ISSN 0139–7893

CONTENTS

FOLIA ZOOLOGICA Volume 60 Number 2 June 2011

Volume 60 / Number 2 / June 2011

FOLIA ZOOLOGICA

An International Journal of Vertebrate Zoology

INSTITUTE OF VERTEBRATE BIOLOGY

Faculty of Environmental Sciences

Folia Zool. – 60 (2): 91–92 (2011)

Special issue: Proceedings of the XII. Czech Ichthyological Conference

Jan KUBEČKA*

Biology Centre of Czech Academy of Sciences, v.v.i., Institute of Hydrobiology, Na sádkách 7, 370 05 České Budějovice, Czech Republic; e-mail: kubecka@hbu.cas.cz

Ichthyological section of Czech zoological society is a relatively young community. It started its history after splitting former Czechoslovakia in 1993. Before both Czech and Slovak Ichthyologists gathered under the framework of the Slovak Zoological Society. The main activity of the new Czech section were the regular meetings – "Czech Ichthyological Conferences" held firstly every second year in Vodňany, South Bohemia, the site of a Fisheries Research Institute with a long tradition. Later the conference venue started to circulate between Vodňany, Prague and Brno and the periodicity was changed to every year and recently back to biennial. Especially when run once a two years ČIK represented a significant event in the life of the community attracting 40-50 lectures from the Czech Republic and also from neighboring countries, especially from Slovakia, Poland, Hungary and republics of former Yugoslavia.

XII Czech Ichthyological Conference took place on 19.-20. May 2010 in Vodňany. 41 lectures covering general ichthyology, fisheries management and ecology, genetics, reproduction, ontogeny, and aquaculture were presented to 80 participants. The abstracts of the conference are available from http://ichtyologie.agrobiologie.cz/data/sbornik_CIK_XII_10.pdf

Usual form of proceedings of the conference was a proceeding book in Czech or in Czech with English summaries (some of them can be downloaded from the section web page: http://ichtyologie.agrobiologie.cz/index.htm). Obviously, the circulation and impact of such proceedings was mostly limited to local audience. Low distribution of the knowledge and increased pressure on research organizations to publish the results in international journals were the main driving forces behind the decision of the steering committee of the Ichthyological Section to encourage submission of the conference papers into a special issue of Folia Zoologica. The announcement of the XII Czech Ichthyological Conference was published some three months before the date and the deadline for the submission was gradually shifted to mid-June 2010. The editorial office has received 13 manuscripts altogether. Unfortunately, many of the manuscripts did not satisfy the requirements for publishing in the international journal and could not be published in their current form. The final number of manuscripts which passed the peer review procedure successfully was five (Havelka,M. et al., Prchalová,M. et al., Kalous,L. & Knytl,M., Stejskal,V. et al., Čech,M. & Vejřík,L.) what is not sufficient for a full size special issue. Therefore, in order not to delay manuscript publishing, the editorial office had to put together other accepted manuscripts submitted in ordinary way. Obviously, there was an effort to put together topics related to ichthyology so the volume is to some extent consistent. Unfortunately only two other "ripe" fish manuscripts were ready for publication, so this volume contains also two herpetological and one mammaliological paper.

So the experiment with creation of international proceedings of the Czech Ichthyological Conference can hardly be judged as a tremendous success due to moderate interest of the authors and high mortality of the papers. The reasons may lie in relatively short notice to the authors prior to the deadline, in lower quality requirements in previous proceeding and also in the fact that truly aquaculture papers were considered as more suitable for other journals. The obvious question of the steering committee is how to proceed on. The pressure on publishing in international journals is unlikely to decrease. In fact, recent system of evaluation of research outputs in the Czech Republic gives zero grading to nonperiodical conference proceedings not reported in the international databases like WOK or Scopus. Therefore, there is a less value for the researchers to contribute to the traditional camera-ready proceedings. Czech Ichthyological Conference may quite well end-up with books-of-abstracts only. Obviously it is possible to give the ichthyologists another chance in XIII Czech Ichthyological

* *The guest editor of a special issue*

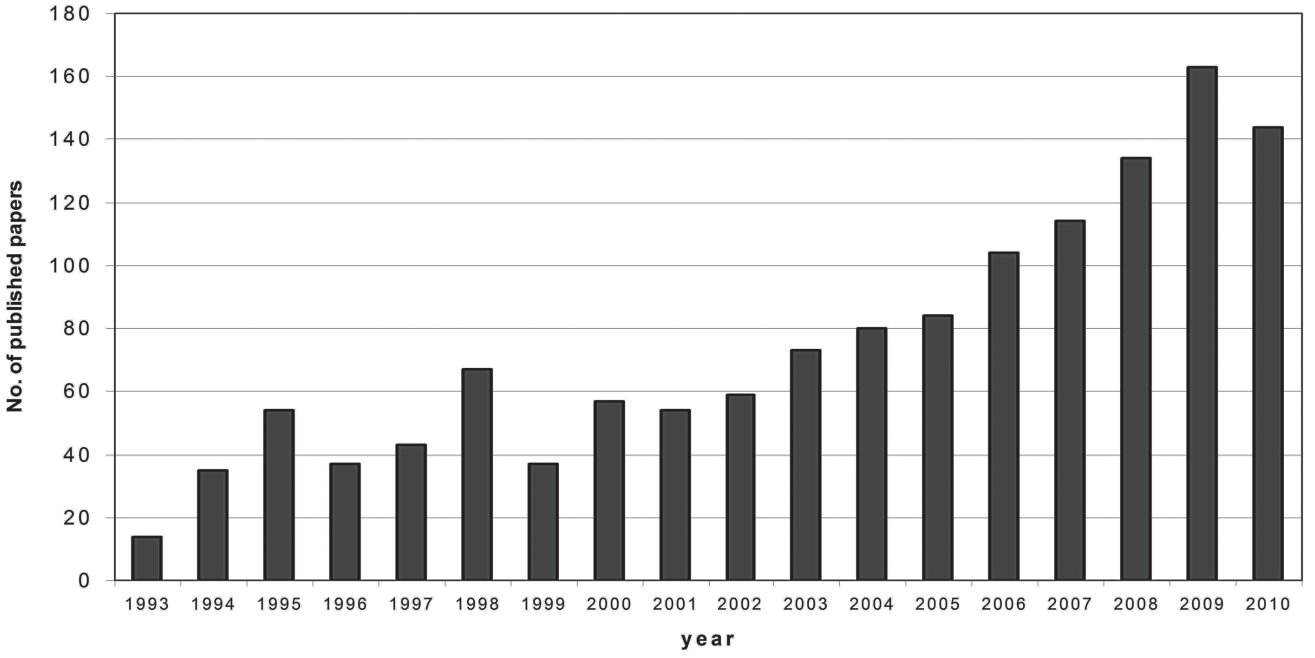

Fig. 1. *Number of papers on Thomson's Web of Knowledge using 'Czech Republic' in the address and 'fish' in the 'topic' excluding genetic method FISH (Fluorescence In Situ Hybridization).*

Conference in 2012. In that case, the steering committee should be prompt in announcing the meeting and the intention to publish meeting proceedings well in advance and in emphasizing the requirements for the quality of manuscripts and following the scope of the chosen journal. The ichthyology is the Czech Republic in fact passes over a historical boom in recent years (Fig. 1) and therefore, also with the contribution of foreign colleagues, there is certainly a potential to produce a strong journal issues with ichthyological results from Czech Ichthyological Conference.

Folia Zool. – 60 (2): 93–103 (2011)

Sturgeon genetics and cytogenetics: a review related to ploidy levels and interspecific hybridization

Miloš HAVELKA*, Vojtěch KAŠPAR, Martin HULÁK and Martin FLAJŠHANS

University of South Bohemia in České Budějovice, Faculty of Fisheries and Protection of Waters, South Bohemian Research Center of Aquaculture and Biodiversity of Hydrocenoses and Research Institute of Fish Culture and Hydrobiology, Zátiší 728/II, 389 25 Vodňany, Czech Republic; e-mail: havelm02@frov.jcu.cz

Received 6 June 2010; Accepted 11 November 2010

Abstract. Sturgeons (Chondrostei: Acipenseriformes) display markedly disjunction distributions with a wide distribution in the northern hemisphere. Their unique benthic specializations and conserved morphology, evolutionary age, the variation in their basic diadromous life history, and the large public interest due to their near extinction or critically endangered status make sturgeons and paddlefishes interesting groups for molecular and cytogenetic studies. From altogether 27 acipenseriform species, seventeen species are supposed to be critically endangered, two species are classified as endangered, four species are vulnerable and other species are near threatened or in low-risk (IUCN Red list 2010). Sturgeons are characteristic by a relatively high number of chromosomes in cell nuclei and differences in ploidy levels. Sturgeons displayed a strong tendency for inter-specific and inter-generic hybridization under altered environmental conditions as well as under conditions of artificial propagation. Almost 20 inter-specific sturgeon hybrids were described. The decrease of natural populations and tendencies leading to restocking may result in uncontrolled restocking, production of hybrid specimens (even with non-native species) and decrease of natural genetic diversity of species in their original distribution area. Identification of parental species of natural hybrids by modern methods of molecular biology is still not easy. Here, we attempt to briefly summarize the major aspects of sturgeon genetics and cytogenetics related to ploidy levels and interspecific hybridization.

Key words: *Acipenser*, polyploidy, conservation, molecular markers

Introduction

Sturgeons are of interest genetically and evolutionarily for a variety of reasons. Firstly, sturgeon fishes are supposed to have evolved about 200 million years ago during the Jurassic period (Bemis et al. 1997). Furthermore, the "living fossils" status of the group makes them important for understanding vertebrate evolution in general and the threatened or endangered status of many of these species indicates that there may be a limited time left to study these organisms. In addition, natural populations of almost all sturgeon species have been seriously affected by overexploitation in combination with a substantial loss and degradation of habitat during the 20th century. These significant changes have invariably initiated conservation efforts and stocking activities. Stocking activities (introductions or reintroductions) with fishes grown in captivity has become a common practice in many countries with the primary aim of getting an increment of angling as well as for the rehabilitation of natural populations. It has been shown that restocking programmes can result in deleterious effects on the natural fish populations that in many cases are the same as those caused by the introduction of exotic species. All this might cause a displacement of the local populations, or in extreme cases, their extinction. Additionally, the natural genetic diversity of species is drastically reduced by uncontrolled

* *Corresponding Author*

restocking, especially because of the fast decline in the number of wild individuals remaining and another dangerous practice like the production of hybrids with non-native species (Fontana et al. 2001).

Approaches employed in conservation genetics can help to prioritize populations for active management, to identify suitable donor and recipient populations for stocking (Welsh & May 2006).

Knowledge of sturgeons' phylogenetic and taxonomic relationships is still limited because of their high morphological variability and a peculiar ability to yield fully fertile hybrids in the wild between taxonomically distant species, sometimes even assigned to different genera (Berg 1962). Standard taxonomical studies and their findings based on morphological characteristics were influenced by availability of genetic and molecular data. For example, two species of the genus *Huso* (*Huso huso* and *H. dauricus*) are genetically more distant between themselves than they are from those of the genus *Acipenser* (Birstein & DeSalle 1998). As a last but not least, sturgeon genetics is important from the viewpoint of any economic issues involving these valuable fishes. These tasks include species identification by genetic tools for needs of trade and identification of caviar.

Here we attempt to briefly summarize the major aspects of sturgeon genetics and cytogenetics related to ploidy levels and interspecific hybridization. Firstly we review these topics and then discuss the present and future of pattern and process discovery in sturgeon genetics.

Sturgeon karyology and ploidy levels

It is clear that, within fishes polyploidy orders are phylogenetically diverse, suggesting that polyploidy has evolved on more than one occasion. Within taxa in which polyploidy is known to occur, it may have evolved independently on more than one occasion. The polyploidy may be a significant phenomenon in the evolution of fishes (Le Comber & Smith 2004).

Species of genera *Acipenser*, *Huso*, *Scaphirynchus* and *Polyodon* are separable into the different classes of chromosome numbers: (A) species with ~ 120 chromosomes including all taxa with between 110 and 130 chromosomes; (B) species with ~ 250 chromosomes including all taxa with between 220 and 276; and the last class (C) is represented only by *A. brevirostrum* having ~ 360 chromosomes (Ludwig et al. 2001). This species has the highest chromosome number and highest amount of DNA among all acipenseriform species. Number of chromosomes for different acipenseriform species are summarized in Table 1.

The first data on sturgeon karyology reported chromosome number 2n = 60 inferred from metaphase plates from blastomeres and cells of branchial mucosa of *H. huso* and *A. ruthenus* which were obtained in the early 1960's (Serebryakova 1972). Other data were published by Ohno et al. (1969), who constructed metaphase plates from squashes of tissue fragments of *Scaphirhynchus platorhynus*. These preparations revealed a diploid number of 112 chromosomes where 48 chromosomes were distinguished as microchromosomes. The high number of chromosomes led the authors to the hypothesis that *S. platorhynus* was tetraploid. Observation of preparations of several tissues of *Polyodon spathula* reported chromosome number 2n = 120 where 72 were microchromosomes (Dingerkus & Howell 1976). These authors arranged chromosomes in groups of fours suggesting tetraploidy of these species.

Another method of ploidy level determination is observation of nuclear organizer regions (NORs). A study of a number of stained active NORs in four different sturgeon species suggested tetraploidy of species with ~ 120 chromosomes and octaploidy of species with ~ 250 chromosomes (Birstein & Vasil'ev 1987). This was particularly discussed by Fontana (1994) who localized NORs in *A. ruthenus* on the telomeric regions of two morphologically different pairs of chromosomes. These NORs are localized on eight chromosomes arranged in two quadruplets in *A. baeri*, *A. transmontanus* and *A. naccarii*. Based on these findings he considered the species with 120 chromosomes to be diploid and species with 240-250 chromosomes to be tetraploid. Varying results concerning the ploidy level of acipenseriform species were also found among studies focused on expressed gene products. Birstein et al. (1997) observed duplicated protein loci in species with ~ 120 chromosomes which they attributed to tetraploidy. If the 120 chromosome species were to be considered tetraploid, then the ones later described with 240 chromosomes should be considered octaploid. On the other hand, a densitometric study of serum albumins performed by Kuzmin (1996) assigned diploidy to species with ~ 120 chromosomes. Other authors hypothesized that all species having ~ 120 chromosomes were tetraploids (e.g. Ohno et al. 1969, Birstein & Vasil'ev 1987, Birstein & DeSalle 1998, Flajšhans & Vajcová 2000).

The theory of genome duplication and subsequent reduction in functional ploidy is frequently applied, even in the evolution of diploid vertebrates; genome quadruplication is discussed (Spring 1997). Piferrer et al. (2009) reviewed that polyploidy has been involved in the speciation of both animals and plants

Table 1. *Chromosome numbers of different acipenseriform species.*

Species	Chromosome number	Reference
Scaphirhynchus platorynchus	112	Ohno et al. (1969)
A. nudiventris	116 ± 4	Nowruzfashkhami et al. (2006)
	118 ± 2	Sokolov & Vasil'ev (1989)
Huso huso	116 ± 4	Fontana & Colombo (1974)
	118 ± 2	Fontana et al. (1998)
A. sturio	116 ± 4	Fontana & Colombo (1974)
	121 ± 3	Tagliavini et al. (1999)
A. ruthenus	118 ± 2	Fontana et al. (1975)
	118 ± 4	Ráb (1986)
	118 ± 2	Birstein & Vasil'ev (1987)
A. stellatus	118 ± 2	Birstein & Vasil'ev (1987)
	146 ± 6	Chicca et al. (2002)
Polyodon spathula	120	Dingerkus & Howell (1976)
A. oxyrinchus	121 ± 3	Fontana et al. (2008b)
A. baerii	229 – 240	Fopp-Bayat et al. (2006)
	246 ± 8	Fontana (1994)
	246 ± 10	Fontana et al. (1997)
	249 ± 5	Vasil'ev et al. (1980)
A. naccarii	239 ± 7	Fontana & Colombo (1974)
	246 ± 8	Fontana (1994)
	248 ± 4	Fontana et al. (1999)
A. transmontanus	246 ± 10	Fontana et al. (1997)
	248 ± 8	Fontana (1994)
	256 ± 6	Wang et al. (2003)
	271 ± 2	Van Eenennaam et al. (1998)
A. mikadoi	247 ± 33	Vishnyakova et al. (2008)
	262 ± 4	Vasil'ev et al. (2009)
A. gueldenstaedtii	249 ± 2	Arefjev & Nikolaev (1991)
	250 ± 8	Birstein & Vasil'ev (1987)
	258 ± 4	Fontana et al. (1996)
A. medirostris	249 ± 8	Van Eenennaam et al. (1999a)
A. persicus	258 ± 4	Nowruzfashkhami et al. (2000)
A. fulvescens	262 ± 6	Fontana et al. (2004)
A. sinensis	264	Yu et al. (1987)
	264	Zhou et al. (2008)
Huso dauricus	268 ± 4	Vasil'ev et al. (2009)
A. brevirostrum	372	Kim et al. (2005)
	372 ± 6	Fontana et al. (2008)

(Mable 2004, Hegarty & Hiscock 2007), and seems to have arisen independently several times during the evolution of fishes with higher incidence in the more primitive groups (Legatt & Iwama 2003). In case of sturgeons, a few theories about polyploidization events were proposed. Ludwig et al. (2001) suggested four polyploidization events in the evolution of sturgeon species. Vasil'eva et al. (2009a) proposed that at least three polyploidization events occurred in acipenserid evolution. Due to functional genome reduction events, some authors (e.g. Fontana 1994, Fontana et al. 1998, Tagliavini et al. 1999, Jenneckens et al. 2000, Ludwig et al. 2001) consider all sturgeons with ~ 120 chromosomes to be modern (functional) diploids. Accordingly, species with ~ 250 chromosomes are then called modern (functional) tetraploids.

Fontana et al. (2007) supposed that the common diploid ancestor of all Acipenseriformes had a karyotype of 60 chromosomes. A genome duplication event must have occurred in this ancestor to produce the acipenserid lineage with species having ~ 120 chromosomes (Birstein et al. 1997). In the first chromosomal duplication the chromosome number probably increased from 2n = 60 to 4n = 120. This event is believed to have occurred at least 200 million years ago since no Acipenseridae species with 60 chromosomes presently survives. Hybridization among the different 120 species resulted in hybrids which, after genome duplication, became allotetraploid or allooctaploid according the ploidy theory. This process may have independently occurred

several times (Fontana et al. 2007). A high number of chromosomes in cell nuclei and high number of microchromosomes among these chromosomes can be considered the main reason of controversial opinion about ploidy levels of sturgeon species.

The amount of nuclear DNA has also been employed to study the ploidy relationship among groups. Vialli (1957) in *A. sturio* detected an amount of nuclear DNA 3.6 picograms per nucleus (pg/N) and Ohno et al. (1969) found 3.2 pg/N in *S. platyrhynchus*. The amount of nuclear DNA of *A. naccarii* (6.26 pg/N) showed to be twice higher than the amount of nuclear DNA in *H. huso* (3.6 pg/N) and *A. sturio* (3.6 pg/N; Fontana 1976). The amount of DNA higher than 13 pg/N was reported in *A. brevirostrum* by Blacklidge & Bidwell (1993) and in *A. mikadoi* by Birstein et al. (1993). In this case Birstein et al. (1993) assumed 500 chromosomes in *A. mikadoi* by the finding of a two times higher DNA content than in species with ~ 250 chromosomes. This finding was discussed many times. Zhou et al. (2009) observed DNA content of 8.0-9.1 pg/N similarly to *A. transmontanus*, which has karyotype containing 270 chromosomes. Similarly, 262 chromosomes were reported in a karyotypic study of *A. mikadoi* by Vasil'ev et al. (2009). Observation of unexpected chromosome numbers in *H. dauricus* (268 chromosomes) published in this study is also interesting. *H. dauricus* was assumed to be a species with 120 chromosomes (Birstein et al. 1993, Birstein & DeSalle 1998, Fontana et al. 2001, Ludwig et al. 2001). Ploidy level of *A. mikadoi* was also discussed by Vishnyakova et al. (2008).

Blacklidge & Bidwell (1993) observed a genome size of 13.08 pg/N in *A. brevirostrum* and suggested dodecaploidy in this species. This was partly supported by Kim et al. (2005) who found 372 chromosomes in *A. brevirostrum*, but they could not discriminate whether this species was hexaploid or dodecaploid. Hexaploidy of *A. brevirostrum* was suggested by Fontana et al. (2008a) by observation of hybridization signal with 5S rDNA probe. They detected six florescent signals in all analyzed metaphases and from previous observation of two florescent signals in 120 chromosome species, four fluorescent signals in 250 chromosome species they made the conclusion that *A. brevirostrum* is hexaploid. In agreement with Kim et al. (2005) they also found 372 chromosomes in *A. brevirostrum* karyotype.

New insight on ploidy levels in Acipenseridae was enabled by methods of molecular biology. The *in vitro* amplification of polymorphic nuclear markers, such as microsatellite loci, permits a direct although partial view of the genome (Ludwig et al. 2001). A pattern of microsatellite alleles' inheritance is more important for studies based on microsatellite genotyping. The study of Ludwig et al. (2001) reported disomic allelic patterns in species with ~ 120 chromosomes and tetrasomic allelic patterns in species with ~ 250 chromosomes. One group of species (*A. brevirostrum*, *A. fulvescens*, *A. gueldenstaedtii*, *A. medirostris*, *A. mikadoi* and *A. naccarii*) was characteristic by microsatellite patterns indicating octosomic or greater allelic band patterns at a minimum of one locus and allelic band patterns in another group of species (*A. baerii*, *A. persicus*, *A. sinensis* and *A. transmontanus*) showed evidence of possible octosomy at a minimum of one locus. Allelic band patterns indicating higher than octosomic inheritance pattern were observed in *A. mikadoi* at two loci (Ludwig et al. 2001). The tetrasomic, double disomic or even octasomic inheritance of the different microsatellite loci was described in various acipenserid species. Pyatskowit et al. (2001) examined twelve microsatellite loci in *A. fulvescens*. They observed five polymorphic loci, LS-19, LS-34 and LS-54 showed two alleles, LS-39 three alleles and LS-68 four alleles. They also suggested tetrasomic inheritance for loci LS-19, LS-34, LS-39, and LS-54. In another study, one of these loci, LS-39, showed no more than two alleles in 501 fishes from six sturgeon species with ~ 120 chromosomes (*A. stellatus*, *H. huso*, *A. nudiventris*, *A. ruthenus*, *A. oxyrinchus* and *A. sturio*) and four alleles in 265 samples from four species with ~ 250 chromosomes (*A. gueldenstaedtii*, *A. baerii*, *A. naccarii* and *A. persicus*) (Jenneckens et al. 2001).

Nine microsatellite loci were developed by Rodzen & May (2002) for *A. transmontanus*. They reported inheritance patterns with a range from disomic system to tetrasomy and octasomy, with some null alleles. Fopp-Bayat (2008) screened microsatellite loci in haploid progeny of *A. baerii*. She suggested that three studied loci segregate disomically (Afu-68, Spl 163 and Spl 168), three tetrasomically (Aox-45, Afu 19 and Afu 39) and one octasomically (Spl-104).

We can conclude that all studies based on microsatellite loci and inherritance of microsatellite alleles supported theory of di- and tetraploidy rather than tetra- and octaploidy in species with ~ 120 and ~ 250 chromosomes, respectively.

Sturgeon sex determination

Generally, fish sex determination is one of the most changeable and diverse among all vertebrates (Vasil'eva et al. 2009b). This problem is more

complicated in acipenseriform species due to high number of polyploidization events in their evolution (Vasil'ev et al. 2009). Sex in vertebrates is generally determined by environmental and genetic factors. To the best of our knowledge, no evidence about environmental effects on sex determination in sturgeons has been found yet. There is no evident view on the sturgeon sex determination system up to now. It is still very difficult to distinguish sex chromosomes in sturgeons' karyotypes because of the large number of microchromosomes (Vasil'eva et al. 2009b). Cytogenetical studies have not revealed any presence of heteromorphic chromosomes in sturgeon species (Van Eenennaam et al. 1999b). In order to reveal sturgeon sex determination in absence of the evidence of heteromorphic chromosomes, genetic approaches can be used based on artificial genome manipulation techniques such as gynogenesis (Arai 2001). This is also of great importance for aquaculture, as potentially monosexual, all-female populations should increase economical feasibility in caviar production. Gynogenesis will produce all-female progeny only in case of sex determination, where the homogamety will be carried by females (XX female/XY male). In the acipenseriform species it has been demonstrated for North American paddlefish (*P. spathula*; Mims et al. 1997). A gynogenetic, all-female population of *P. spathula* was produced by activating eggs with ultraviolet-irradiated shovelnose sturgeon (*S. platorynchus*) spermatozoa and heat shocking. On the other hand the evidence of female heterogamety (WZ female/ZZ male), where gynogenesis did not produce all-female population, was reported several times. Van Eenennaam et al. (1999b) used gynogenesis for the investigation of the sex determination system in *A. transmontanus* and they observed both sexes in 24 month old progeny. This supported the hypothesis of a female heterogametic sex determination system. The female heterogametic sex determination system was also supposed for bester (♀ *H. huso* × ♂ *A. ruthenus*) by Omoto et al. (2005). Flynn et al. (2006) used gynogenesis to study sex determination in *A. brevirostrum*. They received 35 % of males and 65 % of females and based on this observation they suggested female heterogamety in this species. Flow cytometry and microsatellite DNA analysis were used for the verification of gynogenesis induction. Microsatellite DNA analysis was also used by Fopp-Bayat (2010) to verify induction of gynogenesis in *A. baerii*. Histological analysis of gonads of all gynogenetic progeny showed 81 % of females and 19 % of males. This sex ratio provides supposition that *A. baerii* has no female homogametic sex determination system (Fopp-Bayat 2010). The problem of sturgeon sex determination was deeply reviewed by Keyvanshokooh & Gharaei (2010).

An unexpected sex ratio observed in gynogenetic progeny of *A. stellatus* Vasil'eva et al. (2009b) produced hypothesis that sex determination system in *A. stellatus* should be more complicated and connected with the balance of sex chromosomes and autosomes on the one hand or hypothesis that WW homozygotes superfemale should be lethal. Vasil'eva et al. (2009b) mentioned the possibility that *A. stellatus* has ZO female heterogametic sex determination system.

The utilization of molecular markers for sturgeon sex determination would be very profitable but there is no evidence about sex specific markers up to now. Wuertz et al. (2006) did not detect sex specific markers for *A. naccarii*, *A. baerii* and *A. ruthenus* using of RADP techniques. They also used AFLP and ISSR, but no sex specific marker was found using these techniques similarly to RAPD.

RAPD technique used by McCormick et al. (2008) also failed to find sex specific markers in *A. fulvescens* and Keyvanshokooh et al. (2007) obtained similar results by screening the genome of *H. huso*. Wuertz et al. (2006) supposed that gene expression profiling could be used as an alternative method to failed DNA-based techniques. This was partly doubted by Keyvanshokooh et al. (2009). They analyzed protein expression in gonads of mature males and females of *A. persicus*, but they did not find differences in proteins directly linked to the sex determining genes.

Problems of sturgeon diversity inferred from hybridization events

Sturgeon hybrids are being increasingly utilized in aquaculture projects and sport fishing, therefore the ability to detect the accidental introduction of hybrids in the wild becomes extremely valuable. In general, natural interspecific hybridization happens more frequently between closely related fish species. Interspecific hybridization between taxonomically distant verterbrate species differing in their chromosome numbers is a rare event due to genetic incompatibility of parental genomes (Arnold 1997). Even if such interspecific hybrids survive, they are usually sterile because their chromosomes cannot correctly pair during the zygotene stage of prophase I and such impairment interferes with gonadal development and gametogenesis (Piferrer et al. 2009). Due to the unusual genetic structure of acipenserids (all are polyploid); they hybridize more easily than other

vertebrates (Birstein et al. 1997) and this concerns species with the same and/or differing ploidy level. The latter do hybridize both in nature (e.g. Birstein et al. 1997; Ludwig et al. 2002, 2009) and in captivity (Nikolyukin 1964, Arefjev 1997, Flajšhans & Vajcová 2000 and others). Morphological description is not enough to prove that a particular individual is hybrid but only a genetic study can provide necessary proof of sturgeon hybridization. Research efforts on nuclear markers should increase the possibility of hybrid detection and consequently the control of admixture at inter-specific but also at intra-specific level. Interspecific hybridization has a negative effect on outbreeding level. Hybrids are often characterized by greater growth performance which leads to replacing native species and often causing their extinction (Ludwig et al. 2009). There was also demonstrated that hybridization is the most rapidly acting genetic threat to endangered populations, with extinction often occurring in less than five generations (Wolf et al. 2001). Hybrids sometimes resulted from intended release programs (Becker et al. 2007), and in other cases from habitat alterations (Freyhof et al. 2005) or from unintended escape of hatchery fish (Birstein & DeSalle 1998). Most of the time backcross with native species resulted in a genetic cleaning of nuclear genotypes so that evidence for ancestral hybridization is often only detectable in mtDNA (Birstein et al. 2005).

