

## Growth and survival rates, puberty and fecundity in captive common barbel (*Barbus barbus* L.) under controlled conditions

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**ABSTRACT:** Growth and survival rates (specific growth rate – SGR; survival rate – S) of *Barbus barbus* L. were recorded in captivity during three years from the larval period (final body weight –  $W = 0.2 \pm 0.03$  g; SGR =  $13.6 \pm 1.1\%$ /day and cumulative survival –  $S = 76.0 \pm 2.5\%$ ) to the first reproductive season ( $W = 62.55 \pm 13.5$  g; SGR =  $0.89 \pm 0.05\%$ /day;  $S = 59.3 \pm 1.5\%$ ). Final body size and SGR were compared between both sexes. Females reached the significantly higher growth rate (SGR =  $0.84 \pm 0.01\%$ /day) compared to males (SGR =  $0.77 \pm 0.01\%$ /day). Early puberty was observed in 17 and 32 months old males and females, respectively. Multi-stripping activity was found out in both sexes during the first reproductive season. In total, 20%, 25.8%, 30.3%, 14.6% and 9% of females were stripped once, twice and three, four and five times, respectively. But all males produced sperm during the entire reproductive season. The highest and the lowest egg production was recorded in the middle (April) and at the beginning (March) of the reproductive season ( $2155 \pm 925$  vs.  $1279 \pm 298$  eggs per stripping). The highest and the lowest sperm production was observed at the beginning (March) and at the end (May) of the reproductive season ( $7.9 \pm 0.08 \times 10^9$  vs.  $1.9 \pm 0.06 \times 10^9$  per stripping).

**Keywords:** *Barbus barbus*; stripping; egg; sperm; puberty; intensive culture

The common barbel (*Barbus barbus* L.) is regarded as an endangered fish species in Europe (Lusk et al., 2004; Prokeš et al., 2006; Lefler et al., 2008) due to overfishing, water pollution, damming and regulation of rivers (Lusk, 1996; Lusk et al., 1998). This population pressure has led to calls for a restocking programme to increase and enhance wild populations of barbel (Philippart and Mélard, 1983).

The production of barbel for restocking based on the stripping of wild broodstock has been variable from year to year (Philippart, 1982). Therefore, methods for the culture and reproduction of this

species were optimized under controlled conditions during the last three decades (Philippart, 1982; Philippart and Mélard, 1983; Poncin et al., 1987; Poncin, 1989). Poncin et al. (1987) and Poncin (1989) studied the effects of different photoperiod regimes on the reproduction of barbel under controlled conditions. The optimization of larval and juvenile rearing under controlled conditions was described by Labatzki and Fuhrmann (1992), Wolnicki and Górny (1995), Fiala and Spurný (2001) and Policar et al. (2007). However, there is a lack of information about growth and survival rates of barbel juveniles and adults in captivity.

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Recent studies have described the physiology, structure and morphology of barbel sperm, egg and larval quality and their changes during captive breeding (Alavi et al., 2008a,b, 2009b; Policar et al., 2010). The effect of nutrition in barbel males on the sperm quality was described by Alavi et al. (2008c, 2009a). However, there is no information about female and male fecundity and its changes under controlled conditions during the reproductive season.

The aim of the present study was to illustrate growth and survival rates in common barbel under controlled conditions from the beginning of exogenous nutrition to the first reproductive season. Simultaneously, the effect of sex on the growth was found in common barbel during this study. The second aim was to compare egg and sperm production in captivity during three months of the first reproductive season.

## MATERIAL AND METHODS

### Acquisition of larvae

Artificial stripping of wild barbel broodfish was carried out at the Žleby anglers' club hatchery (Central Bohemia region, 49°54'N and 15°30'E) for the production of larvae according to Policar et al. (2007). Fertilized eggs were incubated in three 10 l Zug jars and newly hatched larvae were kept in three Rückl-Vacek incubators (Policar et al., 2004). Active swimming larvae were transported to a fish research facility at the University of South Bohemia, Faculty of Fisheries and Protection of Waters (FFPW). In total 22 500 larvae (13 days after hatching, 236 degree-days post hatch) were obtained for this study.

