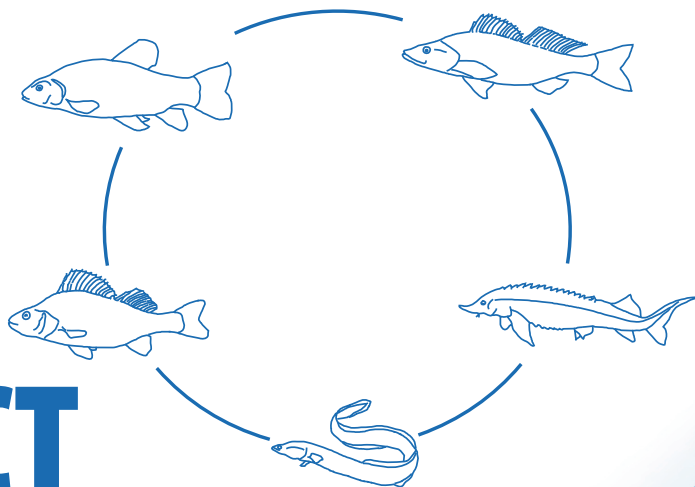




FACULTY OF FISHERIES AND PROTECTION OF WATERS
UNIVERSITY OF SOUTH BOHEMIA IN ČESKE BUDĚJOVICE

DIVERSIFICATION IN INLAND FINFISH AQUACULTURE



ABSTRACT BOOK

MAY 16–18, 2011

PÍSEK, CZECH REPUBLIC



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WELCOME AT DIFA WORKSHOP

Dear friends and colleagues,

The University of South Bohemia, Faculty of Fisheries and Protection of Waters heartily welcome you at the scientific workshop with the title „Diversification in Inland Finfish Aquaculture“ (DIFA) taking place of the modern White Rose Hotel in the ancient town Pisek which was founded on the River Otava by Bohemian kings in the 13th century.

DIFA workshop will address the topic of diversification of inland aquaculture as a tool for expansion and innovation of aquaculture sector.

Generally, global aquaculture has developed at an enormous rate over the last decades and continues to be one the fastest growing food producing sector in the world. The largest producer, on a global scale, is the Asia-Pacific region which supplies 89% of total production. Current world aquaculture produces nearly half of all the fish that are consumed across the globe. The availability of wild catches through capture fisheries has been decreasing over time while continually increasing human population requires higher fish production. Global aquaculture production was increased by approximately 9% per year during the last decade. However, European aquaculture rose only by 3–4% during the same time period. Current freshwater fish production of European aquaculture is mainly based on RAS or flow-through systems in Western parts and on the pond or lake ranching culture in North-East parts of Europe with cultured salmonids, carps, sturgeons and carnivorous fish.

New development and diversification of aquaculture and seafood market including new methods and species with maximum utilisation of their potential in relation to sustainability and safety are very important activities for future high quality fish production. We hope that our scientific discussion during DIFA workshop will be fruitful and some points or remarks will contribute to the development of freshwater inland aquaculture.

Thanks are due to organizing and scientific committees of DIFA workshop for enthusiastic preparation of this event and all sponsors (Aller Aqua, Depur Systems, Fishery Nové Hradý, Fishery Hluboká nad Vltavou and Trout Farm Mlýny) and partners (European Aquaculture Society, Larvanet and Aquaculture Initiative) for financial and promoting support.

Have a nice time in the Czech Republic

Tomáš Polícar & Zdeněk Adámek

WORKSHOP PROGRAM

Monday, 16 May 2011

- 08:00–09:00 Registration, White Rose Hotel, Písek
 09:00–09:30 Welcome for participants and opening ceremonies

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FISH DOMESTICATION, NEW PRACTICE AND SPECIES WITHIN INLAND AQUACULTURE

Chairmen: *Pascal Fontaine & Tomáš Polícar*

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- 10:15–10:30** **Kloas W.**, Van Ballegooy Ch., Rennert B.: Validation of an aquaponic-system for (nearly) emission free tomato and fish production in green houses (Astaf-Pro). (R) **pp. 21**
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- 10:45–11:00** **Skibniewska K. A.**, Guziur J., Zakrzewski J., Wiśniewska A., Szarek J., Białowas H.: Guziur scale as a complex method to assess the effect of production and consumers' value of fish. (R) **pp. 23**
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Chairmen: *Zdeněk Adámek & Denés Gál*

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- 11:45–12:00** **Musil M.**, Potužák J., Pechar L.: Impact of topmouth gudgeon (*Pseudorasbora parva* Schlegel, 1842) on production of common carp (*Cyprinus carpio* L.) – Question of natural food structure. (S)** **pp. 27**
- 12:00–12:15** Gardeur J.-N., Mathé S., Rey-Valette H., Ledoré Y., Simon J., **Fontaine P.**: Ponds in Eastern France: towards an environmentally intensive fishfarming. (R) **pp. 28**
- 12:15–12:30** **Gál D.**, Kosáros T., Pekár F., Kerepeczki É.: Potential of the nutrient reutilisation in a combined intensive-extensive pond system. (R) **pp. 29**
- 12:30–12:45** **Kosáros T.**, Pekár F., Gál D., Lakatos G.: Periphyton utilisation in combined intensive-extensive pond fish farming systems. (R) **pp. 30**
- 12:45–13:00** **Potužák J.**, Duras J., Borovec J.: Nutrient balance of two fishponds of different trophic state and different fishery management. (R) **pp. 31**
- 13:00–13:15** **Všetičková L.**, Adámek Z.: The impact of carp pond management upon macrozoobenthos assemblages in pond canals. (S) **pp. 32**

Note: *(R) – Regular presentation

** (S) – Student presentation

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Chairmen: Damien Toner & Vlastimil Stejskal

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- 17:05–17:20** **Levavi-Sivan B.**, Yom-Din S., Hurvitz A., Degani G.: The involvement of kisspeptin and gonadotropins in puberty in the Russian sturgeon (*Acipenser gueldenstaedtii*). (R) **pp. 46**

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FISH DOMESTICATION, NEW PRACTICES AND SPECIES WITHIN INLAND AQUACULTURE

Chairmen: Pascal Fontaine & Tomáš Polícar

DOMESTICATION OF NEW SPECIES AND DEVELOPMENT OF A SUSTAINABLE AQUACULTURE IN EUROPE

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Within the past three decades, finfish aquaculture increased 14.5-fold from a production of ca. 1.95 millions metric tons (in 1974) to 28.1 (in 2004) and the markets demand is continuously increasing. This important development was mainly based on the production of few species (carps, Atlantic salmon, Nile tilapia) which benefit from a high level of know-how for the control of their life cycle and do not concerned the European countries (except, the production of salmonids in Northern Europe). However the type of development is questionable because it is frequently associated to the introduction of alien species presents environmental risks, such as a loss of biodiversity, local ecosystem disturbances, and introduction of new pathogens. In the same time, consumers, specifically in developed countries, pay more and more attention to the development of a more sustainable aquaculture associated with environment friendly practices. A way to promote the development of a sustainable aquaculture is the diversification of the production based on the farming of native species in order to reduce the environmental impact of fish culture and to respond to demands from local markets. Such development (Mosaic model) could also favour a more integrated local economy and the emergence of resilient systems of production for fish farming. In this context, the building of a generic approach for multi-species domestication is required to optimise the use of the technical and financial means available for fish culture diversification. Means and methods available to achieve this objective will be discussed considering the context of the European Inland aquaculture.

Keywords: *fish culture, diversification, domestication, sustainable development*

CULTURE OF YELLOWTAIL KINGFISH (*Seriola lalandi*) IN RAS IN THE NETHERLANDS

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Hatchery and nursery of yellowtail kingfish (*Seriola lalandi*) are well established in Australia. In the Netherlands a search for new aquaculture species, based on production and reproduction potential, as well as market perspectives, advanced *Seriola* as a species of choice for Recirculation Aquaculture Systems (RAS). Studies were designed to fine-tune husbandry conditions. Fish were stocked in pilot-scale (total volume: 3 m³) RAS (40µm drum filter, 1.5 m³ trickling filter, ozone-injected protein skimmer, UV-unit and thermo-control). Water temperature, pH, salinity and oxygen values were varied; a feeding trial was performed with yellowtail kingfish diets that varied in fish meal content. Growth performance, feed conversion, stress-physiological, microbiological and metabolic parameters were assessed. Trials on water temperature, pH, salinity and oxygen levels allowed definition of excellent husbandry conditions. Clear differences in growth performance and feed conversion, as well as (subtle) changes in stress-physiological parameters under suboptimal conditions, reveal the importance of well-chosen water quality parameters for optimal growth and welfare of this fish. Yellowtail kingfish was successfully cultured from 0.5 g to 4.5 kg in RAS. Our RAS trials confirm the high potential for culture of yellowtail kingfish in Dutch aquaculture. Consumer tests and market studies are presently carried out to substantiate the market opportunities for this species; in a small-scale marketing project the fish was enthusiastically received. Reproduction of yellowtail kingfish in The Netherlands is under study; the first commercial farming of yellowtail kingfish in RAS started in the Netherlands in 2011.

Keywords: yellowtail kingfish, RAS, husbandry, physiology

PARTICULARITIES OF EGG AND LARVAE IN TEMPERATE FRESHWATER FISH SPECIES: IMPLICATIONS FOR AQUACULTURE PRACTICES

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Early life stages are different between freshwater and marine species. Freshwater fish species have generally larger and fewer eggs than marine species. Besides, most freshwater eggs are demersal and develop stuck to diverse substrata, whereas marine eggs are mostly buoyant. These differences have consequences for the evaluation of the quality of eggs, *e.g.*, in marine species with buoyant eggs, non-viable or unfertilized eggs sink to the bottom of the tank while fertilized eggs remain in the water column; in some freshwater fish species fertilized eggs are translucent while unfertilized eggs are whitish or opaque. These differences have also implications for the incubation, particularly the need to use specific products (*e.g.*, Tannic acid, Alcalase) for removing the stickiness of eggs in some species, such as in tench *Tinca tinca* or pikeperch *Sander lucioperca*. Different types of incubators have been developed, *e.g.*, Zoug and Weiss jars, tray-type incubators. Even though the larvae of freshwater species widely vary in their size and their developmental/ontogenetic stage at hatching, most freshwater larvae are larger and more developed at hatching than their marine counterparts, resulting in differences in both the feeding regimes used and the onset of cannibalism. First, because of their small size, most marine fish larvae required to be fed first life preys before being weaned to inert diets. In contrast, in some freshwater species, such as in Northern pike (*Esox lucius*), larvae can be fed inert diets as early as mouth opening. Yet, for numerous species, particularly cyprinids, feeding regimes needs also to begin for few days with live preys (*e.g.*, *Artemia*, rotifers, and zooplankton) before performing weaning. No specific review on the nutritional requirements of freshwater larvae has been realized. Second, cannibalism may emerge sooner in certain freshwater fish species, such as in pike, pikeperch and perch (*Perca fluviatilis*).

Keywords: domestication, early-life stages, egg, larvae, STOREFISH

VALIDATION OF AN AQUAPONIC-SYSTEM FOR (NEARLY) EMISSION FREE TOMATO AND FISH PRODUCTION IN GREEN HOUSES (ASTAF-PRO)

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Aquaculture is the fastest growing sector concerning nutrition of human mankind and the biggest part worldwide resembles aquaculture of freshwater finfish. Due to limited resources an increase of aquaculture production has to meet also the criteria for sustainability, e.g. use of pure freshwater. The trend towards sustainability of the so-called “blue aquaculture” introduced the use of closed recirculation systems for freshwater fish production where the consumption of the limited resource water is restricted and, furthermore, no effluents polluted with fish waste full of nutrients, such as N and P, are released to affect surface water ecosystems. However, the nutrients containing water of closed recirculation systems can be used to supplement hydroponic systems for vegetable culture. Thus, the aim of the presented project was to produce vegetables and fish in greenhouses under emission free conditions by combining a closed recirculation aquaculture system rearing tilapia (*Oreochromis niloticus*) with a vegetable hydroponic system growing tomatoes. Herewith, water from the aquaculture section containing nutrients is mechanically and biologically purified and given to the plants at demand through a one-way valve. Water and soluble nutrients are taken up by plants for growth production and these plants evaporate a good amount of water. Evaporated water is regained from the greenhouse atmosphere via cooling traps and re-fed back into the aquaculture system. Thus, water will be re-used and altogether less than 3% of the make-up water of the whole aquaponic-system has to be added per day. The needed energy is being produced in a sustainable way by a photovoltaic plant. Furthermore, the CO₂ produced by fish is also being absorbed by the plants, which in turn produce O₂ and, thus, the ASTAF-PRO system is nearly emission-free concerning CO₂ combined with the least use of water reported yet. Herewith, the first results of the validation of the new ASTAF-PRO system are reported.

Keywords: *aquaponic, sustainability, emission-free, tilapia, tomato*

COULD PERACETIC ACID BE AN ALTERNATIVE TREATMENT IN AQUACULTURE?

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Peracetic acid (PAA) is an antimicrobial disinfectant used in agriculture, food processing and medical facilities. It has recently been tested as a means to control infestations of *Ichthyophthirius multifiliis* and *Saprolegnia parasitica*. Free-swimming theronts of *I. multifiliis* can be eliminated using concentrations of 0.3 ppm PAA and mature tomites can be treated with 1–1.5 ppm PAA. In flow-through catfish hatcheries, 2.5–5 ppm PAA twice each day until eyes form on the embryos reduced *Saprolegnia* spp. and ensured best hatching rates. However, high concentrations of PAA can be toxic to fish. Chemico-physical water parameters (e.g., water hardness and organics) can modify fish toxicity. The 24 h LC50 of PAA to embryos of zebrafish (*Danio rerio*) ranged between 2.5 ppm in very soft to > 7 ppm in very hard water. In water with higher organic load, PAA degradation is increased. Peracetic acid can be used to reduce the microbial load in aquaculture facilities. Concentrations as low as 1 ppm PAA and an exposure time of 10 minutes reduced the colony forming units (CFUs) to 0.6% *in vivo* and 1.5–3% *in vitro* dependent on the PAA product. Because there are few approved therapeutants that are effective treatments against fish parasites, PAA could become an alternative.

Keywords: peracetic acid, white spot, protozoan parasite, fungus, microbial load, alternative treatment

GUZIUR SCALE AS A COMPLEX METHOD TO ASSESS THE EFFECT OF PRODUCTION AND CONSUMERS' VALUE OF FISH

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Many partial and separate methods of assessment of production and breeding parameters and consumers' value of fish is applied nowadays. Two classic indicators are: nourishment indicator after Fulton and body deep parameter after Walter. Organoleptic method, usually realized as a consumers' test, is usually applied to assess the sensory value. Chemical composition of fish fillet is also important. The aim of the work was to work out the method enabling the complex assessment of production effect and consumers' value of fish. Results obtained during realization of the scientific project on the influence of production technology on quality of consumers' carp (480 fish from 6 Polish farms utilizing semi-extensive, low intensive and highly intensive technology of breeding) and of the realized now project on quality of rainbow trout (240 fish from 6 farms on flowing and recirculating waters). The parameters were used to calculate the Guziur Scale: slaughter efficiency (discriminant weight 40%), content of total protein, total fat and proportion of n-3/n-6 fatty acids (every at 10%), and results of organoleptic assessment (30%). Application of the Guziur Scale indicated the low intensive technology as the most profitable i.e. giving the highest production effect at the highest flesh quality. For trout assessment the parameters were used: slaughter efficiency (discriminant weight 40%), meat colour, content of total protein, total fat and proportion of n-3/n-6 fatty acids (every at 10%) and results of organoleptic assessment (20%). Guziur Scale is a tool making the producers easy to optimize the production of the highest quality of flesh.

Keywords: *carp, fish production, flesh quality, rainbow trout*

SESSION

POND AQUACULTURE AND MANAGEMENT

Chairmen: Zdeněk Adámek & Denés Gál



**BIOTURBATION OF SEDIMENTS BY BENTHIC MACROINVERTEBRATES AND FISH
AND THEIR IMPLICATIONS FOR POND WATER ECOSYSTEMS**

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The sediment-water interface in aquatic systems is a bridge connecting bottom sediments with the overlying water column. This transition zone can be easily disturbed by mechanical mixing (bioturbation) and also sediment layers are frequently affected by this phenomenon which is caused by foraging fish and/or worms, insect larvae, crustaceans and mussels moving around and stirring sediment layers. Different benthic animals have various mechanical activities in sediments, thereby they may have different effects on solute exchange across the water-sediment interface. The ecological and evolutionary dynamics of phytoplankton (cyanobacteria in particular) and zooplankton is in part a function of the numbers and ages of diapausing eggs hatching from aquatic sediments. Successful recruitment from this 'egg bank' must depend upon the eggs being present at or near the sediment surface. Often, however, resting cells of cyanobacteria and zooplankton diapausing eggs are found as deep as 15 to 30 cm in the mud. Bioturbation may thus provide a mechanism for the regular return of buried eggs to the sediment surface. Flux of nutrients from sediment to overlying water is a common phenomenon in aquatic habitats as a result of decomposition and mineralization processes. The sediment of aquatic ecosystems is largely known as a reservoir of nutrients, organic compounds, and a large variety of solutes, mainly through accumulation of organic matter of autochthonous and allochthonous origin. Bioturbation is reworking and mixing of sediment at the sediment-water interface, accomplished collectively by burrowing, feeding, irrigation, resuspension, secretion, excretion and transporting activities of benthic organisms and/or benthivorous fish, which alter the structure and properties of the sediment and thereby influence diffusive and/or advective transport of both solutes and particulate matters.

Keywords: *sediment-water interface, nutrient release, resting cyanobacteria, diapausing zooplankton, burrowing chironomids, benthivorous fish*

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**IMPACT OF TOPMOUTH GUDGEON (*Pseudorasbora parva* SCHLEGEL, 1842)
ON PRODUCTION OF COMMON CARP (*Cyprinus carpio* L.) – QUESTION OF NATURAL
FOOD STRUCTURE**

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Asian cyprinid, topmouth gudgeon (*Pseudorasbora parva* Schlegel, 1842), was spread with stocking material of herbivorous fish during the 1970's and 1980's to the Czech Republic. This species is widespread in many fishponds areas at present. It has an invasive character caused by its successful ecological strategy. Feeding habits of topmouth gudgeon are overlapping with economically significant fish species. Four production fish-ponds were investigated during 2003 and 2004. Stocking density of three-years old carp was 363 ind.ha⁻¹. In 2003, in all investigated fish ponds topmouth gudgeon was outbroken. Topmouth gudgeon got into the fishpond by water inflow from its natural reservoir spontaneously. Estimated population density was 44.3 kg.ha⁻¹ minimally. In 2004 precautions against topmouth gudgeon invasion were taken and it wasn't recorded during the season. Influence of topmouth gudgeon on natural food structure was observed and main production parameters were evaluated. Topmouth gudgeon regulated zooplankton population significantly in 2003, especially large cladocerans of genus *Daphnia*. Negative influence on mean quantity of zoobenthos (mainly chironomid larvae) wasn't detectable but negative influence on macrozoobenthos species frequency was evident during the season 2003. This unfavourable natural food condition in 2003 is well-perceptible on carp production parameters. Total production of four-years old common carp was 283 kg.ha⁻¹ in 2003 and 634 kg.ha⁻¹ in 2004. Food conversion rates were 3.5 in 2003 and 1.6 in 2004. Costs per 1 kg growth were about 57% lower in 2004 than in 2003. Results shows negative influence of topmouth gudgeon on common carp production caused by its grazing pressure on natural food.

Keywords: carp production, natural food, *Pseudorasbora parva*, zoobenthos, zooplankton

**PONDS IN EASTERN FRANCE:
TOWARDS AN ENVIRONMENTALLY INTENSIVE FISHFARMING**

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In the framework of the PISCEnLIT project (2010–2012) focused on the **Ecological Intensification of Fish farming** and funded by the French Research Agency, a survey was conducted on 25 fishfarmers of the region Lorraine, Eastern part of France (May – July 2010). The objectives of the investigation are (i) to characterize pond culture systems (technical, economical, environmental and social points of view) in order to establish a further typology; (ii) to specify the pond services assessment to human populations of aquaculture ponds and their perception by fish farmers, (iii) to study the fish farmer ability to innovate and (iv) to imagine further scenarii of ecological intensification. Fishfarmer are firstly characterized by specific human strategy, in opposition to their know-how and performances, then secondly to the notion of difficulties and the need of help. Production of food, water regulation and maintenance of biodiversity are the 3 main service assessment identified by fish farmers concerning the role of their ponds. The services provided by ponds are generally considered as very important for the activity of the farm and its links with partners outside, although whether they are sometimes in competition with the production activity. The services provided by pond ecosystems are still perceived as interrelated, it means that they are actually perceived as a real complex system. Fish farmers propose ways to enhance financial profitability of the services provided and are ready to change their practices to achieve this objective. A multidimensional approach by multiple correspondence analysis, ascending hierarchical classification and semantic tagging of classes allows to create a typology of ecosystem services in 6 classes, characterized by the farm activity, the pond biodiversity, the production and trade, the business management, the labour and the social relations, the opening to the public, the social networks, the territories and institutions, and the constraints and conflicts.

