

**University of South Bohemia
Research Institute of Fish Culture and Hydrobiology**



Biennial Report
2006–2007

Vodňany, 2008

Biennial report 2006–2007

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INTRODUCTION

I am pleased to be able to present you with our traditional annual report, which summarises the institute's main scientific, research and educational activities over the last two years. We have devoted this time to transform the Institute from a purely nationwide institute on a regional level to a European level, with a stable economy, functional ancillary units, a symbiotic work team including both younger and older staff who are keen and able to make new scientific and research-related discoveries – not only in terms of basic understanding, but also as regarding practical recommendations for fishermen and fisheries practices. Along the way the Institute has made a fundamental change by incorporating workplaces and laboratories into its structure, while retaining a relative degree of autonomy. With regard to its educational programme the Institute has its fishery doctoral course (PhD level) accredited until 2015, with the right to lectureships and professorships until 2011. I can now proudly declare that the institute is becoming a fully-fledged part of the University of South Bohemia, with facilities for the development of scientific and educational activities comparable with those of the university's other faculties, and is independent of these faculties. It no longer needs to count on any "vassal" relations in order to develop, which I see as a crucial factor for the future of the institute.

Science and research, as the institute's main focus, has developed as far as the unprecedented merging of experimental activities between science and research departments, having gradually succeeded in developing new directions of research spanning across the Institute, removing or surpassing interpersonal and economic barriers and interests at the workplace and laboratory level. We have to take account of the sharp increase in the use of molecular biology within the Institute as an important tool in all areas of research, from genetics, through reproduction, to hydrobiology, astacology, ecotoxicology, and ichtyopathology, which will come to realization in 2008–2010. In terms of more traditional research, our results have been published in some of the more worthy periodicals, rather than being merely "buried" in the summaries of various conferences. We have also managed to transform our results into technological methodology, educational films and specialised books. Workplaces and laboratories of a more scholastic nature have managed to acquire new grants from scholastic grant agencies, such as GA ČR or GA AV ČR, while workplaces and laboratories focusing more on fisheries practices have been awarded grants from the Mze ČR agency. If we compare the periods of 2005 and 2007, we can see a twofold increase in the number of acknowledged publications from 28 in 2005 to 48 in 2007. What is even more dramatic is the rise in the number of quotations from our work, increasing from 140 quotations in 2005 to 456 and 428 quotations in 2006 and 2007, respectively. It is clear that the world is interested in our work.

The last two years can be seen as positive for the team as a whole. There has been a gradual increase in the number of science and research, technical, and managerial employees, and a sharp rise in the number of students doing doctorate courses. By incorporating doctorates into laboratories and workplaces and taking on new post-doctorate students. The Institute has managed to reverse the adverse ratio between the institute's creative and provisory elements for the first time since its establishment in 1921. In 2007 the Institute comprised 64 % science and research and academic employees together with doctorate students, 26 % technicians and labourers, and 11 % administrative and managerial staff. The Institute currently employs a staff of 69 people, while in 2005 the figure was only 51. The current number of employees is the highest since 1993. The increase in personnel also reflects an improvement in financial management. During 2005–2007 the institute's revenues increased by 27 %, i.e. from 37 million CZK to 48 million CZK. The sharpest increase came in our entrepreneurial (subsidiary) economic activities, where, compared with revenues of 3.2 million CZK in 2005, we achieved an income of almost 7 million CZK in 2007. This increase was also reflected in remunerations, which were coped by our excellent economic results, and rose by an average of 15-25 % for all categories of employees over the last two years.

In addition to our scientific research activities we have expanded on our education of young scientists-to-be, i.e. our doctorate students. As part of our lifelong learning programme we have organised courses in fisheries practices, broadened the scope of the Institute's editorial work, and begun to organise regular international workshops.

During development we are not only thinking of the time facing the Institute, but are also trying to create good conditions for the future of our employees in the form of a worthy creative environment. We are planning to reconstruct the Institute's entire infrastructure, particularly from the Science and Research for Innovation Operational Programme. We have invested a total of 3 million CZK in planning to obtain permission and buying land. We can say that as far as the planning phase goes, we are ready;

only the financial part is remaining, i.e. to acquire funds and to put all into practice. The implementation in particular, in other words the physical reconstruction, will be the most difficult part, and will test every one of us. I would compare this to the floods of 2002, although these were unplanned, far less destructive and lasted a mere 14 days. Our reconstruction will be planned, but far more complete and longer-lasting (around three years). During this time our scientific and educational work should not be affected.

I would like to thank all my colleagues for this successful and inspiring time we have shared, and most of all I wish you all strong nerves for the next two years.

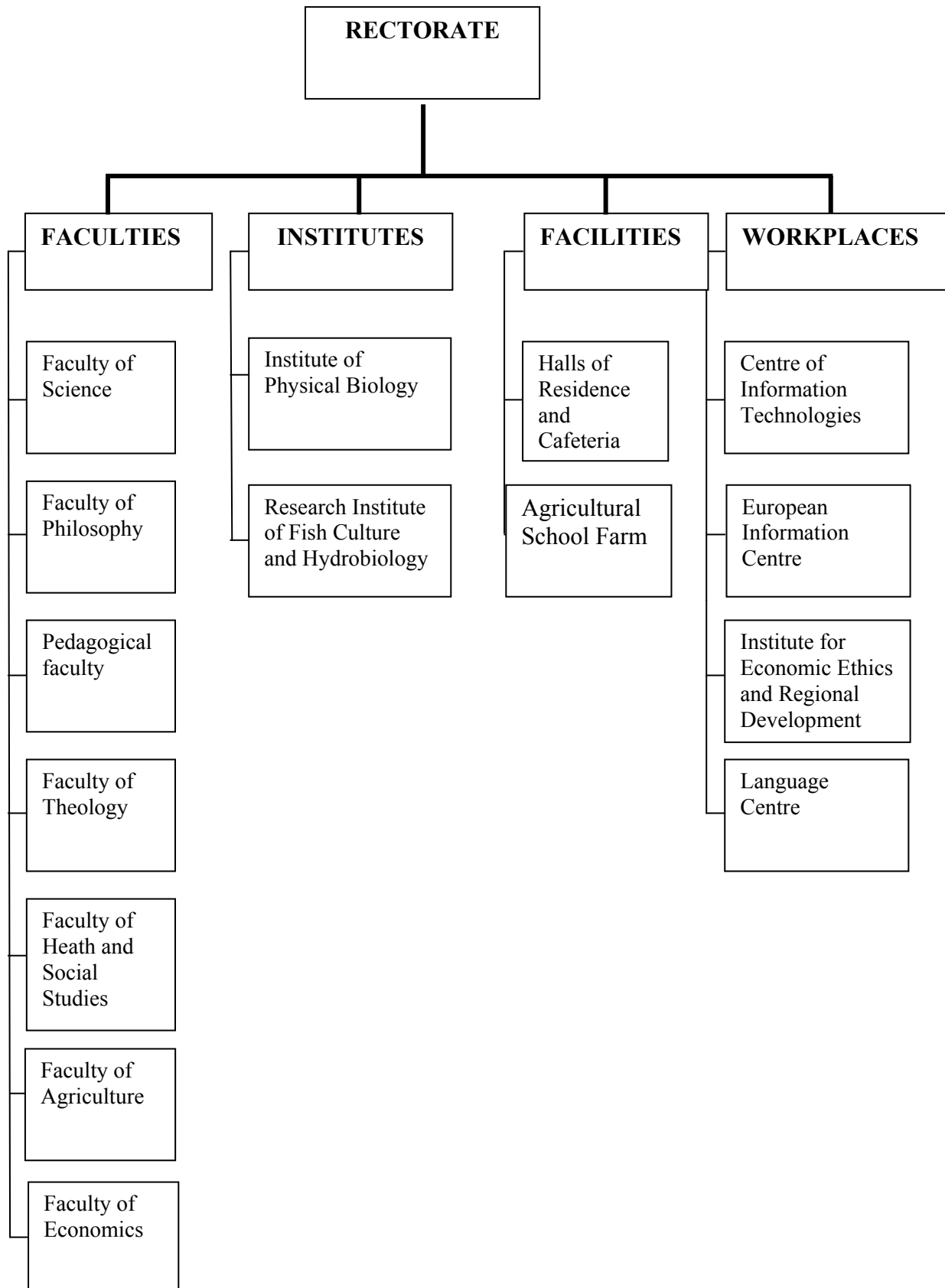
*Prof. Dipl.-Ing. Otomar Linhart, DSc.
Director USB RIFCH*

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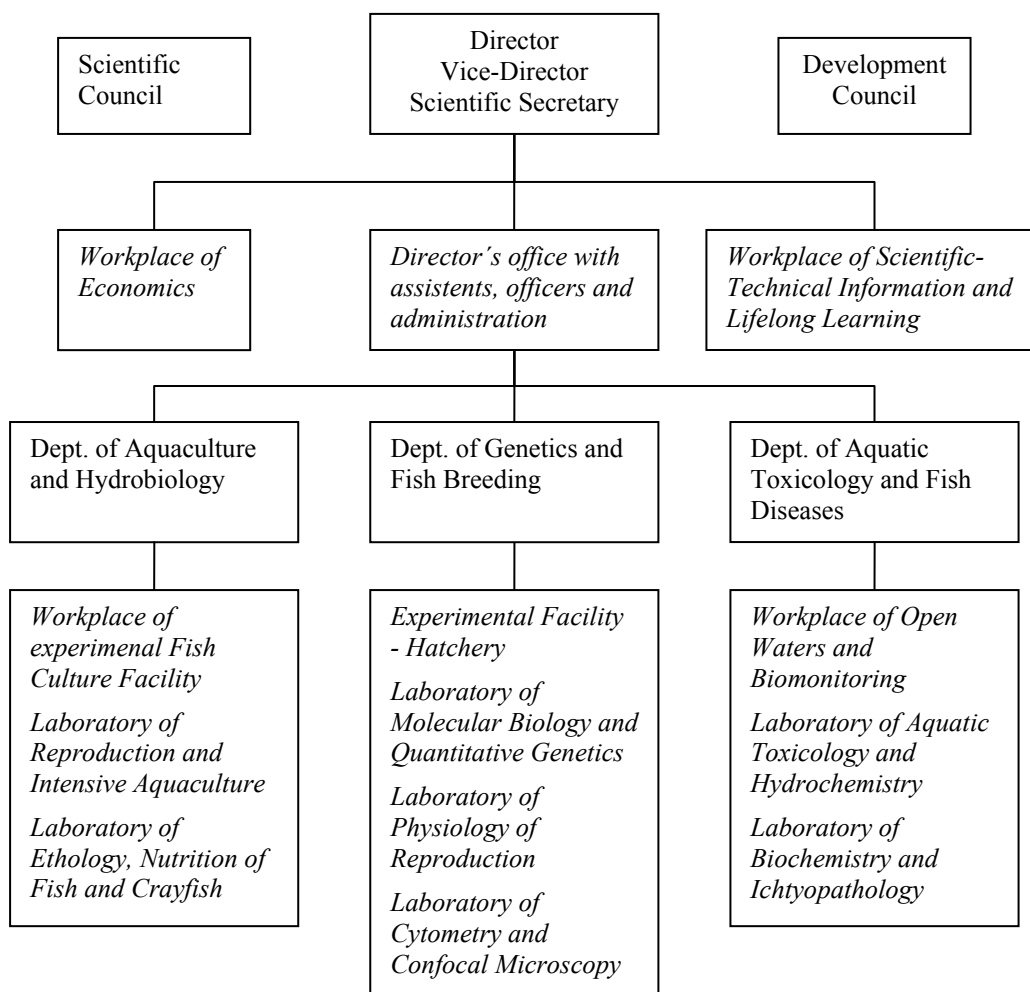
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ORGANIZATION STRUCTURE OF UNIVERSITY AND INSTITUTE

University of South Bohemia in České Budějovice



Research Institute of Fish Culture and Hydrobiology in Vodňany, on the date 1st January, 2008



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SCIENTIFIC COUNCIL

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Internal members:

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RIFCH USB Vodňany

Dipl.-Ing. **Pavel Kozák**, Ph.D.

RIFCH USB Vodňany

Dipl.-Ing. **Martin Flajšhans**, Dr.rer.agr.

RIFCH USB Vodňany

Dipl.-Ing. **Martin Kocour**, Ph.D.

RIFCH USB Vodňany

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USB IPB České Budějovice

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USB FA České Budějovice

Prof. M.Sc. **Libor Grubhoffer**, CSc.

USB FB České Budějovice

Prof. Dipl.-Ing. **Martin Křížek**, CSc.

USB FA České Budějovice

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MUAF Brno

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IAPG ASCR Liběchov

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IHB ASCR České Budějovice

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ENKI o.p.s., Třeboň

Dipl.-Ing. **Martin Dušek**

ANCLP CR Praha

M.Sc. **Pavel Punčochář**, CSc.

MA CR Praha

M.Sc. **Petr Roth**, CSc.

MoE CR Praha

RESEARCH PROJECTS

Year 2006

Projects supported by the Ministry of Education, Youth and Sport

Institutional research plans

- MSM6007665809 - Biological, environmental and breeding aspects in fish culture (2005–2011, responsible researcher prof. Dr. Otomar Linhart)

Research centres

- LC06073 - Centre for research of biodiversity (2006–2010, Institute of Systems Biology and Ecology of CAS in České Budějovice, responsible researcher for a part solved at USB RIFCH prof. Dr. Otomar Linhart)

Bilateral projects – Programme CONTACT

- ME743 - Comparative biological study of chromosomally manipulated and selectively bred populations of tench in China and the Czech Republic (2005–2006, responsible researcher David Gela, Ph.D.)
- ME742 - Short term storage and cryopreservation of fish embryos (2005–2007, responsible researcher prof. Dr. Otomar Linhart)
- ME855 - Utilization of noble crayfish (*Astacus astacus* L.) as bioindicator of open waters and support of its occurrence (2006–2009, responsible researcher Tomáš Polícar, Ph.D.)
- ME853 - New fish species and rearing aspects in aquaculture (2006–2008, responsible researcher Assoc. prof. Jan Kouřil, Ph.D.)

Projects supported by Ministry of Agriculture

Projects of National Agency for Agricultural Research

- QF3028 - Development of new technologies of rearing commercially important riverine species of fish and crayfish endangered by environment degradation (2003–2007, responsible researcher Pavel Kozák, Ph.D.)
- QF3029 - Harmonization with the EU in application of the principles of pharmacovigilancy in aquaculture in the Czech Republic (2003–2007, responsible researcher MVDr. Veronika Piačková, Ph.D.)
- QF4117 - Influence of mass selection in common carp (*Cyprinus carpio* L.) on performance of growth-related traits its progeny with using of DNA genetic methods (2004–2007, responsible researcher Martin Kocour, Ph.D.)
- QF4118 - Development of fish production combining pond cultures and technical aquacultures. (2004–2007, responsible researcher Assoc. prof. Jan Kouřil, Ph.D.)
- 1B44016 - Protection of common carp (*Cyprinus carpio* L.) culture from KHV disease (2004–2006, responsible researcher Veronika Piačková, DVM, Ph.D.)

Other subsidies and programmes

- National programme for conservation and utilization of genetic resources of farm animals – Maintenance of genetic resources in fish (guarantor Martin Flajšhans, Dr.rer.agr.)
- Subsidiary programme 2.A.e.1a): Maintenance and improving of genetic quality of farm animals and plants, Controls of performance – fish (guarantors Martin Kocour, Ph.D., Martin Flajšhans, Dr.rer.agr.)

Projects supported by the Grant agency of the Czech Republic

- 524/06/0817 - Ultrastructure, energetic and competition in spermatozoa: A comparative study using two model species of chondrosteian and teleostean fishes (2006–2008, responsible researcher prof. Dr. Otomar Linhart.)
- 206/05/2159 - Genetic, population and reproductive variability of invasive fish species, *Carassius "gibelio"* with alternating bisexual/asexual reproduction in central Europe (2005–2007, Institute of Vertebrate Biology of CAS Brno, responsible researcher for a part solved at USB RIFCH Martin Flajšhans, Dr.rer.agr.)
- 525/06/P234 - Effect of endocrine disruptors on chub (*Leuciscus cephalus* L.) - experimental studies (2006–2008, responsible researcher Vladimír Žlábek, Ph.D.)
- 523/06/P142 - The evaluation of growth and reproduction in common barbel (*Barbus barbus* L.) under intensive and controlled conditions (2006–2008, responsible researcher Tomáš Polícar, Ph.D.)

Projects supported by Ministry of Environment

- SA/650/5/03 Labe IV. (2003-2006, TGM Water Research Institute Prague, responsible researcher for a part solved at USB RIFCH Tomáš Randák, Ph.D.)

International research projects

6th Framework programme

- COOP-CT-2004 512629 - Securing juvenile production of Eurasian perch by improving reproduction and larval rearing (2005–2006, University of Henri Poincaré, Nancy, France; responsible researcher for a part solved at USB RIFCH prof. Dr. Otomar Linhart)
- COOP-CT-2004 512575-PROTENCH - Intensive and sustainable culture of the freshwater species tench (2005–2007, GESINFIN, Spain; responsible researcher for a part solved at USB RIFCH Martin Kocour, Ph.D.)
- COLL-CT-2006-030384-SUSTAINAQUA - Integrated approach for a sustainable and healthy fresh water aquaculture (2006–2009, Verein zur Foerderung des Technologie Transfers an der Hochschule Bremerhaven E.V., Germany, responsible researcher for a part solved at USB RIFCH Assoc. prof. Dr. Zdeněk Adámek, Ph.D.)
- CSN-INTRAN IPS-2001-42123 - Creating Supporting Network for International Transfer of Innovative Technologies in European Aquaculture (2003–2006, University of Sterling, responsible researcher for a part solved at USB RIFCH Assoc. prof. M.Sc. Zdeněk Adámek, Ph.D.)

Project supported by Grant agency of University of South Bohemia

- 2/2005/P-VÚRH - Ultrastructure of spermatozoa in Siberian sturgeon, *Acipenser baerii*, and tench, *Tinca tinca* (2006, responsible person Martin Pšenička)
- 4/2005/P-VÚRH - Subchronic effects of nitrites on rainbow trout from a view of realistic open water contamination (2006, responsible person Hana Kroupová)

Year 2007

Projects supported by the Ministry of Education, Youth and Sport

Institutional research plans

- MSM6007665809 - Biological, environmental and breeding aspects in fish culture (2005–2011, responsible researcher prof. Dr. Otomar Linhart)

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- ME853 - New fish species and rearing aspects in aquaculture (2006–2008, responsible researcher Assoc. prof. Jan Kouřil, Ph.D.)

Projects supported by Ministry of Agriculture

Projects of National Agency for Agricultural Research

- QF3028 - Development of new technologies of rearing commercially important riverine species of fish and crayfish endangered by environment degradation (2003–2007, responsible researcher Pavel Kozák, Ph.D.)
- QF3029 - Harmonization with the EU in application of the principles of pharmacovigilancy in aquaculture in the Czech Republic (2003–2007, responsible researcher Veronika Piačková, DVM, Ph.D.)
- QF4117 - Influence of mass selection in common carp (*Cyprinus carpio* L.) on performance of growth-related traits its progeny with using of DNA genetic methods (2004–2007, responsible researcher Martin Kocour, Ph.D.)
- QF4118 - Development of fish production combining pond cultures and technical aquacultures. (2004–2007, responsible researcher Assoc. prof. Jan Kouřil, Ph.D.)
- QH71305 - Development of new methods of rearing selected promising species for aquaculture using non-traditional technologies (2007–2011, responsible researcher Pavel Kozák, Ph.D.)
- QH71057 - Monitoring of the koi herpes virus disease (KHVD) occurrence in breeds of common carp in CR and testing of susceptibility of chosen strains of common carp to KHV (2007–2011, Veterinary Research Institute in Brno, responsible researcher for a part solved at USB RIFCH Veronika Piačková, DVM, Ph.D.)

