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PROOF
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Intensive Winter Culture of *Chondrostoma nasus* (Linnaeus, 1758) and *Vimba vimba* (Linnaeus, 1758) for Spring Restocking

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Abstract

The aim of this study was to compare the grow performance of two rheofilic fish species at different water temperatures (WT) under recirculation aquaculture system (RAS) conditions. Two six-month experiments were conducted during two consecutive winter seasons with the five-month-old nase *Chondrostoma nasus* (Linnaeus, 1758) and vimba bream *Vimba vimba* (Linnaeus, 1758) which had been trained on pellet feed and acclimated to four different temperatures. The three experimental groups were reared in closed separate RAS systems with the WT about 15°C, 18°C and 21°C respectively together with a fourth controlled group which were kept in a flow through system simulating ambient outside conditions at an average of approximately 4°C. All monitored parameters, total length (TL), (BW) body weight, specific growth rate (SGR) and food conversion ratio (FCR) were significantly greater with increasing of temperature in all groups for both fish. The nase total length gained 73.37±3.82, 94.11±5.58, 108.68±7.07 and 128.52±7.64 mm and body weight 2.85±0.47, 6.14±1.20, 9.26±2.14 and 16.71±3.46 g in 4, 15, 18 and 21°C, respectively. The same pattern was observed in vimba bream (TL 58.32±2.71, 69.33±6.68, 91.28±7.48 and 102.16±7.80 mm and BW 1.57±0.24, 2.71±0.50, 6.81±1.96 and 8.88±2.52 g).

Introduction

Nase *Chondrostoma nasus* (Linnaeus, 1758) and vimba bream *Vimba vimba* (Linnaeus, 1758) are reophilic cyprinids (Barus & Oliva, 1995) which are listed as endangered species 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

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reproduction (Luszcek-Trojnar, Drag-Kozak, Kleszcz, Popek, & Epler, 2008; Czerniejewski, Rybczyk, Tanski, Keszka, & Antoszek 2011). The populations of both species began decreasing in the 20th century (Lusk,1995; Keckeis, Frankiewicz, & Schiemer 1996; Hliwa, Demska-Zakes, Martyniak, & Krol, 2003; Spurny, Fiala, & Mares, 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 (Witkowski, Kotusz, & Przybylski, 2009; Popovic *et al.*, 2013), and based on the Red List of Threatened Species in the Czech Republic (Lusk, Hanel, & Luskova, 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 as1922 when artificial propagation and rearing were carried out. A relatively substantial amount of information about artificial spawning presently exists (Luszcek-Trojnar *et al.*, 2008; Alavi, Psenicka, Policar, Rodina, Hamackova, Kozak, & Linhart, 2009; Alavi, Kozak, Hatef, Hamackova, & Linhart, 2010; Kamler, Keckeis, & Bauer-Nemeschkal, 1998; Keckeis, Kamler, Bauer-Nemeschkal, & Schneeweiss, 2001; Kouba, Velisek, Stara, Masojidek, & Kozak, 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, Kamler, Wolnicki,, Sikorska, & Walowski, 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 water temperature (WT) for their culture.

Materials 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, 12 000 nase juveniles (total length (TL) = 70.99±3.81 mm; weight (W) = 2.49±0.4g) and 12 000 vimba bream juveniles (TL= 57.05±3 mm; W= 1.41±0.27g) were distributed into twelve fiberglass tanks (1x1x0.8 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 1mm) 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. Table 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.

Table. 1. Experimental groups, used water temperatures, specific weight growth rate and survival in nase and vimba bream at the end of experiment. Different letters indicate significant difference (P<0.05)

Nase	WT [°C]	SGRw [%.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

Observations and Measurements

The biometric measurements of fish 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, Stara, Turek, Kozak, & Velisek, 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, L Xu, Blanchard, & Kestemont, 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

Nase

The total length (Figure 1) and body weight (Figure 2) increased significantly in each group with increasing water temperature. Body weight of fish in Group A remained the same throughout the experiment (Figure 2). Water temperature also had significant effects on SGRw 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.

Vimba Bream

As in case of vimba bream, the total length (Figure 3) and body weight (Figure 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 SGRw 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.

Discussion

Rearing of rheofilic fish species under RAS conditions during the cold winter months provided

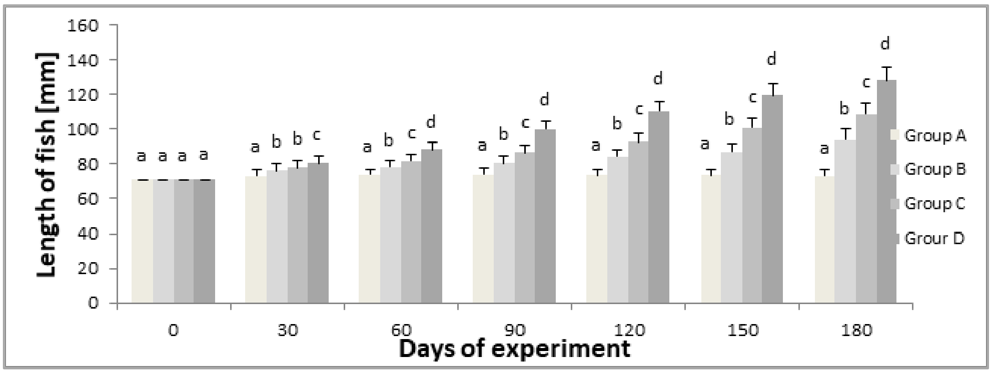

Figure 1. Total length of nase juveniles measured during the experiment. Different letters indicate significant difference (P<0.05).