Keywords: pond ecosystem, service assessment, farm typology

POTENTIAL OF THE NUTRIENT REUTILISATION IN A COMBINED INTENSIVE-EXTENSIVE POND SYSTEM

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The extensive fishponds could be an effective tool for water treatment since they are able to retain high amount of nutrients. The combination of intensive and extensive aquaculture exploits the advantages of both traditional pond farming and intensive fish culture systems. The goals of the combined production system were to increase production capacity; diversify the cultured species and recycle the nutrients within the production system. The investigated combined intensive-extensive system (IES) as a production system operated in a close interaction of the intensive and extensive production units. The key element of the proper operation was the treatment capacity of the extensive unit; hence the investigations were focused on the nutrient utilisation and water quality of the combined production system. In order to increase the nutrient reutilisation capacity of the extensive pond unit the potential of the artificial substrate application for periphyton production were also tested in the frame of the research. In the intensively managed part of the system valuable carnivorous fish can be cultured in controlled conditions and fed with artificial diets. The fish wastes can be utilised in the extensive part and can increase the fish yields. Results proved that combination of intensive aquaculture with extensive fishponds enhances the nutrient utilisation efficiency and fish production in IES. The combined fish production resulted in higher protein utilisation and increased nutrient utilisation efficiency by 5–10% comparing the aquaculture systems without integration.

Keywords: *combined production system, fish production, nutrient utilisation, periphyton, pond*

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PERIPHYTON UTILISATION IN COMBINED INTENSIVE-EXTENSIVE POND FISH FARMING SYSTEMS

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The periphyton development and composition were studied in a combined intensive-extensive pond fish farming system, where the intensive fish producing unit is connected to an extensive fishpond. Since traditional periphyton based aquaculture does not exist yet in Hungary, detailed knowledge on the quantitative and qualitative changes of the periphyton may give possibilities to increase fish yield and improve water quality, even under the temperate climate. The experiment was carried out at the Research Institute for Fisheries, Aquaculture and Irrigation (HAKI) in Szarvas, Hungary in 2007 and 2008. Productivity of extensive units was enhanced by periphyton developed on artificial substrates (PA100%; PA200%). The additional area for periphyton development equalled to 0 (PA0%, control), 100 (PA100%) and 200% (PA200%) of the pond surface area. Comparing the measured parameter of periphyton production (mean dry matter $42.2 \pm 2.96 \text{ g m}^{-2}$), there were no significant differences between the ponds. However, significant quantity of total nitrogen ($5.57 \pm 0.10\%$) and total phosphorous ($17.9 \pm 4.6\%$) were accumulated in the periphyton. Moreover, combined fish production resulted in higher protein utilisation. With periphyton application the protein utilisation in the ponds was increased to 26% in 2007 and to 40% in 2008. The net fish yield of the whole system was the highest in those ponds where periphyton area was 100% (2007 – 0%: 6792; 100%: 7825; 200%: 7083; 2008 – 0%: 16010; 100%: 17837; 200%: 15529 kg ha⁻¹). Our results proved that the combination of intensive and extensive fish farming is a possible solution to reduce environmental pollution of intensive fish farming and increase extensive fish production. The efficiency of the extensive fish farming units can be improved by accelerated periphyton production where the outflow water and waste nutrients from the intensive units can be directly reutilized.

Keywords: artificial substrate, fish pond, periphyton utilisation

NUTRIENT BALANCE OF TWO FISHPONDS OF DIFFERENT TROPHIC STATE AND DIFFERENT FISHERY MANAGEMENT

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Fishponds are an important component of hydrologic system of the Czech Republic surface waters. Their role in transformation of nutrient flows within river-basins (esp. of drinking water reservoirs) is supposedly crucial, but, in fact, the non-empirical evidence for such statement is rather poor. Two different fishponds were studied as a part of the project "Revitalization of Orlík Reservoir". The values of phosphorus (P) retention (R) from the real data were compared to modelled values. The fishpond Dehtář (246 ha, water retention time (Rt) 160 days – in the year 2010) was hypertrophic, intensively stocked with carp fed by grain. The fishpond Hejtman (82 ha, Rt 11 days – in the year 2010) was meso-eutrophic with extensive sport-fishery management. P retention in the fishpond **Dehtář** was positive (R – 10%, 0.42 t P) with measured 1.71 g.m⁻².year⁻¹ specific load of P. The modelled retention of 2.31 t P (R – 55%) suggests that ability of fishpond Dehtář to trap P was utilized from 18%. Despite **Hejtman** was loaded more intensively (3.20 g.m⁻².year⁻¹) with P than Dehtář, P retention calculated from real data (26%, 0.65 t P) well corresponded with the expected value (24%). Different management of both fishponds is considered to be the principal reason of their different functioning although some other factors could support their different behaviour (input of P binding compounds – Fe, character of the sediments, etc.).

Keywords: eutrophication, fishery management, fishpond, nutrient balance, phosphorus retention

THE IMPACT OF CARP POND MANAGEMENT UPON MACROZOOBENTHOS ASSEMBLAGES IN POND CANALS

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Impact of carp pond management upon macrozoobenthos assemblages was studied in inlet and outlet carp pond canals in South Moravia (Czech Republic). Four ponds (two eutrophic and two hypertrophic ones) with different types of fish farming management and intensification measures were selected for the evaluation of the impact of inlet water quality, discharge rate and pond stocking management upon the outlet water quality determinants based on macrozoobenthos evaluation during April – September 2009. Five inlet and four outlet canals were sampled by „kick – sampling” approach and also by using the artificial substrates. The pattern of water quality changes after the flow-through the pond was predominantly influenced by inlet water quality. In ponds supplied with worsened inflow water quality with the saprobic index (SI) according to macrozoobenthos corresponding to alpha-mesosaprobity (SI 2.82–2.89), the outflow water quality was significantly improved by approximately half of the saprobic degree on SI 2.38–2.42. On the contrary, the inflow water quality corresponding to beta-mesosaprobity (SI 2.32) was significantly ($p < 0.01$) deteriorated to alpha-mesosaprobity in the pond outflow. Macrozoobenthos diversity and number of taxa reflected the flow-through the pond identically with saprobic determinants. In ponds with poor inflow water quality, the number of macrozoobenthos taxa and its diversity increased in the outlet canals and vice versa, in ponds with good quality inflow water, the number of taxa and macrozoobenthos diversity decreased in outlet canals.

Keywords: carp farming, water quality, pond aquaculture, pond outlet

PREDICTION OF FISH PONDS MARKET VALUE IN ENVIRONMENTALLY PROTECTED AREAS IN POLAND

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Poland is one of the largest common carp producers in the European Union. Earthen carp ponds are characteristic feature of the traditional rural landscape. They are a space for fish production, but they play also a number of important environmental functions. Ponds, as a habitat of rare plant and animal species, often constitute or are a part of nature reserves and the Natura 2000 network. It enforces low level of production intensity, permanent or temporary production bans in selected ponds, loss of fish and feed caused by preying birds and other animals. The ponds' environmental functions additionally can imply costs that go beyond the limits of aquaculture standard practises. It all reduces production utility and market value of the ponds. On the other side, aqua-environmental compensation should improve their economic performance. The prediction of market value changes in different financial and environmental conditions was presented on the case of fish pond object Tylkowo (165 ha, northern Poland) which is located in environmentally protected areas both of ecological grounds and Natura 2000 network.

Keywords: *aqua-environmental compensation, earthen carp ponds' environmental functions, ponds' market value*

SESSION

PERCID CULTURE AND BIOLOGY

Chairmen: Damien Toner & Vlastimil Stejskal

3

**CULTURE OF EURASIAN PERCH (*Perca fluviatilis* L.) IN IRELAND:
PAST, PRESENT AND FUTURE**

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Bord lascaigh Mhara (BIM) is the state agency charged with the development of Irelands fishing and aquaculture industries. The agency has a worldwide reputation for being innovative, motivated and forward thinking in its support of Irish companies seeking to add value and volume to the industry. A core part of this innovation has been the development of novel species, as a way of creating market niches for product. Species such as Sea Urchins, Abalone, Sea horses, Ornamentals, Arctic Charr and Perch have all been actively investigated by BIM in partnership with commercial operators. In the late 1990's BIM identified European Perch as having potential in marginalised rural communities in Ireland. A publication "Cultivating Perch" was published in 1997 and Irelands first perch farm was established in 2000. Ireland is now at the forefront in development of perch farming in Europe with five licensed farms including two hatcheries. Total licensed production is 175 tonnes. The development of perch culture from initial pond based systems to state of the art RAS is discussed. The potential for further development including major expansion of the industry is outlined.

Keywords: *perch culture, RAS, novel species*

IS IT REASONABLE TO KEEP 0+ PERCH (*Perca fluviatilis* L.) WITH THE PREY FISH?

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Feeding preference and growth of 0+ perch were studied in 4 ponds with the topmouth gudgeon (*Pseudorasbora parva*) and without it. At the end of April, all ponds were stocked by free swimming perch larvae with the stocking density of 120000 ind ha⁻¹. In June, topmouth gudgeon was introduced as forage fish (40 kg ha⁻¹) into two of the experimental ponds. Topmouth gudgeon significantly influenced neither the total abundance of zooplankton nor the abundance of its groups (Rotifera, Cladocera, Copepoda). Abundance of phytophilous macrofauna was higher in the ponds stocked with topmouth gudgeon. However its abundance varied greatly corresponding more with the aquatic macrophyte biomass and species. Nauplius and copepodid stages of copepods (Copepoda) were positively selected by perch larvae (standard length (SL) < 9.5 mm). On the contrary rotifers (Rotifera) did not represent important food component for this stage (25%). Later on, perch (SL > 27 mm) preferred chironomid larvae (Chironomidae), which made 50% of food. Their importance decreased during the rest of season, whereas the importance of copepods increased again and they prevailed in food in the end of season. Cladocerans (Cladocera), especially *Bosmina longirostris*, were only less important food component (20%), although they were positively selected. Other food components, insect water larvae (Ephemeroptera, Odonata, Trichoptera), were ingested occasionally. Fish (smaller perch) appeared in food of a few perch just in the beginning of June and then in September. Specific growth rate of perch was 0.01 mm day⁻¹ in all ponds. Mean standard length (SL) of perch was 61 ± 10 mm in the ponds stocked with topmouth gudgeon, while it was 58 ± 06 mm in the ponds without it. Only 1% of the perch reached higher TL than that recorded in the ponds without the topmouth gudgeon. In this group of perch significant carnivory could be supposed.

Keywords: perch, *Pseudorasbora parva*, feeding preference, growth, pond

**EFFECT OF NUTRITIONAL AND POPULATIONAL FACTORS ON *Perca fluviatilis* L.
OUT-OF-SEASON REPRODUCTIVE PERFORMANCES**

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A way for the development of a sustainable aquaculture in European inland areas is the diversification of the production by the domestication of new species according to consumers demand and local market (mosaic model). The production of a new species requires the control of the reproductive cycle and eggs quality in order to increase larvae and juveniles availability (out-of season spawning) with high quality. However domestication processes highly modify fish reproduction performances. Even if the environmental determinism of the reproductive cycle is well known (photoperiod, temperature), broodstock performances can be influenced by numerous modulating factors, classified into three main groups: nutritional, environmental and “populational” factors. A better knowledge of this multifactorial regulation could be of great interest to optimize the domestication of a new species. Using Eurasian perch, *Perca fluviatilis* as model, we investigate the effects of three “populational” factors (broodstock size, geographical origin and domestication level) and four nutritional factors (type and quantity of food during “summer period”, type and quantity of food during the “wintering period”) using two separate experimentations. The reproduction cycle was artificially driven by photoperiod and temperature manipulations (duration: 10 months). At different sampling dates, several parameters have been measured on breeders (gonado-somatic and hepato-somatic indexes, peri-visceral fat level, condition factor, growth rate). Spawning performances have been estimated at the end of the experimentation (spawning rate, female fecundity, oocytes quality). Important modulating factors influencing out-of-season reproductive performances in Eurasian perch have been identified.

Keywords: Eurasian perch, reproduction performances, modulating factor

**THE EFFECT OF FISH SIZE AND DENSITY ON THE WEANING SUCCESS
IN POND-CULTURED PIKEPERCH (*Sander lucioperca* (L.))**

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Successful weaning of pond-cultured pikeperch juveniles is one possibility to get high-quality juveniles with lower production cost. The aim of this study was to evaluate the effect of fish size and density on the weaning success in pond-culture pikeperch juveniles. Two experiments were performed. The first experiment tested the effect of different initial densities (1; 2; 4; 8 fish.l⁻¹, each density with triplicate) of pikeperch (TL = 40.3 ± 2.3 mm; W = 0.42 ± 0.15 g) on the weaning success. The second experiment observed the effect of different fish sizes (small: TL = 40.3 ± 2.3 mm; W = 0.42 ± 0.15 g; medium: TL = 56.2 ± 2.7 mm; W = 1.66 ± 0.4 g and big: TL = 71.0 ± 3.2 mm; W = 2.95 ± 0.65 g) on the weaning success. After the first 28-days experiment, when complete juvenile weaning was performed, the highest (76.4 ± 2.5%) and lowest survival rate (33.0 ± 3.2%) were found at the highest (8 fish.l⁻¹) and lowest (1 fish.l⁻¹) fish densities. Specific growth rate (SGR) was not affected by the different fish densities. The same SGR (4.25–4.44%.d⁻¹) was observed for all fish densities. The second experiment showed the highest (77.4 ± 2.5%) and lowest (63.8 ± 5.0%) survival rate for small and big fish, respectively. The highest SGR (5.9 ± 0.2%.d⁻¹) and the lowest SGR (1.74 ± 0.1%.d⁻¹) was also found in small and big fish, respectively. No body and fin deformities of weaned pikeperch were found in all groups at the end of both experiments. In total, 0.33–2.7% of weaned pikeperch with both eyes pecked were found in all groups of both experiments. Generally, a higher percentage of pikeperch with pecked eyes were evident in groups with a higher density and smaller fish. The optimal fish density (8 fish.l⁻¹) and size (TL = 40.3 ± 2.3 mm; W = 0.42 ± 0.15 g) were verified in juvenile pikeperch during weaning under experimental intensive rearing conditions. This test confirmed a high survival rate (76.8 ± 2.95%) and a high SGR (6.1 ± 0.1%.d⁻¹) in pikeperch juveniles that were transformed from natural food to an artificial diet over a period of 28-days.

Keywords: artificial food, growth, pikeperch, RAS, survival

EFFECT OF HORMONAL STIMULATION WITH MAMMALIAN GONADOTROPINS ON CULTURED PIKEPERCH (*Sander lucioperca* (L.)) OUT-OF-SEASON SPAWNING

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Three trials of induced hormonal out-of-season spawning of cultured pikeperch were attempted. Two preparations containing human chorionic gonadotropin (hCG) (groups BI and PI – first trial) were used. In the second trial, injections of preparations containing hCG (group PI) or hCG and pregnant mare serum gonadotropin (PMSG) (groups Sr and Pg) were applied. The effectiveness of spawning in four age groups of pikeperch (aged from 2+ to 5+) stimulated with hCG (group PI) was also tested (third trial). The females from all experimental groups were stimulated once with a single dose of hormone at 300 IU kg⁻¹ body weight (BW). Females from the control group were injected with a 0.9% solution of NaCl (group C). The commercial fecundity (CF; % BW), latency time (LT; h), and eyed-eggs survival (EG; %) were determined. Fish aged 2+ matured significantly more slowly than older females (trial 3; $P < 0.05$). No progress in oocyte maturation was noted among the females from group C. Significant differences in trial 2 were noted in LT, which was lower in group PI than in groups Sr and Pg ($P < 0.05$). The youngest fish (2+; trial 3) exhibited significantly lower values of indexes CF and EG in comparison to older fish ($P < 0.05$). The hormonal preparations used had a positive impact on the ovulation and spermiation of the cultured pikeperch. No improvement in the effectiveness of out-of-season spawning was noted when preparations containing hCG and PMSG were used (groups Sr and Pg).

Keywords: *hormonal stimulation, mammalian gonadotropins, out-of-season spawning, percids, pikeperch*

SESSION

STURGEON CULTURE AND BIOLOGY

Chairmen: Harald Rosenthal & Martin Kocour

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**CULTIVATION OF ENDANGERED SPECIES TO ACHIEVE „FITNESS FOR SURVIVAL“
AT RELEASE INTO NATURAL HABITATS**

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Aquaculture for production and culture for stocking natural waters are two VERY different objectives requiring TOTALLY different methodologies to meet specific goals. Most of the technical approaches for both purposes are similar. This is a mistake. While **aquaculture production systems select for** (a) best survival, (b) high food conversion via feeds designed for best biomass gain, (c) fast growth rates to shorten grow-out time (d) disease resistance, (e) cost-effective production and finally (f) for high quality of the end product, **the culturing for restocking** needs to provide progeny with the ability to (a) rapidly respond to changing environmental cues, (b) adjust to behavioural traits (diurnal/nocturnal rhythms, (c) recognize predators to avoid them, (d) display robustness to abruptly changes in water quality (e.g. thermoclines; oxygen depletion; high turbidity and changing micro-light climate and wind/wave action), (e) resist challenges by pathogens/parasites, and (f) finally to effectively interact with behavioural traits of con-specifics (perfect intermingling). In short, culture for release must be designed to allow progeny at the time of release to match the „FITNESS“ required for survival in the receiving habitat while also „MATCHING“ the characteristics in all traits of the con-specifics in natural populations. Although this is logical, most stocking and rehabilitation programmes lack a cohesive concept and systematic approach to meet these requirements. We present here a framework based on recently developed strategies for sturgeons (species of high longevity, low residual population size and specific habitat-dependent traits. The conventional hatchery systems offer largely monotonous life conditions of little use to prepare the progeny for the harsh and challenging environmental conditions which they are facing in nature after release. Step by step adaptation to these challenges in a standardized format can help improving performance, including the transition to natural foods, exposure to predators, as well as acclimation to diurnal rhythmic cues (lunar cycles, tidal currents, wind and wave action, etc).

Keywords: *sturgeon, aquaculture, culture for release, stocking programme, behavioural traits*

**CULTURE OF THE AMERICAN PADDLEFISH (*Polyodon spathula*):
THIRTY-FIVE YEARS OF COLLABORATIVE RESEARCH**

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The American paddlefish is one of two extant species in the family Polyodontidae. It filters feeds on plankton, and is long lived and grows to a large size. It has many primitive and unusual physical and physiological characteristics. These include a skeletal system that is almost entirely cartilaginous, firm shark-like meat and large, dark eggs which are highly valued as caviar. These characteristics, as well as its vulnerability to overfishing have stimulated interest in developing culture techniques. Artificial propagation and manipulations of the reproductive system have been advanced through collaborative research, where the combining of different areas of expertise have facilitated innovative protocols. Ovulation induction has been improved (hypophysis therapy has been replaced by GnRh_a), gamete collection is more efficient (MIST technique has replaced stripping of eggs), nursery techniques have been advanced (intensification), ploidy (gynogenesis) and phenotypic sex manipulation (steroid-induced sex reversal) protocols have been developed, food technology for value-added products (smoking & retort packaging) have been explored, and currently, production optimization during grow-out is being developed (reservoir ranching & polyculture). The American paddlefish will increasingly become a valued foodfish throughout the world as these improvements in culture become adopted.

Keywords: growth, artificial propagation, nursery techniques, gametes, food technology

CORTISOL IMPLANTATION MAY ENHANCE GROWTH PERFORMANCE IN GREAT STURGEON, *Huso huso*

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The objective of current study was to examine the role of cortisol in long term on the growth performance of female great sturgeon, *Huso huso*. Fifteen three-year-old great sturgeons with initial weight and length of 6809.7 ± 72.9 g (mean \pm S.E) and 107.2 ± 1.7 cm, respectively, were raised under natural water temperature and day-length light regime. Three treatments and five fish for each treatment were assigned for this experiment. Fish were intraperitoneally implanted every six week with cortisol in a cocoa butter pellet for 180 days. The implant doses were 0 (as control), 5 and 50 mg cortisol/kg BW. The fish were fed with commercial diet twice a day. At the end of the experiment, body weight was the lowest in fish receiving the high cortisol dose (6350 ± 499.7), intermediate in the control (7354 ± 352.2) and highest in those implanted the low cortisol level (7507 ± 316.4). Similarly, weight gain, body weight increase, specific growth rate, and condition factor were significantly lower in fish implanted with 50 mg cortisol/kg than in either of the other two treatments. These results show that excess cortisol has negative effects on growth performance, while moderate increases in plasma cortisol concentration can stimulate growth in great sturgeon.

