

## Note

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

Tomáš Polícar<sup>a</sup>, Pavel Kozák, Jitka Hamáčková, Andrea Lepičová, Jiří Musil and Jan Kouřil

University of South Bohemia, Research Institute of Fish Culture and Hydrobiology, Department of Aquaculture and Hydrobiology, Zátiší 728/II, 389 25 Vodňany, Czech Republic

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

The effects of the rearing environment on growth performance and survival rate of juveniles were tested under intensive controlled conditions in aquaria and troughs for 84 days following the larval period (from 34 to 118 dph). The environment of the troughs caused significantly decreased growth ( $W = 2079 \pm 433$  mg and  $SGR = 3.1 \pm 0.05\% d^{-1}$ ) of juveniles compared to ones reared in aquaria ( $W = 3236 \pm 264$  mg and  $SGR = 3.6 \pm 0.1\% d^{-1}$ ) at the end of the juvenile rearing period. Nevertheless, rearing environment did not have a significant influence on the cumulative survival rate of barbels ( $S_C = 90 \pm 4\%$  and  $81 \pm 3\%$  in aquaria and troughs, respectively).

**Key words:** *Barbus barbus* / Larval and juvenile rearing / Artificial dry feeding / *Artemia* nauplii / Diet / Rearing environment

## 1 Introduction

The common barbel's (*Barbus barbus*, Cyprinidae) status is nearly regarded as threatened in the Czech Republic (Lusk 1996; Lusk et al. 2004), on the edge of extinction in some Polish rivers (Penczak and Kruk 2000) and as vulnerable in industrialized areas of Western Europe (Poncin 1989). Its abundance has decreased due to stream regulation and canalization, segmentation of rivers, water pollution and subsequent changes in the natural environment of the so-called Barbel Zone, as well as overfishing and predation by introduced exotic species (Jurajda 1995; Lusk 1996; Lusk et al. 1998; Fiala and Spurný 2001; Penczak and Sierakowska 2003). On the

other hand, throughout Western and Central Europe the barbel has been very popular with anglers (Wheeler and Jordan 1990; Penczak and Kruk 2000; Taylor et al. 2004).

Breeding of barbel broodstock under controlled conditions in captivity has been more effective than artificial spawning by the collection of gametes from wild broodfish (Philippart 1982). Females produced 80 000 ova under captive controlled conditions during the spawning season from February to July (Philippart et al. 1989). Poncin et al. (1987) and Poncin (1989) also tried to enhance reproduction under controlled conditions by environmental manipulation including temperature, and light.

In spite of several studies on artificial reproduction of common barbel, few data are available about rearing of larvae and

<sup>a</sup> Corresponding author: policar@vurh.jcu.cz

fingerlings. Wolnicki and Górný (1995a) and Fiala and Spurný (2001) reared larvae under controlled conditions for the first 25 and 15 days of exogenous feeding, respectively. Labatzki and Fuhrmann (1992) described longer rearing of barbel fingerlings under controlled conditions up to 1g of body weight. Philippart (1982) and Philippart and Mélard (1983) reared juvenile barbel for stocking, for 3–4 and 14 mo, respectively. Philippart et al. (1989) described various methods of rearing barbel, namely larvae in small stagnant heated ponds up to age 36–63 days (140–350 mg body weight), juveniles intensively in 4 and 1.5 m<sup>3</sup> tanks at a constant 22–24 °C to age 10 mo (40 g and 150 mm body weight and length, respectively) and adults at high density (94 kg m<sup>-3</sup> = 274 fish m<sup>-3</sup>) in controlled conditions.

Different rearing environments have been used for rearing of barbel larvae and juveniles, such as glass aquaria (Wolnicki and Górný 1995a; Fiala and Spurný 2001), pen facilities (Pyka et al. 2001), and heated (Philippart et al. 1989) and natural ponds (Pyka et al. 2001). However, no author tested the effectiveness of different rearing environments during intensive rearing of barbel.

The first goal of the present study was to determine the effect of short-time *Artemia* feeding during the early stages of exogenous nutrition on the growth and survival rates in barbel under aquaria conditions. The second goal was to test the effectiveness of different rearing environments (aquaria and troughs) during juvenile rearing period in barbel under intensive controlled conditions.

## 2 Materials and methods

Artificial reproduction was carried out at the Žleby anglers' club hatchery (Central Bohemia region) and experiments were performed at the Research Institute of Fish Culture and Hydrobiology at Vodňany, University of South Bohemia, Czech Republic (USB RIFCH).

At night, in May 7, 2005, six female and 13 male barbel were caught by light and dip net in the spawning grounds of the Doubrava river (49° 54' N and 15° 30' E). Four hours after catching of broodstock, sperm and eggs were hand-stripped without spawning induction. Eggs from the six females were mixed and then divided into 13 batches. Each of the 13 batches was fertilized by a single male according to Billard et al. (1995). Using single males during artificial insemination instead of a pool of sperm ensured high genetic variation in the hatched larvae (Linhart et al. 2005). High genetic diversity of barbel broodstock is very important for influencing the diversity of juveniles for stocking and restocking purposes (Wheeler and Jordan 1990). A total of 33 000 fertilized eggs were obtained, 5500 eggs per female (mean Total Length = 355 ± 6 mm and Weight = 261 ± 4 g).

