, when only testicular spermatozoa are available (cases of accidental death of valuable fish individuals) and for improvement of motility parameters of sperm samples.

Keywords: *sturgeon, sperm maturation, fertilization, antioxidant system, proteolytic activity*

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EMBRYONIC STAGES AND PRIMORDIAL GERM CELLS DEVELOPMENT IN PIKEPERCH *SANDER LUCIOPERCA* (TELEOSTEI: PERCIDAE)

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Recently, germ cells transplantation is attracting attention as a potential technique for efficient reproduction of fish. Pikeperch, *Sander lucioperca*, is considered one of the most valuable fish in Europe. To apply the new biotechnology to this species, it is needed to accumulate basic information about an embryonic development, especially primordial germ cells (PGCs) migration. The aim of this study was to describe embryonic development and PGCs migration in pikeperch. Furthermore, we examined if it is possible to use cell transplantation technique in this species. Embryos were obtained by artificial fertilization and dechorionated with 0.01 % trypsin and 0.4 % urea in ringer's solution. Embryos incubated at 15 °C. Embryonic development was characterized into five stages as seen in the other fish species: zygote, cleavage, blastula, gastrula stages, segmentation and hatching. PGCs were visualized by injecting artificially synthesized GFP-*nos*1 3'UTR mRNA at the 1-4-cell stage. GFP-positive PGCs appeared in all embryos at around the 100 % epiboly stage. The average number of the PGCs in each embryo was 12.6 (SD 8.52, Range = 2 to 35). Time-lapse imaging and analysis revealed that their migration pattern from their appearance to the gonadal ridge could be classified into 5 steps. Next, we performed blastomeres transplantation (BT) in this species at the blastula stage. In advance, donor embryos were labeled with GFP-*nos*1 3'UTR mRNA and tetramethylrhodamin in order to label PGCs and all somatic cells. Twelve BT chimeras were produced and 8 chimeras survived until the hatching stage. All of them had red-labeled (donor-derived) somatic cells in the embryonic body. These red-labeled cells were distributed to the whole embryonic body, including somites, brain, and intestine and so on. Two embryos out of 8 had GFP-labeled donor PGCs at the gonadal ridge. In this study, we demonstrated that PGCs from donor embryos were able to migrate toward the gonadal region of host embryos. This result indicates that germline chimera can be produced in pikeperch by BT. Family Percidae include some important species for aquaculture such as *Perca fluviatilis*. The fish belong to this group live not only in freshwater area but also marine water. By using pikeperch as host, it might be possible to produce gametes of marine fish in freshwater. Our results presented here provide useful information that can be a basis for producing germline chimera among Percidae species.

***Keywords*:** *pikeperch, embryogenesis, developmental stage, primordial germ cells, green fluorescent protein*

ANDROGENETIC DEVELOPMENT OF THE BROOK CHARR (*SALVELINUS FONTINALIS*, MITCHILL 1814) IN THE EGGS ORIGINATED FROM THE INTERSPECIFIC CHARR HYBRIDS

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To overcome incompatibility between the egg cytoplasm of one species and the sperm nucleus of another species, application of F1 interspecific hybrids as egg donors for the purpose of androgenesis has been proposed. Here, androgenetic development of the brook charr (*Salvelinus fontinalis*) was successfully induced in the brook charr eggs and the eggs derived from the brook charr × Arctic charr (*Salvelinus alpinus* L.) F1 hybrids. A working androgenesis protocol included inactivation of the maternal nuclear DNA achieved by irradiation of the eggs with 420 Gy of the X-rays, insemination of such treated eggs with the haploid sperm cells and exposition of the haploid androgenetic zygotes to the high hydrostatic pressure shock (51, 711 Mpa for 4 min) applied 420 min. after insemination. Androgenetic larvae that hatched from the brook charr and the hybrid eggs were shown to be homozygous brook charr individuals. Androgenetic individuals exhibited 84 chromosomes and 100 chromosome arms (FN), values characteristic for the brook charr diploid cells. Strategy hybridize first than induce androgenesis should be tested in other salmonids that are able to cross and produce fertile offspring.

Keywords: *androgenesis, chromosomes, egg cytoplasm, fish hybrids, Salmonidae*

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DYNAMICS OF CHANGES IN CYTOLOGICAL CHARACTERISTICS OF BESTER OOCYTES UNDER CONTROLLED CONDITIONS

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Experimental work was carried out in 2010–2012, on two breeds of bester (hybrids of beluga with sterlet BS and sterlet with bester SxBS) at the age of 5 and 6 years which were kept in the RAS aquarial complex. The degree of dehydration intracellular structures of oocyte of sturgeon hybrids was studied during histological processing. All oocytes and envelopes sizes were measured on fresh eggs obtained by a biopsy probe method, and the same cell structures were follow-up sized after histological processing. It was found out that the diameter of the oocyte cytoplasm reduced by 25 % on average, while the oocyte envelope and the nucleus did not change their thickness due to less content of liquid and denser structure. It was revealed that in both hybrids the average diameter of oocytes increased 5 times from the beginning of vitellogenic growth to the germinal vesicle migration towards the animal pole. Interhybrid differences in average diameter were observed in matured oocytes: the germ cells in BS were 14.8 % larger than in SxBS. Nucleus-plasma ratio decreased from III stage to IV matured stage in SxBS and in BS by 8 % and 6.4 %, respectively. It was shown that the oocyte envelopes structure and thickness were corresponded with physiological normal range. At that, the total thickness of the oocyte envelopes was approximated to one of parent-cell (beluga). The average membrane thickness in SxBS was 3 μm lower reliably, compared with these characteristics in BS. During the maturation of oocytes the envelope thickness increases irregularly because of proliferation of the *zona radiata* and chorion. We have analyzed interhybrid differences in the growth characteristics of the structural layers of envelopes oocytes of sturgeon. In general, on the average the envelopes increase from 2 ~ 7 μm to 69 ~ 72 μm during the maturation of cells. It was shown during the ovaries maturation from II to III stage of maturity, the total thickness of the envelopes increases in SxBS in 27 times on the average, while in BS in 7 times. It was deduced during from III up to IV matured stage of maturity the oocytes envelopes grew only in 1.3 ~ 1.5 times for two various hybrids.

Keywords: *bester, maturation, histology, oocytes, oocyte envelope*

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COMPARISON OF THE QUALITY OF PIKEPERCH (*SANDER LUCIOPERCA*) SPERM IN DIFFERENT SPAWNING SEASONS

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In this study were used four groups of pikeperch male (7 fish in each group). All male fish were injected 500 IU of HCG per kilogram before the spawning and they have been spawned in different time (mid of March – group A, the end of April – group B, mid of June – group C). All sperm samples were collected 4th day after spawning.

The highest production of sperm was in group C (28.6 ± 3.92 mld. of sperm). Concentration of sperm was similar in all groups (from 15.73 ± 2.68 to 19.34 ± 3.87 mld.ml^{-1}). The longest motility time has been observed in group A (89.93 ± 10.2 s) and the shortest time in group B (55.18 ± 10.46 s). The highest velocity was in group A (220 ± 22.3 μm.s^{-1}) and the lowest velocity has been measured in group B (159 ± 35 μm.s^{-1}).

The results obtained in this experiment show that the quality of sperm changes during the spawning season.

Keywords: *pikeperch, sperm, motility, concentration, velocity*

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IMPACT OF SEASONAL SOIL FERTILIZERS ON CRAYFISH CARDIAC AND BEHAVIORAL ACTIVITY

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The world natural resources have been being exhausted more and more since last decades due to human activities. In order to improve soil and yield productivity, the various fertilizers are applied on fields, but then washed out with rainfalls into surface waters, where can cause harmful effects to aquatic life. To establish whether nitrogen- and phosphorus-based fertilizers affect such aquatic representative as crayfish, we conducted trial of experiments. Twelve adult signal crayfish Pacifastacus leniusculus males were exposed to two types of fertilizer diluted in water in such way that final ammonium and phosphate concentrations in aquaria were 0.8 mg NH_4 L^{-1}, 1.6 mg NH_4 L^{-1}, 2.4 mg NH_4 L^{-1} and 0.5 mg P_2O_5 L^{-1}, 1.0 mg P_2O_5 L^{-1}, 2.0 mg P_2O_5 L^{-1}, respectively. To evaluate the effects of selected compounds on crayfish, their cardiac and behavioral activities were recorded and analyzed in real time by the developed system based on cardiac activity sensor coupled with camera. Under exposure to the lowest, 0.8 mg L^{-1}, NH_4 concentration, crayfish did not manifest neither cardiac nor behavioral reaction. That confirmed opinion about safety of this NH_4 concentration to aquatic animals. The crayfish showed pronounced increase of the heart rate under exposure to higher NH4 concentrations, where heartbeat variability and movement of selected individuals tended to be higher at 2.4 mg NH_4 L^{-1}. The crayfish showed less pronounced cardiac reaction and motion activity under exposure to P_2O_5, and there was no difference between level of heart rate increase, as well as motion activity, at either concentration. The last indicates lower phosphate impact and related harmful effects for aquatic environment compared with ammonium toxicity. The study confirms crayfish suitability for biological online monitoring. The variance of crayfish reactions says about necessity of preliminary testing before selected biomonitors are applied for water quality control.

Keywords: *biomonitoring, ethology, heart rate, Pacifastacus leniusculus, surface water quality*

Acknowledgements: The study was supported by projects CENAKVA (CZ.1.05/2.1.00/01.0024), by the Ministry of Education, Youth, and Sports of the Czech Republic (CZ.1.05/2.1.00/01.0024) and by the Grant Agency of the University of South Bohemia (084/2013/Z).

ENVIRONMENTAL POLLUTANTS ALTER BOTH REGULATION AND CATALYTIC ACTIVITY OF THE CYP1 SYSTEM IN FISH

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Aquatic wildlife species are increasingly exposed to man-made chemicals in their natural habitats. There is consequently a need to establish early-warning signals, i.e. biomarkers, to determine exposure to certain classes of pollutants and adverse biological responses caused by these pollutants. The objective of the present work was to explore the whole suite of cytochrome P450 1 (CYP1) genes expressed in fish, particularly with regard to inducibility and potential use as biomarkers for environmental monitoring. Complementary DNA (cDNA) of the CYP1A, 1B and 1C transcripts in rainbow trout and three-spined stickleback were cloned and expression levels were determined using quantitative PCR. All transcripts were induced by two aryl hydrocarbon receptor (AhR) agonists (indigo and PCB 126) in both species, suggesting all genes to be AhR regulated. CYP1 mRNA expression showed high and persistent induction by PCB 126 in both gills and liver, while induction by indigo was high only in gills and transient in both organs. In order to determine the ability of pollutants to inhibit CYP1A-catalytic activity, a previously developed gill filament ethoxyresorufin O-deethylase (EROD) assay was adapted to measuring inhibition of EROD activity. Using this method, a set of known CYP-inhibitory pharmaceuticals including antifungal azoles (e.g. ketokonazole) and the proton pump inhibitor omeprazole were characterized with regard to EROD inhibition potency. Moreover, an STP effluent containing a complex mixture of pharmaceuticals and other pollutants was investigated. The same water samples that strongly induced both CYP1 mRNA expression and catalytic EROD activity in gill filaments of fish exposed in vivo also inhibited CYP-catalyzed activity following exposure of gill filaments ex vivo. These seemingly contradictory results were most likely due to a washing out of inhibitors from the in vivo-exposed filaments during the assay procedures. These and other data support the contention that CYP1 mRNA induction could partly be an indirect response resulting from the inhibition of the CYP1A catalytic activity. In conclusion, response patterns of multiple CYP1 mRNAs in gills and liver could provide an improved monitoring strategy. Such patterns (fingerprints) could be used to characterize complex mixtures of AhR agonists/ antagonists and other pollutants in aquatic environments. It is also concluded that the modified ex vivo gill filament EROD assay is useful to screen for waterborne pollutants that inhibit catalytic CYP1A activity in fish gills.

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COMPLEX APPROACH TO MONITORING OF AQUATIC ENVIRONMENT CONTAMINATION IN CZECH REPUBLIC

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Surface water ecosystems become a final reservoir of a majority of extraneous substances arising from anthropogenic activities. At present, an intensive global research focuses on the occurrence of "new" anthropogenic contaminants – above all, pharmaceuticals and personal care products (PPCPs), pesticides and new industrial contaminants. Such pollutants are suspected to harmfull impact on exposed organisms. It is necessary to monitor the surface water quality including identification of the "new" contaminants and to investigate the effects of environmentally relevant concentrations of these compounds on exposed organisms in laboratory and field conditions.

The existing monitoring system of the aquatic environment contamination is based mainly on a point sampling (alternatively 24-hour composite sampling). Moreover, usually only priority pollutants defined by the legislation are monitored. An increase in sampling frequency is extremely economically and logistically demanding. However, the use of passive samplers appears to represent a feasible solution. A passive sampling method represents an innovative tool for a time-integrated measurement of polluting substances in the aquatic environment. The POCIS (Polar Organic Compounds Integrative Sampler) and SPMD (Semipermeable Membrane Devices) type samplers are commonly used in the Czech monitoring programmes. Even a possibility to detect extraneous compounds in the environmental components has been very difficult until lately, which has mainly been caused by a low sensitivity of used analytical methods, a demanding character of samples preparation and also a high cost of special analyses. At present, modern analytical devices are already available that are able to detect environmentally relevant concentrations of monitored compounds. A liquid chromatography with tandem mass detection (LC/MS/MS) and a liquid chromatography with high resolution mass spectrometry (LC/HRMS) are used for these purposes in our lab. At present, an intensive research focusing on development of new more efficient analytical methods is going on.

Investigation of contamination effects on exposed aquatic organisms is a very important part of the monitoring in CR. In case of laboratory studies, the effects of selected extraneous compounds are identified base on toxicological studies with esp. rainbow trout (*Oncorhynchus mykiss* W.), common carp (*Cyprinus carpio* L.) and crustaceans (signal crayfish, *Pacifastacus leniusculus*). With respect to testing of each compound or mixture, a test of subchronic toxicity, or alternatively an embryo-larval test, are carried out on a selected model species. Environmentally relevant concentrations of tested compounds are always included in the tests´ design. The effects are detected by wide spectra of biomarkers. In case of field experiments, studies are focused on identification of pollution sources. The impacts is assessed in ecosystem of model sites (esp. streams and ponds) mainly on fish and invertebrates.

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CONTAMINANT BIOACCUMULATION IN PADDLEFISH AND HYBRID STRIPED BASS FINGERLINGS CULTURED IN RECLAIMED EFFLUENT WATER AT A DECOMMISSIONED WASTEWATER TREATMENT FACILITY

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Reuse of decommissioned wastewater facilities supplied with reclaimed water is being studied to determine if they could become sustainable aquaculture operations. Paddlefish *Polyodon spathula* and hybrid striped bass *Morone chrysops x Morone saxatilis*, chosen as valuable market fish, were cultured in reclaimed, secondary disinfected effluent water at a decommissioned wastewater treatment facility in Frankfort, Kentucky to identify any contaminant bioaccumulation in fish tissues. Both species were cultured from fingerling to stocker size during a 90-day production trial. The tissues were analyzed by the Kentucky Environmental Services Branch Laboratory, Frankfort. Chemical component analyses included heavy metals, organochlorines, commonly known as pesticide components, chlordane products and DDT products, as well as polychlorinated biphenyls (PCB's.) Bioaccumulation of technical chlordane in paddlefish tissues were 39.4 $\mu g.kg^{-1}$ and significantly higher than hybrid striped bass tissues at 5.4 $\mu g.kg^{-1}$. For heavy metals, mercury and selenium were detected at 0.02 and 0.18 $mg.kg^{-1}$ in paddlefish tissues, respectively and 0.04 and 0.26 $mg.kg^{-1}$ respectively in hybrid striped bass tissues. Though low levels of contaminants were detected, they were well below FDA regulated action levels. These results indicated that using reclaimed, effluent water and unused wastewater treatment facilities could be a feasible operation for aquaculture production.

RECENT APPROACHES TO ASSESS ENDOCRINE DISRUPTORS IN AQUATIC ENVIRONMENTS

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During the last decades there is increasing evidence that several compounds contributing to environmental pollution in the aquatic environments display endocrine disrupting activities rather than acute toxicity. These so-called endocrine disruptors (ED) affect mainly the endocrine systems of vertebrates interfering with reproductive physiology and thyroid system of fishes and amphibians. In order to determine endocrine disrupting activities of environmental compounds several OECD test guidelines became established by several fish and amphibian models. Despite reproductive physiology can be covered by fish and amphibian models ED affecting thyroid system is only evaluated in a standardized manner by the amphibian metamorphosis assay (AMA; OECD test guideline no. 231). Beside AMA concerning ED affecting thyroid system recent approaches using the amphibian model *Xenopus laevis* to assess (anti)estrogenic and (anti)androgenic activities of environmental ED by determining their impacts on male mating call behavior of *Xenopus* there is now evidence that these approaches might save time and labor for assessment of ED to fulfill the requests of the 3R-strategy (reduce, replace, refine) for determining ED. The state-of-the-art concerning these innovative developments are presented and being discussed in comparison to existing OECD test guidelines.

Keywords: *endocrine disruptors, reproduction, thyroid system, aquatic vertebrates, fishes, amphibians*

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ANALGESICS, PSYCHOLEPTICS, ANTIDEPRESSANTS AND ILLICIT DRUGS IN AQUATIC ENVIRONMENT OF CZECH REPUBLIC

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The presence of drugs in the aquatic ecosystem is gaining attention because of their possible adverse effects on aquatic life. Several recent studies have already documented that psychoactive drugs can have their effect at environment relevant concentrations. Thus, aquatic drug monitoring is of great importance as it forms a basis for further risk assessment for aquatic organisms.

The goal of this study was to assess bioavailable concentrations of analgesics, psycholeptics, antidepressants and illicit drugs in 21 riverine profiles of Czech Republic regularly monitored by CHMI using passive sampling approach. Passive sampling can overcome limitations of conventional sampling and provide data on time-integrated concentrations. A further advantage of passive sampling is the ability to mimic biological uptake, therefore avoiding use of aquatic organisms for biomonitoring. Polar Organic Chemical Integrative Samplers (POCIS) were deployed for 21 days in spring 2011 and 2012. Thirty seven compounds were analyzed using LC-MS/MS method: 3 analgesics (buprenorphin, codeine, tramadol), 1 anesthetic (ketamine), 6 antidepressants (amitryptiline, citalopram, mianserin, paroxetin, sertraline, venlafaxine), 2 antiepileptics (carbamazepin, clonazepam), 5 drug metabolites (2-oxy-3-hydroxy-LSD, benzoylecgonine, norbuprenorphine glucoronid, norketamine, THC-COOH), 11 illicit drugs (amphetamine, cathinone, cocaine, EDDP, LSD, MBDB, MDA, MDEA, MDMA, mephedrone, metamphetamine), 7 psycholeptics (alprazolam, haloperidol, methylphenidate, midazolam, oxazepam, risperidone, zolpidem), 2 synthetic opioids (methadone, oxycodone). Total amount of measured compounds varied between 463–6447 ng/POCIS. Carbamazepin (196–2690 ng/POCIS) and tramadol (160–2250 ng/POCIS) were the most abundant compounds detected in all monitored sites. Four pharmaceuticals were found at relatively high concentration level: codeine (2.3–110 ng/POCIS), citalopram (18–429 ng/POCIS), venlafaxine (34–605 ng/POCIS) and oxazepam (9.1–276 ng/POCIS). All these compounds were detected in all sites too. The other compounds were detected sporadically. Passive sampling by POCIS is a useful tool providing robust data for the monitoring of pharmaceuticals and illicit drugs in aquatic environment.