Keywords: chronic stress, growth, cortisol, sturgeon

OPTIMALIZATION OF CHROMOSOMAL MANIPULATIONS IN ACIPENSERIDS, A REVIEW

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Present work reviews goals and problem of chromosomal manipulation in sturgeons directed on sex ratios control for optimization of caviar production. Gynogenesis can allow manipulation with sturgeon sex ratios, but available data about sex rates in gynogenetic sturgeon progeny reveal strong differences between sturgeon species studied so far. It can indicate that they have different sex determination systems or autosomes have an influence on sex determination. Percentage of females in gynogenetic progeny of several sturgeon species is in average 65–80%. It could mean that these species have ZW female heterogametic sex determination system, and WW super females can be theoretically obtained. The main problem of gynogenotes production is low survival rates because the methods of diploidy restoration have low efficiency. Therefore sexing should be provided as early as possible. The method that allows the earliest sexing of sturgeon is blood plasma sex steroid analysis, but anyway it can be applied only on 2- to 3-year-old sturgeon. Based on available data about survival and sex rates in gynogenetic sturgeon progeny we suppose that bigger amount of viable gynogenetic progeny of sterlet can be obtained by applying optimized condition of treatments and they might have mainly female progeny.

Keywords: *sturgeon, chromosomal manipulation, gynogenesis, sex determination*

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THE INVOLVEMENT OF KISSPEPTIN AND GONADOTROPINS IN PUBERTY IN THE RUSSIAN STURGEON (*Acipenser gueldenstaedtii*)

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Sturgeon is the major source of black caviar, with 95% of the world supply from the Caspian Sea fisheries. However, sturgeon overfishing, pollution, and habitat loss have led to the decline of the caviar supply and placed these sturgeon fisheries in risk. The high price of caviar, driven by strong international demand, has created the impetus for sturgeon farming which provides an alternative supply of meat and caviar. Sturgeon is late maturing fish, with the females reaching puberty under culture conditions at the age of 6–10 years. The project's long-term goal is to synchronize the puberty in female Russian sturgeon, by studying the endocrine regulation of puberty. We have already cloned and sequenced the three subunits of the pituitary gonadotropins (GTHs) from Russian sturgeon. The first objective of this study was to produce recombinant (r) GTHs and develop a highly specific homologous ELISAs for the measurements of FSH and LH levels. We produced sturgeon (s) rLH and FSH as single-chain polypeptides in the methylotrophic yeast *Pichia pastoris*. Glycoprotein subunit α was joined with sLH β or sFSH β mature protein-coding sequences to form a fusion gene that encodes a "tethered" polypeptide. Recombinant GTHs were used to develop specific and homologous competitive ELISAs for measurements of FSH and LH in the plasma and pituitary of sturgeon, using primary antibodies against rsLH β or rsFSH β , respectively, and rtLH $\beta\alpha$ or rtFSH $\beta\alpha$ for the standard curves. It is recently become clear that kisspeptin/KISS1R system has an important role in initiating GnRH secretion at puberty. We cloned the sturgeon kisspeptin-1 receptor (sKISS1R). A comparison of the sKISS1R with those of the human, tilapia, eel or zebrafish proteins showed identities of 53%–56%. The mRNA expression levels of sKISS1R significantly decreased with the ovarian development.

Keywords: gonadotropins, kisspeptin receptor, puberty, ELISA

SESSION

NUTRITION AND JUVENILE ASPECTS OF FINFISH CULTURE

Chairmen: Jacek Wolnicki & Martin Bláha

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TEMPERATURE-RELATED GROWTH PERFORMANCE, FOOD CONVERSION AND BODY DEFORMITIES IN JUVENILE TENCH *Tinca tinca*

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Effects of temperature on the growth, condition coefficient, food conversion ratio (FCR) and occurrence of body deformities were studied in juvenile tench. The fish were reared at 20; 25 or 30 °C and fed a formulated diet or frozen Chironomidae larvae. To avoid comparing fish of different “physiological age” and contrasting size, daily food rations and duration of rearing were adjusted to different temperatures basing on a correction factor q (1.000; 0.659; 0.444). Thus, at 20 °C fish were reared for 91 days, at 25 °C for 60 days and at 30 °C for 40 days. Daily food rations were also adjusted according to fish biomass and differences in hydration found between the two diets. No mortality occurred during the experiment. Fish size and condition coefficient values were significantly higher in fish fed a formulated diet compared to those fed Chironomidae, irrespective of temperature. The highest relative growth rate values were obtained at 30 °C; 3.41 and 1.91% d⁻¹, for a formulated diet and chironomids, respectively. Thus, the optimum temperature for the growth of juvenile tench was determined to be close to 30 °C, while the optimum temperature for food utilisation was 20–25 °C, where significantly the lowest values of FCR were recorded. Body deformities were found in fish fed a formulated diet. Significantly the lowest share of 31% of fish with visible deformities occurred at 20 °C, while 48% and 45% at 25 and 30 °C, respectively.

Keywords: *deformities, food conversion, growth, temperature, Tinca tinca*

**PASSING TIMES OF TWO TYPES OF FEEDS IN WELS (*Silurus glanis*)
AT THREE DIFFERENT TEMPERATURES**

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Passing times of two types of feeds were determined on wels (*Silurus glanis*) at three temperatures. Fingerlings (38.03 ± 8.04 g) were fed by artificial dry feed (commercial catfish feed) or forage fish. Treatments were carried out (10 fish/aquaria) in 3 replicates. Fish were individually tagged by pit tags. Temperatures applied were as follows: 15 °C, 20 °C and 24 °C. Appetite of fish highly depended on temperature – as was expected. At 15 Celsius grade only 42.4% of the experimental fish accepted the granulated feed, while this ratio was 90.9% at the forage fish fed group. At higher temperatures 100% of the experimental fish fed in both feed groups. The daily mean consumed feed varied between 3.20–10.68% of body weight in case of forage fish and 1.36–4.46% in case of artificial feed. At low temperature (15 °C) the quantity of consumed feed met the the measurement's error treshold, what made the determination of passing time uncertain. At 20 °C the excretion started 26 hours post feeding and lasted 22 hours, while at 24 °C it started 10 hours after feeding and lasted for an other 18 hours.

Keywords: *feed, passing time, Silurus glanis, temperature, wels*

SESSION

REPRODUCTIVE PERFORMANCE IN FINFISH

Chairmen: Zoi Yaron & Mirosława Sokołowska-Mikołajczyk



PAST EXPERIENCE AND FUTURE PROSPECTS IN CARP SPAWNING

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Hypophysation using pituitary homogenate to overcome the dysfunction of the endocrine axis leading to spawning of carp in Israeli hatcheries has frequently failed due to the inconsistent potency of the injected material and the unknown ovarian stage of the recipient fish. Hypophysation has improved since 1984 through the introduction of a standard carp pituitary extract in which luteinizing hormone (LH) activity is calibrated (CCPE). Induction of spawning was successful mainly in females where $\geq 65\%$ of the oocytes has migrating germinal vesicles. Further, due to decreasing quantities of processed carp and the expansion of ornamental carp production (koi and goldfish), the growing demand for CCPE could not be met, and the alternative hypothalamic approach was introduced. Dagin combines a superactive analog of sGnRH (10 $\mu\text{g}/\text{kg}$) and the water-soluble dopamine (D2) receptor antagonist, metoclopramide (20 mg/kg). The progress of oocyte maturation in ovarian biopsies was studied in parallel with changes in hormone levels following a single injection of Dagin. The study indicated that the gradual increase in LH and maturation-inducing steroid (MIS; 17α , 20β , DHP) were similar to those in fish given priming and resolving doses of CCPE. This explains why Dagin is effective even when given in a single injection. Future prospects raise the possibility that by employing molecular tools, a recombinant LH will be produced that will have the regular and expected potency of the hypophyseal approach without the risk of spreading pathogens from donor fish to broodstock. Work along this line is currently in progress.

Keywords: *carp, spawning, hypophysation, Dagin, recombinant gonadotropins*

GHRELIN, LEPTIN AND KISSPEPTIN IN FISH REPRODUCTION

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LH release in teleost fish is controlled by the environmental factors and endocrine system. Gonadoliberin (GnRH) is the main factor stimulating LH secretion and dopamine is the strongest inhibitor of LH release at the level of the hypothalamus and pituitary gland. The mechanism of action of these hormones is well known in different species because of the extensive investigation in the last decades. In fisheries practice GnRH agonists and dopamine antagonists are widely used for the control of spermiation, ovulation or spawning synchronisation. The effectiveness of the methods for spawning induction depends, among others, on the type of hormonal preparations as well as the species of fish. Optimization of the methods used in the controlled reproduction is still possible, not only by searching for the best dosage or timing of hormonal treatment but also by the investigation of the role of other hormones, which may be involved in gonadotropin synthesis and release. Recently the newly discovered hormones (ghrelin and leptin) responsible mainly for the energetic homeostasis were shown to be involved in the control of LH secretion in fish, however their role in this process is not fully elucidated. Another newly discovered peptide – kisspeptin, which has a very strong gonadoliberin release potency in vertebrates, has a potential to become one of the most important factors, besides GnRH, controlling LH synthesis and secretion in fish. The investigations in this field may soon give the new agent, which will be used in the control of reproduction in fisheries practice.

Keywords: *fish reproduction, ghrelin, kisspeptin, leptin*

**INFLUENCE OF SHORT AND LONG-TERM EXPOSITION ON ROUNDUP
ON LH LEVELS IN *Carassius gibelio***

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Roundup is the most extensively used herbicide in world agriculture. It is considered as safe to animals, so it can be used in water environment. However, the recent studies show its harmful effects on animals. In this study we examined the influence of Roundup on reproduction of *Carassius gibelio*. Fish were kept in two glass tanks (control and experimental) with aerated water in temperature 22 ± 1 °C. To the experimental group Roundup was added at the concentration of 15 mg l^{-1} and control group was kept without herbicide. Short-term exposition lasted 10 days, and long-term exposition – 6 months. At the end of the experiments samples of blood were collected and all fish were injected with salmon GnRH analog (sGnRH-A $10 \text{ } \mu\text{g kg}^{-1}$ of b.w.) and pimozide (dopamine antagonist $5 \text{ } \mu\text{g kg}^{-1}$ of b.w.). Six, 12; 24 and 48 hours after injection, blood samples were taken from all fish. LH levels were measured with the use of ELISA method. Also gill samples from decapitated fishes were collected and fixed in 4% formaldehyde. For histopathological investigation they were stained with heamatoxin and eosin (H&E). Analysis of LH concentrations didn't show any significant differences between LH levels in experimental and control group in both times of exposition. However the pathological changes in gills (hypertrophy, epithelial hyperplasia and subepithelial edema) in fish from experimental group in short and long-term exposition were found.

Keywords: *Carassius gibelio*, fish, gills, LH, Roundup

INFLUENCE OF TEMPERATURE ON HORMONAL INDUCTION OF OVULATION IN TENCH (*Tinca tinca* L.)

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One of the specific characteristics of tench (*Tinca tinca*) is the ability to undergo successful ovulation after self-administration of low dose of GnRHa despite the presence of dopamine inhibition of LH release typical for all cyprinids. By *in vivo* trial we investigated which hormonal treatment would be the best option for induction of ovulation under unfavourable temperature conditions and whether dopamine inhibition is more prominent under these conditions. Standard hormonal treatments (CPE, mGnRHa) together with combine treatment (dopamine D2 receptor antagonist metoclopramide with mGnRHa) were applied under two suboptimal (18 °C, 26 °C) and one optimal temperature regimes (22 °C) for tench reproduction. Ovulation success and fecundity index were evaluating. No significant differences were determined for GnRHa and combine treatment under all thermal regimes whether in ovulation success ($\geq 75\%$) or in fecundity index (8.60 ± 1.4). Contrary to other treatments CPE induced the lowest number of ovulation (18 °C = 37.5%, 22 °C = 50% and 26 °C = 62.8%) under all thermal regimes. We can concluded that despite the demonstrated dopaminergic inhibition in tench no improving with regard to ovulation number or fecundity index was recorded after addition of dopamine inhibitor in compare to self-administration of GnRHa under suboptimal conditions. As an optimal hormonal treatment under suboptimal temperature conditions we suggest self-administration of low dose of GnRHa.

Keywords: aquaculture, cyprinid, ovulation, reproduction

**INDUCTION AND ADVANCEMENT OF OVULATION IN BROOK CHARR
(*Salvelinus fontinalis* Mitchell) USING ADMINISTRATION OF EMULSIFIED
D-Arg⁶Pro⁹NEt sGnRH_a**

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Effects of physiological saline (PS) dissolved sGnRH_a (sGnRH_a-PS) or Freund's incomplete adjuvant (FIA) emulsified sGnRH_a (sGnRH_a-FIA) on the induction and advancement of ovulation were assessed in brook charr. Two years old females were randomly divided into 5 groups. Group A and group B were treated with intraperitoneal (ip) injection of sGnRH_a-FIA in dosages 50 and 25 µg.kg⁻¹ body weight (BW), respectively. The females in group C were injected with ip double injection (DI) of sGnRH_a-PS in dosage 25 µg.kg⁻¹ BW spaced 3 days apart, whereas the fishes in group D received only a 25 µg.kg⁻¹ BW ip single injection (SI) of sGnRH_a-PS. Group E was a control group treated with PS, only. After stripping, ovarian plasma pH-level was measured and egg sample was taken from each female to examine one egg weight (OEW), one egg diameter (OED) and survival to the eyed stage (SES). All GnRH_a treated groups ovulated significantly earlier than the control group ($P < 0.01$). There were, however, no significant differences found in ovulation dynamic and latency interval among all GnRH_a treated groups. Ovarial plasma pH level was significantly higher in groups A, B, C compared to group D and E ($P < 0.05$). Significantly lower OEW level than in control group was found in group B ($P < 0.01$). Other GnRH_a treated groups tended to have lower OEW than the control group, too. The OED levels copied the OEW values. The SES level was significantly higher in all GnRH_a treated groups compared to the control. The percentage of eyed eggs was negatively correlated with OEW level ($r = -0.44$; $P < 0.05$). No mortality (prespawning or postspawning) was observed in all groups during 5 month- period. The sGnRH_a-FIA injections appeared to have the same effectivity as the DI protocol with sGnRH_a-PS.

Keywords: induction of ovulation, GnRH_a, Freund's incomplete adjuvant, brook charr

THE EFFECT OF SELECTED OVULATION-INDUCING PREPARATIONS ON POST-STRIPPING MORTALITY AND REPRODUCTIVE INDICATORS OF CULTURED EUROPEAN GRAYLING (*Thymallus thymallus* L.) FEMALES

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Effectiveness of two commercially available hormonal preparations (Gonazon™ and Supergestran) contains mGnRHa was evaluate for induction and synchronization of ovulation and post-spawning mortality in cultured European grayling (*Thymallus thymallus* L.) broodstock. Four years old marked grayling females (n = 80) were divided randomly into four groups. The groups were treated with intramuscular injections as follows: No. 1 with Supergestran (Nordic Pharma s.r.o, Czech republic) at a dose of 30 µg.kg⁻¹ body weight (BW), No. 2 with (Gonazon™ (Intervet International B.V., Boxmeer, the Netherlands, 30 µg.kg⁻¹ BW), No. 3 with physiological solution (0.9% NaCl, Braun Melsungen AG, Germany) and No. 4 was left without any treatment as a control group. No effects of treatment on total number of ovulated females (70–80%) were found at the end of stripping period. Slightly higher (not significant) percentage of ovulated fish in first stripping time (3 days post injection) was observed in Gonazon group. The 30days post-stripping mortality was unaffected by treatment in all groups. The significant differences were found in fertilization rate of egg samples from first stripping time. The highest fertilization (97.6%) was observed in Supergestran group, lower ones (60.9 and 65.1%) in SP and control group and the lowest fertilization (38.8%) in Gonazon group. All other reproductive parameters were unaffected by treatment in all groups.

Keywords: grayling, GnRH, Gonazon, ovulation, Supergestran

ENDOCRINE CHANGES AT THE ONSET OF GAMETOGENESIS IN THE ROACH (*Rutilus rutilus*)

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Understanding the endocrine regulation of reproduction in fish is a prerequisite for sustainability of commercial aquaculture. However, the onset of gametogenesis remains largely unexplored. The present study investigated the seasonal profiles of plasma sex steroids in relation to expression of gonadotropins in the pituitary of adult roach (*Rutilus rutilus*) from Lake Grosser Mueggelsee (Berlin, Germany). Furthermore, given the importance of nuclear steroid receptors and aromatase for sex steroid feedback regulation of gonadotropins, the mRNA-expression of brain-type aromatase (Cyp19b), androgen receptor (AR) as well as oestrogen receptor isoforms was studied at the pituitary level. Investigation by real-time RT-PCR revealed a significant up-regulation of follicle-stimulating hormone β (FSH β) mRNA in the pituitary of both genders at the onset of gametogenesis in July, whereas luteinizing hormone β (LH β) mRNA increased later on in August. In males, the increase of FSH β mRNA in July coincided with rising plasma 11-ketotestosterone concentrations. In females, however, 17 β -oestradiol in plasma increased later, not until August, shortly before vitellogenesis. Expression of sex steroid receptors in the pituitary showed only minor fluctuations in relation to season or gonadal stage, with AR mRNA showing highest level in the pre-spawning period in both genders. On the other hand, Cyp19b mRNA-expression increased in parallel with FSH β already before any changes in plasma 17 β -oestradiol or aromatizable testosterone occurred, suggesting that the expression of both genes in the pituitary is regulated by other factors at this period. In conclusion, the present study investigated, for the first time in roach, the seasonal expression of key genes for reproduction in the pituitary and plasma levels of different classes of sex steroids. The results indicate an important role of FSH and aromatase during early gametogenesis.

Keywords: aromatase, cyprinids, gonadotropins, pituitary, sex steroids

PROGRESS IN THE METHODS USED FOR GRADING FISH SPERMATOZOA QUALITY FOR EVALUATION OF THEIR POTENT SWIMMING PERFORMANCES

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For the purpose of fish artificial reproduction, evaluation of sperm quality varies broadly depending on technologies used for such goal and on needs of fish farmers. In the past, rustic technology for motility estimation has been using for a long time a simple microscope in which case grading is mainly relying on eye appreciation of the proportion of moving cells in the observation field. Two obvious limitations occur when using such approach: 1- fish spermatozoa performances drop down rapidly during the short period following motility activation, 2- the quantification of percentage of moving cells "by eye" is approximate. Therefore, a first improvement has consisted of using recording tools such as photography, cinematography and more recently video-microscopy. In all three cases, such records allow analysis of still frames or visualization on slow-motion cine or video sequences. An additional improvement, mostly resulting from digitized video records, is the possibility to apply automatic evaluation of the swimming performances of a population of spermatozoa, by use of the so-called CASA (Computer Aided Sperm Analysis) after adaptation to specific situations encountered by spermatozoa of different fish species, specially related to their high translational velocity. In contrast to the above mentioned technologies which are focused on movement quantification of sperm heads, a complementary approach appeals the use of higher microscopic magnification allowing visualization of flagella by dark field or phase contrast optics when combined to stroboscopic illumination and leading to the possibility of quantifying flagella parameters in terms of shape, wave length or amplitude, beat frequency but also to detect local damages responsible for the decrease of their swimming performances. These parameters as well as additional ones can nowadays be reached by use of high-speed video cameras providing high resolution, up to 1280 x 1024 pixels in combination with high images rate, up to 2000 frames per second.

Keywords: *fish spermatozoa, sperm velocity, swimming performance, flagella parameters*

HOW DOES BISPHENOL-A MODULATE REPRODUCTIVE PHYSIOLOGY PERFORMANCES IN MALE FISH?

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The main objectives of the present study were to investigate sex steroid synthesis and their regulatory genes, vitellogenin production and sperm quality in male goldfish exposed to bisphenol A (BPA) at environmentally relevant concentrations. Mature males were exposed to BPA in range between 0.0 and 20 ug/L for 30 or 90 days during the spawning season. Blood plasma, tissue (gonad and liver) and sperm samples were collected for analyzing sex steroid and vitellogenin (VTG) levels, gene expression and sperm motility using ELISA kits, qRT-PCR and Micro-image system, respectively. Sperm motility and velocity decreased in fish exposed to BPA at >1.5 ug/L after one month. Adverse effects of BPA might be corresponding to decrease of androgens that regulate sperm maturation. Decrease of androgens, StAR and AR were observed in males exposed to BPA at lower than 2 ug/L for one month. This suggests anti-androgenic activity of BPA through lower delivery of cholesterol into sex steroidogenesis pathway. In males exposed to BPA at 2 or 20 ug/L for longer than 2 months, increase of VTG level, VTG mRNA expression, ER-beta subtypes and CYP19A mRNA expression were observed, but ER-alpha did not show significant increase. Therefore, BPA can also acts as estrogenic mimics at high concentration via ER-beta subtypes agonist which enhances VTG production or via increase of CYP19A, which convert androgens to estradiol. In conclusion, BPA showed both anti-androgenic and estrogenic modes of action depending on concentrations and exposure period.