Other subsidies and programmes

- National programme for conservation and utilization of genetic resources of farm animals – Maintenance of genetic resources in fish (guarantor Martin Flajšhans, Dr.rer.agr.)
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- 525/06/P234 - Effect of endocrine disruptors on chub (*Leuciscus cephalus* L.) - experimental studies (2006–2008, responsible researcher Vladimír Žlábek, Ph.D.)
- 523/06/P142 - The evaluation of growth and reproduction in common barbel (*Barbus barbus* L.) under intensive and controlled conditions (2006–2008, responsible researcher Tomáš Polícar, Ph.D.)
- 524/07/0188 - The role of immune investment in the context of trade-offs: immuno-ecological study of the relationships among reproduction, immunity and parasitism of fresh water fish (2007–2011, Masaryk university in Brno, responsible researcher for a part solved at USB RIFCH Martin Flajšhans, Dr.rer.agr.)

Projects supported by Grant agency of the Czech Academy of Science

- IAA601870701 - Factors influencing negative impact of invasive crayfish in the Czech Republic: migratory capability, reproduction, and pathogen transmission of crayfish plague pathogen (2007–2009, University of Ostrava, faculty of Science Ostrava, responsible researcher for a part solved at USB RIFCH Pavel Kozák, Ph.D.)

Projects supported by Ministry of Environment

- SP/2e7/229/07 - Labe V. (2007–2011, TGM Water Research Institute Prague, responsible researcher for a part solved at USB RIFCH Tomáš Randák, Ph.D.)

International research projects

6th Framework programme

- COOP-CT-2004 512575-PROTENCH - Intensive and sustainable culture of the freshwater species tench (2005–2007, GESINFIN, Spain; responsible researcher for a part solved at USB RIFCH Martin Kocour, Ph.D.)
- COLL-CT-2006-030384-SUSTAINAQUA – Integrated approach for a sustainable and healthy fresh water aquaculture (2006–2009, Verein zur Foerderung des Technologie Transfers an der Hochschule Bremerhaven E.V., Germany, responsible researcher for a part solved at USB RIFCH Assoc. prof. Dr. Zdeněk Adámek, Ph.D.)
- SSA-043150-SUSTAINAQ - Sustainable aquaculture production through the use of recirculation systems (2007–2009, Norwegian Institute of Fisheries and Aquaculture Research, Norway, responsible researcher for a part solved at USB RIFCH Assoc. prof. Dr. Zdeněk Adámek, Ph.D.)

Project supported by Grant agency of University of South Bohemia

- 88/2006/P-VÚRH - Analysis of migrating ability of non-native striped crayfish (2007, responsible researcher Miloš Buřič)
- 86/2006/P-VÚRH - Effect of temperature on growth and early ontogenetic development of weatherfish (*Misgurnus fossilis*) (2007, responsible researcher Bořek Drozd)
- 80/2006/P-VÚRH - Adaptability of in-facility reared stocks of brown trout (*Salmo trutta m. fario* L.) and grayling (*Thymallus thymallus* L.) on conditions of open waters and evaluation of their effect on native populations (2007, responsible researcher Jan Turek)

LIST OF PUBLICATIONS, SCIENTIFIC CITATION INDEX

2006

Papers in journals included in Web of Science (total 39)

- Alavi S.M.H., Cosson, J., 2006. Sperm motility in fishes: (II) Effects of ions and osmotic pressure. *Cell Biology International*. 30(1):1-14
- Alavi S.M.H., Cosson J. a Kazemi R., 2006. Semen characteristics in *Acipenser persicus* in relation to sequential stripping. *Journal of Applied Ichthyology*. 22(1):400-405
- Buchtová H., Svobodová Z., Kocour M., Velíšek J., 2006. Evaluation of growth and dressing out parametres of experimental scaly crossbreds in three-year-old common carp (*Cyprinus carpio*, Linnaeus 1758). *Aquaculture Research*. 37:466-471
- Buchtová H., Svobodová Z., Kocour M., Velíšek J., 2006. Evaluation of the dressing percentage of 3-year-old experimental scaly crossbreds of common carp (*Cyprinus carpio*, Linnaeus 1758) in relation to sex. *Acta Veterinaria Brno*. 75(1):123-135
- Caille N., Rodina M., Kocour M., Gela D., Flajšhans M., Linhart O., 2006. Quantity, motility and fertility of tench *Tinca tinca* (L.) sperm in relation to LHRH analogue and carp pituitary treatments. *Aquaculture International*. 14(1-2):75-87
- Dobšíková R., Svobodová Z., Bláhová J., Modrá H., Velíšek J., 2006. Stress response to long distance transportation of common carp (*Cyprinus carpio* L.). *Acta Veterinaria Brno*. 75(3):437-448
- Dobšíková R., Velíšek J., Wlasow T., Gomulka P., Svobodová Z., Novotný L., 2006. Effects of cypermethrin on some haematological, biochemical and histopatological parameters of common carp (*Cyprinus carpio* L.). *Neuroendocrinology Letters*. 27(2):101-105
- Đuriš Z., Drozd P., Horká I., Kozák P., Policar T., 2006. Biometry and demography of the invasive crayfish *Orconectes limosus* in the Czech Republic. *Bulletin Francais de la Peche et de la Pisciculture*. 380-381:1215-1228
- Đuriš Z., Horká I., Kristian J., Kozák P., 2006. Some cases of macro-epibiosis on the invasive crayfish *Orconectes limosus* in the Czech Republic. *Bulletin Francais de la Peche et de la Pisciculture*. 380-381:1325-1337
- Dušek L., Svobodová Z., Janoušková D., Vykusová B., Jarkovský J., Šmíd R., Pavliš P., 2006. Bioaccumulation of mercury in muscle tissue of fish in the Elbe River (Czech Republic): multispecies monitoring study 1991-1996. *Ecotoxicology and Environmental Safety*. 61(2):256-267
- Flajšhans M., Piačková V., 2006. Difference in blood and water diffusion distance in gill lamellae of diploid and triploid tench *Tinca tinca* (L.). *Journal of Fish Biology*. 69(6):1870-1873
- Gela D., Flajšhans M., Kocour M., Rodina M., Linhart O., 2006. Tench (*Tinca tinca*) broodstock management in breeding station under conditions of pond culture: a review. *Aquaculture International*. 14(1-2):195-203
- Horký P., Slavík O., Bartoš L., Kolářová J., Randák T., 2006. The effect of the moon phase and seasonality on the behaviour of pikeperch in the Elbe River. *Folia Zoologica*. 55(4):411-417
- Kocour M., Linhart O., Vandeputte M., 2006. Mouth and fin deformities in common carp: is there a genetic basis? *Aquaculture Research*. 37(4):419-422
- Kolářová J., Svobodová Z., Piačka V., 2006. Comparison of in vitro and in vivo tests in water toxicology. *Toxicology Letters*. 164(S1):S211
- Kordiovská P., Vorlová L., Borkovcová I., Karpíšková R., Buchtová H., Svobodová Z., Křížek M., Vácha F., 2006. The dynamics of biogenic amine formation in muscle tissue of carp (*Cyprinus carpio*). *Czech Journal of Animal Science : Živočišná výroba*. 51(6):262-270
- Koubek P., Elzeinova F., Sulc M., Linhart O., Peknicova J., 2006. Monoclonal antibody FsC-47 against carp sperm creatine kinase. *Hybridoma*. 25(3):154-157
- Kozák P., Buřič M., Policar T., 2006. The fecundity, time of egg development and juveniles production in spiny-cheek crayfish (*Orconectes limosus*) under controlled conditions. *Bulletin Francais de la Peche et de la Pisciculture*. 380-381:1171-1182
- Kozubíková E., Petrusek A., Đuriš Z., Kozák P., Geiger S., Hoffmann R., Oidtmann B., 2006. The crayfish plague in the Czech Republic - Review of recent suspect cases and a pilot detection study. *Bulletin Francais de la Peche et de la Pisciculture*. 380-381:1313-1323
- Kroupová H., Máchová J., Piačková V., Flajšhans M., Svobodová Z., Poleszczuk G., 2006. Nitrite Intoxication of Common Carp (*Cyprinus carpio* L.) at Different Water Temperatures. *Acta Veterinaria Brno*. 75(4):561-569
- Kroupová H., Máchová J., Svobodová Z., Piačková V., Smutná M., 2006. The ability of recovery in common carp after nitrite poisoning. *Veterinární medicína*. 51(8):423-431

- Linhart O., Mims S.D., Gomelsky B., Cvetkova L.I., Cosson J., Rodina M., Horvath A., Urbanyi B., 2006. Effects of cryoprotectants and male on motility parameters and fertilization rate in paddlefish (*Polyodon spathula*) frozen-thawed spermatozoa. *Journal of Applied Ichthyology*. 22(Suppl. 1):384-388
- Linhart O., Rodina M., Flajshans M., Mavrodiev M., Nebesářová J., Gela D., Kocour M., 2006. Studies on sperm of diploid and triploid tench, *Tinca tinca* (L.). *Aquaculture International*. 14(1-2):9-25
- Linhart O., Rodina M., Kocour M., Gela D., 2006. Insemination, fertilization and gamete management in tench, *Tinca tinca* (L.). *Aquaculture International*. 14(1-2):61-73
- Maršálek P., Svobodová Z., Randák T., 2006. Total mercury and methylmercury contamination in fish from various sites along the Elbe river. *Acta Veterinaria Brno*. 75(4):579-585
- Petrusek A., Filipová L., Ďuriš Z., Horká I., Kozák P., Policar T., Štambergová M., Kučera Z., 2006. Distribution of the invasive spiny-cheek crayfish (*Orconectes limosus*) in the Czech Republic. Past and present. *Bulletin Francais de la Peche et de la Pisciculture*. 380-381:903-918
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- 1st International Workshop on the Biology of Fish Sperm, August, 29 – 31, 2007– worldwide workshop, 46 participants
- Toxicita a biodegradabilita odpadů a látek významných ve vodním prostředí, 2007 – national conference, 45 participants

Scientific Citation Index (SCI):
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RESEARCH PRIORITIES

The department is involved in fundamental and applied research issues, and collaborates with the University of South Bohemia in education and training of under-graduate and Ph.D students. The main objective of research is to optimize the methods of artificial reproduction and intensive culture of freshwater fish (including artificial and semi-artificial propagation, optimization of environmental conditions for nursing of the early fry stages, optimization of food intake in fish reared under intensive culture, assessment of recirculation systems effectiveness, and modern methods of anesthetic application in intensive fish culture). Common barb, perch, catfish, brook trout and sturgeons have been used as model fish in laboratory experimental studies.

Research is focused on a combination of intensive and semi-intensive fish farming technologies in recirculation systems and pond farming facilities, respectively. Applied fisheries hydrobiology issues (including evaluation of the quality determinants of water environment and biological assessment of pond environment degradation, food biology of zooplanktonivorous and piscivorous fish) are explored with particular reference to pond conditions.

Special attention is given to interactions between protected piscivorous predators and fisheries management in still and running waters in the Czech Republic.

In addition to studies on freshwater fish, some research activities are focused on crayfish biology and culture. In this regard research is carried out on the monitoring of autochthonous and alien crayfish species in inland water bodies in the Czech Republic, biology of crayfish species, various

methods of crayfish reproduction and culture, restocking and/or re-introducing of native crayfish to new natural localities, and consultation services in case of crayfish mortalities or crayfish transfers.

There are seven main programmes of research in the department:

- Controlled artificial and semi-artificial fish reproduction
- Nursing of the early fry stages, including optimization of environmental conditions
- Fish culture under intensive conditions
- Use of anaesthetics in aquaculture
- Applied fisheries hydrobiology
- Interactions between protected piscivorous predators and fisheries management of ponds and natural water bodies
- Research in crayfish biology and culture

SELECTED RESEARCH RESULTS

Repeated administration of different hormonal preparations for artificial stripping and their effects on reproduction, survival and blood biochemistry profiles of female tench

The aim of study was to compare physiological statuses of stripped and not stripped tench females with ovulation induced by GnRH α or carp pituitary extract (CPE). The comparison was based on selected biochemical stress indices (glucose), condition (TP, TGA), and cell membrane characteristics (ALT, AST, CK). In the first and in the second year, 37 and 29 tench females were monitored, respectively. No differences in the values of biochemical parameters in either period were found between the two groups of stripped female tench whose ovulation was induced by GnRH α and CPE, respectively. No significant differences in TP, TGA, ALT, AST and CK values were found between the groups of stripped and not stripped females with ovulation induced by GnRH α or CPE. Different values were found in stress indices, specifically in glucose concentrations. A significant increase ($P < 0.01$) in glucose concentrations was found in tench females immediately after stripping and, in not stripped females, about 48 hrs after ovulation induction. Both artificial stripping and the inability to release eggs are important stress factors for female tench

Kouřil J., Svoboda M., Hamáčková J., Kaláb J., Kolářová J., Lepičová A., Sedova M., Savina L., Moreno Rendón P., Svobodová Z., Barth T. 2007. Repeated administration of different hormonal preparations for artificial stripping and their effects on reproduction, survival and blood biochemistry profiles of female tench (Tinca tinca L.). Czech J. Anim. Sci. 54(6): 183-188



Preparation of fish broodstock for artificial propagation in experimental fish facilities

Effects of short-time *Artemia* spp. feeding in larvae and different rearing environments in juveniles of common barbel (*Barbus barbus*) on their growth and survival under intensive controlled conditions.

The effect of short-time *Artemia* spp. feeding on growth performance and cumulative survival rate of barbel (*Barbus barbus*) larvae were studied under controlled aquaria conditions during the 21-day larval period. Three different feeding regimens (presenting reduced *Artemia* feeding) were tested for first exogenous nutrition of larvae (since 13 days post hatch): (1) artificial feed (Asta); (2) *Artemia* nauplii for 7 days followed by artificial feed; (3) *Artemia* nauplii for 14 days followed by artificial feed. The longer period of live food statistically improved growth of larvae ($W = 174 \pm 20$ mg and $SGR = 14.5 \pm 0.5\%d^{-1}$). The artificial dry food Asta without the addition of *Artemia* nauplii caused statistically decreased growth ($W = 135 \pm 22$ mg and $SGR = 13.1 \pm 0.7\%d^{-1}$). However, the growth of larvae with the short period of *Artemia* nauplii ($W = 153 \pm 25$ mg and $SGR = 13.8 \pm 0.7\%d^{-1}$) did not differ compared to either group. All used feeding regimens did not have a significant effect on the cumulative survival rate of larvae ranging from $73 \pm 1\%$ to $74 \pm 1\%$ at the end of the larval rearing period.

The effect of the rearing environment on growth performance and survival rate of juveniles was tested under intensive controlled conditions in aquaria and troughs for 84 days following the larval period. The environment of the troughs caused significantly decreased growth ($W = 2079 \pm 433$ mg and $SGR = 3.1 \pm 0.05\%d^{-1}$) of juveniles compared to ones reared in aquaria ($W = 3236 \pm 264$ mg and $SGR = 3.6 \pm 0.1\%d^{-1}$) at the end of the juvenile rearing period. Nevertheless, rearing environment did not have a significant influence on the cumulative survival rate of barbels ($S_C = 90 \pm 4\%$ and $81 \pm 3\%$ in aquaria and troughs, respectively).

Polícar T., Kozák P., Hamáčková J., Lepičová A., Musil J., Kouřil J. 2007. *Effects of short-time Artemia spp. feeding in larvae and different rearing environments in juveniles of common barbel (Barbus barbus) on their growth and survival under intensive controlled conditions. Aquatic Living Resources 20: 175-183*

Piscivorous fishes diet dominated by Asian cyprinid invader topmouth gudgeon, *Pseudorasbora parva*.

Predation on non-native cyprinid, *Pseudorasbora parva*, was reported during annual study in a small connecting canal about 40 km south of Brno, Czech Republic. Two cyprinid fish species (*Pseudorasbora parva*, and *Rutilus rutilus*) were identified in the diet of 23 piscivores belonging to three native fishes (*Perca fluviatilis*, *Sander lucioperca*, and *Esox lucius*). In environment, relative abundance of prey fishes changed seasonally dominated by topmouth gudgeon in spring and continuously increasing roach incidence. In contrast, topmouth gudgeon was found to be more sized-available prey ($P < 0.001$) and was the most dominant prey species in the diet of all piscivorous fishes the whole season throughout. Strong piscivory towards this alien might be beneficial for its eradication or control to support ecological integrity in natural and modified seminatural habitats.

Musil J., Adámek Z., 2007. *Piscivorous fishes diet dominated by Asian cyprinid invader topmouth gudgeon, Pseudorasbora parva. Biologia, Bratislava 62 (4): 488-490*

Seasonal dynamics in fish assemblage in a pond canal.

The annual dynamics of fish assemblage in a pond-connecting canal of a South Moravian pond aquaculture facility was studied to reveal potential assemblage effects on pond stocks and vice versa. The species richness of fish assemblage and seasonal dynamics of its development were predominantly dependent upon the fish stock of the interconnected ponds (particularly due to escapees during the period of pond harvesting), which contributed to a considerable increase of commercial fish species occurrence. Using a seasonal parameter, the season proved to have a major impact on fish assemblage dynamics ($r = 0.71$, $P < 0.001$). Spring samples were dominated by commercial fishes of aquaculture origin (mainly). Occurrence of several fish species originating from the wild (bleak, *Alburnus alburnus*, chub, *Leuciscus cephalus* and roach, *Rutilus rutilus*) was also reported. The pond canal was found to be an important reservoir site of persistent survival and potential subsequent spreading of some undesirable alien fishes such as topmouth gudgeon (*Pseudorasbora parva*) and gibel carp (*Carrasius auratus*). Moreover, more attention should be paid to the potential negative impacts of

all fishes of aquaculture origin upon the natural ecosystem and to the adverse effects due to the immigration of wild fish into aquaculture pond farming units.

Musil J., Adámek Z., Baranyi Ch., 2007. Seasonal dynamics in fish assemblage in a pond canal. *Aquaculture International* 15: 217-226

Computer-assisted image analysis in the evaluation of fish wounding by cormorant [*Phalacrocorax carbo sinensis* (L.)] attacks.

Fish, which have escaped from a cormorant's (*Phalacrocorax carbo sinensis* (L.)) grasp and/or which could not be swallowed due to their size, suffer from various injuries resulting in consecutive infections and subsequent increased mortality. A computer assisted image analysis was applied to describe the extent of such injuries. Two-year-old mirror carp, *Cyprinus carpio* L. (TL 200 – 300 mm, W 200-300 g) showed injuries ranging over approximately 10% of the total body surface. Immediately after wounding, the damaged epithelium (scars) cover 5 - 35 %, and deeper sub-dermal wounds, caused by the beak tip pervading into muscle tissue, cover an area of 1- 2 % of the total body surface. On the side impacted by cormorant lower mandible, extensive areas of epidermal contusions (scars) occur. As the time progresses, these ratios change – deeper necroses represent up to 10 % of total body surface and healing epithelial scars comprise just 1 – 2 %. In wounded silver carp, *Hypophthalmichthys molitrix* Val. (300 – 400 mm, W 500 – 700 g), the share of sub-dermal wounds usually does not exceed 0.5 % due to their compact scaly cover. During pond draining due to fish harvesting, the size spectrum of wounded fish increases and may also often include bigger fish (e.g. European catfish, *Silurus glanis* L.) up to 2.2 kg.

Adámek Z., Kortan J., Flajšhans M., 2007. Computer-assisted image analysis in the evaluation of fish wounding by cormorant [*Phalacrocorax carbo sinensis* (L.)] attacks. *Aquaculture International*, 15(3-4): 211-216

Fish and macrozoobenthos in the Vlára stream drainage area (Bílé Karpaty Mountains).

Fish fauna and macrozoobenthos were surveyed at 51 and 32 sites, respectively, in mountain and submountain streams of the Vlára stream drainage area in the Bílé Karpaty Mountains. In total, 15 fish species were registered. Brown trout and stone loach were the most frequent species, followed by chub, gudgeon and minnow. The species richness increased downstream in the mainstream of the Vlára stream (maximum of 10 species at the lowest study site). Qualitative data on fish assemblages did not fully correspond with the environmental stress. Macrozoobenthos indicated a minor decrease in water quality downstream of small villages and farms. Larvae of dipterans (Chironomidae and Simuliidae in particular) and mayflies (Ephemeroptera) dominated amongst the temporary water macroinvertebrates, whilst numerous populations of *Gammarus fossarum* (Amphipoda) and in some cases also of *Asellus aquaticus* (Isopoda) were recorded as permanent inhabitants of clean and polluted stretches, respectively.