Species determination and identification of hybrid specimens based on single locus nuclear markers, suitable for different species is very difficult because of the complexity of sturgeon genome and because of different ploidy conditions. Furthermore a single locus marker would be useless in case of second generation hybrids or backcross hybridization. Multilocus nuclear markers could solve the problem (Fontana et al. 2001). There is a need to establish suitable methods for identification at species level of caviar, for population identification, for determination of source of products from sturgeons and for identification of age of caviar according to Ludwig (2008). Utilization of mtDNA – cytochrome *b* sequences was recommended for species identification Ludwig (2008). Correct species identification based on mtDNA is limited due maternal inheritance of mitochondrial DNA and identification of interspecific hybrids is very limited. This method also showed limitation for differentiation of closely related acipenseriform species complex (e.g. *A. gueldenstaedti*, *A. baerii*, *A. persicus*, *A. naccarii*) due to overlapping mtDNA profiles (Birstein et al. 2000). But mitochondrial DNA can be used for species identification as mentioned above. For example, the mtDNA control region has been employed to distinguish the three species of genus *Scaphirhynchus* (*S. albus*, *S. platorhynchus* and *S. suttkusi*) where the first two species are sympatric (Campton et al. 2000). *S. suttkusi* is easy to identify, while the distinction between the two sympatric species is rather difficult because of interferences due to interspecific hybridization. As previously mentioned, mtDNA does not allow recognition of inter-specific hybrids and this problem is particularly relevant to sturgeons, among which hybridization is a common event (Campton et al. 2000). Some species of sturgeon are preferred in aquaculture because they are easy to handle, fast growing and reproduce quickly under hatchery conditions. The escape of farmed sturgeons is often reported especially during flooding events (Maury-Brachet et al. 2008), which have become increasingly frequent in Europe during a last few decades. A clear example of this is reported by Jenneckens et al. (2000) on contamination of *A. gueldenstaedtii* with *A. baerii*. The analysis of mitochondrial cytochrome *b* gene performed on 34 sturgeons captured in the River Volga and morphologically classified as *A. gueldenstaedtii* showed that eleven of them actually had *A. baerii* haplotype. The possibility of molecular identification of sturgeon hybrids, which is often very difficult distinguish by morphological approach, could be a powerful tool for both conservation biology and quality certification of commercial products (Congiu et al. 2001). Ludwig et al. (2009) described a natural spawning of non-native species *A. baerii* in the River Danube basin and hybridization with native *A. ruthenus* by analysis of mtDNA control region and nine microsatellite loci. This hybridization possesses a serious threat for the survival of this native isolated population of *A. ruthenus* in the Danube drainage. The natural hybridization was also demonstrated by combining nuclear (microsatellites) and mitochondrial (cytochrome *b*) markers (Jenneckens et al. 2000, Ludwig et al. 2001), between *A. gueldenstaedti* and *A. stellatus*, *A. gueldenstaedti* and *A. ruthenus* hybrid, as well as five hybrids between *A. gueldenstaedti* or *A. persicus* and *A. nudiventris*. Using the cytochrome *b* sequences, in all these cases Ludwig et al. (2002) identified *A. gueldenstaedti* or *A. persicus* as the maternal species. In all specimens they observed triploid band patterns at several microsatellite loci. The *A. gueldenstaedti*/*A. stellatus* and the *A. gueldenstaedti*/*A. ruthenus* hybrids came from the River Volga and the *A. nudiventris*-hybrids were caught in the Iranian waters of the Caspian Sea near the mouth of the River Safid Rud (Ludwig et al. 2002). Tranah et al. (2004) used microsatellites to observe hybridization between *S. albus* and *S. platorhynchus* in lower part of

the River Mississippi. Using nine microsatellites loci they found evidence of hybridization between these sturgeon species. Congiu et al. (2001) used amplified fragment length polymorphism (AFLP) to separate hybrids (*A. naccarii* × *A. transmontanus*) from their parental species. Ludwig (2008) discussed about AFLP as very useful method for backcross screening (crosses between hybrid and one of its parental species).

Identification of pure species by means of suitable molecular markers is a necessary prerequisite to distinguish hybrids and this concerns also the identification of sturgeon products, either for conservation or forensic purposes. Species specific PCR, which is based on the presence of diagnostic nucleotide differences in mtDNA sequences, was recommended as a very easy, inexpensive and fast method for the identification of two very important caviar producing species *A. stellatus* and *H. huso* (Ludwig 2008). DeSalle & Birstein (1996) proposed the sequencing of parts of cytochrome *b*, 12S and 16S rDNA genes and designing specific primers for diagnostic substitution as a method to identify caviar of three species. Jenneckens et al. (2001) observed one species specific allele on microsatellite locus LS 39 for *A. stellatus*. He recommended microsatellite locus LS 39 to identify caviar origin due to fixed allele of 111 bp, which was absent in the other species. With big probability it is the first genomic marker described for sturgeon with the potential to identify one of the main caviar-producing species and its hybrids.

Many molecular studies have incorporated microsatellite loci in examining genetic variability within and among species. May et al. (1997) demonstrated that primers designed from *A. fulvescens* subgenomic library amplified microsatellites at eight of eleven loci examined in other *Acipenser* species, as well as in *Scaphirhynchus* sturgeon. Furthermore six of the nine loci that amplified in *Scaphirhynchus* species were polymorphic, and *Acipenser* polymorphism ranged from 33 % to 80 %. The dynamics of sturgeon genome appear to mandate using a rapidly evolving marker in lieu of those once used in traditional molecular techniques. Furthermore, the conservation of microsatellite flanking regions among related taxa suggests that the development of microsatellite loci for one species would prove a useful family-wide scale (May et al. 1997). McQuown et al. (2000) developed a large set of microsatellite loci by sequencing of three subgenomic of *S. platorynchus*. Authors designed primers for 113 of sequences and tested against *S. platorynchus*, *S. albus*, *A. transmontanus*, *A. fulvescens* and *A. medirostris*. They observed that 96 % from 113 primer sets amplified on one or more species. Similarly King et al. (2001) described six microsatellite loci isolated from *A. oxyrinchus* and this six microsatellite loci they tested for cross amplification in ten acipenseriform species. Four of six microsatellite primer sets developed for *A. oxyrinchus* produced DNA fragments in all ten of the additional sturgeon taxa (King et al. 2001).

Conclusion

In this review we summarized the main problems on sturgeon polyploidy and interspecific hybridization. A study of sturgeon genetics can show us to what extent the polyploidization events played an important role during the evolution of vertebrates and new results indicate that these events still continue among different sturgeon species. There is still no clear view on the status of sturgeon polyploidy (e.g. paleotetraploidy vs. modern diploidy) even inside the scientific community dealing with this question till now. Polyploidization is closely connected with frequent interspecific hybridization events. At least twelve different types of interspecific hybrids and five intergeneric ones have been described, some of which even fertile, such as the bester (Birstein et al. 1997). Identification of parental species is not easy in natural hybrids. Research efforts on nuclear markers should increase the possibility of detection of hybrids and consequently the control of admixture at interspecific but also intraspecific level. It is evident that this topic is still very complicated and partly unsolved and that it will require further studies in the future.

Acknowledgement

Authors are grateful to CENAKVACZ.1.05/2.1.00/01.0024, research plan of USB RIFCH no. MSM6007665809, project of GAJU no. 046/2010/Z, project of GACR no. 523/08/0824 and project of NAZV no. QH92308.

Literature

Arai K. 2001: Genetic improvement of aquaculture finfish species by chromosome manipulation techniques in Japan. *Aquaculture 197: 205–228.*

Arefjev V.A. 1997: Sturgeon hybrids: natural reality and practical prospects. *Aquaculture Magazine 23: 53–58.*

Arefjev V.A. & Nikolaev A.I. 1991: Cytological analysis of the reciprocal hybrids between low- and high-chromosome acipenserids, the great sturgeon, *Huso huso* (L.), and the Russian sturgeon, *Acipenser*

gueldenstaedti Brandt. *Cytologia 56: 495–502.*
Arnold M.L. 1997: Natural hybridization and evolution. *Oxford University Press, New York.*
Becker L.A., Pascal M.A. & Basso N.G. 2007: Colonization of the Southern Patagonia Ocean by exotic chinook salmon. *Conserv. Biol. 21: 1347–1352.*
Bemis W.E., Findeis E.K. & Grande L. 1997: An overview of Acipenseriformes. *Env. Biol. Fish. 48: 25–71.*
Berg L.S. 1962: Freshwater fishes of the USSR and adjacent countries. *Oldbourne Press, London 1: 52–111. (Translated by Israel Program for Scientific Translation, Jerusalem)*
Birstein V.J. & DeSalle R. 1998: Molecular phylogeny of Acipenserinae. *Mol. Phylog. Evol. 9: 141–155.*
Birstein V.J. & Vasil'ev V.P. 1987: Tetraploid-octoploid relationships and karyological evolution in the order Acipenseriformes (Pisces): karyotypes, nucleoli, and nucleolus-organizer regions in four acipenserid species. *Genetica 73: 3–12.*
Birstein V.J., Doukakis P. & DeSalle R. 2000: Polyphyly of mtDNA lineages in the Russian sturgeon, *Acipenser gueldenstaedtii*: forensic and evolutionary implications. *Conserv. Genet. 1: 81–88.*
Birstein V.J., Hanner R. & DeSalle R. 1997: Phylogeny of the Acipenseriformes: cytogenetic and molecular approaches. *Env. Biol. Fish. 48: 127–155.*
Birstein V.J., Poletaev A.I. & Goncharov B.F. 1993: The DNA content in Eurasian sturgeon species determined by flow cytometry. *Cytometry 14: 337–383.*
Birstein V.J., Ruban G., Ludwig A., Doukakis P. & DeSalle R. 2005: The enigmatic Caspian Sea Russian sturgeon: how many cryptic forms does it contain? *System. Biodivers. 3: 203–218.*
Blacklidge K.H. & Bidwell C.A. 1993: Three ploidy levels indicated by genome quantification in Acipenseriformes of North America. *J. Hered. 84: 427–430.*
Campton D.E., Bass A.L., Chapman F.A. & Bowen B.W. 2000: Genetic distinction of pallid, shovelnose, and Alabama sturgeon: emerging species and the US endangered species act. *Conserv. Genet. 1: 17–32.*
Chicca M., Suciu R., Ene C., Lanfredi M., Congiu L., Leis M., Tagliavini J., Rossi R. & Fontana F. 2002: Karyotype characterization of the stellate sturgeon, *Acipenser stellatus*, by chromosome banding and fluorescent *in situ* hybridization. *J. Appl. Ichthyol. 18: 298–300.*
Congiu L., Dupanloup I., Patarnello T., Fontana F., Rossi R., Arlatis G. & Zane L. 2001: Identification of interspecific hybrids by amplified fragment length polymorphism: the case of sturgeon. *Mol. Ecol. 10: 2355–2359.*
DeSalle R. & Birstein V.J. 1996: PCR identification of black caviar. *Nature 381: 197–198.*
Dingerkus G. & Howell W.M. 1976: Karyotypic analysis and evidence of tetraploidy in the North American paddlefish, *Polyodon spathula*. *Science 194: 842–844.*
Flajšhans M. & Vajcová V. 2000: Odd ploidy levels in sturgeon suggest a backcross of interspecific hexaploid sturgeon hybrids to evolutionary tetraploid and/or octaploid parental species. *Folia Zool. 49: 133–138.*
Flynn S.R., Matsuoka M., Reith M., Martin-Robichaud D.J. & Benfey T.J. 2006: Gynogenesis and sex determination in shortnose sturgeon, *Acipenser brevirostrum* Lesuere. *Aquaculture 253: 721–727.*
Fontana F. 1976: Nuclear DNA content and cytometric of erythrocytes of *Huso huso* L., *Acipenser sturio* L. and *Acipenser naccarii* Bonaparte. *Caryologia 29: 127–138.*
Fontana F. 1994: Chromosomal nucleolar organizer regions in four sturgeon species as markers of karyotype evolution in Acipenseriformes (Pisces). *Genome 37: 888–892.*
Fontana F. & Colombo G. 1974: The chromosomes of Italian sturgeons. *Experientia 30: 739–742.*
Fontana F., Bruch R.M., Binkowski F.P., Lanfredi M., Chicca M., Beltrami N. & Congiu L. 2004: Karyotype characterization of the lake sturgeon, *Acipenser fulvescens* (Rafinesque, 1817) by chromosome banding and fluorescent *in situ* hybridization. *Genome 47: 742–746.*
Fontana F., Congiu L., Mudrak V.A., Quattro J.M., Smith T.I.J., Ware K. & Doroshov S.I. 2008a: Evidence of hexaploid karyotype in shortnose sturgeon. *Genome 51: 113–119.*
Fontana F., Jankovic D. & Zivkovic S. 1975: Somatic chromosome of *Acipenser ruthenus* L. *Arch. biol. nauka, Beograd 27: 33–35.*
Fontana F., Lanfredi M., Chicca M., Congiu L., Tagliavini J. & Rossi R. 1999: Fluorescent *in situ* hybridization with rDNA probes on chromosomes of *Acipenser ruthenus* and *Acipenser naccarii* (Osteichthyes Acipenseriformes). *Genome 42: 1008–1012.*
Fontana F., Lanfredi M., Kirschbaum F., Garrido-Ramos M.A., Robles F., Forlani A. & Congiu L. 2008b: Comparison of karyotypes of *Acipenser oxyrinchus* and *A. sturio* by chromosome banding and fluorescent

in situ hybridisation. *Genetica 132: 281–286.*
Fontana F., Lanfredi M., Rossi R., Bronzi P. & Arlati G. 1996: Karyotypic characterization of *Acipenser gueldenstaedti* with C-, $AgNO_3$ and fluorescence banding techniques. *Ital. J. Zool. 63: 113–118.*
Fontana F., Rossi R., Lanfredi M., Arlati G. & Bronzi P. 1997: Cytogenetic characterization of cell lines from three sturgeon species. *Caryologia 50: 91–95.*
Fontana F., Tagliavini J. & Congiu C. 2001: Sturgeon genetics and cytogenetics: recent advancements and perspectives. *Genetica 111: 359–373.*
Fontana F., Tagliavini J., Congiu L., Lanfredi M., Chicca M., Laurenti C. & Rossi R. 1998: Karyotypic characterization of the great sturgeon, *Huso huso*, by multiple staining techniques and fluorescent *in situ* hybridization. *Mar. Biol. 132: 495–501.*
Fontana F., Zane L., Pepe A. & Congiu L. 2007: Polyploidy in Acipenseriformes: cytogenetic and molecular approaches. In: Pisano E., Ozof-Costaz C., Foresti F. & Kapoor B.G. (eds.), Fish cytogenetics. *Science Publisher, Enfield, Inc. New Hampshire, USA: 385–403.*
Fopp-Bayat D. 2008: Inheritance of microsatellite loci in polyploid Siberian sturgeon (*Acipenser baerii* Brandt) based on uniparental haploids. *Aquaculture Research 39: 1787–1792.*
Fopp-Bayat D. 2010: Meiotic gynogenesis revealed not homogametic female sex determination system in Siberian sturgeon (*Acipenser baeri* Brandt). *Aquaculture 305: 174–177.*
Fopp-Bayat D., Jankun M. & Woznicki P. 2006: Chromosome number and erythrocyte nuclei length in triploid Siberian sturgeon *Acipenser baeri* Brandt. *Caryologia 59: 319–321.*
Freyhof J., Lieckfeldt D., Pitra C. & Ludwig A. 2005: Molecules and morphology: evidence for introgression of mitochondrial DNA in Dalmatian cyprinids. *Mol. Phylogenet. Evol. 37: 347–354.*
Hegarty M. & Hiscock S. 2007: Polyploidy: doubling up for evolutionary success. *Curr. Biol. 17: R927–R929.*
IUCN 2010: IUCN Red list of threatened species. Version 2010.2. *Downloaded on 11 August 2010. www.iucnredlist.org*
Jenneckens I., Meyer J.N., Debus L., Pitra C. & Ludwig A. 2000: Evidence of mitochondrial DNA clones of Siberian sturgeon, *Acipenser baeri*, within Russian sturgeon, *Acipenser gueldenstaedtii*, caught in the River Volga. *Ecol. Lett. 3: 503–508.*
Jenneckens I., Meyer J.N., Horstgen-Schwark G., May B., Debus L., Wedekind H. & Ludwig A. 2001: A fixed allele at microsatellite locus LS-39 exhibiting species-specific for black caviar producer *Acipenser stellatus*. *J. Appl. Ichtyol. 17: 39–42.*
Keyvanshokooh S. & Gharaei A. 2010: A review of sex determination and searches for sex-specific markers in sturgeon. *Aquaculture Research 41: 1–7.*
Keyvanshokooh S., Kalbassi M.R., Hosseinkhani S. & Vaziri B. 2009: Comparative proteomics analysis of male and female Persian sturgeon (*Acipenser persicus*) gonads. *Anim. Reprod. Sci. 111: 361–368.*
Keyvanshokooh S., Pourkazemi M. & Kalbassi M.R. 2007: The RAPD technique failed to identify sex-specific sequences in beluga (*Huso huso*). *J. Appl. Ichthyol. 23: 1–2.*
Kim D.S., Nam Y.K., Noh J.K., Park C.H. & Chapman F.A. 2005: Karyotype of North American shortnose sturgeon *Acipenser brevirostrum* with the highest chromosome number in the Acipenseriformes. *Ichthyol. Res. 52: 94–97.*
King T.L. Lubinski B.A. & Spidle A.P. 2001: Microsatellite DNA variation in Atlantic sturgeon (*Acipenser oxyrinchus oxyrinchus*) and cross-species amplification in the Acipenseridae. *Conserv. Genet. 2: 103–119.*
Kuzmin E.V. 1996: Blood serum albumin system in Acipenseriformes during river period of life. *Vopr. Ikhytiol. 36: 101–108.*
Le Comber S. & Smith S. 2004: Polyploidy in fishes: patterns and processes. *Biol. J. Linn. Soc. 82: 431–442.*
Legatt R.A. & Iwama G.K. 2003. Occurrence of polyploidy in the fishes. *Rev. Fish Biol. Fish. 13: 237–246.*
Ludwig A. 2008: Identification of Acipenseriformes species in trade. *J. Appl. Ichtiol. 24: 2–11.*
Ludwig A., Belfiore N.M., Pitra C., Svirsky V. & Jenneckens I. 2001: Genome duplication events and functional reduction of ploidy levels in sturgeon (*Acipenser*, *Huso* and *Scaphirhynchus*). *Genetics 158: 1203–1215.*
Ludwig A., Debus L. & Jenneckens I. 2002: A molecular approach for trading control of black caviar. *Int. Rev. Hydrobiology 87: 661–674.*
Ludwig A., Lippold S., Debus L. & Reinartz R. 2009: First evidence of hybridization between endangered starlets (*Acipenser ruthenus*) and exotic Siberian sturgeons (*Acipenser baerii*) in the Danube River. *Biol.*

Invasions 11: 753–760.
Mable B.K. 2004: Why polyploidy is rarer in animals than in plants: myths and mechanisms. *Biol. J. Linn. Soc. 82: 453–466.*
Maury-Brachet R., Rochard E., Durrieu G. & Boudou A. 2008: The 'Storm of the Century' (December 1999) and the accidental escape of Siberian Sturgeons (*Acipenser baerii*) into the Gironde Estuary (Southwest France) – an original approach for metal contamination. *Environ. Sci. Pollut. Res. 15: 89–94.*
May B., Krueger C.C. & Kincaid H.L. 1997: Genetic variation at microsatellite loci in sturgeon: primer sequence homology in *Acipenser* and *Scaphirhynchus*. *Can. J. Fish. Aquat. Sci. 54: 1542–1547.*
McCormick C.R., Bos D.H. & DeWoody J.A. 2008: Multiple molecular approaches yield no evidence for sex determining genes in lake sturgeon (*Acipenser fulvescens*). *J. Appl. Ichtyol. 24: 643–645.*
McQuown E.C., Sloss B.L., Sheehan R.J., Rodzen J., Tranah G.J. & May B. 2000: Microsatellite analysis of genetic variation in sturgeon (Acipenseridae): new primer sequences for *Scaphirhynchus* and *Acipenser*. *Trans. Am. Fish. Soc. 129: 1380–1388.*
Mims S., Shelton W., Linhart O. & Wang C. 1997: Induced meiotic gynogynesis of paddlefish, *Polyodon spathula*. *J. World Aquacult. Soc. 28: 334–343.*
Nikolyukin N.I. 1964: Some observations on the histological structure of the gonads of sturgeon hybrids. *Trudy VNIRO 55: 145–157. (in Russian)*
Nowruzfashkhami M.R., Pourkazemi M. & Baradarannoveiri S. 2000: Chromosome study of Persian sturgeon *Acipenser persicus* B. *Cytologia 65: 197–202.*
Nowruzfashkhami M.R., Safaiian S., Bahmani M. & Chubian F. 2006: Karyotype analysis in ship sturgeon *Acipenser nudiventris* in the south Caspian Sea using leukocyte culture. *J. Appl. Ichthyol. 22 (Suppl. 1): 97–98.*
Ohno S., Muramoto J., Stenius C., Christian L. & Kitterell W.A. 1969: Microchromosomes in holocephalian, chondrostean and holostean fishes. *Chromosoma 226: 35–40.*
Omoto N., Maebayashi M., Adachi S., Arai K. & Yamauchi K. 2005: Sex ratios of triploids and gynogenetic diploids induced in the hybrid sturgeon, the bester (*Huso huso* female × *Acipenser ruthenus* male). *Aquaculture 245: 39–47.*
Piferrer F., Beaumont A., Falguiere J.C., Flajšhans M., Haffray P. & Kolombo L. 2009: Polyploid fish and shellfish: production, biology and applications to aquaculture for performance improvement and genetic containment. *Aquaculture 239: 125–156.*
Pyatskowit J.D., Krueger C.C., Kincaid H.L. & May B. 2001: Inheritance of microsatellite loci in the polyploid lake sturgeon (*Acipenser fulvescens*). *Genome 44: 185–191.*
Ráb P. 1986: A note on the karyotype on the sterlet, *Acipenser ruthenus* (Pishes, Acipenseridae). *Folia Zool. 35: 73–78.*
Rodzen J.A. & May B. 2002: Inheritance of microsatellite loci in the white sturgeon (*Acipenser transmontanus*). *Genome 45: 1064–1076.*
Serebryakova E.V. 1972: Some data on the chromosome complexes in Acipenseridae. In: Cherfas B.I. (ed.), Genetics, selection, and hybridization of fish. *Keter'Press Binding, Wiener Bindery Ltd., Jerusalem: 98–106. (Translated from Russian by Israel Program for Scientific Translations)*
Sokolov L.I. & Vasiľev V. 1989: *Acipenser nudiventris* Lovetsky, 1928. In: Holčík J. (ed.), The freshwater fishes of Europe. Vol 1/II General introduction to fishes Acipenseriformes. *Wiesbaden: 206–226.*
Spring J. 1997: Vertebrate evolution by interspecific hybridisation – are we polyploid? *FEBS Letters 400: 2–8.*
Tagliavini J., Williot P., Congiu L., Chicca M., Lanfredi M., Rossi R. & Fontana F. 1999: Molecular cytogenetic analysis of the karyotype of the European Atlantic sturgeon, *Acipenser sturio*. *Heredity 83: 520–525.*
Tranah G., Campton D.E. & May B. 2004: Genetic evidence for hybridization of pallid and shovelnose sturgeon. *J. Hered. 95: 474–480.*
Van Eenennaam A.L., Murray J.D. & Medrano J.F. 1998: Mitotic analysis of the North American white sturgeon, *Acipenser transmontanus* Richardson (Pisces, Acipenseridae), a fish with a very high chromosome number. *Genome 41: 266–271.*
Van Eenennaam A.L., Murray J.D. & Medrano J.F. 1999a: Karyotype of the American green sturgeon. *Transactions of the American Fisheries Society 128: 175–177.*
Van Eenennaam A.L., Van Eenennaam J.P., Medrano J.F. & Doroshov S.I. 1999b: Evidence of female heterogametic genetic sex determination in white sturgeon. *J. Hered. 90: 231–233.*

Vasil'eva E.D., Grunina A.S., Recoubratsky A.V., Barmintsev V.A., Barmintseva A.E., Volkov A.A., Badrtdinov O.A., Kovalev K.V., Chebanov M.S. & Vasil'ev V.P. 2009b: Genetic mechanisms of sex determination of sturgeon. Problems and perspectives. *6th international symposium on sturgeon. October 25-31, Wuhan, Hubei Province, China. Abstracts Oral Presentations: 68–70.*

Vasil'eva E.D., Shedko S.V., Novomodny G.V. & Vasil'ev V.P. 2009a: How many polyploidization events took place in acipenserid evolution? New evidence from karyological study of the sakhalin sturgeon *Acipenser mikadoi* and kaluga *A. dauricus*. *6th international symposium on sturgeon. October 25-31, Wuhan, Hubei Province, China. Abstracts Oral Presentations: 68–70.*

Vasil'ev V.P., Sokolov L.I. & Serebryakova E.V. 1980: Karyotype of the Siberian sturgeon *Acipenser baeri* Brandt from the Lena River and some questions of the acipenserid karyotypic evolution. *Vopr. Ikhtiol. 23: 814–822.*

Vasil'ev V.P., Vasil'eva E.D., Shedko S.V. & Novomodny G.V. 2009: Ploidy levels in the kaluga *Huso dauricus* and sakhalin sturgeon *Acipenser mikadoi* (Acipenseridae, Pisces). *Doklady Biological Sciences 426: 228–231.*

Vialli M. 1957: Volume and content of DNA in nucleus. *Exptl. Cell. Res. (Suppl. 4): 284–293*.

Vishnyakova K.S., Mugue N.S., Zelenina D.A., Mikodina E.V., Kovaleva O.A., Madan G.V. & Yegorov Y.E. 2008: Cell culture and karyotype of sakhalin sturgeon *Acipenser mikadoi*. *Biologicheskie membrany 25: 420–433.*

Wang G., Lapatra S., Zeng L., Zhao Z. & Lu Y. 2003: Establishment, growth, cryopreservation and species of origin identification of three cell lines from white sturgeon, *Acipenser transmontanus*. *Meth. Cell. Sci. 25: 211–220.*

Welsh A. & May B. 2006: Development and standardization of disomic microsatellite markers for lake sturgeon genetic studies. *J. Appl. Ichthyol. 22: 337–344.*

Wolf D.E., Takebeayashi N. & Rieseberg L.H. 2001: Predicting the risk of extinction through hybridization. *Conserv. Biol. 15: 1039–1053.*

Wuertz S., Gaillard S., Barbisan F., Carle S., Congiu L., Forlani A., Aubert J., Kirschbaum F., Tosi E., Zane L. & Grillasca J.P. 2006: Extensive screening of sturgeon genomes by techniques revealed no sex-specicc random screening marker. *Aquaculture 258: 685–688.*

Yu X., Zhou T., Li K., Li Y. & Zhou M. 1987: On the karyosystematics of cyprinid fishes and a summary of fish chromosome studies in China. *Genetica 72: 225–236.*

Zhou H., Fujimoto T., Adachi S., Yamaha E. & Arai K. 2009: Genome size variation estimated by nuclear DNA content flow cytometry in ten sturgeon species and several interspecific hybrids reared in Japan. *6th international symposium on sturgeon. October 25-31, Wuhan, Hubei Province, China. Abstracts Oral Presentations: 61–62.*

Zhou G.Z., Gui L., Li Z.Q., Yuan X.P. & Zhang Q.Y. 2008: Establishment of a Chinese sturgeon *Acipenser sinensis* tail-fin cell line and its susceptibility to frog iridovirus. *J. Fish Biol. 73: 2058–2067.*

Folia Zool. – 60 (2): 104–114 (2011)

Fish occurrence in the fishpass on the lowland section of the River Elbe, Czech Republic, with respect to water temperature, water flow and fish size

Marie PRCHALOVÁ[1,2], Pavel HORKÝ[2], Ondřej SLAVÍK[2], Lukáš VETEŠNÍK[3] and Karel HALAČKA[3]

[1] *Biology Centre of the Academy of Sciences of the Czech Republic, v.v.i., Institute of Hydrobiology, Na Sádkách 7, 370 05 České Budějovice, Czech Republic; marie.prchalova@hbu.cas.cz*
[2] *T G M Water Research Institute, v.v.i, Podbabská 30, 160 62 Prague, Czech Republic; Pavel_Horky@vuv.cz, Ondrej_Slavik@vuv.cz*
[3] *Institute of Vertebrate Biology, Academy of Sciences of the Czech Republic, v.v.i., Květná 8, 603 65 Brno, Czech Republic; vetesnik@ivb.cz, halacka@brno.cas.cz*

Received 7 June 2010; Accepted 4 April 2011

Abstract. The effect of water temperature and flow on the migration of fish was observed using weekly inspections of a fishpass on the lowland section of the River Elbe (Střekov, Czech Republic) from spring to fall 2003 and 2004. The effect was examined separately for immature (up to 2 years old) and adult fish and also the most abundant species (roach *Rutilus rutilus*, bleak *Alburnus alburnus*, chub *Squalius cephalus*, gudgeon *Gobio gobio*). More than 13 thousand fish from 23 species were recorded in the fishpass during both years. The highest levels of fish occurrence in the fishpass were observed during the spring spawning migrations of adults (April-May) as well as during the late summer and fall migrations of adult and immature fish (September-November). While the total number of both fish age categories was significantly related to the interaction of water temperature and flow, however, responses of individual species and age categories differed from each other. The numbers of adult bleak, chub and gudgeon increased with higher temperature. The maximum numbers of adult bleak migrated at medium values of temperature (15-20 °C) and flow (140-270 m^3 s^{-1}). The abundances of adult chub and adult plus immature gudgeon were higher with higher flow. The numbers of immature bleak and chub decreased with increasing flow. The numbers of adult and immature roach were influenced only by water flow with maximal numbers migrating under medium values of flow. Generally, we observed that immature fish and small- and middle-sized species required lower values of water flow than adult fish or large species to facilitate their movement. The exception was gudgeon, which required higher values of flow for its migration, a feature that could be related to its bottom dwelling nature or rheophily.