Keywords: androgens, AR, CYP19a, ER subtype, sperm, StAR, VTG

SESSION

GENETIC AND MOLECULAR APPROACH IN FINFISH AQUACULTURE

Chairmen: Katsutoshi Arai & Martin Flajšhans

**PARENTAGE ASSIGNMENT WITH MARKERS: A PROVEN ENABLING TECHNOLOGY
TO DEVELOP EFFICIENT BREEDING PROGRAMMES IN FISH**

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The implementation of breeding programs in aquaculture remains low, except for a few major species – despite a proven potential to boost productivity. One of the reasons for this is the high investment needed to set up efficient breeding programs using the traditional “separate rearing of families” technology. Genetic markers now permit to recognize families reared together in a single tank or pond, rendering the use of many separate rearing units obsolete. Based on experience, we can now assure that such markers are reliable and effective, and can be used to progressively enable breeding programs for simple and cheap mass selection to more controlled and efficient methods using family information. This is a major step forward to allow the dissemination of breeding programs in species which only have a limited production volume. Both practical results showing the efficiency of parentage assignment and the concept of progressive investment to build elaborated breeding programs will be presented. Limiting factors and the potential of new genotyping methodologies will be discussed.

Keywords: *microsatellites, parentage assignment, selective breeding*

INDUCTION OF VIABLE GYNOGENETIC PROGENY USING EGGS AND UV IRRADIATED SPERM OF CHINESE TETRAPLOID LOACH, *Misgurnus anguillicaudatus*

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When eggs of Chinese tetraploid loach with 100 chromosomes were fertilized with UV irradiated sperm, resultant gynogenetic progeny were viable without any treatment to duplicate maternal chromosomes and they survived beyond the feeding stage to the adult stage. Gynogenetic progeny were diploid because they had 50 chromosomes and two Ag-NORs, CMA₃ positive sites and FISH signals for rDNA loci. In contrast, when gynogens were induced from eggs of diploid loach without chromosome doubling after fertilization with UV irradiated sperm, all the progeny were inviable haploid gynogens with 25 chromosomes and one Ag-NOR, CMA₃ positive site and FISH signal. These observations demonstrate the true genetic tetraploidy of the Chinese loach with 100 chromosomes and thus they are able to use as a source of functional diploid gametes for further expansion of ploidy manipulation.

Keywords: *chromosome, gametes, FISH, polyploid*

CONSERVATION OF FISH GENETIC RESOURCES IN LIVE GENE BANKS. FIFTEEN YEARS OF CZECH EXPERIENCE

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The present National programme of conservation and utilization of genetic resources of farm- and other animals important for nutrition, agriculture and forestry has been launched in the Czech Republic already in 1996 with common carp and trout genetic resources as an integral part. From the very beginning, its goal was to keep old, less productive breeds as a part of national heritage and as a source of genes for contemporary breeding. Nowadays, altogether 12 Czech and Moravian fish farms maintain in total 40 live gene banks of common carp, tench, rainbow trout and wells (with 11; 8; 3 and 2 breeds, respectively), 2 pure populations of brown trout and pure species of great maraena, Northern whitefish, sterlet and beluga. All fish genetic resources have undergone genetic analyses to assess their genetic purity, variability and genetic distances of breeds within each species. Mean annual state subventions to the fish farms comprise 46.55% of direct costs for maintenance of live gene banks. Bank of cryopreserved sperm is operated by the authors' faculty, maintaining at present 4018 insemination doses of 48 fish genetic resources. Fish genetic resources play indispensable role in sustainable development of fisheries and water management of Central European pond ecosystems, contribute to the development of agrotourism and their cultural importance is reflected in regional gastronomy and artistic works.

Keywords: *fish, genetic resources, conservation, live gene banks, sperm cryoconservation*

This study was supported in part by the National Programme of Conservation and Utilization of Genetic Resources of Farm- and Other Animals Important for Nutrition, Agriculture and Forestry of CZ Ministry of Agriculture and by projects CENAKVA CZ.1.05/2.1.00/01.0024 and GAJU 046/2010/Z.

PLOIDY MANIPULATION USING DIPLOID SPERM OF A WILD TETRAPLOID GINBUNA (JAPANESE SILVER CRUCIAN CARP, *Carassius auratus langsdorffii*)

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Tetraploid strains are important sources of diploid gametes available for an expansion of ploidy manipulation. Although artificial induction of tetraploid strains has been attempted by inhibition of a mitotic cell division of zygotes using hydrostatic pressure or temperature treatments at the early developmental stage, successful examples of viable and fertile tetraploid fish were very rare, because most resultant tetraploid progeny exhibited extremely high mortality. Natural tetraploid strains are considered another resource of diploid gametes. In the present study, we tried to induce new strains of tetraploid, triploid and androgenetic diploid using diploid sperm of a tetraploid ginbuna (Japanese silver crucian carp *Carassius auratus langsdorffii*), which caught at the lake Shiro-numa, Gunma Prefecture, Japan. New tetraploid fish were induced by fertilizing eggs of a diploid goldfish *Carassius auratus* with diploid sperm of the tetraploid ginbuna, followed by heat-shock (40 °C for 45; 60 and 75 s) to suppress the release of second polar body at 10 min after fertilization. Although a small number of the new strain of tetraploid fish survived, we could not obtain any functional gametes from them. Triploid fish were successfully induced by fertilizing eggs of a diploid goldfish with diploid sperm of the tetraploid ginbuna. The some of resultant triploid males produced aneuploid sperm at the age of maturation. Androgenetic diploid fish were induced by fertilizing UV irradiated eggs of diploid goldfish with diploid sperm of the tetraploid ginbuna. A mature androgenote produced fertile haploid sperm.

Keywords: chromosome manipulation, cyprinid fish, diploid gamete, polyploid

GROWTH HORMONE GENE IN TENCH, *TINCA TINCA*: DISTRIBUTION OF ALLELES ACROSS POPULATIONS AND POSSIBLE USE OF THE KNOWLEDGE IN BREEDING

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The growth hormone (GH) gene belongs to suitable genetic markers for population and breeding studies in fish. In this study, an attention was paid to tench, species with a potential to diversify global fish market. First, tench GH gene was sequenced in twelve individuals from populations across a microsatellite-based Neighbor-Joining tree to characterize GH gene polymorphisms; second, five polymorphic sites (PSs) were selected to determine distribution of GH gene haplogroups in sixteen tench populations from Europe and Asia, feral and cultured. Sequencing of tench GH gene was performed using an available cloning kit. Haplogroups distribution was detected by RFLP (in SNPs) and fragment length analysis (in indels) at 15–32 individuals per population. In the first step, it was found that the tench GH gene is 1758–1761 bps long and has five exons and four introns. Altogether, 16 PSs were detected. Four of them were located in exons, all as synonymous substitutions. Two main phylogroups of GH gene haplotypes, eastern and western, were identified. During the second part of study, selected PSs enabled distinguishing of five haplogroups, three corresponded to the western and two to the eastern phylogroup. Individual populations bore one to four different haplogroups. Populations from Turkey, China and Spain had only alleles of the eastern phylogroup. The others displayed alleles of both phylogroups with various allelic and genotypic frequencies. The highest degree of differentiation (F_{ST}) of 0.794 was observed between blue coloured tench and a Spanish cultured population, and 67.5% of all pairwise F_{ST} values were significantly different from zero. A Neighbor-Joining tree based on Nei's standard genetic distances divided investigated populations into two major groups. Knowledge gained from the study can serve for testing the effect of GH gene haplogroups on growth performance of tench as also intron polymorphisms associated with growth have been observed.

Keywords: growth hormone, haplotype, molecular biology, sequencing, tench

GENETIC STRUCTURE OF WHITEFISH (*Coregonus lavaretus*) POPULATION IN MIEDWIE LAKE – A „NEW” SPECIES IN AQUACULTURE

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In Poland whitefish used to be present in over 200 lakes as a result of numerous introductions that has started in XIX century. First high scale introductions were reported in 1878. Beside this, whitefish autochthonic populations were found and characterised in Górznińskie, Wigry, Łebsko and Miedwie Lakes, Szczecin Lagoon and Bay of Puck. Presently whitefish populations have shrunk and exist in about 30 lakes. In Europe 92 subspecies of *C. Lavaretus* has been described until 1950, whereas 300 local forms around the world. For instance, taxonomic research conducted in Poland revealed 4 subspecies of whitefish. One of them is Miedwie whitefish (*Coregonus lavaretus maraena*) native to Miedwie and Wigry Lake (*Coregonus lavaretus maraena natio*). After environmental disaster in 1975, due to oxygen deficit below 12 m depth, Miedwie whitefish population has been regarded as extinct. Occasionally single cases have been reported by fishermen until 1985. Subspecies of *C. lavaretus* form Miedwie Lake is well known for its high growth rate. Usually they inhabit deep cold lakes with other species, such as peled (*Coregonus peled*), and are thought to produce hybrids with them. Their offspring with mixed features has been reported in polish lakes. Hybrids are also a result of artificial production by fishermen. The aim of this work was to characterize whitefish population in Miedwie Lake by means of genetic analysis of ND-1 (NADH dehydrogenase 1) gene and ITS1 (internal transcribed spacer 1) region in order to distinguish native forms from hybrids with peled. In analysis, archival samples of *Coregonus lavaretus maraena* from Berlin Museum have been used. Genetic analysis performed using MEGA 4.0 software unequivocally indicated that samples from Miedwie Lake belong entirely to native (fast growing) form of the whitefish. Moreover, this research has also provided a crucial information for *C. lavaretus* management program for Miedwie Lake.

Keywords: *Coregonus lavaretus*, population structure, whitefish, ND-1, ITS1

**INVESTIGATIONS ON DIFFERENT LOCAL STRAINS OF ARCTIC CHARR
(*Salvelinus cf. umbla*) AND THEIR SUITABILITY FOR AQUACULTURE**

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Included in a research project on aquatic genetic resources Arctic charr populations from southern Germany were investigated concerning their genetic characters and their usability for aquaculture. Populations from 9 alpine lakes were sampled and genetically characterized. Moreover, mature spawners were caught, stripped and their eggs were hatched under controlled conditions. After first feeding, samples of 5 selected origins were raised over a period of 7 months under controlled aquaculture conditions. During this experimental period growth, feed conversion ratio, condition, survival and health status were investigated. As a result, first feeding of wild broodstock turned out to be problematic: in the beginning commercial dry feeds offered were not well accepted. Optimal food acceptance could only be reached by additional feeding of frozen zooplankton. After this, fish from all populations continued to grow on quite satisfactory and mortality rates were low. However, growth performance decreased during the ongoing experimental period, which was due to increased gonadal development. At the end of the trial a large variation in the individual growth performance was observed, which is especially true for the origins Lake Tegernsee and Lake Walchensee. In comparison, Arctic charr from Lake Hintersee and Lake Starnberger See demonstrated a better performance. Under the environmental conditions applied, best fish growth was found in the Lake Constance-population. In summary, growth performance of the investigated wild stocks did not reach the performance of Arctic charr populations from aquaculture. However, differences between the southern German populations were found, which gives further perspective for their use in local aquaculture.

Keywords: *aquaculture, arctic charr, genetic characterization, growth performance*

PROTEOMICS ON THE VIABILITY AND FERTILITY IN SALMONID HYBRIDS

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Interspecific crosses of salmonid fish attempted so far in aquaculture and stocking programs are mostly not viable or have low fertility. Comparative proteomics using two-dimensional gel electrophoresis (2-DE), MALDI-TOF mass spectrometry analysis and protein database searching was conducted to elucidate the molecular mechanisms for the inviability of hybrids between female masu salmon and male rainbow trout (MR) and for the sterility of viable hybrids between female brook trout and male masu salmon (BM), with parental species served as controls. Most MR hybrid embryos died rapidly between 10 and 20 days after fertilization (daf), showing severe dwarfism and various numerical and structural chromosome abnormalities. 2-DE protein expression profile was typically stage-dependent in the early MR hybrid embryos, with sharp decrease of discernible protein spots on 20 daf. Such proteins identified in the MR embryos were mostly down-regulated products of house-keeping genes and those involved in nucleic acid metabolism or chromatin replication. These results imply primary association of impairment of nucleic acid metabolism with the occurrence of chromosome abnormalities, which might have initiated the observed inviability through lowered or null expression of house-keeping genes. In the sterile BM hybrids, most females and males had undeveloped gonads, with abnormal germ cell development shown by histological examination. Multiple comparisons of 2-DE profiles between BM hybrids and parental species indicated down-regulation of most differentially expressed proteins in the BM gonads. Proteins with disrupted expression in the sterile hybrids were mostly those implicated in gonadal development and gametogenesis. The present findings suggest direct association of the observed perturbation in protein expression with lethality and sterility of the examined salmonid hybrids, which will provide a novel proteomic approach in fish breeding.

Keywords: *interspecific salmonid hybrid, lethal embryo, abnormal gametogenesis, protein down-regulation*

MORPHOMETRIC AND MOLECULAR CHARACTERISATION**OF *Cyprinus carpio* x *Carassius auratus* HYBRIDS**

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Ovulated eggs of koi (*Cyprinus carpio*) were stripped and fertilized with sperm from adult goldfish (*Carassius auratus*) in Fisheries Research Station of the West Pomeranian University of Technology, Szczecin, Poland during summer period of 2009. One year old *Cyprinus carpio* x *carassius auratus* hybrids as well their “parents” (koi and goldfish groups) have been characterized by means of morphometric and genetic analysis. Totally 25 measurements in 10 fish from each group has been made. Statistical analysis of the obtained results was performed using U-Manna-Whitney’a and principal component analysis (PCA). Genetic analysis has been performed by means of amplified fragment length polymorphism (AFLP) technique and sequencing of internal transcribed spacer 1 (ITS1). Sample of genomic DNA isolated and purified from each 5 fish form group of hybrids and their parents were digested with EcoRI and Mse I restriction enzymes and undergone pre- and selective PCR amplification. Genetic variation among groups has been performed using results obtained after separation and binning of AFLP fragments with BioEdit v7.0.9 and MEGA 5 software. Statistical analysis of measurements reveal that hybrids of carp and goldfish share ten unique features (pD – predorsal length, prO – preorbital distance, poO – postorbital distance, Du – unbranched rays of dorsal fin, lc2 – length of lower caudal lobe, H – maximum body depth, Laco – maximal body width, V-A – distance between V (ventral) and A (anal) bases, P-V – distance between P (pectoral) and V (ventral) bases, lc – length of head) mostly inherited from *C. auratus*. Otherwise, genetic analysis indicate that hybrids are more related to *C. carpio*. These results might be in future used in ongoing studies concerning susceptibility of hybrids to koi herpesvirus disease (KHVD).

Keywords: AFLP, diversity, goldfish, hybrids, morphometry

SESSION

MEAT QUALITY IN FARMED FISH

Chairmen: Jana Pickova & Jan Mráz

8

FOOD, FISH AND FAT

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Studies on lipid composition of animal origin foods have shown that the fatty acid composition of animal, including fish, products is significantly influenced by a number of factors such as diet, species, breed, age and gender of the animals. Diet is most likely the dominating factor in most cases. The say “You are what you eat” is a real truth when it comes to fat composition of animal tissues. In addition, the quality of oils and fats during processing of the foods has a strong influence on the FA composition of servings and thereby the real intake of fat. We know that fish can vary depending on the same variables, and in addition, the water type (fresh- or saltwater) and temperature, as fish are poikilothermic, is of importance. Freshwater fish has shown to be of large importance for the central part of continents for the supply of omega 3 polyunsaturated fatty acids. The diversity of fish in this environment is important as different species can utilize different feed sources and thereby effectively take care of non human intake suitable omega 3 PUFA and convert these to human foods. This presentation will focus on the available sources of omega 3 PUFA for different species, for wild as well as farmed fish and compare this with other animal origin foods.

Keywords: *polyunsaturated fatty acids, animal origin foods, freshwater fish*

CARP FLESH – A HEALTHY PRODUCT FOR PREVENTION OF CARDIOVASCULAR DISEASES

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There is evidence that n-3 highly unsaturated fatty acids (n-3 HUFA), EPA and DHA, are beneficial for human health, especially for cardiovascular health. However, the intake of fish, which is the major source of the EPA and DHA, is low in the Czech Republic (in 2008; only 5.5 kg of fish or fish products per capita per year) and it is far below current recommendations. Other sources of n-3 HUFA are also scarce in the diet consumed by Czech population. Therefore, we aimed to improve the fatty acid composition in the fillet of common carp, which is the major fish produced in the Czech Republic, and to test its health beneficial effects in a clinical trial. We have tested a supplemental diet for common carp based on rapeseed and linseed cake for production of carp. The fillet of the carp contained 300 mg of EPA+DHA in a 200 g serving. The fillets were served to patients in recovery after cardiac surgery. They received meals prepared from these carp fillets twice a week and their blood parameters were evaluated after 4 weeks period. The results from the clinical trial are very promising. Patients ate the carp showed decreased level of LDL cholesterol and increased level of HDL cholesterol compared to the control group. We conclude that carp fillets among other freshwater species serve as a valuable local source of n-3 HUFA for prevention of cardiovascular diseases.

Keywords: cardiovascular disease, common carp, DHA, EPA, nutrition

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**REGULATION OF HUFA BIOSYNTHESIS BY BIOACTIVE COMPOUNDS
IN ATLANTIC SALMON (*Salmo salar* L.) HEPATOCYTES**

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Due to shortage of fish oil and expected increase in aquaculture production, research is focusing on replacement of aqua feeds with increasing amounts of vegetable oils. It is well known that replacement of fish oil with vegetable oils decreases the content of n-3 long chain polyunsaturated fatty acids (LCPUFA) in the fish tissues. In terms of the consumer's health, it would be beneficial to maintain as high as possible amounts of n-3 LCPUFA in fish muscle. Many minor bioactive compounds have been shown to markedly influence lipid homeostasis in rodents and humans, as well as in fish. From this perspective minor bioactive compounds are of interest as potential constituents in fish feed possibly promoting the ability of fish to convert α -linolenic acid (18:3n-3, ALA) into eicosapentaenoic acid (20:5n-3, EPA) and docosahexaenoic acid (22:6n-3, DHA). We have performed in vivo studies examining the effects of different selected bioactive compound specifically – a mixture of sesamin/episesamin, sesamin, lipoic acid and genistein on FA composition and the gene expression profile associated with lipid homeostasis. Hepatocytes from Atlantic salmon (*S. salar* L.) fed with standard fish oil diet were incubated with I) genistein 0.005mM final concentration, II) genistein 0.025mM final concentration, III) lipoic acid 0.2mM final concentration, IV) sesamin/episesamin 0.05mM final concentration, V) sesamin 0.05mM final concentration. Results evaluating the effects on fatty acid composition and gene expression of carnitin palmitoyl transferase I, peroxisome proliferator-activated receptor α , β and γ (PPARs), Δ 6 desaturase, ELOVL5a elongase, Δ 5 desaturase, ELOVL2 elongase, cluster of differentiation 36, acyl-CoA oxidase will be presented and discussed.

Keywords: fatty acid, gene expression, elongases, desaturases

**TEXTURE PROFILE ANALYSES IN TENCH (*Tinca tinca* L., 1758)
FROM EXTENSIVE AND INTENSIVE CULTURE**

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Texture profile analyses (TPA) in tench (*Tinca tinca* L.) flesh was investigated, using instrumental texture profile method, focused on hardness, springiness, cohesiveness and gumminess. One group of fish (A) was raised in natural earth pond conditions (as extensive); the other group (B) was cultured in a recirculation system under feeding on a commercial diet (as intensive). 12 individual samples (proportionally 6 males and 6 females) of tench from each group were disposed for analyses. The results of texture analysis in group A for hardness were 16.01 N and as dimensionless units for springiness, cohesiveness, gumminess and chewiness were 0.72; 0.66; 10.73 and 7.69, respectively. In group B the results were, for hardness 15.16 N; for springiness, cohesiveness, gumminess and chewiness 0.59; 0.52; 8.06 and 4.96, respectively. The TPA effect was determined by analysis of variance and Tukey's mean test ($P < 0.01$). The results proved, that the flesh of fish on natural food is harder, springier, more cohesive and more gummy. Comparing the methods of fish rearing, studies of instrumental textural properties determined fish flesh coming from extensive conditions as more efficient for consumers.