### 2.1 Incubation of fertilized eggs and period of exogenous nutrition

The fertilized eggs were transferred to three 10 L Zug jars (10 000 eggs per jar) and incubated for six days at a mean water temperature of 18.2 ± 0.1 °C (108 degree-days), with 85.7%

hatching success 144 hours after fertilization. Hatching rate was determined as ratio of live hatched larvae and dead larvae and eggs in five representative samples collected from each Zug jar at the end of the egg incubation. Then the larvae were held in three Rückl-Vacek containers (8570 larvae per container) for 9 days (164 degree-days) until the swimming stage (i.e. 272 degree-days after fertilization). Rückl-Vacek containers were also used in Polícar et al. (2004) as flow container with size 470 × 470 × 120 mm, water volume 26.5 L and water flow 5 L min<sup>-1</sup>. Actively swimming larvae were transported to USB RIFCH, and the rearing study with exogenous nutrition started after absorption of the yolk sac one day later (19 days, 344 degree-days after fertilization and 13 days, 236 degree-days post hatch).

### 2.2 Larval rearing

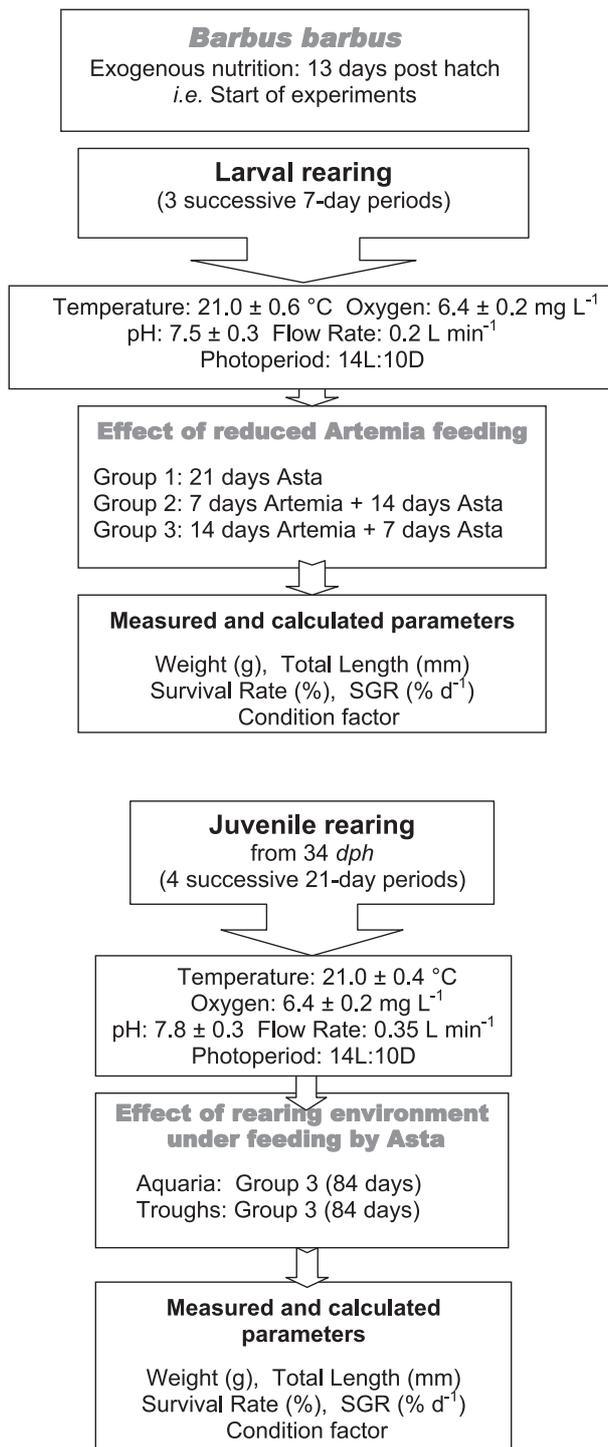
In the present study, metamorphosis (transition from the larval to juvenile stage) was evaluated according to Krupka (1988). Larval rearing lasted from the beginning of exogenous nutrition (13 days post-hatch = day 1) until day 21 and was divided into three 7-day periods (Fig. 1). Initial density of larvae for each larval period was decreased as the fish grew in successive rearing periods (see Table 1).

#### 2.2.1 Diets

For assessment of effect of short time use of *Artemia* feed on larval growth and survival rate, larvae were reared (initial  $W = 10.0 \pm 0.2$  mg and  $L_T = 11.4 \pm 0.3$  mm) in nine 10-L aquaria (400 × 200 × 250 mm; water depth 125 mm) of recirculation system of USB RIFCH. Groups of larvae were separated by three different feeding diets (presenting total or part reduced *Artemia* feeding) with 3 replicates. The exclusive feeding with *Artemia* nauplii was not tested in our study because according Fiala and Spurný (2001) and Wolnicki and Górný (1995a) it is not necessary for successful intensive rearing of barbel larvae. Group 1 larvae were fed from the beginning of exogenous nutrition with Asta food only; group 2 with live *Artemia* nauplii for 7 days followed by Asta food for 14 days; and group 3 with live *Artemia* nauplii for 14 days followed by Asta food for 7 days.