Key words: cyprinids, migration, general linear mixed models, adult fish, immature fish, discharge

Introduction

Considering all possible seasonal movements of fish in rivers, upstream spawning migrations are believed to be the most obvious (Lucas & Baras 2001). Feeding and refuge seeking represent other causes of intensive fish movements (Prignon et al. 1998, Travade et al. 1998). Besides internal mechanisms, fish migrations are controlled by a number of environmental factors, from which water temperature, water flow and photoperiod are listed as the most important (Northcote 1998).

We studied fish migration using catches in a fishpass that is located in the lowland section of the River Elbe, Central Europe. Due to such location, we expected mostly cyprinid species (family Cyprinidae) to occur in our samples. Recently, more attention has been focused especially on the spawning migrations of adult cyprinids in Europe (e.g. Geeraerts et al.

2007, Rakowitz et al. 2008, Slavík et al. 2009), but our knowledge about the effects of water temperature and flow on movements of these fish is still limited and occasionally even inconsistent. The number of cyprinids migrating to spawning areas increases with water temperature. A water temperature threshold that plays a role in initiating spring migrations of cyprinids has been described (6-13 °C, see review provided by Lucas & Baras 2001). Once this threshold is exceeded, further rises in temperature are not significant for the course of spawning migrations (Lucas 2000). Site-specific low and even extremely high water flow may disable the spawning migrations of cyprinids (Santos et al. 2002, Slavík et al. 2009). Besides these extreme values, water flow has not been found to directly influence spawning migrations of cyprinids (Lucas & Batley 1996, Slavík & Bartoš 2004). These conclusions are based mainly on observation of adult fish performing spring spawning migrations. But is there a general effect of water temperature and flow on fish migration, and is this effect the same for different sizes of fish?

The aim of this study was to describe a relationship between fish occurrence in the fishpass and the factors water temperature and flow. We expected to confirm a general temperature threshold is required for the onset of the migration in the spring. Above that threshold the number of fish was hypothesized to be defined by water flow. Furthermore, we separated the observed number of fish into two age categories, adult and immature fish, and we assumed that the effect of water temperature and flow observed would differ between them. Due to the lower kinetic energy of smaller fish, they require lower values of water flow to facilitate their movement (Slavík et al. 2009). For the same previously described reasons, we expected that the effect of water temperature and flow would vary between species. We therefore analyzed the four most abundant species occurring in the fishpass in addition to the total number of migrating fish.

Material and Methods

Study area

The pool fishpass is situated 321 km downstream from the River Elbe spring, Czech Republic (total catchment area 148268 km^2 in Germany, Czech Republic, Austria and Poland, Fig. 1). The fishpass is part of a lock (50°38′ N, 14°02′ E) in Střekov, close to Ústí nad Labem town.

The fishpass consists of 45 concrete pools – 38 standard and seven resting pools, which are extended in length (Fig. 1). The standard chambers are 3 m long, 2 m wide and 1.2 m deep. Dividing screens have two diagonally located orifices (0.3 × 0.3 m). The head between the chambers is 0.2 m. The total length of the fishpass is 250 m and the vertical difference between the downstream entrance and the upstream exit is 9 m. The average flow in the fishpass is 0.4 m^3 s^{-1}. The bottom is flat along the whole trail. The entrance is located approximately 150 m from the dam of a hydropower plant. The water outflow at the entrance forms an angle of ca 45 degrees with the main river flow. The location of the exit is not convenient for downstream fish migration as the exit angle and river flow is 90° and the exit area is negligible with respect to the river flow (Fig. 1).

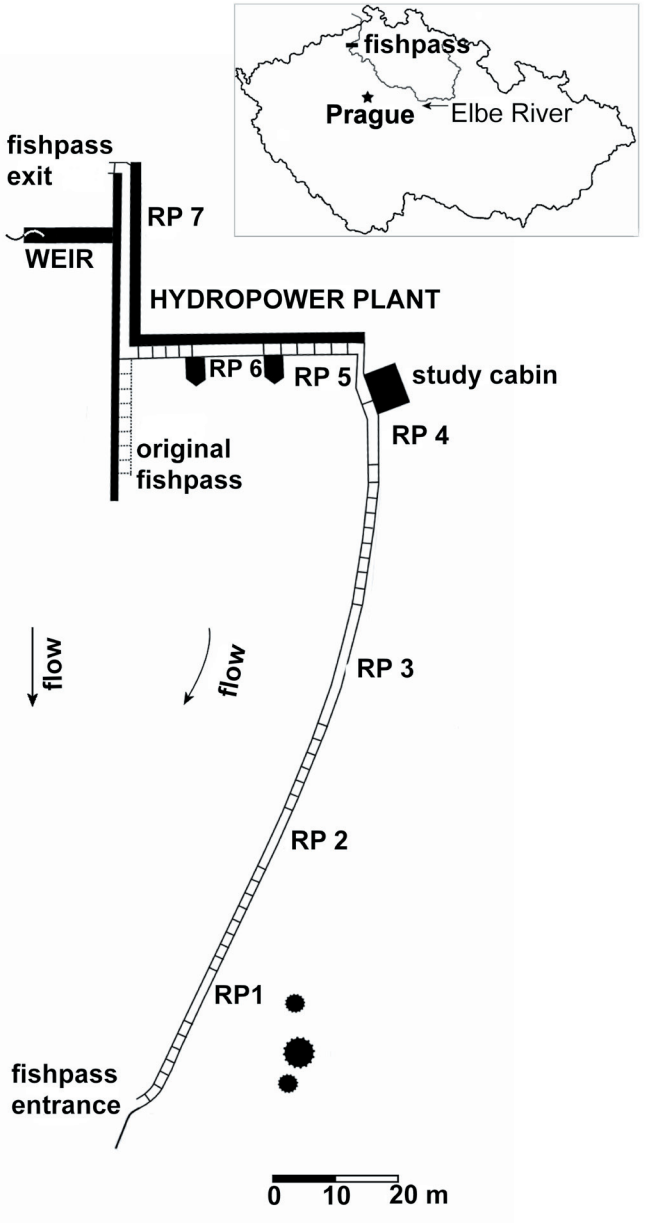

Fig. 1. *Design of the studied fishpass and its location on the River Elbe in the Czech Republic. RP – resting pools.*

Sampling procedures

Fish sampling in the fishpass was conducted weekly from 26 March to 6 November 2003, from 2 April to 1 July 2004 and from 9 August to 16 November 2004. Every sampling started around noon. To sample fish, the water inlet to the fishpass was closed. As the water drained, fish moved downstream to the last pool close to the entrance. Fish were captured using nets at this locality. All fish were identified and measured for standard lengths to the nearest mm and released. The age categories were separated according to species-specific standard lengths into immature and adult fish. The length thresholds were: 150 mm for roach, chub, barbel, perch, white bream, common bream and nase; 80 mm for bleak and gudgeon; 120 mm for dace.

During each sampling period, the water temperature was measured directly in the fishpass (the pool in front of the study cabin, Fig. 1) using the Oxi 340 microprocessor (WTW, Germany). Data on the water flow was provided by the Elbe River Authority, the responsible body for monitoring the water flow at the weir. The window of the upstream entrance (exit) of the fishpass is not regulated, and as such its flow is directly dependent on the flow at the weir. The course of the water flow and temperature over the sampling period is given in Fig. 2.

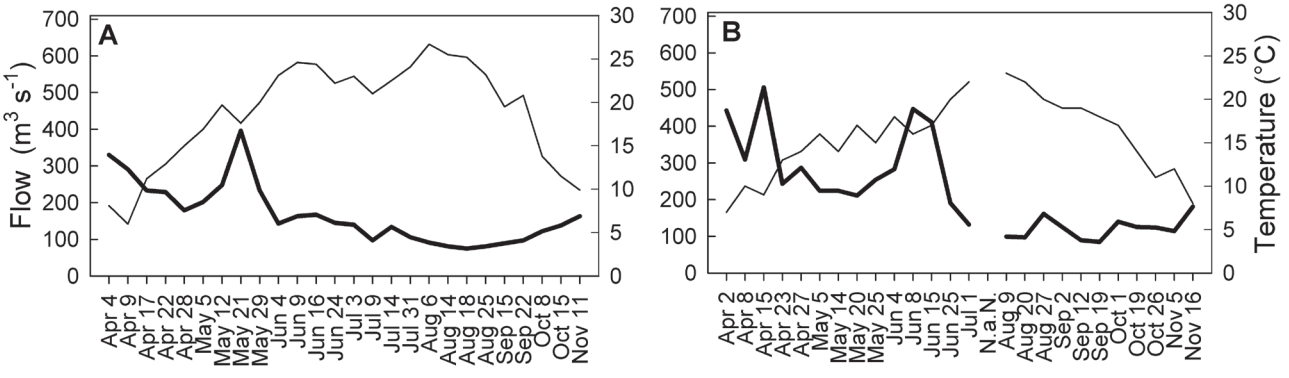

Fig. 2. *The course of water temperature (thin line) and water flow (thick line) during the study period in 2003 (A) and 2004 (B).*

Statistical analysis

Associations between the variables were tested using the General Linear Mixed Model (GLMM). The dependent variables were the numbers of fish recorded in the fishpass. Five separate models were applied for the dependent variables of the total number of fish (GLMM I) and numbers of four most abundant species, roach (GLMM II), bleak (GLMM III), chub (GLMM IV) and gudgeon (GLMM V). The data were $\log_{10}$ transformed for normality before GLMM analyses. The effect of the sampling year was tested first and was not found to be statistically significant in any model (GLMM I-V). Subsequently, to account for the repeated measures across different years, all analyses were performed using mixed model analysis with the year as a random factor using the PROC MIXED software (SAS, version 9.1). Fixed effects were the classes 'age category' – immature, adult and 'month' – April, May, June, July, August, September, October, November and the continuous variables 'temperature' (6-26.7 °C) and 'flow' (75-505 $m^3 s^{-1}$). The significance of each fixed effect, including interaction terms, in the mixed GLMM model was assessed by the F-test, with sequential dropping of the least significant effect, starting with a full model. Fixed effects that were not statistically significant are not discussed further. In the case of unbalanced data with more than one effect, the statistical mean for a group may not accurately reflect the response of that group, since it does not take other effects into account. Therefore we used the least-squares-means (LSM) instead. LSM (further referred to as 'adjusted means') are in effect, within-group means appropriately adjusted for the other effects in the model.

Associations between the dependent variable and other continuous variables were estimated by fitting a random coefficient model using the aforementioned PROC MIXED program as described by Tao et al. (2002). With this random coefficient model, we calculated the predicted values for the dependent variable and plotted them against the continuous variable with predicted regression lines. The degrees of freedom were calculated using the Kenward-Roger method (Kenward & Roger 1997).

Table 1. *List and numbers of fish caught in the fishpass in 2003 during each sampling period.*

Common name	Scientific name	Month	April					May				June				July				August				Sept.		Oct.	Nov.
		Date	4	9	17	22	28	5	12	21	29	4	9	16	24	3	9	14	31	6	14	18	25	15	22	8	15	5
Cyprinidae																												
Bleak	*Alburnus alburnus*		1	2	272	66	224	272	49	37	72		6	2	8		14	4	40		28	3	219	268	90		37	1
Roach	*Rutilus rutilus*		14		253	256	106	10	4	2	4			1		4		1				5	7	11	336	56	14	8
Barbel	*Barbus barbus*							2		3	1				1	3	2	2	11	11	13	35	31	7	4	41	327	1
Dace	*Leuciscus leuciscus*						1		1									1		2	37	80	18	3	13	85	178	2
Chub	*Squalius cephalus*		9	7	7	17	116	7	15	17	23	2	12	17	14	15	13	24	12	8	2	7	5		2	2	1	6
White bream	*Blicca bjoerkna*					24	42	21	125	9	63		7		1	3	3	1								4		
Gudgeon	*Gobio gobio*						2	21	51	43	4	4	7		2	7		3	2	1	8	3	15					
Common bream	*Abramis brama*						7	9	38	6	8	1	3		5	3	3	1	1							1		
Nase	*Chondrostoma nasus*						3	3	16																2	1	30	
Ide	*Leuciscus idus*				6	3	8		5	4	7				2	1		3			5	3	5				2	
Asp	*Aspius aspius*				1	2	3		7	2		2			1													
Crucian carp	*Carassius carassius*								4		3		3								1							
Tench	*Tinca tinca*									1																		
Percidae																												
Perch	*Perca fluviatilis*											1								2	2		41	4	2	1	5	
Pikeperch	*Sander lucioperca*															1					1	1		1				1
Brown trout (Salmonidae)	*Salmo trutta fario*							1		1	1																	
Grayling (Thymallidae)	*Thymallus thymallus*		1																									
Catfish (Ictaluridae)	*Ameiurus nebulosus*								2			7	19	12	1	2		2	1	1			1					
Eel (Anguillidae)	*Anguilla anguilla*											2	12	2	2	5	2											
Total number			25	9	539	368	512	346	317	125	186	19	69	34	37	44	37	42	67	25	97	137	342	294	449	191	594	19
Number of species			4	2	5	6	10	9	12	11	10	7	8	5	10	10	6	10	6	6	9	8	9	6	7	8	8	6
Water temperature (°C)			8.1	6	11.2	12.9	15	16.9	19.7	17.6	20	23.1	24.6	24.4	22.2	23	21	22.5	24.1	26.7	25.5	25.2	23.2	19.5	20.8	13.8	11.5	9.9
Water flow ($m^3 s^{-1}$)			330	291	233	229	179	202	248	396	233	143	163	167	145	140	97	134	106	91	81	75	81	89	97	122	138	163

Table 2. *List and numbers of fish caught in the fishpass in 2004 during each sampling period. Scientific names of species not previously mentioned: Prussian carp* Carassius gibelio, *rudd* Scardinius erythrophthalmus, *Northern whitefin gudgeon* Romanogobio belingi *and rainbow trout* Oncorhynchus mykiss.

Common name	Month	April				May					June				July	August			September			October			November	
	Date	2	8	15	23	27	4	14	20	25	4	8	15	25	1	9	20	27	2	12	19	1	19	26	5	16
Cyprinidae																										
Bleak			125	19	23	200	157	18	7	1	62		1	151	100	5 012	327	88	304	69	12	33	193	34	34	
Roach			51	3	182	167	58	8	1		5					9	8	8	3	18	5	6	12	8	31	5
Gudgeon					11	97	42	11			1			1				1								
Chub			1	1	1	4	10	3			34	1	6	33	24	6	7	4	1	6	3	2	5	4	2	
Dace		1	7	1			2															6	34	6	16	
Common bream					1	4	8	28	12		9	2		1	2		1	1							1	
Prussian carp							13	5	10		11	3	1	12	6	2	1									
White bream					1	8	9	18	7		7	1		1			1	1	1						1	
Barbel																	2	2		4	5	8	11	4	7	4
Ide					2	4	3	1	1		2	1		1			1	1							6	
Nase						2	5	1			8									2						
Asp							1							1		2	1	1		2	1					
Rudd						3	1		1		1															
Nothern whitefin gudgeon															1		1									
Percidae																										
Perch							2				4		2	3		3	4	10		12	7	4	3	14	2	
Pikeperch									1																	
Salmonidae																										
Brown trout						1								1				1								
Rainbow trout													1			1										
Catfish (Ictaluridae)														2	1	5	2									
Eel (Anguillidae)													3	1		3										
Total number		1	184	24	221	490	311	93	40	1	144	8	14	208	134	5043	368	129	313	122	39	65	264	76	109	9
Number of species		1	4	4	7	10	13	9	8	1	10	5	6	12	6	9	12	11	4	7	6	6	6	6	9	2
Water temperature (°C)		7	9.9	8.5	13.3	13.6	16.1	14.4	16.5	15.1	18.3	16	17.3	20.3	21.5	23.1	22.2	20.3	19.4	19.2	18.1	17.4	14.1	11.0	12.3	8.0
Water flow ($m^3\ s^{-1}$)		443	309	506	243	287	224	224	211	254	283	447	411	191	132	99	97	161	125	89	85	140	125	124	114	181

***Table 3**. Type 3 tests of fixed effects for the final GLLM models.*

Effect	Num DF	Den DF	F	P <
For GLMM I				
month*age category	15	83.9	8.16	0.0001
temperature*flow*age category	2	67.3	6.52	0.0026
For GLMM II				
month*age category	15	83.9	4.19	0.0001
flow*age category	2	46	5.28	0.0086
For GLMM III				
month*age category	15	82.4	4.38	0.0001
temperature*flow*age category	2	67.3	3.64	0.0315
For GLMM IV				
month*age category	15	82.5	2.31	0.0085
temperature*flow*age category	2	66.3	7.49	0.0012
For GLMM V				
month*age category	15	83.9	4.02	0.0001
temperature*flow*age category	2	67.3	10.72	0.0001

Results

A total of 13266 fishes representing 23 species were caught in the fishpass (Table 1, 2). Cyprinids (Cyprinidae) represented more than 96 % of the catch during both years. The most abundant and also the most frequent species recorded were bleak, roach and chub. Fish started to occur in the fishpass in significant numbers when the water temperature had reached 8 °C in the spring and fish stopped to utilize the fishpass when water temperature dropped below 10 °C in the autumn.

The final GLMM I, III, IV and V models included fixed factors expressed as interaction between 'month' and 'age category' and interaction between 'flow', 'temperature' and 'age category'. The final GLMM II model of roach occurrence in the fish pass included the fixed factor of interaction between 'month' and 'age category' identical to other models and the interaction between 'flow' and 'age category'. Details of the final GLMM models are shown in Table 3.

The total number of fish of both age categories caught in the fishpass varied according to the month (Fig. 3A). Two peaks were generally found: the first occurred during April and the second during October. The numbers of adult fish were significantly higher than those of immature fish in the April to July period. The numbers of both age categories were equal from August to November. The total number of adult fish increased with water temperature, with an optimum at medium temperatures (15-20 °C) and medium flow conditions (160-300 $m^3 s^{-1}$, Fig. 4A). The occurrence of immature fish decreased with increasing temperature and water flow. Their minimum numbers were recorded at the highest values of both temperature and flow (Fig. 4B).

The pattern of roach occurrence in the fishpass was similar to the general pattern described above, with more roach present in the fishpass during the spring than in the autumn (Fig. 3B). The numbers of adults and immature fish did not differ within individual month. Only flow had a significant influence on roach numbers with the highest numbers of both adult and immature fish recorded during medium values of flow (170-280 $m^3 s^{-1}$, Fig. 5).

The occurrence of bleak in the fishpass was similar to the general pattern described above, with more immature bleak present in the fishpass during the autumn than in the spring (Fig. 3C). The numbers of adult and immature bleak were similar in all monitored months with exception in April. The number of adult bleak peaked at medium values of temperature and flow as well (Fig. 6A). The number of immature bleak was stable across the temperature range and decreased with flow (Fig. 6B). The minimum numbers of immature bleak occurred in the fishpass at the minimum values of temperature and maximum values of flow.

The numbers of both age categories of chub did not differ throughout the season (Fig. 3D). Generally, the numbers of adults were higher than immature chub, which was significant in April, May and July. The number of adult chub increased with both temperature and flow, whereas the response of the immature fish was converse (Fig. 7).

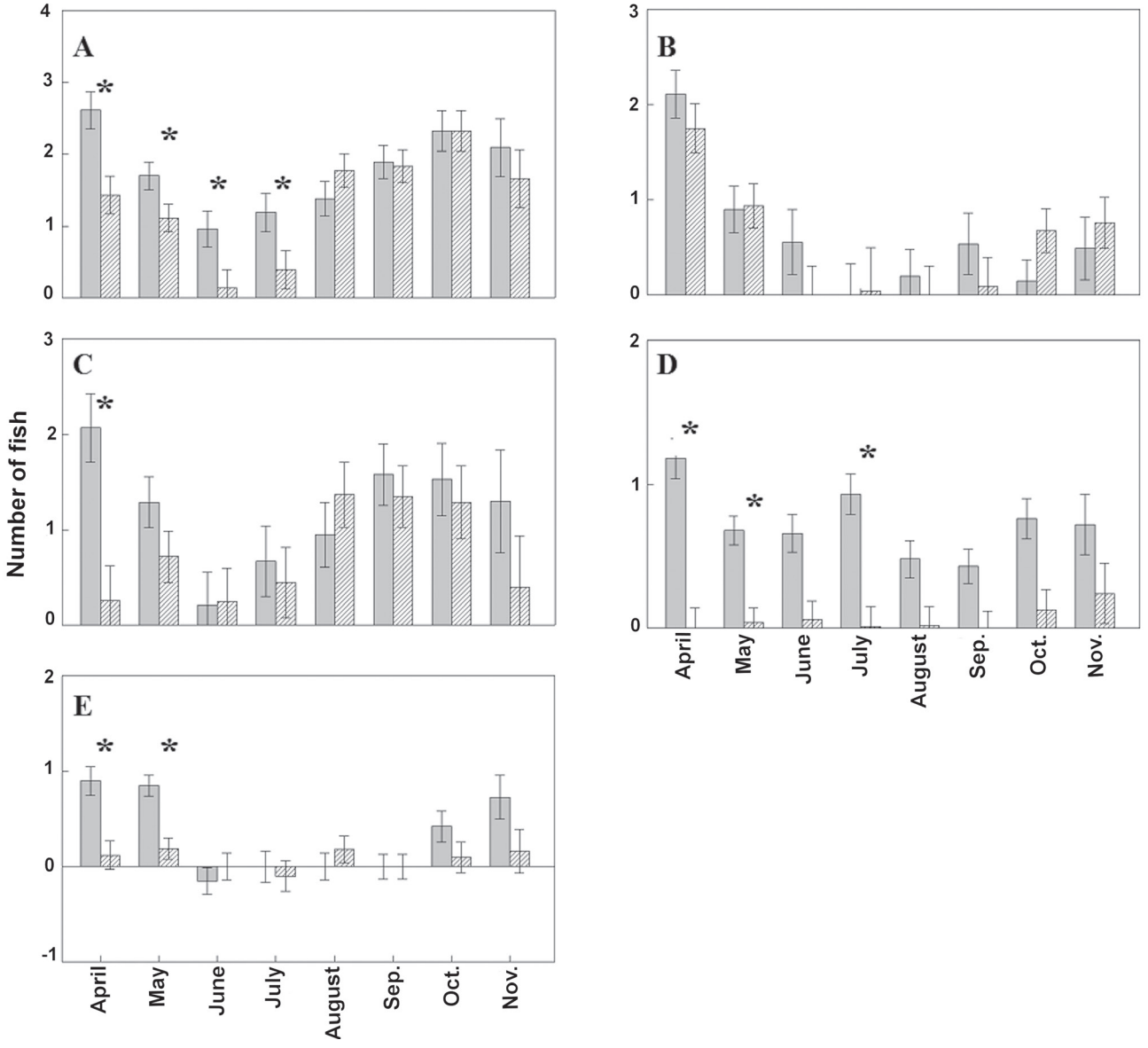

Fig. 3. *Numbers of (A) all fish, (B) roach, (C) bleak, (D) chub and (E) gudgeon caught in the fishpass throughout the months for both years studied. Grey columns refer to adults, striped columns to immature fish. A star above columns indicates a significant difference ($p < 0.05$) in numbers of adult and immature fish. Values are the adjusted means (+/– SE) of log_{10} transformed data.*

The number of adult gudgeon followed the general pattern of fish occurrence in the fishpass throughout the season. The number of immature gudgeon had no clear pattern, however (Fig. 3E). The number of adults was usually higher than that of immature gudgeon, which was significant especially in April and May. Both age categories of gudgeon responded to temperature and flow similarly, with numbers decreasing with both decreasing temperature and flow (Fig. 8).

Discussion

The movements of fish through the studied fishpass followed a clear seasonal pattern in both of the years 2003 and 2004. The numbers of adult and immature fish occurred in the fishpass were significantly influenced by the interaction of water temperature and flow.

The seasonal pattern of migration consisted of the spring spawning migrations of adults (April, May), the summer period of lower activity (June, July) and the late summer and fall refuge seeking migrations of both adults and immature fish (August-October). Fish started to occur in the fishpass in significant numbers when the water temperature had reached 8 °C, which agreed with the temperature threshold for the spring spawning migrations of lowland fish mentioned in other studies (Kotusz et al. 2006, Geeraerts et al.