Keywords: tench, texture profile analyses, muscle, hardness, springiness, cohesiveness

CHEMICAL CONTAMINANTS IN FARMED FISH FROM PONDS IN NORTH EASTERN FRANCE

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The widespread use of chemicals for the industrial and agricultural activities has resulted in contamination of numerous freshwater ecosystems. Some of these contaminants have been documented to be accumulated by fish. Today fish constitutes an important food source and is on the rise globally. In France the rearing system in ponds takes over the second rank after the intensive rearing systems of Salmonids. Extensively managed fishponds were strongly connected to their surrounding watersheds. In consequence, management of the watershed could have an effect on the safety of the fish produced, through bioconcentration and/or trophic transfer of chemicals. Fish farmers are not able to control the chemical inputs in fish, being derived from several phenomena (atmospheric deposition, runoff, effluent, food web). In this context, the purpose of this study was to quantify the levels of some chemical contaminants in the edible part of farmed fish (*Cyprinus carpio*, *Rutilus rutilus* and *Perca fluviatilis*) from five extensive ponds in Lorraine Region (France). The investigated contaminants were indicator PCBs (IUPAC Nos 28; 52; 101; 118; 138; 153; 180), pesticides and trace elements (Cu, Cd, Zn and Pb). The results highlight low PCB and pesticides contents in muscle tissue of fish, contrary to metal concentrations that could be now and then worrying. As an example the mean contamination levels are less than $2.65 \pm 1.37 \text{ ng.g}^{-1}$ wet weight (ww) and $0.71 \pm 0.56 \text{ ng.g}^{-1}$ ww for Σ PCBs and Σ DDT respectively. With regard to the trace elements, Cu and Pb concentrations can exceed the permissible levels for human consumption in Europe. Thus Cu and Pb concentrations in *R. rutilus* muscle tissues were 0.3 mg.kg^{-1} ww and 7.5 mg.kg^{-1} ww, respectively.

Keywords: pond, farmed fish, pesticides, trace elements, PCBs

**WHITE MUSCLE FATTY ACID COMPOSITION OF COMMON CARP (*Cyprinus carpio* L.) –
COMPARISON OF FOUR DIFFERENT CROSSBREDS OF ROPSHA CARP**

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The fatty acid (FA) composition in fish flesh is influenced by many factors such as nutrition and genetic origin. However there are only few data about an influence of genetic origin on FA composition in common carp. The white muscle FA composition of four different 3-years-old crossbreds of Ropsha carp (Rop) reared under the same conditions in an earthen pond with wheat supplementation was investigated. Fish were sampled at the end of vegetation season in September. Used crossbreds were: pure breed of Rop – average weight 1323 ± 233 g, fat content $1.49 \pm 0.57\%$; Rop x Tata scaly carp (Tat) – 1957 ± 346 g, $1.44 \pm 0.68\%$; Rop x Pohorelice scaly carp (Ps) – 1708 ± 150 g, $1.56 \pm 0.72\%$; Rop x Mariánské Lázně scaly carp (ML) – 1761 ± 158 g, $1.44 \pm 0.71\%$. Hungarian mirror carp (M2) x Northern mirror carp (M72) – 1607 ± 160 g, $1.06 \pm 0.40\%$ crossbred was added as a control. FA composition was evaluated in total lipids (TL) as well as in storage lipid (triacylglycerols, TG) and membrane lipid (phospholipids, PL) fractions. Results showed significant differences ($P < 0.05$) in FA composition among the crossbreds. The highest values of polyunsaturated fatty acids (PUFA), especially eicosapentaenoic (EPA) and docosahexaenoic (DHA) acid were found in the muscle of pure Rop (38.3% PUFA; 5.52% EPA; 7.97% DHA in TL). It seems there might be an effect of genetic origin on fatty acid composition in flesh of common carp but it needs other investigation to reveal the reason.

Keywords: muscle, crossbred, Ropsha carp, PUFA, EPA, DHA

SESSION

EUROPEAN AQUACULTURE SOCIETY (EAS)

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THE EUROPEAN AQUACULTURE SOCIETY – OUR ACTIVITIES AND THEIR RELEVANCE TO CEE COUNTRIES

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The European Aquaculture Society (EAS) was founded in 1976 and brings together those working towards the sustainable development of the activity in Europe. EAS also disseminates information and organises events (the annual Aquaculture Europe meetings) to share the latest research findings and to showcase industry products and services. EAS has more than 500 members in 40 countries, although the number of members in Central and Eastern European countries remains low. Apart from its member services, EAS participates and leads EU funded initiatives on aquaculture. The society has also been involved over the past few years in processes with the European Commission and Parliament to develop and enact the 2009 aquaculture strategy. Aquaculture activities in Central Europe may be perceived to have taken a low priority in EU research programmes in the past, and when European policy is developed. EAS Board members and the 2006–2008 EAS President have on many occasions contributed to the debates that have led to the recognition of the European institutions of the importance of freshwater aquaculture and inland fisheries in European policies and research. Back in 1995, EAS organised a conference in Hungary, and the AE2008 event was held in Poland. AQUA 2012 – the joint meeting of EAS and the World Aquaculture Society (WAS) – will be organised in Prague and will be a global event with a very wide spectrum of attendance and subjects. It will provide a showcase for research, technology and innovation in CEE countries. It is also hoped that the EAS and EAS Student Group will attract a significant number of new members during the build up to the Prague event.

Keywords: *EAS, European policy, AQUA 2012*

SESSION

ALLER AQUA

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ALLER AQUA WHO WE ARE, AND WHY WE ARE HERE?

Marcin Walczak

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Feed is the single largest expense item in aquaculture. This is why Aller Aqua feed guarantees profitable production and healthy fish. By working closely with customers, Aller Aqua develops and manufactures the right feed and the right feeding methods to guarantee optimum operations. With more than 40 years in the sector, Aller Aqua is one of the most experienced suppliers of fish feed for freshwater and saltwater species, for example fish feed for barramundi, tilapia, pike perch, carp, catfish, rainbow trout, cod, turbot, rockfish, salmon, seabass, seabream and sturgeon. All our fish feed products are produced by means of extrusion. The fish feed must cover the basic metabolism of the fish and ensure healthy growth. In order to meet these requirements the fish feed composition must meet all needs for nutrients, vitamins and minerals. Aller Aqua fish feed meet all these requirements and have been adapted to various sizes of fish and feeding strategies. The feed have been developed in cooperation with customers and undergo continuous tests, in selected test stations and fish farms. Aller Aqua works both locally and internationally. For decades, we have been a global supplier of fish feed, with production plants in Denmark, Poland and Germany. Aller Aqua is a modern company specialised in the areas of manufacturing, advanced technology, production management and systematic quality control. It is our objective always to be competitive in terms of feed quality and feed economy in order to contribute to the best possible financial results for our customers. The many-sided tasks make heavy demands on our staff as our company is an unbureaucratic organisation. Quick responses and fast decision-making characterise a dynamic organisation, where close and honest customer relations play a key role. This is why Aller Aqua sells total solutions for feed for aquaculture, rather than just feed. The role of the company is also to support the development of aquaculture as a sustainable profitable business. Therefore Aller Aqua takes part in international events such as DIFA conference in Pisek as well as develops such initiatives as International Carp Conference and Sturgeon Conference.

The International Carp Conference will take place 15–16th September 2011 in Kazimierz Dolny, Poland. To find out more, please visit the webpage: www.carpinternational.eu.

The Sturgeon Conference will be held on 23rd November 2011 in Warsaw, Poland. More information on www.aller-aqua.pl.

POSTER SESSION

posters are listed in alphabetical precedence
according to the first author

**AQUACULTURE IN BELGIUM: AQUA-ERF TO INVESTIGATE SPECIES DIVERSIFICATION
IN RAS WITH SPECIFIC INTEREST IN *Lota lota* (LINNAEUS, 1758)**

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The Belgian Aquaculture industry can be described as very small, nevertheless fish consumption is increasing, partially thanks to the positive image fish has as a healthy food source. The world aquaculture production has increased rapidly to respond to these demands for fish and seafood, but local production in Belgium did not follow this expansion, probably because there is the doubt if aquaculture has an economic future in Belgium. Therefore the European Interreg project "AQUAVLAN" has been set up in 2009 to determine which could be the possibilities for an economic viable and sustainable aquaculture in Flanders and Holland. As a partner in this project the Flemish University College, KaHo Sint-Lieven, has opened the Aquaculture Education and Research Facilities (AQUA-ERF) to do research concerning aquaculture, to give formation to their students and external people and to be a reference point and support group for starting fish farmers.

To avoid competition with the increasing and cheaper import and to diversify the finfish supply on the Flemish table market, the AQUA-ERF started to work with the culture of burbot (*Lota lota*). This fish, the only fresh water member of the cod family, has a very good reputation in Scandinavia and Eastern-Europe because of his tasty white fillet. Although in Flanders the burbot has disappeared from the rivers and therefore from the table, it was consumed frequently in the past and a recent taste panel of consumers accredited it with high scores. The current status on the aquaculture production of burbot shows a limited production based on pond culture, with the weaning of larvae being a bottleneck. Currently, trials are running to adjust the weaning strategy but also to optimize the grow out of burbot, investigating the influences of different feed compositions and temperature. Considering the sustainability concept, the AQUA-ERF is 100% based on recirculation technology.

Keywords: burbot, diversification, RAS, sustainability

**MODULATION OF SPERM ACTIVATION IN THE EURYHALINE TILAPIA
(*Sarotherodon melanotheron heudelotii* Dumeril, 1859) ACCLIMATIZED TO FRESH,
SEA OR HYPERSALINE WATERS**

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The effects of osmolality and calcium (Ca^{2+}) in the initiation of sperm activation were investigated in an estuarine tilapia (*Sarotherodon melanotheron*) acclimatized to fresh (FW), sea (SW) or hypersaline waters (HW). Three groups of males were stocked together with females in recycling water systems at salinities set at circa 0 (FW), 35 (SW) and 70 g/L (HW). Intratesticular sperm was collected by squeezing testes and stored in tubes on ice during analysis. In synthetic sea salt solution, sperm activation was triggered between 0–1100; 0–2500 and 0–3300 mOsmol/kg in fish reared in FW, SW and HW, respectively. The maximum sperm motility and velocity were observed at 0–460 and 165–300 mOsmol/kg in FW, at 100–1200 and 0–1700 mOsmol/kg in SW and at 300–1900 and 500–1400 mOsmol/kg in HW, respectively. In sucrose solution, the sperm activation for FW, SW and HW was induced at 0–925; 0–2200; and 0–2200 mOsmol/kg, respectively. In all groups, sperm activation was enhanced when 1 mM Ca^{2+} has been added into the activation medium. Sperm motility was significantly decreased or totally suppressed when EGTA (a chelator of calcium ions) has been added into the activation medium. Free Ca^{2+} concentrations required for triggering sperm activation was measured about 10^{-10}M for FW and about 10^{-9}M for SW and HW. An inhibitor for Ca^{2+} /calmodulin activated phosphodiesterase (*w*-7) inhibits the sperm activation in this species; suggesting Ca^{2+} -dependent mechanism of axonemal beating in sperm activation.

Keywords: calcium, flagella, osmolality, sperm, tilapia

CAN WE USE A SINGLE SAMPLING POINT TO ASSESS SEDIMENTATION RATE IN SHALLOW AQUATIC SYSTEMS?

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Quantitative and qualitative assessments of sedimentation processes in lentic aquatic systems are often based on the analysis of samples collected at a single sampling point. It is generally accepted that the deepest point is the best location to assess sedimentation processes in lakes. As fishponds are generally considered as small and shallow lakes, this method could appear to be perfectly adapted to these systems where sedimentation processes are presumed to be homogeneous. To assess the spatial heterogeneity of sedimentation processes resulting from deposition of allochthonous and autochthonous production (primary flux) and from resuspension of particles already deposited (secondary flux of sedimentation), nineteen sediment traps were exposed in two shallow extensively managed fish ponds (surface areas = 0.09 and 0.37 km²) in North-Eastern France. Traps were collected monthly during 18 months. Sedimentation rate (SR), organic matter (OM) carbon (C), nitrogen (N) and phosphorus (P) contents were measured. In the smallest pond (9 ha) SR, OM and C were not significantly different among traps. However, C/N ratio, N and P contents of particles collected in the upstream part of the pond were significantly different from particles settled in the downstream part. In the largest pond (0.37 km²) quantitative and qualitative spatial variability of sedimentation was highly significant. In order to highlight the spatial variability of sedimentation processes throughout the pond, the C/N ratio was highly discriminant. This study has highlighted quantitative and qualitative spatial variations of settled particles in fishponds. In conclusion, despite the small surface area of ponds, a single sampling point cannot be considered to be sufficient to assess the processes of sedimentation in these shallow systems. Based on C/N ratio of settling particles, ponds must be subdivided into at least three different parts: 1- area close to tributary mouths, 2- area close to banks, 3- central area.

Keywords: extensively managed fishponds, sediment sampling, sedimentation

LARGE CLADOCERANS AS INDICATORS FOR FEEDING IN EXTENSIVE CARP CULTURE

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In Austrian carp culture natural resources are said to be an important component in the diet of common carp and artificial feed, mostly cereals, should only complete the diet. So it is reasonable that feeding with grain should be oriented on the availability of natural resources. However, in practice feeding with grain follows a rather inflexible feeding plan, increasing the amount of feed with water temperature. Natural resources are not considered anyway. In fact, it is difficult for the carp farmer to estimate the amount of natural resources in the pond during the day to day work. As a possible indicator for the natural resources in the pond, we propose the abundance of large cladocerans > 1 mm. With easy to built tools (net and sampler) carp farmers can evaluate the abundance of large cladocerans by using the displacement volume. Basing on this information the grain feed can be adjusted to the available natural feed. This leads to a stable supply of the carp with natural feed and a wise use of grain which can result in a lower consumption of grain. The method was successfully tested in small ponds (1800–2300 m²) leading to a production of up to 743 kg/ha and a saving of grain of up to 764 kg/ha.

Keywords: zooplankton, common carp, pond aquaculture

DANISH MODEL RECIRCULATING SYSTEM FOR SALMONIDS IN THE CLIMATE OF MID-EUROPE: ADVANTAGES, POSSIBILITIES, LIMITATIONS

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The first Danish model recirculating aquaculture system (RAS) for salmonids was built in the Czech Republic in 2007. Surprisingly, there are lacking similar farms also for other fish species. A large skepticism is ingrained within traditional fishermen focused mainly on carp production. We therefore monitored the function and local specific issues for evaluating RAS in the mid-European conditions. The greatest difference compared with the place of Danish model RAS origin is winter season operating, characteristic by 3–4 months of ice coverage and water temperature ranging between 0.9–3.0 °C. However, the use of tested RAS in local Czech Republic conditions was evaluated as very effective, and more beneficial than traditional flow-through systems (FTS) for salmonids. Bio-filtration was effective despite of low temperature and able to remove an amount of metabolites produced in winter season. Compared to FTS placed on the same site, RAS is independent of often seasonal water quality changes caused by land use, sudden weather changes (thaw), flow variation (floods or summer drought), variable content of dissolved matter and possible disease transfer from a river. More stable conditions of water quality, compared to FTS, resulting in better fish condition and growth. Higher increments, feed conversion ratio, and Fulton's condition coefficient were reached in RAS compared to adjoining FTS. However, the long winter period breaks down the total possible production. In general, this question is only one limitation, excluding the primary cost of the RAS, of similar systems in mid-European conditions. The potential production can be largely increased by covering RAS and using oxygenation. Nevertheless, the RAS is functional also in the presented state. The propagation, developing and building of new RAS means to use of more effective techniques and to invest to the future in the frame of sustainable development in aquaculture.

Keywords: aquaculture, evaluation, moderate climate, RAS, fish

MASS PRODUCTION OF IDE (*Leuciscus idus* L.) A NEW SPECIES FOR POLYCURTURAL REARING IN CARP PONDS – THREE SIDES OF THE SAME COIN

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The aim of the paper is to present guidelines for ide (*Leuciscus idus* L.) mass production in polyculture with carp in traditional ponds. Basing on 15 years observations, gathered at Fisheries Research Station Łąki Jaktorowskie Warsaw University of Life Sciences – SGGW, optimal stocking densities for rearing one and two years restocking material were calculated, in correlation to size of harvested ide, survival rate and increase of natural yield. Approximately 60–100 kg.ha⁻¹ of ide could be harvested, basing on natural food resources only. Fish are of excellent value for restocking purposes, as they grow almost in natural environment. On the other hand, the share of ide production should not exceed 20% to 40% dependently to the size of the carp and ide. Higher stocking densities have very negative influence on carp production as natural yield decreases because of interspecies competition. However, since 2005, the ide production at Fisheries Research Station Łąki Jaktorowskie SGGW is highly influenced by great cormorant. The number of birds increased during last six years from 0 before 2005, to 9 individuals in 2005 and to 193 in 2010. As the result, ide production totally collapsed in 2010. Ide survival rate in 2010 was 0–5%, comparing to 50–60% in previous years. The correlation between number of cormorants and additional fish production and “pond resistance to cormorant” was calculated.

Keywords: ide, carp ponds, policulture, natural yield, great cormorant

FOOD COMPOSITION OF WEATHERFISH (*Misgurnus fossilis*) LARVAE AND JUVENILES FROM NATURAL HABITAT

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Food of weatherfish (*Misgurnus fossilis*) larvae and juveniles was studied during one growing season in natural pool (area of 5 m², mean depth 50 cm) with aquatic macrophytes (esp. *Typha latifolia*, *Glyceria fluitans*, *Nymphaea alba*) near experimental facilities of faculty in Vodňany. Weatherfish larvae were released third day after hatching (SL 5.47 ± 0.12 mm). Feeding started third day after stocking (SL 5.54 ± 0.16 mm). First food comprises three main components: ostracods (Ostracoda), free-living amoeba (Arcellinida; *Diffugia* sp.) and rotifers (Rotifera; *Testudinella* sp.). Ostracods were positively selected during whole season composing always at least 40% of ingested food. Cladocerans (Cladocera), especially *Simocephalus vetulus* and *Ceriodaphnia reticulata*, constituted the second important food component of weatherfish juveniles (SL 10–30 mm). This type of food was gradually replaced by copepodid stages and adults of copepods (Copepoda). Copepods as well as ostracods prevailed in food of weatherfish to the end of season. Chironomid larvae (Chironomidae) were detected in food of weatherfish (SL 10.90 ± 0.99 mm) from the beginning of June to the end of the season. Other food components, such as mosquito larvae (Culicidae), waterlouse (*Asellus aquaticus*) and molluscs were ingested occasionally, and were as well as chironomids not the most important food component.

Keywords: feeding, Ostracoda, free-living amoeba, Copepoda, Cladocera

EXTENSIVE FISH FARMING IN THE BOHEMIAN FOREST

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Ostřice pond is located close to village called Horní Planá, 760 m above sea-level. The size of pond is 8030 m² and maximum depth by dyke sluice is 91 cm. Water quality and its temperature vacillated minimally during the year. 2/3 of pond was overgrown with water vegetation (*Typha latifolia*, *Sagittaria sagittifolia*, *Bidens ceria*, *Carex* sp., *Juncus effusus*, *Scirpus sylvaticus*). Water vegetation fuels the development of water spineless (*Culicidae* sp., *Chironomus* sp., *Acisoma*, *Dytiscus marginalis*, *Daphnia* sp.), which had made major part of natural food for fry. Research work is in process since 2009. According to size of the pond and its natural performance, fish stock was fixed to 350 pieces of yearling salmonoid fish. On 3. 4. 2009 a fish stock of rainbow trout (*Oncorhynchus mykiss*) in total number of 175 pieces and fish stock of American char (*Salvelinus fontinalis*) in total number of 175 pieces was implicated into the research. Checking haul was effected after 102 days. In total amount of 16 pieces of rainbow trout and 6 pieces of American char was measured and weighted. The final haul was executed after 167 days. In combination of fish net and electric power was caught 43 pieces of rainbow trout and 33 pieces of American char. A rise of 246.1 g in case of rainbow trout and rise of 190.8 g in case of American char was found. Reason for a small haul of breded fish could be most likely found in high pressure of piscivorous predatos (*Lutra lutra*, *Phalaracrocorax carbo*, *Ardea cinerea*). Research is still in process and will be extended by other fish kinds and ways of fish-scale reading (natural food constitution, meat quality of cultured fish etc.).

Keywords: extensive breeding, rainbow trout, American char, Bohemian Forest

**EFFECTS OF SUBSTITUTING SOYBEAN MEAL WITH BAMBARA NUT MEAL
ON THE FIRST FEEDING AFRICAN CATFISH (*Clarias gariepinus*)**

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Larval African catfish *Clarias gariepinus* of average weight 0.15 ± 0.02 g were fed for 22 days with diets substituting soybean meal with bambara nut meal. The soybean meal: bambara nut meal inclusion levels were as follows, F1, 25:5; F2, 20:10; F3, 15:15; F4, 10:20 and F5, 5:25. A reference diet F6 was also made containing 90% fish meal with no soybean meal or bambara nut meal. Larval specific growth rate (SGR) was best and similar for catfish fed with feed 1, $14.93 \pm 0.31\%$ day⁻¹; feed 2, $14.31 \pm 0.81\%$ day⁻¹ and feed 3, $14.89 \pm 0.0\%$ day⁻¹. There was no significant difference in the SGR of other treatments. Similarly, food conversion ratio (FCR) was lowest for feeds 1, 2 and 3, but increased with increasing bambara nut meal substitution, albeit < 0 for all treatments. The apparent net protein utilization (ANPU) was highest and same for larvae fed with feeds 1, $29.37 \pm 0.49\%$; feed 2, $27.86 \pm 1.44\%$; feed 3, $27.41 \pm 2.2\%$, but lower for those of feed 4 and 5. Larval SGR, FCR and ANPU seem to be a result of increasing levels of essential amino acids like lysine, methionine and isoleucine. However daily feed intake (DFI) was higher for feed 4, 5 and 6 and consequently average weight gain of larvae on these diets was similar with those fed with feed 1. Survivability was best with feed 6, $51.14 \pm 2.4\%$, followed by feed 1, $44.86 \pm 1.21\%$ and lowest for feed 3, 28.57% but otherwise not different from the rest. Consequently bambara nut meal can replace 50% of larval diets without negative growth effects or 82.5% with some reduction in growth.