- *Artemia* nauplii as live food for larvae – *Artemia* spp. nauplii (Sanders Grade A, crude protein 55%, fat 15%, carbohydrates 15%, minerals 5%, dry matter 22%) was used as live food for larvae in our study. *Artemia* nauplii for feeding of larvae were hatched in a pyramid shaped hatcher under controlled conditions (water temperature = 28.0 ± 1.0 °C; salinity = 30 ppt; continuous heavy aeration; light = 2000 lux constant illumination, pH = 8.7 ± 0.2; initial density of *Artemia* cysts = 1.65 g L<sup>-1</sup>) recommended by Hoff and Snell (1987). Under these conditions, hatch of *Artemia* passed after 15 hours. Freshly hatched *Artemia* nauplii (Instar I, body size 400–500 μm, approximately 5 hours after hatching) was used as live food for larvae.

- Asta food – Asta is an artificial dry food for rheophilic fishes reared in Central Europe. This diet was made in 2004



**Fig. 1.** Rearing protocols of common barbel larvae from 13 to 33 days post hatch, and juveniles from 34 to 118 dph.

at the Polish Academy of Sciences, Poland. Its nutrition content was as follows: dry matter (95.8%), crude protein (50.5%), fat (9.1%), carbohydrates (18.6%), fibre (4.5%), ash (9.8%), net energy (18.6 MJ), vitamins: A (24 000 mJ kg<sup>-1</sup>), D3 (300 mJ kg<sup>-1</sup>), B12 (0.7 mg kg<sup>-1</sup>), C (8 g kg<sup>-1</sup>) E (2 g kg<sup>-1</sup>), B1 (0.2 g kg<sup>-1</sup>), B2 (0.32 g kg<sup>-1</sup>).

## 2.2.2 Daily feeding rate and distribution of food

Daily feeding rates of larvae were estimated from their energy requirements or the relationship between food consumption and growth rate according to Kamler (1992) and Keckeis and Schiemer (1992). The daily feeding rate with *Artemia* nauplii was doubled since the energy content of *Artemia* nauplii was lower than that of Asta (Table 1). Food was distributed daily by hand at 07h, 09h, 11h, 13h, 15h, 17h and 19h.

## 2.2.3 Environmental parameters

Larvae were exposed to a 14-h light (from 06h to 20h) and 8-h dark (from 20h to 06h) photoperiod. Mean ( $\pm$  S.E.) rearing conditions were as follows: water temperature =  $21.0 \pm 0.6$  °C; dissolved oxygen =  $6.5 \pm 0.15$  mg O<sub>2</sub> L<sup>-1</sup>; oxygen saturation =  $74 \pm 2\%$ ; water flow = 0.2 L min<sup>-1</sup>; pH =  $7.5 \pm 0.3$ ; ammonia < 0.02 mg L<sup>-1</sup>; nitrites < 0.02 mg L<sup>-1</sup>. Temperature of water, oxygen level, and saturation were measured (WTW MultiLine P4) twice daily (at 07h and 19h), and other parameters of water quality (at chemist's laboratory of USB RIFCH) once weekly.

For requirement of juvenile rearing, three groups of larvae were reared in three troughs (2700 × 445 × 200 mm; water depth 125 mm) without replicates under identical conditions such as larvae reared in aquaria, i.e.: same three different diets, environmental conditions (water temperature =  $21.0 \pm 0.6$  °C; dissolved oxygen =  $6.5 \pm 0.15$  mg O<sub>2</sub> L<sup>-1</sup>; oxygen saturation =  $74 \pm 2\%$ ; water flow = 0.2 L min<sup>-1</sup>; pH =  $7.5 \pm 0.3$ ; ammonia < 0.02 mg L<sup>-1</sup>; nitrites < 0.02 mg L<sup>-1</sup> – same way and frequency of measuring as in larvae from aquaria), density of larvae (Table 1), distribution of food and daily feeding rate (Table 1).

All feeding diets had to be used such as in aquaria, because the larval group of the fastest growers, which continued during juvenile rearing period, was not known at the beginning of larval rearing period. Therefore, this larval rearing in troughs was very demanding for the quantity of larvae and larvae were reared without replicates.

## 2.3 Juvenile rearing

Juvenile rearing lasted from day 22 to day 105 (i.e. from 34 to 118 dph) after larval rearing and was divided into four 21-day stages (Fig. 1).

Only the 3rd group of larvae (representing the fastest growers) continued in aquaria (initial  $W = 174 \pm 20$  mg and  $L_T = 24.3 \pm 1.1$  mm) and troughs (initial  $W = 162 \pm 41$  mg and  $L_T = 23.6 \pm 2.3$  mm) in 3 replicates during juvenile rearing. It means that, significant differences were not found in initial size of juveniles stocked to aquaria and troughs. Initial density of juveniles for each juvenile period was identical for aquaria and troughs, and decreased as the fish grew in successive rearing periods (see Table 1).