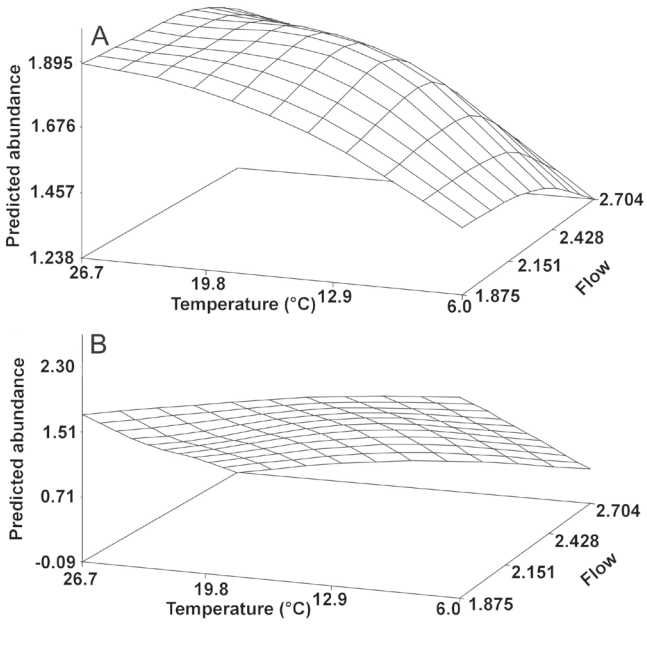

Fig. 4. *Predicted values (log_{10} transformed data) of the total number of adult (A) and immature (B) fish caught in the fishpass plotted against water flow (log_{10} transformed data in $m^3\ s^{-1}$) according to water temperature (°C).*

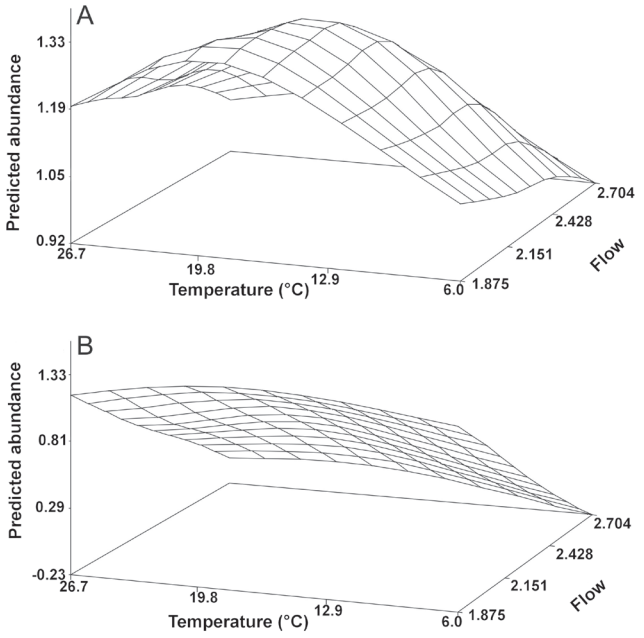

Fig. 6. *Predicted values (log_{10} transformed data) of the number of adult (A) and immature (B) bleak caught in the fishpass plotted against water flow (log_{10} transformed data in $m^3\ s^{-1}$) according to water temperature (°C).*

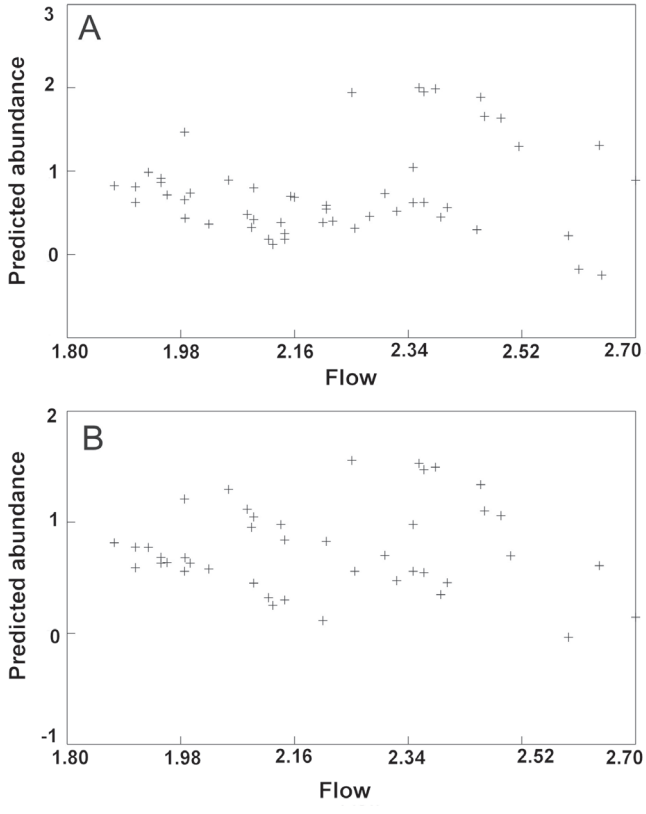

Fig. 5. *Predicted values (log_{10} transformed data) of the number of adult (A) and immature (B) roach caught in the fishpass plotted against water flow (log_{10} transformed data in $m^3\ s^{-1}$).*

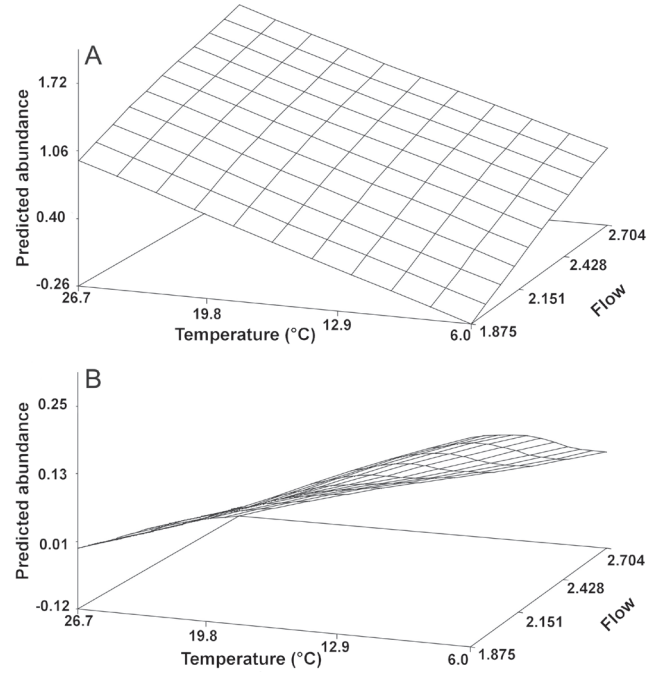

Fig. 7. *Predicted values (log_{10} transformed data) of the number of adult (A) and immature (B) chub caught in the fishpass plotted against water flow (log_{10} transformed data in $m^3\ s^{-1}$) according to water temperature (°C).*

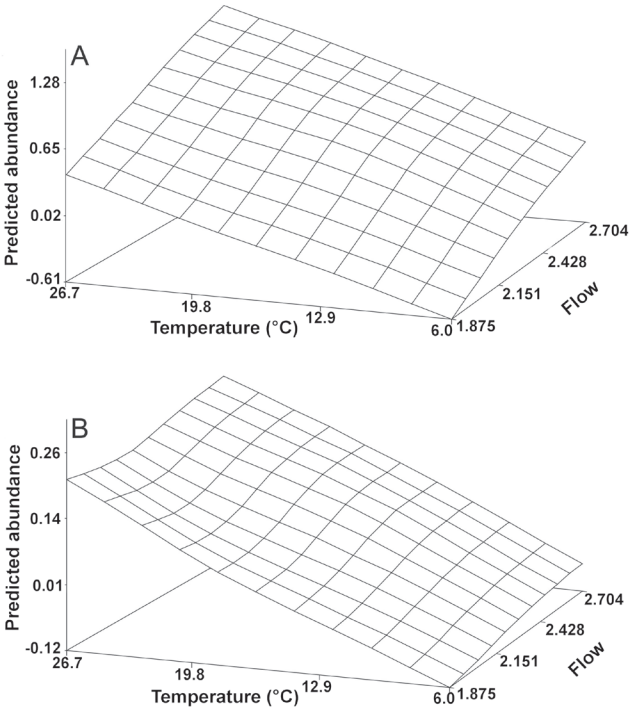

Fig. 8. *Predicted values (log_{10} transformed data) of the number of adult (A) and immature (B) gudgeon caught in the fishpass plotted against water flow (log_{10} transformed data in $m^3 s^{-1}$) according to water temperature (°C).*

2007, Ovidio & Philippart 2008). When the water temperature in the spring dropped below 8 °C, the migration activity decreased markedly (Table 1) as was previously described by Lucas (2000) and Hladík & Kubečka (2003). All 23 fish species registered in the fishpass were recognized as potential migrants (see Lucas & Baras 2001). June and July activities could be most probably considered as local movements (Slavík et al. 2009). Large numbers of immature cyprinids started to migrate in August and this phenomenon has been described in detail by Prchalová et al. (2006a). The number of fish decreased dramatically in the autumn when water temperature dropped below 10 °C. Both adult and immature fish were influenced significantly by water temperature. For adults, increasing temperature was more important, an element connected with the spring spawning migration. On the other hand, immature fish migrated at any temperature (bleak) or preferred lower (chub) or higher temperatures (gudgeon). These responses were the same in varying values of water flow.

During the spring spawning migration, bleak numbers peaked at 10-16 °C temperatures, roach at 11-13 °C, and chub at 15-16 °C. All these values are within the published ranges of temperature requirements for spawning migrations of these European species (e.g. Jurajda et al. 1998, Prignon et al. 1998, Travade et al. 1998, Kotusz et al. 2006). Common bream, white bream, gudgeon and nase exhibited a maximum occurrence in the fishpass during a steep temperature increase in 2003, which corresponds with observations by Lelek & Libosvárský (1960) and Rakowitz et al. (2008). In 2004, these species migrated with the highest intensity in a period of 13-16 °C water temperature, which is in agreement with the finding of Donnely et al. (1998) determining the minimal 13 °C temperature for the migration of common bream. However, it should be noted that reported temperature threshold and ranges optimal for migrations values could be site specific (Jonsson 1991, Jurajda et al. 1998). This stipulation could be valid for the reported values of water flow as well.

Eel occurred in the fishpass only during the summer (June-August) and all individuals were immature fish of similar size (200-400 mm SL). The presence of eel in the fishpass was most probably related to their upstream migration from the sea (Slavík 1996). The ascent of eel into fresh waters runs from April to September, with a peak in May to July (Porcher 2002), which corresponds with the summer occurrence of eel in the studied fishpass located approximately 770 km from the North Sea.

The previously published effects of water flow varied from no effect (Lucas 2000, Kotusz et al. 2006, Geeraerts et al. 2007) to recorded optimal values of water flow for the fish migration and its cessation in extremely high or low water flows (Horký et al. 2007, Slavík et al. 2009). Rakowitz et al. (2008) summed up that most studies on fish migration show the positive effect of decreasing water level on the number of migrating fish. In this study, the effect of water flow was species and size specific. Beach (1984) showed that non-leaping species (e.g. Cyprinidae, Percidae, Esocidae) must swim at least 30 % faster than the opposing flow to progress upstream. As the absolute swimming speed increases with fish size (see Wolter & Arlinghaus 2003), smaller fish are supposed to migrate in conditions of the lower water flow in comparison to adult fish (Slavík et al. 2009). This assumption was supported by our study: Adult bleak (small-sized species) and adult and immature roach (middle-sized species) migrated mostly during medium water flows (Slavík et al. 2009), whereas the number of adult chub (large-sized, rheophilous species) peaked during the highest flows. On the other hand, the number of immature bleak and chub decreased with increasing flow.

However, this pattern had an exception. The numbers of both adult and immature gudgeon (small-sized species) increased with temperature and flow almost linearly (Fig. 8). Gudgeon was the smallest species analyzed, and thus a negative correlation of occurrence with water flow would be expected. Nevertheless, as a benthic and reophilous species, gudgeon could react to increased water flow by increased migration activity, as is the case with the barbel (large-sized species; Baras et al. 1994, Slavík et al. 2009). Water velocity is represented by its relative minimum at the bottom and it is possible that benthic species need higher water flow to reach the same intensity of movements as species occupying the water column (i.e. bleak, roach and chub in this case).

The numbers of fish and the pattern of the spawning migration through the fishpass were unsatisfying for barbel, dace and asp. According to the fall catches in the fishpass and electrofishing in the adjacent river stretch (Prchalová et al. 2006b), these species were abundant in the river. However, nearly no adult specimens appeared in the fishpass during the spring, despite that these species are well known spawning migrants (Lelek & Libosvárský 1960, Lucas & Frear 1997, Jurajda et al. 1998, Lucas 1998). It seems that the fishpass, especially the location of its entrance (Bunt 2001), was unfavorable for the migration of these species, most probably because of their high degree of rheophily (Baras et al. 1994).

Acknowledgements

The authors would like to thank the members of the Elbe River Authority for their valuable help during fieldwork and providing data on water flow. The authors also thank Hassan Hashimi for correcting the language of the manuscript. This study was supported by the grant SP/2e7/229/07 of the Ministry of Environment of the Czech Republic and by the project No. AVOZ60170517 and grant No. 1QS500450513 of the Academy of Sciences of the Czech Republic.

Literature

Baras E., Lambert H. & Philippart J.-C. 1994: A comprehensive assessment of the failure of *Barbus barbus* spawning migrations through a fish pass in the canalized River Meuse (Belgium). *Aquat. Living Resour. 7: 181–189.*

Beach M.A. 1984: Fish pass design. Fisheries technical report 78. *Ministry of Agriculture, Fisheries and Food, Lowestoft, England: 46.*

Bunt C.M. 2001: Fishway entrance modifications enhance fish attraction. *Fish. Mgmt. Ecol. 8: 95–105.*

Donnelly R.E., Caffrey J.M. & Tierney D.M. 1998: Movements of a bream (*Abramis brama* (L.)), rudd × bream hybrid, tench (*Tinca tinca* (L.)) and pike (*Esox lucius* (L.)) in an Irish canal habitat. *Hydrobiologia 371/372: 305–308.*

Geeraerts C., Ovidio M., Verbiest H., Buysse D., Coeck J., Belpaire C. & Philippart J.-C. 2007: Mobility of individual roach *Rutilus rutilus* (L.) in three weir-fragmented Belgian rivers. *Hydrobiologia 582: 143–153.*

Hladík M. & Kubečka J. 2003: Fish migration between a temperate reservoir and its main tributary. *Hydrobiologia 504: 251–266.*

Horký P., Slavík O., Bartoš L., Kolářová J. & Randák T. 2007: Behavioural pattern in cyprinid fish below a weir as detected by radio telemetry. *J. Appl. Ichthyol. 23: 679–683.*

Jonsson N. 1991: Influence of water flow, water temperature and light on fish migration in rivers. *Nordic J. Freshw. Res. 66: 20–35.*

Jurajda P., Hohausová E. & Gelnar M. 1998: Seasonal dynamics of fish abundance below a migration barrier in the lower regulated River Morava. *Folia Zool. 47: 215–223.*

Kenward M.G. & Roger J.H. 1997: Small sample inference for fixed effects from restricted maximum likelihood. *Biometrics 53: 983–997.*

Kotusz J., Witkowski A., Baran M. & Błachuta J. 2006: Fish migrations in a large lowland river (Odra R., Poland) – based on fish pass observations. *Folia Zool. 55: 386–398.*

Lelek A. & Libosvárský J. 1960: Výskyt ryb v rybím přechodu na řece Dyji při Břeclavi [The occurrence of fish in a fish ladder in Dyje River near Břeclav]. *Folia Zool. 9: 293–308. (in Czech with English summary)*

Lucas M.C. 1998: Seasonal movements of coarse fishes in lowland rivers. *Proceedings of 29th IFM ASC, Cambridge, England: 53–72.*

Lucas M.C. 2000: The influence of environmental factors on movements of lowland-river fish in the Yorkshire Ouse system. *Sci. Total Environ. 251/252: 223–232.*

Lucas M.C. & Batley E. 1996: Seasonal movements and behaviour of adult barbel *Barbus barbus* riverine cyprinid fish: implications for river management. *J. Appl. Ecol. 33: 1345–1358.*

Lucas M.C. & Frear P.A. 1997: Effects of a flow-gauging weir on the migratory behaviour of adult barbel, a riverine cyprinid. *J. Fish Biol. 50: 382–396.*

Lucas M.C. & Baras E. 2001: Migration of freshwater fishes. *Blackwell Science, Oxford, England.*

Northcote T.G. 1998: Migratory behaviour of fish and its significance to movement through riverine fish passage. In: Jungwirth M., Schmutz S. & Weiss S. (eds.), Fish migration and fish bypasses. *Blackwell Science, Oxford: 3–18.*

Ovidio M. & Philippart J.C. 2008: Movement patterns and spawning activity of individual nase *Chondrostoma nasus* (L.) in flow-regulated and weir-fragmented rivers. *J. Appl. Ichthyol. 24: 256–262.*

Porcher J.P. 2002: Fishways for eels. *Bull. Fr. Pêche Piscic. 364: 147–155.*

Prchalová M., Vetešník L. & Slavík O. 2006a: Migrations of juvenile and subadult fish through a fishpass during late summer and fall. *Folia Zool. 55: 162–166.*

Prchalová M., Slavík O. & Bartoš L. 2006b: Patterns of cyprinid migration through a fishway in relation to light, water temperature and fish circling behaviour. *Int. J. River Basin Mgmt. 4: 213–218.*

Prignon C., Micha J.C. & Gillet A. 1998: Biological and environmental characteristics of fish passage at the Tailfer dam on the Meuse River, Belgium. In: Jungwirth M., Schmutz S. & Weiss S. (eds.), Fish migration and fish bypasses. *Blackwell Science, Oxford: 69–85.*

Rakowitz G., Berger B., Kubečka J. & Keckeis H. 2008: Functional role of environmental stimuli for the spawning migration in Danube nase *Chondrostoma nasus* (L.). *Ecol. Freshw. Fish 17: 502–514.*

Santos J.M., Ferreira M.T., Godinho F.N. & Bochechas J. 2002: Performance of fish lift recently built at the Touvedo Dam on the Lima River, Portugal. *J. Appl. Ichthyol. 18: 118–123.*

Slavík O. 1996: Migrace ryb v Labi pod Střekovem [Migration of fish in the Elbe River at Střekov]. *Živa 4: 179–180. (in Czech)*

Slavík O. & Bartoš L. 2004: What are the reasons for the Prussian carp expansion in the upper Elbe River, Czech Republic? *J. Fish Biol. 65: 240–253.*

Slavík O., Horký P. & Bartoš L. 2009: Occurrence of cyprinids in fish ladders in relation to flow. *Biologia 64: 999–1004.*

Tao J., Little R., Patetta M., Truxillo C. & Wolinger R. 2002: Mixed the SAS system course notes. *Cary, NC: SAS Institute Inc.*

Travade F., Larinier M., Boyer-Bernard S. & Dartiguelongue J. 1998: Migratory behaviour of fish and its significance to movement through riverine fish passage. In: Jungwirth M., Schmutz S. & Weiss S. (eds.), Fish migration and fish bypasses. *Blackwell Science, Oxford: 3–18.*

Wolter C. & Arlinghaus R. 2003: Navigation impacts on freshwater fish assemblages: the ecological relevance of swimming performance. *Rev. Fish Biol. Fish. 13: 63–89.*

Folia Zool. – 60 (2): 115–121 (2011)

Karyotype diversity of the offspring resulting from reproduction experiment between diploid male and triploid female of silver Prussian carp, *Carassius gibelio* (Cyprinidae, Actinopterygii)

Lukáš KALOUS[1,2] and Martin KNYTL[1]

[1] *Czech University of Life Sciences Prague, Faculty of Agrobiology, Food and Natural Resources, Department of Zoology and Fisheries, 165 21 Praha 6-Suchdol, Czech Republic; e-mail: kalous @af.czu.cz*

[2] *Joint Laboratory of Genetics, Physiology and Reproduction of Fishes of Institute of Animal Physiology and Genetics, Academy of Sciences of the Czech Republic, v.v.i., 277 21 Liběchov and University of South Bohemia in České Budějovice, Research Institute of Fish Culture and Hydrobiology, 389 25 Vodňany, Czech Republic*

Received 8 June 2010; Accepted 25 November 2010

Abstract. Populations of silver Prussian carp (*Carassius gibelio*) are known to exhibit different ploidy levels among their individuals. No consistent information is available regarding chromosome number of triploid biotype. Generally diploids have 100 chromosomes while triploids have 150-160 chromosomes. The karyotype of the *C. gibelio* triploid biotype is characterized by a variable number of small chromosomal elements called supernumerary chromosomes. Here we report the results of a reproduction experiment between a diploid male and triploid female with respect to chromosome numbers of the parents and their offspring. Thirty metaphases of both parents and fifteen individuals of the offspring were investigated. We found variability in chromosome numbers among analysed offspring with a fluctuation from 150 to 159. In comparison, the chromosome numbers of male and female individuals were found to be 100 and 159 respectively. Our results show a high chromosomal plasticity of the *Carassius gibelio* triploid biotype.

Key words: cytogenetic, chromosome number, ploidy level, hybridization

Introduction

Silver Prussian carp (*Carassius gibelio*, Bloch, 1782) occurs in a vast territory of Eurasia in two main biotypes: diploid – evolutionary tetraploid with 100 chromosomes and triploid – evolutionary hexaploid with approximately 150 chromosomes (Kottelat & Freyhof 2007). The triploid biotype is also known for its gynogenetic form of reproduction – sperm dependent parthenogenesis (Golovinskaya et al. 1965, Peňáz et al. 1979). Some authors use the term allogynogenesis to describe a specific form of reproduction of silver Prussian carp wherein the male sperm partially contributes to the genome of offspring (Yi et al. 2003, Zhao et al. 2004).

The original distribution of silver Prussian carp throughout Europe is unclear due to a number of introductions, confusion with feral goldfish (*Carassius auratus*), as well as misinterpretation of the taxonomical status of *C. gibelio* in older literature (Kottelat 1997, Kalous et al. 2004). Although there are many ambiguities of the origin; the expansion of the triploid biotype of *C. gibelio* in Central Europe is well documented (Holčík & Žitňan 1978). Lusk et al. (1977) described the first occurrence of an all female population of *C. gibelio* in a lower stretch of the River Dyje in the territory of the Czech Republic. The study of Peňáz et al. (1979) and Lusk & Baruš (1978) revealed that the fish were all triploids and female,

and therefore should reproduce only gynogenetically. The carp aquaculture in the Czech Republic was then responsible for the expansion of the triploid biotype of *C. gibelio* to two other major European basins of the Elbe and Oder. This was caused by a number of reasons, e.g. the accidental introduction of silver Prussian carp into common carp stock or its use as baitfish by anglers, as well as its occasional escapes during the draining of ponds or due to floods (Lusk et al. 1980, Kubečka 1989, Slavík & Bartoš 2004). Surprisingly, at the beginning of the 1990's, males and diploids started to appear within the population of *C. gibelio* in the River Dyje alluvium (Halačka et al. 2003, Lusková et al. 2004). In a relatively short time, the once all female triploid population transformed to a diploid-polyploid complex with various percentages of males reaching 43 % (Vetešník 2005). Similar complexes were also recorded from other places in Europe (Černý & Sommer 1994, Abramenko et al. 1998, Tóth et al. 2000). Few cytogenetic studies exist on the European population of *C. gibelio* and the results are quite variable especially in the triploid biotype – see Table 1. In any case, cytogenetics can be considered a crucial approach in explaining mechanisms of mysterious phenomena within former all female populations of *C. gibelio* such as a sudden appearance of males and a ploidy level reduction from 3n to 2n in a short period (Ráb et al. 2007). Here we present the results of a reproduction experiment between diploid male and triploid female originating from the locality where the population of silver Prussian carp was originally established within the Czech Republic.

Table 1. *Chromosome numbers of* Carassius gibelio *reported from Europe.*

Locality	Numbers of chromosomes	Reference
Belarus	94, 141	Cherfas (1966)
Former Yugoslavia	160	Vujosevic et al. (1983)
Czech Republic	160 (166)	Peňáz et al. (1979)
Romania	98	Raicu et al. (1981)
Former Yugoslavia	158	Fister & Soldatovic (1989)
Poland	100, 150	Boroń (1994)
Hungary	100, 148–156	Tóth et al. (2005)

Material and Methods

Parental fish were captured during the spring of 2006 in alluvium of the River Dyje close to its confluence with the River Morava, in South Moravia – Czech Republic. Fish were transported to the University of South Bohemia, Research Institute of Fish Culture and Hydrobiology in Vodňany, Czech Republic. Prior to any handling, the fish were anaesthetized with 0.6 $ml.l^{-1}$ 2-phenoxyethanol (Merck Co., Darmstadt, Germany). Hormonal stimulation and gamete collection followed the methodology of Linhart et al. (2003), while fertilization and egg incubation in experimental trays were carried out according to Linhart et al. (2006). Blood was sampled according to Svobodová et al. (1991). Ploidy levels of both specimens were determined as a relative DNA content in erythrocytes by means of flow cytometry (Partec CCA I; Partec GmbH, EU) using 4′, 6-diamidino-2-phenylindol (DAPI). Samples were processed according to Flajšhans et al. (2008). Erythrocytes of a diploid male gave a relative DNA content of 2n as the diploid standard.

A number of the hatched offspring (approximately 100 fish larvae) and both parental specimens were subsequently kept in aquaria until chromosome preparation was carried out.

Parental male and female were investigated using a standard direct procedure for chromosome preparation from the kidneys according to Ráb & Roth (1988). Both male and female nuclei suspensions were dropped on slides and air-dried.

Fifteen specimens of offspring were investigated from 2008 to 2010 using a non-destructive method of chromosome preparation from regenerated tissue of caudal fin; a slightly modified protocol of Völker & Kullmann (2006) was used. The result of a single preparation was, in most cases, composed of two slides with three nuclei rings per specimen. Staining was processed in a buffered 5 % solution of Giemsa-Romanowski for 10 minutes, and 30 best metaphase spreads per individual were examined each time using a system composed of a Microscope Olympus BX41TF (magnification 1000 ×), an Olympus SP-350 digital camera and a computer with QuickPHOTO MICRO version 2.3 software (PROMICRA, s.r.o., Praha, Czech Republic) running on Microsoft® Windows® XP.

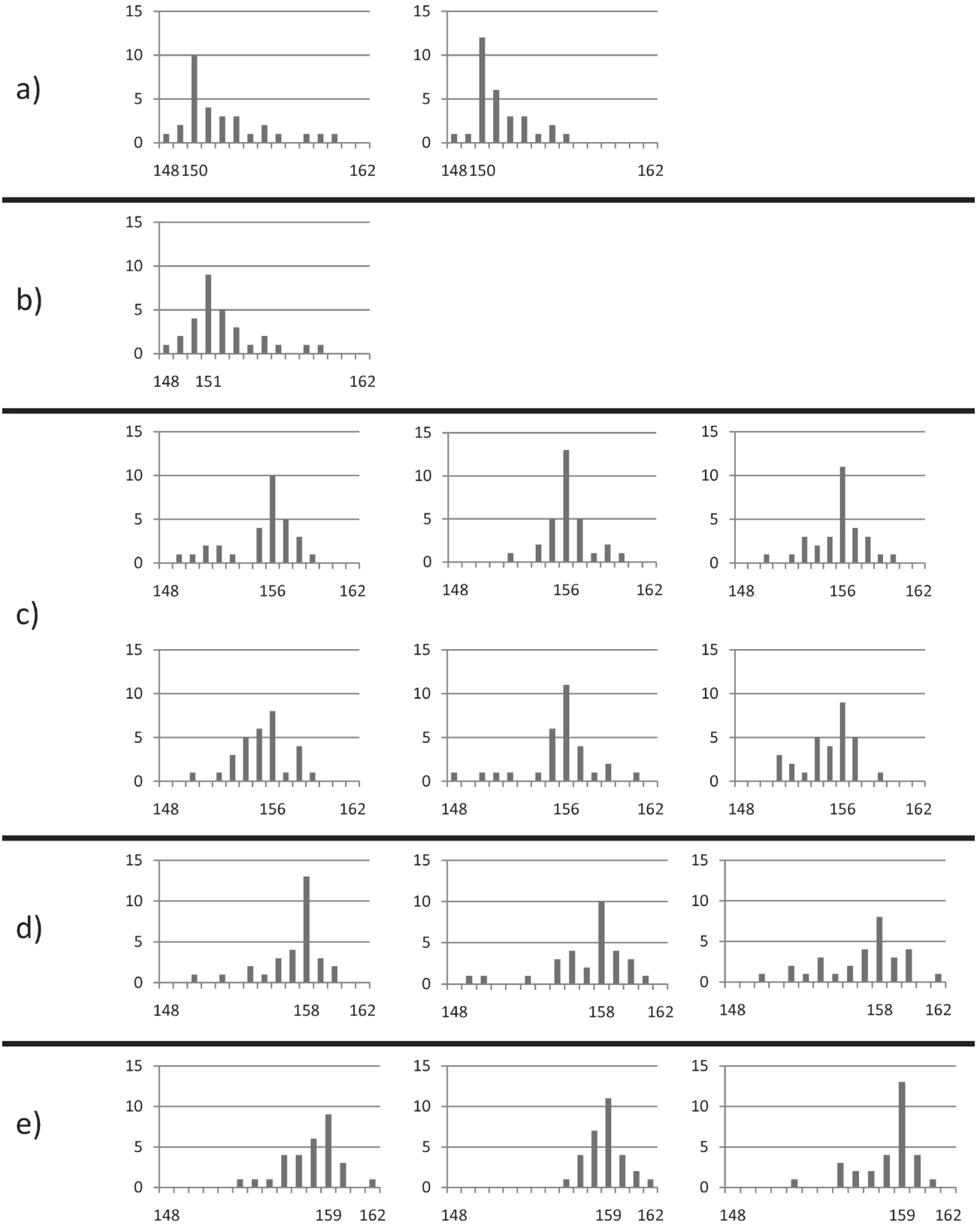

Fig. 1. *Frequency distribution of chromosome numbers of individuals of offspring resulting from reproduction experiment. a) two individuals with modal number of chromosomes 150, b) one individual with modal number of chromosomes 151, c) six individuals with modal number of chromosomes 156, d) three individuals with modal number of chromosomes 158, e) three individuals with modal number of chromosomes 159.*

Chromosome counting was carried out on a PC through the use of QuickPHOTO-MICRO version 2.3 software with a "Counting Points" function. We counted all chromosomes and chromosomal structures, and we did not separate microchromosomes due to their difficult definition and unclear size limits with respect to others chromosomes.

Results

Male and female were identified by flowing cytometry as diploid and triploid respectively. The modal chromosome number of diploid parental male was 100 (70.0 % of investigated metaphases) and the modal chromosome number of triploid female was 159 (46.6 % of investigated metaphases). Fifteen analysed individuals of offspring were divided into five groups with different chromosome numbers – see Fig. 1.

Discussion

Several decades after the appearance of the *C. gibelio* triploid biotype in the territory of the Czech Republic, a fascinating change of ploidy and the male female ratio within the population was observed. A cytogenetic study, conducted shortly after the appearance of all female triploid biotype of *C. gibelio* in South Moravia, recorded 160 chromosomes and six microchromosomes (Peňáz et al. 1979). Unfortunately, no other karyological data from the Czech Republic have been available since 1979.