Jurajda P., Adámek Z., Janáč M., Valová Z., 2007. Fish and macrozoobenthos in the Vlára stream drainage area (Bílé Karpaty Mountains). *Czech J. Anim. Sci.* 52(7): 214-225

Food habits of four bottom-dwelling gobiid species at the confluence of the Danube and Hron Rivers (South Slovakia).

Since 1997, three new *Neogobius* species (*Neogobius kessleri*, *N. fluviatilis* and *N. melanostomus*) have been regularly recorded in the Slovak part of the Danube River, formerly inhabited only by one native gobiid species (*Proterorhinus marmoratus*). The study of their food habits proved that the amphipod *Corophium curvispinum*, chironomid larvae and pupae, caddis fly larvae (*Hydropsyche* sp.) and mayfly nymphs (*Ephoron virgo*, *Potamanthus luteus*) were the most important food items contributing to the similarity of the diets of the gobiid species. Fish (0+ *Zingel zingel* and *Sander lucioperca*) appeared in the diet of *N. kessleri* only.

Adámek Z., Andreji J., Martín Gallardo J., 2007. Food habits of four bottom-dwelling gobiid species at the confluence of the Danube and Hron Rivers (South Slovakia). *Internat. Rev. Hydrobiol.* 92(4-5): 554-563

The effect of inter- and intra-specific competition on survival and growth rate of native juvenile noble crayfish *Astacus astacus* and alien spiny-cheek crayfish *Orconectes limosus*.

Juvenile noble crayfish, *Astacus astacus* and spiny-cheek crayfish, *Orconectes limosus* were reared from the 2nd and the 3rd developmental stage (i.e. since the stage of independence), respectively, until the end of the growing season. The first period revealed very low survival rate of *O. limosus* in the communal stock (7±6.2 %), probably caused by predation of *A. astacus*. Separately reared *O. limosus* proved significantly higher survival rate (49±9.2%) than those in the communal stock with *A. astacus*. The latter species also showed the highest survival rate in this case (90±7.1 %). This was not found to differ from the survival rate of separately reared noble crayfish (86±3.1 %). In general, both the significant effect of bigger size of *A. astacus* than that of *O. limosus* and higher intra-specific cannibalism of *O. limosus* were reflected during the first phase of rearing. Size and weight of both species were equivalent 11 weeks after hatching (8 weeks of rearing). The higher cumulative survival rate was reached in the intraspecific culture of *A. astacus* (90 %) while low cumulative survival of both species was found in communal stock (less than 50 %) in the second phase of rearing. At the end of the growing season, 19-week-old *O. limosus* attained a higher mean size and weight but were more variable in the group (29.3±5.17 mm and 616.4±412.53 mg) than *A. astacus* (21.2±1.82 mm and 229.9±59.45 mg). Single and communal stock did not differ. Growth of *O. limosus* was much faster than that of *A. astacus*.

Kozák P., Buřič M., Polícar T., Hamáčková J., Lepičová A., Lepič P., 2007. The effect of inter- and intra-specific competition on survival and growth rate of native juvenile noble crayfish *Astacus astacus* and alien spiny-cheek crayfish *Orconectes limosus*. *Hydrobiologia* 590: 85-94

Studies on annual gonadal development and gonadal ultrastructure i spiny-cheek crayfish (*Orconectes limosus*).

The aim of this study was to follow the gonadal development in freshwater crayfish *Orconectes limosus* (Rafinesque) by histological examination of ovaries and testes as well as the monthly investigation of the gonadosomatic index over a period of one year. Male gonadosomatic index (I_G) ranged between 0.11 to 0.79% with a minimum in July (0.11%) and maximum in September (0.79%) while in the females it ranged between 0.25 to 6.15% with a minimum in July (0.25%) and maximum in April (6.15%). Between April and May, the histological dissection indicated presence of mature oocytes and oocytes undergoing resorption. Egg extrusion took place in mid April. During the summer months, the volume of oocytes gradually decreased. In winter and spring, the volume of oocytes began to increase during preparation for egg extrusion. The size of oocytes in the ovary increased from 0.2 mm at the beginning of the reproductive cycle to 1.8 mm immediately before egg extrusion. Average number of eggs in the ovary was 140.8±51.63 (76-290). The ovary was of brown colour for the majority of the cycle, but white in June and July and orange in August and September. Mating started in October and continued through the winter. A difference was found in the proportions of testis and *vas deferens* in the male reproductive organ during the year. The male gonadosomatic index increased in September during preparation for mating ($I_G=0.79%$). During the mating season, the male spermatophores were predominantly filled with spermatozoa and sperm was also noted in the *vas deferens*. Two out of 15 males sampled during the winter were found to be intersex, in which atretic oogonia and oocytes were present at the periphery of testicular tissue and occupied <15% of testicular tissue. The evidence of intersex strongly suggested transitional stages of a gradual change of sex, which may be qualified as partial hermaphroditism.

Kozák P., Hulák M., Polícar T., Tichý F. 2007. Studies on annual gonadal development and gonadal ultrastructure i spiny-cheek crayfish (*Orconectes limosus*). *Bulletin Francais de la Pêche et de la Pisciculture* 384: 15-26

Invasive zebra mussel colonisation of invasive crayfish.

We investigated the interaction between two invasive invertebrate species in a shallow Central European flooded sandpit: the epibiosis of Ponto-Caspian zebra mussels *Dreissena polymorpha* on the American crayfish *Orconectes limosus*. Between 2004 and 2005, we followed the seasonal variation in number and size of the mussels attached to crayfish bodies, and microhabitats preferred by mussels. The proportion of crayfish colonised by mussels varied seasonally: in spring and early summer it was

consistently over 75%, afterwards it dropped temporarily due to loss of bivalves during the crayfish moult, and later increased again due to re-colonisation by often relatively large juvenile mussels. Three different pathways of mussel settlement on crayfish hosts are likely: (1) primary settlement of free-swimming pediveliger larvae; (2) secondary settlement of plantigrade mussels and juveniles; (3) active re-attachment of grown mussels from the substrate to crayfish. This epibiosis was promoted by lack of suitable substrates at the studied locality.

Ďuriš Z., Horká I., Petrusek A., 2007. Invasive zebra mussel colonisation of invasive crayfish: a case study. *Hydrobiologia* 590: 43-46

First record of invasive American crayfish *Pacifastacus leniusculus* (Decapoda: Astacidae) in the Morava River (Slovakia).

The signal crayfish, *Pacifastacus leniusculus* (Dana, 1852), was recorded in the Slovak-Austrian stretch of the Morava (March) River in the Záhorie region in August 2006. This is the first confirmed record of a non-indigenous crayfish species of American origin in Slovakia. Most likely, signal crayfish were intentionally released to the river by Austrian fishermen, or invaded from adjacent water bodies in Austria. As this species has a substantial invasive capability and is a potential vector of the crayfish plague pathogen, it represents a threat to the native crayfish species, and its spread should be monitored. Its invasion upstream to the territory of the Czech Republic is not unlikely in near future.

Petrusek A., Petrusková T., 2007. Invasive American crayfish *Pacifastacus leniusculus* (Decapoda: Astacidae) in the Morava River (Slovakia). *Biologia, Bratislava* 62(3): 356-359

***Aphanomyces astaci*, the crayfish plague pathogen, may be a common cause of crayfish mass mortalities in the Czech Republic.**

On three occasions crayfish plague (*Aphanomyces astaci*) has been identified as the cause of mass mortalities of native crayfish in the Czech Republic in 2005 and 2006. The true number of plague outbreaks is likely to be higher since crayfish mortalities often go unnoticed and only the result, the disappearance of a native crayfish population, is noted. Crayfish plague may have a significant negative impact on the successful restoration of indigenous crayfish populations in Central Europe.

Kozubíková E., Petrusek A., Ďuriš Z., Oidtmann B., 2007. *Aphanomyces astaci*, the crayfish plague pathogen, may be a common cause of crayfish mass mortalities in the Czech Republic. *Bulletin of the European Association of Fish Pathologists* 27(2): 79-82

Biometry and demography of the invasive crayfish *Orconectes limosus* in the Czech Republic

Biometry, population data and selected ecological parameters of populations of the spiny-cheek crayfish, *Orconectes limosus*, were measured to evaluate the differences between "marginal" (short-time, situated on margins of an area) and "residential" (longterm, well established inside an area) populations of this invasive crayfish. We selected three types of localities: 1. Large rivers - residential (Labe, Vltava); 2. Brooks (small and shallow running waters) - marginal; 3. Isolated standing waters - residential (old sandpits or flooded quarries). No significant differences in the total body length of crayfish were found between running and standing water localities as a whole, or between marginal and residential populations from running waters. There was, however, a low but distinct difference in the body weight between crayfish from brooks and other waters. Larger specimens (up to 116.5 mm in body length) were found only in isolated standing waters, whereas no crayfish longer than 100 mm were found in the rivers. The sex ratio of the crayfish was almost equal in rivers and isolated waters; males were more numerous in brooks. There was a significant difference between male and female body weight-length relations.

Ďuriš Z., Drozd P., Horká I., Kozák P., Polícar, T., 2006. Biometry and demography of the invasive crayfish *Orconectes limosus* in the Czech Republic. *Bulletin Francais de la Pêche et de la Pisciculture* 380-381: 1215-1228

Some cases of macro-epibiosis on the invasive crayfish *Orconectes limosus* in the Czech Republic

Several cases of epibiosis by macro-invertebrates on *Orconectes limosus* were recorded during our research on the biology of populations of this invasive crayfish species in the Czech Republic. (1) In 2001, we observed a high infestation of *O. limosus* by native species of branchiobdellidan worms (Annelida: Branchiobdellidae) in the river Elbe at Obríství (Central Bohemia). Four European Branchiobdella species were collected and identified from three crayfish specimens: *B. pentodonta* (52%), *B. balcanica* (24%), *B. parasitica* (18%) and *B. hexodonta* (6%). In 2003, only a single crayfish was found bearing branchiobdellidans (5 specimens of *B. parasitica* only) in the same locality. No branchiobdellidans on *O. limosus* have been confirmed since. (2) A flooded sandpit Lhota near Brandýs nad Labem is the only Czech locality where the settlement of *Dreissena polymorpha* (Mollusca, Bivalvia) on an *O. limosus* body was recorded. The crayfish lost the bivalves by moulting in summer; new mussels had settled by late summer and early autumn. (3) Females of the fish louse *Argulus cf. foliaceus* (Crustacea: Branchiura) lay egg-strings on any hard substrate, including the crayfish exoskeleton. Such egg-masses were found on up to 65% of *O. limosus* specimens in the above-mentioned sandpit. (4) Bryozoan colonies of *Plumatella repens* were found twice on crayfish in the rivers Elbe (Labe) and Cidlina.

Ďuriš Z., Horká I., Kristian J., Kozák P., 2006. Some cases of macro-epibiosis on the invasive crayfish *Orconectes limosus* in the Czech Republic. *Bulletin Francais de la Pêche et de la Pisciculture* 380-381: 1325-1337

The fecundity, time of egg development and juveniles production in spiny-cheek crayfish (*Orconectes limosus*) under controlled conditions

We comprehensively describe the fecundity and time of embryonic development of the spiny-cheek crayfish (*Orconectes limosus*). A linear relationship between the female size and ovarian and pleopodal fecundity and production of juveniles at the 3rd stage in spiny-cheek crayfish fecundity was confirmed. The mean value of the ovarian fecundity was found to be 130.8 ± 107.6 (46-505) oocytes. The value of the female gonadosomatic index just before laying was $4.2 \pm 1.8\%$ (0.8-7.7%). Pleopodal fecundity reached 217.8 ± 94.9 (95-492) eggs of a mean diameter 1.8 ± 0.2 mm (1.45-2.15 mm). Statistically significant difference was found between the egg numbers carried on individual pairs of pleopods. Mean time from laying to hatching was 46 ± 3.8 days (37-56 days), i.e. 647 ± 39.9 CTU (Celsius Temperature Units = degrees Celsius x days). Mean production of juveniles at the 3rd stage was found to be 135.7 ± 67.24 (15-243) juveniles. The study shows that the spiny-cheek crayfish has higher fecundity and shorter development time in comparison to the European native crayfish under the same conditions. The higher relative fertility and short time of egg incubation classify spiny-cheek crayfish to rapid selected species in comparison with native crayfish. The early sexual maturation and also high number of juveniles at the 3rd stage per female gives this species a good predisposition for spread quickly to new localities.

Kozák P., Buřič M., Polícar T., 2006. The fecundity, time of egg development and juveniles production in spiny-cheek crayfish (*Orconectes limosus*) under controlled conditions. *Bull. Fr. Peche Piscic*, 380-381: 1171-1182

The crayfish plague in the Czech Republic – Review of recent suspect cases and a pilot detection study.

There are only very limited reports about the occurrence of the crayfish plague in Czechia. In recent years, mass mortalities of *Astacus* spp. with symptoms of possible crayfish plague were noticed in three streams in the country - two in Central Bohemia (1998-9) and one in Silesia (2004). Three dead individuals from the last outbreak were examined for the presence of the crayfish plague pathogen, *Aphanomyces astaci*, by observation of the presence of hyphae in their cuticle and by a PCR-based diagnostic method. In all three cases the detection was positive. Although causes of mass mortalities from two other localities lack such a direct confirmation, the indirect evidence supports the same conclusion. The main potential vector of *A. astaci* in Czechia is the American spiny-cheek crayfish *Orconectes limosus*, widespread in large rivers of the western part of the country and in various isolated standing waters. Using the same molecular method, we investigated the presence of *A. astaci* in living *O. limosus* individuals from six localities (three running and three standing waters).

The analysis indicated the presence of the pathogen in animals from five out of six investigated *Orconectes* populations. One of them is present in the stream where two European *Astacus* species had gone extinct in 1998-9. Our results suggest that the crayfish plague is still present in Czechia, and that populations of *O. limosus* represent a reservoir for the crayfish plague pathogen, which directly endangers populations of the native crayfish.

Kozubíková E., Petrusek A., Ďuriš Z., Kozák P., Geiger Sh., Hoffmann R., Oidtmann B., 2006. The crayfish plague in the Czech Republic – Review of recent suspect cases and a pilot detection study. *Bulletin Francais de la Pêche et de la Pisciculture* 380-381: 1313-1323

Distribution of the invasive spiny-cheek crayfish (*Orconectes limosus*) in the Czech Republic. Past and present.

The American spiny-cheek crayfish, *Orconectes limosus*, was first introduced into European waters in 1890. The first literature record about the occurrence of *O. limosus* on the territory of the Czech Republic was published almost 100 years later - in 1989. The presence of this species in Czechia, however, was first recorded already in the 1960s, when crayfish were observed in the dead arms and pools adjacent to the river Elbe (Labe) in Central Bohemia. In the following few decades the spiny-cheek crayfish has spread into several larger rivers of the Elbe watershed and some of their smaller tributaries. The eastern part of the country (mostly belonging to the watershed of the river Morava) has not yet been colonised by this species. *O. limosus* can be found in lower reaches of a number of watercourses of a low stream order, but does not seem to penetrate far upstream in such localities. Its distribution in standing waters is largely the result of intentional human-mediated translocations. The long-term coexistence of *Orconectes* and native crayfish species has not yet been recorded, although both introduced and native crayfish at least occasionally come into contact. As *O. limosus* is a major carrier of the crayfish plague on the Czech territory, and crayfish plague outbreaks have been recently recorded, the dynamics of *Orconectes* invasion deserves careful monitoring in the future.

Petrusek A., Filipová L., Ďuriš Z., Horká I., Kozák P., Polícar T., Štambergová M., Kučera, Z., 2006. Distribution of the invasive spiny-cheek crayfish (*Orconectes limosus*) in the Czech Republic. Past and present. *Bull. Fr. Peche Piscic.* 380-381: 903-918

Effects of egg bath and daily removal of dead eggs on the hatching success and production of stage 2 juveniles during artificial incubation in noble crayfish (*Astacus astacus* L.)

The effects of egg bath (iodine-detergent preparation) and daily removal of dead eggs on hatching success and production of juveniles in stage 2 were investigated during a short (sixteen days) artificial incubation (AI) of noble crayfish eggs. At the beginning of AI, eggs were in phase XII (pulsating heart appearance) and were incubated in 18 polyethylene 1liter jars (100 eggs/ jar, egg density 4.5 eggs.cm⁻²). Six different treatments were tested during AI: - C: control group without removal of dead eggs and egg bath; - R: daily removal of dead eggs without egg bath; - R-LB: daily removal of dead eggs, low frequency of egg bath (once every five days); - R-FB: daily removal of dead eggs, frequent egg bath (once every three days); - LB: without removal of dead eggs, with low frequency of egg bath; - FB: without removal of dead eggs, with frequent egg bath. Egg bath was performed by iodine-detergent preparation Jodisol (dose 2 ml.l⁻¹ and exposition time 2 minutes). Results showed a better hatching rate after removing dead eggs rather than using the egg bath. Three treatments (R; R-LB; R-FB) showed significantly better survival rates in stage 1 (86.3 ± 5.4%) and 2 (84.2 ± 5.4 %) than control (74.3 ± 0.9 % and 73.3 ± 0.5 %, respectively). Two treatments (LB, FB) showed no statistically different survival rate in stage 1 and 2 (82.5 ± 5.5 and 80.7 ± 5.3 %, respectively).



respectively) than the other treatments. After AI, juveniles from all treatments were reared under controlled conditions. At the end of rearing period, juveniles reached a survival rate of $64.1 \pm 0.5 \%$ with mean body length of 22.0 ± 1.6 mm and mean body weight of 266.0 ± 50.9 mg. Negative effects of AI on growth and survival of juveniles were not evidenced.

*Polícar T., Kozák P., Martín J., 2006. Effects of egg bath and daily removal of dead eggs on the hatching success and production of stage 2 juveniles during artificial incubation in noble crayfish (*Astacus astacus* L.). Bulletin Francais de la Pêche et de la Pisciculture 380-381: 1197-1206*

Effect of culture conditions on reproductive traits of brown trout *Salmo trutta* L.

Progeny from artificial propagation of wild brown trout (*Salmo trutta* L.) of the Blanice river, Czech Republic, were farmed to maturity and spawned at ages three, four and five during 2002-2004. Reproductive parameters and biological quality of eggs in this farmed population were compared to those of the original wild population. ANCOVA showed no differences between wild and farmed fish in weight of eggs per female, total fecundity, or relative fecundity in any year. Significantly higher egg diameter (4.57 mm, $P=0.001$) and weight (69.3 mg, $P=0.0375$) were found in the wild population in 2002 and conversely in 2004, the mean egg weight was higher in the farmed population (94.7 mg, $P=0.0021$). Differences in egg diameter in this year (4.64 ± 0.06 and 4.82 ± 0.06 in wild and farmed trout, respectively) were close to the level of significance ($P=0.079$). Mutual correlations between length or weight and studied reproductive traits were similar in both populations. Fertilization rate, duration of incubation period, egg losses during incubation and mortality of starving hatched fry were monitored in embryos and larvae of farmed population only (FxF), crosses between farmed females and wild males (FxW) and wild population only (WxW). Altogether 6.3 %, 5.8 % and 5.4 % of eggs died during incubation period in FxF, FxW and WxW, respectively. There were also no significant differences in duration of incubation period and mortality of starving fry. It can be concluded that farming conditions did not significantly affect the reproductive parameters and quality of eggs in the first generation of farmed broodstock.

*Randák T., Kocour, M., Žlábek V., Polícar, T., Jarkovský, J., 2006. Effect of culture conditions on reproductive traits of brown trout *Salmo trutta* L. Bulletin Francais de la Pêche et de la Pisciculture 383: 1-12*



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RESEARCH PRIORITIES

The department performs fundamental and applied research and university education in genetics and reproductive physiology of freshwater fish, conservation of fish genetic resources, and enhancement of genetic potential of commercially important species, e.g. common carp, tench, wels and sturgeons.