Keywords: *POCIS, drugs, LC/MS/MS, bioavailable concentrations, CHMI profile*

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PHOTODEGRADATION CHARACTERISTICS OF PHARMACEUTICALS IN WATER UNDER IMPACT OF UV AND SUNLIGHT

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Phototransformation reactions have been suggested to play an important role in the elimination of some of pharmaceuticals from surface waters. Many of these organic pollutants are photo sensitive, resulting in removal of the pharmaceuticals and to the formation of photodegradation products. Identification of photochemical half-lives of pharmaceuticals would improve the understanding of their environmental fate.

The aim of this work was to obtain information about pharmaceuticals photochemical half-lives under UV and Sunlight conditions in Buffered Milli-Q water and water from Labe River and Ume River.

30 pharmaceuticals were included in the study and the results showed that 10 of pharmaceuticals were stable and did not degrade during 8 h of exposure under the UV and sunlight irradiation. 15 pharmaceuticals underwent photodegradation in all water samples and half-lifes were calculated for these. 5 pharmaceuticals showed a varied behaviour.

Keywords: *photodegradation, pharmaceuticals, photofate, half-life time, photostability*

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OCCURRENCE, TOXICITY AND FATE OF NATURAL PROGESTERONE AND SYNTHETIC PROGESTINS IN AQAUTIC ENVIRONMENT: A REVIEW

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Studies show that even at minute level, steroidal hormones in natural waters can be harmful to aquatic organisms. In recent years, concern has been raised about the natural progesterone and its synthetic derivatives (progestins). Synthetic progestins are not only used to treat a number of medical conditions, but also they are often the major active ingredient in oral contraceptives. Some of these synthetic progestins are known to enter surface waters via existing sewage treatment facilities. For example levonorgestrel, norethindrone and progesterone have been reported to be present in surface water at concentration up to 110 $ng.L^{-1}$. Furthermore, there are evidences that some synthetic progestins can contribute to endocrine disruption in fish and other aquatic organisms. For example, at the lowest tested concentration 0.8 $ng.L^{-1}$, levonorgestrel reduced egg production of female fathead minnows by about 95 %. However, there is almost no detail study on the occurrence and distribution of these chemicals in aquatic environment. To address this issue we have selected specific candidates based on their relative consumption and likely potential threat to aquatic environment. For our study, we analyzed a comprehensive Czech Republic medicine distribution database covering natural progesterone and all synthetic progestins distribution with the aim to determine the annual usage of these chemicals in the Czech Republic. In total, 1625 kg of natural progesterone was used in the Czech Republic, which is considerably more than total amount of synthetic progestins (753.9 kg) used in year 2011. We found that the most common synthetic progestin used in contraceptive pills was drospirenone (156.8 kg), followed by Dienogest (74.1 kg) in year 2011. The three most common synthetic progestins currently used in other applications (such as cytotoxic medicine) in the Czech Republic were megestrol acetate (234.1 kg/year), medroxyprogesterone acetate (104.8 kg/year) and medroxyprogesteron (13.9 kg/year). Less information is available about the distribution and effect concentration of these synthetic progestins in Czech aquatic environment. However, concentration assumed from the annual usage of natural progesterone and synthetic progestins could make a significant contribution to improve our knowledge on presence and potential risk to aquatic organisms, especially fish.

Keywords: *natural progesterone, synthetic progestins, annual usage, occurrence, effect*

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SCREENING OF EMERGING POLLUTANTS BY PASSIVE SAMPLING IN MIDDLE AND LOWER REACHES OF YANGTZE RIVER

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In this project, we aim to assess the potential occurrence and impact of biologically active substances on aquatic environment of the Yangtze River, mainly focusing on emerging pollutants. The project is focused on identification and screening of anthropogenic pollution in middle and lower reaches downstream the Three Gorges Reservoir. The special interest of this project is to detect and identify relevant emerging pollutants using passive sampler/mass-spectrometry approach, with specific attention to persistent organic pollutants (POPs), biocides, pharmaceuticals and personal care products (PPCP). This study explores use of integrative passive samplers followed by a novel analytical approach LC-HRMS and GC/GC-TOF HRMS, which can provide additional valuable data to conventional monitoring program.

In general, aquatic monitoring programmers are mostly based on collection of discrete samples of the water phase. The subsequent chemical analysis provides a snapshot of the concentration in the environment at the particular time. In environments where the contaminant concentrations may vary over time, it is desirable to expand the time window and increase the resolution. Passive samplers operate on the principle of the preferential partitioning of contaminants into a device and allow for a combination of the precision, reproducibility and time-integration. These methods allow a time-integrated monitoring of micropollutants directly in the aqueous phase as an alternative to conventional sampling techniques.

Pilot sampling of the Yangtze River was done during September 2012. Following relevant contaminants were detected: pharmaceuticals 56/118 (positive/measured), pesticides 23/52, perfluorinated acids and sulfonates 7/19, drugs and metabolites 4/23, UV blockers 3/4 and others 4/5. Sum of pharmaceuticals, pesticides and bisphenol A show increasing trend downstream from the Three Gorges Reservoir towards the last sampling site in Wuhan. Plan for 2013 includes optimization of the exposure period and consequent sampling at 10 suitable localities.

Keywords: *Yangtze, emerging pollutants; passive sampling; LC-HRMS, GC/GC-TOF HRMS*

Acknowledgements: This study is supported by the MŠMT ČR project Kontakt II – LH 12179, CENAKVA CZ.1.05/2.1.00/01.0024 and the Grant Agency of USB GAJU 087/2013/Z.

BIOCONCENTRATION OF ANTIDEPRESSANT IN FISH EXPOSED TO WASTEWATER TREATMENT PLANT´S EFFLUENT

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Selective serotonin reuptake inhibitors (SSRIs), one group of often prescribed pharmaceuticals, are present in treated wastewaters. Little is known about the bioconcentration and potential effects of these pharmaceuticals on aquatic organisms. Bioconcentration of selected SSRIs was studied on rainbow trout (*Oncorhynchus mykiss*) exposed to 100 % effluent from wastewater treatment plant RyaVerket (Sweden) for 13 days. Citalopram, sertraline and venlafaxine were observed in fish brain and liver. These SSRIs were present below limit of quantification in all samples (for citalopram and sertraline) or in more than 75 % of samples (venlafaxine) in fish plasma.

Keywords: *antidepressant, caged fish, rainbow trout, wastewater treatment plant´s effluent, bioconcentration*

A HISTOLOGY-BASED HEALTH ASSESSMENT OF SELECTED FISH SPECIES FROM TWO RIVERS IN THE KRUGER NATIONAL PARK, SOUTH AFRICA

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The Kruger National Park (KNP) is an important tourist destination in South Africa and the value of river conservation within the park has economic benefits. Two rivers which flow through the KNP were chosen for this study, namely the Olifants River (OR) and the Luvuvhu River (LR). Both of these rivers have upstream activities which are likely to affect the water quality and consequently the health of fish in these rivers. Fish were used as a bioindicator of ecosystem health and histology as a biomarker to determine the effect of pollution on the health status of the selected fish species. Therefore, the aim of this study was to assess the health status of selected fish species from the OR and LR in the KNP using a histology-based health assessment protocol.

The species selected for this study included *Hydrocynus vittatus*, *Labeobarbus marequensis*, *Labeo cylindricus* and *Labeo rosae*. The OR was sampled in September 2009 and May 2010 while the LR was sampled in November 2009 and April 2010. A necropsy was done to determine if there were any internal or external macroscopic alterations. Blood was taken for haematocrit (Hct), leukocrit (Lct) and total plasma protein (TP) determination. Biometric indices including the condition factor, hepatosomatic index, splenosomatic index and the gonadosomatic index were calculated. Age was determined using otoliths for tigerfish and scales for the other species. Samples of selected organs (liver, kidney, gill, testes or ovaries) were taken for histological analysis. Microscope slides were assessed qualitatively to identify any histological alterations present. These results were semi-quantitatively assessed from which an organ index and fish index were calculated. The organ index is an indication of the number and severity of histological alterations in a particular organ of a selected species. The fish index is a sum of all of the organ indices for any given fish sampled.

Biometric indices were within the normal range, however, the hepatosomatic index values were lower than the normal range. Haematocrit values varied with some values being above, below and in the normal range. Leukocrit values were within the normal range apart from *L. marequensis* from the OR in 2009 which were higher than the normal range. *Hydrocynus vittatus* had the highest fish index values of all the species with no significant differences between sites for the species. The mean liver index values of *H. vittatus* from OR was 13 in 2009 and 10.33 in 2010. All mean kidney index, gill index, testis index, and ovary index showed minor histological changes. The results of the biometric indices, blood parameters and histological alterations would indicate that the fish were healthy in terms of these parameters. This may be because the fish have adapted to chronic levels of pollution.

Keywords: *fish health, histology, biomarker, liver, quantitative histological assessment*

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HOW DOES VINCLOZOLIN MODULATE REPRODUCTIVE GENES IN MALE GOLDFISH?

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In mammals, vinclozolin (VZ) is known as anti-androgen, which causes male infertility mediated by disruption in androgenesis via *androgen receptor* antagonism. In fish, the VZ effects on reproductive functions are largely unknown and existed literature show somewhat contradictory results. To understand VZ adverse effects on male reproduction, mature goldfish (*Carassius auratus*) were exposed to three nominal VZ concentrations (100, 400, and 800 μg.L^{-1}) for 30 days. Sperm production and motility kinetics were evaluated. Alternations in neuroendocrine and endocrine reproductive genes, luteinizing hormone (LH), 17β-estradiol (E_2) and 11-ketotestosterone (11-KT) were also investigated compared to solvent control. Sperm production and motility kinetics (including motility and velocity) were decreased at all groups exposed to VZ. In goldfish exposed to 100 μg.L^{-1} VZ, 11-KT and LH were increased in blood plasma. Transcript of *gnrh* mRNA was also increased, while *gnihr* mRNA transcript was decreased coincidently. These data suggest that negative feedback of 11-KT was interfered in fish exposed to 100 μg/L VZ, because of observed increase in LH level and mRNA transcript of *lhr*. At 800 μg.L^{-1} VZ, 11-KT was decreased, while LH was unchanged in blood plasma. Decrease in mRNA transcript of *lhr,* increase in mRNA transcript of *gnihr* and no change in mRNA transcript of *gnrh* suggest that pituitary neuroendocrine function was not disrupted in fish exposed to 800 μg.L^{-1} VZ and therefore VZ acts directly on biosynthesis of androgen in testis. Blood plasma E_2 level was unchanged in all groups exposed to VZ. Taken together, this study suggests different dose-dependent VZ modes of action to disrupt testicular androgenesis mediated by modulation of neuroendocrine functions of pituitary in fish reproduction, which impairs sperm quality.

Keywords: *anti-androgen, 11-ketotestosterone, LH, receptor, goldfish*

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IN VITRO INHIBITION OF HEPATIC CYTOCHROME P450-MEDIATED REACTIONS BY HUMAN PHARMACEUTICALS IN RAINBOW TROUT MICROSOMES

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The primary oxidative enzymes involved in phase I metabolism are superfamily of heme contain proteins, collectively referred as cytochrome P450 (CYP450). First three families of CYP450 play an important role in the metabolism of endogenous and xenobiotic compounds such as drugs, environmental and agricultural chemicals etc.

The clotrimazole (CLO) and dexamethasone (DEX) are human pharmaceuticals which belong to imidazole and glucocorticoid groups of drug, respectively. Literature evidence showed presence of both pharmaceuticals in the aquatic environment. It is generally believed that fish are similar in many of their physiological processes compared with mammals and can serve as sensitive bioindicators for exposure to pharmaceuticals.

The aim of this study was to examine effects of CLO and DEX in fish cytochrome P450 (CYP450) by investigating the activity of three CYP450 isoforms: ethoxyresorufin O-deethylase (EROD; CYP1A), 7-benzyloxy-4-trifluoromethylcoumarin O-debenzylase (BFCOD; CYP3A) and p-nitrophenol hydroxylase (PNPH; CYP2E1). Hepatic microsomes from rainbow trout were exposed to four concentrations of CLO and DEX. CLO at a concentration range of 1 to 100 µM decreased the activity of EROD and BFCOD. EROD activity was non-competitively inhibited with a Ki of 0.5 µM, and BFCOD activity revealed competitive inhibition with a Ki of 0.04 µM. The relatively low Ki for CLO inhibition of EROD and BFCOD activity may indicate that the ability of CYP1A and CYP3A to metabolize xenobiotics *in vivo* is reduced in the presence of CLO. PNPH activity was not affected by CLO. DEX showed no inhibitory potency on any investigated reaction. CLO, but not DEX, interacted with CYP1A and CYP3A and inhibited the catalytic activity by different inhibitory mechanism.

Keywords: *clotrimazole; dexamethazone; rainbow trout; CYP1A; CYP3A*

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DISTRIBUTION OF SOME PHARMACEUTICAL RESIDUES IN THE AQUATIC ECOSYSTEM OF RIVERS

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Intensive use of medicines, cosmetics, household detergents etc. lead to increasing concentration of persistent pollutants in the natural and waste waters as well as in sediments or soil. The organic pollutants maybe present in the aquatic organism, also because of their high bioaccumulation potential. During our investigation we measured some non-steroidal anti inflammatory drugs (NSAID), ex. diclofenac, ibuprofen, ketoprofen, acetaminophen, indomethacine and naproxen in water, sediment, macrophytes, benthic animals and fish. Because the NSAID are the most common used pharmaceuticals and have been detected in the largest amount and frequency in the surface water all around the world we started to study the accumulation and degradation behaviour of these drugs in the aquatic environment and food chain. The analytical measurements were performed with GC-MS method after solvent extraction and derivatization with MTBSTFA reagent.

We have evaluated more than 25 sampling points in the Körös/Crisuri River Basin and also 2 others in the River Bîc, Kisinou, Moldavia. In the studied sampling sites we have detected almost diclofenac residues in the sediment in the range of 0.12 to 32.55 $ng.g^{-1}$ (d.w.), in few cases ibuprofen and naproxen were found. At the Bîc River, ibuprofen and diclofenac were measured in the sediment samples at 0.79 and 0.96 $ng.g^{-1}$ (d.w.) level. In the surface water all the drugs were detected, the highest value was in the case of diclofenac, 4.92 $ng.L^{-1}$, at Szeghalom city, where the sampling point was defined after the waste water treatment plant.

In the vegetation samples NSAID concentration values were located between 0.31 and 88.3 $ng.g^{-1}$ (d.w.). Ketoprofen and naproxen were able to bioaccumulate these drugs with high proportion, in comparation with sediment where diclofenac and ibuprofen were present mainly.

High concentration values were detected in zoobentos samples, also, in the range of 11–600 $ng.g^{-1}$ (d.w.). The obtained NSAID data were significant also in predatory fish; in few cases reached the 100 $ng.g^{-1}$ (dry weight) concentration level. Our studies presented the occurrence of medicines not only in sediment and water, but also in wild fish species.

Keywords: *non-steroidal anti inflammatory drugs, river*

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CRAYFISH MONITORING SYSTEM

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Assessment of water quality is up-to-date problem in today's anthropogenic world. Modern water quality monitoring stations are not working in real-time and not able to warn about the presence of a priori unknown, potentially dangerous chemical agents or their combination in water.

To address the problem it is possible to use aboriginal animals as bioindicators of pollution. It is known that crayfish heart contractile activity changes in response to different chemicals in water. The developed IR light based sensor together with data processing software allows monitoring of crayfish heart contractile activity and sending a warning in case of the presence of dangerous chemical agents in water.

The crayfish based system prototype developed in our laboratory allows recording of cardiac activity of several crayfishes simultaneously. Obtained data is analyzed and processed in order to extract key parameters from the recorded signal, which are then put to the crayfish heart model to make a decision about water quality.

Crayfish based water quality monitoring approach can be used to make cheap, reliable and quick-response front edge pollution warning systems.

Keywords: *crayfish, monitoring, sensor, infrared, bioindicator*

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IMPROVEMENT OF LARVAL REARING IN PERCID FISH THROUGH DIETARY ENRICHMENT WITH NUTRIENTS, IMMUNOSTIMULANTS AND PROBIOTICS

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Despite significant improvements in husbandry and nutrition conditions of percid fish rearing, early life stages of Eurasian perch *Perca fluvialis* and pikeperch *Sander lucioperca* are still considered as a sensitive phase during their production cycle in intensive systems. Low survival rate, resulting from high cannibalism, skeletal deformities but also sensitivity to bacterial diseases, still characterizes the larviculture of these species. The efficiency of different treatments including dietary enrichments of *Artemia* metanauplii with highly unsaturated fatty acids (HUFA) and ascorbic acid (AA), supply of probiotic bacterial mixture through water or enriched *Artemia* metanauplii or dietary supply of prebiotics (Chinese herbal extracts, inulin and oligofructose) was investigated in Eurasian perch and pikeperch larvae and results were evaluated in terms of husbandry variables (survival and growth, cannibalism rate, resistance to challenge test, type and level of deformities) as well as immune (lymphocyte proportions, lysozyme activity and total immunoglobulins, Ig) and/or digestive (trypsin and chymotrypsin, leucine-alanine peptidase, alkaline phosphatase and a-amylase) responses. The interest of *Artemia* metanauplii enrichment with HUFA and AA was demonstrated in pikeperch larvae, particularly regarding the reduction in larval deformities and the increased resistance to stress test. *Artemia* enrichment with high doses of probiotics (mixture of *Bacillus sp*) also increased growth rate, digestive activities (namely a-amylase and chymotrypsin) and some immune markers (lysozyme activity and total Ig) in pikeperch larvae, while phytobiotics treatments did not affect significantly growth and physiological variables. In Eurasian perch larvae, enrichment of *Artemia* nauplii with high doses of *Bacillus* mixture (4×10^{10} CFU L^{-1}) combined or not with *Bacillus* supply through water (4.93×10^{7} CFU L^{-1} day^{-1}) significantly improved the growth rate while it reduced the final growth heterogeneity and, consequently, the cannibalism rate. Lyzozyme activity assayed in fish homogenates was slightly modified by the probiotic treatments whereas total Ig was significantly stimulated by the *Bacillus* mixture, especially when probiotics were supplied through enriched *Artemia* metanauplii). Adose related growth response was observed when Eurasian perch or pikeperch larvae were fed inulin or oligofructose enriched metanauplii, and this was associated with an elevated activity of some digestive enzymes or total Ig level. Combined results from different experiments using husbandry and physiological variables suggest that improvement of larval rearing in percid fish can be achieved through the bioencapsulation of *Artemia* metanauplii with selected nutrients such as HUFA and vitamins, but also with bacterial probiotics or prebiotics.