Our analysis of *C. gibelio* caught in the same locality after almost 30 years revealed different chromosome numbers for male and female, 100 (Fig. 2) and 159 (Fig. 3) respectively. A reproduction experiment of the 2n male and 3n female shows a variability of chromosome numbers in 15 specimens of the offspring (sex undetermined). It is clear that the *C. gibelio* triploid biotype does not bear a defined number of chromosomes, although it does oscillate above 150. This is in agreement with Zhou & Gui (2002) and supports the hypothesis of close relations between East Asian and European populations of triploid biotypes of silver Prussian carp (Kalous & Šlechtová 2004, Kalous et al. 2007) in terms of karyology. The presented findings can also explain inequality in older literature regarding chromosome numbers of *C. gibelio* in Europe (see Table 1). In previous times, authors commonly investigated only few specimens from one locality caught at the same time, and therefore they came to the conclusion that the triploid biotype of *C. gibelio* possesses only one specific number of chromosomes. There is also always a high probability of error when the chromosomes in metaphases are counted; this is primarily due to their high numbers, small size, and the presence of small chromosomal elements called microchromosomes or supernumerary chromosomes (Boroń 1994). Although many authors published karyotypes of C. *gibelio*, they are generally in disagreement. Without additional staining or *in situ* hybridisation it is very complicated to be oriented in karyological structure. We therefore present only chromosome numbers gained from a sizable dataset. Fifteen analysed individuals of the offspring were divided into five groups according to

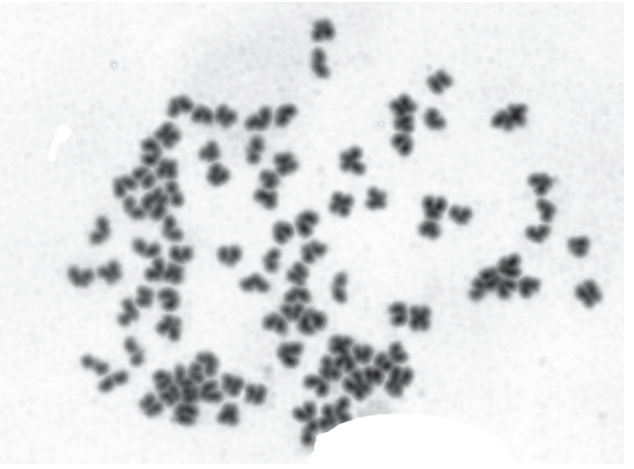

Fig. 2. *Metaphase of diploid male parent of silver Prussian carp (2n = 100) (× 1000).*

Fig. 3. *Metaphase of triploid female parent of silver Prussian carp (3n = 159) (× 1000).*

their modal chromosome numbers: 150, 151, 156, 158 and 159. This variability supports a process of interaction between male and female gametes after the fertilization of eggs from 3n individual with the sperm of 2n male. In any case, this interaction leads to a production of triploid offspring. Since a genetic analysis of progeny was not performed, we can not be sure whether these are recombinant offspring, or that some/all of the individuals are of gynogenetic origin.

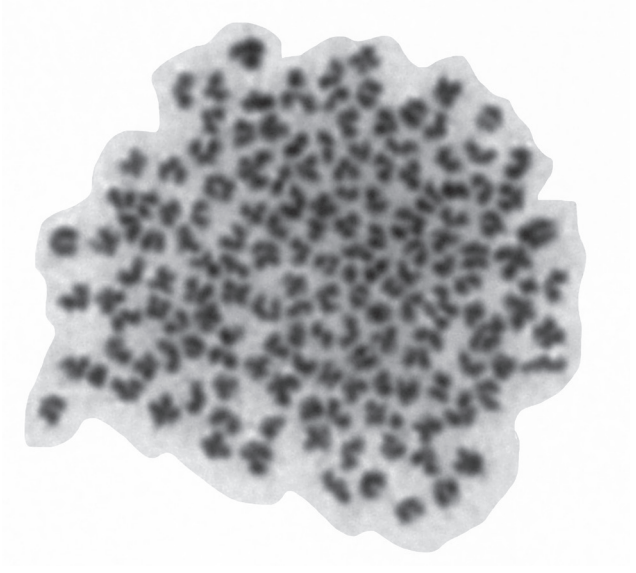

Fig. 4. *Metaphase of triploid offspring specimens of silver Prussian carp with most common chromosome number (3n = 156) (× 1000).*

The most common modal chromosome number of offspring was 156 (Fig. 4), which is also often recorded from other studies (Yi et al. 2003, Zhao et al. 2004). Tóth et al. (2005) found 148-156 chromosomes within the offspring of diploid male and triploid female. Unfortunately, the authors grouped together the chromosome numbers of analysed specimens resulting from the aforementioned reproduction experiment, and it is not clear if the variability was observed among the offspring or just within the metaphases.

The dual reproduction modes, including gynogenesis and sexual reproduction, have been demonstrated to coexist in the triploid silver Prussian carp (Gui & Zhou 2010). In other words, when the eggs are inseminated by heterologous sperm from other species, they produce a clonal lineage of all females by gynogenesis. However, when homologous sperm of triploid (evolutionary hexaploid) male inseminate eggs of triploid (evolutionary hexaploid) female, the responding development mode is sexual reproduction, which produces recombinant offspring. This reproduction strategy requires production of sperm with a "haploid" chromosome number (in triploids this means a 1.5 ploidy level). The discovery of haploid sperm production of diploid *C. gibelio* males and aneuploid sperm production (close to 1.5n) in triploid *C. gibelio* males was proven by Flajšhans et al. (2008). The theoretical fertilization of eggs of 3n female by homologous sperm (1n) of diploid male should result in offspring with approximately 125 chromosomes. Our results do not support this hypothesis because all of the analysed specimens of the offspring were identified to be triploids with at least 151 chromosomes. It seems that silver Prussian carp with 125 chromosomes are rare, or the number of chromosomes is not in accordance with the viability of fertilized egg or even cannot be formed in ova.

The variability in karyological structure was found in the gynogenetic fish *Poecilia formosa*, which is of hybrid origin (Lamatsch et al. 2004). Hybridisation is considered a main evolutionary factor in the occurrence of gynogenetic polyploid animals (Dawley 1989, Vrijenhoek et al. 1989). In the case of triploid biotype of *C. gibelio*, it is not clear which species could have been the parental ones. However, recent findings of a much higher genetic variability of the genus *Carassius* in Eurasia presented by Takada et al. (2010) and Rylková et al. (2010) open new perspectives in finding a potential parental species.

Acknowledgements

We thank Martin Flajšhans and his colleagues from FROV, JČU in Vodňany for the reproduction of fish, flow cytometry analyses, and his valuable advices regarding the problems associated with ploidy levels of silver Prussian carp. We also thank Šárka Pelikánová and Jörg Bohlen for their meticulous care of the fish. We appreciate proof reading of the manuscript made by Brian Kavalir and finally, we are grateful to two anonymous referees for their comments and recommendations. This study was financially supported by grants: GACR 206/05/2159, IRP FAPPZ, CZU No. MSMT 604670901 and IRP IAPG No. AV0Z50450515.

Literature

Abramenko M.I., Poltavtseva T.G. & Vasetskii S.G. 1998: Discovery of triploid males in lower Don populations of the crucian carp *Carassius auratus gibelio* (Bloch, 1782). *Doklady akademii Nauk 363 (3): 415–418. (in Russian)*

Boroń A. 1994: Karyotypes of diploid and triploid silver crucian carp *Carassius auratus gibelio* (Bloch). *Cytobios 80: 117–124.*

Cherfas N.B. 1966: The natural triploidy in females of unisexual form of silver crucian carp (*Carassius auratus gibelio*, Bloch). *Genetika 5: 16–24. (in Russian)*

Černý J. & Sommer N. 1994: Age, growth and production of the silver crucian carp (*Carassius auratus* L.) in a former side-arm of the middle River Danube in 1985-1990. *Biológia (Bratislava) 49: 247–254. (in Slovak)*

Dawley R.M. 1989: An introduction to unisexual vertebrates. In: Dawley R.M. & Bogart J.P. (eds.), Evolution and ecology of unisexual vertebrates. *Bulletin 466, New York State Museum, Albany, New York: 1–18.*

Fister S. & Soldatovic B. 1989: Karyotype analysis of a gynogenetic population of *Carassius auratus gibelio*, Bloch (Cyprinidae) from Pancevacki Rit. *Acta Veterinaria-Beograd 39 (5–6): 259–268.*

Flajšhans M., Rodina M., Halačka K., Vetešník L., Gela D., Lusková V. & Lusk S. 2008: Characteristics of sperm of polyploid Prussian carp, *Carassius gibelio* (Bloch). *J. Fish Biol. 73: 323–328.*

Golovinskaya K.A., Romashov D.D. & Cherfas N.B. 1965: Unisexual and bisexual crucian carp (*Carassius auratus gibelio*, Bloch) forms. *Vopr. Ikhtiol. 5: 614–629. (in Russian)*

Gui J.F. & Zhou L. 2010: Genetic basis and breeding application of clonal diversity and dual reproduction modes in polyploid *Carassius auratus gibelio*. *Sci. China Life Sci. 53: 409–415.*

Halačka K., Lusková V. & Lusk S. 2003: *Carassius "gibelio"* in fish communities of the Czech Republic. *Ecohydrology & Hydrobiology 3 (1): 133–138.*

Holčík J. & Žitňan R. 1978: On the expansion and origin of *Carassius auratus* in Czechoslovakia. *Folia Zool. 27: 279–288.*

Kalous L., Bohlen J. & Ráb P. 2004: What fish is *Carassius gibelio*: taxonomic and nomenclatoric notes. *ECI XI, Tallin 2004, Abstract Book: 26–27.*

Kalous L. & Šlechtová V. 2004: *Carassius gibelio* autochthonous or exotic species in Europe: molecular phylogenetic evidence. *ECI XI, Tallin 2004, Abstract Book: 122.*

Kalous L., Šlechtová V., Jr., Bohlen J., Petrtýl M. & Švátora M. 2007: First European record of *Carassius langsdorfii* from the Elbe basin. *J. Fish Biol. 70 (Suppl. A): 132–138.*

Kottelat M. 1997: European freshwater fishes. *Biologia Bratislava 52 (Suppl. 5): 51–53.*

Kottelat M. & Freyhof J. 2007: Handbook of European freshwater fishes. 1st ed. *Publications Kottelat, Cornol, Switzerland.*

Kubečka J. 1989: Spreading of the German Carp, *Carassius auratus* (Linnaeus, 1758), in the middle course of the Elbe River. *Muzeum a současnost, Roztoky, ser. natur. 3: 43–50. (in Czech)*

Lamatsch D.K., Nanda I., Schlupp I., Epplen J.T., Schmid M. & Schartl M. 2004: Distribution and stability of supernumerary microchromosomes on natural populations of the Amazon molly, *Poecilia formosa*. *Cytogenet. Genome Res. 106: 189–194.*

Linhart O., Rodina M., Gela D., Kocour M. & Rodriguez M. 2003: Improvement of common carp artificial reproduction using enzyme for elimination of eggs stickiness. *Aquat. Living Resour. 16: 450–456.*

Linhart O., Rodina M., Flajšhans M., Mavrodiev N., Nebesářová J., Gela D. & Kocour M. 2006: Studies on sperm of diploid and triploid tench, *Tinca tinca* (L.). *Aquacult. Int. 14: 9–25.*

Lusk S., Baruš V. & Veselý V. 1977: On the occurrence of *Carassius auratus* in the Morava River drainage area. *Folia Zool. 26: 377–381.*

Lusk S. & Baruš V. 1978: Morphometric features of *Carassius auratus* from the drainage area of the Morava River. *Folia Zool. 27: 177–190.*

Lusk S., Baruš V. & Kirka A. 1980: Current spreading and importance of the giebel (*Carassius auratus gibelio*, Bloch) in Czechoslovakia. *Živočišná výroba 25 (11): 871–878. (in Czech with English summary)*

Lusková V., Halačka K., Vetešník L. & Lusk S. 2004: Changes of ploidy and sexuality status of "*Carassius auratus*" populations in the drainage area of the River Dyje (Czech Republic). *Ecohydrology & Hydrobiology 4 (2): 165–171.*

Peňáz M., Ráb P. & Prokeš M. 1979: Cytological analysis, gynogenesis and early development of *Carassius auratus gibelio*. *Acta Sc. Nat. Brno 13 (7): 1–33.*

Ráb P. & Roth P. 1988: Cold-blooded vertebrates. In: Balíček P., Forejt J. & Rubeš J. (eds.), Methods of chromosome analysis. *Cytogenet. Biol. Soc. Brno: 115–124. (in Czech)*

Ráb P., Bohlen J., Rábová M., Flajšhans M. & Kalous L. 2007: Cytogenetics as a tool in fish conservation:

the present situation in Europe. In: Pisano E., Ozouf-Costaz C., Foresti F. & Kapoor B.G. (eds.), Fish cytogenetics 2007. *Science Publishers, Enfield: 215–241.*

Raicu P., Taisescu E. & Bănărescu P. 1981: *Carassius carassius* and *C. auratus*, a pair of diploid and tetraploid representative species (Pisces, Cyprinidae). *Cytologia 46: 233–240.*

Rylková K., Kalous L., Šlechtová V. & Bohlen J. 2010: Many branches, one root: first evidence for a monophyly of the morphologically highly diverse goldfish (*Carassius auratus*). *Aquaculture 302: 36–41.*

Slavík O. & Bartoš L. 2004: What are the reasons for the Prussian carp expansion in the upper Elbe River, Czech Republic? *J. Fish Biol. 65 (Suppl. A): 240–253.*

Svobodová Z., Pravda D. & Paláčková J. 1991: Unified methods of haematological examination of fish. *Manuals of Research Institute of Fish Culture and Hydrobiology, Vodňany 22: 31.*

Takada M., Tachihara K., Kon K., Yamamoto G., Iguchi K., Miya M. & Nishida M. 2010: Biogeography and evolution of the *Carassius auratus* – complex in East Asia. *BMC Evol. Biol. 10: 7.*

Tóth B., Váradi L., Várkonyi E. & Hidas A. 2000: Silver crucian carp (*Carassius auratus gibelio*, Bloch) in the Danube river basin. *Tiscia monograph series 42: 61–65.*

Tóth B., Várkonyi E., Hidas A., Edviné Meleg E. & Váradi L. 2005: Genetic analysis of offspring from intra- and interspecific crosses of *Carassius auratus gibelio* by chromosome and RAPD analysis. *J. Fish Biol. 66: 784–797.*

Vetešník L. 2005: Biological characteristic of silver Prussian carp (*Carassius auratus*) under the aspect of different ploidy level between individuals. *Ph.D. thesis at Mendel University in Brno.*

Völker M. & Kullmann H. 2006: Sequential chromosome banding from single acetic acid fixed embryos of *Chromaphyosemion* killifishes (Cyprinodontiformes, Nothobranchiidae). *Cybium 30: 171–176.*

Vrijenhoek R.C., Dawley R.M., Cole C.J. & Bogart J.P. 1989: A list of known unisexual vertebrates. In: Dawley R. & Bogart J. (eds.), Evolution and ecology of unisexual vertebrates. *Bulletin 466. Albany, New York State Museum: 19–23.*

Vujosevic M., Zivkovic S., Rimsa D., Jurisic S. & Cakic P. 1983: The chromosomes of 9 fish species from the Dunabe basin in Yugoslavia. *Acta. Biol. Jug.-Ichthyologia 15: 29–40.*

Yi M.S., Li Y.Q., Liu J.D., Zhou L., Yu Q.X. & Gui J.F. 2003: Molecular cytogenetic detection of paternal chromosome fragments in allogynogenetic gibel carp, *Carassius auratus gibelio*, Bloch. *Chromosome Res. 11: 665–671.*

Zhao J., Liu L.G. & Chen X.L. 2004: Karyotypic analysis of the multiple tetraploid allogynogenetic Pengze crucian carp and its parents. *Aquaculture 237: 117–129.*

Zhou L. & Gui J.F. 2002: Karyotypic diversity in polyploid gibel carp, *Carassius auratus gibelio*, Bloch. *Genetica 115: 223–232.*

Folia Zool. – 60 (2): 122–128 (2011)

Fin condition in intensively cultured Eurasian perch (*Perca fluviatilis*)

Vlastimil STEJSKAL*, Tomáš POLICAR, Jiří KŘIŠŤAN, Jan KOUŘIL and Jitka HAMÁČKOVÁ

University of South Bohemia in České Budějovice, Faculty of Fisheries and Protection of Waters, South Bohemian Research Center of Aquaculture and Biodiversity of Hydrocenoses and Institute of Aquaculture, Husova tř. 458/102, 370 05 České Budějovice, Czech Republic; e-mail: stejskal@vurh.jcu.cz

Received 9 June 2010; Accepted 15 September 2010

Abstract. Condition of all fins was assessed in intensively cultured perch (n = 300) in comparison with control pond-reared perch (n = 30). Measurements of maximum fins length as well as a four point photographic scale were used. No damage to any fin was visually observed in the pond-reared group. The first dorsal fin showed the least damage in cultured perch with 93 % of fish demonstrating no erosion. The most affected were paired fins, with only 7 % and 2 % of pectoral and ventral fins, respectively, being non-eroded. No difference between culture systems was found in fin length for the first dorsal and the caudal fin. Pectoral, second dorsal, ventral, and anal fins of intensively cultured perch showed reductions up to 52, 49, 35, and 28 %, respectively. The relationship between fin lengths and standard body length (SL) were described for both groups (SL range 104-170 mm). Results of this study are discussed in relation to aesthetic, welfare and fish survival issues.

Key words: intensive culture, percids, fin erosion, fin damage, welfare

Introduction

Eurasian perch are traditionally cultured using an extensive pond polyculture system, but, for the past decade, intensive culture of this species is also increasingly practiced. Currently, intensive culture of perch mainly utilizes recirculation systems with high stocking density (up to 60 kg m^{-3}), at a constant water temperature (23 °C), using commercial feed (Kestemont et al. 1996, Mélard et al. 1996, Fiogbé & Kestemont 2003). Under such conditions, fin damage (FD), or erosion, has been reported in salmonid species (Kindschi et al. 1991, Wagner et al. 1996a, Moutou et al. 1998, Turnbull et al. 1998, MacLean et al. 2000), but no information on similar problems in perch is currently available.

Several authors have presented reasons for FD in fish species, including water parameters (Bosakowski & Wagner 1994a, Winfree et al. 1998), stocking density (Wagner et al. 1996a, Ellis et al. 2002), fish tank design (Bosakowski & Wagner 1995, Wagner et al. 1996b), feeding strategy (Winfree et al. 1998, Gregory & Wood 1999), social rank (Moutou et al. 1998), and interspecific interactions (Abbott & Dill 1985, Kindschi et al. 1991, MacLean et al. 2000) called fin-nipping. Fin damage is generally considered an indicator of fish welfare (Procarione et al. 1999, North et al. 2006). Eroded fins can be a site for microbial infection (Schneider & Nicholson 1980), and may result in partial fin loss (Kindschi et al. 1991). Damaged fins may also interfere with swimming. Fin erosion or absence can also affect acceptance by consumers and reduce the economic value of fish sold whole. Development of a practical means of preventing FD in intensively reared fish is necessary.

The aims of this study were to (1) evaluate the degree of fin damage in cultured perch, (2) compare fin damage in cultured perch with control fish from pond culture, (3) evaluate the relationship between total fin length and standard body length, and (4) evaluate the relationship among total fin score, body weight, and condition coefficient.

Material and Methods

Fish

Two aquaculture systems, differing mainly in the degree of culture intensity and feeding sources, were compared with respect to fish fin condition. The experimental fish included perch reared intensively

* *Corresponding Author*

in a recirculating system and fed with formulated feed (IC, initial body weight BW 1.9 ± 0.5 g, total length TL 55 ± 3 mm, final BW 49.2 ± 16.5 g, TL – 162.4 ± 22.6 mm) and fish from earth ponds fed natural food (CP, final BW – 35.5 ± 7.9 g, TL – 142.3 ± 10.4 mm).

Culture history of the IC group was: pond-reared (60 days) and habituated perch stocked was in six 50-L aquaria (25 July). Stocking density was 1-1.5 individuals per litre. The trial duration was nine months. Water quality during experimental rearing was kept at the following levels (mean ± S.D., T = 22.9 ± 1.9 °C; pH = 6.9 ± 0.7; dissolved oxygen = 6.8 ± 1.4 mg L^{-1}; ammonia (TAN) 0.46 ± 0.24 mg·l^{-1}; nitrite 0.12 ± 0.03 mg·l^{-1}; nitrate 26.7 ± 2.5 mg·l^{-1}). The values were within the optimum range for rearing of Eurasian perch (Mélard et al. 1996). Fish were fed the commercial feed Ecolife 60 (BioMar, Nersac, France) in rations according to Fiogbé & Kestemont (2003). Final density of IC group was 32.7 ± 4.8 kg m^{-3}.

The CP group (n = 30), comprised wild fish obtained at the autumn harvest of Láska pond (Fishery of Trebon a.s.) which were reared at a density of 0.2 individuals m^{-3} in pond polyculture with common carp (*Cyprinus carpio*). The natural production of the pond was 250 kg ha^{-1}. Perch lived on natural prey (zooplankton and zoobenthos). The main forage fish was topmouth gudgeon (*Pseudorasbora parva*). Fish were harvested on 22 October according to usual fish farm practice and immediately transported to the laboratory for measurement.

Analysis of fin condition

Visual assessment of fin condition: The modified method developed by Moutou et al. (1998) for rainbow trout was used to visually assess the degree of fin damage, where 0 = no or minimal visible damage (< 5 % of fin missing), 1 = minor damage (5 to 30 % of fin missing), 2 = moderate damage (30 % to 70 % of fin missing), and 3 = severe damage (> 70 % of fin missing). Assessment was carried out by an experienced operator who was provided with a photographic key. Both dorsal, pectoral and ventral fins as well as anal and caudal fin were assessed for calculating of the total fin scores. Examples of the degrees of damage for each fin are shown in Figs. 1 and 2. In total, 300 fish from the IC group and the 30 from the CP group were examined.

Total fin length: At the end of the growing trial, five fish were netted from each tank (six tanks, n = 30), mildly anaesthetised in a bath of clove oil 33 mg·l^{-1} (Velíšek et al. 2009), and weighed (± 0.1 g). Control fish (1 pond, n = 30) were submited to same procedure. Digital images of anaesthetized fish were produced with a Panasonic Lumix FZ 50 camera fixed on a tripod. Fish were positioned on a white background and photographs (n = 180) were taken for documentation of fin condition (first and second dorsal, caudal, anal, pectoral, and pelvic). Each fish

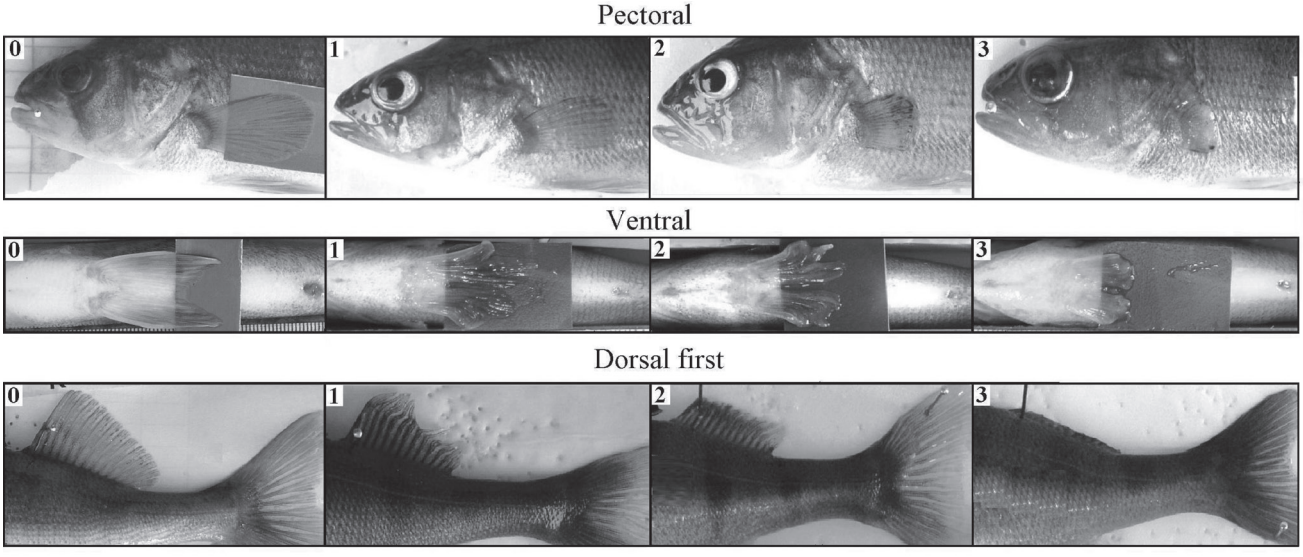

Fig. 1. *Classification of pectoral, ventral and first dorsal fin damage in Eurasian perch. 0 = minimal or no visible damage (< 5 % of fin missing), 1 = minor damage (5-30 % of fin missing), 2 = moderate damage (30-70 % of fin missing), and 3 = severe damage (> 70 % of fin missing).*

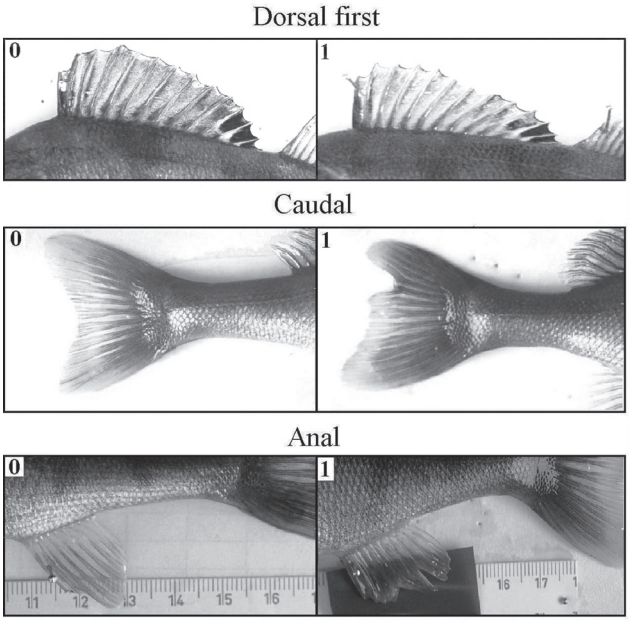

Fig. 2. *Classification of anal, caudal, and first dorsal fin damage in Eurasian perch. 0 = minimal or no visible damage (< 5 % of fin missing), 1 = minor damage (> 5 % of fin missing); 2 and 3 were not found.*

was photographed in left and right lateral views, and ventral view. Images (high-resolution TIFF format) were processed with an image analyzer (Olympus MicroImage v. 4.0 sw) using the manual measurement mode. Data on length measured in milimetres were collected, saved, and transferred to Microsoft Excel 2002 for analysis.

Calculations and statistics: Total fin score for each specimen was calculated as the sum of points for each fin. Relative length of each fin was calculated using followed formula: *RFL = total fin length/standard length × 100.*

Data from IC group was gradually sorted according to body weight and condition factor to obtain two cohorts of fish. Cohort of upper 10 % of fish with best conditon and body weight (probably dominant fish) was compared to the rest population.

Parametric data (body weight, total length, condition factor) were analyzed for normality by the Cochran, Hartley, and Bartlet Test prior to statistical tests. Relative fin lengths (arc-sin transformed), total length, and condition factor were normally distributed, so were compared using Student's t-test. Statistical assessment of all data was carried out with STATISTICA 7.0 (StatSoft Inc., Prague, Czech Republic).

Results

Total fin scores showed high individual variability in intensively cultured perch and ranged from 2 to 17.