### Growth and survival (from the larval period to the end of the first reproductive season)

**Larval culture (26 May 2003–15 June 2003).** In total, 22 500 larvae (total length – TL = 11.4 ± 0.3 mm and body weight – W = 10 ± 0.2 mg) were stocked into three tanks (2.7 × 0.45 × 0.2 m; water depth 0.13 m, volume 150 l). Larval rearing was started from the beginning of exogenous nutrition (13 days post-hatch = day 1) until day 21, when metamorphosis was completed (Peňáz, 1971, 1973; Krupka, 1988).

Larvae received exclusively artificial dry feed Asta made by the Polish Academy of Sciences with the nutrition content: dry matter (95.8%), crude protein (50.5%), fat (9.1%), fibre (4.5%), ash (9.8%), net energy (18.6 MJ) and vitamins: A (24 000 IE/kg), D3 (300 IE/kg), B1 (0.2 g/kg), B2 (0.32 g/kg), B12 (0.7 mg/kg), C (8 g/kg) E (2 g/kg). Artificial feed was distributed daily by hand at 07:00, 09:00, 11:00, 13:00, 15:00, 17:00 and 19:00 h during the light period.

**Juvenile culture to one year of age (16 June 2003–26 May 2004).** In total, 3600 juveniles (age 21 days, W = 175.0 ± 20.5 mg and TL = 24.5 ± 3.5 mm) were stocked into three tanks (2.7 × 0.45 × 0.2 m; water depth 0.166 m, volume 200 l) and reared for 134 days (age of juveniles 155 days). Then, they were moved into larger tanks (1 × 1 × 0.8 m; water depth 0.6 m, volume 600 l) and reared to 366 days of age. Juveniles received exclusively the artificial dry feed Asta described above. Feed was distributed automatically by a feeder during the light period.

**Juvenile culture to two years of age (27 May 2004–26 May 2005).** The subsequent culture of barbel juveniles continued in the same three tanks as the previous culture during 365 days. In total, 1800 juveniles (age 1 year, W = 5.4 ± 0.5 g and TL = 85.2 ± 12.5 mm) were used for this culture. Juveniles received exclusively artificial dry feed Karpico Prime-6 (dry matter 95.0%, crude protein 33.0%, fat 6.0%, fibre 4.2%, ash 8.3%, net energy 15.6 MJ/kg, vitamin A 15 000 IE/kg, vitamin D3 2000 IE/kg, vitamin E 200 mg/kg and vitamin C 150 mg/kg) produced by Coppens International, AM Helmond, Netherlands, as a professional growing diet for cyprinids. Feed distribution was similar like in the previous juvenile culture.

**Culture to three years of age (27 May 2005 to 26 May 2006).** At the beginning of this culture, 180 juvenile fishes (90 non-matured females: W = 39.2 ± 6.4 g; TL = 172.0 ± 18.1 mm and 90 matured males: W = 19.1 ± 2.3 g; TL = 134.1 ± 9.7 mm) were stocked (sex ratio 1:1) into three equivalent tanks like the previous juvenile culture. All stocked fish were individually tagged with PIT tags. The same feed distribution was used during this culture as in the previous juvenile culture. An increasing light regime with the constant water temperature was used during this culture stage (Table 1) to stimulate the reproductive activity of matured barbel (Poncin et al., 1987; Poncin, 1989; Philippart et al., 1989).

