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The use of recirculating systems for rearing of riverine fish species

Pavel Lepič

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CHAPTER 1

GENERAL INTRODUCTION

Recirculation systems

The increasing human population and the growing demand for quality and healthy food, together with troubles related to over-harvesting of oceanic fisheries are the most important factors which make aquaculture one of the most forward-looking food-producing sectors today (Food and Agriculture Organization of United Nations, 2014). To minimize the risk of problems arising and to make aquaculture responsible and sustainable, it is necessary that research workers and farmers cooperate in innovation development and planning of new facilities to ensure the establishment of fully effective, environmentally sound, intensive aquaculture systems (Diana et al., 2013). This is requisite for sustainability and development for meeting the increasing worldwide demand for fish. Recent developments in intensive recirculating aquaculture systems (RAS) provide solutions to limitations of water resources and management of effluents – i.e.providing economic and environmental sustainability (Martins et al., 2010; National Intelligence Council, 2012; Buric et al., 2015). These systems curtail water consumption (Verdegem et al., 2006), culture fish under better hygiene and improved disease management (Summerfelt et al., 2009; Tal et al., 2009), control recycling of nutrients and waste management (Piedrahita, 2003), intensify fish culture, allow year-round culture and completely control the entire rearing process (Martins et al., 2010) as well as preventing escape of fish from breeding facilities (Zohar et al., 2005).

In recent decades, RAS has progressed from abortive pilot systems to becoming a crucial sector of marine and freshwater aquaculture (Martins et al., 2010; Wilfart et al., 2013). Several literature sources (Losordo et al., 1998; Masser et al., 1999; Summerfelt et al., 2004) have affirmed RAS is one of the most important developments e in aquaculture for increased production. To meet growing demands and to limit the potential adverse effects on the environment and climate, aquaculture systems must be efficient and environmentally friendly (Wilfart et al., 2013). RAS wastewater is a concentrated mixture of sludge and dissolved nutrients, which can be used for fertilizer (Schneider et al., 2005; van Rijn, 2013), or compost (van Rijn, 2013),), or cleaned through anaerobic digestion (van Rijn et al., 2006; Mirzoyan et al., 2010), vermicomposting (Schneider et al., 2005, or a system of constructed wetlands (Vymazal, 2013). Currently, waste management is incorporated into planning of new RAS facilities. The simplest method of using sludge is fertilization or composting, but according to the European Directive 86/278/EEC, focused on reducing the quantity of waste for disposal and saving natural resources (reuse, recycling, composting, and energy recovery from waste), sewage sludge must be treated before being used in agriculture.

The basic and most important components of RAS, which in general make a very sophisticated and effective loop, are mechanical and biological filtration of water, oxygenation and disinfection of water with ultraviolet (UV) light and ozone treatment (Martins et al., 2010). The first step of this process is mechanical filtration to remove insoluble particles (uneaten feed, excrements) from the water; mechanical filtration (screen filters, rotary microscreen filters) is needed because accumulation of insoluble particles in RAS can clog a biofilter and decrease its function and effectiveness, increase oxygen demand (Chen, 2000) and damage fish gills (Chapman et al., 1987). The next phase is biological filtration; high-quality and effective biofiltration is an essential part of every RAS. Toxic ammonia (product of fish metabolism) is reduced to nitrates in a process called nitrification. The nitrification process consists of two parts. First, Nitrosomonas bacteria transform ammonia to nitrites, then Nitrobacter bacteria change nitrite to nitrate (Stickney, 2000). In RAS, where the water exchange rate is low (i.e., 30 L per kg of feed in tilapia farms; Martins et al., 2009), nitrate accumulation can present a problem. In this case, denitrification has to be applied (van Rijn et al., 2006; Martins et al., 2010). Denitrification occurs in an anaerobic environment where

nitrate is converted to nitrogen gas (Martins et al., 2010). Finally, treated water is disinfected and sterilized. To disinfect and sterilize water in RAS, UV light and ozone treatment are usually used (Sharrer and Summerfelt, 2007). The UV light at a wavelength of 254 nm has been an effective tool for killing bacterial and viral organisms. The effectiviness of the UV treatment depends on low concentration of suspended solids and very low turbidity of inflowing water. Under optimal UV-treatment conditions can 99.9% of bacteria can be removed (Owsley, 2000). Ozone has been used in RAS for its bactericidal, parasitical and virucidal effects (Bullock et al., 1997; Liltved, 2002). Microbes are killed with ozone by oxidation of their lipid biolayer (Colberg and Lingg, 1978). Ozone treatment can also be applied to eliminate colour, nitrite reduction, algae control and turbidity removal (Tango and Gagnon, 2003; Summerfelt et al., 2009; Martins et al., 2010). The end product of the ozone reaction is dissolved oxygen (Sharrer and Summerfelt, 2007).

RAS can control physical and chemical water parameters such as temperature, pH, oxygen content, as well as being able to monitor the health status of fish and to accurately determine feed rations (Blancheton, 2000; Remen et al., 2008; Zarski et al., 2008, 2010). Recirculating aquaculture systems have been successfully used for the production of fresh water as well as marine fish species (Martins et al., 2010).

Breeding fish in each age group has its own specific characteristics and their respect is a prerequisite for successful culturing. The use of recirculating systems for various life history stages eliminates unfavourable external factors (temperature variations, changes in water chemistry, including oxygen saturation, etc.) (Fiala et al., 2008) while ensuring optimal environmental conditions suitable for rearing the various ages of fish (Kouril et al., 2008). IRearing phases can be divided into the following categories:

1. developing and maintating broodstock
2. artificial reproduction
3. egg incubation
4. fry rearing
5. rearing juveniles
6. fattening fish for the market

In freshwater aquaculture, recirculating aquaculture systems (RAS) are often used to produce species such as African sharptooth catfish (*Clarias gariepinus* Burchell) (Vandecan et al., 2011) and tilapia (*Oreochromis niloticus* L.) (Martins et al., 2011). Freshwater RAS technology has been well established in The Netherlands and Denmark. The Dutch RASs are typically indoor, nearly closed systems (water refreshment rate ranges between 30 $l.kg^{-1}$ $feed.day^{-1}$ and 300 $l.kg^{-1}$ $feed.day^{-1}$, Martins et al., 2009). They are used mainly for freshwater production of African catfish and eel. The Danish model trout farms are outdoor, semi-closed systems for trout on-growing using 3900 $l.kg^{-1}$ feed or 1/13 of traditional trout farming (Jokumsen et al., 2009). In France a trout RAS, designed after the Danish model trout farms was operated at a water refreshment rate of 9000 $l.kg^{-1}$ $feed.day^{-1}$ (Roque d'Orbcastel et al., 2009).

Riverine species of fish

Diversification and expansion of fish species reared in RAS is currently being emphasised. Recently, there has been an increasing interest in diversification of European freshwater aquaculture by the intensification of native species production. The usie of RAS as a part of rescue and restocking programs can produce high quality larvae and fry of endangered species (Targonska et al., 2010; Zarski et al., 2011). Rearing in control conditions during the critical part of year (for example in winter season), or in a critical part of a life cycle (the initial breeding of juveniles) are very effective. The combination of traditional pond aquaculture with modern RAS offers new possibilities for successful rearing of endangered species of fish. Many riverine fishes in Europe have been affected by river engineering and pollution (Kirchhofer, 1996; Ross et al., 2008; Kujawa and Glinska-Lewczuk, 2011) and are endangered or declining (Penaz et al., 2003; Penczak et al., 2004). Bird predation is another factor which contributes to fish decline. The great cormorant (*Phalacrocorax carbo*) is a piscivorous bird occurring at the top of the aquatic food chain (Houserova et al., 2007; Goutner et al., 2011). The cormorant is one of the most dangerous predators of fish, and hunts and catches fish weighing up to 500 g. It is responsible for significant economic losses in pond carp and whitefish (Svobodova et al., 2007). Many native riverine species (nase, barb, vimba, grayling, etc.) are under threat as well. As a result of the unfavourable state of natural populations, research has started in the last two decades focusing on artificial reproduction and rearing for restocking. Restocking programmes have been initiated in a number of countries to prevent further deterioration of its stocks (Kaminski et al., 2010).

Nase (*Chondrostoma nasus*), vimba bream (*Vimba vimba*) and barbel (*Barbus barbus*) are reophilic cyprinids (Barus and Oliva, 1995) which are characteristic endangered species of the lower rhithral (grayling) and the upper potamal (the barbel zone) in European running waters (Barus and Oliva, 1995; Lusk et al., 2005; Schludermann et al., 2009). Nase are widely distributed in central and eastern Europe within the drainages of the Rhine and Danube (Lusk and Penaz, 1995). Nase have a complex life cycle with different life stages specializing in distinct riverine niches. Adults generally live in relatively fast-flowing and deeper parts of rivers with grained (gravel, rubble, rocky, sandy) substratewhere they graze on periphyton; they move upstream into smaller tributaries for reproduction (Hudson et al., 2014). Nase are a long-lived (up to 20 years) and relatively late maturing (three to seven years) species (Hudson et al., 2014). Vimba bream (*Vimba vimba*) occur in freshwater as well as brackish waters and also are migratory (Hanfling et al., 2009). Vimba bream usually engage in short-distance anadromous migrations to barbel or grayling regions with fast-flowing currents and gravel bottoms (Barus and Oliva, 1995). Under natural conditions, vimba bream become sexually mature at 4–5 years of age, but some as early as 3 years or as late as 7–8 years (Luszczek-Trojnar et al., 2008; Czerniejewski et al., 2011). The common barbel (*Barbus barbus* L.) is regarded as an endangered fish species in Europe (Lusk et al., 2004; Prokes et al., 2006; Lefler et al., 2008). Barbel is a fish species which is highly sensitive to adverse environmental changes (Kujawa and Glinska-Lewczuk, 2011). For increasing and enhancing wild populations, methods are used for the culture and reproduction of this species. These methods have been optimized under controlled conditions during the last three decades (Poncin et al., 1996; Policar et al., 2010).

Poor reproduction under natural conditions is one of the main reasons that these species are propagated for stocking, , and broodstock are captured from open waters (Targonska et al., 2011; Cejko et al., 2014). Artificial reproduction under controlled conditions and using of synthetic hormones is one of the solutions to this problem. Thermal condition play

a crucial role during the initial phase of stimulation maturation under controlled conditions. Improperly chosen thermal condition as well as their fluctuation, either delay spawning or cause gametes to mature with defects (Wang et al., 2010; Targonska et al., 2010, 2012; Nowosad et al., 2014a). Successful hormonal stimulation of rheophilic cyprinids has been performed using mainly gonadoliberin analogues (LHRH) or gonadoliberin analogues (LHRHa) applied as a complex with a dopamine receptor antagonist, such as Ovopel or Ovaprim (Zarski et al., 2009; Cejko et al., 2011, 2012a). These products have confirmed a high efficiency of ovulation and economic profitability artificial spawning among barbel bred in captivity (Hakuc-Blazowska et al., 2010; Targooska et al., 2011). Larvae rearing methods are based on the pond aquaculture, rearing in control conditions or combination both of them. Dry diets can be used from the beginning of the feeding period and very high rate of the survival ccan be reached (Kujawa et al., 2010).The stocking density of larvae culture is a very important factor in determining the economic effectiveness of production (Turkowski et al., 2008; Zarski et al., 2011). All of this species tolerate higher stocking densities at the beginning of rearing.

Objectives of the thesis

The main goal of this study was to develop and evaluate sustainable management of RAS for the rearing of riverine species, with an emphasis on economical sustainability and profitability. In my study, I used our previous long-term research on rearing barbel, in combination with RAS and pond culture, and also applied it to vimba and later on to nase. There are many possibilities for use of recirculating aquaculture system. One of them is a winter seasonal rearingcombining RAS and pond rearing to gain larger and more viable fish for restocking in the spring time. We tested this technology on both species in the following seasons and compared it with previous barb rearing. The next study was to try use vimba bream in training pikeperch fingerlings to a commercial diet. . Because of stress during the manipulation of fish during RAS rearing and the fact that the welfare of fish is one of the most important factors, we also made a short experiment on vimba bream to compare the impact of stress from each offour anaesthetics.

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CHAPTER 2

INTENSIVE WINTER CULTURE OF NASE (*CHONDROSTOMA NASUS*) AND VIMBA BREAM (*VIMBA VIMBA*) FOR SPRING RESTOCKING

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INTENSIVE WINTER CULTURE OF NASE (*CHONDROSTOMA NASUS*) AND VIMBA BREAM (*VIMBA VIMBA*) FOR SPRING RESTOCKING

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ABSTRACT

Two six-month experiments were conducted during two consecutive winter seasons with the five-month-old nase (*Chondrostoma nasus*) and vimba bream (*Vimba vimba*) which had been trained on pellet feed and acclimated to four different temperatures. The WT fluctuated in parallel with ambient outside conditions at an average of approximately 4 °C for the inflow system and approximately 15, 18 and 21 °C in closed, recirculation systems; these experimental groups were designated as A, B, C, and D, respectively. The total length and weight were measured at two-week intervals and SGR_w, FCR and survival were monitored.