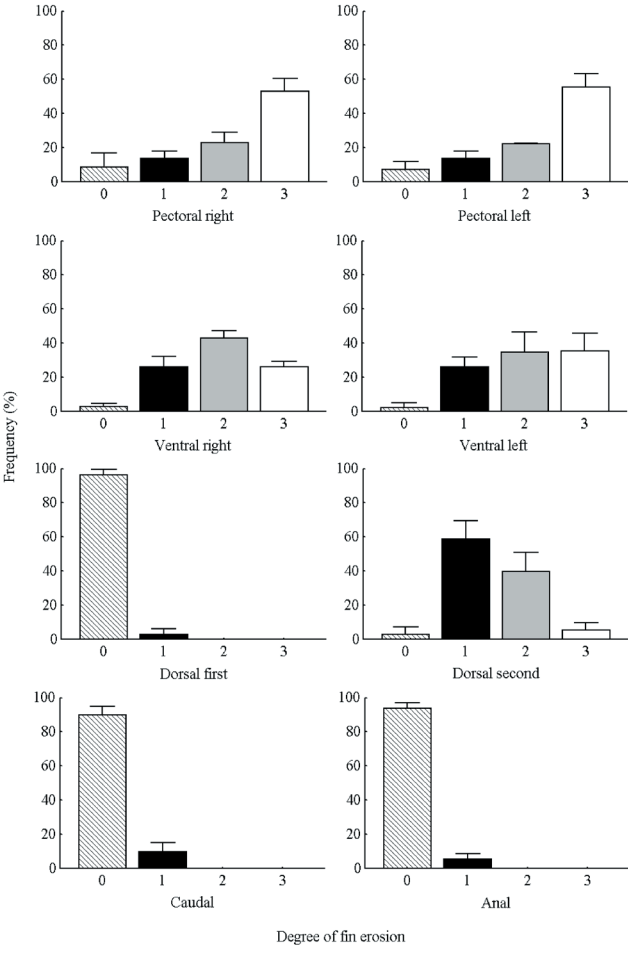

Fig. 3. *Occurrence of fin damage categories in intensively cultured Eurasian perch. 0 = minimal or no visible damage (< 5 % of fin missing), 1 = minor damage (5-30 % of fin missing), 2 = moderate damage (30-70 % of fin missing), and 3 = severe damage (> 70 % of fin missing). Whiskers indicate S.D. (n = 300).*

No FD was found by visual assessment in CP for any fin. The first dorsal fin was intact (category 0) in 93.3 % of IC fish (Fig. 3). In IC fish, no damage to 90.8 % of anal fins and 83.3 % of caudal fins was observed. No instances of FD categories two and three to the first dorsal, caudal, or anal fin were recorded. The most affected were paired fins; only 7 % of pectoral and 2 % of ventral fins were classified as category 0. The second dorsal fin was undamaged in only 4 % of IC fish. There was found no difference ($t = -0.59$, $P = 0.558$) between dominant fish and the rest of population in IC group. A 3D plot was constructed for demonstration of relationships among condition coefficient, body weight, and total score for FD using the method of least squares (Fig. 4). Using linear regression, the relationship can be described with the formula: *Total score = 17.629 + 0.0236BW – 7.8144CC* where BW is body weight (g) and CC is condition coefficient.

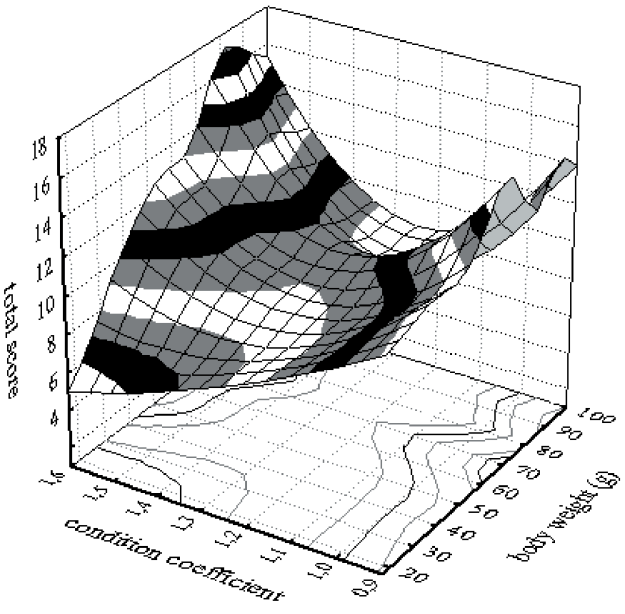

Fig. 4. *Relationships among total body weight, condition coefficient, and total score for fin erosion in cultured Eurasian perch using method of least squares n = 300.*

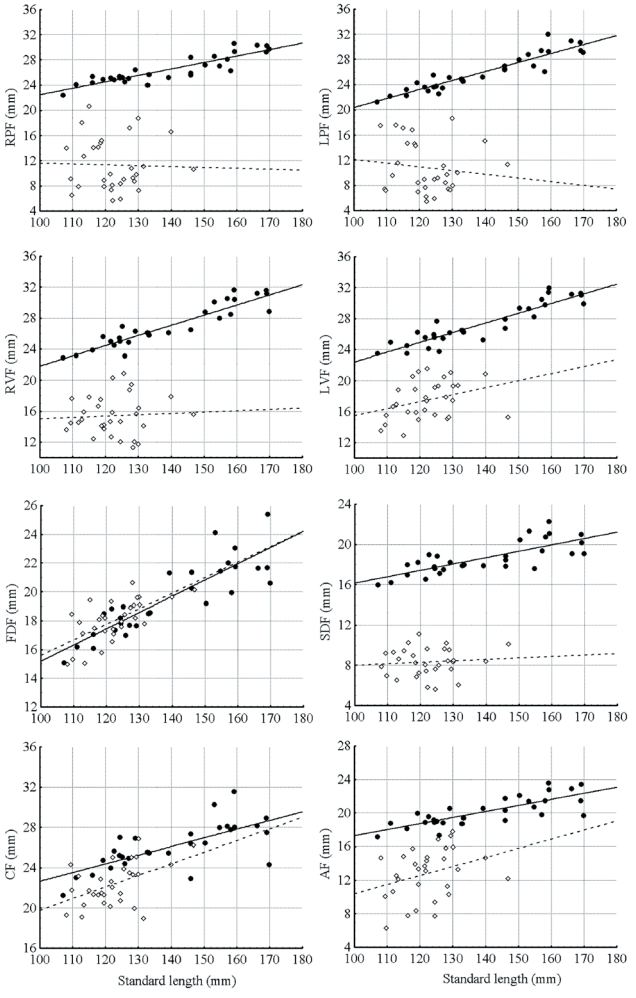

Fig. 5. *Linear regression of maximum fin length to standard body length in control (black circles) and cultured (open diamonds) Eurasian perch (n = 60). LPF = left pectoral fin, RPF = right pectoral fin, RVF = right ventral fin, LVF = left ventral fin, FDF = first dorsal fin, SDF = second dorsal fin, CF = caudal fin, AF = anal fin.*

Linear regression plots were drawn using standard body length to eliminate the effect of eroded caudal fins (Fig. 5). Results demonstrated clear linear relationships between standard length and total fin length for all fins in the control group fish within the sampled range: SL 108-170 mm. Correlation coefficients were greater than 0.72 indicating a strong correlation for all fins in the control group (Table 1). In contrast, in cultured perch, a huge variability in fin length was found for all fins, with the exception of first dorsal and caudal fins.

Comparison of relative fin lengths revealed significant differences between groups in pectoral (left: $t = 14.66$, $P < 0.001$; right: $t = 14.23$, $P < 0.001$), ventral (left: $t = 12.99$, $P < 0.001$; right: $t = 15.07$, $P < 0.001$), second dorsal ($t = 21.45$, $P < 0.001$) and anal ($t = 8.40$, $P < 0.001$) fins (Fig. 6) with lower values for cultured perch. On the other hand, there were no differences in first dorsal ($t = 1.45$, $P < 0.062$) and caudal fin ($t = 1.31$, $P = 0.194$). In addition, both group differ significantly in condition factor ($t = –2.56$, $P = 0.012$), total length ($t = 4.27$, $P < 0.001$) and body weight ($t = 4.37$, $P < 0.001$).

Discussion

Fin damage was observed in the majority of intensively cultured perch, but there was no or minimal damage to the first dorsal and caudal fins. No FD was found in the control pond-reared group for any fin, which confirms other reports (Bosakowski & Wagner 1994a, b). Fin damage was most frequent in the right and left pectoral fin of the IC group. Bosakowski & Wagner (1994a, b) reported the dorsal fin to be the most affected in intensively cultured salmonids, such as brook trout (*Salvelinus fontinalis*), brown trout (*Salmo trutta*), rainbow trout (*Oncorhynchus mykiss*), and cutthroat trout (*Oncorhynchus clarki*). These authors reported that 46-90 % of salmonids (with species specific differences) had no affected pectoral fins, in contrast to the fish in this study (Fig. 3). Intensively cultured perch showed a reduction of up to 52 % in pectoral fin length, while salmonids show less reduction of

Table 1. *Regression of maximum fin length with standard body length in cultured (n = 30) and control (n = 30) Eurasian perch. r = correlation coefficient, p = ANOVA probability that slope equals zero, m = slope, b = y-intercept, LPF = left pectoral fin, RPF = right pectoral fin, RVF = right ventral fin, LVF = left ventral fin, FDF = first dorsal fin, SDF = second dorsal fin, CF = caudal fin, AF = anal fin.*

	RPF	LPF	RVF	LVF	FDF	SDF	CF	AF
control	r = 0.89	0.93	0.92	0.91	0.87	0.76	0.72	0.80
	r^2 = 0.79	0.86	0.84	0.83	0.76	0.58	0.51	0.63
	p = < 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
	m = 0.103	0.143	0.131	0.125	0.113	0.063	0.086	0.072
	b = 12.164	6.021	8.725	9.858	3.915	9.790	14.100	10.039
cultured	r = 0.03	0.13	0.06	0.33	0.65	0.09	0.48	0.32
	r^2 = < 0.01	0.02	< 0.01	0.10	0.42	< 0.01	0.23	0.10
	p = 0.875	0.495	0.753	0.075	< 0.001	0.630	0.001	0.084
	m = –0.014	–0.059	0.017	0.091	0.108	0.014	0.115	0.107
	b = 13.001	17.996	13.282	6.414	4.808	6.567	8.327	–0.366

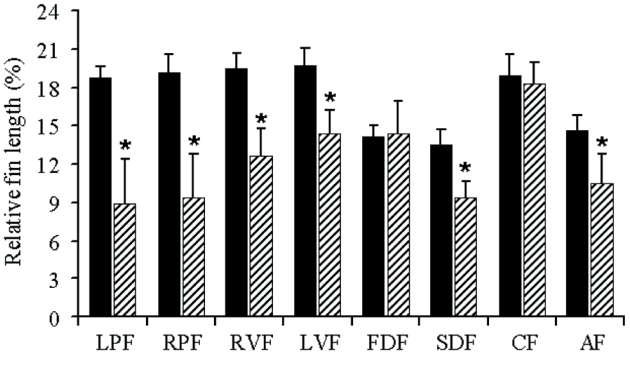

Fig. 6. *Comparison of mean values of relative fin length between control (dark bars) and cultured (striped bars). Asterisks indicate significant differences between bars (p < 0.05). LPF = left pectoral fin, RPF = right pectoral fin, RVF = right ventral fin, LVF = left ventral fin, FDF = first dorsal fin, SDF = second dorsal fin, CF = caudal fin, AF = anal fin. Whiskers indicate S.D. (n = 30).*

length in these fins (Bosakowski & Wagner 1994b). No or minimal FD to the first dorsal fin was observed, probably due to the bony structure of this fin. The second dorsal fin was affected, but with more than 58 % of fish having only minor damage (degree 1). The dorsal fin is also reported to be affected in salmonids at similar levels (40-74 %) (Bosakowski & Wagner 1994a). We found size reductions of up to 49 % in the second dorsal fin, in agreement with results for salmonids (Bosakowski & Wagner 1994b). Only 2 % of perch examined were without damage to ventral fins. Hence, the ventral fin was the most frequently affected, while reduction of length (35 %) was lowest in comparison to pectoral fins. Bosakowski & Wagner (1994b) reported a smaller reduction in ventral fins in salmonids. The present study found no or minimal incidence of FD to caudal fins, similar to results in rainbow trout. On the other hand, 30 % of brook trout and brown trout examined were found to show damage to this fin (Bosakowski & Wagner 1994a).

The suggestion that the largest perch, in best condition (probably dominant fish), would show less FD compared to smaller fish in poorer condition was not confirmed (Fig. 4). On the contrary, a high degree of FD was observed in these fish, possibly as a result of aggressive feeding behaviour and strong competition for food. A similar effect has been observed in Atlantic salmon (*Salmo salar*) where dominant fish compete aggressively and incur fin damage, while less aggressive individuals adopt alternative feeding strategies, which result in lower food intake and growth but reduce the risk of FD (MacLean et al. 2000). Several social cohorts with varying feeding strategies were observed in the perch population. The lowest total score for FD was observed in fish with a CC ranging from 1.1 to 1.3, irrespective of body size (fish growth). Fast-growing fish with significantly higher or lower CC incurred a higher total FD score (Fig. 4). On the other hand, the lowest total FD score was observed in groups of poorly-growing perch with high CC. These were probably less aggressive individuals with alternative feeding strategies resulting in lower food intake and growth, but probably reduced the risk of FD. On the other hand, Moutou et al. (1998) reported that subordinate rainbow trout had higher FD on the dorsal fin.

Fin damage in other intensively cultured fish species has been attributed to high stocking density (Wagner et al. 1996a, Procarione et al. 1999, Ellis et al. 2002), fin-nipping (Abbott & Dill 1985), or abrasion from

rough tank surfaces (Bosakowski & Wagner 1995, Wagner et al. 1996b, Moutou et al. 1998). The glass aquaria used in our study minimized tank abrasion. Stocking density was lower than the maximum reported by Mélard et al. (1996), and water quality parameters were kept at optimal levels for perch (Kestemont et al. 1996, Mélard et al. 1996). There was no observed bacterial or fungal disease which could be a causative agent of FD, as has been previously reported (Schneider & Nicholson 1980). Intraspecific fin-nipping behaviour was observed. Therefore, we suggest that fin-nipping was the major cause of FD in our study.

Fin erosion in intensively reared salmonids can affect post-stocking survival, increase the likelihood of disease, and reduce the aesthetic appeal of fish to the consumer (Schneider & Nicholson 1980, Bosakowski & Wagner 1994a). In perch, eroded fins could be niches for secondary pathogens such as saprophytic fungi or bacteria as well as sites of ion loss, especially under wild conditions. Signs of bacterial or fungal diseases were observed when perch with eroded fins were kept in flow-through systems at lower temperatures (8-12 °C); however, no signs were observed in recirculating aquaculture systems, probably due to higher water salinity (Stejskal, unpublished data).

Food fish with FD can have reduced marketability. In some cases it also reduces osmoregulatory control and disrupts homoeostasis. Therefore fin damage is considered an indicator of welfare in a variety of cultured fish (Procarione et al. 1999, European Commission 2004, North et al. 2006). According to Latremouille (2003) there are several possible means of reducing FD in fish (salmonids), including increasing water speed, feeding to satiation, and tank design. Significantly reduced or no FD was observed in perch reared in a recirculating system with lower efficiency of solid waste removal resulting in higher turbidity (Stejskal, unpublished data). Accordingly, water turbidity may play a role in reduction of FD, and the use of clay or other substances to artificially increase turbidity should be evaluated as a protective treatment. Future research on perch should be focused on the impact of water turbidity, rearing density, feeding level, and water velocity, to reduce fin damage. Our study showed that FD is more frequent in intensively cultured perch. Fish size and condition does not seem to be a substantial factor explaining occurrence and intensity of FD. Pectoral, ventral, and second dorsal fins are the main sites of FD in perch.

Acknowledgements

This work was supported by MSM project (No. 6007665809), NAZV projects (No. QI101C033 and No. QH91310), GAJU project (No. 047/2010/Z), KONTAKT project (No. ME 10126) and project CENAQUA (CZ.1.05/2.1.00/01.0024).

Literature

Abbott J.C. & Dill L.M. 1985: Patterns of aggressive attack in juvenile steelhead trout (*Salmo gairdneri*). *Can. J. Fish. Aquat. Sci. 42: 1702–1706.*

Bosakowski T. & Wagner E.J. 1994a: Assessment of fin erosion by comparison of the relative fin length in hatchery and wild brown trout in Utah. *Can. J. Fish. Aquat. Sci. 51: 636–641.*

Bosakowski T. & Wagner E.J. 1994b: A survey of trout fin erosion, water quality, and rearing conditions at state fish hatcheries in Utah. *J. World Aquac. Soc. 25: 308–316.*

Bosakowski T. & Wagner E.J. 1995: Experimental use of cobble substrates in concrete raceways for improving fin condition of cutthroat (*Oncorhynchus clarki*) and rainbow trout (*O. mykiss*). *Aquaculture 130: 159–165.*

Ellis T., North B., Scott A.P., Bromage N.R., Porter M. & Gadd D. 2002: The relationships between stocking density and welfare in farmed rainbow trout. *J. Fish. Biol. 61: 493–531.*

European Commission 2004: Farmed fish and welfare. *European Commission, Directorate-General for Fisheries, Research and Scientific Analysis Unit (A4). Brussels, Belgium.*

Fiogbé E.D. & Kestemont P. 2003: Optimum daily ratio for Eurasian perch *Perca fluviatilis* L. reared at its optimum growing temperature. *Aquaculture 216: 234–252.*

Gregory T.R. & Wood C.M. 1999: Interactions between individual feeding behaviour, growth, and swimming performance in juvenile rainbow trout (*Onchorhyncus mykkis*) fed different rations. *Can. J. Fish. Aquat. Sci. 56: 479–486.*

Kestemont P., Mélard C., Fiogbé E.D., Vlavonou R. & Masson G. 1996: Nutritional and animal husbandry aspects of rearing early life stages of Eurasian perch *Perca fluviatilis*. *J. Appl. Ichthyol. 12: 157–165.*

Kindschi G.A., Shaw H.T. & Bruhn D.S. 1991: Effect of diet on performance, fin quality and dorsal lesions in steelhead. *J. Appl. Aquacult. 1: 113–120.*

Latremouille D.N. 2003: Fin erosion in aquaculture and natural environments. *Rev. Fish. Sci. 11: 315–335.*
MacLean A., Metcalfe N.B. & Mitchell D. 2000: Alternative competitive strategies in juvenile Atlantic salmon *Salmo salar*: evidence from fin damage. *Aquaculture 184: 291–302.*
Mélard C., Kestemont P. & Grignard J.C. 1996: Intensive culture of juvenile and adult Eurasian perch (*Perca fluviatilis*): effect of major biotic and abiotic factor on growth. *J. Appl. Ichthyol. 12: 175–180.*
Moutou K.A., McCarthy I.D. & Houlihan D.F. 1998: The effect of ration level and social rank on the development of fin damage in juvenile rainbow trout. *J. Fish. Biol. 52: 756–770.*
North B.P., Turnbull J.F., Ellis T., Porter M.J., Migaud H., Bron J. & Bromage N.R. 2006: The impact of stocking density on the welfare of rainbow trout (*Oncorhynchus mykiss*). *Aquaculture 255: 466–479.*
Procarione L.S., Barry T.P. & Malison J.A. 1999: Effects of high rearing densities and loading rates on the growth and stress response of juvenile rainbow trout. *N. Am. J. Aquac. 61: 91–96.*
Schneider R. & Nicholson B.L. 1980: Bacteria associated with fin rot disease in hatchery-reared Atlantic salmon (*Salmo salar*). *Can. J. Fish. Aquat. Sci. 37: 1505–1513.*
Turnbull J.F., Adams C.E., Richards R.H. & Robertson D.A. 1998: Attack site and resultant damage during aggressive encounters in Atlantic salmon (*Salmo salar* L.) parr. *Aquaculture 159: 345–353.*
Velíšek J., Stejskal V., Kouřil J. & Svobodová Z. 2009: Comparison of the effects of four fish anaesthetics on biochemical blood profile of perch (*Perca fluviatilis* L.). *Aquacul. Res. 40: 354–361.*
Wagner E.J., Intelmann S.S. & Routledge D. 1996a: The effects of fry rearing density on hatchery performance, fin condition, and agonistic behaviour of rainbow trout *Oncorhynchus mykiss* fry. *J. World Aquac. Soc. 27: 264–274.*
Wagner E.J., Routledge M.D. & Intelmann S.S. 1996b: Fin condition and health profiles of albino rainbow trout reared in concrete raceways with and without a cobble substrate. *The Progressive Fish-Culturist 58: 38–42.*
Winfree R.A., Kindschi G.A. & Shaw H.T. 1998: Elevated water temperature, crowding, and food deprivation accelerate fin erosion in juvenile steelhead. *Prog. Fish. Cult. 60: 192–199.*

Folia Zool. – 60 (2): 129–142 (2011)

Winter diet of great cormorant (*Phalacrocorax carbo*) on the River Vltava: estimate of size and species composition and potential for fish stock losses

Martin ČECH* and Lukáš VEJŘÍK

Biology Centre of the Academy of Sciences of the Czech Republic, Institute of Hydrobiology, Na Sádkách 7, 370 05 České Budějovice, Czech Republic; e-mail: carcharhinusleucas@yahoo.com

Received 22 June 2010; Accepted 5 November 2010

Abstract. The winter diet of the great cormorant (*Phalacrocorax carbo*) was studied by means of examining regurgitated pellets, individual fish bones and fish remains collected from below the roosting trees in two sites on the River Vltava in Vyšší Brod and at Slapy Reservoir, Czech Republic, and by analysis of stomach contents of birds shot on the River Vltava in Prague. Using diagnostic bones (*os pharyngeum*, *dentale*, *maxillare*, *praeoperculare*) and own linear regression equations between measured dimension of the diagnostic bone and fish total length (L_T), a total of 1152 fish of 22 species and 6 families were identified in the diet of great cormorants and their sizes were reconstructed. At all three localities on the main stream of the River Vltava, roach (*Rutilus rutilus*), bream (*Abramis brama*), bleak (*Alburnus alburnus*), European chub (*Squalius cephalus*), European perch (*Perca fluviatilis*) and ruffe (*Gymnocephalus cernuus*) made up at least 74.2 % of the cormorants' diet. A great potential for fish stock losses was identified for the River Vltava at Vyšší Brod and in Prague where the loss of fish due to overwintering great cormorants was estimated to be 22 kg ha^{-1} and up to 79 kg ha^{-1} respectively, i.e. belonging among the highest ever published figures for fish withdrawal caused by great cormorants from any inland waters (carp fishponds excluded). Most probably, both great cormorants and anglers are responsible for the decrease in catches of brown trout (*Salmo trutta* m. *fario*) and grayling (*Thymallus thymallus*) from the River Vltava in Vyšší Brod.

Key words: diagnostic bones, European chub, European perch, fish withdrawal, grayling, regurgitated pellets, roach, ruffe, Slapy Reservoir, trout spp.

Introduction

The great cormorant (*Phalacrocorax carbo*) as highly efficient avian fish predator (Grémillet 1997, Grémillet et al. 2001) is able to cause serious losses to both marine (e.g. Barrett et al. 1990, Leopold et al. 1998, Johansen et al. 1999, Lilliendahl & Solmundsson 2006) and farm fisheries (Lekuona 2002) as well as to freshwater fisheries (Stewart et al. 2005). The large increase in the number of great cormorants in continental Europe since 1980 has provoked widespread conflict with both commercial and sport/recreational fisheries, which in turn has led to an upsurge in diet analyses. Excluding carp fishponds, blaming great cormorants for negative effects on wild freshwater fish populations and yields has many times had surprisingly little support in the results of dietary studies carried out in various European countries and on various types of waters (for exception see Mous 2000). Mostly, it is considered unlikely that birds impose a serious threat to either commercial or recreational fisheries, since there is only a small overlap between the cormorants' diet and valuable prey, suggesting minimal competition with human interests (e.g. Keller 1995, Keller 1998, Engström 2001, Carss & Ekins 2002, Wziątek et al. 2005, Liordos & Goutner 2007, Liordos & Goutner 2008). In the

* *Corresponding Author*

cases of eutrophic lakes and water supply reservoirs, by taking away large amounts of zooplanktivorous fish, great cormorants are, moreover, considered to have a positive influence on water quality by reducing the overexploitation of zooplankton (Dirksen et al. 1995, Veldkamp 1995, Čech 2004, Čech & Čech 2009). Besides juvenile flatfish (Pleuronectiformes) and European eel (*Anguilla anguilla*) in coastal habitats (Leopold et al. 1998, Carss & Ekins 2002), the only fish threatened by great cormorants feeding in inland waters seems to be grayling (*Thymallus thymallus*) (Suter 1997, Staub et al. 1998). However, the real effect of great cormorants on the annual yields and population dynamics of this vulnerable species is still a subject of heated debate (cf. Suter 1995, Staub et al. 1998, Suter 1998).

In the Czech Republic, excluding carp fishponds again, the main problem with great cormorants is undoubtedly concentrated on rivers below reservoirs, where secondary salmonid stretches have been found due to the discharge of relatively cold – hypolimnetic water from the reservoirs upstream (the whole year round discharge of relatively cold water, resulting, however, in ice-free conditions during winter). These river stretches are very interesting localities for both anglers (open season from mid-spring to mid-fall) and great cormorants (winter). A typical example is the River Vltava below Lipno I and Lipno II Reservoirs – i.e. the River Vltava in Vyšší Brod (Fig. 1), where the fisheries Vltava 28 and Vltava 27 are among the best Czech sport fisheries for trout spp. and grayling. Since the mid-90s these river stretches have also been visited by overwintering great cormorants (at a maximum of up to > 200 birds; K. Křivanec, unpublished data). At the same time, anglers started to blame the great cormorants for reduced yields of the two native fish species – brown trout (*Salmo trutta* m. *fario*) and grayling. Their opinion was well supported by the official catch statistics of the Czech Anglers Union. For example, in the fishery Vltava 28 the catches of grayling have decreased significantly since 1996 (regression analysis: $r^2 = 0.96$, $F_{1, 5} = 129.75$, $P < 0.001$; Fig. 2) from 597 fish caught in 1996 to only 52 in 2002 (> 91 % reduction). In 2003, fishing for grayling was completely prohibited in this fishery. Similarly, the catches of brown trout have also decreased dramatically since 1999 (regression analysis: $r^2 = 0.88$, $F_{1, 3} = 21.86$, $P < 0.05$; Fig. 2) from 1236 fish caught in 1999 but only 254 in 2003 (> 79 % reduction). The same trends in catches of grayling and brown trout were observed for the neighbouring fishery Vltava 27 (Czech Anglers Union, unpublished data).

The aim of this study was to analyze the winter diet of great cormorants on the River Vltava in Vyšší Brod (species and size composition) and evaluate the losses caused to the recreational fisheries (total fish consumption, fish withdrawal per ha of targeted fisheries). The results were compared with two other stretches of the River Vltava where the predation pressure and losses caused by overwintering great cormorants are supposed to be either less important (the River Vltava in Prague) or even negligible (Slapy Reservoir).

Study Area

The study was carried out at three great cormorant roosting sites on the main stream of the River Vltava, Czech Republic – the River Vltava in Vyšší Brod (*c*. 320 river km; winter 2004/05), Slapy Reservoir (*c*. 108 r. km; winter 2005/06) and the River Vltava in Prague – Troja

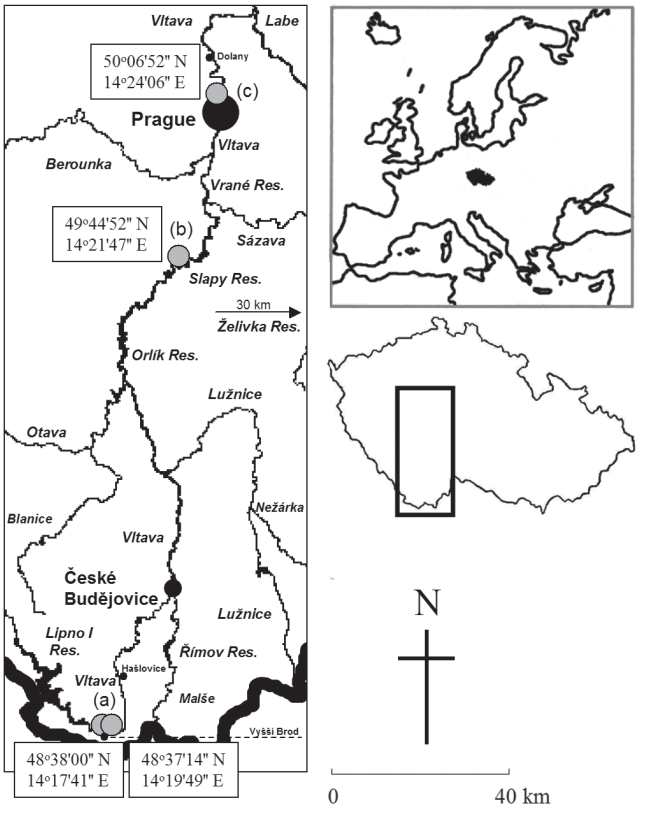

Fig. 1. *A map of the main River Vltava basin, its locations in the Czech Republic and positions of individual roosting places (grey dots) of great cormorant (*Phalacrocorax carbo*) where regurgitated pellets, fish bones and sporadic fish remains were collected (a), (b) or where birds were shot (c). Latitude and longitude is given for each roosting place. (a) the River Vltava in Vyšší Brod (roosting place at Lipno II Reservoir and at the River Vltava); (b) Slapy Reservoir; (c) the River Vltava in Prague – Troja.*

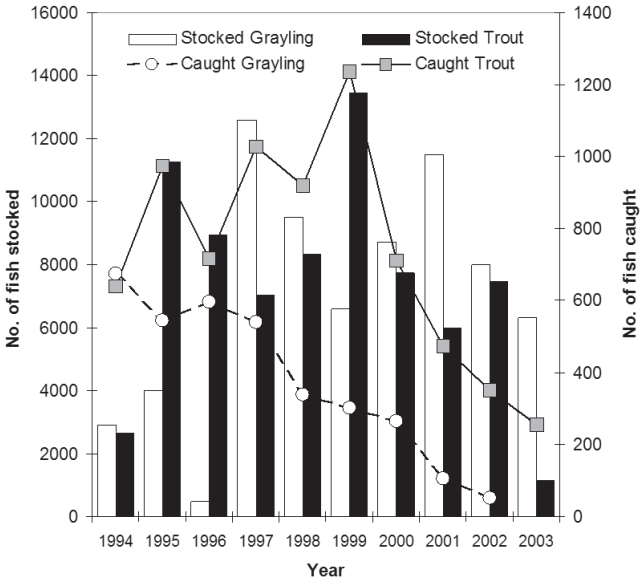

Fig. 2. *An example of stocking and catch statistics of grayling (*Thymallus thymallus*) and brown trout (*Salmo trutta *m.* fario*) at one of the best trout fisheries in the Czech Republic – Vltava 28 (Czech Anglers Union, unpubl. data), where heavy predation by overwintering great cormorants on fish stock has occurred since mid 90s. Note that in 2003 fishing for grayling was completely prohibited in this fishery.*

(*c*. 45 r. km; winter 2006/07 and 2007/08) (Fig. 1).