Keywords: *Eurasian perch, pikeperch, HUFA, ascorbic acid, probiotics, phytobiotics*

EFFECT OF AGE, SIZE AND DIGESTIVE TRACT DEVELOPMENT ON WEANING EFFECTIVENESS IN CRUCIAN CARP, *CARASSIUS CARASSIUS* (L.), UNDER LABORATORY CONDITIONS

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The aim of the study was to determine the optimum age, body weight (WBW) and body length (TL) in the crucian carp, *Carassius carassius* (L.), which ensures the highest effectiveness of switching from live feed to artificial feed under controlled conditions. Moreover, the degree of development of the digestive tract was analyzed based on the height of enterocytes in a histological analysis. The experimental rearing was started 5 days post hatching (DPH), when the crucian carp reached the average WBW of 2.21 ± 0.64 mg and TL 6.12 ± 0.05 mm, and it was conducted for 40 days in a recirculating aquaculture system. The fish were divided into six experimental groups: control (C), fed with *Artemia* nauplii, and F15, F20, F25, F30 and F35, for which live feed was replaced with artificial fodder on 15, 20, 25, 30 and 35 DPH, respectively. The highest enterocytes at the time of the feed type switching were found in the individuals in groups F30 and F35 (34.79 µm and 35.42 µm on average, respectively). The highest values of TL (42.12 ± 0.70 mm) and WBW (905.34 ± 50.25 mg) were featured by the individuals in the control group. The lowest survival rate was recorded in group F15 (90.70 ± 1.15 %). The results indicate that an effective food type switching in the crucian carp could not be performed before 30 DPH at a temperature of 27 °C, when the fish reached TL = 31.07 (± 0.41) mm and WBW = 436.91 (± 13.71) mg.

Keywords: *crucian carp, larvae, feed, weaning, early development, laboratory conditions*

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

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Wels (European) catfish, *Silurus glanis* L., is a high valued fish of European pond aquaculture. Recently, the quality of its flesh, suitability of very good growth performance in high stocking densities and ability to ingest artificial pelleted diets, led to its wider utilisation under conditions of intensive warm-water farming units including recycling systems. The evaluation of processing yields of 18-month-old catfish (2.1–2.5 kg) was performed using fish cultured in two different farming units – (1) outdoor pond aquaculture system (PAS) with flow-through regime (24.6 ± 0.2 °C) and (2) the indoor tank aquaculture system (RAS) with recirculation regime (26.0 ± 1.0 °C). Both RAS and PAS fish were siblings originating from the spawning batch (28 g at stocking). Both groups were cultured for one year and fed identically with pellets Ecolife 15No8 Biomar (45 and 16 % protein and fat, respectively). Significant differences with respect to their processing traits were recorded in relative weights (% total weight) of skinned trunk, fillets and skin which were higher in RAS fish. PAS fish had significantly higher head and female gonadosomatic index. Visceral, ventral and dorsal fat deposits were significantly bigger (amounting the twofold weight percentage) in RAS fish in comparison to PAS fish.

Keywords: *European catfish, recirculation system, pond aquaculture, processing yield*

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STURGEON AND CAVIAR PRODUCTIONS AND MARKETS: AN OVERVIEW

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One of the most interesting family of fish for freshwater aquaculture in the last decades was that of the Acipenseridae. High prices and market demands for caviar, together with the dramatic decline of natural sturgeon stocks and the subsequent restrictions on their fishery, provided the incentive since the early nineteen-eighties to rear sturgeons for meat and caviar production. At the same time and for the same reasons, people started to seek for alternatives products that could fill the gap in caviar supplies. Additionally, innovative product developments were initiated to not only to meet the demand for caviar but also to even create several new market sectors while hitch-hiking on the traditional image of caviar. Historic demand for caviar on traditional export markets (Europe, US, Japan, plus Russia, China etc.) can be assessed based on trade data from the 1980s and 1990s. The estimate accounts for an amount up to 3000 t annually. Main activities of farming sturgeons have been in Russia, Iran, the European Union, China and the USA. Besides these major producers and consumers, other entities in other countries show up on the scene, also outside the natural range of sturgeons, not only contributing to the production, but having a substantial influence on the structure of the industry and the markets (examples: Uruguay, Arabic countries, Israel, and more recently Vietnam). At present, total estimated caviar output from aquaculture is about 260 t. This production is expected to increase quickly to 500–750 t in the next 5–10 years. If these figures are a realistic reflection of the market, the future of caviar trade will mainly be determined by demand-driven price structures, and a new evolving "mass market" will appear, while the traditional luxury and expensive market will persist. At the same time, other so-called "caviar" products are presently offered on the markets, sometimes misleading the consumers. The various type of these products have been divided into six categories: (a) true caviar; (b) substitutes for caviar (eggs from other fish species or from other animals); (c) caviar imitations (caviar-like products derived from other biological substances, imitating and/or reconstructing caviar in appearance and taste); (d) caviar simulations (using other biological substances to simulate only the taste but not necessarily the appearance); (e) derivates (products that contain true caviar as a component of their recipes); (f) products with emotional associations to caviar (these are not related to caviar at all, but incorporate the name as a marketing strategy used to evoke a luxury image and infer a high value). Presently, the eggs of more than 38 species of fish, besides sturgeons, and 3 species of other animals are used to produce substitutes; about 15 "caviar like" preparations are known on the market using as raw material fish flesh, seaweed and others mixtures of materials of various origin; five products can be considered simulations; few use caviar as a component in their production recipes; a lot of different objects are presently marketed which use the name caviar as a brand. Some uncertainties have already appeared in the market place (e.g. rapid production increase and market sensitivities to overproduction with price structure effects, stress effects in production costs, drastically reduced profit margins, additional costs to promote product diversification; growing competition from alternative products). All these issues indicate that this relatively new branch of the aquaculture industry faces similar growth pains as previous developments (e.g. salmon farming) but within a shorter time frame.

Keywords: *sturgeons, caviar, caviar substitutes, productions, markets*

DIFFERENCES IN ANTIOXIDANT STATUS AMONG IMPORTANT REPRESENTATIVES OF CYPRINIDAE, ACIPENSERIDAE AND SILURIDAE REARED IN CAGE CULTURE

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Nowadays a cage cultures enable an intensive form of farming of many fish species. However, the culture conditions of this type of farming are often significantly different from these observed in natural environment. Higher density and reduced space may significantly affect physiological and biochemical parameters of fish blood, what might reflect health status in fish. Therefore, the aim of the study was to compare oxidative status between three important representatives of Cyprinidae, Siluridae and Acipenseridae families, reared in cages.

In the experiment, samples of blood serum of *Tinca tinca*, *Silurus glanis* and *Acipenser ruthenus* farmed at a cage culture were collected and several biochemical parameters (antioxidant status, activity of antioxidant enzymes, superoxide dismutase, glutathione peroxidase, catalase and level of lactate dehydrogenase, alanine and aspartate transaminase) were assayed. Additionally, water parameters and feed composition were analysed for the purpose of the experiment.

The result of assays showed that antioxidant status of blood serum was significantly higher in tench by comparison with catfish *and* sturgeon. Additionally in tench samples, activity of glutathione peroxidase had significantly higher values in comparison to two other groups of analysed samples. The activity of other enzymes measured in samples of the blood serum was at comparable level in all groups.

The results of studies describe different response of antioxidant defence that occur in fish form diverse families farmed in cages.

Keywords: *bioindicators, Tinca tinca, Silurus glanis, Acipenser ruthenus, antioxidant status*

FIRST DETECTION OF *HERPESVIRUS ANGUILLAE* (HVA) IN POLAND FROM VARIOUS EEL (*ANGUILLIDAE SP.*) SAMPLES

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Herpeswirus anguillae (HVA) belongs to the most abundant group of DNA viruses of fish and pose a real treat to wild and cultured eel species. In Europe HVA was isolated for the first time from cultured eels in 1998. In previous years, virus presence was also described in Japan and Taiwan. Eel mortality form HVA ranges from 1 to 10 %, however might increase and reach 50 % as an effect of stress. Since 2007, European eel is listed on Annex II of CITES (Convention on International Trade in Endangered Species), thus restoration of eel population is mainly achieved by restitution, introduction of the size of fish that can legally be harvested or other regulations in catches. Therefore the aim of the study was to detect HVA in eels and assess risk associated with its presence.

The study focused on 61 fish in total from *Anguilla* genus, that were collected during 2009–2013 from open waters of the north-west Poland (Dąbie Lake, Szczecin Lagoon), China (food product) and Denmark (glass eels for farming). During laboratory analysis eels were barcoded (cytochrome oxidase, COI) and fish samples (gills and gill swabs) were analysed for the presence of HVA virus. The study identified three species of eel (*A. anguilla*, *A. japonica*, *A. rostrata*) among, from which only *A. japonica* samples were free from HVA. Additionally, analysis revealed that origin of the analysed fish samples is not related to the type of the water (marine or freshwater) or stage of the eel lifecycle (adults or glass eel).

This is the first study, which confirm presence of HVA in Poland. HVA is dangerous to fish cultured in fish farms as well as for natural stocks, therefore it is advisable to monitor eel health in relation to HVA.

Keywords: *Anguilla sp., HVA, herpesvirus anguillae, eel, PCR*

NURSING OF PIKEPERCH (*SANDER LUCIOPERCA* L.) FRY IN SHALLOW FISHPONDS

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Pikeperch (*Sander lucioperca*) appearing in European markets can derive from three sources: from catches of natural waters, from pond farms (produced as associated species) and in the last few years also from intensive monocultures. In all three cases supply of stocks can be ensured most efficiently by pre-rearing (nursing) pikeperch larvae deriving from hatcheries in ponds. Pond rearing of pikeperch applied by the present experiments was based on the selection of zooplankton. On the basis of average results of six years in a shallow, 2.0 hectares rearing pond during the four weeks of nursing the survival of larvae was 13.4 ± 4.37 %.

Keywords: *pikeperch, advanced fry rearing in ponds, Hungary*

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PRELIMINARY RESULTS OF ON-GROWING DURING HATCHING OF BURBOT (*LOTA LOTA*) USING CHIRONOMIDAE LARVAE AND CHICKEN LIVER

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In recent years burbot (*Lota lota*) has aroused increasing interest among fish breeders. Main reasons of such situation are its limited abundance in natural waters and tasty meat which can attract consumers.

Nowadays the methods of controlled hatching of this fish are well known and commonly applied. However, the on-growing poses more problems due to difficulties with feeding the hatch. Usually rotifers or nauplii of the brine shrimp *Artemia* sp. are used as the first food items. In feeding with rotifers, a system for their production is necessary while the purchase price of brine shrimps is relatively high.

Therefore, in the Department of Aquaculture (West Pomeranian University of Technology, Szczecin) an attempt was made at on-growing of burbot during hatching using Chironomidae larvae and chicken liver as substitutes for the above mentioned food items.

The experiment was conducted in custom-built closed systems for the on-growing of fish brood and lasted 28 days. The stocking density amounted to 15 indiv. dm^{-3}. Various configurations of brine shrimp, Chironomidae larvae and chicken liver gave 8 feeding variants which were tested throughout the investigation. At the end of the experiment fish were counted, measured and weighed which allowed determining basic indices, i.e. fish survival, increments of total length growth in a time unit (ITL), specific growth rate (SGR), condition factor (K) and the factor of average unit weight gain and survival (WS).

This study indicated the highest survival for fish fed with *Artemia* sp. for the whole experiment (18.67 %). The survival in the feeding variant with blood worms was on average 50 % lower but the WS index was in that case higher which implied the production of larger biomass from identical starting conditions. Results obtained in this investigation suggest that the attempts at finding at least partial food substitute for *Artemia* sp. in the breeding of juvenile burbots are justified.

Keywords: *on-growing during hatching, burbot, Chironomidae, chicken liver, Artemia sp.*

EFFECT OF TEMPERATURE AND STOCKING DENSITY ON FOOD UTILIZATION AND GROWTH OF EUROPEAN WHITEFISH (*COREGONUS LAVARETUS* L.) FROM LAKE CONSTANCE

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Due to decreasing yield of European whitefish (*Coregonus lavaretus* L.) in Lake Constance the attention to produce this local form (called "Felchen") in aquaculture is increasing. However, little is known about optimal temperature and preferred stocking density of this form under aquaculture conditions. Therefore the effect of two temperatures (10 °C and 14 °C) and three stocking densities (initial: 3.75 $kg.m^{-3}$, 7.5 $kg.m^{-3}$, 15 $kg.m^{-3}$) on mortality, feed intake, growth and feed conversion ratio of whitefish fingerlings (initial weight 11.2 g) was investigated. The fish being offspring of wild-caught near-shore spawning whitefish from Lake Constance. They were reared for 70 days in plastic tanks (50 L) under 24 h constant light and fed ad libitum on a commercial dry diet. The trial was run in triplicate. Mortality was recorded daily. At the end of the trial, individual body weight and body length of all fish were determined. Mortality was low and influenced neither by the water temperature, nor by the stocking density. The feed conversion ratio (FCR) of whitefish reared at 14 °C was significantly better than at 10 °C. At high stocking density the FCR was inferior to medium and low densities, independent of water temperature. At 14 °C the thermal growth coefficient (TGC) at high stocking density was significantly lower compared to the other. However, at 10 °C no influence of densities on TGC was found. In conclusion, rearing at 14 °C resulted in higher growth rate and better feed conversion ratio than rearing at 10 °C. Nevertheless for the whitefish of Lake Constance, high stocking densities should be avoided to prevent slower growth and low feed conversation ratios.

Keywords: *Coregonus lavaretus, temperature, stocking density, whitefish, density-dependent*

Acknowledgements: This study was financed by the Deutsche Bundesstiftung Umwelt (DBU) (project No. 28134 – 34).

EFFICIENCY OF TWO SPAWNING TECHNIQUES EVALUATED BY REPRODUCTIVE PERFORMACE IN PIKEPERCH (*SANDER LUCIOPERCA* L.)

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Pikeperch (*Sander lucioperca* L.) is new promising fish species for diversification of European aquaculture. This species is highly valuated freshwater fish in Europe thanks to flesh quality. Current European market is undersupplied with marketable pikeperch. Therefore pikeperch intensive aquaculture has been developed in Europe during last fifteen years. This culture system requires some reliable mechanism for controlled reproduction and larval production. Protocol of hormonally induced ovulation of two spawning techniques (artificial or semiartificial spawning) was already optimized in pikeperch. The aim of this study is to compare the efficiency of both techniques and recommend one of them for fish farming.

Hormonal injection of maturated pikeperch females (TL = 511.4 ± 31.5 mm, W = 1219.9 ± 232.0 g) in final stages and males (TL = 492.5 ± 35.6 mm, W = 1109.8 ± 178.0 g) was used with hCG (Chorulon, 500 IU.kg^{-1}) for this study. In total, two groups (each 40 pairs of broodstock) were created for the comparison of efficiency of two spawning techniques – artificial (included: egg and sperm stripping, artificial fertilization, egg stickiness elimination and artificial incubation in jars) and semiartificial spawning (included: natural ovulation, fertilization, egg laying and incubation on the nest with male present). The efficiency of tested techniques was compared by the following reproductive performance of broodstock under controlled conditions (temperature: 15.0 ± 0.5 °C): ovulation and spermiation rates (%), latency time (hours), rate of spontaneous spawning (%), fertilization and hatching rates (%), egg diameter 24 h after fertilization (mm), egg incubation time (°d) and production of larvae per one female (pcs).

Same spermiation rate (100 %) and statistically the same egg diameter (1.33 ± 0.1 for semiartificial and 1.38 ± 0.3 mm for artificial spawning) and egg incubation time (118.6 ± 6.3 for semiartificial and 126.4 ± 9.5 °d for artificial spawning) were found after both spawning techniques. Other reproductive performances were significantly different between artificial and semiartificial spawnings. Artificial spawning with higher demand for labor provided lower ovulation rate (88.75 %), higher rate of spontaneous spawning with production of unfertilized eggs (35 %), shorter latency time (69.1 ± 5.2 h), lower fertilization and hatching rate (78.7 ± 5.5 % and 63.5 ± 4.5 %) and lower production of fresh larvae (81,600 larvae per female) compared to semiartificial spawning with following parameters: higher ovulation rate (100 %), higher latency time (81.7 ± 10.7 h), lower rate of spontaneous spawning (0%), higher fertilization and hatching rate (91.5 ± 3.0 % and 75.6 ± 2.7 %) and higher larval production (97,300 fresh larvae per female). According these results we prefer to use semiartificial spawning within pikeperch culture which is more efficient in production term with lower demand for labor.

Keywords: *pikeperch, reproduction, incubation, egg and larvae*

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POSTER SESSION

POSTERS ARE LISTED IN ALPHABETICAL PRECEDENCE ACCORDING TO THE FIRST AUTHOR

EFFECTS OF DIFFERENT SUPPLEMENTAL FEED ON CLADOCERAN ASSEMBLAGES AND CARP YEARLINGS IN FISH PONDS

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In semi-intensive carp production different supplemental feed are used in order to fulfill the nutritional requirements of cultured fish together with natural food present in ponds. Depending on the level of intensification, added feed can be given in an unprocessed form – row cereals or different degrees of processed feed as pelleted and extruded feed. However, except on fish growth different supplemental feed can affect the amount and structure of natural food for carp yearlings and have a further effect on fish production. In this study, we have investigated the effect of three different types of supplemental feeds: row cereals (corn, wheat and barley), commercial pelleted and extruded feed (25 % proteins and 7 % fat) on cladoceran assemblages and fish growth. The experiment was carried out from June to October in nine earthen ponds (three per treatment) at the fish farm "Kapetanski rit". Ponds were stocked with common carp yearlings in medium density (appox. 2000 ind.ha^{-1}). Overall, the diversity of Cladocerans was higher in ponds with pelleted feed compared to ponds fed cereals and extruded feed, where only 9 and 4 species out of 17 were found, respectively. Additionally, supplemental feed had a considerable effect on the Cladoceran abundance and structure. In ponds with pelleted feed the average number of large cladocerans was significantly higher compared to ponds where cereals or extruded feed were applied. Due to low protein content in corn, wheat and barley, fish depleted the available natural sources from ponds, leaving only one dominant cladoceran species *Bosmina longirostris* unattractive for carp yearlings due to its small size. Contrary to this, pelleted feed provided enough proteins for fish, and furthermore a sufficient amount of nutrients for cladoceran production. However, extruded feed apparently had no additional effect on cladocerans. Type of supplemental feed also had an effect on fish production parameters, where pelleted feed provided significantly higher average individual weight and total fish yield than cereals. Since fish production was the highest during the maximal cladoceran production in ponds fed pelleted feed it can be assumed that fish extensively used the natural proteins available in the ponds.

In conclusion, it can be said that pelleted feed provides not only good growth of carp yearlings compared to row cereals and extruded feed but can also serve as a way to replenish large cladocerans, and in turn provide an additional source of proteins for fish in the form of natural food organisms.