Growth was significantly greater in higher temperature conditions; total length by nase (A – 73.37 ± 3.82, B – 94.11 ± 5.58, C – 108.68 ± 7.07 and D – 128.52 ± 7.64 mm) and body weight (A – 2.85 ± 0.47, B – 6.14 ± 1.20, C – 9.26 ± 2.14 and D – 16.71 ± 3.46 g). The same pattern was observed in vimba bream (TL A – 58.32 ± 2.71, B – 69.33 ± 6.68, C – 91.28 ± 7.48 and D – 102.16 ± 7.80 mm and body weight A – 1.57 ± 0.24, B – 2.71 ± 0.50, C – 6.81 ± 1.96 and D – 8.88 ± 2.52 g). The values of SGR_w at the end of the experiment were significantly different in all groups in both species of fish. The ranges were 0.001 ± 0.014 – 0.930 ± 0.014 for the vimba bream and 0.077 ± 0.009 – 0.924 ± 0.004 for nase, respectively. FCR at the end of experiment were A – 14.79 ± 3.50, B – 2.79 ± 0.19, C – 2.43 ± 0.11 and D – 2.16 ± 0.07 and A – 16.95 ± 4.67, B – 3.37 ± 0.33, C – 1.65 ± 0.03 and D – 1.59 ± 0.04 in case of nase and vimba bream, respectively. Survival ranging between 97.7 ± 0.5 and 99.0 ± 0.5 %, in all groups except that survival in group A (95.9 ± 1.3%) of vimba bream was significantly lower.

Intensive winter rearing of nase and vimba bream is an effective way to prepare fish for spring restocking.

Keywords: *Vimba bream, Nase, SGR_w, FCR, RAS*

Introduction

Nase (*Chondrostoma nasus*) and vimba bream (*Vimba vimba*) are reophilic cyprinids (Barus & Oliva, 1995) which are characteristic endangeredspecies of the lower rhithral (grayling) and the upper potamal (the barbel zone) in European running waters (Barus & Oliva, 1995; Lusk, Luskova, Halacka, Slechtova, & Slechta, 2005; Schludermann, Keckeis, & Nemeschkal, 2009). Nase are widely distributed in the central and eastern Europe within the drainages of the Rhine and Danube catchments (Lusk & Penaz, 1995). Nase have a complex life cycle with different life stages specializing in distinct riverine niches. Adults generally live in relatively fast- flowing and deeper parts of rivers with grained (gravel, rubble, rocky, sandy) substrate, where they graze on periphyton; they move upstream into smaller tributaries for reproduction (Hudson, Vonlanthen, & Seehausen, 2014). Nase are a long-lived (up to 20 years) and relatively late maturing (three to seven years) species (Hudson et al. 2014). Vimba bream occur in freshwater as well as brackish waters and also are migratory (Hanfling, Dumpelmann, Bogutskaya, Brandl, & Brandle, 2009). Vimba bream usually engage in short-distance anadromous migrations to

barbel or grayling regions with fast-flowing currents and gravel bottoms (Barus & Oliva, 1995). Under natural conditions, vimba bream become sexually mature at 4–5 years of age, but some as early as 3 years or as late as 7-8 years for initial reproduction (Luszczek-Trojnar et al., 2008; Czerniejewsk et al., 2011). The populations of both species began decreasing in the 20th century (Lusk, 1995; Keckeis, Frankiewicz, & Schiemer, 1996; Hliwa et al., 2003; Spurny et al., 2004) not only because of overfishing, but also from other anthropogenic effects such us loss of gravel bank areas, water pollution, destruction of spawning areas or eutrophication of open waters. According to the IUCN/WCU (2008) species endangered in many European countries (Witkowsk et al., 2009; Popovic et al., 2013), and based on the Red List of Threatened Species in the Czech Republic (Lusk et al., 2004), vimba bream is categorised as a vulnerable, while nase is classified as an endangered fish species in Czech nature. As a result of the unfavourable state of natural populations, research has been started in the last two decades focusing on artificial reproduction and rearing for restocking; captive breeding programs are widely used in conservation of many endangered fish species.

Nase culture in Europe began as early as 1922, when artificial propagation and rearing were carried out. A relatively substantial amount of information about artificial spawning presently exists (Luszczek-Trojnar et al., 2008; Alavi et al., 2009; Alavi et al., 2010; Kamler ,et al., 1998; Keckeis et al., 2001; Kouba et al., 2014), as well as different aspects of larvae and juvenile rearing of vimba bream and nase (Hliwa et al., 2003; Spurny et al., 2004; Hamackova et al., 2009; Kwasek et al., 2009) and other cyprinids (Kaminski ,et al., 2010; Policar et al., 2011).

Further, nase larvae have been reared in ponds (Schludermann et al., 2009) or dike ponds (long and narrow ponds) (Lusk, 1997). It seems that controlled culture of juvenile stages of nase and vimba bream for restocking could be one of the promising ways of increasing the abundance of this fish species in running waters (Hliwa et al., 2003).

The aim of this study was to rear the nase and vimba bream in recirculation aquaculture system (RAS) for 180 days during the winter season to find the optimal (economically and effectively) water temperature (WT) for their culture.

Material and methods

Experimental fish and rearing conditions

Five-month-old nase and vimba bream juveniles were obtained from Experimental Fish Culture Facility of the Faculty of Fisheries and Protection of Waters at the University of South Bohemia in Vodnany and were trained to accept pellet feed. Experiments were run during two consecutive winter seasons, 2012/2013 and 2013/2014 for vimba bream and nase. Each experiment lasted for six months. At the beginning of rearing, 12000 nase juveniles [total length (TL) = 70.99 ± 3.81 mm; weight (W) = 2.49 ± 0.4g] and 12000 vimba bream juveniles (TL = 57.05 ± 3 mm; W= 1.41 ± 0.27 g) were distributed into twelve fiberglass tanks (1 x 1 x 0.8 m; usable volume 250 L, water column 0.25 m) at density of 4 individuals/L (1000 individuals/tank). Illumination intensity at the water surface was 40 lux and the light regime was 12L:12D. The water temperature (WT) and oxygen saturation (OS) were measured twice daily with an oximeter (OxyGuard) throughout the experiment. Fish were fed during the light cycle using a belt feeder (FIAP GmbH). Feed (Aller Futura MP EX, sice 0.7 mm and 1 mm) contained: protein – 60%, fat – 17%, NFE – 5.3%, ash – 10.5%, fiber – 0.5%. The daily feeding rate was 2–3% of fish biomass.

Experimental groups

Twelve tanks were used for each species were divided into four groups with different water temperatures (Groups A; B; C and D – with three repetitions. Tab. 1). Tanks in groups B; C and D were connected to a close recirculation system with the WT approx. 15, 18 and 21 °C, respectively while tanks in group A were connected and filled with water from local river to provide ambient, fluctuating winter WT conditions with an average of approximately 4 °C.

Observations and measurements

The control harvesting of all tanks were made every thirtieth day of experiment. Fifty juveniles from each tank were anaesthetised with Clove oil (0.03 $ml.l^{-1}$) (Lepic et al., 2014) to measure total length (TL) to the nearest 1 mm and weighed to the nearest 0.1 g. Specific growth rate (SGR_w) was calculated using the formula SGR_w (% day^{-1}) = 100 (Ln W_f – Ln W_i)ΔT^{-1}, where W_i and W_f are initial mean weight and final mean weight in mg, and T is growing period in days (Nyina-wamwiza et al., 2005). The daily feed ration was adjusted for the next period based on total weight of fish in each tank and established for group A – 0.5–1.5%, B – 1.5%, C and D – 2% of total weight stock, respectively. Dead juveniles were removed every day.

Statistical analysis was based on one-way Analysis of Variance (ANOVA, Statistica 12, StatSoft, Inc.). Significant differences between groups were estimated using Tukey's post-hoc test. The level of significance was set at $P < 0.05$.

Results

Tab. 1. *Experimental groups, used water temperatures, specific weight growth rate and survival in nase and vimba bream. Different letters indicate significant difference (P < 0.05).*

Nase	WT [°C]	SGR_w [%.day^{-1}]	Survival [%]
Group A	3.9 ± 2.4	0.077 ± 0.009a	98.3 ± 0.5a
Group B	14.7 ± 0.58	0.367 ± 0.060b	98.2 ± 1.2a
Group C	17.7 ± 0.61	0.590 ± 0.034c	98.3 ± 0.9a
Group D	21.0 ± 0.29	0.924 ± 0.004d	97.7 ± 0.5a
Vimba bream			
Group A	4.4 ± 3.2	0.001 ± 0.014a	95.9 ± 1.3a
Group B	15.0 ± 0.47	0.306 ± 0.032b	98.7 ± 0.8b
Group C	18.5 ± 0.59	0.758 ± 0.036c	99.0 ± 0.5b
Group D	21.1 ± 0.3	0.930 ± 0.014d	98.9 ± 0.2b

Nase

The total length (Fig. 1) and body weight (Fig. 2) increased significantly in each group with increasing water temperature. Body weight of fish in Group A remained the same throughout the experiment (Fig. 2). Water temperature also had significant effects on SGR_w of nase juveniles among each group (Tab. 1); however, there were no significant effects of water temperature on survival of nase juveniles (Tab. 1). FCR at the end of experiment were 14.79 ± 3.50, 2.79 ± 0.19, 2.43 ± 0.11 and 2.16 ± 0.07 for the group A, B, C and D, respectively.

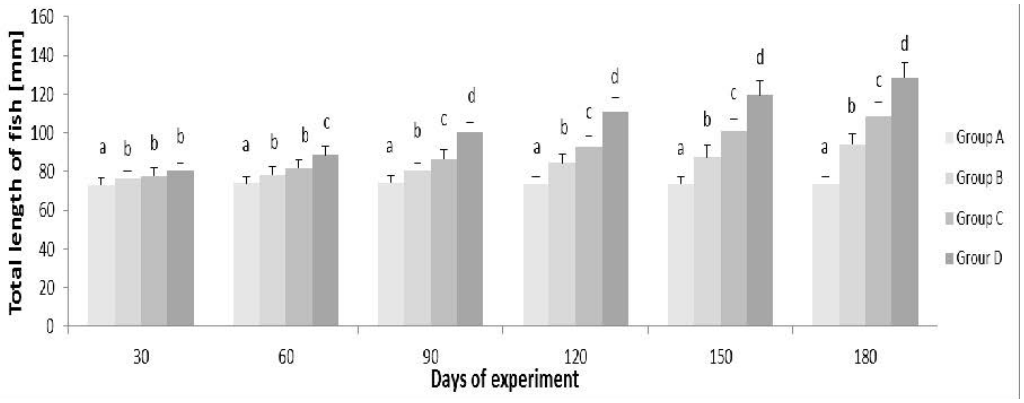

Figure 1. *Total length of nase juveniles measured during the experiment. Different letters indicate significant difference (P < 0.05).*

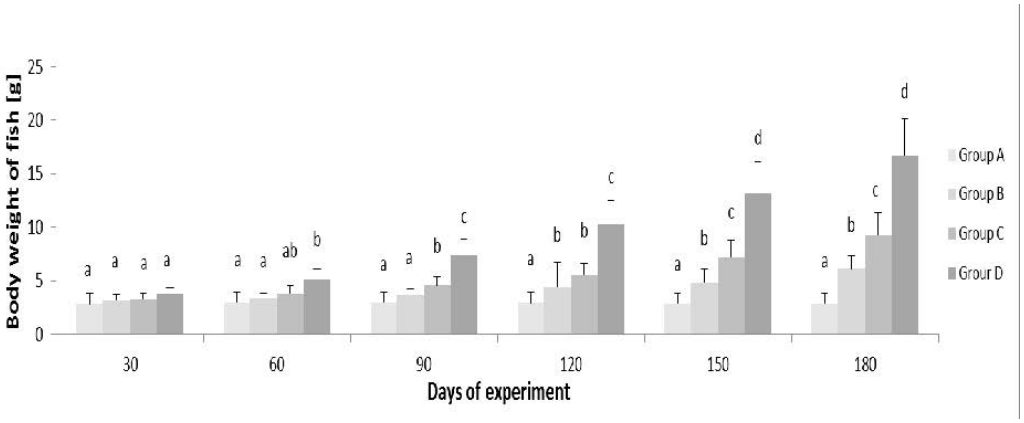

Figure 2. *Body weight of nase juveniles weighed during the experiment. Different letters indicate significant difference (P < 0.05).*

Vimba bream

As in case of vimba bream, the total length (Fig. 3) and body weight (Fig. 4) also were significantly influenced by water temperature regime. There was a strong difference in growth between 15 °C (Group B) and 18.5 °C (Group C). WT had major effect on SGR_w in experimental groups, however, growth of fish decreased in the low WT, Group A (Tab. 1). Moreover, compare to the nase, survival rate was significantly lower in the lowest water temperature (3.9 °C; Group A) (Tab. 1). Values of FCR at the end of experiment were 16.95 ± 4.67, 3.37 ± 0.33, 1.65 ± 0.03 and 1.59 ± 0.04 for the group A, B, C and D, respectively.