At Vyšší Brod, the great cormorants roost on one large spruce (*Picea abies*) at Lipno II Reservoir (area 47 ha, length 2 km, mean and maximum depth 3.6 m and 9 m, volume 1.7 × 10^6 m^3, meso- to eutrophic, 190 km south of Prague) and on spruces and pines (*Pinus sylvestris*) near the River Vltava (mean river depth 0.9 m) approximately 3 km further downstream (on average 73 birds per winter 2004/05, M. Čech, M. Hladík, unpublished data). Lipno II Reservoir serves to regulate water level fluctuations when the hydropower station of Lipno I Reservoir is in operation. Consequently, ice coverage of the reservoir itself and of the river further downstream is very rare. The potential fishing habitat for great cormorants at the River Vltava in Vyšší Brod was Lipno II Reservoir (fishery Vltava 29) and two other river fisheries – Vltava 28 (area 29 ha) and Vltava 27 (area 40 ha) down to the Hašlovice, 15 km north of the roosting colony. Birds have never been observed fishing upstream of the Lipno II Reservoir in the shallow, fast flowing river (M. Hladík, K. Křivanec, pers. comm.). Due to a relatively high altitude (> 500 m a.s.l.) and "normal" winter conditions (mean ± S.D. air temperature –1.3 ± 5.5 °C; Czech Hydrometeorological Institute, unpublished data) other fishing localities, especially ponds and the large Lipno I Reservoir (area 4820 ha, altitude 726 m a.s.l.) were covered by ice for the whole winter, i.e. fish were not accessible to cormorants from these localities.

At Slapy Reservoir (area 1392 ha, length 42 km, mean and maximum depth 19.3 m and 58 m, volume 269 × 10^6 m^3, meso- to eutrophic, 40 km south of Prague), great cormorants roost on pines and oaks (*Quercus robur*) on a steep bank below the Vymyšlenská pěšina natural reserve (on average 60 birds per winter 2005/06, Čech et al. 2008). For most of the winter, the reservoir is filled with relatively warm, hypolimnetic water (7.7-8.5 °C; Čech et al. 2007) discharged from the reservoirs upstream. Because of its high mean annual inflow of 85 m^3 s^{-1}, resulting in a theoretical retention time of only 38.5 days, and low altitude (271 m a.s.l.) (Hrbáček & Straškraba 1966), even in severe winters (winter 2005/06 lasted for 4.5 months; mean ± S.D. air temperature –2.5 ± 4.3 °C; Czech Hydrometeorological Institute, unpubl. data) the reservoir is covered by ice for less than two weeks. Since all other water bodies near to Slapy Reservoir were covered by ice for the whole winter of 2005/06 the great cormorants were forced to forage exclusively on this reservoir.

On the River Vltava in Prague – Troja (mean river depth 1.8 m, eu- to hypertrophic), great cormorants roost on poplars (*Populus tremula*) in close proximity to the sewerage plant. Extremely warm weather during the winter of 2006/07 (the warmest winter since year 1922; Czech Hydrometeorological Institute, unpublished data) and very similar weather during the winter of 2007/08 (mean ± S.D. air temperature 6.6 ± 1.8 °C in winter 2006/07 and 4.2 ± 2.5 °C in winter 2007/08) prevented ice covering the River Vltava in Prague and enabled roosting and foraging of over 1000 great cormorants there (P. Musil, pers. comm. and unpublished data). For the purpose of this study, great cormorants roosting on the River Vltava in Prague – Troja were considered to be foraging on the fisheries Vltava 3-7 (528 ha), i.e. from the dam of Vrané Reservoir, 20 km south of the roosting colony, to the weir at Dolany 15 km north of the roosting colony. Due to the lack of valuable telemetric data, however, foraging on other localities further upstream or further downstream could not be completely excluded (Š. Rusňák, J. Andreska, pers. comm.).

Material and Methods

The species composition and sizes of fish prey in the diet of great cormorants were investigated from

regurgitated pellets, individual bones and sporadic fish remains collected below the roosting trees on 1 and 21 January, 4 February and on 1 April 2005 (the River Vltava in Vyšší Brod) and on 8 April 2006 (Slapy Reservoir). In detail, *c*. 100 m^2 of the ground was searched each time in the case of the River Vltava in Vyšší Brod, and *c*. 250 m^2 at Slapy Reservoir, from which 1150 ml and 2000 ml of food remains, respectively, were collected. Whole regurgitated material was immersed for one week in concentrated detergent solution, then washed through a sieve (mesh size 1 mm), dried at room temperature and analyzed under a binocular magnifying glass (magnification 8 times and 16 times).

Similar to the findings of Carss et al. (1997) and Čech et al. (2008) at both roosts studied (the River Vltava in Vyšší Brod, Slapy Reservoir), the regurgitated pellets, fish bones and remains are, immediately after the roosting season (frequently even during the roosting season), scavenged by red foxes (*Vulpes vulpes*), feral pigs (*Sus scrofa*) and pine martens (*Martes martes*). Contamination of the samples by regurgitated pellets and fish remains from previous years is therefore negligible.

At the River Vltava in Prague, great cormorants were shot before sunset when arriving at the night roosting trees, i.e. after the second foraging peak of the day, on 27 February (n = 5), 1 March (n = 2), 1 December (n = 8), 2 December (n = 3), 3 December (n = 1), 4 December (n = 1) and 31 December (n = 3) 2007 and on 2 January (n = 1) and 5 January (n = 2) 2008. Permission to shoot was granted by the Department of Nature Conservation of the Prague City Hall. Immediately after the shooting, birds killed were picked up from the water using boats, then measured, weighed and sexed. The stomach and oesophagus were dissected from each bird and deep frozen for later analysis. In the laboratory, analysis of stomach contents was carried out in a similar way to that used for the pellets, since the soft tissues of all fish were at least partly digested (40 % digestion at least; oesophaguses were empty).

To identify the species and sizes of fish preyed upon, a reference collection of diagnostic bones was constructed for each of the potential prey species (see Čech et al. 2008 and this study). For dissection of the bones, fish were taken from gill net and seine net catches from Římov Reservoir and from dip net and fishing rod catches from various streams, rivers and ponds belonging to the River Vltava basin in the years 2000-2008. In total, 254 fish were measured (total length, L_T, to the nearest 0.1 cm), boiled, dissected and the diagnostic bones, selected according to Hallet (1977, 1982), Reynolds & Hinge (1996), Čech & Čech (2006) and Čech et al. (2008), were measured to the nearest 0.1 mm (Fig. 3). Pharyngeal bones (*os pharyngeum*) were selected for cyprinid species (Cyprinidae), lower jaws (*dentale*) for European eel, grayling, trout spp., northern pike (*Esox lucius*) and percid species (Percidae), upper jaws (*maxillare*) for trout spp. and preopercular bones (*praeoperculare*) for bullhead (*Cottus gobio*) and percid species. The measurements selected were the pharyngeal bone tip, PhT, for cyprinid species, dental length, DeL, for European eel, trout spp., northern pike and percid species, maxilar length, MxL, for trout spp., preopercular length, PpL, for bullhead and the preopercular gape, PpG, for percid species (Fig. 3, see also Čech et al. 2008). From the reference material collected, a linear regression equation was established for each of the seven prey species and one hybrid, between the measured dimension of the diagnostic bone and fish total length (Table 1). For the rest of the fish species linear regression equations were taken from the work of Čech et al. (2008; another 357 fish originated from the River Vltava basin dissected for diagnostic bones).

The species-specific identification of salmonid fishes (Salmonidae) is possible only using the praevomer (*praevomer* – a relatively small, fragile bone from the top of the mouth cavity), while the habitus of lower and upper jaws as well as e.g. intermaxilar (*intermaxillare*) and palatal (*palatinum*) bones appear to be the same. Unfortunately, no praevomers were found in the samples, so the category "trout spp." refers to both native brown trout and non-native rainbow trout (*Oncorhynchus mykiss*) and brook trout (*Salvelinus fontinalis*).

Mass estimates for the fish prey were obtained by using a length-weight regression equation for each fish species from either Želivka Reservoir (Prchalová et al. 2005) or Římov Reservoir and its tributary (J. Kubečka, M. Prchalová, unpublished data), both belonging to the River Vltava basin.

To estimate winter (16 December-15 March) fish withdrawal caused by great cormorants in individual fisheries, the following equation was used: $FW = (DFI \times N \times D) / A$ where *FW* is the fish withdrawal caused by a roosting colony of great cormorants in the targeted fisheries during the appropriate winter, *DFI* is the daily food intake of individual bird (calculation see below and Fig. 4), *N* is the average number of great cormorants in the roosting colony during the appropriate winter, *D* is the number of foraging

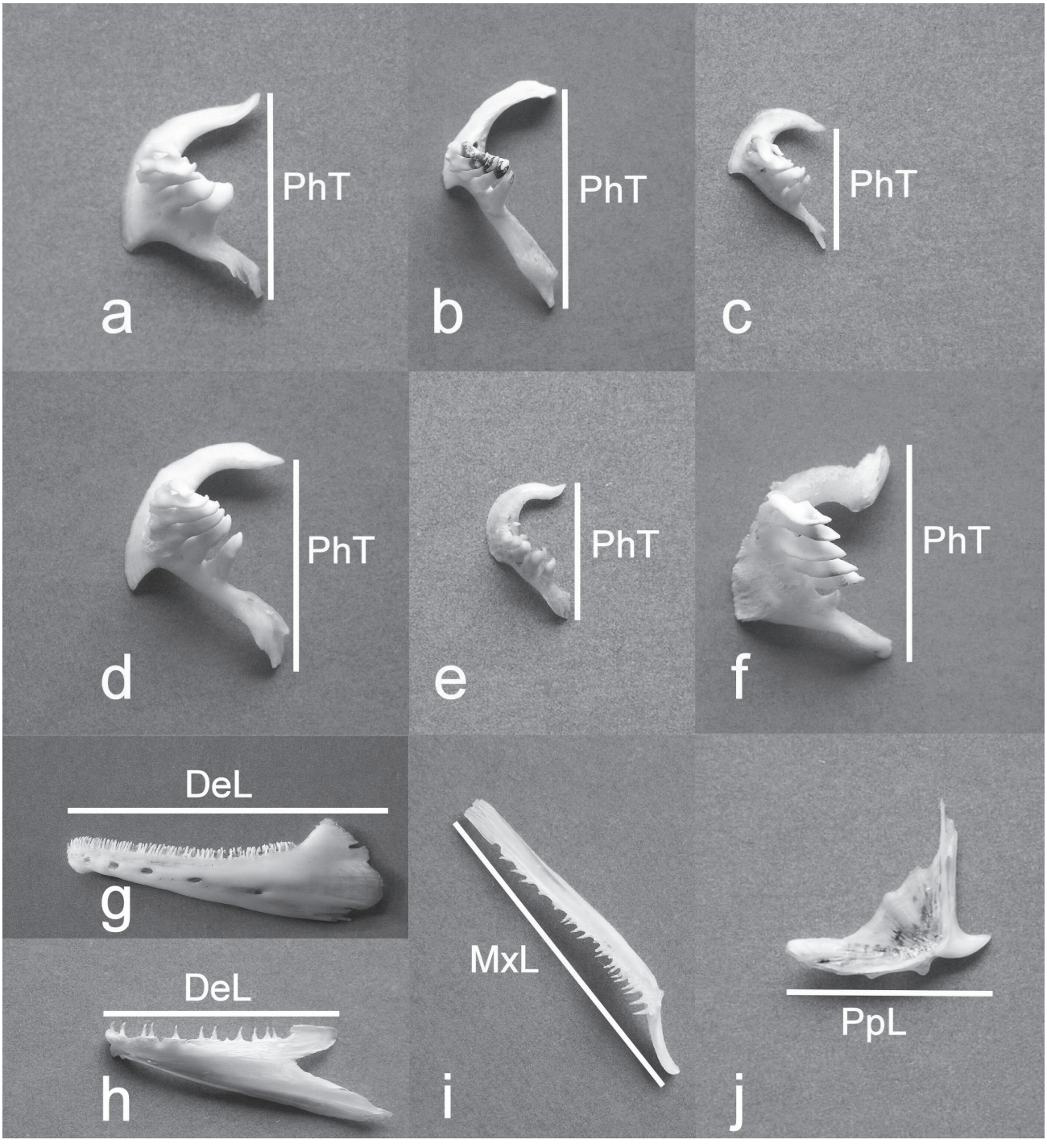

Fig. 3. *Diagnostic bones of selected fish species: pharyngeal bone (os pharyngeum) of (a) roach, (b) bream, (c) common dace, (d) roach × bream hybrid, (e) gudgeon, (f) nase; lower jaw (dentale) of (g) European eel, (h) trout spp.; upper jaw (maxillare) of (i) trout spp.; preopercular bone (praeoperculare) of (j) bullhead. The white line indicates the measurement. PhT, pharyngeal tip; DeL, Dental length; MxL, maxilar length; PpL, preopercular length. Photo M. Čech.*

days (note that $DFI \times N \times D$ is equal to the total fish consumption per roosting colony) and A is the area of potential foraging habitats, i.e. the area of targeted fisheries.

For estimation of the daily food intake (DFI) of great cormorant, the assumption of Carss et al. (1997) that pellets, the stomach contents of shot birds and direct feeding observations cannot be used to derive good estimates of DFI because of the associated biases in estimating diet, was taken into consideration.

Table 1. *Regression equations of total length (L_T; cm) on bone dimensions (mm) for the seven prey fish species and one hybrid. Numbers in parentheses represent the range of fish lengths (cm) from which the equations are derived. PhT, pharyngeal tip; DeL, dental length; MxL, maxilar length; PpL, preopercular length (for details see Fig. 3).*

Species	n	Equation
Roach (*Rutilus rutilus*)	75	$L_T = 1.5658PhT + 0.2805$ $r^2 = 0.9918$ (4.1 – 38.0)
Common dace (*Leuciscus leuciscus*)	10	$L_T = 1.8579PhT - 0.9119$ $r^2 = 0.9858$ (9.9 – 18.4)
Gudgeon (*Gobio gobio*)	110	$L_T = 1.9278PhT - 0.0653$ $r^2 = 0.9592$ (2.8 – 14.0)
Nase (*Chondrostoma nasus*)	7	$L_T = 1.4818PhT + 7.5102$ $r^2 = 0.931$ (18.0 – 48.0)
Roach × bream hybrid (*Rutilus rutilus* × *Abramis brama*)	30	$L_T = 1.743PhT + 0.943$ $r^2 = 0.9749$ (13.0 – 38.5)
European eel (*Anguilla anguilla*)	10	$L_T = 1.7691DeL + 8.800$ $r^2 = 0.9799$ (24.0 – 88.0)
Trout spp.	5	$L_T = 1.3191DeL + 2.0274$ $r^2 = 0.9901$ (12.4 – 36.0) $L_T = 1.0755MxL + 1.8713$ $r^2 = 0.9917$ (12.4 – 36.0)
Bullhead (*Cottus gobio*)	7	$L_T = 1.203PpL - 0.9609$ $r^2 = 0.9805$ (5.4 – 15.0)

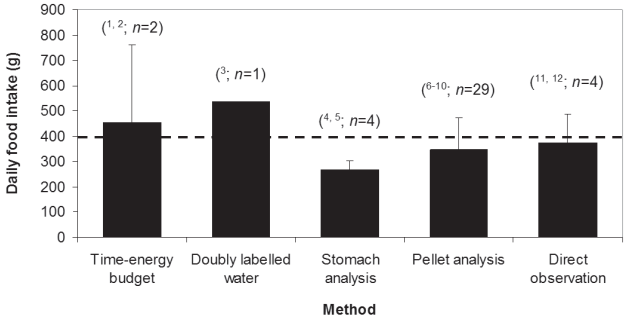

Fig. 4. *Daily food intake (mean + S.D.) of great cormorant (*Phalacrocorax carbo *and* P. c. sinensis*) calculated using different methods according to the work of [1]Grémillet et al. (1995), [2]Grémillet et al. (2003), [3]Keller & Visser (1999), [4]Opačak et al. (2004), [5]Liordos & Goutner (2007), [6]Dirksen et al. (1995), [7]Keller (1995), [8]Platteeuw & van Eerden (1995), [9]Leopold et al. (1998), [10]Gagliardi et al. (2007), [11]Voslamber et al. (1995), [12]Lekuona (2002). Note that values of DFI calculated specifically for the larger, primarily marine subspecies of great cormorant (*P. c. carbo*) were not included. Numbers in parenthesis refer to individual published works (see above), n refers to number of stated values of DFI. Dashed line shows calculated average DFI (397 g), which was used in the present study for the estimate of the total fish consumption in case of the River Vltava in Vyšší Brod, Slapy Reservoir and the River Vltava in Prague.*

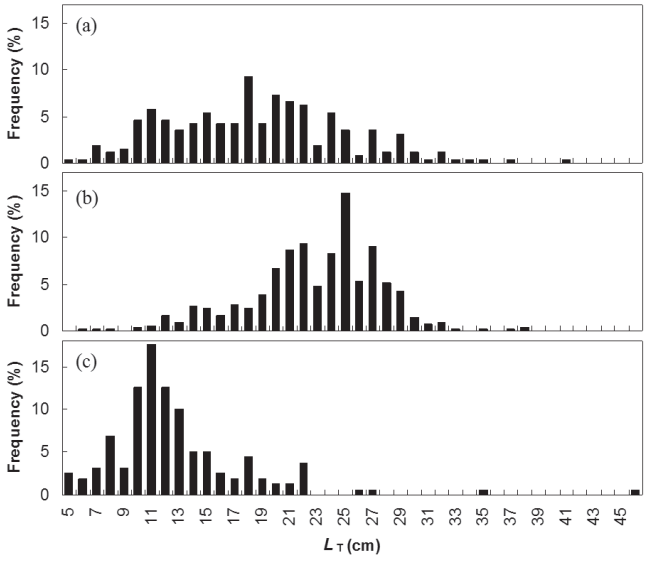

Fig. 5. *Frequency distributions of total length (L_T) of all fish species found in the diets of great cormorants (*Phalacrocorax carbo*) hunting on a) the River Vltava in Vyšší Brod (winter 2004/05; n = 389), b) Slapy Reservoir (winter 2005/06; n = 604) and c) the River Vltava in Prague (winter 2006/07, winter 2007/08, pooled data; n = 159).*

However, calculations of DFI from bioenergetic models and time-energy budgets also include great potential for biases in each step of the calculation

Table 2. *Fish species composition in the diet of great cormorants (*Phalacrocorax carbo*) hunting on the River Vltava in Vyšší Brod, on Slapy Reservoir and on the River Vltava in Prague. Note that in all cases roach was the dominant prey species in terms of weight. n, number of individuals; $\%_a$, percentage of abundance; W, total weight (kg) of all fish of appropriate species caught and digested by great cormorants in the sample; $\%_W$, percentage of total weight. Values of the three most numerous fish species in each individual locality are in bold.*

	Vyšší Brod¶				Slapy Reservoir¶				Prague§							
	winter 04/05				winter 05/06				winter 06/07				winter 07/08			
Species	n	$\%_a$	W	$\%_W$	n	$\%_a$	W	$\%_W$	n	$\%_a$	W	$\%_W$	n	$\%_a$	W	$\%_W$
Roach (*Rutilus rutilus*)	**132**	**33.9**	**16.6**	**41.0**	**502**	**83.1**	**74.2**	**83.4**	**17**	**25.8**	**1.1**	**45.3**	**23**	**24.7**	**0.71**	**29.2**
Bream (*Abramis brama*)	5	1.3	0.9	2.3	1	0.2	0.1	0.1	1	1.5	0.12	4.8	**11**	**11.8**	**0.3**	**12.4**
White bream (*Blicca bjoerkna*)	1	0.3	0.1	0.3	6	1.0	1.1	1.3	-	-	-	-	3	3.2	0.15	6.1
Bleak (*Alburnus alburnus*)	25	6.4	0.3	0.6	5	0.8	0.2	0.2	1	1.5	0.05	2.2	6	6.5	0.19	7.9
European chub (*Squalius cephalus*)	**99**	**25.4**	**10.0**	**24.8**	**18**	**3.0**	**2.9**	**3.3**	4	6.1	0.18	7.4	3	3.2	0.1	4.3
Common dace (*Leuciscus leuciscus*)	4	1.0	0.1	0.3	-	-	-	-	4	6.1	0.09	3.8	4	4.3	0.03	1.2
Gudgeon (*Gobio gobio*)	-	-	-	-	-	-	-	-	**12**	**18.2**	**0.17**	**7.0**	7	7.5	0.07	3.1
Rudd (*Scardinius erythrophthalmus*)	-	-	-	-	2	0.3	0.07	0.07	-	-	-	-	-	-	-	-
Nase (*Chondrostoma nasus*)	-	-	-	-	-	-	-	-	-	-	-	-	1	1.1	0.01	0.5
Tench (*Tinca tinca*)	-	-	-	-	6	1.0	1.0	1.2	-	-	-	-		-	-	-
Common carp (*Cyprinus carpio*)	1	0.3	0.3	0.7	8	1.3	1.3	1.4	-	-	-	-	-	-	-	-
Grass carp (*Ctenopharyngodon idella*)	-	-	-	-	6	1.0	0.5	0.6	-	-	-	-	-	-	-	-
Prussian carp (*Carassius auratus*) †	-	-	-	-	-	-	-	-	1	1.5	0.23	9.6	-	-	-	-
Roach × bream hybrid (*R. rutilus* × *A. brama*)	-	-	-	-	-	-	-	-	-	-	-	-	6	6.5	0.07	3.1
European eel (*Anguilla anguilla*)	-	-	-	-	-	-	-	-	-	-	-	-	1	1.1	0.19	7.7
Grayling (*Thymallus thymallus*) †	1	0.3	0.2	0.4	-	-	-	-	-	-	-	-	-	-	-	-
Trout spp.	3	0.8	0.3	0.8	-	-	-	-	-	-	-	-	-	-	-	-
Northern pike (*Esox lucius*)	2	0.5	0.04	0.09	5	0.8	1.4	1.6	-	-	-	-	1	1.1	0.13	5.2
Bullhead (*Cottus gobio*)	4	1.0	0.01	0.03	-	-	-	-	-	-	-	-	-	-	-	-
European perch (*Perca fluviatilis*)	**96**	**24.7**	**10.6**	**26.2**	**38**	**6.3**	**5.1**	**5.8**	3	4.5	0.22	9.0	3	3.2	0.05	2.2
Ruffe (*Gymnocephalus cernuus*)	14	3.6	0.4	0.9	1	0.2	0.01	0.01	**23**	**34.8**	**0.26**	**10.8**	**24**	**25.8**	**0.42**	**17.2**
Zander (*Sander lucioperca*)	2	0.5	0.7	1.7	6	1.0	0.9	1.0	-	-	-	-	-	-	-	-
Total	389	100	40.5	100	604	100	89.0	100	66	100	2.43	100	93	100	2.41	100

† Species classification and size estimates of one Prussian carp and one grayling was done using own reference collection of diagnostic bones of these species, which originated from ponds, streams and rivers belonging to the River Vltava basin.

¶ Results based on pellets analysis.

§ Results based on stomachs analysis.

(see e.g. Grémillet et al. 1995, Grémillet et al. 2003) and the values of DFI obtained are highly variable (cf. Grémillet et al. 1995 and DFI 238 g day^{-1} for adult *P. carbo sinensis* and Grémillet et al. 2003 and DFI 672 g day^{-1} for adult *P. carbo* – subspecies not stated). The only attempt to calculate DFI of wild great cormorants (*P. c. sinensis*) using a doubly labelled water technique and stable isotopes provides an estimate of DFI of 539 g day^{-1} (Keller & Visser 1999). The main disadvantage of both these methods (time-energy budget, doubly labelled water) is the extremely limited number of birds in the analysis (<< 10 birds). Since all the other methods (pellets and stomachs analysis, direct feeding observations) are often based on hundreds of samples (cf. e.g. Dirksen et al. 1995, Lekuona 2002, Opačak et al. 2004, Gagliardi et al. 2007) for the purpose of this study the DFI was calculated as an average from all the methods mentioned above, i.e. as 397 g day^{-1} (Fig. 4). The data were analyzed using linear regression and one-way ANOVA.

Results

At the River Vltava in Vyšší Brod site during the winter of 2004/05, the regurgitated diet remains of the great cormorants included 389 fish (after pairing the diagnostic bones) of 14 fish species of 5 families (Cyprinidae, Salmonidae, Esocidae, Cottidae, Percidae). Roach (*Rutilus rutilus*), bream (*Abramis brama*), bleak (*Alburnus alburnus*), European chub (*Squalius cephalus*), European perch (*Perca fluviatilis*) and ruffe (*Gymnocephalus cernuus*) represented 95.3 % (numerically) of the diet (Table 2). From the dominant species, roach taken were in the length range 10-29 cm

Table 3. *Estimated winter fish consumption (total, dominant species – by weight, species of anglers' interest) by great cormorants (*Phalacrocorax carbo*) at the River Vltava in Vyšší Brod, Slapy Reservoir and the River Vltava in Prague. nds, not dominant species, i.e. species presented in the diet but here included in the category "Others". Note that results from Vyšší Brod and Slapy Reservoir are based on pellets analysis while results from Prague are based on stomachs analysis.*

	Vyšší Brod	Slapy Res.	Prague	
	winter 2004/05	winter 2005/06	winter 2006/07	winter 2007/08
Average No. of great cormorants	73*	60†	1000‡	1150‡
No. of foraging days	90	90	90	91
No. of cormorant days	6 570	5 400	90 000	104 650
Daily food intake (g)		397		
Fish consumption – total (kg winter^{-1})	2 608	2 144	35 730	41 546
Area of potential foraging habitats, i.e. area of targeted fisheries (ha)	116	1 392	528	
Fish withdrawal (kg ha^{-1} winter^{-1})	22	2	68	79
Fish of anglers' interest withdrawal (kg ha^{-1} winter^{-1})	1.0	0.07	0	10.2
Consumption of selected species (kg)				
Roach (*Rutilus rutilus*)	**1 069**	**1 788**	**16 186**	**12 131**
Bream (*Abramis brama*)	*nds*	*nds*	*nds*	**5 152**
European chub (*Squalius cephalus*)	**647**	**71**	*nds*	*nds*
Common carp (*Cyprinus carpio*)	18	30	-	-
Grass carp (*Ctenopharyngodon idella*)	-	13	-	-
Prussian carp (*Carassius auratus*)	-	-	**3 430**	-
European eel (*Anguilla anguilla*)	-	-	-	3 199
Grayling (*Thymallus thymallus*)	10	-	-	-
Trout spp.¶	21	-	-	-
Northern pike (*Esox lucius*)	23	34	-	2 160
European perch (*Perca fluviatilis*)	**683**	**124**	*nds*	*nds*
Ruffe (*Gymnocephalus cernuus*)	*nds*	*nds*	**3 859**	**7 146**
Zander (*Sander lucioperca*)	44	21	-	-
Others	91	62	12 255	11 758
Fish of anglers' interest (total)	117	99	0	5 359

** M. Čech, M. Hladík, unpublished data.*

† Čech et al. (2008).