Keywords: *carp yearlings, natural food, cladocerans, supplemental feed, pelleted feed*

Acknowledgments: The present study was supported by the Ministry of Education, Scinece and Technological Devolopment, through the project improvement of production capacities of carp (Cyprinus carpio) through nutrition and selective breeding programs (TR 31075) and project AREA (EU – FP7*).*

THE EFFECT OF SUPPLEMENTARY FEEDING OF COMMON CARP (*CYPRINUS CARPIO* L.) WITH MODIFIED CEREALS ON WATER QUALITY AND NUTRIENT BALANCE

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A four month study was conducted in eight storage ponds to evaluate the effect of the addition of supplementary feeds with different treatments as a management tool for common carp growth. At the same time, water quality, natural food availability and balance of the most important nutrients were monitored in this semi-intensive system. In addition, nitrogen and phosphorus budget were calculated as the difference in output (effluent water and fish harvested) and input (food, fish stocked and effluent water). Three types of supplementary feed were used: wheat; wheat thermally treated; and wheat thermally treated and pressed. One additional group provided only with naturally available food served as control. The type of supplementary feed did not influence water quality, except dissolved oxygen and also did not significantly affect abundance of natural food availability. The use of modified cereals (especially thermally treated and pressed wheat) improved common carp growth, and these diets were related to lower nutrient concentration in effluent water. According to our results, thermally and thermally-pressed cereals improved the balance of phosphorus and these diets can be helpful for farmers, not only as a source of nutrients for carp growth but also indirectly as a management tool for maintaining ecological stability in ponds.

Keywords: *common carp, nitrogen, phosphorus, water quality, wheat*

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EVALUATION BY METABOLOMIC MEANS OF FULL FAT SOY IN FEEDS TO SALMONIDS

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The aim of the present study is to evaluate differences in response of salmonids to metabolize soy meal of different qualities. Unprocessed soy meal is known to cause some undesirable by-effects as it contains some anti-nutritional compounds. Therefore in our study, salmonids have been fed defatted and full fat soy meal as a main protein source to their diets. The soy meals were combined with meal based on some microorganisms to contradict the soy effect in the intestine. We have also compared to commercial feeds aimed for salmonids. Fat content of the different diets have been equalized to be able to compare the nutritional value and evaluate the fish growth and the feed conversion ratio. Lipid quality and fatty acid profile has been analyzed together with metabolomics (NMR) of the fish livers to be able to evaluate the different feed qualities. The processing of raw materials requieres a lot of resources and we aim to optimize the use and also needs of energy. This study is a part of a larger trial where also gut health is evaluated. The results of this study will be of importance for the sustainability of aquaculture practises.

SHELF-LIFE EVALUATION OF COMMON CARP (*CYPRINUS CARPIO* L.) FILLETS USING THE TORRY SCALE

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Seafood has a significant place in the human diet due to the high nutritional composition offered by fatty acids (HUFA, PUFA), essential amino acids and a high protein content. However, rich nutritional composition together with high water content, pH values (6–7) and weak connective tissue leads to decreased shelf-life in seafood. Although microbiological and biochemical analysis plays an important supporting role, sensory analysis is of utmost importance to the consumer. Several methods are available for the evaluation of freshness, with torry scale being used most widely due to its user-friendly nature. Fish from semi-intensive and extensive culture systems were harvested and processed. Fillets were aerobically stored in plastic bags under refrigerated conditions at 2 °C for 8 days. Each day of sampling, fillets were evaluated in terms of texture, odor, discoloration and overall acceptability by a panel of ten trained panelists. Scores were based on a scale from 1 to 5 with 1 being extremely undesirable and 5 being extremely desirable. Regarding the sensory analysis, fillets were rejected after 8 days of storage. Significant differences ($p < 0.05$) were found between the fillets obtained from fish grown in the two cultured systems. Fillets from semi-intensively cultured fish had a mean score of 9.5 while extensively culture fish had a mean score of 5.3 for the last day of the storage period respectively. However, these differences may be primarily due to the different scores for the texture parameter which were 2.5 and 1.7 respectively for semi-intensive and extensively cultured fish. This study showed that the end point for the shelf-life of common carp fillets under the above mentioned storage conditions is 8 days. A score of 8 for the sensory analysis can be regarded as the lower limit for acceptability of common carp fillets.

Keywords: *common carp, processing, shelf-life, torry scale, texture*

Acknowledgements: The study was supported by the South Bohemian Research Center of Aquaculture and Biodiversity of Hydrocenoses CENAKVA CZ.1.05/2.1.00/01.0024 and projects GA JU 047/2010/Z University of South Bohemia.

REPLACEMENT OF FISH MEAL BY SOY PROTEIN CONCENTRATE IN COMPLETE FEED FOR CARP AND EFFECTS ON CARP FRY GROWTH

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As a consequence of increased need for fishmeal (FM) and stagnation in its production, the price of this important component in complete fish feed is constantly raising. How successful are efforts to efficiently replace FM depend on species feeding habits and ingredients used as FM replacement. Ingredients of soy origin occupy an important position among possible FM alternatives. One of the most important soy products that can replace FM is soy protein concentrate (SPC) obtained from a higher rate of soy grain processing. Owing to the processing technology that include: oil extraction using hexane as solvent, solvent removal system, and soluble sugars rinsing by diluted alcohols from degreased soy flocks, proteins remain preserved and their content in SPC vary from 65 to 70 %. In order to study effects of FM replacement by SPC a 120 days feeding experiment with carp fry was carried out. The trial was realized in the Laboratory for fish nutrition of the Faculty of Agriculture University of Belgrade. In the complete feed containing 38 % proteins and 7% lipids FM was replaced by SPC in following quantities: 25 % in the first treatment, 50 % in the second and 100 % in the third treatment. Carp fry growth rate was compared with growth rate of fry fed complete feed containing FM as main protein supply. All treatments were done in triplicates. After 120 days the lowest growth rate was found in the group fed 100 % FM diet. With the increase of SPC content growth rate increased, so that the highest was obtained using a complete feed with 100 % SPC. According to the results obtained in this study, in complete feed for carp as an omnivorous species, FM can be successfully replaced by SPC.

Keywords: *carp fry, fish meal, soy protein concentrate*

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EVALUATION OF THE TEXTURAL PROPERTIES OF COMMON CARP (*CYPRINUS CARPIO* L.) FROM DIFFERENT CULTURE SYSTEMS

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Common carp is one of the most widely cultivated fish species in Central and Eastern Europe. Nowadays, with the quality of fish flesh having a more significant role in consumer demands, textural properties namely hardness, cohesiveness, springiness and gumminess play a vital role in developing consumer perceptions and maintaining a high demand. Fish with a mean body mass of 1640 ± 336 g and 811 ± 104 g, and a fat content of 6.2 ± 2.2 % and 3.8 ± 0.3 % were harvested from semi-intensive (SI) and extensive (E) systems respectively. The textural properties of the fillets were measured after being stored at 2 °C for a period of 24 h. No significant differences were observed in the cohesiveness values ($P > 0.05$) between the different culture systems. However, significant differences ($P < 0.05$) were observed in the following other textual parameters measured and calculated. Fish from SI and E systems had a mean hardness value of 199.62 ± 52.08N and 126.10 ± 29.90N respectively. Springiness values were 0.62 ± 0.03 and 0.72 ± 0.07 for SI and E systems respectively. The value of gumminess for SI systems was 4825.23 ± 1386.27 and 7202.70 ± 2277.46 for E systems. This study shows that the textural properties, especially the hardness of common carp fillets are therefore particularly dependent on the culture system used. Feeding strategies as well as general pond management is therefore of critical importance to enhance the textural properties of common carp fillets and meet consumer demands.

Keywords: *common carp, culture system, fillet, hardness, textural properties*

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INFLUENCE OF DIFFERENT SUPPLEMENTAL FEEDS ON LIVER AND INTESTINE OF COMMON CARP (*CYPRINUS CARPIO*, L. 1758) IN SEMI-INTENSIVE SYSTEM: A HISTOLOGICAL STUDY

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In order to determine effects of feeding with different types of supplementary feed on liver and intestine histology of carp reared in a semi-intensive system, a six-month field experiment was carried out. Carp yearlings were fed different supplements: cereals (CF), pelleted (PF) and extruded (EF) compound feed. No severe histopathological alterations of the liver and distal intestine were observed during the experiment. The most frequent changes in the liver were congestion of sinusoids, fibroses with increased number of eosinophilic granule cells, and infiltration of leucocytes, while in the pancreas the most frequent finding was the presence of adipocytes. A histomorphometry revealed larger nuclear profile area of EF-fed fish compared to other two groups, while cytoplasmic profile area of the hepatocytes decreased in following sequence: CF > EF > PF. Lower nucleus/citoplasm ratio was found in CF-fed fish compared to EF- and PF-fed fish. Regardless of the type of supplementary feed, the number of lipid droplets in hepatocytes was highest in July and August, lipid depletion occurred in September and October, while glycogen was present in hepatocytes throughout the experiment. No size difference between groups was observed for enterocytes height, absorptive surface height and number of goblet cells in *tunica mucosa*, but a strong relationship between length of intestinal folds and fish weight was found. Histological analysis revealed a difference among three types of supplemental feed used in this field study: extruded or steam-pelleted feed induced better nutritional status of common carp in comparison to cereals. At the same time, histomorphometry indicated differences between EF- and PF-fed fish, even though the weight gain of fish in those groups was similar.

Keywords: *hepatocytes, enterocytes, histomorphometry, histopathology, lipid droplets*

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INCREASING NUTRITIONAL VALUE OF TRADITIONAL MEAT PRODUCTS WITH AN UNDERUTILIZED BY-PRODUCT FROM CARP PROCESSING

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The aim of our study was to find a way to use the minced fish flesh that is separated from the bones of carp after filleting. This by-product is a good quality source for both fish protein and omega 3 long chain polyunsaturated fatty acids (n-3 PUFA). However this raw material is problematic to use as it is prone to oxidation and bacterial breakdown. Until now, on the Czech market there are quite few applications for this product. Together with the industry we evaluated what type of products would be accepted by the customers and experimented with traditional recipes for barbecue 53.5, vienna and hotdog type sausages 46.5 %, and liver pate 31.8 by exchanging a part of meat with the fish separate. To ensure high quality sensory, microbial and storage stability analyses were carried out beside the general composition and fatty acid composition to determine the final proportion of nutritional valuable n-3 PUFA. The proportion of combined EPA and DHA was 74, 54, 109 and 77mg/100 g in barbecue sausage, hotdog, Vienna sausage and pate respectively. This means 29.6 %, 21.6 %, 43.6 % and 30.8 % of the minimal daily recommended intake according to EFSA [1]. These two FA are usually not found in this type of products according to both various databases [2,3] as well as analyses [4]. With the proper processing technique and careful handling both oxidation and microbial counts were below the limits and the products had comparable storage time to the traditional ones. We concluded that the separate is a cheap and easy available raw product to increase the nutritional value of traditional meat products without compromising taste or storage stability.

Keywords: *n-3; EPA; DHA; TBARS; fish product*

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THE DIGESTIBILITY OF FEED MIXTURES CONTAINING DIFFERENT LEVEL AND ORIGING OF PROTEINS FOR COMMON CARP

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Determination of apparent digestibility coefficient (ADC) of nutrients in fish feed is important not only for proper diet formulation but also for estimating the potential degradation of environmental conditions. Digestibility of nutrients is variable and depends on a range of factors, where the level and origin of proteins in the diet is one of the most important. The aim of this study was to determine the apparent digestibility coefficient of concentrate mixtures with different content and origin of proteins for carp yearlings. The experiment was carried out at the Laboratory for fish nutrition of the Faculty of Agriculture, University of Belgrade, for 90 days. Fish were fed with concentrate mixtures having 38 % (Ap), 41 % (Bp) and 44 % (Cp) of proteins. The bigger share of the protein part in Ap were plant proteins (PP), in Cp fish meal (FM), while in Bp the share of FM and PP was approximately the same. The results of feces analysis showed significantly higher ($p \geq 0.027$) values of apparent digestibility coefficient of proteins (74.66 %) and fat (84.76 %) in fish fed with mixture Cp compared to fish fed with Ap. The digestibility of NFE-nitrogen free extracts (from 66.22 % to 67.50 %), energy (from 64.86 % to 74.50 %) and digested energy (from 14.37 % to 13.30 %), did not differ significantly ($p > 0.05$) in fish fed mixtures with different protein content.

The results suggest that with the increase of proteins in mixtures, their digestibility increases. A higher content of fish meal in Cp mixture provided significantly higher digestibility of proteins compared to mixture Ap with higher content of plant proteins. It is known that the utilization, digestibility and availability of plant proteins are lower than in proteins of animal origin.

Keywords: *digestibility, carp, protein levels, fish meal, plant proteins*

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COMPARISON OF THE EFFICIENCY OF COMMON CARP AND SILVER CARP PITUITARY IN THE BREEDING OF COMMON CARP (*CYPRINUS CARPIO*) AND NORTHERN PIKE (*ESOX LUCIUS*)

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For many years fish farmers have been using hormone preparations for the artificial propagation of different fish species. In practice, acetone-dried common carp pituitary is the most commonly used agent to induce ovulation (i.e. hypophysation technique). In the last two decades, following the political and economic changes in Eastern Europe, many big fish factories were closed or divided up. This has reduced the supply of glands from the region; world supply has been reduced, and the market price has soared. The aim of our research was to compare the effects of silver carp pituitary (SC) to those of common carp pituitary (CC) on different reproductive traits. Experiments were performed over the large-scale breeding of northern pike (*Esox lucius*) and common carp (*Cyprinus carpio*).

Ovulation rate of northern pike females for both groups was 70.4 % in the first experimental year. In the second year, ovulation rates were 94.4 % and 87 % for the CC and SC groups, respectively. PGSI values for the two groups were similar (14.3 %) in the first year. In the second year PGSI values were 18.7 ± 3.43 % and 20.1 ± 3.92 % for the CC and SC groups, respectively. Fertilization rates for the two groups were similar in each experimental year. Ovulation rate and PGSI for common carp females induced by common carp pituitary were 66.7 % and 13.7 ± 5.49 %, respectively. In the SC group ovulation rate and PGSI were 100 % and 14.5 ± 2.32 %, respectively. With the exception of ovulation rate of common carp, all reproductive traits for the two treatments were statistically similar. According to our research, silver carp pituitary could be suitable to have wide application for induced ovulation of fish in the future.

***Keywords**: fish pituitary, induced breeding, common carp, northern pike*

PARTIAL SUBSTITUTION OF DRY DIET WITH FROZEN CHIRONOMIDS CAN COUNTERACT BODY DEFORMITIES IN JUVENILE *LEUCISCUS CEPHALUS* (L.) UNDER CONTROLLED CONDITIONS

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Under controlled conditions, intensively fed to juvenile cyprinids commercial dry starter feeds often result in high fish growth rates, but also in body malformations including skeletal deformities. In contrast, natural diets as chironomid larvae do not induce body malformations irrespective of feeding intensity. Hence, partial substitution of dry diet with natural food might be a simple method to mitigate incidence of deformities without diminution in fish growth.

In the present 60-d experiment at 25 ºC, efficiency of the substitution technique for juvenile *L. cephalus* (species of considerable susceptibility to body deformities among Cyprinidae) was evaluated. Initial fish mean TL was 44.8 mm, mean BW was 0.61 g, and fish stocking density amounted to 1.7 individ. dm^{-3}. Six triplicated experimental groups were set. In group F intensive feeding with exclusively commercial dry diet (Aller Futura, Denmark; mean daily feeding rate 3.9 % of fish biomass) was performed whereas frozen chironomid larvae (44 % daily) were fed in the control group C. In the remaining groups, fed as in group F, full daily ration of dry feed was substituted with full daily ration of chironomids (as in group C) every fifth, fourth, third and second day, respectively in groups 4F1C, 3F1C, 2F1C and 1F1C.

The first spinal deformities in fish were recorded on day 30, in all groups receiving dry diet, and then their share ranged from 4 % to 8 %. On the final day of the experiment, juveniles with spinal deformities constituted from 55 % (maximum, group F) to 0 % (minimum, group C). In groups 4F1C, 3F1C, 2F1C and 1F1C, the respective values equalled to 42, 37, 20 and 8 %. The final fish size was the larger the more frequent was substituting of dry diet with natural food, with the maximum values of 75.4 mm TL and 3.47 g BW recorded in group 1F1C substituted every second day. In this group, the lowest fish condition coefficient value of 0.80 was found, whereas the highest value of 0.83 was recorded in group F. The final survival rates were 100 % in all groups.

In group F, individuals with deformities proved to be significantly larger than those without (71.8 *v.* 69.6 mm TL; 3.16 *v.* 2.83 g BW). The former had significantly higher fat content (9.91 *v.* 8.25 %), but significantly lower ash content (2.36 *v.* 2.93 %), at similar content of macrominerals responsible for skeleton ossification (Ca, P, Mg) and similar Ca:P ratio.

In view of all the above, partial substitution of used intensively commercial dry feed with frozen chironomid larvae can be considered a simple and useful tool to counteract spinal deformities in juvenile cyprinids. However, further studies would be desired to optimize this technique of feeding also with a view to the economical use of both formulated and natural diets.

Keywords: *chub, deformities, dry feed, juveniles, substitution*

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CARP FARMING UNDER CONDITIONS OF CZECH REPUBLIC WITH FOCUS ON FILLET FATTY ACID COMPOSITION

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Common carp (*Cyprinus carpio*) is the most reared fish species in the Czech Republic. The annual production of 20 000 tons is the highest in Europe. Farming takes place in ponds in semi-intensive system and is based on the utilisation of natural feed (plankton and benthos) with cereal supplementation. Lipids of carps reared in this way contain high amount of monounsaturated fatty acids (MUFA) (oleic acid) at the expense of polyunsaturated fatty acids (PUFA). N-3 PUFA are known to have positive effects in human organism as for example preventing cardiovascular diseases. However, their intake by Czech population is low and the incidences of cardiovascular diseases are high. This study describes experiences with farming of carp supplemented by linseed/rapeseed (rapeseed mouldings) pellets (group P) which are a good source of alpha-linolenic acid as the precursor of n-3 PUFA. Lipid quality of the group P was compared with carps supplemented with cereals (group C) and carps kept on natural feed only (group N). Fish from group C have the highest fillet lipid content with high proportion of MUFA, fish from group P have moderate lipid content with increased proportion of n-3 PUFA (especially alpha-linolenic acid) as well as the longer chain PUFA eicosapentaenoic (EPA) and docosahexaenoic (DHA) acid. Fish from group N have the lowest lipid content and high proportion of n-3 PUFA. Using the fish from group P, we realized a clinical study in which patients recovering from major cardiac surgery received 2 x 200 g portion of carp with elevated content of n-3 fatty acids for 4 weeks. There was a significant improvement in healing processes, reduced content of triacylglycerols (TG; -26 %), total (TC; -27 %) and low-density (LDL; -26 %) cholesterol and increased level of high-density (HDL; +30 %) cholesterol in the blood plasma compared to the control group of patients. Based on the results and several years of experiences in farming and marketing we can recommend to include the meat of carp with increased content of n-3 fatty acids to the diet of Czech population.