Keywords: replacing, soybean meal, with bambara nut meal, in larval African catfish

SPAWNING INDUCTION IN KUTUM *Rutilus kutum* USING DIFFERENT HORMONAL ADMINISTRATIONS: ANALYSIS OF HORMONE PROFILE AND SUCCESS OF INDUCTION

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The objective of this study was to evaluate the effectiveness of different hormones on spawning success and sex steroids changes in Kutum, *Rutilus kutum*, as an endemic fish of the Caspian Sea. Fish were captured from the coastal of the Caspian Sea during the spawning migration. Groups of five fish were injected intramuscularly as follows: 2 mg kg⁻¹ common carp pituitary (CPE) as a positive control, 100 IU kg⁻¹ human chorionic gonadotropin (hCG), 2 µg kg⁻¹ luteinizing hormone releasing hormone analog (LHRH_{a2}), 10 µg kg⁻¹ sGnRH, 0.2 ml kg⁻¹ ovaprim, the combination of 2 µg kg⁻¹+100 IU kg⁻¹ LHRH_{a2} with hCG, 10 µg kg⁻¹+100 IU kg⁻¹ sGnRH with hCG and saline solution as negative control. The blood samples were taken synchronous with injection and after ovulation. Results showed that no fish ovulated in the negative control group. In CPE- treated fish, 4 of 5 fish ovulated. In other treatments, no response was observed in hCG treatment, while the lowest response was in sGnRH, LHRH_{a2} and sGnRH+hCG injected females which only one of five fish ovulated. In utilization of combination of LHRH_{a2}+hCG, three females spawned. Of the hormones tested, ovaprim was found to have the most effective, so 100% females treated with this hormone were ovulated. There was no significant difference in sex steroids concentrations among females in the different groups, while pre injection and post ovulation, there were significant differences in testosterone concentration in negative control, LHRH_{a2}, hCG, DHP and estradiol 17β concentrations in all treatment except ovaprim treated fish. This study revealed that ovaprim was an effective agent for the induction of ovulation in Kutum. In conclusion, because of ovaprim is cheaper than CPE and caused higher number of ovulated females, it preferred over CPE for inducing of ovulation for this species.

Keywords: Kutum, induction, hormonal manipulation, sex steroids

**HEAT-SHOCK INDUCED TETRAPLOID AND DIPLOID-TETRAPLOID MOSAIC
IN THE LOACH, *Misgurnus anguillicaudatus***

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Tetraploid fish, which are considered as key resources of diploid gametes for further breeding and ploidy manipulation, can be artificially induced by inhibition of the mitotic cell division with hydrostatic pressure or temperature treatments. Although many attempts have been performed to induce artificial tetraploid strains, successful establishment and maintenance of viable and fertile tetraploid strains are rare. In the loach, *Misgurnus anguillicaudatus*, tetraploid individuals are distributed in natural populations and diploid gametes from the tetraploid fish have been used for induction of various kinds of polyploids, but artificially induced tetraploid strains have not been established yet. In the present study, we optimized starting timing of the heat-shock treatment (41 °C for 2 min) to inhibit a mitotic cell division in fertilized eggs of the common diploid loach between 21 and 51 min after insemination at 20 °C incubation temperature. After the treatment, we observed external appearance of hatching larvae and flow-cytometrically determined ploidy status of the resultant larvae. Although tetraploid and diploid-tetraploid mosaic larvae were obtained, the treating timings inducing these progeny were different among the crosses. Various kinds of polyploids such as haploid, diploid, triploid, pentaploid, hexaploid, aneuploids and mosaics were detected in non-optimum timings for tetraploidization. A tetraploid and a diploid-tetraploid mosaic male grew up and matured, but they produced functional haploid spermatozoa.

Keywords: biotechnology, chromosome, Cobitidae, polyploid

**BODY TRAITS AND CHEMICAL COMPOSITION OF PADDLEFISH (*Polyodon spathula*),
A POTENTIAL AQUACULTURE SPECIES**

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In many regions of the world non-native species have been introduced for aquacultural purposes and have contributed significantly to the expansion of the industry. The American paddlefish (*Polyodon spathula*) has been introduced into Bulgarian aquaculture in 2003 as a species suitable for breeding in polyculture with common carp in ponds and small manmade dam-lakes. The aquaculture of this species is still in the research and developing phase but its potential is unquestionable. The results from this study showed that when raised in ponds in polyculture with common carp, paddlefish reach 0.8–2.0 kg in two years depending on the pond size and the available natural food. Paddlefish yields with viscera removed, but with head and fins, decrease from 90 to 83% with increasing the body weight. Dressed fish (with viscera, head and fins removed) and dressed fish without skin increase, respectively from 48 to 60% and from 46 to 57%, with increasing the body weight. Paddlefish meat from fish weighing 0.8–2.0 kg contain 77.13–76.6% moisture, 17.78–19.81% protein, 4.18–2.61% fat, and 0.97–0.98% ash, respectively. With increasing the body weight the proteins increase, and the water content and fats decreases. The energy value of the meat varies from 591.10 to 577.21 kJ.100 g⁻¹, and results mostly from proteins (71.90–84.17%).

Keywords: *paddlefish, body traits, chemical composition*

MODES OF ACTION OF MERCURY ON FISH SPERM

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The main objective of the present study was to investigate modes of action of HgCl₂ on fish sperm (perch, *Perca fluviatilis*). In the 1st experiment, sperm performance (motility, velocity, and ATP content) was measured in sperm that had been exposed to different concentrations of HgCl₂ in an activation medium (AM; 50 mM NaCl, 20 mM Tris, pH 8.5). The 2nd experiment studied the effects of HgCl₂ in AM on sperm, which has been incubated in an immobilizing medium (IM; 180 mM NaCl, 2.68 mM KCl, 1.36 mM CaCl₂, 2.38 mM NaHCO₃, pH8.5) with or without HgCl₂ during 24 h exposure. In the 3rd experiment, the role of the plasma membrane was investigated using demembrated sperm after re-activation in the presence of various HgCl₂ concentrations. Direct exposure of non-incubated sperm decreased motility and velocity in a dose-dependent manner. Sperm motility was suppressed at 250 μM HgCl₂, but ATP content of sperm was similar to that of control at HgCl₂ > 125 μM. Motility and velocity of demembrated sperm decreased after activation in AM containing 62 μM HgCl₂, and was suppressed at 250 μM HgCl₂. Sperm motility of incubated sperm in IM without HgCl₂ was suppressed at 125 μM HgCl₂ after 3 h incubation. In case of incubated sperm in IM containing HgCl₂, sperm motility was suppressed at 31 μM HgCl₂. Sperm ATP content was lower in IM containing HgCl₂ > 3 μM compared with those of the control (no HgCl₂) and lower HgCl₂ concentrations. Damage to the plasma membrane and axoneme were observed in sperm incubated in an IM containing HgCl₂ compared with the control, when HgCl₂ concentration and incubation time increased. In conclusion, HgCl₂ acts on sperm through disruption of function of the plasma membrane, axoneme, and ATP content.

Keywords: axoneme, mitochondria, plasma membrane, sperm

INFLUENCE OF TEMPERATURE ON PUBERTY AND MATURATION OF PIKEPERCH, *Sander lucioperca*

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Among external factors, temperature is known to exhibit a prominent role in reproduction of temperate fish species. Here, temperature related induction of puberty in pikeperch *Sander lucioperca* was investigated. For the first time the key factors of the pikeperch brain-pituitary-gonad axis, targeting the mRNA expression of the luteinising hormone (LH) and the follicle stimulating hormone (FSH), as well as the plasma sex steroids estradiol (E2), testosterone (T), 11-ketotestosterone (11-KT) and 17 α ,20 β -dihydroxy-4-pregnen-3-one (17,20 β -P) were addressed in the experiment. Concomitant the maturational stages were described histologically. After 3 months, female pikeperch kept at 12 °C revealed significant increases in the GSI and plasma E2 concentration and 90% of the females were mid vitellogenic. After 5 months, females kept between 9 °C and 15 °C exhibited significant up-regulation of E2 and GSI as well as comparable histological outcome. At 6 °C and 23 °C in nearly all females stagnation of oogenesis was recorded. Congruently, T was increased at 12 °C and 15 °C. Expression analysis revealed a significant up-regulation of LH β and FSH β mRNA in females from early vitellogenesis, and from mid spermatogenesis in males, correlated to elevated plasma concentrations of steroids (except for E2 in males). In conclusion, moderate temperatures (12–15 °C for) for at least 3 months were required to proceed with first maturation in juvenile pikeperch. The most efficient effect was observed at 12 °C, while high (23 °C) or low (6 °C) temperatures prevented gonadal maturation. So, temperature was identified as a prime factor in the induction of puberty in pikeperch, as revealed by histological as well as endocrine parameters.

Keywords: oogenesis, spermatogenesis, gonadotropins, sex steroids, teleost

**REPRODUCTIVE BIOLOGY OF THE CHANNEL CATFISH (*Ictalurus punctatus* Raf.)
REARED IN WARMED WATERS**

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Size and age at which the channel catfish reared in the conditions of a thermal power station cooling lake have reached sexual maturity were studied. The fish were reared in net cages situated in the cooling lake. The water temperature during the winter months does not fall under 10 °C, which allows the fish to feed during the autumn-winter period and to generate growth. The established body weight of the one-summer old catfish is 100.4 g for males and 81.2 g for females. For the one-year old catfish, the body weight was 163.4 g for males and 133.2 g for females. Differentiation of the gonads was clearly pronounced as early as at age of one-summer. The GSI was 0.19% for the females and 0.04% for the males. By the age of one year, the ovaries doubled in size with an average weight of 0.21 g, and the testes of 0.04 g. The GSI had values of 0.23% for the females and 0.02% for the males. The maturity stage of the ovary and the testis was determined as stage II. For the two-summer old catfish (16–17 months of age), an average body weight of 897.8 g for the males and 958.9 g for the females was established. The GSI was 1.68% for females and 0.35% for males. The ovary was in the III-IV stage of maturity and their predominant fraction consisted of early vitellogenic follicles. At an age of 18–19 months, the ovary was in the IV stage of maturity, and the vitellogenic follicles were the main fraction in the ovary. The spawning takes place during late May-mid June, and the fish that reached the age of two years, is with an average body weight of 1007.8 g for the males and 985.2 g for the females, and can participate successfully in the reproduction process. The values of the absolute (7583 eggs) and relative (5900 eggs kg) fecundity for the two-year old matured channel catfish were lower than those typical for the older sexually mature fish. The weight and diameter of the eggs were 0.0071 g and 2.12 mm, respectively.

Keywords: channel catfish, first maturity, fecundity, egg size

IMMUNOSTIMULATORY AND DISEASE RESISTANCE PROPERTIES OF *Achyranthes aspera*, A HERB (AMARANTHACEAE) IN CARP *Labeo rohita*

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Indian major carp *Labeo rohita* (rohu) larvae were fed with diets containing seeds of *Achyranthes aspera* at various doses; diet without seeds served as control. Fish were immunized with c-RBC after 30 days and blood and tissue samples were collected after immunization. Significantly ($P < 0.05$) higher average weight and SGR were found in seeds incorporated diet fed fish compared to the control one. The average weight of fish showed a direct relationship with the increasing dose of seeds in the diets. FCR was significantly ($P < 0.05$) lower in seeds incorporated diet fed group. Total serum protein, albumin and globulin levels were always higher in treated groups compared to the control one. The antigen-specific antibody titre levels were significantly ($P < 0.05$) lower in control diet fed fish compared to the treated groups throughout the study period. Serum glutamic oxaloacetic transaminase (SGOT), Serum glutamate pyruvate transaminase (SGPT) and Alkaline phosphatase (ALP) levels were significantly ($P < 0.05$) higher in control diet fed fish compared to the treated groups throughout the study period. Higher myeloperoxidase level was found in all treated rohu compared to the control one. Fish were challenged with live *Aeromonas hydrophila* and mortality of fish was recorded. After 7 days post challenge, significantly ($P < 0.05$) higher mortality was recorded in the control diet fed group compared to the treated groups. The present study documented the immunostimulatory and disease resistance properties of seeds of *Achyranthes aspera* in carp.

Keywords: carp, challenged test, rohu, immunostimulation

**CHARACTERISTICS OF EARLY ONTOGENETIC DEVELOPMENT STAGES OF LAKE OHRID
BELVICA (*Salmo ohridana* Staind.) IN CONTROLLED CONDITIONS**

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Salmo ohridana is one of the five endemic trout species in Lake Ohrid and till present day it is a native population – artificial spawning was not performed of this species for restocking purposes. But, due to its high commercial value in our days it is exposed to a big fishing pressure. In order to preserve the native population and its gene pool the following investigations were made with aim of its aquaculture. With successful farming of this species the pressure of the native population should be reduced and the market demand will be satisfied. The investigation on ontogenetic development was done in several successive experiments within a period of 250 days from fertilization of the eggs, in which 4 deferent groups of specimens were surveyed. The first group was consisted of crossings between males and females of *Salmo ohridana* from two natural spawning peaks in the year. Two other groups were crossings of *Salmo ohridana* and *Salmo letnica* and as a control group same number of fertilized eggs of *Salmo letnica* obtained for the restocking purposes of Lake Ohrid was used. All groups were examined on a daily basis for differences in the ontogenetic development during the period of the incubation of the eggs as well as after the hatching, until the end of the investigated period.

Keywords: *Lake Ohrid, Salmo ohridana, Salmo typicus, hybridization ontogenetic development*

SUSTAINABLE TREATMENT OF AQUACULTURE EFFLUENTS BY CONSTRUCTED WETLANDS

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Several intensive flow-through fish production plants were established in Hungary in the last decades, representing a significant share of the total fish production. However the full management of the discharged waste from such systems is still unsolved. A pilot-scale constructed wetland system was built for the treatment of the effluents of an intensive flow-through African catfish farm in Szarvas (Hungary). Besides the water treatment the constructed wetland system was able to transform the waste nutrients into valuable by-products such as fish and energy plants. The aim of this study is to present the nutrient removal efficiency and the potential of the wetland system to transform discharged nutrients into valuable by-products. The wetland system was constructed by the combination of a stabilisation pond, a fishpond and macrophyte pond units. The effluent from the African catfish farm was pumped into the aerated stabilisation pond, and then was introduced into the fishpond unit stocked in carp polyculture. The nutrient removal capacity of the wetland system was in average 1300; 180 and 3000kg ha⁻¹ year⁻¹ for nitrogen, phosphorus and organic carbon, respectively. The average efficiency of water treatment exceeded the 90% removal rate for nitrogen and organic carbon, and 84% was found for phosphorus in 2007 and 2008. On average, 4% of the total input of nitrogen and 6% of the phosphorus input were reutilised in the by-products (including both fish and plants).

Keywords: *effluent, energy plant, fishpond, nutrient utilisation, wetland*

Financial support for the research work was provided by the SustainAqua EC-project (COLLECT-2006-030384).

INTEGRAL EVALUATION OF PHYSIOLOGICAL STATE OF CRAYFISH (*Pontastacus leptodactylus* Esch.) BASED ON RECORDING AND ANALYSIS OF CARDIAC ACTIVITY

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Using of species-bioindicators for biomonitoring of the environment requires the development of methods to evaluate their physiological state (health status). In our ecotoxicological studies crayfish *Pontastacus leptodactylus* Esch. were used as bioindicators and their cardiac activity characteristics (heart rate – HR and stress index – SI) as physiological biomarkers. It was observed that in the rest state and especially under toxic treatment the variability of individual cardiac characteristics is obviously due to the differences of crayfish physiological state. Taken this into account, we concluded that to improve the reliability and accuracy of the results of biomonitoring it is important to select a reference group of healthy animals, i.e. most homogeneous in their physiological state, in particular with the lowest background scatter characteristics of cardiac activity. Based on these points special test-treatments were developed and approbated. Responses to such treatments allowed to select animals more applicable for the purposes of water quality bioindication. The following parameters were chosen as the most consistent: stable expressed circadian rhythm in HR, clear change in HR under handling, suspension test, in test with salinity changes of medium or under natural food exposure. We consider that use of such characteristics together with well-known morphological characteristics and some behavioural reactions allow to obtain integral evaluation of crayfish physiological state. All mentioned above give an opportunity to form reference groups of animals not only to improve the effectiveness of bioindication, but also to form crayfish micropopulation more resistant for the conditions of aquacultural breeding and reproduction.

Keywords: *crayfish, physiological state, cardioactivity, reference group, biomonitoring*

**INDUCED ARTIFICIAL PROPAGATION OF BARBEL (*Barbus barbus*):
EFFECT OF SOME HORMONAL PREPARATES AND DIFFERENT TEMPERATURES
ON SPAWNING PERFORMANCE AND LATENCY TIME**

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An experiments was conducted to induce ovulation in barbel originated from river by a single injection of preparates: carp pituitary extract (CPE, dose 6 mg.kg⁻¹), Supergestran (contains GnRH analogue Lecirelin, dose between 1 to 125 µg.kg⁻¹), Ovopel and Dagin (contains GnRH analogues and dopaminergic inhibitor). The best results were found in Supergestran (in dose 100 or 125 µg.kg⁻¹ of GnRH analogue) and preparates Dagin and Ovopel (100% ovulated of females). The relative weight of eggs, obtained from the females treated with highest dose of Lecirelin, were 10.54 ± 4.51%. The dependence upon temperature was proved for a latency time between injection (of prepare Ovopel) and ovulation within the mentioned water temperature range (from 15 to 19 °C).

Keywords: *ovulation, reproduction, GnRH α , CPE, dopaminergic inhibitor, latency time*

This study was supported by the Czech projects of KONTAKT ME 10126 and NAZV QH91310.

**A WIDE DIFFERENCE IN SUSCEPTIBILITY TO NITRITE BETWEEN EURASIAN PERCH
(*Perca fluviatilis*) AND LARGEMOUTH BASS (*Micropterus salmoides*)**

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Influence of nitrite on two fish species: Eurasian perch (*Perca fluviatilis*) and largemouth bass (*Micropterus salmoides*) was assessed in two acute toxicity tests. In the first one, lethal concentrations (48hLC50) of nitrite were estimated at 11 mg.l⁻¹ NO₂⁻ for perch and 882 mg.l⁻¹ NO₂⁻ for bass. In the second test, fishes were exposed for 48 h to concentrations representing ¼ and ½ value of 48hLC50 for each species. This test showed that the higher nitrite concentration in the water was the higher methemoglobin content in the blood and nitrite levels in the blood plasma were observed in both species. On the other hand, leukocyte count showed opposite trend. Activity of NADH-methemoglobin reductase was markedly lower in largemouth bass compared to European perch and was not stimulated by nitrite exposure in neither of the species.

Keywords: fish, haematology, lethal concentration, methaemoglobin, NADH-methemoglobin reductase

This study was supported by CENAQUA project CZ.1.05/2.1.00/01.0024, project USB (GAJU) no. 047/2010/Z, and project no. QH82117.