Research combines laboratory analyses and experiments with pilot testing in experimental ponds or indoor/outdoor tanks, and/or with regular performance testing in ponds on productive fish farms. Many results derived from our studies have wide implications for applied aquaculture.

There are seven main programmes of research in the department:

- Genetic, biological, physiological and reproductive aspects of polyploid and unisexual fish populations.
- Cytogenetic, haematologic and reproductive aspects of hybrid diploid-polyploid complexes of selected fish genera (*Cobitis*, *Carassius*).
- Breeding programmes based on determination of heritability of performance traits and comparison of performance among different genetic groups (hybrids, breeds, lines).
- Endocrine effects on gametogenesis and gamete quality for controlled reproduction.
- Male competition during the fertilization process and its effects on population genetic variability.
- Short-term preservation and cryopreservation of fish spermatozoa and embryos.
- Sperm ultrastructure and characteristics of the fertilization process.



Department of Genetics and Fish Breeding – from the left standing: Otomar Linhart, Ping Li, Martin Flajšhans, David Gela, Filip Raab, Martin Kahanec, Sergey Boryshpolets, Zdeněk Elšnic, Ivana Samková, Martin Kocour, Marie Pečená, Sayyed Mohammad Hadi Alavi, in the front line: Vojtěch Kašpar, Marek Rodina, Martin Hulák

SELECTED RESEARCH RESULTS

Autotriploid tench *Tinca tinca* (L.) larvae obtained by fertilization of eggs previously subjected to post - ovulatory ageing *in vitro* and/or *in vivo*.

Eggs of diploid tench *Tinca tinca* L. were half-stripped out and stored for 0 (control batch), 1, 3 and 5 hours at 17.0 ± 0.4 °C and 21.9 ± 0.5 °C or for 0, 1, 2, 3, 4 and 5 hours at 24.0 ± 0.0 °C *in vitro* prior to fertilization. The eggs remaining *in vivo* in the fish kept at 17.0 ± 0.4 °C and 21.9 ± 0.5 °C were collected and fertilized in the same time intervals. Fertilization rate and larval yield mostly decreased after 3 – 5 h storage of eggs both *in vitro* and *in vivo* and only the diploid larvae were found in all control batches. Triploid larval yields increased to maxima 5.26 % after 5 h *in vitro* storage at 24.0 °C and 1.07 % and 1.60 % after 3 h *in vitro* storage at 21.9 °C and 17.0 °C, respectively. Triploid larval yield during *in vivo* storage at 21.9 °C reached its maximum 0.91 % after 5 h. As the spontaneous autotriploid larvae arose solely from fertilized eggs previously subjected to post – ovulatory egg ageing by means of prolonged storage, the autotriploidy was probably caused by failure of extrusion of the second polar body.

Flajšhans M., Kohlmann K., Ráb P., 2007. Autotriploid tench Tinca tinca (L.) larvae obtained by fertilization of eggs previously subjected to post - ovulatory ageing in vitro and/or in vivo. Journal of Fish Biology 71: 868-876

Studies on sperm of diploid and triploid tench, *Tinca tinca* (L.).

The tench *Tinca tinca* is an interesting fish from the viewpoint of polyploidy and related atypical reproduction aspects. Triploid tench were produced artificially. Studies of spermiation as well as of sperm motility and structure were performed on several triploid and diploid males simultaneously with individual experimental crosses with diploid females to define their reproductive capacities. The testes of triploids visually looked less developed in the most of cases with lower sperm production (0.05 cm^3 sperm per male), GSI and weight of testes compared to diploids (0.58 cm^3 sperm per male). Analysis of variance showed significant influence of ploidy level on the percentage of motile spermatozoa. Triploidy did not change percentage of live spermatozoa and velocity of spermatozoa at the first time of sperm movement. The study of sperm structure by scanning electron microscopy revealed that most sperm cells were of normal structure with some anomalies. Sperm heads of triploid and diploid males were mostly round-shaped, 1.86 ± 0.2 and 1.6 ± 0.18 µm in diameter. The midpiece of triploid spermatozoa was slightly narrower than that of diploid ones with typical cylindrical shape. Flow cytometry revealed sperm cells of triploids to be largely aneuploid (1.47 n) with high mosaic DNA, oscillating from haploid DNA content (1.0 n) to diploid DNA content (1.9 n). Experimental crosses between triploid males and diploid females revealed that these males were capable to stimulate effective development with relatively high level of fertilization and hatching rates from 0 to 70 %. In conclusion, triploidization does not seem to guarantee sterility of tench.

Linhart O., Rodina M., Flajšhans M., Mavrodiev N., Nebesarova J., Gela D., Kocour M., 2006. Studies on sperm of diploid and triploid tench, Tinca tinca (L.). Aquaculture International 14(1-2): 9-25

Difference in blood and water diffusion distance in gill lamellae of diploid and triploid tench *Tinca tinca* (L.).

Image analysis of sagittal sections of gill lamellae of diploid and triploid tench *Tinca tinca* revealed the blood and water diffusion distance in diploids (2.07 µm) to be significantly higher than that of their triploid siblings (1.46 µm; $P < 0.01$). Lamellae of diploids compared to triploids were found to be significantly shorter (105.84 v. 132.11 µm) and thicker (18.47 v. 14.21 µm; all at $P < 0.05$) than those of their triploid siblings but with similar mean sectional areas (1965.44 v. 1910.86 µm²).

Flajšhans, M., Piackova, V., 2006. Difference in blood and water diffusion distance in gill lamellae of diploid and triploid tench Tinca tinca (L.). Journal of Fish Biology 69(6): 1870-1873

Diversity of European spined loaches (genus *Cobitis* L.): an update of the geographic distribution of the *Cobitis taenia* hybrid complex with a description of new molecular tools for species and hybrid determination

Although the unique features of asexual reproduction and hybridization among European spined loaches (genus *Cobitis*) have recently attracted the attention of conservation biologists, faunists

and evolutionary biologists, the research has suffered from uncertain identification of specimens and their genomes because of the extreme morphological similarity of all the species within the hybrid complex. In this article, a Europe-wide study is reported, which was performed on samples collected by several research teams. Several complementary methodologies, such as allozyme analysis, karyotyping, flow cytometry and DNA sequencing allowed us to confirm or reject the existence of all previously reported species and their hybrids as well as to uncover several new hybrid biotypes. The biogeography of all the known biotypes, that is, parental species and hybrid biotypes, has been summarized here and the taxonomic position of two undescribed putative species mentioned in previous publications has been established. New polymerase chain reaction restriction fragment length polymorphism markers for species determination have further been developed and applied, which would allow the unambiguous identification of parental species and their genomes in the known hybrid biotypes within the complex.

Janko K., Flajšhans M., Choleva M., Bohlen J., Šlechtová V., Rábová M., Lajbner Z., Šlechta V., Ivanova P., Dobrovolov I., Culling M., Persat M., Kotusz J., Ráb P., 2007. Diversity of European spined loaches (genus *Cobitis* L.): an update of the geographic distribution of the *Cobitis taenia* hybrid complex with a description of new molecular tools for species determination. *Journal of Fish Biology* 71 (Suppl. C): 387-408

Heritability estimates for processing and quality traits in common carp (*Cyprinus carpio*) using a molecular pedigree

Heritability estimates with a microsatellite parentage assignment based pedigree are of special interest in common carp cultured under traditional pond conditions. This method reduces common environment effects as all families can be grown immediately after hatching mixed in the same pond. We applied this method to study genetics of growth and processing traits in common carp at market size (1.5 kg and more). The experimental progeny was established by crossing 147 two-year old males and 8 females (six to eight-year old) of Hungarian synthetic mirror carp population (HSM). The fish grew up through three vegetation seasons and at the end a sample of 331 fish was examined for biometrical traits (standard length, body weight, relative head length, relative body height, relative body width), percent fat and processing traits (% processed body, % fillets with skin, % fillets without skin). It was shown that sex had a significant effect on most traits: females were larger and fatter than males, and they had both higher percent processed body and percent fillet with skin. Standard length, body weight, percent fat and relative head length had a high heritability (>0.5), while relative body height, relative body width, percent processed body and fillet yields had a medium heritability ($0.2 - 0.5$). We found relatively high positive genetic correlations between body size (standard length and body weight) and percent of fat (0.71 and 0.59, respectively), favourable genetic correlations between body size and percent processed body (0.69 for standard length and 0.74 for body weight) and between body size and fillet yields (0.50 – 0.77). Genetic correlations between body size and body shape (relative head length, relative body height and relative body width) were weak to moderate, thus selection for better growth should have little impact towards more rotund shape. Relative head length had strong negative correlation (-0.7 to -0.9) with percent fat, percent processed body and percent fillet yields. This means that indirect selection for reduced relative head length should be effective in improving of fillet yield.

Kocour M., Mauger S., Rodina M., Gela D., Linhart O., Vandeputte M., 2007. Heritability estimates for processing and quality traits in common carp (*Cyprinus carpio*) using a molecular pedigree. *Aquaculture* 270: 43-50

Semen of *Perca fluviatilis* L.: Sperm volume and density, seminal plasma indices and effects of dilution ratio, ions and osmolality on sperm motility

The objectives of the present study were to characterize sperm volume and density, seminal plasma indices (ionic contents and osmolality) and to study the effects of dilution ratio, ions and osmolality on sperm motility parameters (percentage of motile sperm and sperm velocity) in farmed European perch (*Perca fluviatilis* L.). The means of sperm volume (ml), sperm density (-109 sperm ml^{-1}) and total number of sperm (volume \times density) per fish were 2.75 ± 0.51 , 29.19 ± 3.15 and 82.19 ± 15.26 . The seminal plasma osmolality (mOsm kg^{-1}), sodium, chloride, potassium and calcium ions concentrations (mM) were measured to be 298.07 ± 5.09 , 130.97 ± 2.19 , 106.75 ± 2.37 , 10.70 ± 0.61 and 2.41 ± 0.09 , respectively. At 15 s post-activation of stripped sperm, the percentage of motile

sperm (%) and sperm velocity (mm s^{-1}) were 91.90 ± 1.27 and 115.54 ± 1.25 , respectively, and decreased significantly following sperm activation ($P < 0.05$). The optimal sperm motility was observed when the sperm was prediluted in immobilizing solution (IS) at a ratio 1:50. Prediluted sperm showed the maximum velocity when activated in 2.5 mM Ca^{2+} , 50 mM K^{+} and sucrose with osmolality 100 mOsm kg^{-1} . Neither Ca^{2+} nor K^{+} showed a significant effect on the percentage of motile sperm at 15 s post-activation. Osmolality higher than 200 mOsm kg^{-1} significantly decreased the percentage of motile sperm, while osmolality of 300 mOsm kg^{-1} or above totally suppressed sperm motility.

Alavi S.M.H., Rodina M., Policar T., Kozak P., Psenicka M., Linhart O., 2007. Semen of Perca fluviatilis L: Sperm volume and density, seminal plasma indices and effects of dilution ratio, ions and osmolality on sperm motility. Theriogenology 68: 276-283

Fish Spermatology

Aside from their immense importance in intensive commercial aquaculture, chondrosteian and teleostean fishes are vital laboratory animals and are widely used by reproductive physiologists as model species. Over recent years, the control of fertility in commercially –important fish has become a subject of great economic importance in terms of manipulating valuable fisheries resources by adopting broodstock management strategies appropriate to the reproductive traits of the species concerned. This book, with chapters written by the world's leading authorities, reflects the theoretical and practical importance of studies concerning the reproductive physiology of male broodfish. Significant advances and future trends in global research in fish spermatology are presented and discussed in four sections: 1) General Biology - Principles and Practices; 2) Gonadal development and Spermatogenesis; 3) Physiology and Biochemistry; 4) Aquaculture.

Alavi S.M.H., Cosson, J.J., Coward, K., Rafiee, Gh., 2007. Fish Spermatology. Alpha Science Ltd, Oxford, UK. 460 pp.

Equalizing sperm concentrations in a common carp sperm pool does not affect variance in proportions of larvae sired in competition

Proportions of offspring from five common carp males contributing to a sperm pool composed of equalized sperm concentrations (N-progeny) or equal sperm volumes (V-progeny) were each compared to a uniform distribution. Four microsatellite markers (*MFW1*, *MFW6*, *MFW7*, *MFW28*) were used to determine the paternity of the progeny. The homogeneity of offspring numbers from each male for the two types of progeny, were tested using an exact test for the likelihood-ratio chi-square. Numbers of offspring in the progeny groups were highly variable (0.4-50 % in V-progeny, 2.4-41.2 % in N-progeny) and highly significantly different as shown by Pearson chi-square statistics ($\chi^2 = 189$, 4 d.f., $p < 0.0001$ in V-progeny and $\chi^2 = 139$, 4 d.f., $p < 0.0001$ in N-progeny). Significant heterogeneity between treatments ($p < 0.05$) together with reduction of χ^2 value from 189 to 139 showed that equalization of sperm concentration reduced heterogeneity in numbers of offspring. Number of sperm per male, sperm motility (71-98 %), sperm velocity ($97\text{-}155 \mu\text{m s}^{-1}$) and control hatching rates (81-91 %) all affected the observed number of offspring sired by each of the males, but the high variability in proportion of progeny among the 5 males remained unexplained.

Kaspar V., Kohlmann K., Vandeputte M., Rodina M., Gela D., Kocour M., Alavi S.M.H., Hulák M., Linhart O., 2007. Equalizing sperm concentrations in a common carp sperm pool does not affect variance in proportions of larvae sired in competition. Aquaculture 272S: S204-S209

Cryopreservation of tench, *Tinca tinca*, Sperm: Sperm motility and hatching success of embryos

The aim of the present study was to elaborate cryopreservation methods for ex situ conservation of tench. Success of cryopreservation was tested during two series of experiments. The first set of experiments studied the effects of two types of cryoprotectants (DMSO and a combination of DMSO with propanediol at ratio 1:1) at concentrations of 8 and 10% and three different equilibration times in two different immobilization solutions (IS) (Kurokura 180 and Kurokura) before freezing (0.0, 2.0 and 4.0 h after T0). The K4 cooling programme was used to freeze 1 ml of cryoextended sperm using 1.8 ml cryotubes. Main monitored parameter was hatching rate after using of cryopreserved sperm.

The second set of experiments studied the volume effect of 0.5, 1 and 5 ml straws and compared these with 1.8 ml cryotubes as well as the effect of the cooling programme (K4 and L1). Following the results of the first study, a combination of DMSO and propanediol (ratio 1:1) at concentration of 10% was added to extended sperm in Kurokura 180 IS. Main monitored parameter was hatching rate after using cryopreserved sperm, supplementary parameters were sperm velocity and motility percentage assessed at 10 s post-activation.

Sperm was collected directly into IS and stored at 4 °C for 2.5 h. Thereafter were sperm samples pooled, equilibrated in IS (first set of experiments) or directly mixed with cryoprotectants (DMSO or a mixture of DMSO with propanediol at ratio 1:1) and transferred to 1.8 ml cryotubes or straws (0.5, 1 and 5 ml). Then the cryotubes/straws were directly transferred to preprogrammed PLANER Kryo 10 series III and cooled using two different cooling programmes including a slow cooling programme (a) named K4 (from +4 to -9 °C at a rate of 4 °C min⁻¹ and then from -9 to -80 °C at a rate of 11 °C min⁻¹) and a rapid cooling programme (b) named L1 (directly from +4 to -80 °C at a rate of 20 °C min⁻¹). Both slow (K4) and rapid (L1) cooled samples were held 6 min at -80 °C. Finally, samples were transferred into liquid N₂.

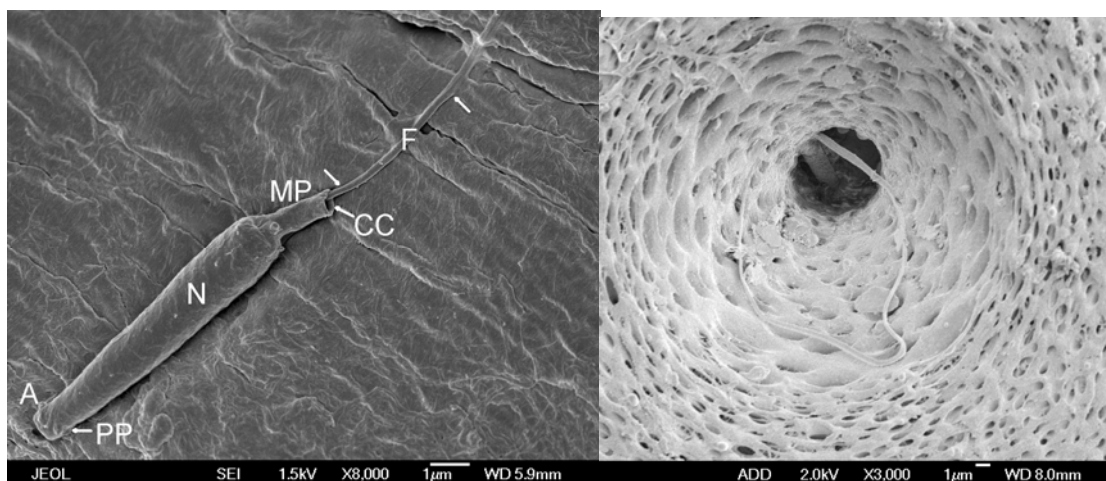
The frozen spermatozoa were thawed in a water bath (40 °C) according to the frozen volume and checked for fertilization and hatching rates. Percentage of sperm motility and sperm velocity were measured using video recorded frames. ANOVA showed a significant influence of frozen and fresh sperm in all treatments. The hatching rates of 33.8% were obtained when sperm was equilibrated for 0 h before freezing in IS of Kurokura 180 and frozen with a 10% of mixture 1:1 of DMSO and propanediol into straws of 5 ml and cooled using program L1. The velocity of frozen-thawed spermatozoa ranged from 31 to 46 µm.s⁻¹ and in post-thawed sperm was not significantly different according to frozen sperm volume, but a higher velocity was obtained when sperm was fast frozen using programme L1. A large volume of frozen sperm could reveal the best procedure for freezing, but also for simulating methods of artificial propagation for future practical use of frozen tench sperm at a large scale.

Rodina M., Gela D., Kocour M., Alavi S.M.H, Hulak M., Linhart O., 2007. Cryopreservation of tench, Tinca tinca, Sperm: Sperm motility and hatching success of embryos. Theriogenology 67: 931-940

Morphology and ultrastructure of Siberian sturgeon, *Acipenser baerii*, spermatozoa using scanning and transmission electron microscopy

Available data concerning the sperm morphology of teleost fishes demonstrate wide variation. In present study, the spermatozoa of Siberian sturgeon, *Acipenser baerii* (Brandt, 1869) belonging to chondrosteian fishes was investigated. Contrary to the teleost fishes, chondrosteian spermatozoa has a head with a distinct acrosome, while other structures such as a midpiece and a single flagellum are presents in spermatozoa of most species. The average length of the head including the acrosome and the midpiece was 7.01 ± 0.83 µm. Ten posterolateral projections derived from the acrosome were present on a subacrosomal region with mean length of 0.94 ± 0.15 µm and width of 0.93 ± 0.11 µm. The nucleus consisted of electrodense homogeneous nuclear chromatin. Three mingled endonuclear canals bounded by membranes traversed the nucleus longitudinally from the acrosomal end to the basal nuclear fossa region. There were three to six mitochondria, two types of centrioles (proximal and distal) in the midpiece and two vacuoles composed of lipid droplets. The flagellum (44.75 ± 4.93 µm long), originating from the centriolar apparatus, had a typical 9 + 2 eukaryotic flagellar organization. In addition, there was an extracellular cytoplasm canal between the cytoplasmic sheath and the flagellum. A principal components analysis explained the individual morphological variation fairly well. Of the total accumulated variance, 41.45 % was accounted for by parameters related to the head and midpiece of the sperm and the length of the flagellum. Comparing the present study with previous studies of morphology of sturgeon spermatozoa there were large inter- or intra-specific differences that could be valuable taxonomically.