Keywords: *carp, cardiovascular disease, linseed, n-3 fatty acids, rapeseed*

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EFFECT OF LOW-LEVEL OPTICAL IRRADIATION ON STURGEON SPERM MOTILITY

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Milt quality is a measure of the ability of sperm to successfully fertilise an egg which such ability mostly depends on qualitative parameters of milt i.e. composition of seminal fluid, milt volume, sperm density and sperm motility. Sperm motility is a key factor in allowing us to determine semen quality and fertilizing capacity. Improving quality of milt – this is an important direction in science of aquaculture.

Low-level optical irradiation (LLOI – irradiation of laser and light-emitting diode) is a light source treatment that generates light of a single wavelength. LLOI emits no heat, sound, or vibration. Instead of producing a thermal effect, LLOI may act via nonthermal or photochemical reactions in the cells, also referred to as photobiology or biostimulation. While the exact mechanism of its effect is unknown, it is theorized that LLOI can penetrate deeply into the tissues where it may have a photobiostimulation effect. For example, these types of LLOI have been advocated for use in a wide range of medical conditions encompassing: wound healing; smoking cessation; tuberculosis and many others. The use of LLOI has become popular in animal husbandry and veterinary medicine. Our previous studies have shown that LLOI is effective for influence on sturgeon embryos and on the blood system of female sturgeon.

In this paper, we show the results of the effect of LLOI on the quality of milt of Bester sturgeon (*Huso huso × Acipenser ruthenus*). Male fish were selected randomly from each group and all milt were stripped in a graduated dry and cool glass vials. Before stripping, anal region of fish were cleaned by dry towel for avoiding urea and faces contamination. All sperm manipulations were performed on ice. Statistical analyses were carried out using STATISTICA software. As the radiation sources, we used semiconductor laser and quasi-monochromatic light-emitting diode (LED) with a wavelength from 450 nm to 1280 nm, in continuous and modulation regimes, with radiation power density from P = 0,5 mW/cm^2 to P = 100 mW/cm^2. In all the experiments with lasers or LEDs, its radiation was defocused by a lens in such a way that the size of the light spot corresponded to the area of a monolayer of the irradiated sperm. Sperm irradiated for energy intake from 60 mJ/cm^2 to 180 mJ/cm^2 at a temperature of 16 °C ± 1 °C. Control (untreated) sperm specimens were also placed in the Petri dish and experienced the same conditions (except for irradiation) as the test specimens.

Our results showed that the LLOI has a high stimulating effect on sperm motility. The maximum effect of stimulation in the experimental group was 83.9 %. It is shown that sturgeon sperm are convenient objects for estimating the biological activity of the radiation. The dose dependence of the stimulating effect for each irradiation regime is characterized by a curve with a pronounced extremum, whose magnitude and energy position are determined by the frequency modulation. The collection of dose curves obtained using continuous and modulated radiation covers an energy range in which the photobiological effects for various operating regimes are virtually no different or lie within the limits of experimental error.

Keywords: *sperm motility, sturgeon, low-level optical irradiation*

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SUCCESSIVE IMPROVEMENT OF BROOK TROUT SPERM MOTILITY BY INCREASING OF CA^{2+} CONCENTRATION IN ACTIVATION MEDIA

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The semen quality in fishes is primarily examined by determining the sperm motility duration and percentage of motile sperm. It is well known, that for salmonids sperm quality decreases to the end of spawning season. For generating sperm motility, presence of external Ca^{2+} is described as essential in salmonids. We hypotized that increasing of Ca^{2+} in activation media could improve low motility parameters of sperm samples collected in the end of spawning season.

Sperm form brook trout were collected during start (19. 10. – 30. 10. 2011) middle (14. 11. – 4. 12. 2011) and end (18. 1. – 19. 1. 2012) of spawning season. Motility percentage; sperm velocity and motility duration were evaluate in solutions of different osmolalities. Activation media (AM: 125 mM NaCl, 0.1 mM $CaCl_2$, 20 mM TRIS-HCl), 150mM NaCl, and distilled water (DW) were used for motility parameters evaluations. All solution contained 10 mM TRIS-HCl, pH 8.5. Motility was recorded for 1 min post-activation using video microscopy techniques till motility cessation. Video records were analyzed to estimate spermatozoa curvilinear velocity (VCL), percentage of motile cells (motility) by microimage analyzer (Olympus Micro Image 4.0.1. for Windows). For sperm motility activation in the end of spawning season, 10mM Ca^{2+} was added to all activation solutions. Concentrations of Na^+ and K^+ were measured by potentiometry using ion selective electrodes (ISE) (Bayer HealthCare, NY). Calcium ion concentration was measured by absorption photometry using the o-cresolphthalein complexone method. Osmolality of seminal fluid (SF) and all studied media was monitored using a vapor pressure osmometer (Wescor, USA).

Results showed lower values of motility parameters at the end of the spawning period. Addition of 10 mM Ca^{2+} to the activation medium led to significantly increased motility variables ($P < 0.05$) from a spermatozoa motility rate of 5 ± 1.86 % and VCL of 25 ± 8.2 $\mu m.s^{-1}$ to a motility rate of 95 ± 5.6 % and VCL of 130 ± 14.8 $\mu m.s^{-1}$ at the end of spawning. No significant differences in Na^+ or Ca^{2+} concentration or seminal fluid osmolality in samples from the middle and end of the spawning season were detected ($P > 0.05$). Seminal fluid K^+ concentration ($P < 0.05$) was significantly higher in the middle (23.945 mM) from that found the end of the spawning period (10.94 mM). Possibility of using of the phenomenon of motility parameters increasing by adding of Ca^{2+} to activation media for fisheries practice requires future study.

Keywords: *sperm motility, Ca^{2+} ions, brook trout, spawning season*

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STUDIES OF 3-D STRUCTURE, VOLUME, AND DNA CONTENT IN ERYTHROCYTE NUCLEI OF POLYPLOID FISH

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Feulgen image analysis densitometry, image cytometry, and confocal laser scanning microscopy were used to study the relationships among ploidy level, DNA content (pg DNA nucleus^{-1}), and dimensional characteristics such as volume (μm^3), nuclear surface (μm^2), and 3-D structure of erythrocytes in a series of fish ploidy level models. Species models comprised diploid tench (*Tinca tinca*) (2n), Cuban gar (*Atractosteus tristoechus*) (2n), triploid tench (3n), evolutionary tetraploid sterlet (*Acipenser ruthenus*) (4n), evolutionary octaploid Siberian sturgeon (*A. baerii*) (8n), triploid Siberian sturgeon exhibiting dodecaploidy (12n), evolutionary 12n shortnose sturgeon (*A. brevirostrum*), and experimentally obtained sturgeon hybrids exhibiting tetraploidy, hexaploidy (6n), heptaploidy (7n), octaploidy, decaploidy (10n), dodecaploidy and/or tetradecaploidy (14n). Standards used for FIA were blood smears of diploid and induced triploid tench, *Tinca tinca* (2.04 and 3.1 pg DNA nucleus^{-1}, respectively). All ploidy levels were first verified by flow cytometry. Species of the same ploidy level, but differing in DNA content, exhibited a similar mean erythrocyte nuclear volume (V_{voxel}), as was demonstrated in sterlet and a hybrid of 4n sterlet and 4n Beluga (48.3 and 48.9 μm^3, respectively) with a respective mean DNA content of 3.74 and 3.1 pg DNA nucleus^{-1}. A similar relationship was found for the ploidy levels 6n, 10n, 12n. Volume (V_{voxel}) of erythrocyte nuclei, as sum of voxels calculated from live cells, appears to be more accurate than volume (V_{axis}) calculated from measuring the major and minor axis, especially at higher ploidy levels. With increasing ploidy level, the surface-to-volume ratio decreased non-linearly. The DNA in erythrocyte nuclei appeared to be more densely packed with increase in ploidy level. Expansion in ploidy level was accompanied by growth of the nucleus and an increase in the number of flattened ellipsoid nuclei with increased transverse diameter at higher ploidy levels.

Keywords: *sturgeon, erythrocyte, DNA content, ploidy, nuclear volume, DNA cytometry, 3D confocal analysis*

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MORE SECRETS ABOUT FISH SPERM FLAGELLA REVEALED BY HIGH-SPEED VIDEO-MICROSCOPY AND STROBOSCOPY

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Fish spermatozoa are propelled by a tiny flagellum which beats at high frequency: this feature as well as the high velocity of forward sperm movement makes recording of the moving sperm cells a challenge; this challenging goal becomes nowadays attained by use of stroboscopic illumination or by high speed video imaging combined with high resolution image captured at high performance level on modern optical microscopes.

We propose to gather and show our best results obtained in the recent past years when using such high-tech equipments. Presentation will be designed in a dynamic way, showing selected video sequences of spermatozoa collected from many fish species.

The end of the presentation will be devoted to briefly explain which additional descriptions are allowed by using such technology.

Keywords: *fish, spermatozoa, sperm movement, flagella, high-speed video, optical microscopy*

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ATP CONTENT IN STURGEON SPERMATOZOA DURING SPERM MATURATION

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Sperm maturation is a final physiological stage of spermiogenesis. Testicular efferent ducts of sturgeons are in close contact with kidneys. During the process of maturation testicular sperm is diluted with hypotonic urine during passing through kidney to mesonephric (Wolffian) duct. Maturation process exists in sturgeon. It occurs outside of testes after dilution of testicular sperm by seminal fluid. But, these processes are still unclear now. Study of sperm maturation opens new possibilities in conservation programs when we have to work with testicular sperm. Measuring of the ATP content during sperm maturation will probably give us new possibility to examine the involvement of spermatozoa breathing in sperm maturation.

Keywords: *fish, spermatozoa, testicular sperm, testes, ATP, maturation, sturgeon*

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DYNAMIC CELLULAR CHANGES DURING FISH SPERM ACTIVATION AS MEASURED BY FLOW CYTOMETRY

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Flow cytometry and time parameter analysis offer a unique tool to study the dynamics of subcellular changes over time. The aim of the present pilot study was to detect the changes of mitochondrial membrane potential after carp sperm activation. Milt from three carp males was collected via stripping, transferred to the laboratory and subsequently pooled. Pooled sperm was split into two aliquots. After an initial 100x dilution in PBS, from both aliquots 100–100 µl was suspended further in 450 µl PBS in a 5 ml polypropylene test tube and mitochondria were labelled with the potentiometric dye JC-1. This stain exhibits potential-dependent accumulation in mitochondria, indicated by an emission shift from green to red/orange (from monomers to aggregates). Mitochondrial depolarization is indicated by a decrease in the red/green fluorescence intensity ratio. After 10 min incubation at room temperature in the dark, sperm activation was induced with adding 450 µl distilled water to one aliquot (activated) immediately before the start of flow cytometric analysis. To the other aliquot 450 µl PBS was added in a similar manner (control – not activated). Flow cytometric measurements were done on a Beckman Coulter FC-500 flow cytometer. JC-1 was excited with a 488 nm Ar ion laser (20 mW) fluorescent signal of JC-1 monomers (green) was collected on detector FL1 (525 nm BP), while the signal of JC-1 aggregates (orange) on detector FL2 (575 nm BP). Acquisitions were continuously running for 5 minutes. List mode files were analyzed with the free "Flowing" software (version 2.5.0). Two dimensional plots were drawn to measure the changes of the orange:green ratio vs. time. The time axis was divided into 10, 30-sec intervals and the orange:green ratios were recorded for each interval. Somewhat surprisingly, the orange:green ratio showed a slight increase over time in both the activated and control samples, indicating that mitochondria retained their membrane potential and function even after activation during the studied time frame. Although further studies are needed to investigate the subcellular effects of sperm activation; we feel that time parameter analysis with flow cytometry provides a useful approach to such studies.

Keywords: *flow cytometry, time parameter analysis, fish sperm activation*

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THE IMPACT OF RECENT HUMAN MEDIATED STOCK TRANSFER ON GENETIC STRUCTURE OF EUROPEAN GRAYLING *THYMALLUS THYMALLUS* L. IN THE CZECH REPUBLIC

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Combination of mitochondrial DNA and microsatellite markers were firstly used in order to estimate impact of the human mediated stock transfer on population genetic structure of European grayling (*Thymallus thymallus*) in three main drainages in the Czech Republic. Supportive breeding activities have long tradition in Central Europe and have been rapidly increased during last 20 years with the primary aim of counteracting strong decline in natural populations. Unfortunately, these efforts were done without any respect to highly divergent population structure of European grayling among three main European drainages of Elbe, Oder and Danube. Moreover, contact zones for these three drainages exist in the Czech Republic which makes local populations very sensitive for human mediated gene flow. In total, 350 individuals from 17 populations including wild and hatchery stocks were sampled across Elbe, Oder and Danube drainages. The results confirmed a high level of admixture of the analyzed populations among studied drainages. Extensive introgression was observed from Elbe gene pool to Danube while reciprocal gene flow was not detected. Almost all analyzed populations contained haplotype TH1 originating from Elbe drainage. This haplotype was probably widely spread among hatchery stocks and subsequently distributed during reintroduction processes by fish managers. Correspondence analysis based on individual multilocus genotypes did not reveal any distinctive group. However, slight diversification was observed in the Danube cluster, which corresponded to the results of mtDNA analysis. Small genetic differentiation of analyzed populations was supported by low total fixation index Fst (0.088).

In conclusion, presented results supported the expectation that all populations of European grayling in Czech rivers were strongly affected by introgressive gene flow caused by fisheries management and unprecedented restocking. Losing of intraspecific diversity of this species could have negative effect on survival of its natural and stocked populations and might still contribute to permanent decreasing of European grayling populations in Czech rivers.

Keywords: *admixture, restocking, European grayling, introgression, conservation*

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A MARKER-ASSISTED BREEDING SYSTEM FOR BROWN TROUT

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A marker-assisted breeding system based on individual identification was developed for a brown trout broodstock in North-Eastern Hungary. Brown trout is cultured throughout Europe for restocking into natural waters managed by anglers therefore stocking of fish belonging to the local population is of primary importance. In this study, individuals of the only brown trout broodstock in Hungary were PIT tagged and fin clips were collected from each one. Fish were genotyped for three PCR-RFLP (mtDNA control region, lactate dehydrogenase and somatolactin genes) and five microsatellite (BFRO002, OMM1064, Ssa408uos, SsoSL417, SsoSL438) markers. Markers were selected for the ability to distinguish between Danubian (native to Hungary) and Atlantic (exotic) populations of brown trout. Genotyping revealed the prevalence of genes characteristic of the Atlantic lineage with 99.8 % on the mitochondrial markers and 57–81 % on the nuclear ones. This has indicated that the original founding population was probably of Atlantic origin to which locally captured individuals were added over the decades of its existence. Following genotyping individual values were assigned to each particular marker. The highest value (10 points) was assigned to those female individuals that carried the mitochondrial haplotype specific for the Danubian lineage of brown trout. In addition, each Danubian allele on any of the markers was given 1 point and each rare allele – an allele that occurs on the mitochondrial markers less than 5 times in the entire broodstock of around 400 individuals – was also given 1 point. The points were then summarized and uploaded into the PIT tag reader device. This allows the farmer to select individuals that have a value higher than the average and to apply selection pressure towards creating a population that has a higher share of the Danubian genotype. This system can be employed as a model for any fish farm that wants to carry out selective breeding if markers related to selection criteria are available.

Keywords: *brown trout, genotype, haplotype, selection, molecular markers*

Acknowledgements: The work was supported by the project GOP-111-09/1-2010-0141.

MARKER DEVELOPMENT AND POPULATION GENETIC STUDIES IN PERCH (*PERCA FLUVIATILIS*)

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In Europe, many research teams are developing intensive farming technologies for perch *(Perca fluviatilis)*. However, little is known about the genetic background of this species, their populations, farmed stocks or varieties, these technologies could further be improved with molecular biological tools.

Our aim is to isolate new, specific genetic markers – microsatellites and sex specific markers – from perch allowing the genetic analysis of the species and close relatives.

two microsatellite enriched libraries were generated with a slightly modified method of Travis C. Glenn and N. A. Schable (2005. Meth. Enzymol. 395:202-222).

98 clones were sequenced. 94.8 % of the sequences contained microsatellite type repeats. The redundancy was less than 3 %. Until now 40 new microsatellite markers were isolated for the perch.

12 microsatellite markers were chosen to analyse the population of the Lake Biatorbágy (Hungary) (41 individuals), the collection of Olstyn (Poland) (59 individuals) and Lake Dunaföldvár (Hungary) (43 individuals).

All populations showed relatively high allelic diversity (Mean number of alleles/locus = 8.3–9.1; number of alleles = 3–48) and expected heterozygozity (H_e =0.64, 0.56 and 0.69) and significantly lower observed heterozygozity (H_o = 0.53, 0.41 and 0.6). PIC value of the 12 screened microsatellite loci varied widely between 0.033 and 0.954. The F_{ST} values showed significant genetic difference among populations.

In order to identify sex specific markers comparative Fluorescent Motif Enhanced Polymorphism assays (FluoMEP) are being performed on sexed and pooled DNA samples of Olstyn and Biatorbágy populations. Until now 48 primer combinations were tested and 5 putative sex specific markers were identified. The confirmation of sex specificities are in progress on individual samples.

The information on the genetic structure obtained in this study will be used for setting up new broodstock in Hungary. In addition the newly isolated markers could be of great use in genetic management and stock improvement through selective breeding, as well as for a wide range of other genetic studies that disclose domestication effects or evolutionary relationships.

Keywords: *microsatellite markers, genetic diversity, population structure, perch, Perca fluviatilis*

Acknowledgements: This work was supported by OTKA (K105365) and GOP 1.3.1-11/A-2012-0187.

THE PERIOD OF PIKEPERCH, *SANDER LUCIOPERCA* (L.), EGGS ABILITY TO FERTILIZATION AND ITS DEPENDENCE ON EGG QUALITY

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Pikeperch, *Sander lucioperca* (L.), is one of the very important candidates for intensive freshwater aquaculture diversification. To date, there is lack of standardized methods of *in vitro* fertilization protocol which is dependent, among others, on the time of contact of gametes with each other. The period of time during which the gametes retain the fertilization capability is of high importance. The aim of this study was to evaluate the period of pikeperch eggs ability to fertilization following exposure to hatchery water in relation to its quality. The eggs obtained from 10 hormonally stimulated (hCG, 500 IU kg^{-1}) wild pikeperch females were used in this study. After the ovulation occurred, eggs were stripped into a dry plastic container and immediately afterwards covered. For the fertilization trial, about 200 eggs were placed on the Petri dish (50 mm diameter) with 10 mL of hatchery water (14 °C) and next the pooled (n = 3) sperm sample (50 µL) was added at 0 (control), 15, 30, 60, 90, 120, 150 and 180 s post eggs activation. Eggs were incubated on a Petri dishes in a recirculating aquaculture system at 14 °C. Embryos survival was determined after 24 h of incubation. The results of the embryonic survival were divided into two groups where over 50 % (group 1) and below 50 % (group 2) of survival was recorded. Among the groups the regression analysis was performed and differences between the groups were investigated. In group 1 highest (67.9 %; $P < 0.05$) survival rate as compared to group 2 (36.9 %) was recorded. In both groups significant decrease ($r = -0.99$ and $r = -0.96$ for group 1 and 2 respectively; $P < 0.05$) in survival rate of embryos was recorded within the entire period considered. However, in group 2 after 90 s less than 10% of viable embryos were noted, whereas in group 1 even after 180 s over 30 % could be recorded. There were significant differences at each time-point between the groups. The results of the present study for the first time clearly shows, that the eggs of 'good quality' (with over 50 % survival rate) remain capable of fertilization for longer period than the eggs of 'lower quality' (with less than 50 % fertilization rate). The correlation among the eggs of 'good quality' ($r = -0.99$; $p < 0.001$) clearly showed that eggs of the pikeperch should be inseminated immediately after activation and every delay in sperm addition may negatively affect the fertilization result.