### 2.3.1 Feeding and distribution of food

All juveniles were fed Asta food (identical daily feeding rates in each period, Table 1) under intensive controlled

**Table 1.** Daily feed rate and initial density of larvae and juvenile *Barbus barbuis*.

Rearing		Daily feeding rate, Asta particle diam. (% W of fish <sup>-1</sup> , mm)			Initial density (fish L <sup>-1</sup> )	Volume (litres of water)	
		Group	%	mm		Aquaria	Troughs
Larvae	1 <sup>st</sup> period (May 26-June 1) (13–19 dph)	1	30	0.2	50		
		2*	60	-			
		3*	60	-			
	2 <sup>nd</sup> period (June 2-8) (20–26 dph)	1	20	0.3	44	10	
		2	20	0.3			
		3*	40	-			
		1	15	0.5			
		2	15	0.5			
		3	15	0.5			
3 <sup>rd</sup> period (9-15 June) (27–33 dph)	3	15	0.8	6			
	3	10	1.0				
	3	5	1.2				
	3	2.5	1.5				
Juveniles	1 <sup>st</sup> period (June 16-July 6)	3	15	0.8	6		
	2 <sup>nd</sup> period (July 7-27)	3	10	1.0	5	50	
	3 <sup>rd</sup> period (July 28-Aug. 17)	3	5	1.2	3		200
	4 <sup>th</sup> period (Aug. 8-Sept. 7)	3	2.5	1.5	2.5	100	

(\*) Larval feeding with *Artemia* nauplii (dph) days post hatching.

conditions, in aquaria and troughs. Daily feeding rates of juveniles were estimated according to same principles as during larval rearing. Food was distributed daily by hand at 07h, 11h, 15h and 19h, in both aquaria and troughs.

### 2.3.2 Environmental parameters

Fifty litre aquaria (550 mm×350 mm×300 mm; water deep 260 mm) were used for the 1st and 2nd periods of juvenile rearing and then 100-L aquaria (760 mm×506 mm×355 mm; water depth 260 mm) for the 3rd and 4th periods. The same troughs were used for juveniles as for larvae, but the water was increased to a depth of 166 mm (200-L). Aquaria and troughs were connected to the recirculation system of USB RIFCH.

Environmental conditions were again similar in aquaria and troughs and were measured by identically like during larval rearing period (water temperature = 21.0 ± 0.4 °C; dissolved oxygen = 6.4 ± 0.2 mg O<sub>2</sub> L<sup>-1</sup>; oxygen saturation = 72 ± 2%; water flow rate = 0.35 L min<sup>-1</sup>; pH = 7.8 ± 0.3; ammonia < 0.03 mg L<sup>-1</sup>; nitrites < 0.02 mg L<sup>-1</sup> and photoperiod 14L:10D – same way and frequency of measuring as during larval rearing period). Data of all parameters of water quality are presented as Mean (± S.E.).

### 2.4 Growth performance and survival rate

Individual body weight ( $W$ ) was measured by a Mettler electronic balance (model AE 200) to the nearest 0.1 mg for larvae and 1 mg for juveniles) and total length ( $L_T$ ) was measured by a digimatic calliper (to the nearest 0.5 mm) in representative samples of 33 fish from each replicates at the beginning of both rearing periods and at the end of the each period of larval and juvenile rearing period at 7-day and 21-day intervals, respectively. The surviving fish were counted in all replicates of both rearing periods at the same intervals as the

biometric measuring and cumulative survival rate were calculated at the end of the each period.

Specific growth rate,  $SGR = 100 (\ln W_2 - \ln W_1)/t$ , where  $W_1$  and  $W_2$  are initial and final weights, and  $t$  is the period of time between  $W_2$  and  $W_1$  (in days),

Condition level (Fulton's condition coefficient  $C_F = 100 W_2 L_T^{-3}$ ) of larvae and juveniles were calculated at the end of the larval and juvenile rearing period.