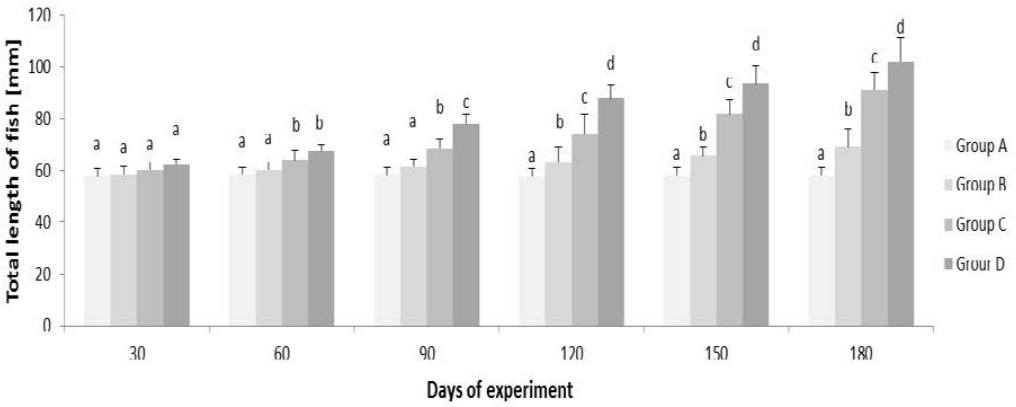

Figure 3. *Total length of vimba bream juveniles weighed during the experiment. Different letters indicate significant difference (P < 0.05).*

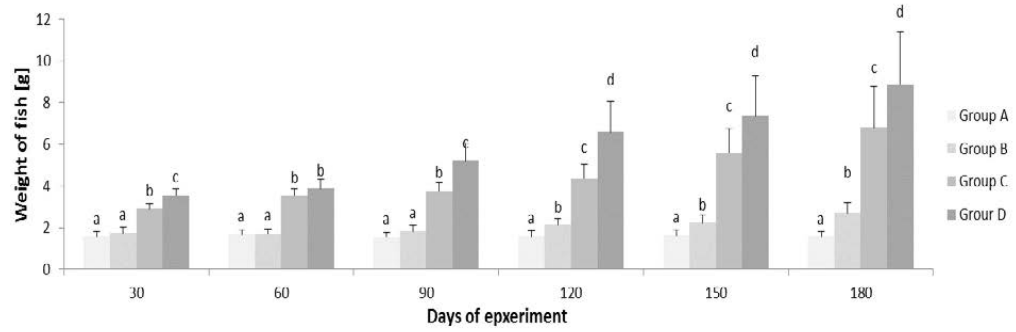

Figure 4. *Body weight of vimba bream juveniles weighed during the experiment. Different letters indicate significant difference (P < 0.05).*

Discussion

Rearing of rheophilic fish species under RAS conditions during the cold winter months provided enhanced restocking material during the spring when the water temperature and hydrological conditions are favourable and the larger size should increase post-stocking survival. Philippart et al. (1989) restocked juvenile common barbel (*Barbel barbel*) after intensive culturing. The main reason for using of the intensive rearing methods in rheophilic fish species is to optimize the environmental conditions during the cold months of the year and the main advantage could be acceleration of their growth. Fish growth rate is mainly influenced by feed, water quality (Molnar et al., 2004), and water temperature (Wang et al., 2009; Ott et al., 2012).

Temperature aspects

We observed a positive effect of higher water temperatures on their growth rate of each species. Both groups D (WT 21 °C) had the most rapid growth and highest SGR_w. Predictably, growth rate and SGR_w were lowest for each species in group A, under the ambient temperature conditions. There were no differences between individual weights at the beginning and end of this treatment. Also the poor FCR indicates that the fish did not use the feed that was provided.

Nevertheless, to specify the best WT for production is not unambiguous because several aspects. The best growth was achieved under the 21 °C temperature conditions. Individual weight of fish at the end of rearing approached the size of two-year-old fish. Not only was growth higher, but also the time to reach sexual maturity was reduced. Integrating conventional pond culture (Luszczek-Trojnar et al., 2008) with indoor overwinter culture can enhance stocking material. Further, broodstock management can be improved by advancing sexual maturation of vimba bream and nase. Also values of FCR (2.16 ± 0.07 and 1.59 ± 0.04 for nase and vimba bream, respectively) approached levels normally achieved in the RAS and demonstrates the ability to effectively use the feed in this temperature. Based on these results, despite the higher costs of operating the RAS at this temperature, advantages can be realized. Several authors (e.g. Kwasek et al., 2009; Kaminski et al., 2010) have recommended even higher WT (24–25 °C) as optimal, although this would result in even higher energy cost. However, several RAS fish farms are use the waste heat energy from e.g. biogas power plants, which could make it effective.

Lower temperature at the level of 18 °C gave good results in comparison to rearing in cold water. Individual weight was 3.25 and 4.13 times higher (9.26 ± 2.14 g and 6.81 ± 1.96 g) for nase and vimba bream, respectively. Values of FCR (2.43 ± 0.11 and 1.65 ± 0.03 for nase and vimba bream, respectively) indicate that fish utilize the feed better. From the point of view of energy cost, restocking advantages might outweigh this negative aspect.

The temperature 15 °C was chosen with regard to the functioning of the biofilter and in this experiment, it proved to be a minimal temperature suitable for rearing of river fish species in RAS. On one hand the economic costs to maintain this temperature is relatively low, but on the other hand the effect of production (low individual weight and high FCR) considered this level of WT as very less effective in several aspects.

Conclusion

Intensive winter rearing of nase and vimba bream is a good way to increase the potential to survive the winter, but also gain some additional weight and energy for the spring restocking phase. Of course, it is needed to find the optimal rearing conditions (for high survival, sufficient growth rate, low energy and cost demand) which would make this method as effective as possible and would not be too expensive. In this case, the rheophilic fish could play a role of the additional fish species to fulfil the capacity of the whole RAS system during the winter. It is also possible to rear the juveniles under current water temperature of the RAS and decrease the cost of the rearing.

Additionally, the multidisciplinary approach combining not only aquaculture production data but also welfare and healthy aspects, genetic, phenotypic and geographic structuring of phenotypic and genetic diversity across populations both natural and captive) should be given strong attention (Rabova et al., 2003; Hanfling et al., 2009; Popovic et al., 2013; Hudson et al., 2014). Rearing riverine fish under non-flowing conditions may be stressful and lead to adverse growth but also can increase the potential to the diseases (Recek et al., 2009). Using of local fish stocks instead of non-native stocks often use in commercial hatcheries seems to be preferred for many aspects in conservation captive breeding restocking programs of many endangered species (Laikre, 2010; Luikart et al., 2010; Popovic et al., 2013; Vetesnik et al., 2009).

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CHAPTER 3

ADAPTATION TO PELLETED FEED IN PIKEPERCH FINGERLINGS: LEARNING FROM THE TRAINER FISH OVER GRADUAL ADAPTATION FROM NATURAL FOOD

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SHORT COMMUNICATION

Adaptation to pelleted feed in pikeperch fingerlings: learning from the trainer fish over gradual adaptation from natural food

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Abstract – Pikeperch (*Sander lucioperca*) is commercially important as well as a valued culinary fish with potential for intensive culture. One of the basic problems in pikeperch culture in recirculating aquaculture systems is adapting early life stages to pelleted feed (PF). Our work compares four different ways of adapting 6-week-old pikeperch fingerlings (standard length, SL = 29.30 ± 2.14 mm; weight, $W = 0.38 \pm 0.08$ g) to feeding on a commercial diet. The methods are designated, A – use of trainer fish (*Vimba vimba*; SL = 36.88 ± 3.28 mm; $W = 0.77 \pm 0.26$ g) and direct application of PF; B – use of trainer fish and gradual addition of PF with natural food (natural feed (NF), chironomid larvae); C – direct application of PF only; D – gradual addition of PF with NF. The growth trial experiment (including adaptation to PF) lasted 14 days by which time all experimental groups were accepting PF. Pikeperch fingerlings in group A grew significantly faster and PF was more readily accepted compared to other groups (SL = 46.11 ± 4.09 mm; $W = 1.44 \pm 0.38$ g; specific growth rate = 9.48 ± 0.83% day^{-1}). Other characteristics observed (total weight increment, feed conversion ratio) also support the use of trainer fish and direct application of PF for pikeperch in their adaptation to intensive culture. It is also an illustration of applicable use of learning potential in fish which can be usable for other fish species.

Keywords: Aquaculture / Pikeperch / Feed / Social learning

1 Introduction

The growing human population and the increasing demand for quality and healthy food together with problems related to over-harvesting of oceanic fisheries are the most important factors which make aquaculture one of the most progressive food-producing sectors today (Food and Agriculture Organization of United Nations, 2014). Recent developments in aquaculture are exemplified by the use of intensive recirculating aquaculture systems (RASs) which provide solutions to limitations of water resources and management of effluents – i.e. to provide economic and environmental sustainability (Martins et al., 2010; National Intelligence Council, 2012; Buřič et al., 2015). On the other hand, considerable effort is spent on implementation of intensive culture for new species in RAS. Pikeperch (*Sander lucioperca*) is commercially important, attractive and valued for its culinary attributes and is increasingly being reared in RAS in Europe, with potential for further use in intensive aquaculture (Philipsen and van der Kraak, 2008; Frisk et al., 2012; Hermelink et al., 2013). This is valid also for the related North American species, walleye (*Sander vitreus*), which tends to be a more important aquaculture species in Canada and USA (Summerfelt, 2004; Zarnoch et al., 2010). Reproduction, both artificial and semi-artificial, is well mastered and when combined with early pond culture of larvae and fingerlings it is possible to obtain an adequate supply of fish for adaptation to pelleted feed (PF) (Bódis et al., 2007; Policar et al., 2013a). There have been other considerable advances in intensive perciform fish culture and research, but a basic problem is to adapt the early life stages to PF so as to take full advantage of their growth capacity (Summerfelt, 2004; Policar et al., 2013a).

There have been different approaches to adapting a predatory fish such as pikeperch to commercial PF. Various species differ in the time of adaptation (age of adapted fish), method (density, feed type, use of natural feed (NF) or cultured live feed etc.) and their combinations (Summerfelt, 2004; Molnar et al., 2004; Ostaszewska et al., 2005; Hamza et al., 2007; Policar et al., 2013b). In walleye, clay turbidity is considered essential for enhancing intensive walleye culture in tanks, as it provides visual contrast for the prey and leads to reduction of cannibalism and clinging behavior in larval stages (Clayton et al., 2009). Taking growth capacity into account in

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relation to the technical difficulties, the most probable optimization would be to adapt 6-week-old pond raised fingerlings to a formulated pelleted diet and then habituate them to it (Steffens et al., 1996; Summerfelt, 2004; Bódis et al., 2007; Policar et al., 2013a). Six-week-old juveniles are 30–50 mm and initiate predatory feeding (including cannibalism) on fish (Antalfi, 1979; Steffens et al., 1996), which is the optimal stage for training to a nutritious PF. Usually PF is gradually and increasingly given so as to replace NF. Good results have been achieved using chironomid larvae initially with gradual addition of PF (Bódis et al., 2007; Policar et al., 2013b). Recently, "trainer fish" such as previously trained pikeperch and perch were used to facilitate the adaptation or training to PF (Horváth et al., 2013) but this approach has been demonstrated only for yearlings and was unsuccessful.

However, the use of trainer fish seemed a promising avenue for future research and practice especially for younger fish, as the 6-weeks-old pikeperch described above. There is a possibility to gain from the usual behavioral patterns of earlier developmental stages of fish such as shoaling behavior (Hager and Helfman, 1991; Pitcher, 2001) which can increase protection against predators and optimal food gathering (Pitcher, 1986, 2001). When fish form a compact shoal, then, apart from the above mentioned benefits, they can learn from each other in space and time, e.g. in the case of feeding behavioral patterns (Lachlan et al., 1998; Chapman et al., 2008). Similar patterns, called social learning, are known from different animal taxa and in a not negligible extent also in teleost fish species (Laland and Williams, 1997; Brown and Laland, 2003). Social learning was successfully tested in hatchery reared fish for improving their post-release survival (Brown and Laland, 2001). Our work could show how to practically apply this behavioral concept in commercial aquaculture.