Keywords: *gamete management, in vitro fertilization, reproduction, egg biology*

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REACTIVATION OF DNA IN STURGEON SPERMATOZOA DURING GYNOGENESIS INDUCTION

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Chromosomal manipulations in sturgeons, particularly gynogenesis could be used as the way to change female ratio in progeny hence could be useful for caviar production. Analysis of published data on the UV irradiation protocol for gynogenesis induction in sturgeon faced with great diversity of irradiation parameters. The most common way to inactivate spermatozoa's DNA in order to induce gynogenesis is to irradiate them by UV light; however UV irradiation treatment showed overwhelming impact on motility of sturgeon sperm as well as the optimization of UV treatment is complicated due to high optical density of sperm and reactivation of DNA under light. The DNA reactivation in UV-irradiated sperm measured by comet assay showed significant level of DNA restoration in UV-irradiated sperm under artificial light condition. The results of induction of gynogenesis with sperm subjected to the light of an incandescent bulb showed significant difference in percentage of putative gynogenotes caused by reactivation. Additionally we investigate effects of infra red light on UV irradiated sperm. The results indicate that the infra red light do not induce light-depended reactivation of DNA. Therefore we propose to use infra red light for lightening of working places during gynogenesis induction.

SEASONAL CHANGES IN THE ANDROGEN RECEPTOR GENE EXPRESSION IN TISSUES OF THE SPINED LOACH, *COBITIS TAENIA* (PISCES, COBITIDAE)

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Androgens influence in a multidirectional way on the male fish organism in which these hormones regulate processes of gonadal differentiation and spermatogenesis as well as initiate the sexual behavior. However, these steroid hormones are also produced in females, in which in specific physiological stages plasma testosterone concentration reaches even higher values than in males. Furthermore, it has been shown that androgens regulate the process of oogenesis and vitellogenesis and, taking part in pheromones synthesis, trigger the specific spawning behavior in female fish. Androgens delivers a broad range of physiological effects by binding to the intracellular androgen receptor (AR) which acts as a hormone-inducible transcription factor modulating the expression of target genes. The androgenic response is thus limited by the availability of ARs which can be determined by numerous factors including the level of its cognate ligand. Seasonal changes in circulating androgens in many fish species have been observed. The aim of this study was to test whether the expression of AR gene occurs in the tissues of *Cobitis taenia* males (n = 8) and females (n = 8), and if so whether the AR expression is subjected to seasonal changes. All the loaches under study have been karyologically verified as *C. taenia* species by possessing 2n = 48 chromosomes. Tissues (liver, muscle and gonads) were collected from the fish during the spawning season and after it. The level of AR gene expression was analyzed using semi-quantitative RT-PCR method. The partial sequence of the AR gene in *C. taenia* has been described among the results of the presented study. The current experiment for the first time provides an evidence on the presence of AR transcript in the liver, muscle, testis and ovary of *C. taenia* during the spawning season and after. Furthermore, the seasonal changes in the level of AR gene expression in the examined tissues of *C. taenia* males and females has been documented. The level of AR gene expression was influenced by the season as well as the type of tissue and sex of the fish. Seasonal changes in the AR gene expression in different tissues may suggest an important function of AR as a factor modulating the effect of androgens on the spined loach organism.

Keywords: *androgen receptor, Cobitis taenia, gene expression, tissue expression, spawning season*

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ISOLATION AND CRYOPRESERVATION OF SPERMATOGONIA OF TENCH (*TINCA TINCA*)

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Spermatogonia (Spg) are testicular germ cells with potential to transmit genetic information to the next generation. That is why these cells have also potential to be of value for gene banking and cryopreservation and can generate the germ-line chimeras by the production of donor gametes. To obtain germ-line chimeras, germ cells as Spg are isolated from the donor and transplanted into the host. The host becomes germ-line chimera if the transplanted germ cells succeed to become correctly localized to the genital ridge. Therefore this new technique will have high application in biotechnology because it can lead e.g. to the production of gametes of species with later sexual maturation using species with earlier sexual maturation as surrogate hosts. In future study we will use in the role of host our economically important cyprinid fish, the tench (*Tinca tinca*), and transplant its Spg to a close related fish species, white cloud mountain minnow (*Tanichthys albonubes*) (in the role of host), because of small body size, easy reproduction and shorter reproduction cycles than tench. Therefore in this study we established the system of isolation and cryopreservation of tench Spg by testing different conditions for later transplantation of these cells into host to generate germ-line chimeras.

In first part of this project we established isolation and dissociation of tench Spg. We isolated Spg from immature testes of 2 years old four tench males, cut these testes into small pieces and incubated in mediums with enzymes. For isolation and dissociation of tech Spg we used phosphate buffer saline with 0.1 % enzymes of collagenase and trypsin. We filtered this homogeneous suspensions through 50 µm filters, sorted the cells by using percoll gradient (5, 10, 20, 30 %) and stained with specific antigen DDX4 combined with secondary antibody Anti-Rabbit IgG–FITC to identify germ cells.

In the second part we focused on cryopreservation of tench Spg as a source for gene banking. For cooling we used an automatic cooler Kryo 10 serie III (Planner), cooled the Spg with speed 1 °C / min until the temperature reached -80 °C and stored our samples in liquid nitrogen. We tested 6 cryoprotectants (methanol, glycerol, dimethyl sulfoxide, dimethyl sulfoxide + 1,2 propandiol, ethylene glycol and N,N dimethyl acetamid) to find out the best one by comparison the highest viability of Spg before and after cryopreservation by counting the percentage of live/dead cells (Cell stain double staining kit, Fluka).

Future aim of this study would be to focus on transplantation of tench Spg into close related white cloud mountain minnow to generate germ-line chimera.

Keywords: *tench, spermatogonia, isolation, cryopreservation, germ-line chimera*

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MOLECULAR AND CYTOGENETIC ANALYSIS OF THE ANDROGENETIC BROOK CHARR *SALVELINUS FONTINALIS* (MITCHILL, 1814)

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Molecular and cytogenetic examination of the androgenetic progenies is usually applied to confirm only paternal chromosome inheritance and efficiency of the radiation-induced maternal nuclear genome inactivation and diploidization of the haploid set of chromosomes in the androgenetic zygotes. Here, androgenetic brook charr embryos, larvae and fry were studied using microsatellite DNA analysis and mitotic chromosome examination. Androgenetic development of the brook charr was induced in the eggs subjected to 420Gy and 450Gy of X-rays. Diploidization of the haploid genome in the androgenetic zygotes was performed by application of the high-pressure shock (51.711MPa for 4 minutes) 420 minutes after fertilization. For the molecular analysis microsatellite DNA loci *Sfo*-12 and *Sfo*-292 were chosen. Two brook charr males (B1 and B2.1) used in the androgenetic experiments were heterozygous and showed two allelic forms at locus *Sfo-1* (200bp and 260bp). Brook charr male B2.2 exhibited two alleles at locus *Sfo*-292 (190bp and 220bp). As expected, all studied androgenetic individuals were homozygous. In all examined diploid and haploid androgenotes number of the chromosomes equalled 84 and 42, respectively and these values are characteristic for the brook charr species. Apart from the paternally inherited intact chromosomes, small chromosome fragments were identified in about 57 % and 36 % of the cytogenetically examined androgenetic brook charr developing in the eggs irradiated with 420Gy and 450Gy, respectively. Results presented here confirmed only paternal inheritance of the nuclear genome among the brook charr androgenetic offspring. However, as far as conditions of the high-pressure shock applied to double the haploid set of chromosomes in the brook charr androgenetic zygotes are appropriate, the protocol for inactivation of the maternal brook charr chromosomes must be improved. Radiation-induced maternal chromosome fragments observed in the androgenetic brook charr may interfere with the ontogenetic development of the androgenetic fish and increase their mortality.

Keywords: *androgenesis, brook charr, chromosomes, microsatellite DNA, Salmonidae*

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SPERMATOZOON ULTRASTRUCTURE OF SPINY-CHEEK CRAYFISH *ORCONECTES LIMOSUS* (RAFINESQUE, 1817)

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The spiny-cheek crayfish *Orconectes limosus* is the oldest invasive species in Europe. The basic reproductive information such as spermatozoon morphology might be useful for future plans against non-indigenous species. The acrosome is a cup shaped and electron-dense structure at the anterior of the spermatozoon and comprises three layers of differing electron densities filled with parallel filaments that extend from the base to the apical zone. Three to four short protrusions are present at the anterior of the acrosome body. Radial arms are visible on each side of the acrosome or nucleus in sagittal view and wrap around the spermatozoon. Each radial arm comprises a parallel bundle of microtubules arranged along the long axis within a sheath. The nucleus, with decondensed material, is located in the posterior of the cell. All parts of the spermatozoon are tightly enclosed within an extracellular capsule. The similarities and differences of spermatozoon morphology of spiny-cheek crayfish with other crayfish are discussed.

Keywords: *acrosome, morphology, spiny cheek crayfish, spermatozoon*

CONTROL MECHANISMS OF FISH SPERM ACTIVATION: BREAKING SOME RULES

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From immotile in the seminal fluid, fish spermatozoa immediately (< 20 msec) trigger their motility according to two modes of activation: due to ion concentrations (K^+, Na^+, and Ca^{2+}) and osmolality (general case) of the spawning environment. While exploring effects of organic solvents (DMSO as example) on sperm motility activation, we observed two unexpected effects which lead to partly break the ubiquitous rules above-mentioned. During the study, semen of *Acipenser ruthenus* was diluted in hypertonic solution (5–10 % DMSO or 2.5 % glycerol and 10 mM TRIS-HCl, pH 8.5) for 5 s; after this pre-incubation, sperm suspension was transferred into 50 µl of 15 mM KCl on a glass slide. As controls, semen was also diluted in either 15 mM KCl or hypertonic solution mentioned above. Motility activation of sterlet sperm occurred immediately after contact with 10 mM TRIS-HCl as swimming solution. In contrast, spermatozoa were completely immotile in the 2.5 % glycerol solution; the same was observed in the presence of 15·mM extracellular K^+. When sperm was dispersed into a swimming medium containing 5 or 10 % of DMSO, we observed a significant 6 to 9 seconds delay of the motility activation between time of mixing spermatozoa and initiation of motility. A similar delay due to high DMSO (or ethylene glycol) concentration was observed on sperm of most freshwater fishes (trout, carp as examples) tested whereas this effect was not confirmed in the case of euryhaline and marine fish sperm. CASA analysis show that motility percentage and velocity of motile cells in 10 mM TRIS-HCl reached maximal value immediately after mixing, while in presence of 10 % DMSO maximal values were reached 30 sec. later. Other compounds such as glycerol lead to similar effect: glycerol-treated sperm initiated motility in 10 mM TRIS-HCl with a delay of 7 s and motility period lasted twice longer than for intact sperm (about 4 min). Independently, we observed a complementary effect due to sperm exposure to high osmolality: when sterlet sperm was pre-incubated in solution containing 2.5–5 % of glycerol or 5–10 % of DMSO for 5 s, then immediately transferred into K^+-rich swimming solutions (10 to 50 mM K^+ with 10 mM TRIS-HCl), spermatozoa were fully activated. Motility parameters after pre-incubation in glycerol or DMSO followed by activation in 15 mM KCl were similar to control. Our results show that exposure of sterlet spermatozoa to an osmotic shock leads to two kinds of effects: 1- a delay (8 to 15 sec) in the appearance of first waves propagated along flagella, leading to full motility activation; 2- the ability for sturgeon sperm, after transfer in swimming solution, to swim in presence of high K^+ ions concentration previously demonstrated as fully repressive for motility. Experimentally, this delay offers a very useful tool for observation of flagellum activation in real time, thus allowing obtainment of detailed record by high speed video of the first flagellar waves at activation of individual spermatozoa, a situation which cannot be reached in regular swimming solutions.

Keywords: *sperm motility initiation, organic solvents, osmotic shock, potassium (K^+), sterlet*

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DETERMINATION OF THE BEST POST-OVULATORY STRIPPING TIME FOR COMMON CARP, *CYPRINUS CARPIO*

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To determine the best time interval between ovulation and artificial egg stripping in female common carp, *Cyprinus carpio*, unfertilized eggs were retained inside the fish body for 12–4 Hours Post Ovulation (HPO) at 20.5 °C. Eggs of 4 female carp were stripped with 2 hour intervals after ovulation for 12–14 HPO and fertilized with mixed milt obtained from 3 males. Eyeing, hatching and eyed-egg mortality rates as well as the occurrence of the malformations and ploidy anomalies were considered as indexes for egg quality. The results indicated that post-ovulatory retention time in the ovarian cavity has significant effect on egg viability rates and their development into normal embryos. The eyeing and hatching rates remained around 90 % and 80 % of the initial rates, respectively, throughout 4–6 HPO. Although not significantly different, viability rates increased to about 7.5 % of the initial rates at 2–4 HPO compared to 0–2 HPO. Thereafter, the eyeing and hatching rates decreased over time linearly and finally dropped to 11 % and 2.5 % at 12–14 HPO, respectively. Eyed-egg mortality and malformation rates also did not show any marked increase during 4 hours of egg retention, but then significantly increased, so that more than 50 % of eyed eggs died and around 20 % of hatched larvae were malformed at 8–10 HPO. Post-ovulatory oocyte ageing did not affect the incidence of triploid larvae. Based on the results obtained in this study, common carp egg quality is retained inside the fish body for at least 4 HPO. The best post-ovulatory stripping time was estimated to lie within 2–4 HPO and therefore we recommend that the eggs be retained inside the fish body for this period after ovulation. Complete loss of egg viability also occurs from 12–14 hours after ovulation.

Keywords: *Cyprinus carpio, egg viability, Fertilization, ovulation, retention time*

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ANTIOXIDANT RESPONSES OF SPERM IN DIFFERENT FISH SPECIES

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Spermatozoa are protected from oxidative damage by various antioxidants and antioxidant enzymes, which are present in the seminal plasma or in spermatozoa itself. Low levels of antioxidants cause oxidative stress and may damage or kill sperm cells. In fish sperm, an antioxidant system consists in enzymatic and non-enzymatic components which have important consequence in maintaining the semen viability. The aim of this study was to compare antioxidant status of the seminal plasma with that of spermatozoa in Russian sturgeon *(Acipenser gueldenstaedtii)*, Siberian sturgeon (*Acipenser baerii),* sterlet (*Acipenser ruthenus),* common carp (*Cyprinus carpio*), and brook trout (*Salvelinus fontinalis*) and to assess the capacity of fish seminal plasma to protect spermatozoa from oxidative stress. Significant differences in total superoxide dismutase (SOD) were detected between spermatozoa and seminal plasma in all studied species. The maximum spermatozoa SOD activity was found in Siberian sturgeon (3.6 ± 0.6 mU.mg.$^{-1}$ protein), whereas the minimum was detected in sterlet (1.08 ± 0.3 mU.mg.$^{-1}$protein). The mean value of SOD activity in seminal plasma was 1.06–1.7 mU.mg.$^{-1}$ protein. Moreover, there was no significant difference in SOD among species. Glutathione reductase (GR) activity showed a trend similar to that of SOD, and statistical differences in activity of GR between spermatozoa and seminal plasma in all studied species were obtained. The highest value of GR activity was present in the spermatozoa of brook trout (47 ± 7.79 mU.mg.$^{-1}$ proteins) and carp (40 ± 6.33 mU.mg.$^{-1}$ proteins). No significant differences in GR level of seminal plasma were detected among fish species. High variability in glutathione peroxidase (GPx) activity in seminal plasma and spermatozoa was observed. Analysis revealed no significant differences in GPx level between seminal plasma and spermatozoa in carp, sterlet, or Siberian sturgeon. The lowest value of GPx activity (6.81 ± 1.56 mU.mg.$^{-1}$ proteins) was found in brook trout seminal plasma and the highest (12.56 ± 3.23 mU.mg.$^{-1}$ proteins) in brook trout spermatozoa. Our investigation indicated significantly lower seminal plasma SOD and GR activity in all studied species, as compared to the activity found in spermatozoa. It can be concluded that reduced SOD and GR activity in fish seminal plasma might result in reduced protection against ROS. On the other hand, little differences between the level of GPx activity in spermatozoa and that in seminal plasma were demonstrated in the present study. This is likely to be an adaptive response to toxicant stress and serves to neutralize the impact of increased ROS generation.

Keywords: *seminal plasma, spermatozoa, antioxidant activity*

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DEVELOPMENT OF WATER PARAMETERS IN LOWER-AUSTRIAN FISH PONDS OVER THE LAST 30 YEARS.

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Fishponds play an important role in the landscape and culture of north-western Lower Austria. Ponds are closely connected to their environment, therefore, existence and cultivation of ponds are dependent on environmental changes. Our objective was to assess possible effects of environmental changes on ponds. We analyzed data of temperature, pH, oxygen, ammonium and total phosphorous of pond water over a time span of 30 years (1983–2012). Data-sets of 25 representative ponds were used for our analysis (n = 1824). Our results show an increase of temperature over the investigated time frame, especially from April to June (+2.68 °C, +2.59 °C and +2.53 °C, respectively). The pH value follows a clear trend, decreasing from a mean of pH 8.2 in 1983 to pH 7.0 in 2012. Particularly in the last ten years, measured values > pH 8.5 became rare. Ammonium and total phosphorous also decreased. Mean concentrations currently are about 0.05–0.2 $mg.l^{-1}$. Overall, the results of this study show that ponds have to deal with changing water temperature and chemistry. Causes and effects of this development on pond aquaculture are discussed.

Keywords: *Lower-Austria, fish pond, carp, water quality, climate change*

BISPHENOL A EFFECTS ON PROTEIN PROFILE AND DNA INTEGRITY OF SPERMATOZOA OF BROOK TROUT (*SALVELINUS FONTINALIS*)

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Bisphenol A (BPA), a xenobiotical compound used to harden polycarbonate plastics and epoxy resin, is the focus of a growing number of research studies and legislative actions. BPA is a one of endocrine disrupting chemicals (EDC) that can act as a xenoestrogen modulating the endocrine pathways via a receptor-mediated process. Concentrations of BPA in surface waters were shown to vary significantly from 0.5 ng.l^{-1} up to 21 µg.l^{-1} and exposure of fish gametes to the pollutant could be detrimental for the fertilization success. In the present study we investigated the effect of short-term in vitro exposure (2 h) to BPA on brook trout (*Salvelinus fontinalis*) sperm with respect to spermatozoa motility parameters, DNA integrity and protein composition.