*Psenicka M., Alavi S. M. H., Rodina M., Gela D., Nebesarova J. Linhart O., 2007. Morphology and ultrastructure of Siberian sturgeon, *Acipenser baerii*, spermatozoa using scanning and transmission electron microscopy. Biology of the Cell 99(2): 103-115*



Left: Sperm cell structures of Siberian sturgeon using scanning electron microscopy showing acrosome (A), posterolateral projections (PP), nucleus (N), midpiece (MP), cytoplasmic channel (CC) and flagellum (F). The arrows show development of fins along the flagellar length (scale bar = 1 µm).

Right: The micrograph shows spermatozoon penetration into the egg through a micropyle using scanning electron microscopy (scale bar = 1 µm).

Ultrastructure of spermatozoa of tench *Tinca tinca* observed by means of scanning and transmission electron microscopy

Structure of tench (*Tinca tinca* L.) spermatozoa was investigated by means of scanning electron microscopy (SEM) and transmission electron microscopy (TEM). Spermatozoa of 26.1 ± 3.8 µm total length possessed typical primitive simple structure, called "aqua sperm", without acrosomal head structures. It was probably the smallest spermatozoon described among cyprinid fishes. Heads were mostly composed of dense and slightly granular material, which appeared to be fairly homogeneous except for the occasional appearance of vacuoles. The midpiece remained separated from the flagellum by the cytoplasmic channel; it was cylindrical/cone-shaped, 0.86 ± 0.27 µm in length and 1.17 ± 0.24 µm in width at proximal part. The proximal centriole was located in the "implantation fossa". The distal centriole appeared almost tangential to the nucleus and it functioned as a basal body for the flagellum. It had an orientation of 140° with respect to the distal centriole. The sperm flagellum with 25.45 ± 2.47 µm of total length had no any fin. The diameter of the flagellum perpendicular to the plane of the doublet of central microtubules was 173.67 ± 20.45 nm and horizontal plane of the central microtubules was 200.71 ± 20.45 nm. Peripheral doublets and the central doublet of microtubules measured 23.39 ± 3.18 and 35.88 ± 4.44 nm in width, respectively. The diameter of a microtubule was only 9.14 ± 2.97 nm. A vesicle was attached to the most basal region of the flagellum and located just under plasma membrane of the flagellum.

Psenicka M., Rodina M., Nebesarova J., Linhart O. 2006. Ultrastructure of spermatozoa of tench Tinca tinca observed by means of scanning and transmission electron microscopy. Theriogenology 66: 1355-1363

APPLICATIONS OF RESEARCH RESULTS

Tench (*Tinca tinca*) broodstock management in breeding station under conditions of pond culture

Breeding work in tench has been recently focused on survey of genetic variability among different populations, maintenance of pure breeds, evaluation of breeding performance and reproduction level of crossbreds, purebreds and genome - manipulated populations. The basic assumption to carry out the breeding work is to have well-organised broodstock management. Taking into account the number of populations bred and the limited number of ponds available, it is impossible to avoid rearing several juvenile or adult breeds in mixed stocks. Fish of individual breeds are group-marked with regular renewal of the freeze-branded mark and adults should be marked individually when involved into the broodstock category. In order to avoid inbreeding or losses in genetic variability, at least 120 fish per strain are reared and when the strain is restored, factorial cross

of at least 15 females and 25 males is to be applied. All activities are individually registered in "Evidence 2003" data-recording software and data can be sorted upon the origin of fish population, of the strain or of individual fish; reproductive and performance parameters are recorded individually also. Further breeding operations with tench which deserve special attention comprise selection after fish overwintering and rearing of fish before, during- and after reproductive season.

Gela D., Flajšhans M., Kocour M., Rodina M., Linhart O., 2006. Tench (*Tinca tinca*) broodstock management in breeding station under conditions of pond culture. *Aquaculture International* 14(1-2): 195-203

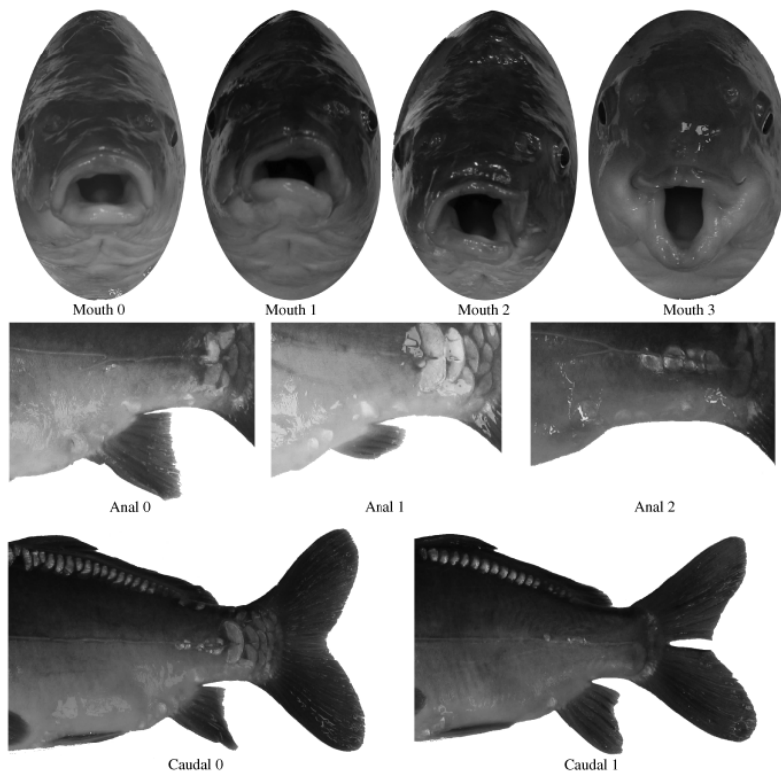
Application of image analysis for the determination of nucleus position in sturgeon oocytes

The technique to determine the stage of oocyte maturation in sturgeon by using the nuclear position was proposed by Truskov (1964) and improved by Kazanskii et al. (1978). Practical applications of these methods differ only slightly with the equipment used for the observation. The goal of this study is to describe a computer-assisted image analysis technique for precise assessment of nuclear position while employing digital images of cross-sections of biotically sampled oocytes fixed in Serra solution, a technique used in the University of South Bohemia, Research Institute of Fish Culture and Hydrobiology in Vodnany, (USB RIFCH), Czech Republic to measure the nucleus position in oocytes of Siberian sturgeon, sterlet, stellate sturgeon and Russian sturgeon. This method is proposed for general use in sturgeon research.

Rodina M., 2006. Application of image analysis for the determination of nucleus position in sturgeon oocytes, *Journal of Applied Ichthyology* 22: 373-374

Mouth and fin deformities in common carp: is there a genetic basis?

Mouth and fin deformities have an impact on marketing value of common carp. Major opinion of carp farmers that the deformities are of the genetic origin was investigated during selective breeding experiment in common carp under pond conditions of the Central Europe using a molecular pedigree,



where a significant proportion of deformed fish was observed. A stock of 452 uniquely assigned two-summers fish from two-generation pedigree was individually assessed for mouth deformities coded 0 (normal mouth), 1 (deformed but able to close), 2 (unable to close completely), 3 (unable to close); anal fin deformities coded 0 (normal), 1 (reduced, with first bony ray missing), 2 (missing) and caudal fin deformities coded 0 (normal) or 1 (split in two parts). The severity of mouth deformity significantly ($P < 0.0001$) affected growth, and body weight between groups with normal and missing anal fin was also different ($P < 0.05$). Heritabilities (observed scale) were low (< 0.1) and only the one for anal fin deformity ($0.09 \pm$

0.04) was significantly different from zero. Genetic correlations between the deformities were mostly negative and unprecise, and phenotypic correlations were low (≤ 0.12). The influence of inbreeding calculated from regressions of the severity of deformities on the proportion of homozygous loci did not explain a significant part of variance for any trait ($R^2 = 7.10^{-4}$ for mouth, $R^2 = 7.10^{-5}$ for anal fin and

$R^2=0.017$ for caudal fin). It can be concluded that no genetic effect on mouth deformities was observed, but the situation in fin deformities remains unsure.

Kocour M., Linhart O., Vandeputte M., 2006. Mouth and fin deformities in common carp: is there a genetic basis? *Aquaculture Research* 37(4): 419-422

INTERNATIONAL COOPERATION

Effects of cryoprotectants and male on motility parameters and fertilization rate in paddlefish (*Polyodon spathula*) frozen-thawed spermatozoa

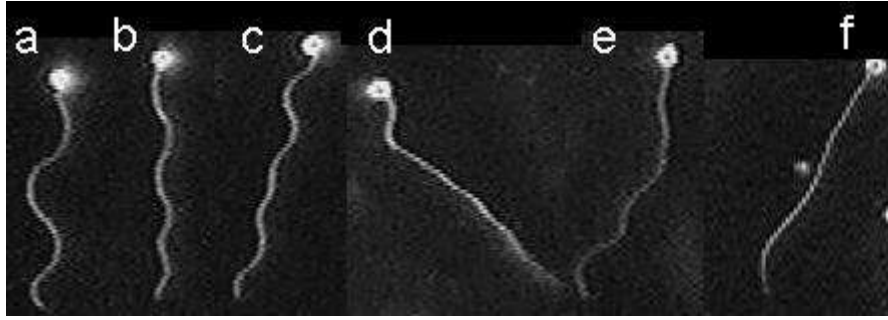
A sperm cryopreservation method using different cryoprotectants and sperm from different males was developed. Different percent of pure cryoprotectant (methanol and DMSO) was added in extender 1 or 2 (20 mM tris pH 8 + 30 mM sucrose + 0.5 mM KCl or 20 mM tris pH 8 + 50 mM sucrose + 0.5 mM KCl, dilution 1:1) or non-extended sperm and every 1 ml of mixture was transferred to a 2-ml cryotube. The cryotubes were directly transferred to a pre-programmed PLANER Kryo 10 series III at 0 °C and cooled from 0 °C to -5 °C at a rate of 3 °C.min⁻¹, from -5 °C to -15 °C at a rate of 5 °C.min⁻¹, from -15 °C to -25 °C at a rate of 10 °C .min⁻¹, from -25 °C to -80 °C at a rate of 20 °C .min⁻¹, then held for 5 min at -80 °C and finally transferred into LN2 until next morning. The sperm was thawed in a water bath at 40 °C for 105 s. Fertilization rate of control sperm (unfrozen) samples after 24-h storage at 3 °C was 81.5 % it indicated that the gametes were of good quality. Percentage and the velocity of motile sperm from video frames using image analysis were evaluated in fresh and post-thawed sperm. The results of hatching rate significantly correlated with post-thawed sperm motility ($r=0.49$, $P=0.035$) and velocity ($r=0.55$, $P=0.014$) and none correlated with velocity of post-thawed spermatozoa ($r=0.32$, $P=0.177$). The best fertilization rates 64-75 % in post-thawed sperm (3.6×10^5 spermatozoa per egg) were obtained, when sperm either without any extender or with both extenders was treated with methanol in concentration 8 or 10 %. These results were not significantly different compared with the fresh sperm control sample. Hatching rate was very low, only 8-15 %, when sperm was frozen with 8 or 10 % DMSO. ANOVA showed a significant effect of males on sperm motility, velocity and fertilization rate in post-thawed sperm.

Linhart O., Mims S., Gomelsky B., Cvetkova J., Cosson J., Rodina M., Horvath A., Urbanyi B., 2006. Effects of cryoprotectants and male on motility parameters and fertilization rate in paddlefish (*Polyodon spathula*) frozen-thawed spermatozoa. *Journal of Applied Ichthyology* 22(Suppl. 1): 384-388

Ultrastructure and morphology of spermatozoa in Chinese sturgeon (*Acipenser sinensis* Gray 1835) using scanning and transmission electron microscopy

The Chinese sturgeon (*Acipenser sinensis* Gray 1835) is an endangered anadromous sturgeon inhabiting the Yangtze River in China. In this study, the ultrastructure and morphology of spermatozoa was studied using transmission and scanning electron microscopy with a cryo-holder. The spermatozoon consisted of an elongated head with a distinct acrosome and nucleus region, a midpiece and a flagellum. The mean length of the head and midpiece, the flagellum and total length of spermatozoon were 4.48, 33.3 and 37.8 μm , respectively. The nucleus was an elongated trapezoid shape with anterior (acrosome) end narrower than the posterior. Granular material and an actin filament were observed within the anterior acrosome. Three to five endonuclear canals were present. The midpiece was eudipleural along its longitudinal axis. Compared to other sturgeon species, the data from the present study suggest more recent evolutionary linkage between Chinese sturgeon and white sturgeon (*Acipenser transmontanus*, Richardson 1836).

Wei Q., Li P., Psenicka M., Alavi S. M. H., Shen L., Liu J., Peknicova J., Linhart O., 2007. Ultrastructure and morphology of sperm in Chinese sturgeon, *Acipenser sinensis*, using scanning and transmission electron microscopy. *Theriogenology* 67: 1269-1278



*Motility demonstration of barbel (*Barbus barbus*) spermatozoon in 10(a), 15(b), 35(c), 45(d) a 60(e) seconds after activation with activating solution of osmotic concentration $100 \text{ mOsmol kg}^{-1}$. In the course of sperm motility, there is a gradual change from total wave motion of the entire flagellum towards to partial wave motion.*

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RESEARCH PRIORITIES

The department performs fundamental and applied research, and participate in the academic program in courses on fish diseases and aquatic toxicology at the University of South Bohemia České Budějovice. The Aquatic Toxicology and Fish Diseases Department focuses in general on laboratory researches in parallel with aquacultural and environmental studies.

There are six main research programmes:

- Surveillance of xenobiotics effects that load the aquatic environments on fish.
- Assessment of contamination of fish and other components of the aquatic ecosystem with xenobiotics.
- Toxicity of substances and wastes to aquatic organisms.
- Improvement of the prevention and elimination of health risks in fish culture – Principles of pharmacovigilancy and implications for aquaculture in Czech Republic (harmonisation with EU): Protection of common carp (*Cyprinus carpio* L.) from KHV disease.
- Assessment of technological properties of fish flesh, fish processing, organoleptic assessment, and determination of polyunsaturated fatty acids (PUFA, EPA, DHA), amino acids and fat deposition, and effects of fish farming technology to fish flesh quality.
- Management of brown trout and grayling culture, interaction between wild and reared fish.



Department of Aquatic Toxicology and Fish Diseases – from the left standing: Josef Velišek, Jindřiška Čížková, Hana Kroupová, Anna Kocová, Veronika Piačková, Jana Máchová, Olga Valentová, Eliška Sudová, Petra Beránková, Tomáš Randák, Jan Turek

SELECTED RESEARCH RESULTS

Radio-telemetry monitoring behaviour of the different species in river

The first telemetry study (Slavík et al., 2007) analyzed behaviour of the largest freshwater predator in European rivers, *Silurus glanis*. It was performed bimonthly during the years 2002-2004. Movement of juveniles and adults occurred mostly in the same time intervals. The only statistical differences occurred for nocturnal activity in spring and autumn. In spring and winter, activity was low with the peaks during daylight, and in autumn, maximal movement was recorded during dusk. In summer, the European catfish were active across the whole 24 h even during high-flow conditions. During all other seasons, movement was inversely related to flow rate. Maximal home ranges occurred in summer, being larger for adults. Juveniles and adults were spatially segregated. With increasing flow, fish were displaced, and individuals from both groups got closer to each other. Only juveniles migrated downstream from the upstream isolated river stretch suggesting a negative impact of lateral obstructions on the fish population structure.

The next study (Horký et al., 2007) tested whether docksides in channelised rivers can provide winter habitats for fish, 19 specimens of chub and 8 specimens of pikeperch were radio-tracked for more than 12 months. Locations of fish were obtained weekly along a 40-km long stretch of the Elbe River in the Czech Republic. Occurrence of chub in docksides was associated with a decrease in water temperature, whilst pikeperch occurrences within docksides were distributed randomly across all seasons. Fish entrance into docksides was not significantly influenced by the flow regime of the river. Both species displayed size-dependent spatial segregation in the adjacent river stretch. Our results showed that the docksides were not permanently occupied by pikeperch or chub across seasons. We suggest that docksides enhance habitat diversity in channelised rivers.

The third study (Horký et al., 2007b) observed changes in the behaviour of cyprinids belonging to different ecology groups. These changes in behaviour were observed below a weir that was restricting their upstream migrations during the spawning period May–July. Positions of chub *Leuciscus cephalus* (L.), Prussian carp *Carrasius auratus gibelio* (Bloch 1783), bream *Abramis brama* (L.) and white bream *Blicca bjoerkna* (L.) were recorded every 12 min over a 24-h period using an automatic data logger (ALS) in the upper Elbe River, Czech Republic. For all observed species, two activity peaks occurred; however, the interspecies timing of these peaks differed. Peak times for chub, bream and white bream occurred at night and during daylight, whereas Prussian carp was active mostly at night. Specimens belonging to the rheophilous group suggested more efficient behavioural tactics in passing the weir, staying below the obstacle one month longer than did eurytopic species.

Slavík O., Horký P., Bartoš L., Kolářová J., Randák T., 2007. Diurnal and seasonal behaviour of adult and juvenile European catfish as determined by radio-telemetry in the River Berounka, Czech Republic. *Journal of Fish Biology* 71: 101-114

Horký P., Slavík O., Bartoš L., Kolářová J., Randák T., 2007a. Docksides as winter habitats of chub and pikeperch in the channelised Elbe River (*Zimoviště jeliců tloušťů a candátů obecných v kanalizované řece Labe*.) *Fundamental and Applied Limnology, Archiv für Hydrobiologie* 168(3): 281-287

Horký P., Slavík O., Bartoš L., Kolářová J., Randák T., 2007b. Behavioural pattern in cyprinid fish below a weir as detected by radio telemetry. *Journal of Applied Ichthyology* 23(6): 679-683

Contamination assessment by means of multiple biomarker detection

The Elbe River is the most polluted aquatic ecosystem in territory of Czech Republic. The most important contaminants are heavy metals and persistent organic compounds. A whole series of contaminants may affect negatively the endocrine systems of aquatic animals. The chub (*Leuciscus cephalus* L.) was selected as a bioindicator for the field study. Blood plasma and tissue samples were collected from wild chub (*Leuciscus cephalus* L.) from 9 localities of the Elbe and Vltava rivers and from 1 locality of the Blanice river (control locality) in 2003. Biochemical markers were used to evaluate the influence of aquatic pollution on fish population. Fish muscle samples were analysed for toxic metals (Hg, Cd, Pb, As), persistent organochlorine pollutants (PCB, DDT, HCH, HCB, OCS) and alkylphenols (Randák et al., 2006).

Biochemical markers, specifically enzymes of the first phase of xenobiotic transformation - cytochrome P450 and ethoxyresorufin-O-deethylase (EROD) –were in studies (Havelkové et al., 2007a, b) used to determine the quantities of persistent organic pollutants (POPs) in fish muscle (PCB, HCB, HCH, OCS, DDT). Eight rivers were monitored (Orlice, Chrudimka, Cidlina, Jizera, Vltava,

Ohře and Bilina; and the River Blanice was used as a control). The indicator species selected was the chub (*Leuciscus cephalus* L.). There were no significant differences in cytochrome P450 content between the locations monitored. The highest concentration of cytochrome P450 in fish liver was in the Vltava (0.241 nmol mg⁻¹ protein), and the lowest was in the Orlice (0.120 nmol mg⁻¹ protein). Analysis of EROD activity showed a significant difference between the Blanice and the Vltava ($P < 0.05$), and also between the Orlice and the Vltava ($P < 0.01$), the Orlice and the Bilina ($P < 0.01$), and the Orlice and the Ohře ($P < 0.05$). The highest EROD activity in fish liver was in the Vltava (576.4 pmol min⁻¹ mg⁻¹ protein), and the lowest was in the Orlice (63.05 pmol min⁻¹ mg⁻¹ protein). In individual locations, results of chemical monitoring and values of biochemical markers were compared. A significant correlation ($P < 0.05$) was found between biochemical markers and OCS, and PCB. Among the tributaries studied those that contaminated the Elbe most were the Vltava and the Bilina. These tributaries should not be considered the main sources of industrial contamination of the River Elbe, because the most important contamination sources were along the river Elbe itself.