We designed an experiment for 6-week-old pond-raised pikeperch fingerlings which compared the gradual transition from NF to PF, direct application of PF, and both approaches, using a cyprinid "trainer fish" in RAS. The main goal of the experimental work was to explore the potential benefits, advantages or disadvantages, so as to find the most appropriate method for pikeperch adaptation to PF. The results should outline how to minimize inputs and maximize outputs in the production of pikeperch fingerlings intended for further intensive culture in RAS.

2 Materials and methods

2.1 Experimental animals and conditions

Fish were used from separate cultures of two species, pikeperch and vimba bream (*Vimba vimba*). Pikeperch were raised by a semi-artificial breeding approach using hormonal stimulation and semi-natural spawning on provided transferable substrates. Prior to hatching, nests with eyed eggs were transferred to prepared ponds in May 2015 (Policar et al., 2008; Krist'an et al., 2013; Policar et al., 2013b). After 6 weeks of pond rearing, fingerlings (standard length, SL = 29.30 ± 2.14 mm; weight, $W = 0.38 \pm 0.08$ g) were harvested, acclimated to experimental conditions and stocked in tanks. The trainer fish vimba bream, a cyprinid inhabiting lower or middle reaches of warm rivers in Europe and western Asia (Kottelat and Freyhof, 2007), was chosen due to its availability in time and good experiences with feeding with PF (Hamackova et al., 2009). One-year-old vimba bream were harvested from ponds in April 2015 and stocked in a flow-through system for training to PF. After the PF acceptance, vimba bream (SL = 36.88 ± 3.28 mm; $W = 0.77 \pm 0.26$ g) were moved for acclimation to experimental conditions before stocking in the experimental set-up.

Table 1. Experimental conditions in the recirculating aquaculture system during experiment. Data are presented as mean ± standard deviation.

Parameter	Mean ± standard deviation
Water temperature (°C)	21.39 ± 1.07
Dissolved oxygen (mg L^{-1})	8.48 ± 0.85
pH	6.99 ± 0.33
Ammonia, NH_4^+ (mg L^{-1})	0.22 ± 0.06
Nitrite, NO_2^- (mg L^{-1})	0.22 ± 0.12

Four types of experimental conditions were used: A – pikeperch with trainer fish and direct application of PF, B – pikeperch with trainer fish and gradual addition of PF to natural food (NF, chironomid larvae), C – pikeperch without trainer fish and direct application of PF, and D – pikeperch without trainer fish and gradual addition of PF to NF. Each group consisted of 3600 pikeperch fingerlings and, in groups A and C, also of 720 vimba bream juvenile trainer fish. The initial biomass was 2.39 kg m^{3-1} with trainer fish and 1.69 kg m^{3-1} without them. Each experimental group was stocked in triplicate.

The experimental work, including fingerling rearing and acclimation, was carried out in the RAS of the Experimental Fish Culture Facility of Research Institute of Fish Culture and Hydrobiology. Oxygen saturation levels and water temperatures (oximeter Oxi 3205 with CellOx® 325, WTW Gmbh, Weilheim, Germany) were monitored twice daily and pH (pH meter pH 330i with SenTix 41, WTW Gmbh, Weilheim, Germany), ammonia (NH_4^+) and nitrite (NO_2^-) (by colorometric analysis) content was monitored daily (Table 1). Experimental tanks were 1 m × 1 m × 0.8 m (0.8 m^3); they were cleaned daily. Light regime was set to 16 h light and 8 h dark.

Fish were fed to satiation (feeding was stopped when fish did not react to feed) hourly during the light period. That means that feed was applied in total 15 times per day – feeding started 1 h after the beginning of light period and the last feeding was conducted 1 h before its end. Visual observations of fish behavior were made during each feed application. Uneaten feed was removed and the feed ration for the next hour was weighed. Due to the feeding procedure used the amount of uneaten feed was negligible and was not included in the calculations, where only the amount of feed provided is used. This complicated system of feed dosage was necessary to maintain the pre-determined ratio between NF and PF, when both diet components were applied. The daily feed rations of PF and NF for all experimental groups are presented in Table 2. Frozen chironomid larvae (NF) and a commercial PF (Inicio Plus, Biomar A/S, Denmark) were used. The training or adaptation period lasted 14 days, by which time all experimental groups were well trained to the PF, and thereafter only PF was given.

Table 2. Daily feed rations in grams (wet weight was used for natural feed) in four experimental set-ups used for pikeperch adaptation to commercial pelleted feed: pikeperch with trainer fish and direct application of pelleted feed (A); pikeperch with trainer fish and gradual addition of pelleted feed to natural food (B); pikeperch without trainer fish and direct application of pelleted feed (C); pikeperch without trainer fish and gradual addition of pelleted feed to natural feed (D). The proportion of pelleted feed (% of PF) in the total weight of applied feed is presented in groups where natural (NF) and pelleted feed (PF) was used.

Day	Pikeperch with trainer fish				Pikeperch without trainer fish			
	A	B			C	D		
	PF	NF	PF	% of PF	PF	NF	PF	% of PF
1	90	630	0	0	45	500	0	0
2	135	595	0	0	65	485	0	0
3	170	780	0	0	100	520	0	0
4	220	780	187.5	20	115	780	188	20
5	210	520	216	30	125	520	216	30
6	190	407	275	40	120	407	275	40
7	165	261	261	50	100	261	261	50
8	195	163	243	60	130	163	243	60
9	210	112	267	70	140	112	267	70
10	190	81	325	80	125	81	325	80
11	210	42	392	90	140	42	392	90
12	275	0	255	100	205	0	205	100
13	300	0	300	100	215	0	215	100
14	185	0	190	100	185	0	195	100
Total feed amount (g)	2745	4371	2911	X	1810	3871	2781	X

2.2 Data acquisition

The amount of feed and mortality were recorded daily. In both fish species, pikeperch and vimba bream, SL was measured to an accuracy of 1 mm and weight to the nearest 0.01 g with an electronic balance (Kern & Sohn GmbH, Balingen, Germany), initially and at the end of the experiment. A random sample of fifty specimens per replicate was measured and weighed for each species. Calculations included:

Total weight increment (TWI, g):

$$\mathrm{TWI} = \mathrm{TBf} - \mathrm{TBi},$$

where TBf = total final biomass (g) and TBi = total initial biomass (g) in the experimental stock.

Feed Conversion Ratio (FCR) using the following formula:

$$\mathrm{FCR} = \mathrm{Wf}/\mathrm{TWI},$$

where Wf = amount of PF applied (g) and TWI = obtained TWI (g) in the experimental stock. In experimental groups B and D the FCR calculation is based on PF only, without the contribution of NF.

Specific growth rate (SGR, % per day)

$$\mathrm{SGR} = (\ln(\mathrm{Wt}) - \ln(\mathrm{Wi})) \times 100/T,$$

where Wt = weight at time t (g), Wi = initial weight (g) and T = time (days).

2.3 Data analysis

Data were analyzed using Statistica 12.0 (StatSoft, Inc.). Results were first examined for normal distribution (Kolmogorov–Smirnov test). Non-parametric Kruskal–Wallis test with multiple comparisons post hoc test was used to analyze the mortality, FCR, SGR and TWI; nested analysis of variance (ANOVA) was used for to analyze SL and weight of pikeperch and vimba bream in different experimental groups at the end of experiments (replicate tanks as a random factor, experimental group as a fixed factor) with Tukey's post-hoc test, t-test was used for comparison of vimba bream standard length (SL) and weight, and Mann–Whitney test was used for comparison of vimba bream TWI and SGR at the end of experiment. The null hypothesis was rejected at $\alpha = 0.05$. Data are presented as means ± standard deviation (STD).

3 Results

Mortality did not differ among experimental groups (Kruskal–Wallis test, $H = 3.10$; df = 3; $P = 0.38$) and ranged among 7.3–15.9%, 9.4–13.5%, 9.8–14.7%, and 5.5–11.0% in experimental groups A, B, C, and D, respectively (Table 3). Final size and weight of pikeperch fingerlings was significantly higher in group A (ANOVA, $F = 44.99$; df = 3; $P < 0.05$ and $F = 34.43$; df = 3; $P < 0.05$, respectively). There were no detected significant differences between replicates within experimental groups regarding SL and W (ANOVA, $F = 1.99$; df = 2; $P = 0.14$ and $F = 0.03$; df = 2; $P = 0.97$). SGR was

Table 3. Mortality, standard length (SL), weight (*W*), total weight increment (TWI) reached at the end of the experiment and feed conversion ratio (FCR) obtained in all experimental groups of pikeperch fingerling: pikeperch with trainer fish and direct application of pelleted feed – PF (A); pikeperch with trainer fish and gradual addition of pelleted feed to natural food – PF + NF (B); pikeperch without trainer fish and direct application of pelleted feed – PF (C); pikeperch without trainer fish and gradual addition of pelleted feed to natural feed – PF + NF (D). Data are presented as mean ± standard deviation. Values in the same row with different superscripts differ significantly ($\alpha = 0.05$).

Parameter	Pikeperch with trainer fish		Pikeperch with trainer fish	
	A PF	B PF + NF	C PF	D PF + NF
Mortality (%)	9.41 ± 3.80^{a}	9.65 ± 1.81^{a}	12.16 ± 2.46^{a}	7.63 ± 2.96^{a}
SL (mm)	46.11 ± 4.09^{a}	41.07 ± 3.37^{c}	42.92 ± 4.20^{b}	42.80 ± 3.86^{b}
W (g)	1.44 ± 0.38^{a}	1.09 ± 0.25^{c}	1.17 ± 0.32^{bc}	1.19 ± 0.32^{b}
TWI (g)	3252 ± 357^{a}	2130 ± 162^{b}	2352 ± 230^{b}	2603 ± 204^{b}
FCR	0.76 ± 0.07^{a}	1.13 ± 0.11^{b}	0.77 ± 0.08^{a}	1.07 ± 0.08^{b}

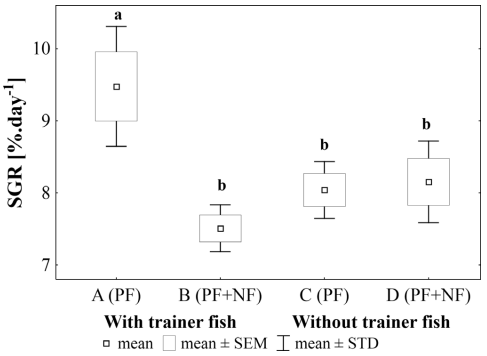

Fig. 1. Specific growth rate (SGR) achieved in pikeperch fingerlings in all experimental groups of pikeperch fingerling: pikeperch with trainer fish and direct application of pelleted feed – A (PF); pikeperch with trainer fish and gradual addition of pelleted feed to natural food – B (PF + NF); pikeperch without trainer fish and direct application of pelleted feed – C (PF); pikeperch without trainer fish and gradual addition of pelleted feed to natural feed – D (PF + NF). Data are presented as mean, standard error of mean (SEM), and standard deviation (STD). Values with different superscripts differ significantly ($\alpha = 0.05$).

significantly poorer in group B (for detailed information see Table 3). The apparently higher SGR in group A was also significantly different (Kruskal–Wallis test, $H = 8.91$; df = 3; $P < 0.05$) from other groups (Fig. 1).

TWI was obviously also significantly greater (Kruskal–Wallis test, $H = 8.64$; df = 3; $P < 0.05$) in group A, but with no difference among the other three groups (Table 3). The FCR, calculated for PF only, was significantly higher (Kruskal–Wallis test, $H = 9.51$; df = 3; $P < 0.05$) in groups where only PF was given (group A and C) with no difference between them, despite the overestimation of FCR in groups with application of NF (not included in calculations) (Table 3). Group C had very good FCR, but the amount of feed applied in accordance to fish foraging behavior was lower (Table 2).

Table 4. Standard length (SL), weight (*W*), total weight increment attained (TWI) and specific growth rate (SGR) for vimba bream used as trainer fish at the end of the experiment in group A (pikeperch with trainer fish and direct application of pelleted feed – PF) and B (pikeperch with trainer fish and gradual addition of pelleted feed to natural food – PF + NF). Data are presented as mean ± standard deviation. Values in the same row with different superscripts differ significantly ($\alpha = 0.05$).

Parameter	A PF	B PF + NF
Mortality (%)	2.69 ± 0.76^{a}	2.73 ± 0.63^{a}
SL (mm)	43.14 ± 6.66^{a}	44.42 ± 4.04^{a}
W (g)	1.32 ± 0.41^{a}	1.46 ± 0.45^{a}
TWI (g)	371 ± 31^{a}	470 ± 116^{a}
SGR (% day^{-1})	3.85 ± 0.24^{a}	4.60 ± 0.74^{a}

The growth of trainer fish was identical among groups – SL and *W* (ANOVA, $F_{1,90} = 1.33$; df = 1, 90; $P = 0.25$ and $F = 2.33$; df = 1, 90; $P = 0.13$ respectively), and SGR and TWI did not differ significantly (Mann–Whitney test, $Z = -1.31$; $P = 0.19$ and $Z = -0.87$; $P = 0.38$, respectively). There were no detected significant differences between replicates within experimental groups regarding SL and *W* (ANOVA; $F = 1.76$, df = 2, 90; $P = 0.18$ and $F = 1.48$; df = 2, 90; $P = 0.23$ respectively) (Table 4).