The brook trout sperm was used to investigate the potential deleterious effects of BPA on spermatozoa physiology, DNA integrity, oxidative stress indices and protein profile. Sperm samples were diluted to obtain spermatozoa density of 5 × 10^{7} cells × ml^{-1}. The sperm samples (n = 6) were then exposed for 2 h to following concentrations of BPA (1.75, 2.5, 5 and 7.5 µg.l^{-1}). The percentage of motile spermatozoa and spermatozoa velocity were examined by dark-field microscopy at 20x objective magnification (Olympus BX 50, USA). DNA damage was evaluated using the Comet assay procedure. TBARS assay was used for screening of lipid peroxidation (LPO) and CP (Carbonyl Proteins) assay was used to determine the level of carbonyl proteins in spermatozoa. SDS-PAGE and two-dimensional (2D) gel electrophoresis were used to determine changes in protein profile induced by BPA.

Percentage of motile spermatozoa, spermatozoa velocity as well as duration of sperm cells movement were decreased after 2 h exposure to solutions containing BPA in concentrations 5–7.5 µg.l^{-1}. Presence of xenobiotic in immobilization solution also led to a higher level of DNA fragmentation and increased level of lipid peroxidation and carbonyl proteins compare to control. The highest concentration of BPA also led to changes in protein profiles of trout spermatozoa. In response to the presence of xenobiotic 11 protein spots changed their position or concentration. The overall results of in vitro experiment demonstrated that oxidative stress induce by BPA treatment is the major factor responsible for dramatic increase of DNA fragmentation and changes in protein profile observed in the present study.

Keywords: *sperm, DNA integrity, protein profile, spermatozoa motility, oxidative stress*

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MANAGING WATER QUALITY OF THE HS DTD AS A PREREQUISITE FOR SUPPORTING FISH FUND

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The Hydrosystem Danube – Tisa – Danube (HS DTD) is an important waterway in the region since it consists of huge canals of 960 km in length of which 600 km are navigable enlisting it to the biggest waterways in the world. Its primary role is to drain sufficient water, and provide water for irrigation when necessary. Beside its hydro technical function is a wildlife habitat for various aquatic organisms and significant fish fund comprising of 30 fish species from 8 families. The canal network receives waste waters from point sources like food processing industries and municipal waste waters and diffuse sources of pollution such as agricultural runoff. Freshwater fishes are susceptible to various water quality parameters if their tolerable limits are exceeded, and especially on ammonia. The paper focuses upon changes in ammonium and ammonia concentrations in canal water along 14.58 km reach of the Becej-Bogojevo canal, because high water pollution within the reach occasionally led to euthrophic processes and fish kills. Since insufficiently purified waste waters originating from a pig farm, four food processing industries and a municipal collector are main sources of pollution, nitrogen and phosphorus compounds were considered to be the main pollutants. Complex methodology was applied comprising of application of Geographic Information System, hydraulics modeling using HEC-RAS, water quality modeling using QUAL2K. First of all, model calibration and validation were performed using different data sets obtained by hydrometric and water quality measurement. Secondly, all data were inserted into GIS attribute tables. It enabled presentation of each water quality parameter on map by coloring the canal reach differently corresponding to its concentration/value in accordance with Water Framework Directive coloring scheme. Further more, simulations were performed for different scenarios using historical data of canal flow rates and concentrations of polluting substances. The results of simulations have shown that excluding waste water from two biggest polluters (the pig farm and a meat processing industry) lead to significant improvement of canal water and good status for ammonium and ammonia in line with the requirements of Fish Directive. Simulation outputs were presented in form of colored canal reach on GIS maps and maximum permissive loads for nitrogen and phosphorus compound were determined. The methodology applied to the canal reach of the Bečej-Bogojevo canal could be used within the whole network of the HS DTD for management and planning for achieving good water quality. Beside nitrogen compounds, other water quality parameters of importance for supporting fish fund could be analyzed in a similar manner.

Keywords: *water quality, fish fund, water pollution, integral methodology, QUAL2K*

TISSUE AND SEX SPECIFIC MRNA EXPRESSION OF ARYL HYDROCARBON RECEPTOR TYPE 2 IN SPINED LOACH, *COBITIS TAENIA* (PISCES, COBITIDAE)

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The aryl hydrocarbon receptor type 2 (AhR2) is an intracellular transcription factor best known for mediating the toxicity of dioxins in fish. Bony fishes are one of the most sensitive vertebrates to dioxin and exhibit a wide range of developmental defects. Spined loach, *Cobitis taenia* is a freshwater protected species of deceasing range of distribution due to the deterioration of its environment and hybridization with related species. The examination of functional roles of AhR2 in *C. taenia* has never been done. The aims of the study were: 1/ to determine a partial nucleotide sequence of *C. taenia* AhR2 (CtAhR2) and 2/ to investigate the mRNA expression level of the AhR2 in the liver, gills, muscles, gonads and heart of *C. taenia* females (4 individuals) and males (3 individuals). Tissues were dissected from fish, immediately frozen in liquid nitrogen and stored at -80 °C. Total RNA was extracted and reverse transcribed. To determine the partial nucleotide sequence of CtAhR2 polymerase chain reaction (PCR) was used with primers designed on the basis of highly conserved regions of zebrafish (*Danio rerio*; AF063446.1) and goldfish (*Carassius auratus*; FJ554572.1) AhR2 nucleotide sequences. To determine the mRNA expression of the CtAhR2 quantitative real-time PCR was used with SYBR green dye intercalation in 7500 Fast Real-Time PCR System. In the study, the partial nucleotide sequence of CtAhR2 containing 387 bp was determined. The sequence exhibited 84 % homology with the zebrafish and goldfish AhR2 sequences. The CtAhR2 transcript was found in all examined tissues of both females and males. In the females higher CtAhR2 transcript level was detected in gills and heart in comparison with liver, muscles and ovary. In the males higher CtAhR2 mRNA expression was found in gills, heart and testis in comparison with muscles. Interestingly, CtAhR2 mRNA content in gills and muscles in the female was 3-fold higher than in males. The presence of CtAhR2 transcript in the examined tissues may account for their sensitivity to some environmental pollutants. Moreover, CtAhR2 expression profiles were tissue and sex specific, indicating distinct roles of this gene in particular organs of female and male *C. taenia*.

Keywords: *aryl hydrocarbon receptor, Cobitis taenia, molecular biology, gene expression, tissue expression*

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GENETIC MONITORING AND BIOINDICATION OF WATER RESOURCES ON BASIS OF SNAIL *LYMNAEA STAGNALIS*

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Weakness of the environmental monitoring in general, and monitoring of water resources in particular, applied today is a "one-sided" approaches used to carry out it (abiotic factors are mainly studied and very little attention is paid to biological monitoring and bioindication). Undoubtedly, a simple extension of the list of accountable abiotic parameters improves to some extent reliability of the environment quality estimate too. But for adequate environmental regulation it is necessary not only to choose the most representative abiotic characteristics of the ecosystem, but also to take into account the biota reaction to changes in the environment. Monitoring based on the chemical analysis of water and sediment is not sufficient to assess the biological damage and predict the environmental effects.

We propose the algorithm for ecologo-genetic monitoring, which includes the following steps: the first step is genetic evaluation of populations of indicator species which inhabit the studied biotops; the second step is implementation of bioindication procedure.

As an indicator species in our study, we have chosen the snail *L. stagnalis*. Thus, at the first stage of the study we conducted a genetic assess of F_1 individuals of populations inhabiting biotops with different environmental load: inlet of the Pripyat river (37–49 kBq/m^2) and Perstok lake (678–1975 kBq/m^2) (Gomel region, Belarus). Intrapopulation and interpopulation polymorphism, the presence of unique amplicons for populations were evaluated on the basis of the PCR profiles for 154 RAPD-loci. Differences in the population structure were assessed by conversion of the PCR profiles to the gene frequency. Population genetic analysis was performed on the basis of molecular genetic data using computer approach (programs MEGA, POPGENE, Phylip): intrapopulation gene diversity H_s and the total gene diversity H_t of populations, gene differentiation G_{st}, effective allele number n_e, gene flow N_m, Shannon's index *SI*, linkage disequilibrium *LD*, genetic identity and genetic distance were calculated; Ewens-Watterson test of neutrality was carried out. It was shown that *L. stagnalis* population samples "Perstok" and "Pripyat" are characterized by high genetic similarity.

The second step of the study included bioindicator test using the comet assay. It was revealed that hemocytes of F_1 snails from ecologically unfavorable Perstok lake population contained double DNA amount. That is, polyploidization was observed in mollusks from "Perstok" as adaptation to radiation exposure. In turn, heavy metal Sr inhibited the proliferative processes: DNA amount in the hemocytes decreased in both population groups under its influence.

Since we have shown high genetic similarity of "Perstok" and "Pripyat" snail samples in terms of the genetic analysis of population structure, this fact suggests that the observed differences in the hemocyte DNA content may be due to differences in environmental conditions and are not associated with genetic variation. Thus the hemocyte DNA content of the snails is an effective bioindicator parameter of aggressive environmental effects.

The proposed approach (genetic monitoring in combination with bioindication) enables quick identification, objective and comprehensive assessment of environmental changes for adverse effects on the ecosystem, biodiversity, and implementation of adequate measures for effective environmental management. *L. stagnalis* can be used as an indicator species in the territory of Belarus and as the object of genetic monitoring in the network of environmental monitoring of water systems.

Keywords: *genetic monitoring, bioindication, molecular markers, DNA-comets, L. stagnalis*

EFFECT OF SUPPLYING WATER QUALITY ON CARP FINGERLING SURVIVAL IN PONDS

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Water quality in the Vistula has improved during the last decades significantly. This is mainly due to the effect of investments in wastewater treatment, forced by EU and local law regulations. For example, annual average total ammonia nitrogen (TAN) concentration in upper stream of Vistula, decreased from 0.43 $mgN.L^{-1}$ in 1994 to ~ 0,08 $mgN.L^{-1}$ in 2009. Similar trend pertain to other forms of nitrogen and phosphorus.

Upper stream of Vistula river is a main source of water for more than 2000 ha of carp ponds. As the result of river water quality improvement between 1980 and 2010, nutrient loads delivered to the ponds at the time of spring filling has been reduced by 84 %, 58 % and 67 % in case of TAN, total nitrogen and phosphates, respectively. These compounds are main source of N and P for primary production in carp fingerling ponds at early spring season and hence development of zooplankton and benthos. Limited supply of natural food for carp, before the food supplementation and fertilisation begins, may lead to malnutrition and further developmental disorders, including increased mortality.

The most commonly applied pond fertilisation regime (*S*) has been compared with two alternative: early mineral (*M*), and early manure fertilisation (*O*). In both experimental treatments alternative fertilisation has been initiated about two weeks prior pond filling. The field experiment has been conducted in 12 earthen ponds (1500 m^2 each), four replications per treatment. Ponds were filled with water in March and stocked with 20 g one-year-old common carp fingerling, 600 individuals per pond. Ponds were harvested in July to determine survival rate, individual body weight and biomass gain.

Survival rate of the fish stock in ponds where mineral fertilisation started before stocking (*M*) (72.3 %) was significantly higher than in standard (*S*) and manure (*O*) treatments, 44.8 % and 39.9 %, respectively. Significantly lower individual body weight was observed in treatment *M* (193.0 g), compared to treatment *S* (263.5 g)and treatment *O* (273.4 g). However the biomass gain of treatment *M* (499.6 $kg.ha^{-1}$) was higher than in remaining treatments (*O* – 392.3 $kg.ha^{-1}$ and *S* – 420,3 $kg.ha^{-1}$).

Observed differences in results of survival rate, individual weigh and biomass gain as well as water quality parameters suggest that enhanced availability of biogenic compounds in carp fingerling ponds during early spring period allows to limit stock mortality and hence to increase the fish biomass production.

Keywords: *common carp, fingerling ponds, fertilisation, malnutrition, natural water quality*

EFFECT OF ZETA-CYPERMETHRIN ON OXIDATIVE STRESS BIOMARKERS OF RAINBOW TROUT (*ONCORHYNCHUS MYKISS*)

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The aim of this study was to assess the effect of pesticide product Fury 10 EW with containing zeta-cypermethrin (active substance 100 $\mu g.L^{-1}$) on rainbow trout (*Oncorhynchus mykiss*).

Rainbow trout were exposed to the acute concentration (96hLC50) 13 $ug.L^{-1}$ for 96 h. The following biomarkers were evaluated: levels of thiobarbituric acid reactive substances (TBARS) and activity of the antioxidant enzymes – superoxide dismutase (SOD), catalase (CAT) and glutathione reductase (GR). These parameters were measured in liver, muscle and gills.

Results indicate that the enzymatic activity were changes in all tested tissues with compare the control group. The SOD activity was higher in liver, muscle and gills ($p < 0.05$), the CAT was observed lower activity only in liver, and in activity GR were significantly differences ($p < 0.05$) in muscle and liver. The levels TBARS were observed significantly higher ($p < 0.1$) only in muscle with respect to the control group.

In conclusion, zeta-cypermethryn induces oxidative stress and antioxidants changes in selected tissues on rainbow trout, the liver is the organ most affected by this damage.

Keywords: *pyrethroids, oxidative stress, antioxidants enzymes, fish*

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SUB-LETHAL EFFECTS OF THE HUMAN PHARMACEUTICAL DILTIAZEM ON RAINBOW TROUT (*ONCORHYNCHUS MYKISS*)

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Diltiazem is a human pharmaceutical belonging to the group of calcium channel blockers. It's used in the treatment of angina pectoris and hypertension or for prevention of migraine and chronic anal fissure. It has been detected in concentrations of ng.L^{-1} in waste and surface waters. The present study aimed to assess sub-lethal effects of diltiazem on rainbow trout (*Oncorhynchus mykiss*). Juvenile trout were exposed for 21 and 42 days to three concentration levels of diltiazem (0.03 µg.L^{-1} – environmentally relevant concentration, 3 µg.L^{-1} and 30 µg.L^{-1}). The effect on morphology, haematology, histology (highest tested concentration), the enzymatic activities of selected isoforms of CYP450 family and the *reactive oxygen species* (ROS*)* defence system were studied. At all tested concentrations, the haemoglobin content of the blood was significantly reduced. In the liver and gills, diltiazem was found to significantly affect activity of the antioxidant enzyme superoxide dismutase (SOD) in fish exposed to 0.03 µg.$L^{-1}$and 3 µg.L^{-1}. Moreover, in both tissues, the activity of glutathione peroxidase (GPOX) was affected in fish exposed to 0.03 µg.L^{-1}. In the liver, the catalase (CAT) activity was significantly affected in fish exposed to 0.03 µg.L^{-1}, while in the gills the activity of CAT was not affected. In the gills, but not in the liver, the activity of glutathione-S-transferases (GSTs) and lipid peroxidation (LPOX) were affected at 0.03 µg.L^{-1}. These differences in the enzymatic activities indicate a higher sensitivity of the gills to diltiazem. The highest concentration caused histopathological changes in the liver and kidney. In summary, at environmental relevant concentration, diltiazem was found to alter physiological indices in the blood, gills and liver suggesting a potential hazard to non-target species such as fish in the aquatic environment.

Keywords: *calcium channel blockers, fish, haematology, histology, oxidative stress*

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THE EFFECTS OF ZINC EXPOSURE ON THE HISTOMORPHOLOGY OF THE GONADS OF *OREOCHROMIS MOSSAMBICUS*

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In a recent study done in the Okavango Delta Panhandle, Botswana high levels of five endocrine disrupting metals were found in the surface water. These metals were selenium, mercury, aluminium, lead and zinc. Despite the presence of elevated concentrations of these heavy metals found in an area described as 'near pristine', no histopathology was found in the gonads of the selected fish species assessed in this study, except for one case of intersex in *Serranochromis angusticeps* fish species. The current study examined the effects of the zinc on the histopathology of the gonads of *Oreochromis mossambicus* in order to determine whether this metal is capable of causing gonadal histomorphological alterations. The study incorporated two exposures: 10 % of LC_{50} of zinc chloride and 15 % of LC_{50} of zinc chloride. The fish were exposed over a period of 96 hours. The gonads were excised, fixed in 10 % buffered formalin and prepared for staining using standard histological techniques. Three stains utilised included, namely the Hematoxylin and Eosin Stain to identify key structures and cells, the Perl's Prussian Blue Stain for identification of pigments within melano-macrophage centers, and the Gordon and Sweets Silver Stain to identify the connective tissue within the gonadal samples. Intersex or testicular feminisation denoted by the presence of ovarian tissue inside the testes is the most pronounced histopathological alteration identified in the current study. However, no pathology was found in the females. Other histomorphological alterations found in the testes included pyknosis, invasion of red blood cells in the seminiferous lobules, general reduction of mature spermatozoa within the seminiferous lobules and an increase of connective tissue around the gonads. The pigment hemosiderin was reduced in exposed fish. Results of the current study have reinforced the negative effects of elevated levels of zinc within an ecosystem. Furthermore, exposure to zinc may have a direct correlation to the intersex identified in *S. angusticeps* in the Okavango Delta study and *O mossambicus* in the current study. Further research needs to be done to identify the effects of the other four metals (selenium, mercury, aluminium and lead) on the gonads of several fish species and the possible interactions between them.

Keywords: *fish histology, histopathology, zinc, endocrine disrupting metals, gonads*

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SERUM AMINOTRANSFERASE ACTIVITY OF STURGEON BROODSTOCK IN RECIRCULATING AQUACULTURE SYSTEMS

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Liver diseases are common among fish grown in intensive aquaculture. As we know the importance of the liver plays in the sexual maturation of sturgeon. Serum aminotransferase activities, including aspartate aminotransferase (AST) and alanine aminotransferase (ALT), are commonly referred to as "liver enzymes," because they are abundantly present within hepatocytes, catalyzing transfer of amino groups to generate products in gluconeogenesis and amino acid metabolism. The blood was collected from sturgeon fish in recirculating aquaculture systems of Belarus in 2011–2013. For research use broodstock of sturgeon fish: sterlet (*Acipenser ruthenus*), siberian sturgeon (*A. baerii*), russian sturgeon (*A. gueldenstaedtii*) and sturgeon hybrids (bester, sib. sturgeon x rus. sturgeon). In serum studied AST and ALT. Our results showed that serum AST-activity of sturgeon broodstock depend on different physiological conditions. Thus, the serum AST-activity of normally maturing sturgeons broodstock was 97.0 % higher than that of serum AST-activity of not normal maturing sturgeons broodstock. Serum AST-activity of the female sturgeons, a negative reacting on stimulating injections during the spawning season, was on average 46.7 % higher than that of serum AST-activity of female sturgeons, a positive reacting to stimulating injection during the spawning season. These differences depend on the type of sturgeon: the serum AST-activity of bester, a negative reacting on stimulating injections during the spawning season, was 82.8 % higher; the serum AST-activity of siberian sturgeon, was 92.8 % higher. We have established significant differences between the serum AST-activity of broodstock during late autumn assessment and broodstock after spawning. Thus, the serum AST-activity of broodstock after spawning was higher by an average of 56.0 % than that of the serum AST-activity of normally maturing sturgeons broodstock, and 213.2 % higher than that of not normal maturing sturgeons broodstock. That the ALT-activity in serum of sturgeon broodstock depend on different physiological conditions. The serum ALT-activity of females of siberian sturgeon, a negative reacting on stimulating injections during the spawning season, was on average 70.5 % higher than that serum ALT-activity of females of siberian sturgeon, a positive reacting to stimulating injection during the spawning season.We have established significant differences between the serum ALT-activity of during late autumn assessment and broodstock after spawning. Thus, the serum ALT-activity of broodstock after spawning was higher by an average of 120.2 % than that serum ALT-activity of normally maturing sturgeons broodstock, and 35.5 % higher than that of not normal maturing sturgeons broodstock.