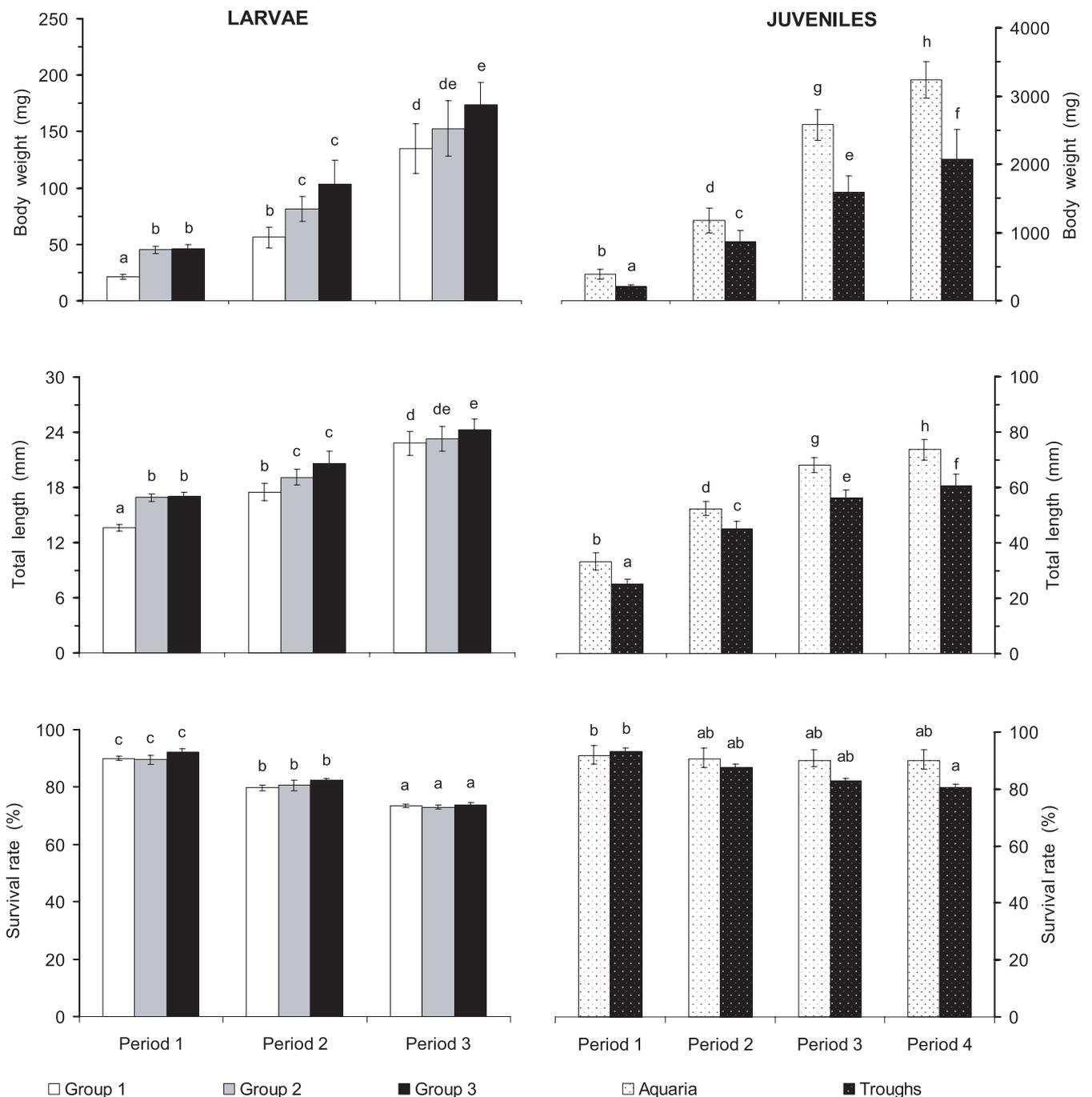
### 2.5 Data analysis

Growth and survival data are presented as Mean (± S.E.). Statistical assessment of all data was carried out by means of Statistica software 6.1 (StatSoft, Inc. Czech Republic). Data were firstly tested for normality (Kolmogorov- Smirnov test) and homoskedasticity of variance (Bartlett's test). Then two-way analysis of variance (ANOVA), Tuckey's multiple comparison test ( $p < 0.05$ ) was used for comparison of growth data ( $W$ ,  $L_T$ ,  $C_F$ ) and the non-parametric Kruskal-Wallis's test was applied to identify significant differences in specific growth rate (SGR) and survival rate among different feed groups of larvae and among juveniles reared under different rearing environments. The effects of short-time *Artemia* feeding in larvae and of rearing environments in juveniles on their growth parameters ( $W$ ,  $L_T$ , SGR,  $C_F$ ) and survival rates were determined.

## 3 Results

### 3.1 Effect of short-time *Artemia* feeding during larval rearing period

Final growth parameters ( $W$ ,  $L_T$ , SGR, and condition factor) and survival rates of larvae are summarized in Table 2.



**Fig. 2.** The effects of short-time *Artemia* feeding during larval rearing period and different rearing environments during juvenile rearing period on body weight (mg), total length (mm) and cumulative survival rate (%) in common barbel (*Barbus barbus*) under intensive controlled conditions. Data are presented as Mean ( $\pm$  SE). Groups without a letter in common are significantly different ( $p < 0.05$ ).