4 Discussion

In the present study we used 6-week-old pond raised fingerlings for the training-period of 14 days. We tested trainer fish to facilitate the efficiency of acceptance of PF, with and without gradual transition from NF. Trainer fish were selected following three basic criteria: larger size at the beginning of experiment (to avoid predation), fish well adapted to PF (to facilitate pikeperch acclimation to PF), and slower growth (to avoid increased competition with pikeperch). Following these criteria, we used 1-year-old pond cultured vimba bream which had been trained to PF. Other species can be used as trainers, depending on availability. A similar design was used

by Horváth et al. (2013), but they were not successful, probably due to the use of yearling pikeperch which had been feeding as predators for at least one growing season, and probably also because of aggressive trainers (adapted pikeperch and perch, *Perca fluviatilis*) or unsuitable rearing conditions (small scale experimental aquaria). The other reason could be the individualistic behavioral patterns in such old specimens, which probably revoked the effect of social learning which can easily occur in a compact shoal of fish (Lachlan et al., 1998; Kelley et al., 2003).

We demonstrated the positive effect of trainer fish in the present study when PF was applied from the first day. Pikeperch fingerlings fed on PF were positively stimulated by the foraging activity of vimba bream. It is surprising how early pikeperch specimens adapted to the new situation, formed a compact shoal with vimba bream and fed similarly to them on the new food item. This behavioral imitation illustrates the capability of fish for social learning, as suggested also by Brown and Laland (2001), and its practical use in aquaculture. In the present study this ability led to direct acceptance of PF which resulted in better growth and therefore bigger size, better SGR, and higher TWI at the end of the experiment. Moreover, the direct application of PF did not affect survival in this group. On the other hand, combining trainer fish with gradual application of PF to NF was not beneficial. This is probably due to active feeding by pikeperch on chironomid larvae, while the PF was predominantly utilized by trainer fish. Generally, the learning effect from the trainer fish was therefore reduced by selection for a preferred food item. Prolongation of the test period affected the growth of pikeperch. Surprisingly, similar results were observed in both experimental groups without trainer fish, whether with or without application of NF. The only negative effect in the group receiving direct application of PF to pikeperch fingerlings was lower growth. That was partly compensated by better FCR which did not differ from group A (trainer and direct application of PF). Notwithstanding, group A fish fed more actively (subjective personal observation), which led to significantly better growth parameters.

Pikeperch fingerlings equaled the higher weight of vimba bream at the end of the experiment and there was no predation on trainer fish. At that time the trainer fish could be removed and the species reared separately. Trainer fish can therefore be used in production of this species, or could potentially be used as natural food for brood stock. We can therefore harvest large pikeperch fingerlings adapted to dry PF together with another "by-product", vimba bream, e.g. for restocking. Probably different non-aggressive PF-trained and valuable cyprinid species could also be used, such as tench (*Tinca tinca*) (Pantazis and Hatzinikolaou, 2011) or even commercially unimportant common species like roach (*Rutilus rutilus*) or rudd (*Scardinius erythrophtalmus*).

The present approach may provide a new way to obtain well-adapted pikeperch fingerlings for intensive culture and overcome the present limiting factor of supply for pikeperch aquaculture in RAS using dry PF. The use of trainer fish could also be applicable to different fish species to streamline their fingerling production. Finally, the described approach demonstrates the applicability of the concept of social learning in fish. The simplicity with which the trained fish accepted the behavior of trainer fish extends the possibility of wide applicability of the trainer-fish approach in general. Further experimentation is needed to evaluate how broadly the "trainer approach" could be applied to different species.

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CHAPTER 4

THE EFFECTS OF FOUR ANAESTHETICS ON HAEMATOLOGICAL AND BLOOD BIOCHEMICAL PROFILES IN VIMBA BREAM, *VIMBA VIMBA*

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The effects of four anaesthetics on haematological and blood biochemical profiles in vimba bream, *Vimba vimba*

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ABSTRACT: The aim of this study was to compare the effect of four anaesthetics on the haematological and blood biochemical profiles of vimba bream (*Vimba vimba*). The haematological and blood biochemical profiles of vimba bream were evaluated 10 min and 24 h after anaesthesia with MS 222 (100 mg/l), clove oil (33 mg/l), 2-phenoxyethanol (0.4 ml/l), Propiscin (1.0 ml/l) and compared to non-anaesthetised controls. The 10 min exposure to any of the anaesthetics did not show any effects on haematological profiles. The exposure to 2-phenoxyethanol and Propiscin significantly ($P < 0.01$) influenced levels of glucose and ammonia, and the activity of aspartate aminotransferase compared with the control group. The level of triacylglycerols was significantly ($P < 0.01$) increased and the activity of lactate dehydrogenase was significantly ($P < 0.01$) decreased by exposure to MS 222. The use of clove oil showed no effects on the haematological and blood biochemical profiles and is recommended as a suitable anaesthetic for vimba bream. Other anaesthetics tested affected blood biochemical profiles to some extent.

Keywords: anaesthesia; tricaine methane sulphate; clove oil; 2-phenoxyethanol; Propiscin

The use of non-stressful anaesthetics is common practice in modern aquaculture. Such substance are used during handling, sorting, tagging, artificial reproduction procedures, and surgery, thus reducing stress-induced problems such as reduction in feeding and immune function (Ross and Ross 1999; Kolarova et al. 2007).

A variety of anaesthetics with differing properties have been used in aquaculture (Cho and Heath 2000; Kazun and Siwicki 2001; Velisek and Svobodova 2004a,b; Velisek et al. 2005a,b, 2006, 2007, 2009, 2011). Chemicals used in aquaculture are subject to strict control, particularly with regard to safety and efficacy (Taylor and Roberts 1999). The anaesthetics most commonly used are tricaine methane sulphonate (MS 222), benzocaine, quinaldine sulphate, methomidate, clove oil, and 2-phenoxyethanol (Velisek and Svobodova 2004a,b). Currently, only MS 222 is licensed for use in food fish in the USA and the European Union. However, compounds such as 2-phenoxyethanol, clove oil, and Propiscin have been evaluated experimentally and are being used in non-food fish and in research (Coyle et al. 2004). Their use on food fish remains illegal under EEC Regulation 2377/90, as no maximum residue levels (MRL) have been established.

Tricaine methane sulphonate is an isomer of benzocaine with an additional sulphonate radical, making it more soluble but also more acidic in solution (Congleton 2006). It is the most commonly used anaesthetic for fish, with a recommended concentration of 100 mg/l water (Marking and Meyer 1985).

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Clove oil is derived from the stems, leaves, and buds of the *Eugenia aromatica* and *Eugenia caryophyllata* clove trees (Sato and Burhanuddin 1995; Keene et al. 1998). The active ingredient is eugenol (4-allyl-2-methoxyphenol), which constitutes 70–90% of the oil weight. It is used as a disinfectant and analgesic in dentistry (Curtis 1990) and as an additive in perfumes (Maura et al. 1989). The recommended concentration for anaesthesia of vimba bream is 33 mg/l water (Hamackova et al. 2008). 2-phenoxyethanol (ethylene glycol monophenyl ether) is used in the Czech Republic for short-term immobilisation of fish before artificial spawning at a recommended concentration of 0.40 ml/l water (Hamackova et al. 2008). Propiscin was developed at the Inland Fisheries Institute in Poland and is routinely used for immobilisation of fish in that country (Szkudlarek and Zakes 1996). The active substance of Propiscin is etomidate [etomidate (1)-ethyl 1-(α-methylbenzyl) imidazole-5-carboxylate] (Kazun and Siwicki 2001). The recommended concentration is 1.0 ml/l water (Szkudlarek and Zakes 1996).

Although anaesthesia of fish may mitigate against the biochemical and physiological stress due to handling, the anaesthetic can itself induce alterations in haematological and biochemical values. The purpose of this study was to compare the effects of clove oil, 2-phenoxyethanol, Propiscin, and MS 222 on haematological and blood plasma biochemical profiles in vimba bream, with particularly reference to stress.

MATERIAL AND METHODS

Anaesthetics. MS 222 was purchased from Sigma-Aldrich Chemicals Ltd. Clove oil (eugenol concentration 78%) was obtained from the Kulich Company (Jan Kulich, Hradec Kralove/Ricany, Czech Republic), and 2-phenoxyethanol from MERCK-Schucherd, Hohenbrunn, Germany. Propiscin was supplied by the Division of Fish Pathology and Immunology at Zabieniec (Inland Fisheries Institute in Olsztyn, Poland). Other chemicals were obtained from Sigma-Aldrich Corporation (USA).

Experimental procedures. For assessment of the haematological profiles and the biochemical profiles of blood plasma, 54 vimba bream (339.21 ± 75.79 g body weight and 34.94 ± 3.56 cm total length) were used. During a 10-day acclimatisation period, an suitable amount of food was offered at a rate appropriate to maintain growth. Fish were not fed for 24 h before the experiments. Water temperature was maintained at 17.6–18.2 °C throughout the experimental period, and fish were maintained on a 12L : 12D regime. Nine groups of three fish each were compared:

For the control – no anaesthetic group blood was sampled prior to the treatment of anaesthetised groups. Blood was sampled immediately after 10 min of anaesthesia in the four experimental groups: MS 222 (10 min) (100 mg/l), clove oil (10 min) (33 mg/l), 2-phenoxyethanol (10 min) (0.40 ml/l), and Propiscin (10 min) (1.0 ml/l). In a further four groups blood was sampled 24 h after 10 min anaesthesia: MS 222 (24 h), clove oil (24 h), 2-phenoxyethanol (24 h), and Propiscin (24 h).

Each group was held in a separate tank containing freshwater plus the anaesthetic for experimental groups. Each treatment was duplicated. There were no mortalities during the study.

Blood was drawn from the *vena caudalis* using an 18G 1½ in syringe with heparin as anticoagulant (Heparin inj., Leciva, Czech Republic) at a concentration of 5000 IU heparin sodium salt in 1 ml. Erythrocyte count (RBC), haematocrit (PCV), haemoglobin (Hb), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), and leukocyte count (Leuko) were determined according to Svobodova et al. (2012).

Blood was separated in a cooled centrifuge (4 °C, 837 × g), and the plasma was stored at –80 °C until analysis on a VETTEST 8008 analyzer (IDEXX Laboratories Inc., USA). Biochemical indices determined in plasma included glucose (GLU), total protein (TP), albumin (ALB), total globulins (GLOB), ammonia (NH_3), calcium (Ca^{2+}), magnesium (Mg), inorganic phosphate (PHOS), triacylglycerols (TAG), aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH), creatine kinase (CK), alkaline phosphatase (ALP), and lactate (LACT). Biochemical indices were assayed using the method of Kolarova and Velisek (2012).

Statistical analysis. Statistical analysis was carried out using Statistica software 10.0 for Windows (StatSoft, Czech Republic). Data were first tested for normality (Kolmogorov-Smirnov test) and homoscedasticity of variance (Bartlett's test). If those conditions were satisfied, one-way analysis of vari-

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ance (ANOVA) was employed to reveal significant differences in measured variables among control and experimental groups. When a difference was detected ($P < 0.05$), Tukey's multiple comparison test was applied to identify which treatments were significantly different. If the conditions for ANOVA were not satisfied, the non-parametric Kruskal-Wallis test was used (Zar 1996).

RESULTS

Haematological profile

The 10 min exposure to the anaesthetics (MS 222, 2-phenoxythenalo, clove oil, and Propiscin) were not different from the control with respect to erythrocyte count, hematocrit, haemoglobin, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, or leukocyte count (Table 1).

Biochemical blood profile

The biochemical profiles are given in Table 2. The level of glucose was significantly ($P < 0.01$) greater with 2-phenoxyethanol (10 min and 24 h) and Propiscin (10 min and 24 h) compared to controls.

Ammonia levels were significantly higher ($P < 0.01$) with 2-phenoxyethanol (10 min and 24 h) and Propiscin (24 h) compared with the control group.

The levels of triacylglycerols were significantly increased ($P < 0.01$) with MS 222 (24 h) compared with the control group.

The activity of aspartate aminotransferase showed a significant increase ($P > 0.01$) with 2-phenoxyethanol (10 min and 24 h); however after anaesthesia with Propiscin (10 min and 24 h) the activity of aspartate aminotransferase was significantly decreased ($P < 0.01$) compared to the control group.

Immediately after anaesthesia induced by MS 222 (10 min), fish showed significantly lower ($P < 0.01$) lactate dehydrogenase activity compared with the control group and with all other treatments. Lactate dehydrogenase activity returned to the control level within 24 h (MS 222 24 h).