Keywords: *serum, liver enzymes, sturgeon,* broodstock*, recirculating aquaculture systems*

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EFFICIENCY OF HORMONAL TREATMENTS FOR STIMULATION OF OVULATION IN NORTHERN PIKE (*ESOX LUCIUS* L.)

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Optimization of hormonal stimulation of broodstock fish is one of the most important issues in nowadays aquaculture. Despite that it has been studied during last decades, the certain suggestion has not yet concluded. Therefore, we tested the effectiveness of gonadotropin-releasing hormone analogues: Gonazon – doses 25 $\mu g.kg^{-1}$ (GnRHa-G), Supergestran – doses 25 $\mu g.kg^{-1}$ (GnRHa –S), carp pituitary (CP) (3 $mg.kg^{-1}$) and CP (3 $mg.kg^{-1}$) emulsified in Freund´s incomplete adjuvant (CP-FIA) in northern pike (*Esox lucius* L). In total, 100 % female in positive control group (CP) and 86.5 % females in the CP-FIA group ovulated 96 hours after injection. Whereas, neither hormonally treated (GnRHa – G and S) nor control fish (saline solution injection) ovulated. The two mentioned GnRHa preparations were ineffective under the protocol used in the study. In present study, the following parameters were evaluated in successfully ovulated fish. Pseudogonadosomatic index (pGSI) in the CP and CP-FIA groups showed significant differences 18.62 ± 8.76 % and 22.74 ± 2.75 %, respectively (mean ± s.d.). The fertilization rate in the CP group was not significant and reached 27.51 ± 0.97 %, while it was 25.4 ± 1.05 % for CP-FIA group. Hatching in group CP and CP-FIA was 25.86 ± 1.01 and 24.08 ± 1.09, respectively without any significant differences. Ovarian fluid pH was significantly higher in the CP-FIA group, 8.24 ± 0.02, than in CP group, 8.11 ± 0.02. Ovarian fluid pH positively correlated with fertilization rate ($r = 0.63$; $p < 0.05$; $r^2 = 0.40$) in each individual of female. The females with higher ovarian fluid pH than 8.39 showed the highest eggs fertilization rate. We did not find significant differences between eggs size in CP and CP-FIA group, 2.7 ± 0.16 mm and 2.8 ± 0.14 mm, respectively. The present study results showed that only CP is effective hormonal treatment for ovulation in Northern pike. Any positive effect of the combination of FIA with CP was found for the improving of ovulation, fertilization and hatching rates, except stabilization and increase of pseudogonadosomatic index.

Keywords: *GnRHa, carp pituitary, Freund´s incomplete adjuvant, northern pike, reproduction*

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APPLICATION OF CERAMIC MEMBRANES FOR THE TREATMENT OF POST-PRODUCTION WATER FROM FISH FARMING IN CLOSED CIRCUITS

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Fisheries economics focusing on raising and breeding fish is one of the sources of ground water pollution. Presently, alongside traditional methods of using water in aquacultures based on open circuits (flow-through systems), closed circuits, i.e. the so-called Recirculating Aquaculture Systems (RAS) are used more and more frequently. They are gaining popularity as they not only allow for saving up water and minimalizing the discharge of wastewater but also make it possible to control and manipulate physicochemical parameters of the used water. The RAS water treatment systems utilize biological treatment combined with conventional separation techniques such as sedimentation, flocculation and filtration. According to literature on the subject, membrane filtration can also be utilized in the process of biological treatment.

The aim of the study was to analyze and evaluate the possibilities of using a pressure membrane installation as one of the stages of treating post-production water from fish farming in closed circuits (exemplified by African sharptooth catfish (*Clarias gariepinus*) farming). The tested treatment system had the form of a membrane installation consisting of a feed tank (50 dm^3), pump, heat exchanger and a module equipped with a commercial ceramic membrane with 1 kDa cut-off (transmembrane pressure: TMP 0.25 Mpa). In the feed tank, the values of water quality indicators were measured in the permeate (filtrate) and in the retentate (concentrate). Those indicators included: $BZT_{5,}$ $ChZT_{Cr}$, total suspension, turbidity, conductivity, ammonium nitrogen (NH_4), nitrate nitrogen (III), nitrate nitrogen (V), total nitrogen, phosphates, total phosphorus, and total iron. Then, after completing the process of water treatment, the degree of pollution reduction was evaluated and the efficiency and selectivity of applied membranes were analyzed.

Results of the research into the process of filtration of post-production water in a three-hour cycle (with cleaning of the membrane module taking place every hour) using the above described ceramic membrane revealed that as a result of the conducted process the highest level of retention was achieved in the case of general suspensions (the degree of pollution retention: the retention coefficient R equaled 100.0 %), followed by turbidity (R = 99.4 %), total iron content (R = 89.2 %), BZT_5 (R = 76.0 %), and nitrate nitrogen (III) (R = 62.3 %). As the process was progressing, the values of all water quality indicators were dropping (to various degrees), meaning that the degree of pollution retention on the membrane was increasing. Obtained results have proven that the process of membrane separation using the membrane with the 1 kDa cut-off can constitute one of the stages of treating wastewater from fish farming in closed circuits.

Keywords: *membrane filtration, ceramic membranes, water treatment, fish farming*

ARTIFICIAL PROPAGATION OF ASP (*ASPIUS ASPIUS* L.) FEMALES USING HORMONAL STIMULATION.

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In 2009 the asp broodstock was captured using electrofishing in the Želivka Dam, and then kept in fish pond with sufficient amount of prey fish. Following spring on 31 March 2010 the fish pond was harvested and the asps were transported to the hatchery. Selected females were randomly placed into 1 m^3 flow-through tanks (5 individuals per each tank). Average water temperature was 8.1 (7.5–9.1) °C and oxygen was kept at 12.2 (11.3–12.6) $mg.l^{-1}$. Males were placed into separate tank. Females weighted 1804±322 g (within range 1220–2620g) and fork length was 550–705 mm. Females were injected as follows: 1. group – double injection of carp pituitary at 1+3mg/kg body weight (BW) 24 h apart; 2. group received Ovopel (1 $pellet.kg^{-1}$ BW containing GnRHa with dopaminergic inhibitor); 3. group was injected with Dagin (producer recommended GnRHa dose, Dagin also contains dopaminergic inhibitor); 4. group with Supergestran (25$\mu g.kg^{-1}$ BW GnRHa); 5. group was an untreated control. In group 1 and 3, ovulation commenced and all females were stripped. Pseudogonadosomatic index (pGSI = weight of stripped eggs/female weight × 100) reached 7.25 ± 2.19 and 5.5 ± 1.18 %, respectively. Latency (time from injection to ovulation followed by stripping) was 39 ± 1.2 and 43.4 ± 1.7 h, respectively. In groups 2 and 4, 60 % of females ovulated. pGSI was 5.45 ± 1.18 and 7.44 ± 1.64 %, respectively. Latency was 42 ± 0.7 and 41.8 ± 0.4 h, respectively. In controls, no ovulation could be detected five days after start of experiment. Average egg weight from all females (n = 16) was 2.74 ± 0.26 mg.

SYSTEM FOR FISH MONITORING IN BIG TANKS

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Monitoring of water quality is one of up to date problems in fish related research areas. The control is especially crucial for the recirculation units, since they are very vulnerable to infections, poisoning, etc. In case of failures, the consequences quickly propagates over the whole system. Since cultured fish itself is a perceptive sensor of water quality, systems based on analysis of fish behavior can be used for monitoring of water quality.

The current study is focused on the development of robust and cheap system for automatic non-invasive fish tracking in the real conditions of the clear water tanks used in aquaculture. The approach used to calculate the 3D fish coordinates is based on estimation of amount of infrared light reflected from the fish surface. The system uses external source of eye-safe near-infrared. The advantage of using IR illumination is that it doesn't affect the fish day/night cycle.

Our experiments show that the variance of the reflected light caused by the differences between individual fish is much lower than the variance caused by absorption of water layer. Therefore after performing calibration the system is able to estimate the distance to the fish from the camera based on the amount of reflected light. The in-plane position of the fish is determined by image processing method.

The developed system can be used during the research in the field of nutrition, welfare, health and pathology, environmental interaction or aquaculture systems design.

Keywords: *fish, monitoring, tracking, infrared, aquaculture*

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GROWTH RATE OF DIFFERENT PERCH (*PERCA FLUVIATILIS* L.) POPULATIONS UNDER CONTROLLED CONDITIONS OF RAS: A REVIEW

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The intensive culture of Eurasian perch is one of perspective directions of European aquaculture. It occurs mainly because of high meat quality and increasing interest of perch market. Therefore operation of accessible water resources represents real possibility for intensive development of perch aquaculture. The use of recirculating aquaculture systems (RAS) with biological treatment of water is optimal for this purpose.

Wild perch populations are characterized by dynamic and annual fluctuations of abundance that depend on environmental and intra-stock mechanisms. Variations in these features may be the reason for differences in the biological characteristics of perch originating from various geographic regions. It is well known that Eurasian perch abundance levels are higher in northern biotopes than in southern ones, although thermal conditions in the latter would seem to be more optimal for perch growth.

It is important to note that research conducted on growth of several Eurasian perch wild strains originating from different regions of Europe reared in RAS suggested marked differences between strains starting when grown from larvae to marketing size: in body weights, survival, morphometric parameters and other biological characteristics. Therefore, knowledge about survival and growth differences between different perch strains is necessary as a tool for improving of perch aquaculture. The identification of the best strain adapted to intensive culture conditions (recirculation aquaculture systems (RAS) is the first step in starting a selection program.

The purposes of this review are summarizing the available information about biology of different wild perch populations and evaluating the possibility their using in intensive conditions of RAS.

Keywords: *recirculating aquaculture systems, morphology, geographical origin*

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EFFECT OF WATER TEMPERATURE ON EARLY LIFE HISTORY OF AFRICAN CATFISH, *CLARIAS GARIEPINUS* (BURCHELL, 1822)

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Early life history of *Clarias gariepinus* from egg fertilization to full yolk sac resorption was studied within thermal range 17.4–38.6 °C. The viable temperature ranges for embryonic (17.5–35.5 °C; t_0 = 15.4 °C; $D°_{eff}$ = 12) and larval (19–33 °C; t_0 17.14 °C; $D°_{eff}$ = 27.32) periods were found. It was observed that the early life history of *Clarias gariepinus* is a thermal dependent process in terms of the onset of key ontogenetic events, developmental rate, survival rate and size of larvae at any key ontogenetic event. Length of the incubation period, hatching period, period up to the first intake of exogenous food and length of the period up to the full yolk sac resorption (with or without exogenous feeding), as well as size of larvae at the hatching, the first intake of exogenous food and the full yolk sac depletion, were inversely proportional to the incubation temperature within an optimal temperature range for early life history (23–30 °C). In term of survival, the zone of thermal tolerance for early life history of *Clarias gariepinus* ranges from 19 to 33 °C (with thermal optimum between 23 and 30 °C), i. e. this fish belongs to the typical thermophilous species. The suboptimal temperature lies within intervals 21–23 °C and 30–33 °C, respectively. Temperatures below 17.5 °C as well as above 35.5 °C can be considered lethal temperatures already during embryonic period. And those below 19 °C and above 33 °C are lethal ones during larval period.

Keywords: *abiotic factors, clariidae, incubation, ontogeny, thermal tolerance*

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ECOLOGICAL CONDITIONS OF INVASIVE BRYOZOANS *PECTINATELLA MAGNIFICA* IN PROTECTED LANDSCAPE AREA TŘEBOŇSKO

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In most reservoirs in Protected area Trebonsko, where this species occurs, the temperature, conductivity, oxygen, pH and transparency of water in the period from July to August during the years 2006 to 2012 were measured. In this period was also conducted sampling of colonies, determined their biomass and their abundance.

Recorded were following ranges of specified physical parameters: water temperature 16.4 °C – 29.2 °C, conductivity of 4.9 to 20.5 $mS.m^{-1}$, pH 4.6 to 10.2, the oxygen content of 2.3–16, 1 $mg.l^{-1}$ and transparency of water from 0.2 to 3.4 m. Slightly linear dependence was recorded between conductivity and abundances of colonies. For the other factors, linear relationship, even in relation to the numbers or to the weight of the colonies, does not been detected. The results indicate, that this species has rather thermophilic character. It appears, that *P. magnifica* prefers oligotrophic to mesotrophic type of water with a slightly alkaline pH (7–8), with a lower conductivity (less than 200 $\mu S.cm^{-1}$) and it has a high tolerance to content of oxygen in the water.

Keywords: *The Protected Landscape Area Třeboňsko, physical parameter of water, water quality, linear dependence, Pectinatella magnifica.*

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FIRST STEPS OF ARCTIC CHARR (*SALVELINUS ALPINUS*) AND ITS INTERSPECIFIC HYBRID ELSÄSSER CHARR (*SALVELINUS FONTINALIS* × SALVELINUS ALPINUS) IN THE CZECH SALMONID AQUACULTURE

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The Arctic charr is the northernmost living salmonid on the Earth. Rearing of this species restricted mostly to Arctic and subarctic regions with summer water temperatures not exceeding 16 °C has become of increased interest in the last decades especially in Iceland, Canada, and Norway. Since couple of years, having an inspiration in German salmonid farming, we have been playing with an idea to introduce these two salmonids to the Czech Republic in order to supplement or completely replace Brook trout (*Salvelinus fontinalis*) production. Looking for the best place in the Czech Republic corresponding with its water conditions to boreal climate, choice for one of the oldest salmonid farm, the Vacek´s trout farm in Nedošín (Litomyšl, Eastern Bohemia, 49°53'3.104"N, 16°17'9.362"E), has been made. Controversially, this farm located on the Loučná River in the middle of the upper Elbe River basin belongs to areas with "warm climate" according to the official climatic classification of the Czech Republic. The highest summer temperatures 16.5 °C and the lowest winter temperatures around 5 °C at this farm fit superbly the physiological requirements of Arctic charr for efficient growth performance over most of the year. In January 2013, under a pilot project supported by the Czech Ministry of Agriculture (CZ.1.25/3.4.00/11.00366), 15,000 and 20,000 eyed eggs (including also some fingerlings) of both the purebred Swedish Hornavan Arctic charr strain and its interspecific hybrid with brook trout called Elsässer have been purchased from Bavarian State Exemplary Fish Farm in Lindbergmühle. The Swede Hornavan belongs along with Canadian Nauyuk to the most suited strains for aquaculture purposes (Gavin Johnston 2002, Arctic Charr Aquaculture). After the hatch and six month of parallel rearing of the three charrs (Arctic charr, Elsässer hybrid, and brook trout), both newly introduced charrs showed to be superior to the particular Czech brook trout strain in survival and growth. Especially in 1+ Hornavan strain (app. 27 mm in length, 200g+) no early sexual maturity could be detected. In brook trout and Elsässer hybrid sexual maturation was observed in 1+ males. Our preliminary results suggest that a) provided adequate water conditions Arctic charr farming can be successful even in warmer European regions, b) deployment of photoperiod manipulation will be essential in the future in order to increase rearing efficiency in genus *Salvelinus* in general. We are currently in the half of the production cycle. Summary results are going to be presented after fish have reached market size in late summer or autumn 2014.

Keywords: *Arctic charr, brook trout, Elsässer charr, growth, aquaculture*

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EFFECTS OF DIFFERENT DIETARY ASTAXANTHIN LEVEL ON PIGMENTATION, GROWTH, SURVIVAL RATE OF THE FRESHWATER CRAYFISH (*ASTACUS LEPTODACTYLUS,* ESCH, 1823)

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In this study, the effects of astaxanthin that is added the feeds of crayfish (*Astacus leptodactylus) (*0, 18 g initial body weight and 20, 12 mm total length second stage) at different ratios, on the growth, survival rate and pigmentation are studied. The experiments lasted 90 days and four diet groups containing different rates of astaxanthin (50 $mg.g^{-1}$, 100 $mg.g^{-1}$, 150 $mg.g^{-1}$ and the one without any astaxanthin) are applied for three times. The maximum and the minimum effects of adding astaxanthin to the feeds at different rates on the relative increase of weight and length are observed from the A100 group and K0 group, respectively. However, the significance of the growth rates is determined to be low ($p > 0.05$). While the best result in terms of survival is attained from K0 group (66.67 %), the worst survival rates belong to A100 group (40.35 %). When the pigmentation is examined, the highest rate belongs to A150 group (meat sample: 1.9 $mg.g^{-1}$; shell sample: 20.3 $mg.g^{-1}$) while the minimum belongs to K0 group (meat sample: 0.9 $mg.g^{-1}$; shell sample: 13.5 $mg.g^{-1}$).

Keywords: *Astacus leptodactylus, astaxanthin, HPLC, growth, survival rate, Pigmentation*

GROWTH PATTERN EVALUATION OF SPINY-CHEEK CRAYFISH BASED ON AGE AND SEX DIFFERENCES

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The spiny-cheek crayfish (*Orconectes limosus*) is originated from the North American continent, but has been introduced in last century, and has widely spread in European waters since last decades. Compared to other species, spiny-cheek crayfish have several characteristics such as rapid maturation, short-lifespan, high fecundity and second mating period, that facilitate its fast population growth and high tolerance to water pollution. *Orconectes limosus* have easily spread to the various areas due to the mentioned adaptations. Additionally, the very limited data are available in the literature on its growth patterns. In this context, the present study was conducted in order to determine the growth patterns of spiny-cheek crayfish of different age (juvenile J, adult A) and sex (male M, female F). Seven crayfish groups (1 juvenile male, 1 juvenile female, 1 adult male, 1 adult female – each observed separately, 2 juvenile males + 2 juvenile females, 2 juvenile males + 2 juvenile females with 1 adult big-size male, 2 juvenile males + 2 juvenile females with adult big-size female) were evaluated in terms of their carapace (CL) and post-orbital carapace lenght (POCL), and weight (W) as well, within 90 days, where growth parameters were measured each 30 days. Differences between individual growth and group-growth performance were statictically significant as well ($p < 0.05$). Growth performance were the highest for individually kept crayfish and CL, POCL and W of the individuals were found: 17.62 ± 0.99 mm (mean ± SD), 12.79 ± 0.78 mm and 1.37 ± 0.31 mm at the beginning, and 31.62 ± 1.34 mm, 22.97 ± 0.85 mm and 7.94 ± 0.69 mm at the end of the study, respectively. Juvenile male kept together with mature male have shown statistically the highest growth rate compare with other groups ($p < 0.05$).

Keywords: *Spiny-cheek crayfish, Orconectes limosus, growth performance*

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COMMENTS

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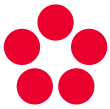

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