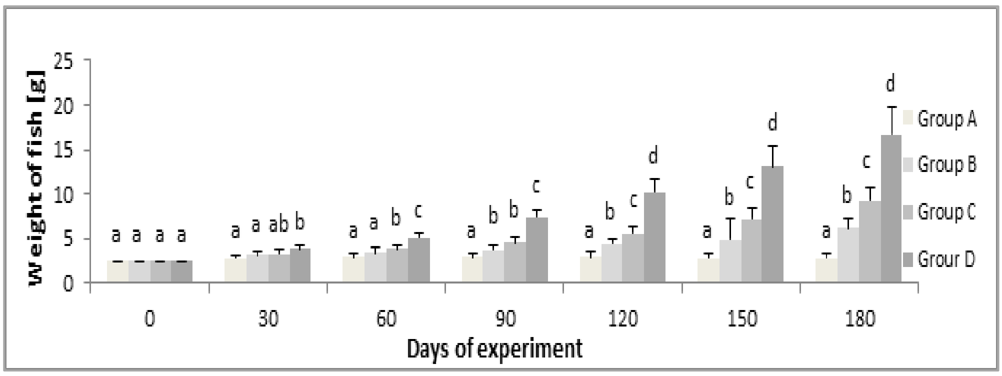

Figure 2. Body weight of nase juveniles weighed during the experiment. Different letters indicate significant difference (P<0.05).

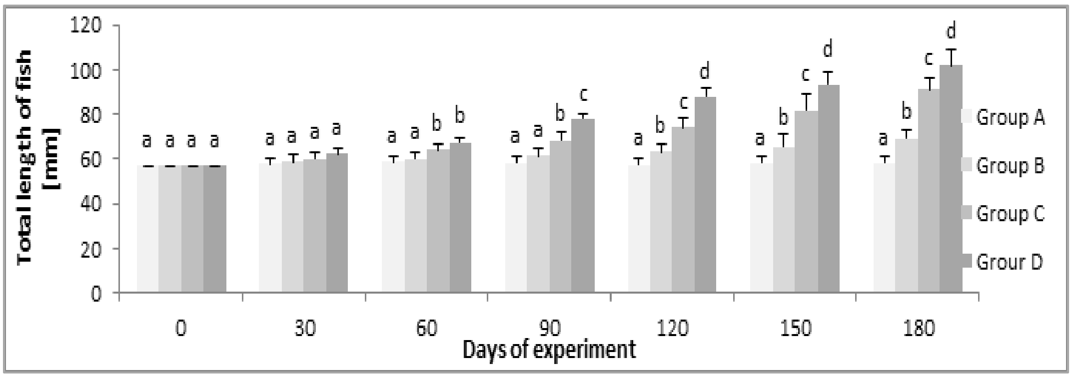

Figure 3. Total length of vimba bream juveniles weighed during the experiment. Different letters indicate significant difference (P<0.05).

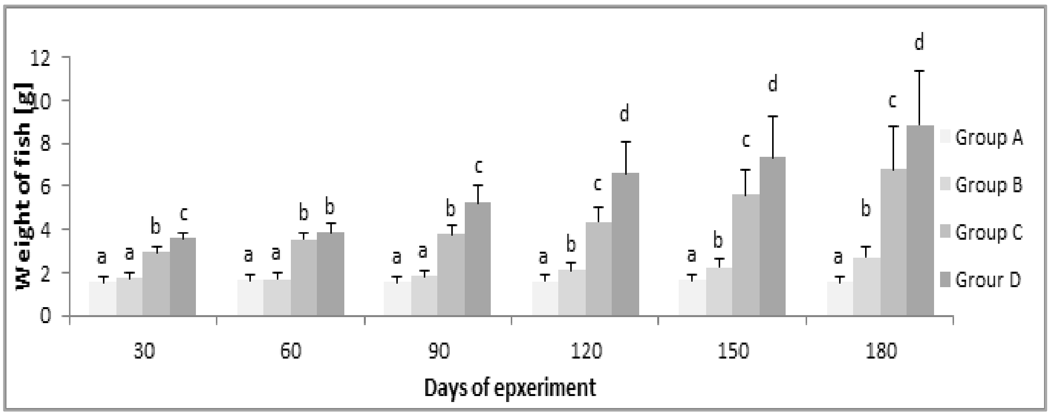

Figure 4. Body weight of vimba bream juveniles weighed during the experiment. Different letters indicate significant difference (P<0.05).

enhanced restocking material during the spring when the water temperature and hydrological conditions are favourable and the larger size should increase post-stocking survival. Philippard, Melard, & Poncin, (1989) restocked juvenile common barbel (*Barbel barbel*) after intensive culturing. The main reason for using of the intensive rearing methods in rheofilic 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, Xuliang, & Kestemont, 2009; Ott, Loffler, Ahnelt, & Keckeis, 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. Predictably, growth rate and SGR 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 (Luszcek-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 for 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±2.14g and

6.81±1.96g) 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 rheofilic 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, Rab, Ozo uf-Costaz, Ene, & Wanzebock, 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, Palikova, Lojek, & Navratil, 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, Ryman, Tallmon, Schwartz, & Allendorf, 2010; Popovic *et al.*, 2013; Vetesnik, Halacka, Papousek, Mendel, & Simkova, 2009).

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