The values for total protein, albumin, total globulin, alanine aminotransferase, creatine kinase, calcium, magnesium, inorganic phosphate, alkaline phosphatise, and lactate were similar among all groups.

Table 1. Effects of MS222, clove oil, 2-phenoxyethanol, and Propiscin anaesthesia on haematological indices of blood plasma in vimba bream

Indices	Control	MS222		Clove oil		2-phenoxyethanol		Propiscin	
		10 min	24 h	10 min	24 h	10 min	24 h	10 min	24 h
RBC (T/l)	1.68 ± 0.15	1.58 ± 0.12	1.53 ± 0.35	1.52 ± 0.20	1.70 ± 0.32	1.54 ± 0.22	1.66 ± 0.40	1.55 ± 0.12	1.65 ± 0.24
Hb (g/l)	83.38 ± 6.92	89.28 ± 5.79	93.52 ± 6.37	81.64 ± 11.68	94.30 ± 8.15	88.33 ± 11.95	95.49 ± 9.52	87.67 ± 9.78	91.13 ± 12.63
PCV (l/l)	0.45 ± 0.06	0.52 ± 0.05	0.47 ± 0.06	0.46 ± 0.06	0.49 ± 0.04	0.48 ± 0.03	0.48 ± 0.04	0.46 ± 0.05	0.49 ± 0.04
MCV (fl)	268.02 ± 51.67	326.46 ± 29.29	334.92 ± 135.3	306.45 ± 40.69	295.39 ± 59.03	317.47 ± 35.62	309.30 ± 77.21	299.64 ± 46.33	301.20 ± 41.95
MCH (pg)	49.93 ± 5.23	56.72 ± 4.65	65.73 ± 19.61	54.39 ± 8.29	57.77 ± 13.87	57.74 ± 5.85	60.16 ± 11.62	57.19 ± 9.56	57.25 ± 16.04
MCHC (g/l)	191.81 ± 33.24	174.63 ± 16.57	203.96 ± 34.75	177.87 ± 17.09	195.30 ± 23.32	182.73 ± 15.90	197.17 ± 12.05	190.67 ± 11.46	188.88 ± 34.97
Leuko (G/l)	14.47 ± 3.49	14.58 ± 3.11	11.82 ± 5.15	11.57 ± 3.57	13.22 ± 3.07	14.32 ± 2.27	12.53 ± 2.94	13.45 ± 3.95	12.15 ± 2.67

All values are mean ± SD, $n = 6$

Table 2. Effects of MS222, clove oil, 2-phenoxyethanol, and Propiscin anaesthesia on biochemical indices of blood plasma in vimba bream

Indices	Control	MS222		Clove oil		2-phenoxyethanol		Propiscin	
		10 min	24 h	10 min	24 h	10 min	24 h	10 min	24 h
GLU (mmol/l)	4.50 ± 0.67	6.50 ± 1.24	4.78 ± 1.04	6.95 ± 1.63	4.90 ± 2.22	7.54 ± 1.22**	8.34 ± 1.51**	8.72 ± 0.78**	11.21 ± 2.56**
TP (g/l)	42.33 ± 2.74	43.33 ± 2.87	44.00 ± 3.92	42.50 ± 2.36	43.67 ± 2.36	46.00 ± 1.41	42.67 ± 2.56	44.17 ± 2.27	44.67 ± 1.60
ALB (g/l)	8.17 ± 1.77	7.83 ± 0.37	7.67 ± 0.75	8.17 ± 1.67	7.83 ± 0.69	9.33 ± 1.25	8.00 ± 0.58	8.50 ± 0.96	8.00 ± 0.03
GLOB (g/l)	34.33 ± 2.21	34.67 ± 1.60	36.00 ± 3.51	34.33 ± 1.80	35.83 ± 2.11	36.67 ± 1.87	34.67 ± 2.69	35.67 ± 2.36	36.67 ± 1.60
NH_3 (µmol/l)	277.67 ± 99.93	351.83 ± 141.37	267.67 ± 89.86	393.83 ± 75.19	285.50 ± 52.36	410.83 ± 55.01**	506.17 ± 112.43**	368.67 ± 101.16	620.3 ± 110.7**
TAG (mmol/l)	3.49 ± 0.57	4.17 ± 0.80	5.68 ± 1.16**	3.41 ± 0.55	3.68 ± 1.16	3.38 ± 0.46	3.84 ± 1.66	3.16 ± 1.04	3.52 ± 1.06
AST (µkat/l)	1.61 ± 0.59	1.21 ± 0.50	1.46 ± 0.41	1.03 ± 0.13	1.58 ± 0.44	2.00 ± 1.52**	2.87 ± 0.62**	0.90 ± 0.25**	0.73 ± 0.31**
ALT (µkat/l)	0.85 ± 0.31	0.53 ± 0.13	0.77 ± 0.29	0.68 ± 0.14	0.81 ± 0.45	0.72 ± 0.28	0.73 ± 0.35	0.75 ± 0.30	0.76 ± 0.29
LDH (µkat/l)	22.13 ± 1.27	14.56 ± 3.60**	21.96 ± 2.60	20.23 ± 2.58	21.64 ± 2.73	22.30 ± 2.77	23.15 ± 3.18	23.61 ± 3.56	20.67 ± 1.39
CK (µkat/l)	16.79 ± 1.09	16.93 ± 1.28	16.75 ± 1.79	16.43 ± 1.26	16.90 ± 2.25	16.48 ± 1.17	17.57 ± 1.27	17.25 ± 1.60	17.39 ± 2.37
Ca^{2+} (mmol/l)	2.29 ± 0.16	2.26 ± 0.14	2.41 ± 0.34	2.36 ± 0.19	2.56 ± 0.56	2.39 ± 0.24	2.62 ± 0.29	2.46 ± 0.28	2.70 ± 0.25
Mg (mmol/l)	1.13 ± 0.12	1.15 ± 0.06	1.33 ± 0.35	1.19 ± 0.08	1.41 ± 0.32	1.27 ± 0.07	1.24 ± 0.21	1.38 ± 0.25	1.16 ± 0.40
PHOS (mmol/l)	2.26 ± 0.38	2.44 ± 0.16	2.45 ± 0.34	2.68 ± 0.39	2.67 ± 0.54	2.52 ± 0.25	2.64 ± 0.42	2.58 ± 0.30	2.74 ± 0.57
ALP (µkat/l)	0.57 ± 0.22	0.53 ± 0.34	0.57 ± 0.25	0.64 ± 0.20	0.59 ± 0.25	0.59 ± 0.22	0.56 ± 0.18	0.59 ± 0.21	0.60 ± 0.23
LACT (mmol/l)	5.18 ± 1.31	5.23 ± 0.81	5.27 ± 1.01	5.48 ± 0.98	5.17 ± 1.11	5.57 ± 0.73	5.39 ± 1.13	5.47 ± 0.80	5.36 ± 0 51

Significance levels observed are *$P < 0.05$, **$P < 0.01$ in comparison with the control group. All values are mean ± SD, $n = 6$

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DISCUSSION

Because fish species may differ widely in their response to anaesthetics, the screening of dosages is often necessary. The anaesthetics tested in this study were effective as sedatives for routine weighing and measuring procedures and handling for spawning of vimba bream.

The analysis of blood parameters is one of the most valuable methods available for modern diagnostics (Anver Celik 2004), and can provide important information about the internal environment of the organism (Anver Celik 2004; Velisek et al. 2005a,b, 2006, 2007, 2009, 2011; Kristan et al. 2012). Haematological and biochemical profiles are frequently used for evaluation of the effect of anaesthetics (Iwama et al. 1989; Velisek and Svobodova 2004a,b; Velisek et al. 2005a,b, 2006, 2007, 2009, 2011; Kristan et al. 2012). To our knowledge, no other data on biochemical and haematological profiles in vimba bream anaesthetized with MS 222, Propiscin, 2-phenoxyethanol, or clove oil are available.

Anaesthesia with MS 222, Propiscin, 2-phenoxyethanol, and clove oil showed no effect on the haematological profile of vimba bream. These results correspond with the results of Velisek et al. (2005b) and Velisek et al. (2006) who found no changes with clove oil anaesthesia in common carp (*Cyprinus carpio* L.) and European catfish (*Silurus glanis* L.). However, our data differ with findings reported in other species of fish. Velisek et al. (2007) observed changes in MCHC and PCV with 2-phenoxyethanol anaesthesia on European catfish. Similar results were obtained by Kristan et al. (2012) in their study of the effects of anaesthesia on pikeperch (*Sander lucioperca*).

Biochemical indices of blood plasma were affected by the action of anaesthetics. The level of glucose was significantly higher with 2-phenoxyethanol (10 min and 24 h) and Propiscin (10 min and 24 h) compared with the control group. Increases in glucose concentrations after 2-phenoxyethanol were also detected by Ortuno et al. (2002) in gilthead sea bream, *Sparus aurata*, and Park et al. (2008) in kelp grouper (*Epinephelus bruneus*). Increased glucose levels were also reported by Kristan et al. (2012) with MS 222 and clove oil anaesthesia in pikeperch. On the other hand, Iversen et al. (2003) found no change in blood glucose concentrations in Atlantic salmon (*Salmo salar*) following clove oil anaesthesia. Velisek and Svobodova (2004a) and Velisek et al. (2007) also found no changes in the concentration of glucose in common carp and European catfish following 2-phenoxyethanol (0.30 ml/l) anaesthesia. The increases in blood glucose concentrations found in the present study reflected the response of anaesthetised fish to metabolic stress. Increases in plasma glucose are mediated by the release of catecholamines, presumably in response to the hypoxia caused by cessation of respiration in anaesthetised fish (Gingerich and Drottar 1989; Iwama et al. 1989).

2-phenoxyethanol (10 min and 24 h) and Propiscin (24 h) were associated with higher levels of ammonia compared to the control group. Alteration in levels of NH_3 in blood indicates a change in protein catabolism and/or disturbance in NH_3 elimination (Svoboda 2001). In contrast to our results, Kristan et al. (2012) reported lower ammonia levels in pikeperch following anaesthesia with 2-phenoxyethanol (0.3 ml/l), MS 222 (150 mg/l), clove oil (33 mg/l), and Propiscin (1.5 ml/l). Gomulka et al. (2008) also reported decreased ammonia levels in Siberian sturgeon (*Acipenser baerii*) after eugenol (0.075 ml/l) and MS 222 (125 mg/l) anaesthesia. No changes in the levels of ammonia in rainbow trout, carp, European catfish, or perch following clove oil (30 mg/l) and 2-phenoxyethanol (0.30 ml/l) anaesthesia were observed by Velisek and Svobodova (2004a), and Velisek et al. (2005b, 2007, 2009). Our differing results may be associated with the concentrations of the tested substances and the fish species used.

In the present study, the triacylglycerol levels were significantly increased 24 h after MS 222 anaesthesia. Changes in blood triacylglycerol levels indicate a change in protein metabolism. Similar results were reported by Gomulka et al. (2008) after MS 222 (125 mg/l) and eugenol (0.075 ml/l) anaesthesia in Siberian sturgeon. Velisek et al. (2006) reported increases in triacylglycerol levels in European catfish after clove oil (0.3 mg/l) anaesthesia. Conversely, Kristan et al. (2012) found decreased triacylglycerol levels after 2-phenoxyethanol (0.3 ml/l), MS 222 (150 mg/l), clove oil (33 mg/l), and Propiscin (1.5 ml/l) anaesthesia in pikeperch.

Enzyme activity in blood plasma can be a stress indicator. The enzymes analysed for this purpose were LDH, CK, and the transaminases ALT and AST. A significant change in the activity of the fore-mentioned enzymes indicates tissue damage, which may be stress-induced (Svoboda 2001). In

our experiment AST activity was significantly increased with 2-phenoxyethanol (10 min and 24 h) and significantly decreased with Propiscin (10 min and 24 h) compared with the control group. The activity of LDH was significantly lower with MS 222 compared with controls. The altered transaminase activity observed in the present study suggests amplified or attenuated transamination processes. Velisek and Svobodova (2004a) and Velisek et al. (2005a) reported decreased AST activity in rainbow trout after clove oil and 2-phenoxyethanol anaesthesia. A similar change in LDH activity was reported by Velisek et al. (2009) with clove oil anaesthesia in perch. Velisek et al. (2011) reported increased activity of AST with clove oil and 2-phenoxyethanol anaesthesia in rainbow trout. Velisek and Svobodova (2004a,b) and Velisek et al. (2007) found no changes in LDH activity in rainbow trout, common carp, and European catfish with 2-phenoxyethanol (0.30 ml/l) anaesthesia.

