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PROOF
SHORT PAPER

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Survival and Growth Rates of Juvenile Grass Carp *Ctenopharyngodon idella* Overwintered in Ponds and Recirculating Aquaculture Systems Including a Comparison of Production Economics

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Abstract

The survival of grass carp juveniles overwintered in ponds and RAS were compared in this study. The grass carp, W = 2.49 ± 0.34g, were distributed among five 350 L tanks connected to a RAS and/or into three (1500 m^2) ponds (P group). After 165 days, the survival rate was significantly higher in the RAS group, 97.8 ± 0.5%, compared to 10.9 ± 11.4% in P group. The grass carp in a RAS during the first winter significantly improved survival and good conditions of juvenile for subsequent ongrowing period. The RAS provided production profit compare to loss in P group.

Introduction

Grass carp *Ctenopharyngodon idella* are widely cultured Chinese freshwater fish (Du *et al.*, 2006) the rearing of which began more than a thousand years ago (Fu, Shen, Xu & Li, 2016). They have been cultured in c. 40 countries worldwide (FAO 2016; Pípalová, 2006) and were introduced to Europe in 1963 as a low-cost biological alternative for pond maintenance (Krupauer, 1971), particularly for control of aquatic plants (Dibble & Kovalenko, 2009; Murphy *et al.*, 2002; Schoonbee, 1991; Silva, Cruz, Pitelli & Pitelli, 2014). The major producers of grass carp are China, Bangladesh, Taiwan, Iran, and the Russian Federation (FAO 2016).

Grass carp production is likely to increase, because of their ability to utilize plant protein sources to produce high quality meat commanding a good market price (Brožová, 2005; Lin, Zeng, Zhu & Song, 2012; Lin *et al.*, 2016). Currently, production is limited by inadequate and fluctuating production of juvenile stock for ongrowing in ponds. In Central Europe, the primary challenge to commercial grass carp production is the generally low survival rate and growth in ponds during the first winter, especially in fish of total length 50–70 mm (Able & Curran, 2008).

Recirculating systems have been used for winter production of juvenile common carp in Germany (Rümmler *et al.*, 2011). A combination of pond and intensive culture in a recirculating aquaculture system (RAS) is used for producing high-quality juvenile pikeperch, especially in regions with large pond areas (Blecha, Křišťan & Policar, 2016; Policar *et al.*, 2013; Policar *et al.*, 2016). Application of such technologies to grass carp can be positive as limitation of low winter temperatures which are not well tolerated by this species. Nevertheless, the economic benefits of rearing an herbivorous fish, with high flesh quality and a potential developing market, suggest that RAS culture over winter, followed by pond culture under higher water temperatures would be profitable and effective. The aim of this study therefore was to investigate survival of 3-month-old grass carp in a RAS group compared with pond group overwintering.

Materials and Methods

Thirty thousand juvenile grass carp were obtained from experimental ponds at the University of South Bohemia, Faculty of Fisheries and Protection of Waters (USB FFPW). Body weight (W) (2.49 ± 0.34 g) was measured to the nearest 0.01 g (PCB 1000-2, Kern, Germany), and total length (TL) (59.5 ± 1.9 mm) was measured with callipers to the nearest 0.1 mm (Policar *et al.*, 2011a). At the end of October, the experimental fish were divided into two equal groups (P and RAS) for 163 – 165 day culture during wintering.