‡ Estimated according to published results of Fišerová & Bergmann (2004), Mourková & Bergmann (2005) and corrected for winter 2006/07 and 2007/08 by P. Musil (unpubl. data and pers. comm.).

*¶ Category "Trout spp." includes both native brown trout (*Salmo trutta *m.* fario*) and non-native rainbow trout (*Oncorhynchus mykiss*) and brook trout (*Salvelinus fontinalis*).*

(average L_T 21.5 cm), European chub in the length range 7-35 cm (average L_T 18.7 cm) and European perch in the length range 9-37 cm (average L_T 18.4 cm). The largest fish taken by the great cormorants was a 41 cm zander (*Sander lucioperca*), the heaviest was a 734 g European perch (regurgitated prior to complete digestion of the soft tissues). The average size of fish captured and ingested by great cormorants was 18.6 cm L_T and 114 g. Fish ≤ 20 cm L_T comprised 60.3 % of the cormorants' diet at this site (Fig. 5a).

The great cormorants at the River Vltava in Vyšší Brod were estimated to have consumed 2608 kg of fish during the winter of 2004/05 (Table 3). During this period, the estimated roach consumption was 1069 kg, while corresponding values for European perch were 683 kg and for European chub 647 kg. From the fish of interest to anglers, the cormorants consumed 44 kg of zander, 23 kg of northern pike, 21 kg of trout spp., 18 kg of common carp (*Cyprinus carpio*) and 10 kg of grayling. The overall fish withdrawal considering the fisheries Vltava 29 (Lipno II Reservoir), Vltava 28 and Vltava 27 was 22 kg ha^{-1} (Table 3).

In Slapy Reservoir during the winter of 2005/06, the regurgitated diet remains of great cormorants included 604 fish of 13 fish species of 3 families (Cyprinidae, Esocidae, Percidae). Roach, bream, bleak, European chub, European perch and ruffe again represented 93.6 % of the diet (Table 2). From the dominant species, roach in a length range of 6-35 cm (average L_T 22.9 cm), European perch in a range of 11-29 cm (average L_T 21.2 cm) and European chub of 7-31 cm (average L_T 24.3 cm) were taken. The largest fish taken by the great cormorants at this site was a 38 cm northern pike, and the heaviest was a 575 g roach. The average size of fish captured and ingested by great cormorants was 22.8 cm L_T and 157 g. Fish ≤ 20 cm L_T made up only 26.5 % of the cormorants' diet (Fig. 5b).

The great cormorants at Slapy Reservoir were estimated to have consumed 2144 kg of fish during the winter of 2005/06 (Table 3). During this period, estimated roach consumption was 1788 kg, while the corresponding values for European perch were 124 kg and for European chub 71 kg. From the fish of anglers' interest, cormorants consumed 34 kg of northern pike, 30 kg of common carp, 21 kg of zander and 13 kg of grass carp (*Ctenopharyngodon idella*). Thus the overall fish withdrawal when considering the fisheries Vltava 10-14 (Slapy Reservoir) was 2 kg ha^{-1} (Table 3).

On the River Vltava in Prague during the warm winter of 2006/07, the stomachs of seven great cormorants killed included 66 fish of nine species of two families (Cyprinidae, Percidae). Roach, bream, bleak, European chub, European perch and ruffe represented 74.2 % of the diet (Table 2). From these dominant species, ruffe were taken in the length range of 6-12 cm (average L_T 9.2 cm), roach in the range of 8-35 cm (average L_T 15.4 cm) and gudgeon (*Gobio gobio*) 11-14 cm (average L_T 12.6 cm). The largest and heaviest fish taken by the great cormorants at this site was a 35.2 cm and 578 g roach (an exceptional catch by a ringed, large, 5 year old male cormorant of 3700 g net weight). The average size of fish captured and ingested by great cormorants was 13.0 cm L_T and 37 g. Fish ≤ 20 cm L_T comprised 92.4 % of the cormorants' diet. The daily food intake reconstructed from the stomach contents of individual birds was estimated to be 347 ± 193 g of fish (mean ± S.D.; not used for final estimate of total fish consumption).

Great cormorants at the River Vltava in Prague were estimated to have consumed 35730 kg of fish during the winter of 2006/07 (Table 3). During this period, the estimated consumption of roach was 16186 kg, while corresponding values for ruffe were 3859 kg and Prussian carp (*Carassius auratus*) 3430 kg. There were no fish of anglers' interest in the stomachs of great cormorants analysed, but the sample was limited. The overall fish withdrawal considering fisheries Vltava 3-7 was 68 kg ha^{-1} (Table 3).

At the River Vltava in Prague during the warm winter of 2007/08, the stomachs of 19 great cormorants killed included 93 fish of 13 species and 4 families (Cyprinidae, Anguillidae, Esocidae, Percidae). Roach, bream, bleak, European chub, European perch and ruffe represented 75.2 % of the cormorants' diet (Table 2). Of the dominant species, ruffe were taken in the length range 7-13 cm (average L_T 11.1 cm), roach in the range of 5-22 cm (average L_T 12.4 cm), bream in the range of 7-22 cm (average L_T 12.9 cm) and gudgeon 10-14 cm (average L_T 11.3 cm). The largest and heaviest fish taken by the great cormorants was a 46 cm and 185 g European eel. The average size of fish captured and ingested by great cormorants was 12.5 cm L_T and 26 g. Fish ≤ 20 cm L_T comprised 92.5 % of the cormorants' diet at this site. The daily food intake reconstructed from the stomach contents of individual birds was estimated to be 127 ± 129 g of fish (mean ± S.D.). When excluding birds found with empty stomachs (n = 5; two males, three females) from the calculation, this weight increased to 173 ± 121 g of fish (mean ± S.D.; not used for final estimate of total fish consumption). Surprisingly low value of DFI was most probably caused by highly turbid, flood water running in the River Vltava on 1-4 December 2007 (13 out of 19 birds shot during these days) causing the

visual hunting for fish extremely problematic.

Great cormorants at the River Vltava in Prague were estimated to have consumed 41546 kg of fish during the winter of 2007/08 (Table 3). During this period, estimated consumption of roach was 12131 kg, while corresponding values for ruffe were 7146 kg and bream 5152 kg. From the fish of anglers' interest, cormorants consumed 3199 kg of European eel and 2160 kg of northern pike. The overall fish withdrawal considering the Vltava 3-7 fisheries was 79 kg ha^{-1} (Table 3).

At the River Vltava in Prague, eight fish species – roach, bream, bleak, European chub, common dace (*Leuciscus leuciscus*), gudgeon, European perch and ruffe – were found in the diet of great cormorants during both the 2006/07 and 2007/08 winters. The most hunted fish species was ruffe (made up 34.8 % and 25.8 % of the cormorants' diet in 2006/07 and 2007/08 winters respectively) followed by roach (25.8 % and 24.7 % in the 2006/07 and 2007/08 winters respectively; Table 2). The proportions of roach, bream, bleak, European chub, European perch and ruffe, as well as the proportion of fish ≤ 20 cm L_T, in the diet of great cormorants remained the same in both winters (see above). Similarly, the sizes of fish hunted by the great cormorants in 2006/07 and 2007/08 winters also did not differ significantly (ANOVA: $F_{1, 157} = 0.26$, $P = 0.61$; for pooled data see Fig. 5c).

Discussion

At all three roosting localities on the main stream of the River Vltava, six fish species – roach, bream, bleak, European chub, European perch and ruffe made up at least 74.2 %, i.e. the gross majority, of the cormorants' diet. These results correspond well with most findings throughout large European non-salmonid inland waters (see e.g. Dirksen et al. 1995, Keller 1995, Suter 1997, Keller 1998, Engström 2001, Wziątek et al. 2005, Čech et al. 2008, Fonteneau et al. 2009). Since all these fish species are highly gregarious (Suter 1997, Čech et al. 2005, Čech & Kubečka 2006), moreover, highly abundant in large waters of the Czech Republic, it is not surprising that they form the majority of the diet of piscivorous birds. The similarity of the diets of great cormorants roosting at the River Vltava in Vyšší Bord, Slapy Reservoir and on the River Vltava in Prague (at first sight completely different localities) could be, on the other hand, a great surprise for anglers, since it resembles the diet of cormorants fishing in lakes and reservoirs (for details see references above). However, the River Vltava in Prague is dammed by a series of weirs (six weirs from Vrané Reservoir to the weir in Dolany), and, in reality, this lowland river operates like a cascade of small reservoirs. Similarly, in the case of the River Vltava in Vyšší Brod, there is the small Lipno II Reservoir and another three weirs down to the Hašlovice, giving the river, at least to some extent, reservoir-like characters. From the second view, those stretches of the River Vltava together with Slapy Reservoir are more similar than expected.

The only surprise is the very low presence of trout spp. and grayling in the diet of great cormorants at Vyšší Brod when anglers were certain that the birds are responsible for brown trout and grayling populations being close to collapse and for the significant decrease in their catches. Moreover, Suter (1995) and Keller (1995, 1998) have shown that in cases when salmonids, and especially grayling, are abundant in a river, they are also abundant in the diet of great cormorants. The results of the present study have therefore two possible explanations:

1) The dietary study in Vyšší Brod was commissioned by the Czech Anglers Union – the South Bohemian Board too late, when populations of both native salmonids had already been reduced dramatically. As with roach and the other fish species, grayling is highly gregarious (Suter 1995, Staub et al. 1998) and is, moreover, "stupid fish" (Suter 1997, M. Čech, pers. observation) with very poor avoidance reactions and less tendency to seek shelter. For that reason, this fish species is highly vulnerable to cormorant predation and overwintering great cormorants could easily have decimated the population in the late 90s and at the beginning of the new millennium (i.e. prior to this study). In contrast to grayling, brown trout is a solitary, territorial fish (Sundstrom et al. 2003) with very strong avoidance reactions and a strong tendency to seek shelter. Fishing for hidden brown trout in the cold, fast flowing river must be less profitable for great cormorants (Grémillet et al. 2001). This seems to be the main reason why great cormorants do not fish on the River Vltava upstream of the Lipno II Reservoir (where it is a shallow, fast flowing river; M. Hladík, K. Křivanec, pers. observation). Definitely, fishing for brown trout in a cold, fast flowing river with many boulders/shelters would have a completely different impact on the birds' daily energy requirement than fishing for brown trout in lakes like Loch Leven (Stewart et al. 2005). Therefore, predation pressure by great cormorants has little potential to explain the decrease of brown trout in anglers' catch statistics between the years 1999 and 2003. However, this decrease corresponds well with the decrease in stocking of brown trout in the same years (regression

analysis: $r^2 = 0.80$, $F_{1,3} = 12.03$, $P < 0.05$; see Fig. 2).

2) Anglers themselves are responsible for the decrease of brown trout and grayling catches. Over the last two decades, the anglers' ability to catch a fish has increased dramatically (new technologies and materials, new know-how from the literature, films and internet). This significant improvement is well documented in the catch statistics of two non-native but heavily stocked salmonid species – rainbow trout and brook trout. For example, the stocking of rainbow trout and brook trout into the fishery Vltava 28 in the years 1994-2003 revealed no trend (regression analysis: $P > 0.65$ for both species; the fish stocking size exceeds the minimum size limit for put and take fishery). However, their catch increased significantly over the years (regression analysis: $r^2 = 0.48$, $F_{1,8} = 7.26$, $P < 0.05$ for rainbow trout and $r^2 = 0.42$, $F_{1,8} = 5.73$, $P < 0.05$ for brook trout). Moreover, in the years 1994-2003, the average anglers' yield of all fish species from the fishery Vltava 28 exceeded 60 kg ha^{-1} to which the yield of salmonids contributed over 45 kg ha^{-1} (Czech Anglers Union, unpublished data). On the other hand, in the neighbouring fishery, Vltava 27, the average anglers' yield of all fish species (years 1994-2003) exceeded only 18 kg ha^{-1} to which the yield of salmonids contributed over 13 kg ha^{-1} (Czech Anglers Union, unpublished data).

In accordance with the stocking strategy of the Czech Anglers Union – the South Bohemian Board (M. Hladík, pers. comm.), it could also be possible that anglers fishing on the River Vltava in Vyšší Brod, although they catch all the salmonids, they take only non-native species and selectively release the native brown trout and grayling, especially in a situation when it is generally supposed that populations of those two species are close to collapse. This shift in anglers' behaviour in recent years could have a similar effect on the catch statistics of brown trout and grayling as the predation pressure by great cormorants (biasing the real state of the brown trout and grayling populations). The above mentioned put and take strategy to protect populations of native species, could also have a negative effect, since heavily stocked rainbow trout and brook trout could impose food and space competition on both brown trout and grayling, and cause further decline of their populations (Blanchet et al. 2007, Fausch 2007).

Most likely, the truth will be somewhere between explanations 1 and 2: most probably a cumulative effect of both great cormorants and anglers on the populations of brown trout and grayling, exacerbated by river fragmentation and degradation of spawning and nursery habitats. No doubt, one can also hypothesized that both anglers and cormorants are responding to, rather than being responsible for, changes in fish populations (Davies 1997). Unfortunately, the data to test this assumption are missing in case of targeted study sites. On the other hand, restrictions, which have been applied to Vltava 28 and Vltava 27 fisheries since 2005 (fly-fishing only, barbless hooks, catch and release stretches, minimum size limit for brown trout 45 cm) seem to have led to a noticeable recovery of both brown trout and grayling populations (M. Hladík, pers. comm.). Despite this stock improvement there is still a persistent idea of the need to somehow protect the brood stock of grayling against the overwintering great cormorants. One possibility is to catch most of the adult fish prior to the cormorants' arrival and place them in the store-ponds. A part of this brood stock would be used for artificial spawning and yearling production, and the rest of the adult fish will be restocked into the fishery in the same manner as rainbow trout and brook trout, i.e. after the great cormorants leave the river in mid-March (M. Hladík, pers. comm.).

The present study shows that great cormorants hunting on the main stream of the River Vltava prey mostly on coarse fishes of low or even no- interest to anglers. Therefore, it could be easily concluded that the competition between great cormorants and anglers is of minor importance, which would be consistent with findings of other authors (e.g. Keller 1995, Keller 1998, Engström 2001, Wziątek et al. 2005, Liordos & Goutner 2007). This statement is particularly true in the case of Slapy Reservoir where overall fish withdrawal caused by overwintering great cormorants (2 kg ha^{-1}) is similar to the published withdrawal from Lake Veluwemeer, The Netherlands (2.1 kg ha^{-1}; Dirksen et al. 1995), or Želivka Reservoir, Czech Republic (2 kg ha^{-1} $month^{-1}$; Čech & Čech 2009). The removal of mostly zooplanktivorous fish is considered to have a positive influence on water quality of those large water bodies – the top down effect of great cormorants is a substitute for human biomanipulation interventions into the lake/reservoir ecosystem (Dirksen et al. 1995, Čech & Čech 2009). On the other hand, the estimated withdrawal of 22 kg ha^{-1} of fish from the River Vltava in Vyšší Brod (fishery Vltava 27-29) and of 68-79 kg ha^{-1} from the River Vltava in Prague (fishery Vltava 3-7) belong among the highest ever published figures for withdrawal caused by great cormorants from any inland waters (carp fishponds excluded; cf. Dirksen et al. 1995, Suter 1995, Staub et al. 1998, Engström 2001). Therefore, the potential for competition and conflict with anglers is substantial.

This study also shows a peculiar preference for much smaller fish than expected (cf. Čech et al. 2008) for cormorants hunting on the River Vltava in Prague. This finding has at least two possible explanations:
1) The great cormorants are hunting preferentially in Prague's harbours (e.g. under the Charles Bridge) or below Prague's sewerage plant outlet, where extremely high abundance of these small fish was observed many times (Š. Rusňák, J. Andreska, pers. observation).
2) Another reason could be extremely warm winters (both 2006/07 and 2007/08 winters), which could have changed the daily energy budget of great cormorants significantly (Grémillet et al. 2001). Both alternatives result in a situation where cormorants are not forced by natural conditions to prey on larger fish. It must be also taken into account that results from the River Vltava in Prague are based on stomachs analysis while the results from the River Vltava in Vyšší Brod and Slapy Reservoir as well as published relationships between the size of fish taken by great cormorants and air/water temperature (Čech et al. 2008) are based on pellets analysis. It seems that by using pellets, especially very small fish could be to some extent underestimated in the diet of great cormorants (Carss et al. 1997).

Acknowledgement

The authors thank M. Hladík and P. Čech for help with data collection at the River Vltava in Vyšší Brod (MH) and Slapy Reservoir (PČ), J. Andreska for shooting the birds in Prague – Troja, experts from the Prague Zoo for dissecting the birds and all the biometric measurements and Š. Rusňák for providing the stomachs of 26 killed birds and for the initiation of the study in Prague. M. Hladík, the head manager of the fish stocking program of the Czech Anglers Union – the South Bohemian Board, provided the long term data on stocking and catch statistics as well as valuable comments to the situation at the River Vltava in Vyšší Brod. M. Morris carefully read the latest version of the manuscript and corrected the English. The study was supported by the Grant Agency of the Czech Republic (projects No. 206/06/1371 and 206/09/P266), the Academy of Sciences of the Czech Republic (projects No. 1QS600170504 and AVOZ60170517), the Czech Anglers Union – the South Bohemian Board and Š. Rusňák. The paper is dedicated to the boss of the Fish Ecology Unit (FishEcU), J. Kubečka, to his 50th birthday.

Literature

Barrett R.T., Røv N., Loen J. & Montevecchi W.A. 1990: Diets of shags *Phalacrocorax aristotelis* and cormorants *P. carbo* in Norway and possible implications for gadoid stock recruitment. *Mar. Ecol. – Prog. Ser. 66: 205–218.*

Blanchet S., Loot G., Grenouillet G. & Brosse S. 2007: Competitive interactions between native and exotic salmonids: a combined field and laboratory demonstration. *Ecol. Freshw. Fish 16/2: 133–143.*

Carss D.N. & Ekins G.R. 2002: Further European integration: mixed sub-species colonies of great cormorants *Phalacrocorax carbo* in Britain – colony establishment, diet, and implications for fisheries management. *Ardea 90: 23–41.*

Carss D.N., Bevan R.M., Bonetti A., Cherubini G., Davies J., Doherty D., El Hili A., Feltham M.J., Grade N., Granadeiro J.P., Grémillet D., Gromadzka J., Harari Y.N.R.A., Holden T., Keller T., Lariccia G., Mantovani R., McCarthy T.K., Mellin M., Menke T., Mirowska-Ibron I., Muller W., Musil P., Nazirides T., Suter W., Trauttmansdorff J.F.G., Wilhelminenberg O., Volponi S. & Wilson B. 1997: Techniques for assessing cormorant diet and food intake: towards a consensus view. *Supplementi di Ricerche Biologia Selvaggina 26: 197–230.*

Čech M. 2004: The diet of great cormorant on reservoirs. *Rybářství 2: 14–15. (in Czech)*

Čech M. & Čech P. 2006: Diet of kingfisher at various types of waters. In: Čech P. (ed.), Proceedings of 1th international kingfisher (*Alcedo atthis*) seminar. *Czech Union for Nature Conservation, Vlašim, Czech Republic: 55–71. (in Czech with extended abstract in English)*

Čech M. & Čech P. 2009: The diet of great cormorants (*Phalacrocorax carbo*) at Želivka water supply reservoir. *Sborník vlastivědných prací z Podblanicka 48: 91–105. (in Czech with English summary)*

Čech M. & Kubečka J. 2006: Ontogenetic changes in the bathypelagic distribution of European perch fry *Perca fluviatilis* monitored by hydroacoustic methods. *Biologia 61: 211–219.*

Čech M., Čech P., Kubečka J., Prchalová M. & Draštík V. 2008: Size selectivity in summer and winter diets of great cormorant (*Phalacrocorax carbo*): does it reflect season-dependent difference in foraging efficiency? *Waterbirds 31 (3): 438–447.*

Čech M., Kratochvíl M., Kubečka J., Draštík V. & Matěna J. 2005: Diel vertical migrations of bathypelagic perch fry. *J. Fish Biol. 66: 685–702.*

Čech M., Kubečka J., Frouzová J., Draštík V., Kratochvíl M., Matěna J. & Hejzlar J. 2007: Distribution of the

bathypelagic perch fry layer along the longitudinal profile of two large canyon-shaped reservoirs. *J. Fish Biol. 70: 141–154.*

Davies J.M. 1997: Impacts of cormorants (*Phalacrocorax carbo* L.) on the Lower River Ribble Fishery, Lancashire. *Ph.D. thesis, Liverpool John Moores University, United Kingdom.*

Dirksen S., Boudewijn T.J., Noordhuis R. & Marteijn E.C.L. 1995: Cormorants *Phalacrocorax carbo sinensis* in shallow eutrophic freshwater lakes: prey choice and fish consumption in the non-breeding period and effects of large-scale fish removal. *Ardea 83: 167–184.*

Engström H. 2001: Long term effects of cormorant predation on fish communities and fishery in a freshwater lake. *Ecography 24: 127–138.*

Fausch K.D. 2007: Introduction, establishment and effects of non-native salmonids: considering the risk of rainbow trout invasion in the United Kingdom. *J. Fish Biol. 71: 1–32.*

Fonteneau F., Paillisson J.-M. & Marion L. 2009: Relationships between bird morphology and prey selection in two sympatric great cormorant *Phalacrocorax carbo* subspecies during winter. *Ibis 151: 286–298.*

Gagliardi A., Martinoli A., Preatoni D., Wauters L.A. & Tosi G. 2007: From mass of body elements to fish biomass: a direct method to quantify food intake of fish eating birds. *Hydrobiologia 583: 213–222.*

Grémillet D. 1997: Catch per unit effort, foraging efficiency, and parental investment in breeding great cormorants (*Phalacrocorax carbo carbo*). *ICES J. Mar. Sci. 54: 635–644.*

Grémillet D., Schmid D. & Culik B. 1995: Energy requirements of breeding great cormorants *Phalacrocorax carbo sinensis*. *Mar. Ecol. – Prog. Ser. 121: 1–9.*

Grémillet D., Wanless S., Carss D.N., Linton D., Harris M.P., Speakman J.R. & Le Macho Y. 2001: Foraging energetics of Arctic cormorants and the evolution of diving birds. *Ecol. Lett. 4: 180–184.*

Grémillet D., Wright G., Lauder A., Carss D.N. & Wanless S. 2003: Modelling the daily food requirements of wintering great cormorants: a bioenergetics tool for wildlife management. *J. Appl. Ecol. 40: 266–277.*

Hallet C. 1977: Contribution a l´étude du régime alimentaire du Martin-pêcheur (*Alcedo atthis*) dans la vallée de la Lesse. *Aves 14: 128–144.*

Hallet C. 1982: Etude du comportement de predation du Martin-pêcheur *Alcedo atthis* (L.): taille preferentielle de capture du Chabot *Cottus gobio* L. et de la Truite *Salmo trutta* L. *Rev. Ecol. (Terre Vie) 36: 211–222.*

Hrbáček J. & Straškraba M. 1966: Horizontal and vertical distribution of temperature, oxygen, pH and water movement in Slapy Reservoir (1958-1960). In: Hrbáček J. (ed.), Hydrobiological studies 1. *Academia Publishing House of the Czechoslovak Academy of Sciences, Prague: 7–40.*

Johansen R., Pedersen T. & Barrett R.T. 1999: Cormorants (*Phalacrocorax carbo carbo*) as predators in a cod (*Gadus morhua* L.) enhancement area in north Norway. In: Howell B., Moksness E. & Svåsand T. (eds.), Stock enhancement and sea ranching. *Fishing News Books, Oxford: 334–349.*

Keller T. 1995: Food of cormorants *Phalacrocorax carbo sinensis* wintering in Bavaria, southern Germany. *Ardea 83: 185–192.*

Keller T. 1998: The food of cormorants (*Phalacrocorax carbo sinensis*) in Bavaria. *J. Ornithol. 139: 389–400.*

Keller T.M. & Visser G.H. 1999: Daily energy expenditure of great cormorants *Phalacrocorax carbo sinensis* wintering at Lake Chiemsee, southern Germany. *Ardea 87: 61–69.*

Lekuona J.M. 2002: Food intake, feeding behaviour and stock losses of cormorants, *Phalacrocorax carbo*, and grey herons, *Ardea cinerea*, at a fish farm in Arcachon Bay (southwest France) during breeding and non-breeding season. *Folia Zool. 51: 23–34.*

Leopold M.F., van Damme C.J.G. & van der Veer H.W. 1998: Diet of cormorants and the impact of cormorant predation on juvenile flatfish in the Dutch Wadden Sea. *J. Sea Res. 40: 93–107.*

Lilliendahl K. & Solmundsson J. 2006: Feeding ecology of sympatric European shag *Phalacrocorax aristotelis* and great cormorants *P. carbo* in Iceland. *Mar. Biol. 149: 979–990.*

Liordos V. & Goutner V. 2007: Spatial patterns of winter diet of the great cormorant in coastal wetlands of Greece. *Waterbirds 30: 103–111.*

Liordos V. & Goutner V. 2008: Habitat and temporal variation in the diet of great cormorant nestlings in Greek colonies. *Waterbirds 31: 424–437.*

Mourková J. & Bergmann P. 2005: Winter waterfowl census in central Bohemia in winter 2003/2004. *Zprávy ČSO 60: 5–18. (in Czech with English summary)*

Mous P.J. 2000: Interactions between fisheries and birds in Ijsselmeer, the Netherlands. *Ph.D. thesis, Wageningen*

University, The Netherlands.

Opačak A., Florijančić T., Horvat D., Ozimec S. & Bodakoš D. 2004: Diet spectrum of great cormorants (*Phalacrocorax carbo sinensis* L.) at the Donji Miholjac carp fishponds in eastern Croatia. *Eur. J. Wildlife Res. 50: 173–178.*

Platteeuw M. & van Eerden M. 1995: Time and energy constraints of fishing behaviour in breeding cormorants *Phalacrocorax carbo sinensis* at Lake Ijsselmeer, The Netherlands. *Ardea 83: 223–234.*

Prchalová M., Kubečka J., Jůza T., Říha M., Jarolím O. & Tušer M. 2005: Complex fish stock assessment of Želivka Reservoir in year 2004. *Report of the Hydrobiological Institute, Academy of Sciences of the Czech Republic: 1–22. (in Czech)*

Reynolds S.J. & Hinge M.D.C. 1996: Foods brought to the nest by breeding kingfisher *Alcedo atthis* in the New Forest of southern England. *Bird Study 43: 96–102.*

Staub E., Egloff K., Krämer A. & Walter J. 1998: The effect of predation by wintering cormorants *Phalacrocorax carbo* on grayling *Thymallus thymallus* and trout (Salmonidae) populations: two case studies from Swiss rivers. Comment. *J. Appl. Ecol. 35: 607–610.*

Stewart D.C., Middlemas S.J., Gardiner W.R., Mackay S. & Armstrong J.D. 2005: Diet and prey selection of cormorants (*Phalacrocorax carbo*) at Loch Leven, a major stocked trout fishery. *J. Zool. 267: 191–201.*

Sundstrom L.F., Lohmus M. & Johnsson J.I. 2003: Investment in territorial defence depends on rearing environment in brown trout (*Salmo trutta*). *Behav. Ecol. Sociobiol. 54: 249–255.*

Suter W. 1995: The effect of predation by wintering cormorants *Phalacrocorax carbo* on grayling *Thymallus thymallus* and trout (Salmonidae) populations: two case studies from Swiss rivers. *J. Appl. Ecol. 32: 29–46.*

Suter W. 1997: Roach rules: shoaling fish are a constant factor in the diet of cormorants *Phalacrocorax carbo* in Switzerland. *Ardea 85: 9–27.*

Suter W. 1998: The effect of predation by wintering cormorants *Phalacrocorax carbo* on grayling *Thymallus thymallus* and trout (Salmonidae) populations: two case studies from Swiss rivers. Reply. *J. Appl. Ecol. 35: 611–616.*

Veldkamp R. 1995: Diet of cormorants *Phalacrocorax carbo sinensis* at Wanneperveen, The Netherlands, with special reference to bream *Abramis brama*. *Ardea 83: 143–155.*

Voslamber B., Platteeuw M. & van Eerden M. 1995: Solitary foraging in sand pits by breeding cormorants *Phalacrocorax carbo sinensis*: does specialized knowledge about fishing sites and fish behaviour pay off? *Ardea 83: 213–222.*

Wziątek B., Martyniak A., Hliwa P., Kozłowski J., Krzywosz T., Osewski M., Sobocki M., Szymańska U. & Gomułka P. 2005: Great cormorant predation on coregonid fishes at seven sites in Poland. *Advanced Limnology – Biology and Management of Coregonid Fishes 60: 285–297.*