Randák T., Žlábek V., Kolářová J., Svobodová Z., Hajšlová J., Šíroková Z., Jánková M., Pulkrabová J., Čajka T., Jarkovský J. 2006. *Biomarkers Detected in Chub (Leuciscus cephalus L.) to Evaluate Contamination of the Elbe and Vltava Rivers, Czech Republic. Bull. Environ. Contam. Toxicol.* 76: 233-24

Havelková M., Randák T., Leontovyčová D., Krijt J., Svobodová Z., 2007a. *Use of biochemical markers for the assessment of contamination of the river Elbe Tributaries. Toxikology letters, Abstracts, 44rd International Conference EUROTOX, Amsterdam, Holland, 172: 160*

Havelková M., Randák T., Žlábek V., Krijt J., Kroupová H., Pulkrabová J., Svobodová Z., 2007b. *Biochemical markers for assessing aquatic contamination. Sensors 7 (11): 2599-2611*

Hygienic quality of marketable common carp from selected ponds of the Czech Republic

The aim of the study (Čelechovská et al., 2007) was to investigate the distribution of selected metals (As, Cd, Pb, Hg, Cr, Cu and Zn) in tissues of the common carp (*Cyprinus carpio* L.) grown in ponds. A total of 125 market-ready carp (aged 3 - 4 years, weight 2312 ± 583 g) from 10 fishponds in the Czech republic were analysed. The tissues analysed included muscle, liver, kidney, spleen, gills, ovaria and testes. Metal concentrations were determined by the AAS, mercury was determined using a cold-vapour AAS analyse (AMA 254). Results showed different affinities of metals to different tissues. The highest concentrations of As and Hg were in muscle tissue, Cu in the liver, and Cd and Zn in the kidneys, compared with levels in other tissues ($p < 0.01$). The lowest metal concentrations (particularly of Cd) were found in the spleen, gills and gonads. Lead concentrations in the individual tissues were low and practically identical. Chromium concentrations were also balanced with sporadic significant differences ($p < 0.05$). Significantly higher concentrations of Cu, Cr ($p < 0.05$) and Zn ($p < 0.01$) were found in the ovaria compared with the testes. The study showed that meat and gonads of pond carp in the Czech republic are safe from the point of view of contamination with the metals investigated.

The aim of the second study (Maršálek et al., 2007) was to investigate concentrations of total mercury (THg) and methylmercury (MeHg) in the common carp from Czech ponds named Rožmberk, Spolský, Nezmar, and Velký Bědný. Seven common carps (*Cyprinus caprio*) from each of the ponds were caught. Muscle tissue, the liver, and the soft and hard roe were used for the tests. Total mercury and methylmercury were found in all the muscle tissue samples examined. Detection limits for total mercury and methylmercury determination methods were 0.001 mg kg⁻¹ and 0.013 mg kg⁻¹ respectively. Methylmercury levels in the liver and gonads were below the method's limits of detection. THg and MeHg concentrations in muscle tissues were 0.018–0.063 mg kg⁻¹ w.w. and 0.019–0.063 mg kg⁻¹ w.w. respectively. MeHg made up 90–100% of THg in muscle tissues. Total mercury and methylmercury concentrations were significantly ($P < 0.05$) higher in fish from the Spolský pond than from the Nezmar pond. MeHg/THg ratios were significantly ($P < 0.05$) higher in fish from the Rožmberk, Spolský and Nezmar ponds compared with fish from the Velký Bědný pond.

Čelechovská O., Svobodová Z., Žlábek V., Macharáčková B., 2007. *Distribution of metals in tissues of common carp (Cyprinus carpio L.). Acta Veterinaria Brno 76(suppl. 8): 93-100*

Maršálek P., Svobodová Z., Randák T., 2007: *The content of total mercury and methylmercury in common carp from selected Czech ponds. Aquaculture International 15(3-4): 299-304*

Contamination assessment of fishies and other components of water ecosystem by heterogeneous material

The study (Hajšlová et al., 2007) charted brominated flame retardants (BFRs). They are widely used industrial chemicals, residues of which can be nowadays found in all environmental compartments. The widespread presence of BFRs in various environmental compartments and food chain is a consequence of both their broad application area and physico-chemical properties, such as resistance to degradation and high lipophilicity. Alike in the case of other halogenated persistent organic pollutants (POPs), fish can be used as a bioindicator of aquatic environment pollution. In presented study, conducted in the year 2005, altogether 80 samples representing the most abundant fresh water fish species, viz. chub (*Leuciscus cephalus*), bream (*Abramis brama*), and perch (*Perca fluviatilis*) collected in 11 sampling sites located at Elbe and Vltava (Moldau) rivers were examined for levels of major BFRs. Without any exception, BFRs were detected in all fish samples. BDE 47 was the dominating congener in all fish species. This fact was not surprising, since it used to be the main component in various kinds of technical mixtures. With regard to relatively high levels of BDE 47 in fish tissue, as compared to other BFRs, and considering strong correlation with the total PBDEs content, simplified laboratory examination and, consequently, increased samples throughput can be obtained when only this congener is monitored. The potential of comprehensive two-dimensional gas chromatography coupled to time-of-flight mass spectrometry (GC · GC–TOFMS), to provide more comprehensive information on the bioaccumulating chemicals occurring in fish samples, has been demonstrated in this study.

The paper of Křížková et al. (2007) report on the use of adsorptive transfer stripping technique in connection with chronopotentiometric stripping analysis for metallothionein determination and of differential pulse anodic stripping voltametry for lead detection in tissues of wild perch (*Perca fluviatilis*, n = 6) from the Svatka River in Brno, Czech Republic. Primarily, we determined the content of MT in tissues (muscles, gonads, liver and spleen) of perch. We measured the highest content of MT in spleen and liver (100–350 ng MT per gram of fresh weight). We assume that the content of MT determined in perch tissues is probably related with the age of the fish and, therefore, with their exposition to heavy metals naturally occurring in the Svatka River. We detected a lead concentration in the tissues of one perch. It clearly follows from the results that the content of MT well correlates with the concentration of lead.

The next study (Hájková et al., 2007) search synthetic musk fragrances, which are contained in almost all scented consumer products, enter aquatic environment mainly by way of wastewater paths. To monitor contamination of the Vltava River by these relatively persistent chemicals in the surroundings of Prague industrialized agglomeration, chub (*Leuciscus cephalus*) was employed as a bioindicator. Validated gas chromatography– mass spectrometry method was used for fish sample examination. Polycyclic musks, represented by 1,3,4,6,7,8- hexahydro-4,6,6,7,8,8-hexamethylcyclopenta-(c)-2-benzopyran (galaxolide) and 1-(5,6,7,8-tetrahydro-3,5,5,6,8,8- hexamethyl-2-naphthalenyl)-ethanone (tonalide) were the most abundant representatives of this group; their levels in fillets were in the range of 1.7 to 105.9 lg/kg and 0.9 to 19.3 lg/kg wet weight, respectively. Nitro-musks, musk ketone, and musk xylene were also detected in most samples; nevertheless, their levels were lower, £ 2 lg/kg. Significant contamination of resident fish was recognised in the locality of Klecany, which is located 6 km downstream from a large municipal sewage-treatment plant (STP). High levels of musks found in the locality of Vraňany, 32 km downstream from Prague STP, were caused not only by this emission source but probably also by the effluents from the local plant that produces cleaning agents in Velvary.

Hájková K., Pulkrabová J., Hajšlová J., Randák T., Žlábek V., 2007. Chub (*Leuciscus cephalus*) as a Bioindicator of Contamination of the Vltava River by Synthetic Musk Fragrances. *Archives of Environmental Contamination and Toxicology* 53(3): 390-396

Hajšlová J., Pulkrabová J., Poustka J., Čajka T., Randák T., 2007. Brominated flame retardants and related chlorinated persistent organic pollutants in fish from river Elbe and its main tributary Vltava, *Chemosphere* 69(8): 1195-1203

Křížková S., Zítka O., Adam V., Beklová M., Horna A., Svobodová Z., Sures B., Trnková L., Zeman L., Kizek R., 2007. Possibilities of electrochemical techniques in metallothionein and lead detection in fish tissues. *Czech J. Anim. Sci.* 52(5): 143-148

Nitrite influence on fish

The aim of the first study was to discover the influence of water temperature on the nitrite toxicity mechanism in common carp (*Cyprinus carpio* L.) (Kroupová et al., 2006a). Carp were exposed to nitrite ($1.45 \text{ mmol l}^{-1} \text{ NO}_2^-$) for 48 hours at $14 \text{ }^\circ\text{C}$ and $20 \text{ }^\circ\text{C}$. Nitrite exposure produced high levels of methaemoglobin compared to controls at both water temperatures. High fish mortality occurred in the experimental groups (30% and 51%) compared with controls (0%). Exposure also resulted in an accumulation of nitrite in the fish body. The highest levels developed in the blood plasma, followed by liver and muscle. Carp concentrated nitrite in the blood plasma and tissues to markedly higher levels at higher temperature ($20 \text{ }^\circ\text{C}$). At this temperature plasma nitrite concentrations were more than 7 times that in the environment. At $14 \text{ }^\circ\text{C}$ the concentration was 3.5 times ambient levels. In both cases, plasma K^+ levels increased. Plasma Na^+ levels decreased slightly only at the higher temperature. Nitrite exposed fish showed lower haematocrit values at both experimental temperatures compared with controls. At $20 \text{ }^\circ\text{C}$, the blood haematocrit decrease was accompanied by a low erythrocyte count and by a low haemoglobin level. At $14 \text{ }^\circ\text{C}$, the haematocrit decrease was the result of a low mean corpuscular volume. In conclusion, our experiment indicates that mortality and methaemoglobinaemia during nitrite poisoning are not related to water temperature. However, other haematological and biochemical parameters (NO_2^- in the blood plasma, muscle, and liver; plasma Na^+ concentration; Hb; PCV; RBC) were more altered during nitrite exposure at higher temperature.

The aim of the second study was to assess the ability of recovery in common carp after nitrite poisoning and to distinguish the difference in nitrite poisoning of carp at two different chloride concentrations in water (Kroupová et al., 2006b). Two groups of carp (1 and 2) were exposed to an environmental nitrite concentration of $1.45 \text{ mmol l}^{-1} \text{ NO}_2^-$ at different chloride concentrations (group 1, $0.31 \text{ mmol l}^{-1} \text{ Cl}^-$ and group 2, $3.73 \text{ mmol l}^{-1} \text{ Cl}^-$) for 48 hours, after which they were transferred into nitrite-free water. In group 1, mortality of 51% occurred during nitrite exposure and a further 11% mortality after 24 hours in nitrite-free water. No mortality occurred in group 2 and controls. Nitrite and methaemoglobin concentrations increased markedly in group 1 compared to controls. Nitrite exposure also resulted in an increase in plasma K^+ , ammonia, urea, and uric acid concentrations in group 1, while values of haematocrit, erythrocyte count, and haemoglobin were markedly lower than in controls. Most of these changes were reversed by the end of the recovery period; only plasma potassium continued to drop to concentrations below the control values. Minimal changes were observed in group 2 compared with controls during the duration of the experiment. This showed the positive effect of chlorides on fish resistance to nitrite toxicity. The experiment also verified the ability of carp to recover from nitrite intoxication.

Kroupová H., Máchová J., Piačková V., Flajšhans M., Svobodová Z., Poleszczuk G., 2006a. Nitrite intoxication of common carp (Cyprinus carpio L.) at different water temperatures. Acta Vet- Brno 75(4): 561-569

Kroupová H., Máchová J., Svobodová Z., Piačková V., Smutná M., 2006b. The ability of recovery in common carp after nitrite poisoning. Vet. Med. – Czech 51 (8): 423-431

Koi herpesvirus (KHV)

A disease called koi carp causing serious losses in culture of common carp (*Cyprinus carpio* L.) and namely of its ornamental form has spread nearly all over the world since 1998. Koi herpesvirus (KHV) was designated as its agens. The outbreak of the disease is always accompanied with high mortality of fish (80-90%) and water temperature ranging from 18 to 28°C is a predisposition factor for emergence of the disease. The KHV is clinically manifested by fish disorientation, irregular swimming and respiration, and by mass kill. Large necrosis of gill lamellae is the most important pathological alteration. The virus can be cultivated on KF-1 cell line, creating a cytopathic effect (CPE). The approach of polymerase chain reaction (PCR) appears to be the best method for virus detection in tissues. World research workplaces are looking for possibilities of carp culture protection. A protocol of natural fish immunisation has been elaborated, application of attenuated vaccine has been tested, and resistant carp strains are being bred.

In 2006, the monitoring of the presence of KHV in common carp and koi carp cultures in the Czech was carried out in the frame of the ending grant project „Protection of common carp culture from disease caused by KHV”. Samplings took place in the Research Institute of Fish Culture and Hydrobiology in Vodnany as well as haematological and biochemical examinations. Virological investigations by means of PCR and cultivation on cell cultures were carried out in Veterinary

Research Institute in Brno. Bacteriological investigations were performed in Veterinary and Pharmaceutical University Brno.

In 2007, the new grant project „ Monitoring of the koi herpesvirus disease (KHVD) occurrence in breeds of common carp in CR and testing of susceptibility of chosen strains of common carp to KHV“ was started. During this first year, the virological as well as complementary investigations of fish from Czech fish farms were carried out.

Pokorová D., Piačková V., Čížek A., Reschová S., Hůlová J., Václavík M., Veselý T., 2007. Tests for the presence of koi herpesvirus (KHV) in common carp (Cyprinus carpio carpio) and koi carp (Cyprinus carpio koi) in the Czech Republic. Veterinarni Medicina 52(12): 562-568

Findings of good antiparasitics

Malachite green has been used as an effective compound of external fungal and protozoan infections of fish since 1933 but it was never registered as a veterinary drug for use in food fish, because of its potential carcinogenicity, mutagenity and teratogenicity in mammals. The paper reviews negative side effects of malachite green including its cummulation and persistence in fish that have been treated and describes other alternative substances for the treatment of fish and fish eggs.

Sudová E., Máchová J., Svobodová Z., Veselý T., 2007. Negative effects of malachite green and possibilities of its replacement in the treatment of fish eggs and fish: a review. Vet. Med. – Czech 52(12): 527-539

Anaesthetics

Assessment of the effect of anaesthetics (2-phenoxyethanol and clove oil) was performed on the three important fish species including common carp (*Cyprinus carpio* L.), trout (*Oncorhynchus mykiss*) and European catfish (*Silurus glanis*). The effect was assessed on the basis of acute toxicity tests and effects of anaesthetics on haematological, biochemical blood plasma profile and histological examination of tissues.

The fish were divided into four groups for haematological and biochemical examinations of blood and histological examinations of tissues. The groups were as follows: Control I (before the anaesthetic administration), Experiment I (immediately after 10 min anaesthesia), Experiment II (24 hrs after 10 min anaesthesia) and Control II (controls examined in parallel with Experimental II). The indices used to evaluate the haematological profile included the erythrocyte count (Er), haemoglobin concentration (Hb), haematocrit (PCV), mean erythrocyte volume (MCV), mean corpuscular haemoglobin concentration (MCHC), mean corpuscular haemoglobin content (MCH), leukocyte count (Leuko) and the differential leukocyte count (Leukogram). Biochemical indices determined in blood plasma included glucose (GLU), total protein (TP), albumins (ALB), total globulins (GLOB), ammonia (NH₃), triacylglycerols (TRIG), aspartate aminotransferase (AST), alanin aminotransferase (ALT), lactate dehydrogenase (LDH), creatinkinase (CK), calcium (Ca²⁺) and inorganic phosphate (PHOS). Samples of gills, skin, liver, cranial and caudal kidney and spleen were histologically examined.

Results of the examinations suggest that the use of anaesthetics 2-phenoxyethanol (0.30 ml.l⁻¹) and clove oil (30 mg.l⁻¹) are safe in the recommended concentration for common carp (*Cyprinus carpio* L.), rainbow trout (*Oncorhynchus mykiss*), and European catfish (*Silurus glanis*).

Velíšek J., Wlasow T., Gomulka P., Svobodová Z., Novotný L., Ziomek E., 2006. Effects of clove oil anaesthesia on european catfish (Silurus glanis L.). Acta Veterinaria Brno 75(1): 99-106

Velíšek J., Wlasow T., Gomulko P., Svobodová Z., Novotný L., 2007. Effects of 2-phenoxyethanol anaesthesia on sheatfish (Silurus glanis L.). Vet Med-Czech 52(3): 103-110

Velíšek J., Svobodová Z., Piačková V., 2007. Effects of 2-phenoxyethanol anaesthesia on haematological profile on common carp (Cyprinus carpio) and rainbow trout (Oncorhynchus mykiss). Acta Veterinaria Brno 76(3): 487-492

The toxic effect of pesticide on fish

The assessment of the toxic effect of pesticide preparations Decis EC 50 (active substance deltamethrin), and Alimethrin 10 EC (active substance cypermethrin), was performed on common carp (*Cyprinus carpio* L.) and rainbow trout (*Oncorhynchus mykiss*). The effect was assessed on the basis of acute toxicity tests and effects of anaesthetics on haematological, biochemical blood plasma profile and histological examination of tissues.

Examination of haematological and biochemical profile and histological tissue examination was performed on the control group and experimental group after of exposure to 96hLC50. The indices used to evaluate the haematological profile included the erythrocyte count (Er), haemoglobin concentration (Hb), haematocrit (PCV), mean erythrocyte volume (MCV), mean corpuscular haemoglobin concentration (MCHC), mean corpuscular haemoglobin content (MCH), leukocyte count (Leuko) and the differential leukocyte count (Leukogram). Biochemical indices determined in blood plasma included glucose (GLU), total protein (TP), albumins (ALB), total globulins (GLOB), ammonia (NH₃), triacylglycerols (TRIG), aspartate aminotransferase (AST), alanin aminotransferase (ALT), lactate dehydrogenase (LDH), creatinkinase (CK), calcium (Ca²⁺), inorganic phosphate (PHOS) and glutathione S-transferase. Samples of gills, skin, liver, cranial and caudal kidney and spleen were histologically examined.

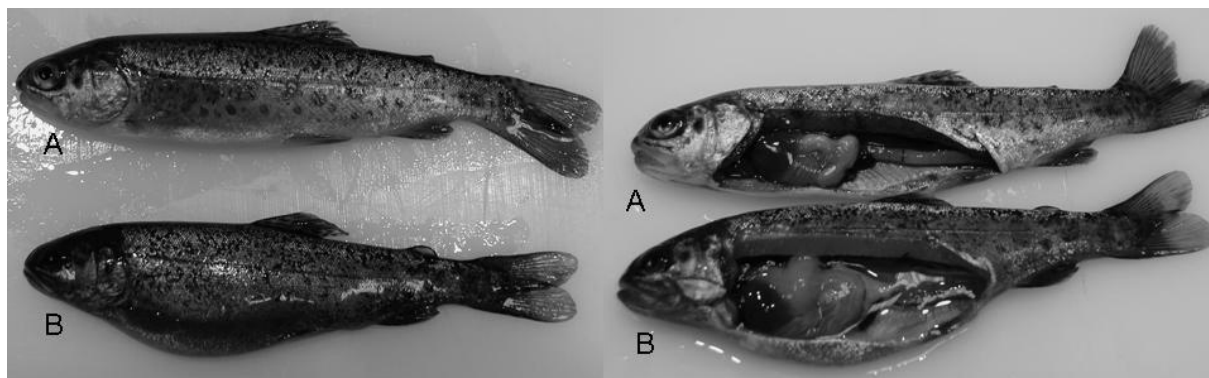


Figure : A – control rainbow trout; B – experimental rainbow trout after acute exposure to metribuzin – dark colour body, transudate in the body cavity (foto J. Velišek)

Results of the examinations suggest that the pesticide preparations Decis EC 50 (active substance deltamethrin) and Alimethrin 10 EC (active substance cypermethrin) for common carp (*Cyprinus carpio* L.) and rainbow trout (*Oncorhynchus mykiss*) were classified as highly toxic.