At the end of the larval rearing period, statistical differences in body weight and total length were only found between group 1 ( $W = 135 \pm 22$  mg,  $L_T = 22.8 \pm 1.3$  mm) and group 3 ( $W = 174 \pm 20$  mg,  $L_T = 24.3 \pm 1.1$  mm). The body weight and total length of larvae were not statistically different between groups 1 and 2 ( $W = 153 \pm 25$  mg,  $L_T = 23.3 \pm 1.4$  mm) and between groups 2 and 3 at that point of their rearing. Specific growth rates showed the same results as values of  $W$  and  $L_T$ . Statistically, the highest value of SGR was achieved in

groups 3 ( $15 \pm 1\%$   $d^{-1}$ ). However, no statistically differences were noted between groups 3 and 2 ( $14 \pm 1\%$   $d^{-1}$ ). Statistically the lowest value of SGR was observed in group 1 ( $13 \pm 1\%$   $d^{-1}$ ), without differences between groups 1 and 2. The final condition of larvae was the same in all groups ( $1.2 \pm 0.1$ ), without differences between any group of larvae. The highest and the lowest cumulative survival rate was found in group 3 (80%) and group 2 (73%), respectively, without statistical differences between all groups at the end of the larval rearing period.

**Table 2. Larval rearing.** The effect of short-time *Artemia* feeding on growth performances: body weight; total length ( $L_T$ ); specific growth rate (SGR); Fulton condition factor, and survival rate of common barbel (*Barbus barbus*) at the end of larval rearing period (33 dph). Data are presented as Mean ( $\pm$  S.E.).

Protocol	Group 1	Group 2	Group 3
Fish Weight (mg)	135 $\pm$ 22 <sup>a</sup>	153 $\pm$ 25 <sup>ab</sup>	174 $\pm$ 20 <sup>b</sup>
$L_T$ (mm)	22.8 $\pm$ 1.3 <sup>a</sup>	23.3 $\pm$ 1.4 <sup>ab</sup>	24.3 $\pm$ 1.1 <sup>b</sup>
SGR (% d <sup>-1</sup> )	13.1 $\pm$ 0.7 <sup>a</sup>	13.8 $\pm$ 0.7 <sup>ab</sup>	14.5 $\pm$ 0.5 <sup>b</sup>
Condition factor	1.2 $\pm$ 0.1 <sup>a</sup>	1.2 $\pm$ 0.1 <sup>a</sup>	1.2 $\pm$ 0.1 <sup>a</sup>
Survival rate (%)	74 $\pm$ 1 <sup>a</sup>	73 $\pm$ 1 <sup>a</sup>	80 $\pm$ 1 <sup>a</sup>

Within a row, values without a letter in common are significantly different ( $p < 0.05$ ).

**Table 3. Juvenile rearing.** The effect of different rearing environments on growth performances: body weight ( $W$ ); total length ( $L_T$ ); specific growth rate (SGR); Fulton condition factor, survival rate of common barbel (*Barbus barbus*) at the end of the juvenile rearing period. Data are presented as Mean ( $\pm$  S.E.).

Rearing environment	Troughs	Aquaria
Fish Weight (mg)	2079 $\pm$ 433 <sup>a</sup>	3236 $\pm$ 264 <sup>b</sup>
$L_T$ (mm)	61 $\pm$ 4 <sup>a</sup>	74 $\pm$ 4 <sup>b</sup>
SGR (% d <sup>-1</sup> )	3.1 $\pm$ 0.1 <sup>a</sup>	3.6 $\pm$ 0.1 <sup>b</sup>
Condition factor	0.9 $\pm$ 0.1 <sup>b</sup>	0.8 $\pm$ 0.1 <sup>a</sup>
Survival rate (%)	81 $\pm$ 3 <sup>a</sup>	90 $\pm$ 4 <sup>a</sup>

Within a row, values without a letter in common are significantly ( $p < 0.05$ ).

The courses of  $W$ ,  $L_T$  and survival rate of larvae during their rearing are summarized in Figure 2. After two periods of larval rearing, significantly greater body weight and total length were found in both group 2 ( $W = 45 \pm 4$  mg and  $81 \pm 11$  mg,  $L_T = 16.9 \pm 0.4$  mm and  $19.1 \pm 0.9$  mm) and group 3 ( $W = 46 \pm 4$  mg and  $103 \pm 21$  mg,  $L_T = 17.0 \pm 0.5$  mm and  $20.6 \pm 1.4$  mm) with partial (7 days) and total (14 days) live feeding by *Artemia* nauplii compared to group 1 ( $W = 21 \pm 2$  mg and  $56 \pm 9$  mg,  $L_T = 13.6 \pm 0.4$  mm and  $17.5 \pm 1.0$  mm) with artificial dry feeding. Statistical differences in body weight and total length were not found between groups 2 and 3 after these periods of larval rearing. After the third period of larval rearing, achieved values of  $W$  and  $L_T$  in larvae of groups 1 and 2 were more well-balanced and did not have significant differences. The feeding diet in all groups of larvae had no significant influence on their cumulative survival rates during the whole larval rearing period. The highest cumulative survival rate of larvae was achieved in group 3 from beginning ( $92 \pm 1\%$ ) to the end ( $74 \pm 1\%$ ) of larval rearing period. However, these values of cumulative survival rate were not statistically different between groups 1 and 2, which achieved values of survival rate from the beginning to the end of the larval rearing period of  $90 \pm 1\%$ – $74 \pm 1\%$  and  $90 \pm 2\%$ – $73 \pm 1\%$ , respectively.