The P group was maintained under traditional pond monoculture in three ponds, each stocked with 5000 fish (3.3 fish for 1 m^2). Ponds (1500 m^2, 1.5 m average depth) in two locations (Vodňany and Horažďovice, Czech Republic) with varying hydrographic characteristics were chosen. Water was supplied from channel of local river Blanice in Vodňany or river Otava in Horažďovice. The diet consisted of naturally occurring food (winter and spring zooplankton and benthos). Temperature was recorded every 2 h with an automatic temperature sensor (Minikin T, Environmental Measuring Systems, Brno, Czech Republic) in each pond 1 meter below water surface. Water quality and pond control (oxygen = 69.0 ± 7.5 %, pH = 6.89 ± 0.35, NH^{4+} = 0.26 ± 0.1 mg l^{-1}, and NO_{3-} = 0.48 ± 0.2 mg l^{-1}) was recorded once a week. After 163-165 days, fish were harvested using a net cage in the outlet channel according to Policar, Blaha, Kristan & Stejskal (2011b) and Stejskal, Kouril, Policar, Hamackova & Musil (2009). The survival rate (SR %) was calculated as SR = (SF2/SF1) x 100, where SF2 is number of harvested fish, and SF1 is number of stocked fish. A sample of 50 representative juveniles was collected from each pond for biometric measurements. Fish were anaesthetized with clove oil before handling according to Kristan, Stara, Turek, Policar & Velisek (2012). Specific growth rate (SGR) and condition were calculated by the formulae: SGR = 100 t^{-1} $\ln(W_f W_i^{-1})$, where W_i and W_f are initial and final body weight in g, and t is the growing period in days, and Fulton's condition coefficient $K = (W/TL^3) \times 100$, where W is final body weight and TL is final total length.

Fish of the RAS group were stocked into a recirculating aquaculture system at USB FFPW comprising five cylindrical 350 l tanks (~8 fish l^{-1}, 3000 fish per tank) fitted with mechanical (Ratz Aqua Polymer Technik, Remscheid, Germany) and biological filters (Nexus 310, Evolution Aqua, Wigan, UK). Water temperature and quality parameters (oxygen level = 80.0 ± 5.5%, pH = 6.94 ± 0.22, NH^{4+} = 0.12 ± 0.05 mg l^{-1} and NO_{3-} = 0.33 ± 0.3 mg l^{-1}) were measured daily. Fish were fed a commercial diet (Inicio 917, size 1.1 mm BioMar Ltd) at 1% of current biomass. All fish were collected and weighed at 30-33 day intervals. A sample of 30 representative juveniles was collected from each tank for biometric measurements. The SR, SGR, and *K* were calculated as for the P group assessment. The feed conversion ratio (FCR) was calculated as FCR = $F/(W_f – W_i)$, where F is total feed consumption, and W_i, W_f are initial and final body weight, respectively.

At the conclusion of the trial, an economic analysis of culture methods was conducted in cooperation with an economist of the USB FFPW, taking into consideration total costs (stocking material, salaries, feed, overheads, and depreciation), revenue, and profit or loss of each production group.

Statistical Analysis

Data were expressed as mean ± SD. First, datasets were checked for normal distribution using the Kolmogorov-Smirnov test and homoscedasticity of variance (Bartlett's test). Data meeting normality criteria were then analysed with single-factor ANOVA. When a significant ($p < 0.05$) difference was found, a Tukey's multiple comparison tests was used. Statistical assessment of all data was carried out with Statistica 7.0 (StatSoft, Inc., Czech Republic).

Results

Pond temperatures fluctuated throughout the experimental period with the lowest value being 0.9°C. The mean temperature was 4.2 ± 2.6°C (range 0.9-13.1 °C) in the ponds and a stable 20.8 ± 0.4°C in the RAS

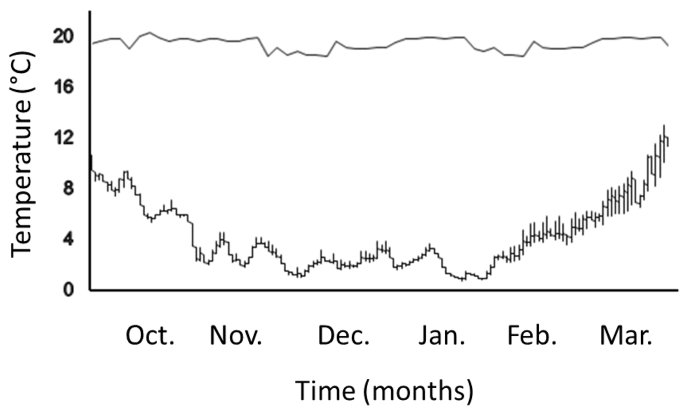

Figure 1. Temperature in ponds and RAS during the juvenile grass carp rearing trial.