HORMONAL INDUCTION OF PIKEPERCH (*Sander lucioperca* (L.)) USING DIFFERENT SINGLE DOSES OF HUMAN CHORIONIC GONADOTROPIN (HCG) AND MAMMALIAN GnRH WITHOUT DOPAMINE INHIBITOR

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The main aim of the experiment was to compare an efficiency and to found an optimal single doses of Chorulon preparation containing the human chorionic gonadotropin (HCG) and Supergestran preparation containing a mammalian GnRH ([D-Ala⁶]GnRHProNhet) without dopamine inhibitor in artificial spawning of pikeperch. The three years old females were divided into eleven experimental groups, each contained seven fish. The females were injected once with HCG and mGnRHa. Four doses of HCG [250; 500; 750; 1000 IU.kg⁻¹ body weight (BW)] and six doses of mGnRHa (1; 2.5; 5; 10; 25; 50 µg GnRHa.kg⁻¹ BW) were tested under experimental conditions of University of South Bohemia, Faculty of Fisheries and Protection of Waters. The last group was injected with one dose of 0.9% NaCl solution (control group, 0.9 cm³. kg⁻¹ BW). The average percentage of ovulating females was 88.5 ± 12.3% and 80.8 ± 11.9% in groups treated with HCG and mGnRHa without effect of hormonal doses, respectively. Average latency was 82 ± 9.9 and 85 ± 17.7 hours in groups treated with HCG and mGnRHa, respectively. The synchronization of spawning in female groups treated with HCG was higher compared to groups with mGnRHa. Average diameter of eggs was 0.949 ± 0.055 mm and 0.976 ± 0.057 mm in all groups treated with HCG and mGnRHa, respectively. All these parameters were not statistically different between all groups treated with HCG and mGnRHa. In case of hatching rate (HR) statistically different average values were found (HR = 73.6 ± 14.4% and 50.6 ± 17.7% in groups treated with HCG and mGnRHa, respectively). The experiment indicated that hormonal injection of pikeperch with HCG and as well as with mGnRHa is possible, but the HCG is more effective. The highest percentage of spawned females, synchronization and HR were obtained in groups treated with HCG after application of doses (500; 750 IU.kg⁻¹) and in Supergestran after application of dose 25 µg GnRHa.kg⁻¹.

Keywords: hormonal stimulation, Chorulon, Supergestra, synchronization, spawning

ARTIFICIAL REPRODUCTION OF BURBOT (*Lota lota* L.) UNDER CONTROLLED CONDITIONS

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Burbot (*Lota lota* L.) is one of the most endangered fish species in Poland and in many others European countries. Additionally, potentially might be a new species for freshwater coldwater aquaculture. For this reason it is necessary to develop methods of artificial spawning and rearing larvae under controlled conditions. The data on spawning of burbot in captivity are very limited. The aim of this study was investigated the artificial reproduction of burbot in captivity. Spawners survival was very high (over 97%) when fish were kept even for four months under the hatchery conditions. Only about 50% of burbot spawners ate trout pellets, whereas more than 70% individuals fed forage fish (frozen). Oogenesis in all fish, which took food, proceeded properly what resulted in ovulation after hormonal stimulation. Less than 30% of spawners were not mature; it might be connected with spawning behaviour of this species what was reported earlier that in natural burbot populations some part (sometimes even about 50%) of spawners do not mature during spawning season. In present study from all stimulated specimens (over 70 individuals) viable and good quality gametes were obtained. First females were ready to spawn 4–6 days after the last injection, independently of applied spawning agents and their doses. Males generally produced over 10 ml of semen per 1 kg of body with 30–90% motile spermatozoa. But the results among treatment groups were very variable. Females produced a great number of small eggs (over 800000 per kg of female). The egg survival to the eyed-egg-stage (water temperature 3–4 °C) after fertilization was high: average over 70% (ranging from 30 to 95%). Obtained results: high spawners survival, high percentage of matured fish and good quality of gametes, showed that it is possible to propagate successfully burbot in captivity, under controlled conditions.

Keywords: burbot *Lota lota*, coldwater aquaculture, hormonal stimulation, induced spawning

WATER POLLUTION BY COMMON CARP ORIGINATING FROM DIFFERENT POND MANAGEMENT SYSTEMS

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Water pollution by common carp originating from different farming conditions (semi-intensive with supplemental feeding by wheat and extensive without supplemental feeding) was evaluated. Two fish from each pond with different feeding regime were placed into 100 L aquaria filled with tap water. Water temperature was conformed to water temperature in ponds (21 ± 2 °C). The experiment was carried out in five replicates per treatment and took 72 hours. Every 24 hours, the fish were moved into an aquarium with clean water. Water from the original aquarium was filtered through a screen with 40 µm mesh size. Both, filtered water and solids were analysed. Solids were analysed for a content of dry matter, total phosphorus (P) and nitrogen (N). Filtered water was analysed for a concentration of suspended solids, total nitrogen, total ammonia, total phosphorus, and chemical oxygen demand (COD_{Mn} , and COD_{Cr}). Pollution detected in solids and in filtered water was related to live weight of fish per day. Observed results showed, that fish fed on wheat excreted higher amount of suspended solids, organic matter, nitrogen and phosphorus compared to fish without supplemental feeding. Fish secreted the main part of suspended solids during the first 24 hours of the experiment. Fish fed on wheat and without supplemental feeding excreted 1674 and 1055 $mg.day^{-1}.kg^{-1}$ of solids given in dry matter, respectively. These values decreased markedly during the second two days of the experiment. The similar time pattern showed values of COD_{Mn} and COD_{Cr} . The values of total nitrogen and total phosphorus, produced by fish fed on wheat were 563 and 22 $mg.day^{-1}.kg^{-1}$, resp. and by fish without supplemental feeding 424 and 17 $mg.day^{-1}.kg^{-1}$, resp. Comparable levels were measured during the rest of the experiment.

Keywords: *COD, BOD, nitrogen, phosphorus, ammonia, common carp, excrements, feed pellets, wheat, natural food*

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IMPORTANCE OF BEHAVIOUR STUDIES TO THE DIVERSIFICATION OF SPECIES IN AQUACULTURE

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Fish behavior studies are an important contribution to aquaculture industry and its regulation. In this study the patterns of agonistic behaviour and space occupation are investigated in captive groups of juveniles of rainbow trout (*Oncorhynchus mykiss*) and brown trout (*Salmo trutta fario*) under conditions that simulate their natural habitat, namely water temperature, light exposure and shelter availability. To compare the aggression levels and the use of space in nonospecific and mixed groups of juveniles, behavioural observation were made using focal observations of mixed groups (3 rainbow trout and 3 brown trout) or monospecific groups (6 brown trout or 6 rainbow trout) in a total period of 120 hours of observation. This type of studies can contribute to the diversification of finfish species in aquaculture and its sustainable development by ensuring that the culture techniques respect the normal behaviour patterns of this species and by evaluating the environmental impact of the introduction of invasive salmonids. Behaviour studies are also needed to evaluate the possibility of polyvalent aquaculture with different species.

Keywords: aggression, behaviour, salmonids, shelter use, interspecific

CURRENT STATUS OF STURGEON FARMING IN ROMANIA

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Started in Romania around the year 2000, the idea of sturgeon farming for caviar production hadn't the expected trend. Many small or middle size entrepreneurs invested different sum of money in sturgeon facilities, but most of them, because of lack of knowledge, stopped the activity even from incipient stages. Those who had correct knowledge about investment costs had a relative success in sturgeon farming, but not for caviar production. Today, only few sturgeon farms are running in Romania, and most of them are producing fingerlings for restocking the Danube river. These sturgeon nurseries rely on wild catch of broodstock, from which gametes are harvested and then the parents are released in natural waters. The fingerlings are farmed to different sizes, starting from 10–20 grams for *Acipenser ruthenus* and ending up to 300–600 grams for *Huso huso*. After reaching the requested size, the fingerlings are released in Danube in locations that are specified by officials. The caviar production farms are just started in Romania and most of them are based on recirculation systems. Unfortunately many of these farms were closed from the first months of activity and just a few are still running. The main reason is the bad understanding of necessary size of investment. However, currently over ten caviar farms are constructed and expected to run in the following year. These farms are better dimensioned having a holding capacity of over 5000 sturgeons per generation, and up to 10 generations are hosted in these farms.

Keywords: *sturgeon farming, trends, investment, caviar production, restocking*

THE CHANGES IN MATERNAL PHYSIOLOGICAL TRAITS OF ENDANGERED CASPIAN BROWN TROUT DURING CAPTIVITY AND THEIR RELATIONSHIPS WITH REPRODUCTION SUCCESS

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The reproductive success of two groups of captive Caspian brown trout was compared with a wild group. The results showed a higher hatching rate for wild group ($90.3 \pm 2\%$) compared to captive-wild ($63.5 \pm 10.1\%$) and captive reared ($18.1 \pm 9.7\%$) groups. The analysis of ovarian fluid as a non-invasive method demonstrated higher concentrations of basic ions in the ovarian fluid of captive-wild (Calcium: $1.8 \pm 0.06 \text{ mM.l}^{-1}$, Sodium: $166.7 \pm 5.1 \text{ mM.l}^{-1}$, Magnesium: $14.6 \pm 0.9 \text{ mM.l}^{-1}$) and captive-reared (Calcium: $2.2 \pm 0.2 \text{ mM.l}^{-1}$, Sodium: $169.9 \pm 5.6 \text{ mM.l}^{-1}$, Magnesium: $13.8 \pm 1.8 \text{ mM.l}^{-1}$) in comparison with wild group (Calcium: $0.8 \pm 0.07 \text{ mM.l}^{-1}$, Sodium: $117.9 \pm 9.9 \text{ mM.l}^{-1}$, Magnesium: $7.7 \pm 0.9 \text{ mM.l}^{-1}$). Also, higher levels of cholesterol and protein observed in ovarian fluid of captive-wild (cholesterol: $177.5 \pm 17.7 \text{ mg.l}^{-1}$, protein: $7.8 \pm 0.6 \text{ mg.l}^{-1}$) and captive-reared (cholesterol: $242.3 \pm 32.7 \text{ mg.l}^{-1}$, protein: $7.1 \pm 1.5 \text{ mg.l}^{-1}$) compared to wild group (cholesterol: $119 \pm 20.9 \text{ mg.l}^{-1}$, protein: $4.2 \pm 0.7 \text{ mg.l}^{-1}$). Significant negative correlations were found between hatching rate and calcium ($r = -0.64$, $p < 0.0001$), magnesium ($r = -0.40$, $p = 0.025$), sodium ($r = -0.43$, $p = 0.016$) and cholesterol ($r = -0.48$, $p = 0.007$) contents of ovarian fluid. Also, significant ($p < 0.05$) positive correlations were existed between eyed eggs mortality and calcium ($r = 0.65$, $p = 0.001$), magnesium ($r = 0.41$, $p < 0.05$), cholesterol ($r = 0.56$, $p = 0.005$) and total protein ($r = 0.41$, $p < 0.05$) contents of ovarian fluid. In conclusion, the perturbations in the levels of components of ovarian fluid in captive groups as indicators of stress and low exercise were considered as two of the possible factors affecting reproductive success of captive groups of endangered Caspian brown trout.

Keywords: *Caspian brown trout, captivity, hatching, maternal traits*

THE INFLUENCE OF SWALLOWED AIR BUBBLES ON BURBOT, *Lota lota* (L.), LARVAE – A CASE STUDY

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Burbot, *Lota lota* (L.), is one of the most perspective species for freshwater aquaculture diversification. However, the effective culture procedures are still developed. The main obstacle is the initial larvae rearing, where very high (up to 70%) mortality was reported. Therefore, any worrying aspect should be carefully studied. The aim of the study, was to investigate the effect of swallowed air bubbles by burbot larvae, on their growth, survival and detailed investigation of dynamic of this phenomenon. The 10 days investigation was conducted. On the 16 day post hatch (DPH), during controlled rearing in the collective tank (at stocking density 150 ind. L⁻¹), some of the larvae were seen to swam irregularly near the surface and some of them were oriented upside down or aside. Under the stereoscopic microscope it was certified that these otherwise swimming larvae had air bubbles in their digestive tract. Thus, randomly chosen larvae were separated for further observations. Additionally, duration and factors affecting the presence of the air bubbles in the digestive tract were reconsidered. The highest percentage of larvae with air bubbles were certified 5 hours after the first (in each day) feeding. After the next 3 hours all larvae dismissed bubbles from the digestive tract. It was also found that the feeding did not affect dismissing of the air bubbles positively. Results obtained indicate that swallowed air bubbles were not very harmful for burbot fish larvae. However, after the swallowing of air bubbles larvae did not eat and this phenomenon could be very high stress inducing factor. It could potentially affect further growth differentiation and in consequence cannibalism intensification. Thus, the presence of the air bubbles in the rearing tanks (e.g., caused by aeration or water inlet) should be limited during initial rearing of burbot larvae under controlled conditions.

Keywords: air bubbles swallowing, burbot *Lota lota*, controlled rearing, larviculture

EFFECT OF A DIET BASED ON RAW MATERIAL FROM NON-HUMAN FOOD SYSTEM ON FATTY ACIDS COMPOSITION IN WHITE MUSCLE OF ARCTIC CHARR (*Salvelinus alpinus*)

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To reduce the use of fish meal and oil in feed and maintain the n-3 polyunsaturated fatty acid (PUFA) portion in fish muscle is of highest interest among fish feed manufacturers and fish producers. Therefore, new sources of oils and proteins are tested in fish diets. An experimental diet (ED) where the protein source consisted of zygomycete (*Rhizopus oryzae*) meal 23%, mussel meal 20%, fish meal 24% and lipid source consisted of fish oil 13%, rapeseed oil 7% and sesame oil 1% of total diet was fed to Arctic charr (*Salvelinus alpinus*). A commercial diet (CD) with similar lipid content as ED was used as control. ED group resulted in higher 18:3n-3 portion ($P < 0.05$) but lower levels of 20:5n-3 (EPA) both in triacylglycerols (TAG) and phospholipids (PL) ($P < 0.05$) and 22:6n-3 (DHA) in TAG (but higher in PL and this cannot be well explained) compared with CD group. The fish growth was similar in both groups ($P > 0.05$). ED did cause a decrease of total n-3 PUFA portion ($P < 0.05$) and a lowered n-3/n-6 ratio in white muscle. However, the portion of EPA, DHA and lipid content in wild Arctic charr white muscle were around 9.4%, 28.4% in PL, 3.6%, 3.2% in TAG and 1.2% (average value of three groups of wild Arctic charr in another study) while that in our ED group were 9.6%, 41.0% in PL, 3.9%, 7.3% in TAG and 2.1% and in CD group 10.3%, 36.5% in PL, 4.9%, 8.3% in TAG and 2.4%. We concluded that ED decreased n-3 PUFA portion compared with CD but still able to maintain a satisfied portion of n-3 PUFA in Arctic charr white muscle.

Keywords: Arctic charr, EPA, DHA, n-3/n-6

RAPID DETECTION OF FISH PATHOGENS USING POLYMERASE CHAIN REACTION

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The polymerase chain reaction (PCR) has become an integral tool for the molecular biologist. It represents a workhorse which is readily adaptable for a wide variety of new procedures and applications in biological researches and especially in fish science. In a short time since PCR was invented, it has revolutionized our approach to investigation of genes and genomes. We can now isolate essentially any gene from any organisms using PCR. The undisputed success of detection assays based on PCR has been largely due to its rapidity in comparison to many conventional diagnostic methods. For instance, detection and identification of bacterial and viral fish pathogens can be accelerated from several days to a single working day when clinical samples are directly examined. Other microbial agents that are difficult to propagate outside their natural host often remain undetected by techniques relying on cultural enrichment, thus rendering PCR the only viable alternative to demonstrate their presence. Using nucleotide sequences of fish iridoviruses and herpesviruses and also sequence of 16S RNA gene of *Pseudomonas* sp. we designed the sets of oligonucleotide primers for rapid identification of these infectious agents. After amplification of pathogens genomic DNA, PCR products were visible on agarose gels. Thus, the PCR developed in this study provides rapid and simple identification of fish iridovirus, herpesvirus and *Pseudomonas* sp.

Keywords: DNA amplification, fish pathogens, PCR

**THE INFLUENCE OF SPERM CRYOPRESERVATION ON PROGENY
OF RUSSIAN STURGEON (*Acipenser gueldenstaedti* Brandt)**

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Cryopreservation of fish gametes is very important for reserving of genetic pool of endangered species. So in April 2010 we investigated the influence of using of freeze-thawed sperm (FTS) on viability and genetic-biochemical parameters of progeny of Russian sturgeon at Alexandrovsky Fishery (in Volga delta). We used high-quality mature eggs (appr. 50 g) from one female with length 132 cm and weight 16.9 kg. One portion of eggs was fertilized by sperm of 3 males (1:1:1) after freezing in liquid nitrogen and thawing after 24 hours with using original methodic (Lunev, 2009, Trenkler, Lunev, 2010). Control portion of eggs was fertilized by natural sperm of same males. The procedures of insemination, de-adhesion and incubation of both portions of eggs were identical. The fertility rates determined at stage of "small yolk-plug" were equal to 66% (FTS) and 99% (control). The hatchling rates were 44.8% and 89.6%, respectively. The rates of survival of larvae before beginning of active feeding were very high in both variants – 97%. So the main mortality of progeny after using FTS took place during first stages of embryogenesis (appr. 33%). Electrophoretic analysis of genetic markers (Ryabova etc, 2008) was carried out in tissues of larvae at stage of transition to active feeding. The heterozygotes (H_{ob}) of malate dehydrogenase (MDH) were equal to 0.649 in FTS-larvae ($n = 137$) and 0.619 in control larvae ($n = 142$). The rates of most frequent allele (pA) of MDH were 0.383 and 0.436, respectively. The values of H_{ob} of esterase (Est) were equal to 0.431 in FTS-larvae and 0.144 in control species, pA Est were 0.776 and 0.927, respectively. So we could not observe significant changes in MDH locus but noticed almost 3-fold increase of number of heterozygotes in Est locus. χ^2 -test demonstrated significant differences in H_{ob} Est between FTS- and control larvae ($P < 0.001$). These result suggested that sperm cryopreservation could be factor of selectivity of adaptive genotypes.

Keywords: *cryopreservation of sperm, genetic markers, progeny, sturgeon production*

**DOMESTICATION OF STELLATE STURGEON (*Acipenser stellatus* Pallas)
IN WARM-WATER FARM**

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The stellate sturgeon refers to one of most difficultly cultivating species of Acipenseridae under artificial conditions. Complex moments in the technological chain are: the onset of active feeding and transition to using of artificial feeds; low feeding efficiency of artificial feeds; high requirements for the quality of feeds and thermo-hydrochemical conditions. Therefore broodstocks of Volga stellate sturgeon are not numerous, both at private and state fish-farming enterprises. Meanwhile formation of broodstocks of stellate sturgeon under controlled conditions besides of contemporary natural area of inhabiting is important for conservation of the natural gene pool. In 2009 formation of broodstock of stellate sturgeon began in recirculation systems of Mozhaisk industrial-experimental fish-farming plant (MIEFFP). The fish-farmers used progeny from 3000 eggs received from different breeders in Astrakhan region. The experience of industrial cultivation of this species obtained earlier on different sturgeon fisheries was used. Now at MIEFFP nearly 900 of one-year juveniles are reared, average length of fishes is 48.5 cm (min. – 32.5 cm, max. – 62 cm) and average weight is 243.9 g (min. 60 g, max. 615 g). In process of domestication of stellate sturgeon we worked out optimal methods which gave steady results at most dangerous stages of early ontogenesis: hatchling rate is equal to 70–80%; survival rate (SR) of larvae at stage of transition to active feeding – 85%; SR of fingerlings with weight 2 g – 70%; SR of one-year juveniles – 85%. So the many-year experience of the cultivation of stellate sturgeon in recirculation systems could be used in process of domestication of this species.

Keywords: *domestication, broodstocks, recirculation systems, stellate sturgeon*

SPONTANEOUS AND GnRH-STIMULATED LH SECRETION FROM DISPERSED CELLS OF MALE AND FEMALE COMMON CARP (*Cyprinus carpio* L.) PITUITARY UNDER THE INFLUENCE OF TAMOXIFEN AND AROCLOR 1254

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The aim of this study was to examine the impact of PCB mixture – aroclor 1254 and tamoxifen (estrogen antagonist) on the spontaneous or GnRH analogue stimulated LH secretion from dispersed pituitary cells of sexually mature male and female common carp (*Cyprinus carpio* L.) at the spawning season. Aroclor 1254 (5; 10 ng ml⁻¹) and/or tamoxifen (10⁻⁵ M) and/or GnRH analogue (10⁻⁸ M) were added to the incubation medium. After incubation, which lasted 4 and 24 hours LH levels were determined by ELISA method. Aroclor 1254 given alone (at both tested concentrations) after 4 and 24 hours of incubation had no effect on LH secretion neither in males nor in females. Similarly aroclor caused no statistical changes in GnRH – stimulated LH release at 4 and 24 hours in female cells incubation and after short incubation of male cells. But after 24 hours in male cells incubation significant increase in LH release were observed. The highest increase of LH was detected in groups treated with tamoxifen in both sexes. Tamoxifen strongly increased spontaneous and GnRH stimulated LH secretion from male carp pituitary cells only after 24 hours and in females cells incubations these effect were observed also earlier (after 4 hours). Obtained results confirm the possibility of PCBs to act directly at the level of pituitary gland in fish. Administration of tamoxifen alone or in combination with PCB and/or GnRH caused a very high increase in LH secretion from male and female common carp pituitary cells.