### 3.2 Effect of rearing environment during juvenile rearing period

Final growth parameters and survival rates of juveniles are summarized in Table 3. Juveniles from aquaria were statistically heavier ( $W = 3236 \pm 264$  mg) and longer ( $L_T = 74 \pm 4$  mm) than those from troughs ( $W = 2079 \pm 433$  mg and  $L_T = 61 \pm 4$  mm) at the end of the juvenile rearing period. Juveniles from aquaria reached about 55.7% heavier body weight and about 21.4% longer total length than ones from troughs at the end of their rearing period. A statistically higher specific growth rate was found in juveniles from aquaria (SGR =  $3.6\%$  d<sup>-1</sup>) compared to ones from troughs (SGR =  $3.1\%$  d<sup>-1</sup>). Statistically, a higher final condition of juveniles was observed in juveniles reared in troughs ( $0.9 \pm 0.1$ ) compared to ones from aquaria ( $0.8 \pm 0.1$ ). At the end of the juvenile rearing period, juveniles reared in aquaria had a higher survival rate ( $90 \pm 4\%$ ) than those from troughs ( $81 \pm 3\%$ ), but these differences were not significant. The courses of  $W$ ,  $L_T$  and survival rate of juveniles during their rearing are summarized in Figure 2. Juveniles reared in aquaria achieved statistically higher body weight and total length compared with those from troughs during all periods of juvenile rearing. Juveniles from aquaria had statistically higher body weight ( $W = 2576 \pm 230$  mg) and total length ( $L_T = 68 \pm 3$  mm) at the end of the third period of their rearing compared to older juveniles in troughs at the end of the fourth period of their rearing ( $W = 2079 \pm 433$  mg and  $L_T = 61 \pm 4$  mm). Cumulative survival rates fluctuated from  $93 \pm 3\%$  to  $81 \pm 3\%$  in juvenile reared in troughs and from  $92 \pm 4\%$  to  $90 \pm 4\%$  in juveniles from aquaria. This means that high cumulative survival rates were found in both groups from different rearing environments without statistical differences during the whole juvenile rearing period.

## 4 Discussion

Decreasing barbel abundance and high angler demands require conservation measures either by improvement of the habitat for natural reproduction (Peñáz et al. 1999) or by artificial reproduction, rearing and restocking (Wheeler and Jordan 1990; Lusk et al. 1998). Nowadays, thousands of yearlings and adult barbel are stocked annually into streams, rivers (Wheeler and Jordan 1990; Lusk 1996) and ponds (Taylor et al. 2004).

In our experimental conditions, the larval period lasted 21 days at  $21.0 \pm 0.3$  °C (441 degree-days), between days 19–39 after fertilization. Krupka (1988) found a longer larval period – 24 days, between days 13–36 after fertilization, at  $19.2$  °C (460.8 degree-days). Generally, water temperature is an important factor determining the length of the larval period in aquatic animals- higher water temperature causes a shorter larval period and decreases the number of degree-days during this period (Peñáz 1973; Hessen et al. 1987; Policar et al. 2004).

At the end of the larval rearing period, the lowest  $W$ ,  $L_T$  and SGR were found in the group of larvae not fed *Artemia* feeding (group 1). Of groups fed *Artemia* nauplii, a higher growth rate of larvae was found in group 3, which had the longer period of live feed (14 days). Similarly, Wolnicki and

Górny (1995a) and Fiala and Spurný (2001) found the highest  $W$ ,  $L_T$  and SGR in larvae fed *Artemia* nauplii when compared to larvae fed dry diets or a combination of *Artemia* nauplii and dry diets during 15- and 25-day rearing periods. After 25 days, larvae fed dry diets achieved only 37.5–70.3% of the body weight and 69.3–81.7% of the total length of those fed *Artemia* nauplii (Fiala and Spurný 2001). During the 15 days after the start of exogenous nutrition, larvae fed mixed diet grew considerably slower than larvae fed *Artemia* nauplii, and faster than groups of larvae receiving dry food (Wolnicki and Górny 1995a). We recorded the highest SGR in larvae fed *Artemia* nauplii (groups 2 and 3), showing that larvae initially need to receive live food to maintain their high growth (Rust 2002).

The lower growth of larvae receiving just dry food had two causes. Firstly, the larvae of many species of fish (especially cyprinids) have low digestive enzyme activity which increases with age. Trypsin and chemotrypsin activity increases substantially in cyprinid larvae from about 20 mg body weight. At this point, the first gut loops have been formed and the secretory activity of the exocrine pancreatic cells has increased. This development of the gut stops the resorption of body proteins, and the nutrient and energy needs and the content of body proteins are increased markedly (Kamler et al. 1987; Kamler 1992). Secondly, in larvae fed an artificial dry food, tryptic activity increases and trypsin production is twice as high as in larvae fed natural foods. However, increased tryptic activity impedes digestion and causes a lack of essential substances, leading to increased losses of body proteins (Hofer 1985; Kamler et al. 1990; Kolkovski 2001).