The results of this study suggest that the internal organs and tissue of vimba bream are not altered by clove oil anaesthesia, but are slightly affected by MS 222, 2-phenoxyethanol, and Propiscin anaesthesia. However, the effects of MS 222, 2-phenoxyethanol, and Propiscin anaesthesia did not significantly differ in any measured variable. Although on the basis of this experiment, it seems that clove oil would be preferred to MS 222, the final choice of anaesthetic must take into account legislation, availability, cost-effectiveness, ease of use, and safety for the user and the environment. As clove oil, 2-phenoxyethanol, and Propiscin are not approved for use on food fish, we do not advocate their use on any fish until MRL (EEC Regulation 2377/90) standards are determined and proper licensing is enacted.

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CHAPTER 5

GENERAL DISCUSSION
ENGLISH SUMMARY
CZECH SUMMARY
ACKNOWLEDGMENTS
LIST OF PUBLICATIONS
TRAINING AND SUPERVISION PLAN DURING THE STUDY
CURRICULUM VITAE

GENERAL DISCUSSION

Rearing of riverine species under control conditions during the cold winter months leads to better restocking material during the spring when the water temperature and hydrological conditions are favourable and the larger size should improve post-stocking survival. Philippart et al. (1989) restocked juvenile common barbel (*Barbel barbel*) after intensive culturing. The main reason for the use of the intensive rearing methods in rheophilic fish species is to optimize the environmental conditions during the cold months of the year and so as to accelerate their growth. Fish growth rate is mainly influenced by feed, water quality (Molnar et al., 2004) and water temperature (Wang et al., 2009; Ott et al., 2012).

In our first study, two six-month experiments were conducted during two consecutive winter seasons starting with five-month-old nase (*Chondrostoma nasus*) and vimba bream (*Vimba vimba*) which had been trained on pellet feed and acclimated to four different temperatures. The WT were fluctuated in parallel with ambient outside conditions at an average of approximately 4 °C for the inflow system and approximately 15, 18 and 21 °C in closed, recirculation systems. The total length and weight were measured at two-week intervals and SGR_w, FCR and survival were monitored. We observed a positive effect of higher water temperatures on their growth rate of each species. Both groups (WT 21 °C) had the most rapid growth and highest SGR_w. Predictably, growth rate and SGR_w were the lowest for each species in a group under ambient temperature conditions. There were no differences between individual weights at the beginning and the end of this treatment. Also the poor FCR indicates that the fish did not use the feed that was provided.

Nevertheless, to specify the best WT for production is not unambiguous because of several aspects. The best growth was achieved under 21 °C temperature conditions. Individual weight of fish at the end of rearing approached the size of two-year-old fish. Not only was the growth higher, but also the time to reach sexual maturity was reduced. Integrating conventional pond culture (Luszczek-Trojnar et al., 2008) with indoor overwinter culture can enhance stocking material. Further, broodstock management can be improved by advancing sexual maturation of vimba bream and nase. Values of FCR (2.16 ± 0.07 and 1.59 ± 0.04 for nase and vimba bream, respectively) approached levels normally achieved in RAS and demonstrate the ability to effectively use the feed in this temperature. Based on these results, despite the higher costs of operating RAS at this temperature, advantages can be realized. Several authors (e.g. Kwasek et al., 2009; Kaminski et al., 2010) have recommended even higher WT (24–25 °C) as optimal, although this would result in higher energy cost. However, several RAS fish farms use the waste heat energy from e.g. biogas power plants, which could make it more cost effective.

Lower temperature at the level of 18 °C gave good results in comparison to rearing in cold water. Individual weight was 3.25 and 4.13 times higher (9.26 ± 2.14 g and 6.81 ± 1.96 g) for nase and vimba bream, respectively. Values of FCR (2.43 ± 0.11 and 1.65 ± 0.03 for nase and vimba bream, respectively) indicate that fish utilize the feed better. From the point of view of energy cost, restocking advantages might outweigh this negative aspect.

The temperature of 15 °C was chosen with regard to the functioning of the biofilter and in this experiment, it proved to be a minimal temperature suitable for rearing of river fish species in RAS. On the one hand, the economic costs to maintain this temperature is relatively low, but on the other hand the effect of production (low individual weight and high FCR) considered at this level of WT affects effectiveness in several aspects.

To conclude it, we found that intensive winter rearing of nase and vimba bream is a good way to increase the potential to survive the winter, but also gain some additional weight and energy for the spring restocking phase. Of course, it is important to find the optimal rearing conditions (for highest survival, sufficient growth rate, low energy and cost demand) which

would make this method as effective as possible and would not be too expensive. In this case, the rheophilic fish could play a role of the additional fish species to fulfil the capacity of the whole RAS system during the winter. It is also possible to rear the juveniles under current water temperature of RAS and decrease the cost of the rearing.

On the other hand, the multidisciplinary approach combining not only aquaculture production data but also welfare and health aspects, genetic, phenotypic and geographic structuring of phenotypic and genetic diversity across populations both natural and captive) should be given strong attention (Rabova et al., 2003; Hanfling et al., 2009; Popovic et al., 2013; Hudson et al., 2014). Rearing riverine fish under non-flowing conditions may be stressful and lead to adverse growth but further can increase the potential for diseases (Recek et al., 2009). Using of local fish stocks instead of non-native stocks as is often done in commercial hatcheries, seems to be preferred by many aspects in conservation captive breeding and restocking programs of many endangered species (Laikre, 2010; Luikart et al., 2010; Popovic et al., 2013; Vetesnik et al., 2009).

In our second study we tested the use of vimba bream as a training fish for adapting, highly valuable market fish, pikeperch fingerlings, to a commercial diet. Percide species as pikeperch (*Sander lucioperca* L.) and perch (*Perca fluviatilis* L.) are highly valuable, suitable for diversification of European inland aquaculture (Samarin et al., 2015) which needs rapid technological growth for sustainable production of fish and seafood products (Policar and Adamek, 2013; Bondarenko et al., 2015). This species has delicate flesh and are highly sought by the European market and anglers (Blecha et al., 2015). Nowadays, a lot of the marketable pikeperch are captured from wild populations of East or North European waters (Policar et al., 2013a). A negative impact of industrial fishing on these populations is results in overfishing and decreasing of wild stocks (Dil, 2008). Development and technical support is applied with the aim to increase and improve production of marketable pikeperch by different ways of culture such as pond aquaculture in Central, North and East Europe and intensive aquaculture using recirculation aquaculture system (RAS) in Western and Central Europe over more than 20 years (Hilge and Steffens, 1996; Steenfeldt, 2015). Nowadays, there are dozens of specialized intensive pikeperch farms throughout Europe which have still not solved main bottlenecks related to broodstock management, controlled reproduction and production of high-quality juveniles (Overton et al., 2015).

In total, three different methods of pikeperch juvenile culture up to the body weight around 8–10 g (common size of pikeperch for the beginning of ongrowing phase) are used in throughout Europe (Steenfeldt, 2015). Exclusive pond culture of pikeperch up to summer fingerling (TL = 80–120 mm) is widely used for pond ongrowing culture, stocking to lakes and dams and/or for adaptation to RAS and pellet feed (Ruuhijarvi and Hyvarinen, 1996; Policar et al., 2011). Production of pikeperch juveniles using a combination of pond (including larval and juvenile phase up to advanced summer fry) and RAS aquaculture (covering the adaptation of the advanced or summer fry to artificial feed and RAS and following culture up to the body weight of 8–10 g) is applied for ongrowing intensive (Policar et al., 2014) or pond aquaculture (Blecha et al., 2016).

Together with intensive pikeperch juvenile culture, the combination of pond and RAS aquaculture is a prospective alternative of juveniles production for the ongrowing phase (Zakes and Demska-Zakes, 1998; Policar et al., 2013a, 2014; Overton et al., 2015; Steenfeldt, 2015) mainly in countries with large pond areas such as the Czech Republic, Hungary, Germany and Austria (Blecha et al., 2016). This approach which has been more or less stable from year to year, provides good survival of around 19% from fresh larvae up to 8–10 g juveniles (Policar et al., 2013b, 2014); this pattern was confirmed in the present study (17.9 ± 5.6%). Large-scale juvenile production optimized in the Czech Republic using this method with low production

cost and a high sustainability has supplied several production pikeperch farms in the Czech Republic, Belgium, Denmark, France, Bulgaria and Netherland during last 5 years (Policar et al., 2014) and currently also in Switzerland and Germany (Policar, unpublished data).

The main disadvantages of this system are as follows: the use of one batch production per year and the necessity to use several small suitable ponds which are not available in all European countries. A critical component of this combination of pond-RAS technology is the conversion of juveniles from live feed to pellet feed. In this dissertation work we used 6-week-old pond-raised fingerlings for a training-period of 14 days. We tested trainer fish to facilitate the efficiency of acceptance of PF, with and without gradual transition from NF. Trainer fish were selected following three basic criteria: larger size at the beginning of experiment (to avoid predation), fish well adapted to PF (to facilitate pikeperch acclimation to PF), and slower growth (to avoid increased competition with pikeperch). Following these criteria, we used one-year-old pond cultured vimba bream which had been trained to PF. Other species also can be used as trainers, depending on availability. A similar design was used by Horvath et al., (2013), but using yearling pikeperch which had been feeding as predators for at least one growing season;, they were not successful, probably because of aggressive trainers (adapted pikeperch and perch, *Perca fluviatilis*) or from unsuitable rearing conditions (small scale experimental aquaria). The other reason could be the individualistic behavioural patterns in such old specimens, which probably revoked the effect of social learning which can easily occur in a compact shoal of fish (Lachlan et al., 1998; Kelley et al., 2003).

We demonstrated a positive effect of trainer fish in the present study when PF was applied from the first day. Pikeperch fingerlings fed on PF were positively stimulated by the foraging activity of vimba bream. It was surprising how quickly pikeperch adapted to the new situation, formed a compact shoal with vimba bream and fed similarly to them on the unfamiliar food item. This behavioural imitation illustrates the capability of fish for social learning, as suggested also by Brown and Laland (2001), and its practical use in aquaculture. In the present study this ability led to direct acceptance of PF which resulted in better growth and therefore, bigger size, better SGR, and higher total weight increment at the end of the experiment. Moreover, the direct application of PF did not affect survival in this group. On the other hand, combining trainer fish with gradual application of PF to NF was not beneficial. This is probably due to active feeding by pikeperch on chironomid larvae, while the PF was predominantly utilized by trainer fish. Generally, the learning effect from the trainer fish was therefore reduced by selection for a preferred food item. Prolongation of the test period affected growth of pikeperch. Surprisingly, similar results were observed in both experimental groups without trainer fish, whether with or without application of NF. Slower growth was the only negative effect in the group that received direct application of PF to pikeperch fingerlings. That was partly compensated by better FCR, which did not differ from group A (trainer and direct application of PF). Notwithstanding, group A fish fed more actively (subjective personal observation), which led to significantly better growth parameters.

Pikeperch fingerlings equalled the higher weight of vimba bream at the end of the experiment and there was no predation on trainer fish. At that time, the trainer fish could be removed and the species reared separately. Trainer fish can therefore be used in production of this species, or could potentially be used as natural food for brood stock. We can therefore harvest large pikeperch fingerlings adapted to dry PF together with another "by-product", vimba bream, e.g. for restocking. Probably different non-aggressive PF-trained and valuable cyprinid species could also be used, such as tench (*Tinca tinca*) (Pantazis and Hatzinikolaou, 2011) or even commercially unimportant common species like roach (*Rutilus rutilus*) or rudd (*Scardinius erythrophtalmus*).

The present approach may provide a new way to obtain well-adapted pikeperch fingerlings for intensive culture and overcome the limiting factor of supply for pikeperch aquaculture in RAS using dry PF. The use of trainer fish could also be applicable to different fish species to streamline their fingerling production. Finally, the described approach demonstrated the applicability of the concept of social learning in fish. The simplicity with which the trained fish accepted the behaviour of trainer fish extends the possibility of wide applicability of the trainer-fish approach in general. Further experimentation is needed to evaluate how broadly the "trainer approach" could be applied to different species.