(Figure 1).

The mean survival rate in the RAS was significantly higher (97.8 ± 0.5%) compared to 10.9 ± 11.4% in P group (Table 1).

The SGR at the conclusion of the trial was significantly higher ($p < 0.05$) in RAS at 0.49 ± 0.08% d^{-1} than -0.12 ± 0.10 % d^{-1} in P group (Table 2). The mean final TL of juveniles cultured in RAS was 70.1 ± 5.2 mm, compared 61.4 ± 1.8 mm for P group. Significantly higher ($p < 0.05$) body weight was recorded in RAS (5.59 ± 0.89 g) compared to P group (2.07 ± 0.53 g) (Table 1, Figure 2).

In P group, Fulton's condition coefficient was significantly ($p < 0.05$) lower at the end of the culture period than at the beginning, while, in the RAS group, final *K* was higher (Table 1). The feed conversion ratio calculating in the RAS group only, was FCR = 1.69 ± 0.07.

Results of the economic study are shown in Table 2. The total production costs of equal-sized fish groups were lower for P group (€1,301) compared to rearing in RAS group (€2,364). Revenue of €2,714 was obtained from RAS compared to €304 for P group. Production profit of €350 was obtained in RAS group compared to a production loss of -€997 in the P group.

Discussion

The ability of fish to survive the first winter is an important determinant of success in fish production from the perspective of having enough juveniles for ongrowing to market size category. Survival of grass carp juveniles in RAS was significantly higher than in ponds. The low pond survival rate of grass carp was probably influenced primarily by low temperatures (Hurst, 2007; Binder *et al.*, 2015). Grass carp tolerate a range of water temperatures from 0 to 33°C (Cudmore & Mandrak, 2004). Similar survival rate achieved Jones *et al*. (2017) in different Canadian lakes.

Limited food availability also plays a major role in survival (Ludsin & DeVries, 1997; Post, Kitchell & Hodgson, 1998). Grass carp feeding activity begins at 7-8°C, intensive feeding occurs only when water temperature is at least 20°C (Cudmore & Mandrak, 2004). Chilton & Muoneke (1992) stated that grass carp rarely fed at temperatures below 3°C and, while in their over-winter habitat, they do not feed at all (Fischer & Lyakhoich, 1973). Under pond conditions, fish not only lose weight, but are in relatively poor condition when water temperatures begin to rise (David, 2006). The weakened fish are vulnerable to bacterial and fungi contamination and disease (Binder *et al.*, 2015) and are not suitable for further aquaculture production. According to available published data the immune system is non-functional in grass carp for most of the overwintering period. Water temperature only reached 14 °C at the end of the culture period of this study. Predation by birds is an additional problem with pond culture during winter and spring (Adamek, Kortan & Flašhans, 2007; Kortan, Adamek, Flajshans & Piackova, 2008). Grass carp may be attacked by mammalian or even insect predators-dragonfly larvae (Akpona *et al.*, 2015). Injured juveniles may also contract secondary bacterial and fungi infection and disease (Binder *et al.*, 2015).