- Dobšíková R., Velišek J., Wlasow T., Gomulka P., Svobodová Z., Novotný L., 2006. Effects of cypermethrin on some haematological, biochemical and histopathological parameters of common carp (*Cyprinus carpio* L.). *Neuroendocrinology Letters* 27(2): 101-105
- Velišek J., Dobšíková R., Svobodová Z., Modrá H., Lusková V., 2006. Effect of deltamethrin on the biochemical profile of common carp (*Cyprinus carpio* L.). *Bulletin of Environmental Contamination and Toxicology* 76(6): 992-998
- Velišek J., Wlasow T., Gomulka P., Svobodová Z., Dobšíková R., Novotný L., Dudzik M., 2006. Effects of cypermethrin on rainbow trout (*Oncorhynchus mykiss*). *Vet Med-Czech*, 51(10): 469-476.
- Bláhová J., Slatinská I., Kružiková K., Velišek J., Svobodová Z., 2007: The effect of deltamethrin on activity of glutathione S-transferase of common carp (*Cyprinus carpio*). *Chemické listy* 101(14): 168-169
- Velišek J., Jurčíková J., Dobšíková R., Svobodová Z., Piačková V., Máchová J., Novotný L., 2007. Effects of deltamethrin on rainbow trout (*Oncorhynchus mykiss*). *Environmental Toxicology and Pharmacology* 23(3): 297-301

The use of Diazinon 60 EC in fishery

During the years 2006-2007, acute toxicity of Diazinon 60 EC was assessed for guppy (*Poecilia reticulata*) and common carp (*Cyprinus carpio*). Furthermore, the influence of this biocide was evaluated for early-life stage of common carp (Máchová et al., 2007). Subsequently, methodology of the using of Diazinon 60 EC in pond fishery to suppress excessive propagation of large daphnian zooplankton was finished (Faina et al., 2007).

Diazinon 60 EC is a chemical insecticide (biocide) with diazinon [(O,O-diethyl-0-2-isopropyl-4-methylpyrimidinyl-4-yl)-thiophosphate] as its active substance. Diazinon 60 EC may be used in well-grounded cases as a biocide in fishery operations, and it must be done in compliance with the law of the Czech Republic.

Final results of the acute toxicity test (96hLC50) of Diazinon 60 EC for adult guppies (*P. reticulata*) are: 96hLC50 = 3 mg l⁻¹ and for juveniles of the common carp (*C. carpio*) 96hLC50 = 10 -

25 mg l⁻¹. The effect of Diazinon 60 EC on early life stage of carp was evaluated by means of the embryonic and larval toxicity tests. During the embryonic test, the fertilized eggs of carp were exposed to Diazinon 60 EC in the concentrations of 10, 50, 100, 500 and 1000 µg.l⁻¹. No significantly negative effects of Diazinon 60 EC at concentrations tested for hatching and embryo viability were demonstrated. During the larval toxicity test, the larvae after swimming and resorption of the sac were exposed to the preparation in concentrations 10, 100, 1000 and 3000 µg l⁻¹ for 10 days and then they were transferred to water without the substance tested. The retardation of the longitudinal growth of larvae was observed in the concentrations of 100, 1000 and 3000 µg l⁻¹ at the end of the period of exposure to the toxicant. No differences were observed at the concentration of 10 µg l⁻¹.

As demonstrated by embryonic and larval tests of toxicity for the common carp (*C. carpio*), Diazinon 60 EC at the concentration of 10 µg l⁻¹ causes no harm to the tested individuals even in these sensitive (critical) stages of ontogeny.

Máchová J., Prokeš M., Svobodová Z., Žlábek V., Peňáz M., Baruš V., 2007. Toxicity of Diazinon 60 EC for *Cyprinus carpio* and *Poecilia reticulata*. *Aquaculture International* 15 (3-4): 267-276

Faina R., Máchová J., Svobodová Z., Kroupová H., Valentová O., 2007. Použití přípravku Diazinon 60 EC v rybníkářské praxi k tlumení nadměrného rozvoje hrubého dafniového zooplanktonu. [The using Diazinon 60 EC in pond fishery to suppress excessive propagation of large daphnian zooplankton]. *Edice Metodik, VÚRH JU, Vodňany*, č. 80, 18 s.

Processing technology and hygienic quality of fish flesh

This research was focussed on supplemental cereal feeding (maize, wheat and triticale) compared to control group with natural food and its effect on fatty acids (FA) expression in the flesh during long-lasting storage of common carp (*Cyprinus carpio*). The content of fatty acid was investigated in the flesh of carp during 8 months long-lasting storage without an additional feeding. The weight of the fish (marketable fish) ranged from 1358 to 2221 gr. Fatty acid (PUFA, n-3) content and composition in fish flesh were determined by gas chromatography (VARIAN 3300). Supplemental cereals caused lower level of PUFA and n-3 PUFA in fish fat. The content of these fatty acids did not decrease even during eight months of fish storage. The average percentage of PUFA in total fat from edible parts was as follows: 13.7% ± 1.58 for maize, 11.6% ± 1.17 for wheat and 10.7% ± 1.00 for triticale. The percentage of n-3 PUFA for maize was 2.5% ± 0.36, for wheat 3.38% ± 0.44 and for triticale 3.1% ± 0.39.

Vácha F., Vejsada P., Hůda J., Hartvich P., 2007: Influence of supplemental cereal feeding on the content and structure of fatty acids during long-lasting storage of common carp (*Cyprinus carpio* L.). *Aquacult. Int.* 15: 321-329



The examination of fish health

INTERNATIONAL CO-OPERATION

The department co-operated with the following foreign institutions:

Faculty of Environmental Science and Fisheries, University of Warmia and Mazury Olsztyn, Poland (T. Wlasow and P. Gomulka) – Anaesthetics.

Szczecin University, Szczecin, Poland (G. Poleszczuk) – Nitrite toxicity for fish.

APPLICATION OF RESEARCH RESULTS AND OTHER PRIORITIES

- Monitoring of surface water pollution.
- Eco-toxicological assessment of preparations, substances, and wastes in the toxicological Laboratory accredited by the Czech Institute of Accreditation registered under No. 1052 (Head – Dipl.-Ing. J. Máchová, Quality manager – Dipl.-Ing. O. Valentová). Acute toxicity test are performed out on fish (guppy *Poecilia reticulata*, zebra fish *Brachydanio rerio*, common carp *Cyprinus carpio*, rainbow trout *Oncorhynchus mykiss*), green chlorococcal alga (*Scenedesmus subspicatus*), aquatic arthropods (*Daphnia magna*), and plant (*Sinapis alba*).
- Chronic toxicity test on fish – common carp (*Cyprinus carpio*) and rainbow trout (*Oncorhynchus mykiss*).
- Basic chemical analysis of drinking-, surface- and waste water, and determination of total mercury in biological materials in the Central Hydro-chemical Laboratory. Production and service of field kits for quick basic chemical analysis of water (Head – Dipl.-Ing. O. Valentová).
- Parasitological, pathological, biochemical and haematological examination of fish (V. Piačková, DVM; J. Kolářová, DVM, E. Sudová, DVM and Prof. Z. Svobodová, DVM).
- Management of Blanice Vodňanská 4 B special trout angling ground.
- Teaching of eco-toxicology at the Higher Professional School of Water Management and Ecology of the Fisheries High School in Vodňany.



View to Hydro-chemical Laboratory

INTERNATIONAL ACTIVITIES OF USB RIFCH

Cooperation

International co-operation runs on several levels:

1. Cooperation in mutual exchange of publications, experience, results and short-term stays aiming at resolving related research subjects. This co-operation is based on signed contracts between USB RIFCH and the specific foreign institution. In the period 2006–2007 we had valid agreements with following institutions:
 - Key Laboratory of Freshwater Fish Germplasm Resources and Biotechnology, Ministry of Agriculture, Jingzhou, China
 - INRA-IFREMER Amélioration génétique des poissons“, Chemin de Maguelone, Palavas les Flots, France
 - Department of Zootechnical Science, Agricultural faculty, Università degli Studi di Torino, Torino, Italy
 - Shimoda Marine Research Centre, University of Tsukuba, Shimoda, Japan
 - Szent István University of Gödöllő, Faculty of Agricultural and Environmental Sciences, Department of Fish Culture, Gödöllő, Hungary
 - Research Institute of Fisheries, Aquaculture and Irrigation, Szarvas, Hungary
 - Institute of Ichthyobiology and Aquaculture, Polish Academy of Sciences, Golysz, Poland
 - Federal Bureau of Water Management, Station of Ecology, Schrems, Austria
 - All-Russian Scientific Institute of Fisheries and Oceanography, Moscow, Russia
 - Scientific Research Centre of Ecological Safety, Russian Academy of Science, Sankt Peterburg, Russia
 - Department of Genetics, Developmental and Molecular Biology, Aristotle University of Thessaloniki, Thessaloniki, Greece
 - Department of Food Science, Swedish University of Agricultural Science, Uppsala, Sweden
 - National Research Center for Environment and Health, Institute of Ecological Chemistry, Neuherberg, Germany
 - University of Florence, Department of Animal Biology and Genetics, Florence, Italy
 - University of Extremadura, Department of Vegetal Biology, Ecology and Earth Sciences, Badajoz, Spain
 - Institute of Animal Reproduction and Food Research of the Polish Academy of Sciences, Olsztyn, Poland
2. Bilateral cooperation in scope of countries which sign contracts on governmental level regarding mutual co-operation in research and development. These programmes are available through the Ministry of Education, Youth and Sport of the Czech Republic (see capture “Research projects”)
3. Direct cooperation with several partners from Europe concentrating on research and development in scope of the programmes of the European Union (see capture “Research projects”)

International scientific meetings organized by USB RIFCH

1. First International Workshop on Biology of Fish Sperm, Vodňany, Czech Republic, August 29 – 31, 2007

Under guidance of prof. Otomar Linhart, director of USB RIFCH, a Laboratory of Physiology of Reproduction was established at our institute. The laboratory is recognized worldwide due to a high number of results published in the scientific literature, developed international cooperation, and the broad background of students attending the doctorate study program „Fisheries“ accredited at our institute. The dominant position of this laboratory cumulated by organizing of the 1st International meeting of scientists from the field of fish sperm biology. The workshop took place in Vodňany in the conference hall of the main building of the University of South Bohemia, Research Institute of Fish Culture and Hydrobiology.

The programme of the workshop was divided over three days, and contributions were presented in oral as well as poster form. The contributions covered a wide range of topics from basic sperm

morphology and physiology through histological studies to biochemical and functional control of sperm quality and cryopreservation of sperm in fresh water as well as marine fishes. At the workshop contributions of two key speakers were presented:

- Cosson, J. (France) - Traits of sperm motility in marine fishes
- Horvath, A. (Hungary) - Cryopreservation of sperm in sturgeon: membrane integrity and fertilizing capacity

The workshop clearly demonstrated the progress made in recent years to better understand the general biology of fish sperm. A more heterogeneous group than fish within reproduction strategy, biology, and species cannot be found in vertebrates. It was also obvious that our knowledge in this field is still highly fragmented, although the importance of this subject is presently accentuated not only in terms of basic biological disciplines, but also in its practical application in aquaculture and, equally important, in global species conservation.



At the end of workshop possibilities of other mutual cooperation among participants were discussed, and the following conclusions and recommendations were highlighted:

- There is a need for continued research efforts in this area, which seems not to gain sufficient attention in the international science community, and subsequently does not receive adequate support on the research agenda of national and international sponsoring agencies.
- There is a need to continue with fish sperm biology workshops in a timely fashion. The next meeting should be held no later than 2009.
- It is recommended to establish a committee that explores the options to form and fund an International Society on Aquatic Gametes (ISAG).

The workshop was attended by about 50 participants from 16 countries (Czech Republic, Poland, France, Germany, Spain, Portuguese, Israel, Italy, Hungary, Ukraine, Ireland, Iran, South Africa, Canada, Taiwan and Japan) including several familiar names. The participants had the opportunity to visit the experimental facilities and laboratories of USB RIFCH, and enjoyed a social evening with traditional Czech banquet and music. The accompanying people could join an additional program with sightseeing in the near region.

2. International meeting of 12 colleagues from 5 institutions (Czech Republic, Poland, France, Ukraine, Japan) to work on methodology of cryopreservation of sturgeon sperm and electroforetic separation of sperm and sperm plasma proteins in chondrosteian and teleostean fishes, Vodňany, Czech republic, March 26 – 29, 2007

Participation of international conferences and symposiums by USB RIFCH employees and students Year 2006

- AQUA2006 – Linking of tradition and technology, Firenze, Italy, May 9 – 13
- Hygiena Alimentorum XXVII, Štrbské Pleso, Slovakia, May 18 – 20
- IXth International Symposium on Genetics in Aquaculture, Montpellier, France, May 25-30
- 23rd World Congress of the Czechoslovak Society of Arts and Science, České Budějovice, Czech Republic, June 25 – July 2

- 1st European Congress of Conservation Biology “Diversity in Europe”, Eger, Hungary, August 21–28
- 5th International Conference on Reservoir Limnology and Water Quality, Brno, Czech Republic, August 27 – September 2
- 10th International Symposium on Spermatology, Madrid, Spain, September 17 – 22
- 43rd Congress of the European Society of Toxicology „EUROTOX“, Cavtat, Croatia, September 21 – 24
- IXth International conference of German-speaking pathologists, Murten, Switzerland, October 11 – 13
- International Conference on Chemical Ecology in Aquatic Systems, Firenze, Italy, October 16 – 18

Year 2007

- IIIrd International Conference and Minifair „Fisheryí“, Beograd, Serbia, February 1 – 4
- 8th International Symposium on Reproductive Physiology of Fish, St. Malo, France, June 3 – 8
- 5th Symposium of European Freshwater Sciences, Palermo, Italy, July 8 – 13
- XIIth European Congress of Ichthyology, Dubrovnik, Croatia, September 9 – 13
- 13th International EAFP Conference on Disease of Fish and Shellfish, Grado, Italy, September 17 – 21
- 15th International Conference on Aquatic Invasive Species, Nijmegen, Netherlands, September 24 – 27
- 4th International Conference on Aquaculture and Water Ecosystems, Plovdiv, Bulgaria, October 10 – 12
- Conference on Fisheries Management of Water Reservoirs, Neum, Bosnia and Herzegovina, October 23 – 26

Memberships of USB RIFCH in international networks and organisations

- NACEE (Network of Aquaculture Centres in Central-Eastern Europe)
- AQUA-TNET (European thematic network in the field of aquaculture, fisheries and aquatic resources management)
- EAS (European Aquaculture Society)

Memberships of USB RIFCH employees and students in international organisations

Adámek Z.	European Aquaculture Society (EAS), member of board till end of 2006
Flajšhans M.	Network of Tropical Aquaculture Scientists
Kozák P.	International Association of Astacology
Kouřil J., Linhart O.	European Aquaculture Society
Linhart O.	International Society of Cryobiology World Sturgeon Conservation Society
Svobodová Z.	European Association of Fish Pathologists OECD – Ecotoxicology

Memberships of USB RIFCH employees in editorial boards

Adámek Z.	Aquaculture International Ribarstvo Zagreb Agriculae Conspectus Scientificus Zagreb
Svobodová Z.	Acta Veterinaria
Linhart O.	Journal of Applied Ichthyology Czech Journal of Animal Science Bulletin VÚRH Vodňany
Kozák P.	Bulletin VÚRH Vodňany
Randák T.	Bulletin VÚRH Vodňany
Flajšhans	Bulletin VÚRH Vodňany
Vykusová B.	Bulletin VÚRH Vodňany

EDUCATION AND TEACHING ACTIVITIES

Pedagogical activities are concentrated on **doctoral study of subject Zootechnics - 4103V003 Fisheries**, which has been accredited at the USB RIFCH in 2004. RIFCH staff members also participate in education of students at the USB and other universities, Secondary Piscatorial School and College of Water Management and Ecology.

Doctoral study of the subject Fishery can be studied either in a full-time form, or in a combined form, in Czech or in English, for a standard period of three years.

The purpose of the doctoral studies in Fisheries is a research-oriented education of fish culture specialists. The study field involves thorough training in ecology, reproduction, physiology, genetics, ecotoxicology, diseases, and culture of fish. Students acquire knowledge in various fishery segments and elaborate a doctoral thesis in English focused on a specific segment within the study field. The study is completed by a final state examination consisting of examinations in the field of study and a defence of the candidate's doctoral dissertation. At least part of the results of the candidate's own research included in the thesis must be published (or accepted for publication) prior to the defence.

The Fisheries doctoral study programme graduates possess general knowledge of fish, i.e. of the system, morphology, and physiology of fish, they have a good grasp of the general fishery, agricultural, and biological subjects (ecology, genetics, cell and molecular biology), and are able to use the theoretical background for the environmental management in the area of fish culture and water management.

1. Number of students

Academic year	Total number	3 rd year of study	2 nd year of study	1 st year of study	Completed study	Aborted study	Interrupted study
2005/06	10		2	7 + 1C		1C	1
2006/07	16	2	6	7	2	1	
2007/08	21	5	7	6 + 3k			

C – number of students in the combined form of study

In September 2007 two Ph.D students successfully completed their study and passed state doctoral examination and defended their Ph.D theses.