Keywords: PCB, LH, carp, reproduction, tamoxifen

**ARTIFICIAL SPAWNING AND BREEDING OF THE ENDEMIC TROUT SPECIES
(NATIVE POPULATION) FROM LAKE OHRID FOR ITS RESTOCKING PURPOSES**

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The native ichthyofauna of Lake Ohrid is represented with 21 species among which five are endemic trout ones. For the purpose of this investigation the following three were considered: *Salmo typicus* (Karaman), *Salmo aphelios* (Stefanovic) and *Salmo ohridana* (Staindachner). Those three were chosen like most abundant and most phenotypically distinguished; in the same time they are with highest commercial value. Naturally, these three species, in the lake are spawning in different seasons and at different depths and substrates. The fertilization of the eggs was made on the sampling sites, from where they were transferred to the hatchery installations in the Hydrobiological institute in Ohrid, with permanent water temperature (10.5 ± 0.5 °C) and rich in oxygen. The incubation and their further breeding till age 240 days after fertilization was in the same conditions for all the species, when they were released into the lake. Female's fecundity was based on eggs counts of 100 specimens for each species and it is expressed in number of eggs per kg wet weight. The average volume of the sperm is expressed in ml.kg^{-1} . Differences were found on a species level. The fertilization rate was significantly high (> 94%) among all species and the ratio of fresh milt volume and number of eggs was calculated for obtaining the highest fertilization rate. The survival rates till hatching was in range of 87–98%, while till the releasing in the lake the losses were maximum 8.5%.

Keywords: *Lake Ohrid, Salmo typicus, Salmo aphelios, Salmo ohridana, artificial spawning*

IN VITRO SURVIVAL OF *Gyrodactylus salaris* AND TRANSMISSION OF *G. salaris* AND *G. thymalli* IN CO-HABITATION EXPERIMENT WITH ATLANTIC SALMON (*Salmo salar*) AND GRAYLING (*Thymallus thymallus*)

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In Norway, since the first records in mid-1970ies, the monogenean ectoparasite *Gyrodactylus salaris* has caused gyrodactylosis which has resulted in mass death of wild Atlantic salmon (*Salmo salar*). In contrast, the sibling species *Gyrodactylus thymalli* infecting grayling (*Thymallus thymallus*), is non-pathogenic. Two experiments were performed: (1) *In vitro* survival of *G. salaris* was tested in microtitre plates in 200µl charcoal filtered de-chlorinated water at 4 °C. Both active (in the search mode) and passive parasites were individually removed directly to the plates from killed salmon. After 20 h, 50% of the parasites had died. The maximal survival time of *G. salaris* at 4 °C was 49 h. No difference in survival time between previously active and passive parasites was found. (2) In the second experiments, two juvenile fish, one infected and one naïve fish, were co-habituated at 12 °C for 24 h in small plastic tanks (17 cm x 23 cm x 7cm). (i) A *G. salaris*-infected salmon or a (ii) *G. thymalli*-infected grayling were co-habituated with a naïve grayling or a naïve salmon. Mean intensities of *G. thymalli* and *G. salaris* on donor fish were 17 and 15, respectively. The four tests were run 24 times each. Results: (i) the *G. salaris*-donor-salmon infected 23.1% of the salmon and 4.2% of the grayling; (ii) the *G. thymalli*-donor-grayling infected 40.9% of the grayling and 20.8% of the salmon. After the host switch, the infected naïves were transferred separately into small boxes at 12 °C. The infection was regularly checked for more than one month. *G. thymalli* survived on four infected salmons for a mean time of 15 d (max. 35 d).

Keywords: ectoparasites, fish, parasite transmission, Platyhelminthes, salmonids

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EFFECT OF STOCKING DENSITY ON GROWTH AND FIN CONDITION IN INTENSIVELY CULTURED EURASIAN PERCH (*Perca fluviatilis* L.)

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The effect of stocking density (500; 1000; 1500; 2000 fish.m⁻³) on growth parameters and fin condition was investigated in perch juveniles reared under intensive culture conditions (recirculating aquaculture system). Moreover, the fin condition was compared to control group from pond culture at the end of trial in intensive culture. The body weight (mean ± S.D.) and total length of perch at the beginning of experiment was 19.1 ± 5.1 g and 106.8 ± 8.5 mm, respectively. After 90 days of rearing, the mean body weight was 48.5 ± 14.5; 35.9 ± 10.5; 32.1 ± 8.3 and 31.8 ± 8.1 g in densities 500–2000 fish.m⁻³, respectively. The results of this study showed that body weight, condition factor, feed conversion rate and fin condition were significantly different among treatments. The mean final body weight, condition coefficient and total fin length/standard length ratio (TFLR) were significantly higher ($P < 0.05$) at density 500 fish.m⁻³, than at other densities. On the other hand, there were no significant differences in survival and coefficient of variation for weight. There was a reduction of 15; 22; 24 and 26% (for particular stocking density) in TFLR compare to control group of perch. It is concluded that juvenile perch, within the range of tested stocking densities belong to category of fish which is characterised by an inverse correlation between stocking density and growth rate. With respect to the various effects that density causes on growth and fin condition, better understanding of these phenomena could have impact on many rearing steps of this species.

Keywords: *intensive culture, percids, fin erosion, fin damage, welfare*

This study was supported by CENAQUA project CZ.1.05/2.1.00/01.0024, project GAJU (No. 047/2010/Z), project KONTAKT no. ME 10126 and NAZV projects (QH91310 and QI101C033).

FIRST REPORT ABOUT SIBLING CANNIBALISM IN CHUB *Leuciscus cephalus* (L.) LARVAE

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Cannibalism is a type of feeding usually observed in predators. In omnivorous fish, like most cyprinids, it is noted sporadically. In the present study, the first report about sibling cannibalism in chub *Leuciscus cephalus* (L.) larvae was described. Chub larvae were reared under controlled conditions. After 9 days of rearing at a water temperature of 25 °C, cannibalism type I was observed (mortality caused by body damage). At this time, the larvae total length ranged from 10 to 11 mm. Additional observations on the preserved dead larvae showed that many of the first attacks were not towards the tail part but on the fin-fold located near the anal fin. Total mortality caused by cannibalism type I and II (totally swollen preys) was over 35% of initial number of stocked larvae. The greater heterogeneity size of the larvae influenced the higher mortality affected by cannibalism. However, it is not clear which factors induced cannibalism in chub. Thus, further observations of cannibalistic behavior, including post-mortem feed are required.

Keywords: *cannibalism; cyprinids; larviculture; Leuciscus cephalus, mortality*

HATCHING TIME EFFECT ON MORPHOLOGY AND GROWTH OF LARVAE FROM A SINGLE EGG BATCH OF AN EARLY SPRING SPAWNER *Esox lucius* AND A LATE SPRING SPAWNER *Cyprinus carpio*

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Hatching is a major turning point from ecological and behavioral points of view, yet it has little significance from morphological and physiological aspects. Indeed, it is not correlated with a fixed developmental stage and is not associated with qualitatively important morphological or physiological changes. Significant size differences were observed between larvae hatching at different times (*i.e.*, at the beginning or the end of the hatching period) both between and within egg batches. The ecological significance of such variability is suggested to be a bet-hedging strategy by parents to ensure that some of their offspring are able to survive in changeable environments (food, temperature). Based on the analysis of nine morphological variables, this study aims to evaluate the consequence of early and late hatching on morphology and growth of larvae of two species having different reproductive strategies, namely pike *Esox lucius* and carp *Cyprinus carpio*. First, results show that hatching time structures larval morphology of body and yolk sac in different ways for the two species. In pike, early hatching larvae were smaller and grew slower compared to late hatching ones. In carp, the inverse was observed and in the two cases, larval size at hatching was negatively correlated to growth rate. Initial body size differences between groups of larvae were no more significant at the total yolk sac resorption stage for the two species. In conclusion, both larval morphology and growth seem to be modulated by hatching times, possibly in order to create variability between larvae coming from a single egg batch. The onset of initial larval size variability at hatching seems also to be important for the further development of individuals. Hatching time effect appears to be linked to the spawning season, which is characterised by different environmental conditions (temperature and food).

Keywords: hatching time, morphology, growth, pike, carp

THE IMPACT OF POND MANAGEMENT INTENSITY ON WATER QUALITY

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The impact of a pond management on water quality was studied during 3 years under pilot production conditions at the Research Institute of Fish Culture and Hydrobiology at Vodňany (Czech Republic). Extensive (initial stocking density 50–310 kg per hectare without supplementary feeding) and semi-intensive carp production systems (initial stocking density 186–1506 kg per hectare with supplementary feeding with wheat or pellets) were carried out in experimental ponds (0.16 ha each). The efficiency of supplementary feeding was evaluated by relative feeding coefficient, which ranged between 1.2 and 6.6 kg.kg⁻¹. During the experiment, water quality determinants were monitored. The results proved that increasing fish stocking density and their supplementary feeding had negative impact on water quality, especially on organic loading (COD, BOD) and suspended solids and total phosphorus concentration.

Keywords: *common carp, water pollution, zooplankton, supplementary feeding, fish production*

This study was supported by CENAQUA project CZ.1.05/2.1.00/01.0024, project USB (GAJU) no. 047/2010/Z and project no. QH82117.

**TOLBUTAMIDE METABOLISM BY LIVER MICROSOMES
FROM ATLANTIC SALMON (*Salmo salar* L.)**

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In the present study we investigated an ability of liver microsomes from Atlantic salmon to metabolize tolbutamide to 4'-hydroxytolbutamide (TBOH). The hepatic microsomes were prepared from the salmon liver by Ca-aggregation method. The incubation mixtures (0.25 ml) contained 0.5 mg of microsomal protein in 50 mM Tris-HCl, 10 mM MgCl₂, 0.1m M EDTA buffer (pH 7.4). The reaction was initiated with NADPH (1mM) and was allowed to proceed at 25 °C for 40 min. The reactions were terminated with 100% cold acetonitrile. The concentration of TBOH was measured by HPLC with fluorescence detector. Formation of TBOH demonstrated single enzyme Michaelis-Menten kinetics with an apparent Km of 0.09 mM and Vmax of 49.5 pmol/min/mg. Addition of sulfaphenazole, a specific inhibitor for mammalian CYP2C9, in a range from 1 to 200 μM decreased formation of TBOH in a concentration-dependent manner. The highest inhibition (30%) was observed at the highest concentration of sulfaphenazole of 200μM. To further characterize tolbutamide metabolism by salmon microsomes, tolbutamide was incubated in the presence of specific inhibitors of fish CYP1A1, ketoconazole and ellipticine. Ketoconazole did not noticeably affect TBOH formation. In contrast, ellipticine inhibited TBOH formation with the IC50 value of 12.1μM. The rate of TBOH formation was altered in the presence of sesamin in incubations (from 10 to 1000μM). Kinetic analysis using 100μM of sesamin in the incubations revealed competitive-type inhibition. However, the degree of inhibition of tolbutamide metabolism by sesamin did not increase with increased sesamin concentration. In conclusion, the results provide evidence that tolbutamide is metabolized by microsomes from Atlantic salmon with formation of TBOH. This metabolism is at least partly mediated by CYP1A1 as suggested by decreased TBOH formation in the presence of specific CYP1A1 inhibitor ellipticine. Contribution of other CYP450 isoforms on tolbutamide metabolism in fish must be further investigated.

Keywords: *A. salmon, hepatic microsomes, sesamin, sulfaphenazole, tolbutamide*

DIETARY EFFECT OF OILS IN ATLANTIC SALMON (*Salmo salar*)

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Sesamin has been shown to affect levels of DHA (22:6n-3) in freshwater stage of salmonids. The effects of sesamin supplementation to vegetable oil-based diets fed to large Atlantic salmon in saltwater were examined. Sesamin was included to the diets with different concentrations (high 5.8 g/kg, low 1.16 g/kg and 0 g/kg). The oil mixture used in the feed contained rapeseed, linseed and palm oil with an n-6/n-3 fatty acid ratio of either 0.5 or 1. Fish were fed for 4 months and no mortality occurred during the experiment. The fish grew from an average weight of 105 ± 10 g to 554 ± 88 g. The fat content in fillets were similar in most groups (average $9.1 \pm 0.9\%$), although the diet without sesamin and low n-6/n-3 ratio had significant higher fillet fat ($10.0 \pm 0.9\%$) than the other groups. Addition of sesamin (5.8 g/kg) to diets fed to Atlantic salmon had a significant negative effect on growth rate and live weight. Sesamin supplementation did not significantly affect the percentage of DHA in triacylglycerol and phospholipid fraction of white muscle or liver, but it decreased the percentage of EPA (20:5n-3) and DPA (22:5n-3) in the fish fed high concentration of sesamin. We conclude that high concentrations of sesamin have negative effects on fish growth. Therefore it is important to explore the mechanisms of the metabolic actions of this lignan added in different concentrations and in different life stages of fish.

Keywords: dietary sesamin, DHA, fatty acids, salmonid, vegetable oil

**EFFECTS OF TEMPERATURE ON SURVIVAL, GROWTH AND BODY COMPOSITION
IN BARBEL *Barbus barbus* LARVAE**

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Larval barbel were reared for 21 days at 21.0; 24.1; 27.2 or 30.0 °C, and fed *ad libitum* live *Artemia* nauplii for 16 h a day. The final survival rates exceeded 99%. The highest mean daily growth rates, 0.97 mm TL and 17.3% BW, were found at 27.2 °C. Optimum temperature calculated for the growth of larvae was 26.9 °C for TL and 26.5 °C for BW. The lower was water temperature the higher was increase in fish TL in relation to the effective day-degrees age-scale. This indicates that an increase of water temperature to a higher extent accelerated developmental rate than the growth rate. Thus, long-term rearing of barbel larvae at temperatures different from those occurring under natural conditions could potentially result in undesired changes in fish size at maturity, their fecundity and mean size of eggs, embryos and larvae at hatching. Temperature significantly influenced the final chemical composition of fish body. The lowest dry matter content (19.1%) was found at 24.1 °C. The highest values of ash content were determined for 27.2 and 30.0 °C, 11.3 and 11.0% of dry matter, respectively. The caloric value of fish body was the highest at 30.0 °C (30.0 J mg⁻¹). However, when changes of body chemical characteristics were analysed against the effective day-degrees age-scale, no effect of temperature was observed. Thus, the effects of temperature seem to be related mainly to barbel development.

Keywords: *Barbus barbus*, body composition, growth, larvae, temperature

**IMMUNOMODULATORY EFFECT OF DIETARY BREWER'S YEAST EXTRACT IN PIKEPERCH
(*Sander lucioperca* (L.)) JUVENILES AGAINST THE CHALLENGE OF *Aeromonas salmonicida***

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Juvenile European pikeperch (initial body weight of 8.7 g) were fed commercial feed (group C) or commercial feed supplemented with *Saccharomyces cerevisiae* yeast extract (NuPro®, Alltech Inc., Nicholasville, KY, USA) in dose of 4 g 100 g⁻¹ feed (group N4) for 8 weeks. Blood and pronephros samples for the study of nonspecific cellular and humoral defense mechanisms were collected from 10 fish from each group. At the end of the experiment a challenge test was performed by injecting the fish with live pathogenic *Aeromonas salmonicida*. Increases in blood serum lysozyme activity and total Ig levels in serum were noted in group N4 ($P < 0.05$), which indicate the activation of non-specific humoral defense mechanisms. Experimental diet also stimulated non-specific cellular mechanisms, as indicated by the increased metabolic activity of blood phagocytes and pronephric macrophages, and in the increased intracellular killing activity of phagocytes and proliferative response of lymphocytes stimulated by mitogens compared with the control-fed pikeperch ($P < 0.05$). After *in vivo* challenge test, mortality in the control group was higher by 15% compared to the N4 group. Additionally, the supplementation of feed with yeast extract did not have a significant impact on the fish growth, condition or the feed conversion ratio. The proximate composition of the fish bodies were also very similar ($P > 0.05$). In conclusion, eight weeks of supplementation with 4 g of NuPro® 100 g⁻¹ feed strongly stimulated the pikeperch innate immune system.

Keywords: *Aeromonas salmonicida*, brewer's yeast extract, growth parameters, immune response, pikeperch

EFFECT OF DIFFERENT COMMERCIAL SPAWNING AGENTS AND THERMAL REGIME ON THE EFFECTIVENESS OF PIKEPERCH, *Sander lucioperca* (L.), REPRODUCTION UNDER CONTROLLED CONDITIONS

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Pikeperch, *Sander lucioperca* (L.), was pointed out as a one of the most perspective candidate for diversification of freshwater aquaculture. However, some aspects of production are still developed where controlled reproduction is one of the bottlenecks. The aim of the present study was to compare effectiveness of different commercial spawning agents in induction of final oocyte maturation (FOM) and ovulation in wild spawners. Within the study four spawning agents (human chorionic gonadotropin [hCG], mixed human and horse gonadotropin [PG-600], carp pituitary [CPH] and mammalian GnRH analogue combined with metoclopramide [Ovopel]) in different thermal regimes (13 and 15 °C) were tested. In both thermal regimes the highest ($P < 0.05$) ovulation rate among the treatment groups was certified after stimulation with hCG (100% in both cases). Latency time was the shortest in groups where CPH was used (2–3 and 3–4 days for 15 and 13 °C) and similar in the remaining groups (3–4 and 4–5 days for 15 and 13 °C, respectively). Embryo survival was the highest in groups treated with hCG (78.9 and 81.3% at hatching stage for 15 and 13 °C, respectively). Hormonal stimulation did not affect spermiation rate and spermatozoa motility significantly ($P > 0.05$). On the base of the obtained results it could be recommended to use hCG for induction of FOM and ovulation in pikeperch. Also, the thermal regime within the tested range seemed to have no negative effect on the reproduction outcome and application of lower temperature only prolonged time of ovulation.

Keywords: artificial spawning, hormonal stimulation, pikeperch, *Sander lucioperca*, spawning agents

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AQUA 2012: GLOBAL AQUACULTURE – SECURING OUR FUTURE

The Boards of Directors of the European Aquaculture Society (EAS) and the World Aquaculture Society (WAS) have the pleasure to announce that the AQUA 2012 event will take place in the Czech Republic capital, Prague, from September 1–5, 2012.

The AQUA events are co-organised by EAS and WAS every 6 years, and AQUA 2012 succeeds the 2006 event in Florence, Italy and the 2000 event held in Nice, France. The event comprises an international scientific conference, an international trade exhibition, workshops for aquaculture producers, forums organised by students and by the European Commission Directorate General for research and many other satellite workshops and meetings.

The previous AQUA events each attracted more than 2000 participants from over 50 countries, showing the global importance of aquaculture and specifically this event.

The theme “**Securing our Future**” has several aspects. It has obvious implication in global and regional food security and aquaculture trade, placing aquaculture products in the global fisheries market. It also refers to economic and environmental sustainability and the image of aquaculture activities. Our future is what we make of it now – how we alleviate poverty; how we manage our future resource needs and especially how we educate, train and manage knowledge for the next generation of aquaculture researchers, producers and other stakeholders.

AQUA 2012 Programme co-chairs Marco Saroglia, José Polanco and Zdenek Adamek have put together a vast programme of more than 50 technical sessions that will be open for abstract submission. Major pillars of the technical programme include Environment, Biodiversity and Climate Change; Aquaculture Certification; Aquaculture and Human Health; Production Systems; Feedstuffs, Feeds and Feed additives; Molluscs & other Invertebrates; Marine Shrimp; General Finfish Culture; Freshwater Fish Culture; Marine Fish Culture; Seaweeds; Ornamentals; Aquaculture Economics; Animal Welfare, Health and Diseases; Breeding and Genetics and other specialised topics. The programme therefore covers many aspects of the global aquaculture value chain for contribution by authors from all over the world.

Following the approval of the location by EAS and WAS Boards, Michael New OBE, Chair of the AQUA 2012 Steering Committee commented: *“After somewhat delicate discussions over the past 2 years with our partners and authorities in St. Petersburg, Russia and subsequently in Sharm El Sheikh, Egypt, the approved location in Prague is for me definitely not a ‘third choice’ location. We just hadn’t considered it in our earliest discussions. After visiting Prague for the first time early this year, I was taken in by its beauty and fascinated by the centuries-old tradition of aquaculture, pond and water management in the Czech Republic. Prague is at the centre of Europe. It is an easy location to fly or drive to and the congress centre is close to the centre, with excellent metro links stopping in front of the building and with an extremely wide choice of luxury and budget hotels within easy reach”.*

AQUA 2012 will be organised with the local partnership of the University of South Bohemia, the home of Czech aquaculture and pond fisheries. Professor Otomar Linhart, Dean of the Faculty of Fisheries and Protection of Waters at the university expressed his support, saying that *“the University of South Bohemia is exceptionally proud to host the organisation of AQUA 2012 in our country. This year, we are celebrating the 90th birthday of the Research Institute of Fish Culture and Hydrobiology and the chance that we have to announce AQUA 2012 at the upcoming inauguration of the South Bohemian Research Center of Aquaculture and Biodiversity of Hydrocenoses is one that we will not miss. Our Institute staff members are young, dynamic and motivated and we will do our utmost to support the success of AQUA 2012.”*

Further information on AQUA 2012 will be published on the web sites of EAS and WAS, with abstract submission and registration available online within the coming weeks.

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