Table 1. The number of stocked and harvested fish, survival rate, initial and final weight, total length, Fulton's condition coefficient *K*, and specific growth rate of grass carp overwintered in ponds and RAS

	Stocked fish		Harvested fish	Survival rate		Initial		Final		SGR
	(n)	(n)	(%)	W (g)	TL (mm)	*K*	W (g)	TL (mm)	*K*	(% d^{-1})
Ponds	15 000	1642	10.9 ± 11.4	2.49 ± 0.41	59.9 ± 1.7	1.17 ± 0.28	2.07 ± 0.53	61.4 ± 1.8	0.90 ± 0.31	-0.12 ± 0.10
RAS	15 000	14 669	97.8 ± 0.5	2.49 ± 0.33	59.3 ± 2.1	1.22 ± 0.30	5.59 ± 0.89	70.1 ± 5.2	1.62 ± 0.14	0.49 ± 0.08

Table 2. Economic comparison of juvenile grass carp overwintering practices

		Ponds €	Pond characteristics	RAS €	RAS characteristics
Costs	Cost of stock	555	15 000 fish	555	15 000 fish
	Salaries	483	0.5 h per day	965	1 h per day
	Feed (kg)	0		229	80
	Overheads	156	15% of costs	262	15% of costs
	Depreciation	107		422	
	Total costs	1301	Total production 1642 fish	2364	Total production 14 669 fish
Revenue		304	Price per fish € 0.185	2714	Price per fish € 0.185
Profit/loss		€ -997		€ 350	

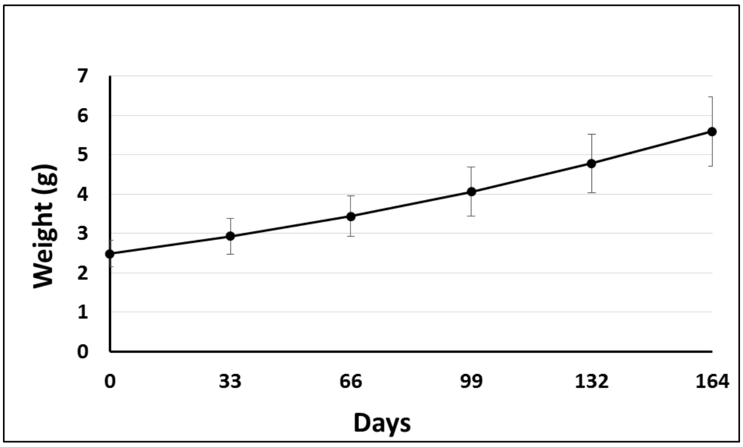

Figure 2. Growth rate in recirculation aquaculture system.

Rearing in RAS positively affected survival rate and health and provided protection against predators. David (2006) also reported these benefits in the culture of European catfish *Silurus glanis* in RAS during winter. Similar results were observed in grass carp by Du *et al.* (2006) who reported SGR of 0.67% at a feeding rate 1% of biomass and a temperature of 25°C. Du *et al.* (2006) recommended an optimum feeding rate of 1.97% body weight d^{-1} for fish around 3 g. Shireman, Colle & Rottmann (1977) recommended 2.1 fish per litre for optimal growth rate. In this experiment, the high density of fish (8 fish per litre) was not a problem for wintering storage time in RAS. The daily feeding rate was only 1% in comparison with *ad libitum* in study Shireman, Colle & Rottmann (1977). The condition of fish reared in RAS was higher than pond reared fish. Similar results were obtained by Cai, Fang, Johnson, Lin, Tu, Liu & Huang (2014), who demonstrated *K* of 1.4–1.6 in RAS cultured grass carp of TL 130 mm. In this study, the feed conversion ratio was similar (FCR = 1.69 ± 0.07) and compared to Ming, Ye, Zhang, Xu & Xie (2015), who reported FCR of 1.7–2.1 in grass carp.

The results of economic analysis showed a profit in the RAS of €350, compared to loss of -€997 for the pond group (Table 2), stressing the importance of including RAS in the grass carp production cycle.

This seems to be the first study comparing overwintering of grass carp juveniles in ponds with their maintenance in RAS. On the basis of better obtained production data (e.g. survival rate, Fulton's condition coefficient) and economic analysis, the recirculating aquaculture system showed potential to increase the production and profitability of grass carp juveniles for ongrowing culture in Central Europe which can significantly increase production of this species in mentioned region.

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