In the last part of my dissertation, thestress impact of four anaesthetics were compared f on vimba bream. The welfare of fish is one of the most important factors in RAS because they are stressed during manipulations such as handling, sorting, tagging, artificial reproduction procedures. Anti-stress agents such as anaesthetics are commonly used in modern aquaculture to reduce the impact, however, anaesthesia itself imparts some stress which reduces feeding and immune function (Ross and Ross, 1999; Wagner et al., 2002; Pirhonen and Schreck, 2003; Acerate et al., 2004; Roubach et al., 2005). We compared the effect of MS 222 (tricaine methane sulphonate), clove oil, 2-phenoxyethanol and Propiscin on haematological and blood biochemical profiles in vimba bream. A 10-min exposure to each of the anaesthetics had no effect on the haematological profile. However, the effect of the anaesthetics on biochemical indices of blood plasma was demonstrated. The exposure of the anaesthetics 2-phenoxyethanol and Propiscin significantly ($P < 0.01$) influenced levels of glucose, ammonia and the activity of aspartate aminotransferase compared with the control group. The level of triacylglycerols and the activity of lactate dehydrogenase was significantly ($P < 0.01$) affected by exposure of MS 222. Clove oil had no effect on the haematological and blood biochemical profiles, therefore, can be recommended as a suitable anaesthetic for vimba bream. Other anaesthetics tested more or less affected the value of blood biochemical profiles. These results correspond with the results of the authors Velisek et al. (2005) and Velisek et al. (2006) which observed no changes with clove oil anaesthesia in common carp (*Cyprinus carpio* L.) and European catfish (*Siluru glanis* L.), respectively. But these results contrast with the results of other authors who reported on other species of fish. Velisek et al. (2007) observed the changes of MCHC and PCV in 2-phenoxyethanol anaesthesia on European catfish. Similar results were achieved also Kristan et al. (2012) in their study of the effects of anaesthetics at pikeperch (*Sander lucioperca*).

Additionally, it should be remembered that the final choice of anaesthetic must take into account legislation, availability, cost-effectiveness, ease of use, and safety for the user and the environment. Furthermore, as clove oil, 2-phenoxyethanol and Propiscin are not approved for the use on food fish, we do not advocate use on any fish until MRL (EEC Regulation 2377/90) standards are determined and proper licencing is acquired.

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ENGLISH SUMMARY

The use of recirculation system for rearing of riverine fish species

The use of recirculation systems (RAS) for the rearing of economically significant species of fish is commonly practiced in Western Europe countries. These modern systems are highly effective and successful for rearing under controlled conditions for recovering programs of endangered species of fish. In this work we examined the suitability of RAS for rearing Nase (*Chondrostoma nasus* L.), Vimba bream (*Vimba vimba* L.) and Barbel (*Barbus Barbus* L.).

In the first part, two six-month experiments were conducted during two consecutive winter seasons with the five-month-old nase (*Chondrostoma nasus*) and vimba bream (Vimba vimba), which had been trained on pellet feed and acclimated to four different temperatures. The WT fluctuated in parallel with ambient outside conditions at an average of approximately 4 °C for the inflow system and approximately 15, 18 and 21 °C in closed, recirculation systems. Total length and weight were measured in two-week intervals and SGR_w, FCR and survival were monitored. We observed a positive effect of higher water temperatures on their growth rate of each species. Both groups (WT 21°C) had the most rapid growth (16.71 ± 3.46 g for nase and 8.88 ± 2.52 g for vimba) and the highest SGR_w. Predictably, the growth rate and SGR_w were the lowest for each species in the group with ambient temperature conditions. Lower temperatures at the level of 18 °C gave good results in comparison to rearing in cold water. The individual weight was 3.25 and 4.13 times higher (9.26 ± 2.14 g and 6.81 ± 1.96 g) for nase and vimba bream, respectively. The temperature of 15 °C (final individual weight 6.15 ± 1.20 g nase and 2.72 ± 0.50 g vimba) was chosen in consideration of the functioning of the biofilter; in this experiment, it proved to be a minimal temperature suitable for rearing of river fish species in RAS. On the one hand, the costs to maintain this temperature was relatively low, but the effect of production (low individual weight and high FCR) is considered the least effective in several aspects. To conclude this part, we found intensive winter rearing of nase and vimba bream was an effective way to prepare fish for spring restocking.

In the second part we tested the suitability of Vimba bream as a trainer fish for pikeperch to facilitate acceptance of a commercial pellet diet. Pikeperch (*Sander lucioperca*) is commercially important, as well as a valued culinary fish with potential for intensive culture. One of the basic problems in pikeperch culture in RAS is adapting early life stages to pelleted feed (PF). Our work compared four approaches for adapting 6-week-old pikeperch fingerlings to feeding on a commercial diet. The methods were designated, A – use of trainer fish (*Vimba vimba*; SL = 36.88 ± 3.28 mm; W = 0.77 ± 0.26 g) and direct application of PF; B – use of trainer fish and gradual addition of PF with natural food (NF, chironomid larvae); C – direct application of PF only; D - gradual addition of PF with NF. The growth trial experiment (including adaptation to PF) lasted 14 days by which time all experimental groups were accepting PF. Pikeperch fingerlings in group A grew significantly faster and PF was more readily accepted compared to other groups. Other characteristics observed (total weight increment, FCR) also support the use of trainer fish and direct application of PF for pikeperch in their adaptation to intensive culture. It is also an illustration of applicable use of learning potential in fish which can be usable for other fish species.

The last part of this work focused on the use of anesthetics as a means of preventing injury by manipulation of fish (sorting, artificial spawning, transporting etc.).The aim of the study was to compare the effect of anaesthetics MS 222 (tricaine methane sulphonate), clove oil, 2- phenoxyethanol and Propiscin on haematological and blood biochemical profiles in vimba bream (Vimba vimba). The haematological and blood biochemical profiles of vimba bream anaesthetized with MS 222 (100 $mg.l^{-1}$), clove oil (33 $mg.l^{-1}$), 2- phenoxyethanol (0.4 $ml.l^{-1}$),

Propiscin (1.0 ml.l^{-1}) and non-anaesthetized control group were tested. Each group was divided into two subgroups. The first subgroup was sampled immediately after a 10-min exposure. The second subgroup was sampled 24 h after a 10-min anaesthesia. The 10-min exposure to the anaesthetics (MS 222, clove oil, 2-phenoxyethanol and Propiscin) had no effect on the haematological profile of vimba bream; however, the effect of anaesthetics on the biochemical indices of blood plasma was demonstrated. The exposure of the anaesthetics 2-phenoxyethanol and Propiscin significantly ($P < 0.01$) influenced levels of glucose, ammonia and the activity of aspartate aminotransferase compared with the control group. The level of triacylglycerols and the activity of lactate dehydrogenase was significantly ($P < 0.01$) affected to exposure of MS 222. The use of clove oil had no effect on the haematological and blood biochemical profiles and can be recommended as a suitable anaesthetic for vimba bream. Other anaesthetics tested, more or less affected the value of blood biochemical profiles.

CZECH SUMMARY

Využití recirkulačních systémů při odchovu říčních druhů ryb

Odchov ekonomicky významných druhů ryb v recirkulačních systémech je běžnou praxí v zemích západní Evropy. Výhody těchto moderních systémů, jako jsou vysoká úspěšnost a efektivita odchovu v kontrolovaných podmínkách, jsou využívány v záchranných chovech ohrožených druhů ryb. V této práci jsme se zaměřili na vhodnost recirkulačních systémů pro odchov ostroretky stěhovavé (*Chondrostoma nasus* L.), podoustve říční (*Vimba bream* L.) a parmy obecné (*Barbus barbus* L.).

Jako první byly provedeny dva šestiměsíční experimenty ve dvou, po sobě následujících, zimních sezónách. K pokusům byl použit plůdek ostroretky a podoustve ve stáří pěti měsíců, který byl adaptován na příjem komerčně vyráběného krmiva a aklimatizován na čtyři různé teploty vody. V průtočném systému teplota vody kolísala v závislosti na venkovních podmínkách a v průměru dosahovala hodnot 4 °C. V recirkulačních systémech byly teploty udržovány na úrovni 15, 18 a 21 °C. Ve dvoutýdenních intervalech byla měřena individuální hmotnost a celková délka těla. Dále byly vyhodnoceny hodnoty SGR_w, FCR a přežití. Vyšší teplota vody pozitivně ovlivnila rychlost růstu u obou testovaných druhů. Obě skupiny (při teplotě vody 21 °C) dosáhly nejrychlejšího růstu (16,71 ± 3,46 g u ostroretky a 8,88 ± 2,52 g u podoustve) a nejvyšších hodnot SGR_w. Podle očekávání, nejnižších rychlost růstu a SGR_w bylo dosaženo u každého druhu při teplotách simulujících venkovní podmínky prostředí. Ve skupinách odchovávaných při teplotě 18 °C bylo dosaženo velmi dobrých výsledků v porovnání se skupinami odchovávanými v chladné vodě. Individuální hmotnost byla 3,25 a 4,13x vyšší (9,26 ± 2,14 g a 6,81 ± 1,96 g) u ostroretky a podoustve. Teplota 15 °C (konečná individuální hmotnost 6,15 ± 1,20 g u ostroretky a 2,72 ± 0,50 g u podoustve) byla zvolena s ohledem na funkčnost biofiltru a v tomto experimentu se ukázala jako minimální teplota vhodná pro chov druhů říčních ryb V RAS. Pozitivem této teplotní úrovně jsou nízké náklady na její udržení. Naproti tomu, z hlediska horších výsledků chovu (nízká individuální hmotnost a vysoké hodnoty FCR) se tato teplotní úroveň jeví jako neefektivní. Závěrem této části je konstatování, že intenzivní odchov ostroretky a podoustve v kontrolovaných podmínkách může být velmi účinnou cestou, jak zvýšit efektivitu vysazování ryb do volných vod.

Druhá část práce byla zaměřena na testování podoustve říční jako vhodného „pomocníka" při adaptaci plůdku candáta na komerční granulované krmivo. Candát říční (*Sander lucioperca*) je z komerčního i kulinářského hlediska velmi ceněnou rybou s velkým potenciálem pro intenzivní akvakultury. Jedním ze základních problémů odchovu candáta v recirkulačních systémech je adaptace plůdku na peletované krmivo (PK). V této práci jsou porovnány čtyři rozdílné způsoby adaptace šestitýdenního plůdku candáta (pocházejícího z rybničního chovu) na komerčně vyráběné krmivo. Každý způsob byl zastoupen jednou skupinou. A – využití podoustve (*Vimba vimba*; SL = 36,88 ± 3,28 mm; W = 0,77 ± 0,26 g) a přímá aplikace PK; B – využití podoustve a postupný přechod k PK v kombinaci s přirozeným krmivem (NF, larva patentky); C – přímá aplikace PK; D – postupná adaptace na PK v kombinaci s patentkou. Délka experimentu byla 14 dní (adaptace na PK) a po této době byly všechny skupiny adaptovány na komerčně vyráběné krmivo. Plůdek candáta ve skupině A rostl podstatně rychleji a předkládané krmivo bylo přijímáno ochotněji v porovnání s ostatními skupinami. Také výsledky dalších hodnocených parametrů (celkový hmotnostní přírůstek, FCR) dokazují vhodnost používání "pomocných" ryb v kombinaci s přímým předkládáním PK jako vhodnou alternativu adaptace plůdku candáta na podmínky intenzivního chovu. Tyto výsledky zároveň ilustrují určitou míru učenlivosti těchto ryb, které lze využít i u jiných rybích druhů.

V poslední části byla tato práce zaměřena na používání anestetik jako opatření proti poškození ryb při manipulaci (při třídění, umělém výtěru, transportu ryb apod.). Cílem studie bylo porovnání vlivu čtyř druhů anestetik (MS 222, hřebíčkový olej, 2-phenoxyethanol a Propiscin) na ematologické a biochemické parametry krve u podoustve říční. Tyto parametry byly testovány u skupin, které byly anestezovány v dávkách: MS 222 (100 $mg.l^{-1}$), hřebíčkový olej (33 $mg.l^{-1}$), 2-phenoxyethanol (0,4 $ml.l^{-1}$), Propiscin (1,0 $ml.l^{-1}$) a u kontrolní skupiny, která anestezií neprošla. Každá z těchto skupin byla rozdělena do dvou podskupin, přičemž první z nich byla vzorkována bezprostředně po 10minutové anestezii, druhá pak po 24 hodinách.

Z výsledků vyplývá, že 10minutová expozice anestetik (MS 222, hřebíčkový olej, 2-fenoxyethanol a propiscin) neměla žádný vliv na hematologický profil podoustve říční. Dále bylo prokázáno působení anestetik na biochemické ukazatele krevní plazmy. Expozice anestetik 2-fenoxyethanol a propiscin významně ($P < 0,01$) ovlivnila hladiny glukózy, amoniaku a aktivity aspartátaminotransferázy v porovnání s kontrolní skupinou. Úroveň triacylglycerolů a aktivita laktátdehydrogenázy byla významně ($P < 0,01$) ovlivněna expozicí MS 222. Na základě výsledků tohoto experimentu lze konstatovat, že použití hřebíčkového oleje nemělo žádný vliv na hematologické a biochemické profily v krvi a lze jej doporučit jako vhodné anestetikum pro podoustev říční. Ostatní testovaná anestetika více či méně ovlivňují hodnoty parametrů biochemických profilů v krvi.

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Lepič, P., Buřič, M., Hájíček, J., Kozák, P., 2017. Adaptation to pelleted feed in pikeperch fingerlings: trainer fish over gradual adaptation from natural food. Aquat. Living Resour. 30, 8. (IF 2015 = 1.327)

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Application of methodologies, patents, pilot plants, verified technologies

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