2. The Fisheries doctoral study programme graduates (2007)

Supervisor	Ph.D. student	Doctoral thesis
Prof. Dipl.-Ing. Otomar Linhart, DSc.	Dipl.-Ing. Martin Hulák	Sex control of common carp (<i>Cyprinus carpio</i> L.)
Assoc. Prof. Dipl.-Ing. Jan Kouřil, Ph.D.	Dipl.-Ing. Hana Kroupová	A study of the effects of nitrite on fish and possible ways to reduce its negative impact

3. Actual Ph.D student's and topics of their theses in period 2006–2007

Supervisor	Ph.D. student	Doctoral thesis
Assoc. Prof. Zdeněk Adámek, Ph.D.	Dipl.-Ing. Jiří Kortan	Evaluating of secondary losses on fish stock caused by feeding activity of great cormorant (<i>Phalacrocorax carbo sinensis</i>) on the ponds
	Dipl.-Ing. Karel Dubský	Utilization of natural food sources in nutrition of fry in intensive fish culture
	M.Sc. Eva Nyklová	Importance and indicative weight of selected groups of larvae of water insects in pond ecosystem with regard to fisheries management
Prof. M.Sc. Zdeněk Brandl, Ph.D.	Dipl.-Ing. Martin Bláha	Ecology and biology of three species <i>Acanthocyclops vernalis</i> - <i>robustus</i> group and their differentiation using molecular methods
Assoc. Prof. Dipl.-Ing. Jan Kouřil, Ph.D.	Dipl. Ing. Vlastimil Stejskal	Intensive culture and nutrition of Eurasian perch (<i>Perca fluviatilis</i> L.) fingerlings
	M.Sc. Bořek Drozd	Critical population parameters in weatherfish (<i>Misgurnus fossilis</i> , L. 1758)
	M.Sc. Peter Podhorec	Artificial reproduction of barbel (<i>Barbus barbus</i> , L.)
	Dipl.-Ing. Tomáš	Effect of commercial diets in brook trout (<i>Salvelinus fontinalis</i>)

	Pávek	and ranbow trout (<i>Oncorhynchus mykiss</i>) feeding
Dipl.-Ing. Pavel Kozák, Ph.D.	Dipl.-Ing. Miloš Buřič	Biology of spiny-cheek crayfish <i>Orconectes limosus</i> (Rafinesque, 1817) in conditions of Czech Republic and the study factors influencing its invasive spread
	Dipl.-Ing. Antonín Kouba	Optimalization of noble crayfish breeding and estimation of adaptability and migration of crayfish after reintroduction
Prof. Dipl.-Ing. Otomar Linhart, DSc.	M.Sc. S. M. H. Alavi	Behavior and competition of sperm in chondrosteian compared to teleost fish
	Dipl.-Ing. Martin Pšenička	Ultrastructure of spermatozoa and eggs during fertilization using electron and konfokal microscopy
	Dipl.-Ing. Vojtěch Kašpar	Genetic variability of common carp (<i>Cyprinus carpio</i> L.) broodstocks
	M.Sc. Sergey Boryshpolets	Energetics and motility of fresh and frozen thawed spermatozoa in models of teleost and chondrosteian fish
	M.Sc. Ping Li	Characterisation of the protein in seminal plasma of fresh and frozen fish spermatozoa
	M.Sc. Ivo Přikryl	Correlation between nutrients and species composition of fishpond zooplankton
	M.Sc. Petra Vorlíčková	Impact of breeding conditions on quality of spawners, their gamets and progenies of barbel (<i>Barbus barbus</i> L.)
Assoc. Prof. Jana Picková, Ph.D.	Dipl.-Ing. Jan Mráz	Enrichment of fatty acid composition of common carp (<i>Cyprinus carpio</i>)
Assoc. Prof. Dipl. Ing. Petr Ráb, DSc.	Dipl.-Ing. Jan Kohout	Population genetic structure of brown trout (<i>Salmo trutta</i>) in the Czech republic and Slovakia
M.Sc. Ondřej Slavík, Ph.D.	Dipl.-Ing. Jan Turek	Adaptability of hatchery - reared brown trout (<i>Salmo trutta</i> m. <i>fario</i> L.) and European grayling (<i>Thymallus thymallus</i> L.) in natural watters
	Dipl.-Ing. Radek Hanák	Interactions between wild and hatchery fish in salmonid waters
Prof. Zdeňka Svobodová, DVM DSc.	Eliška Sudová, DVM	Pharmacovigilance in fish culture focused to antibiotics and antiparasitics
	M.Sc. Petra Beránková	Genotoxic potential of extraneous substances in surface water ecosystems
	Dipl.-Ing. Jana Máchová	Prevention of fish mortality in highly eutrophic ponds

4. Undergraduate students who worked in the Institute and were supervised by the Institute's fellows in 2006–2007

<i>Supervisor</i>	<i>Student</i>	<i>Study</i>	<i>2006</i>	<i>2007</i>	<i>Defended</i>	<i>University/Faculty</i>
Z. Adámek	J. Kortan	M.Sc.	+		2006	USB FA
	K. Kořínek	M.Sc.	+		2006	USB FA
	P. Kabilka	M.Sc.	+	+	2007	USB FA
	J. Sikora	M.Sc.	+	+		USB FA
	J. Ťuk	M.Sc.	+	+		USB FA
	J. Zeman	Bc.	+	+		USB FA
	T. Blinka	M.Sc.	+	+		USB FA
	D. Hercig	M.Sc.	+	+		USB FA
	K. Němec	M.Sc.	+	+		USB FA
	J. Strapina	M.Sc.	+	+		USB FA
M. Flajšhans	J. Kašpar	M.Sc.	+			USB FA
	O. Fořt	Bc.		+		USB FA
	M. Marek	Bc.		+		USB FA
	L. Štěch	M.Sc.		+		USB FA
M. Hulák	P. Sova	M.Sc.	+	+		USB FA
M. Kocour	M. Kříž	M.Sc.		+		USB FA
J. Kouřil	J. Turek	M.Sc.	+		2006	USB FA
	J. Hájek	M.Sc.	+	+	2007	USB FA
	J. Mráz	M.Sc.	+	+	2007	USB FA

	J. Nocar	M.Sc.	+	+	2007	USB FA
	J. Škeřík	M.Sc.	+	+	2007	USB FA
	T. Borkovec	M.Sc.	+	+		USB FA
	K. Olbert	M.Sc.		+		USB FA
	K. Raška	Bc.		+		USB FA
P. Kozák	M. Buřič	M.Sc.	+		2006	USB FA
	M. Musil	M.Sc.	+	+	2007	USB FA
	J. Kanta	M.Sc.	+	+	2007	USB FA
	A. Kouba	M.Sc.	+	+	2007	USB FA
	L. Kočí	M.Sc.	+	+		USB FA
	P. Vích	M.Sc.	+	+		USB FA
O. Linhart	J. Hušek	M.Sc.	+		2006	USB FA
	V. Kašpar	M.Sc.	+		2006	USB FA
	R. Slabý	M.Sc.	+		2006	USB FA
	R. Vach	M.Sc.	+			USB FA
	M. Aldorf	M.Sc.	+	+	2007	USB FA
	T. Gavenda	Bc.		+		USB FA
J. Máchová	P. Dvořáková	M.Sc.	+		2006	USB FA
	Š. Hrabánková	M.Sc.	+		2006	USB FA
	P. Tomešek	M.Sc.	+			USB FA
T. Polícar	P. Benedikt	Bc.	+	+	2007	USB FA
	A. Vavrečka	M.Sc.	+	+		USB FA
Z. Svobodová	M. Gřunděl	M.Sc.	+	+		USB FA
	M. Podlesný	M.Sc.	+	+		USB FA
	P. Scheiner	M.Sc.	+	+		USB FA
	H. Dort	M.Sc.		+		USB FA
J. Velíšek	V. Malý	M.Sc.		+		USB FHSS
Total	47		38	35		

5. PhD students from other universities or departments with supervisor from USB RIFCH

<i>Supervisor</i>	<i>Student</i>	<i>2006</i>	<i>2007</i>	<i>Defended</i>	<i>University/Faculty</i>
Z. Adámek	D. Kortan	+		2006	USB FA
	J. Musil	+	+	2007	USB FA
	V. Prášek	+		2006	MU FS Brno
	J. Sychra	+	+		MU FS Brno
J. Kouřil	Z. Stupka	+		2006	USB FA
O. Linhart	M. Kocour	+		2006	USB FB
	M. Rodina	+	+	2007	USB FA
Z. Svobodová	T. Randák	+		2006	USB FA
	J. Velíšek	+		2006	USB FA
Total	9	9	3		

6. Courses at universities

<i>Supervisor</i>	<i>Subject</i>	<i>2006 hours</i>	<i>2007 hours</i>	<i>University/Faculty</i>
Z. Adámek	Hydrobiology	56	56	USB FA
	Fishery Hydrobiology	56	56	USB FA
	Fundamentals of Fisheries	28		MU FS Brno
	Ichthyology and Fundamentals of Fisheries		28	PU FS Olomouc
M. Flajšhans	Fish Breeding	56	48	USB FA
J. Kouřil	Trout and Fish Culture in Warm Waters	26	56	USB FA
	Nutrition and Feeding of Fish		12	USB FA
P. Kozák	Protection and Culture of Crustaceans and Clams	30		USB FA
O. Linhart	Directed Fish Reproduction	56	56	USB FA
T. Polícar	Aquarium Fish Culture	56	48	USB FA
	Basic Biology of Aquarium Fishes		42	USB FA
Z. Svobodová	Fish Diseases	56	56	USB FA
	Total	420	458	

LIFELONG LEARNING

Educational seminars, courses and studies cofinanced by EU and the Czech state budget:

ADVISORY, INFORMATION AND TRAINING FISHERY CENTER AT VÚRH JU VODŇANY

(CZ.04.1.03/3.3.03.3/0002)

responsible solutioner: Dipl.-Ing. Blanka Vykusová, CSc., administrator: Dipl.-Ing. Petra Plachtová,
provider: South Bohemian Regional Authority

Within the project solution, **the cycle of thematic seminars** (2007–2008) has been held. Seminars are especially designed for applicants among consultants, educators, tutors and methodists of fishing companies, fishing organizations, schools and state institutions.

The project target is increasing the knowledge regarding the present status of science and research in fishing, improving tutorial skills at transmitting data, creating educational materials (applicable even after the project is finished; e-learning), teaching skills in obtaining data, required for further advancements in the branch (where and how).

Target groups: Tutors, consultants, teachers and employees of fishing organizations in the South Bohemian region.

1st seminar – Controlled reproduction in fish: 20 – 21.3.2007

2nd seminar – Hatching the seed including its nutrition: 2 – 3.5.2007

3rd seminar – Recirculation systems in fish cultures: 13 – 14.11.2007

4th seminar – Veterinary and toxicological aspects in fishing: 11 – 12.12.2007

5th seminar – Genetics and breeding the fish: 11 – 12.3.2008

6th seminar – Perspectives of the Czech fishing: 15 – 16.5.2008



INTRODUCING THE COMBINED TWO-SEMESTER SPECIALIZATION STUDY IN THE FIELD OF FISHERIES AT THE UNIVERSITY OF SOUTH BOHEMIA

(CZ.04.1.03/3.2.15.2/0358)

responsible solutioner: Assoc. Prof. Dipl.-Ing. Jan Kouřil, Ph.D., administrator: Zuzana Dvořáková
project provider: the Ministry of Education, Youth and Sport Czech Republic, Prague

In the academic year 2007–2008, two-semester specialization study of fishing was organized, dedicated to employees of fishing production companies, professional employees of fishing sport associations, employees of the state administration and other applicants. The study was organized as a

block of lectures and seminars (total 150 hours of tuition). This education, performed by foremost Czech experts, was targeted to selected chapters on the technology of traditional and non-traditional fish breeding technologies, fish genetics and breeding, controlled reproduction in fish, feeding the fish, an intensive aquaculture, technical news, fish processing, fishery marketing, preparing applications of national and European grant titles in fishing, the Czech and European legislative regarding fishing, water management and protecting the environment or other hot themes.

IMPROVING THE BACHELOR, MAGISTER AND DOKTORAL STUDY IN THE FIELD OF FISHERIES AT THE UNIVERSITY OF SOUTH BOHEMIA

(CZ.04.1.03/3.2.15.3/0427)

responsible solutioner: Assoc. Prof. Dipl.-Ing. Jan Kouřil, Ph.D., administrator: Zuzana Dvořáková
project provider: the Ministry of Education, Youth and Sport Czech Republic, Prague

The first and second degree (bachelor - three years; magister - two years) are provided by the Faculty of Agriculture, České Budějovice; the doctoral degree is then performed at the Research Institute of Fish Culture and Hydrobiology in Vodňany. The education is shared by both cited parts of the University of South Bohemia. The project essential is improving both the theoretical and applied section of training students. It includes instruction aids (lecture notes, compendiums, DVD films) and improving the current laboratory equipment. As a part of this solution, selected subjects are innovated, including introducing new ones (Feeding the fish, breeding the fish in recirculation systems). Particular subjects shall be enriched via lectures by several foreign experts. In order to work individually and to present all reached results, the so called "Student's seminar week" shall be introduced. As an integral project part, the special library shall be complemented.

In the project framework, a lot of educational publications shall be issued, being at call of the study participants. Following study publications are planned: Fishpond management, Essentials of molecular biology in fishing, Utilizing the topmouth gudgeon in producing the predatory fish, Artificial reproducing and breeding the ide stock, Artificial reproducing and breeding the vimba stock, Breeding sturgeons, Regardful pond farming, Methods of the crayfish fishery, Breeding the noble crayfish, Utilizing heat pumps in the aquaculture, Using the Diazinone 60EC for suppressing an excessive development of the daphnia zooplankton. Following educational courses shall be issued on DVD: Processing the fish, Artificial fish reproduction, Breeding the American char.

THE NETWORK OF INFORMATION AND ENVIRONMENTAL CENTERS FOR THE CARE OF WETLANDS AND LAND WATER

(CZ.04.1.03/4.2.16.4/0016)

responsible solutioner: M.Sc. Ivo Přikryl, ENKI Třeboň, USB RIFCH: solutioner: Dip.-Ing. Blanka Vykusová, CSc., provider: the Ministry of Agriculture Czech Republic, Prague

The project aim is to increase the knowledge of people, who affect the status of wetlands and the water circulation/function in the landscape in various ways. A special attention shall be devoted to ponds and their reasonable and regardful management, to renewing wetland biotopes and the way of applying the General directive on waters (Directive 2000/60/ES). Water is especially important for the land condition and for people, living there. However, the whole structure of environmental education, training and culture (EVVO) considers water in an inadequate and ex-parte way. This project should contribute to a better understanding in experts and general public regarding the water circulation, properties of various water bodies and possibilities to affect them positively.

The aim shall be realized by series of lectures and seminars, adapted for needs of particular target groups. Regarding these groups, electronic and printed materials (designed for courses) shall be accessible and available as separate compendiums. In the project framework, the network of advisory and information centers shall be formed; lectures shall be possible at these sites. Target groups can obtain relevant information or a competent specialist can be immediated there.

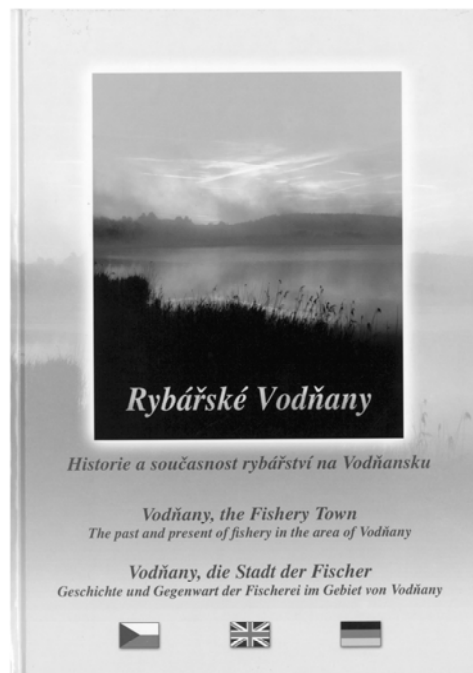
The project target groups are employees of civil offices on the level of ministries, regions and municipalities in charge, employees of CHKO and NP administrations, ČIŽP, hygienists, owners of ponds and minor waters as well as subjects, managing them; members and employees of NNO, municipal representatives, designers of minor waterworks and water revitalizations or other applicants.

traditional biennial exhibition *FISHTECH* (Thursday - Saturday) was visited by about 1000 of visitors, both laics and experts.

It is necessary to mention an excellent cultural program, including folklore choruses from partnerhip cities, fireworks and the Saturday fish market in the square.



The members of FDV (Vodňany, FHS and College in Vodňany, USB RIFCH) has prepared the popularization paper called ***Fishery Vodňany; History and present fishing in the Vodňany area***, utilizing the financial aid from EU and the South Bohemian Region (GS SROP 4.1.2). This teamwork (B. Vykusová, M. Merten, J. Velková, Z. Klimeš and A. Cepáková) informs on the history and present status of town fishery, fishery schools and the research institute in Czech, English and German language. The publication includes lots of photographs and one CD-ROM.



FURTHER FEATURES REGARDING OUR ACTIVITIES & REWARDS

The living fossil as a perspective genus for aquaculture delivered to the Research Institute of Fish Culture and Hydrobiology

After several years of effort, Dipl.-Ing. David Gela, Ph.D. (the head of hatchery of USB RIFCH) was successful at importing paddlefish (*Polyodon spathula*) fertilized eggs in the first half of 2006. Artificially spawned and fertilized eggs were purchased (after all needed formalities, i.e. veterinary certificates, CITES) from an American breeder due to the USB RIFCH research aim (MSM6007665809). Having been transported by air, eggs were incubated in the fish hatchery of the Department of Fish Genetics and Breeding (RIFCH USB Vodňany). Eggs were incubated in troughs (1 month), and hatched fish intensively fed with live zooplankton. When fish reached total length 4 cm they were transferred into prepared ponds. Individuals about total length 15 - 25 cm were found there before the autumn controlled fishing season.



Dipl.-Ing. Martin Kocour, Ph.D. significantly awarded



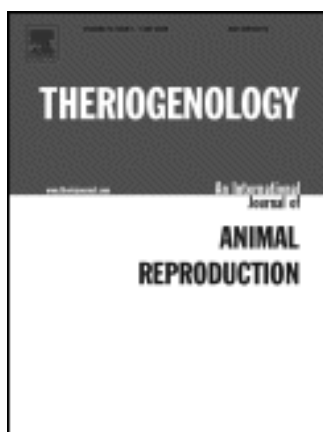
Prof. M.Sc. Libor Grubhoffer, CSc., dean of the Faculty of Biology, University of South Bohemia, granted the Award of Dean to Dipl.-Ing. Martin Kocour, Ph.D. upon his excellent scientific results presented in a dissertation thesis entitled “Utilizing genome manipulations as well as additive and non-additive genotype components in order to increase the efficiency of common carp (*Cyprinus carpio* L.)“, defended in the field of molecular and cell biology and genetics at the Faculty of Biology, USB.

The Award of Albrecht Daniel Thaer in Vodňany

Several academic awards were granted during the summer academic festival „LGF – Innovation und Wandel“, arranged by the Faculty of Agriculture and Gardening, Humboldt University of Berlin (July, 2007). Prof. Dr. Dr.h.c. Otto Kaufmann (the faculty Dean) and prof. Dr. Dr.h.c. Ernst Lindemann granted the Albrecht Daniel Thaer award (for the best 2006 dissertation thesis) to Dipl.-Ing. Martin Flajšhans, Dr.rer.agr., USB RIFCH, Vodňany. The ceremony was visited by the Czech chancellor in Germany, Dipl.-Ing. Jan Zlický.



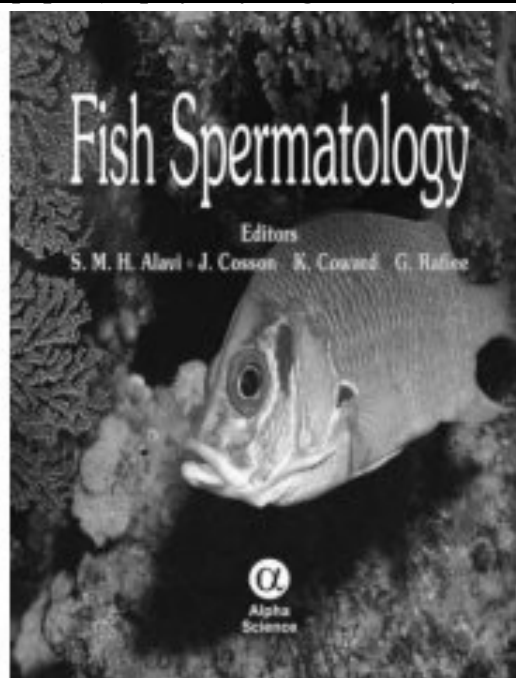
The Director's Award for the best 2006 scientific paper (employees younger than 35 years)



The scientific council evaluated 8 scientific papers at the 1/2007 meeting; the one by Dipl.-Ing. Martin Pšenička (a 1st year doctoral student of fisheries at RIFCH USB; promoter prof. O. Linhart) was chosen. The paper describes the ultrastructure of tench spermatozoa and provides valuable information regarding its reproduction. It also evaluates intra and interspecies differences, which may contribute to solving some taxonomic and phylogenetic questions. Besides the moral award, the RIFCH USB director rewarded the author with an extra scholarship of 12 000 CZK (paper abstract – see p. 42).

The Director's Award for the best 2007 scientific paper (employees younger than 35 years)

The scientific council (1/2008 meeting) rewarded M.Sc. Sayyed Mohammad Hadi ALAVI (Ph.D. student in the 2nd year; promoter prof. O. Linhart) with the Director's Award for the best 2007 scientific paper. Mr. ALAVI was the editor of the first book on fish spermatology (name book: Fish Spermatology, editors: Sayyed Mohammad Hadi ALAVI, Jacky COSSON, Kevin COWARD, Gholamreza RAFIEE), published by the prestige Alpha Science International Ltd. editorship, Oxford, UK (484 pages). S.M.H. Alavi, the main editor of the book, provided one free copy to the USB RIFCH library. Several important scientists shared in preparation of this book. We are pleased that researchers of USB RIFCH Vodňany took part as well. The chapter 12 of book Fish Spermatology: Implications for Aquaculture Management) was mainly compiled by three



scientists (S. M. H. Alavi, O. Linhart and M. Rodina) of the USB RIFCH Vodňany (Department of Fish Genetics and Breeding). In addition to, the moral award main editor (S. M. H. Alavi) was rewarded with an extra scholarship of 20 000 CZK (the abstract – see p. 40).

USB RIFCH in the “Planet science” TV serial

During 2007 the Czech TV filmed a special program, targeted to river fish and crayfish (Planet Science). These programs were broadcasted in June and November (ČT 2, ČT 24).



Shooting takes