Larvae fed *Artemia* nauplii (groups 2 and 3) achieved higher specific growth rates at the end of the larval rearing period (SGR = 13.8 and 14.5% d<sup>-1</sup>) than those fed *Artemia* nauplii by Fiala and Spurný (2001) up until 25 days after start of exogenous nutrition (SGR = 10.2% d<sup>-1</sup>). In addition, larvae of group 1 (fed with artificial dry Asta diet) had higher SGR values (13.1% d<sup>-1</sup>) than those fed *Artemia* nauplii in the study published by Fiala and Spurný (2001). Large differences in SGR values were found between our larvae (13.1% d<sup>-1</sup>) and Fiala and Spurný's larvae which were fed only with artificial diets (SGR = 8.7–6.0% d<sup>-1</sup>). Lower growth rates of larvae during experiment of Fiala and Spurný (2001) was probably caused by initial low daily food rate (in *Artemia* less than 50% and in artificial food less than 10% of larvae biomass) under higher temperature (26.1 ± 0.3 °C). Beside, our results shows that Asta food is a high-quality artificial diet which can be used without the need for supplementing with live food for intensive rearing of barbel larvae under controlled conditions. Wolnicki and Górny (1995a) confirmed the successful use of artificial dry diets and the ability of barbel larvae to receive an artificial dry diet without additional live food during intensive rearing under controlled conditions. The relatively advanced ontogenic development of larval stage in common barbel (compared to other members of the Cyprinidae family) supports to accept an artificial food of suitable size and composition (Fiala and Spurný 2001).

The positive effect of the feeding diet with a longer period of live food was significantly determined by the achieved values of  $W$ ,  $L_T$  and SGR during larval rearing period. On

the other hand, current annually high consumption of *Artemia* cysts (over 2000 metric tons of dry *Artemia* cysts) and dramatic decrease of natural stocks increase current high price of *Artemia* cysts (Sorgeloos et al. 2001). Therefore, in terms of larval growth, each hatchery manager can decide whether to use *Artemia* nauplii or artificial feed. The using of *Artemia* nauplii will result in better growth rates of barbel larvae, but also higher production costs caused by high price of *Artemia* cysts, demand for labour and energy for its cultivation. The using of a suitable artificial dry diet will result in lower growth rates of barbel larvae, and the added benefit of decreased labour and energy requirements associated with the application of this feed.

Cumulative survival rates did not differ significantly in all groups throughout larval rearing. Hence, barbel larvae can be reared successfully not only on live but also on mixed and on dry food (Wolnicki and Górny 1995a). The effect of different feeding diet on the survival rate of reared larvae was not evident during our experimental larval rearing period. Therefore, in terms of larval survival, we recommend the use of an artificial dry diet for intensive rearing of barbel larvae, which is cheaper and less demanding for labour and energy costs than the combination of an artificial dry diet with the addition of *Artemia* nauplii.

Nevertheless, initial feeding with live food has been necessary for successful intensive rearing of larvae of other cyprinids. The recommended periods for weaning of some cyprinids fish are: 4 days for common carp, *Cyprinus carpio* (Dabrowski 1984), 5 days for tench, *Tinca tinca* (Wolnicki and Górny 1995b), 4 days for vimba, *Vimba vimba* (Wolnicki 1996), and 5–10 days for ide *Leuciscus idus* (Wolnicki and Górny 1995c). Dabrowski (1984) also states that a body weight about 4.3 mg is sufficient for the transfer from live to dry diets in common carp larvae.

At the end of a 25-day larval feeding experiment, Fiala and Spurný (2001) found the highest cumulative survivals in larvae fed with *Artemia* nauplii (99.7%) and with artificial flaked feed for aquarium fish (98%). These values were higher than our achieved values of  $S_C$  (73–80%) at the end of the larval rearing period. During 15-day larval rearing period, Wolnicki and Górny (1995a) achieved a similar survival rate in larvae fed the artificial dry diet Danish Aller Krystal 1 (73%). However, these authors found a still higher survival rate (about 99%) in larvae fed *Artemia* nauplii, by a combination of *Artemia* nauplii with artificial dry diets (Russian Ekvizo and Danish Aller Krystal 1) and by the artificial dry diet Russian Ekvizo.

During the juvenile period, barbel grew faster in aquaria than in troughs. This was probably related to the lower depth of water in troughs (165 mm) compared to aquaria (250–305 mm). Vilizzi and Copp (2001) and Vilizzi (2002) noted that the behaviour and activity of juvenile barbel is affected by water depth in an artificial channel.

## 5 Conclusion

During larval and juvenile rearing periods, high growth performance and survival rates of barbel were found. Even early stages of barbel are suitable for intensive culture under controlled conditions.

A longer period of live food had a positive effect on the growth of larvae compared to feeding with artificial dry feed. However, feeding with *Artemia* nauplii did not have a significant effect on survival rate of larvae. Even early stages of barbel can be successfully fed with Asta or another suitable food, without the addition of live food.

A significant effect of different rearing environments (aquaria and troughs) on the growth of juveniles was observed during the juvenile rearing period. Nevertheless, the different rearing environments did not influence the survival of juveniles.

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