**Environmental conditions of each culture stage.** Detailed information about environmental conditions (water temperature, light regime, wa-

Table 1. Detailed environmental conditions, daily feeding rate, size of pellets and fish density used for common barbel (*Barbus barbus* L.) during intensive culture from the larval period to the end of three-year culture (the first reproductive season)

Culture	Water temperature (°C)	Light regime (h/day)	Water flow (l/min)	Oxygen saturation (%)	pH	Ammonia (mg/l)	Nitrites (mg/l)	Feed, size pellets (mm)	Daily feeding rate (% from biomass)	Fish density (fish/l)
26 May 03 –15 June 03	21 ± 0.6	14L/10D	0.2	80.0 ± 3.0	7.3 ± 0.3	< 0.02	< 0.02	Asta, 0.2–0.5	1–7 days: 30 8–14 days: 20 15–21 days: 15	50
16 June 03 –26 May 04	20.0 ± 1.0	12L/12D	10	78.5 ± 2.5	7.3 ± 0.3	< 0.01	< 0.01	Asta, 0.8–1.5	22–155 days: 10 156–366 days: 5	6
27 May 04 –26 May 05	19.5 ± 1.2	12L/12D	10	77.5 ± 2.0	7.2 ± 0.2	< 0.02	< 0.01	Karpico, 1.5–2.0	367–550 days: 2.5 551–731 days: 1.5	1
27 May 05 –26 May 06	21.0 ± 0.4	May 05–Jan.06: 10L/14D Feb.06–March 06: 12L/12D April 06: 14L/10D May 06: 16L/8D	12.5	75.5 ± 2.9	7.3 ± 0.2	< 0.02	< 0.02	Karpico, 1.5	constant: 1.5	0.1

L = light, D = dark

ter flow, oxygen saturation, pH, concentration of ammonia and nitrites), daily feeding rate, size of used pellets and fish density during each culture is summarized in Table 1.

Water temperature and oxygen saturation were measured twice daily (at 07:00 and 19:00 h) by WTW MultiLine P4 (WTW GmbH, Weilheim, Germany). The other parameters of water quality (pH, ammonia, nitrites) were analysed at the chemical laboratory of FFPW (Vodňany, Czech Republic) once a week during each culture.

**Collection of growth and survival data.** At the end of each culture, all fish tanks were harvested and all survived fish were counted and a representative sample of 33 fishes was collected from each tank.

Survival rate (S) was calculated as follows:

$$S = (L_2/L_1) \times 100$$

where:

$L_2$  = number of survived larvae

$L_1$  = number of stocked fish

Individual body weight (W) was measured with a Mettler electronic balance (model AE 200) to the nearest 0.0001 g (in larvae) or 0.01 g (in juveniles and broodstock) and total length (TL) was measured with a calliper (to the nearest 0.1 mm) in fish samples from each tank. Specific growth rate [SGR =  $100/t \ln (W_2/W_1)$ , where  $W_1$  and  $W_2$  are initial and final weights, and  $t$  is the growing period in days] and condition level (Fulton's condition coefficient  $FC = 100W/TL^3$ , where  $W$  is the final weight and  $TL$  is the final total length of fish) were calculated after the biometric analysis of fish from representative samples. At the end of the three-year culture, final growth parameters of fish (W, TL, SGR and FC) were measured and calculated in all survived fish ( $n = 178$ ).

### Effect of sex on growth rate

SGR of captive females and males was calculated according to biometric data on both sexes after

three-year culture. Specific growth rate of farmed females was compared to SGR of farmed males at the end of the three-year culture.

### Fish puberty

During the last two years of the culture, all captive fish were harvested, fish condition and the onset fish puberty were checked quarterly. The first matured fish releasing eggs or sperm were recorded during this inspection.

### Reproductive performance and fecundity

Reproductive performance and average fecundity as production of eggs and sperm were found in all tagged farmed females and in 18 selected males in all tanks from March (beginning) to May (end) of the reproductive season of the last culture, respectively. Female and male reproductive activity and fecundity were recorded weekly and monthly, respectively.

During each control, all females were anesthetized with clove oil (0.033 ml/l) (Hamáčková et al. 2001) before egg stripping. Stripping frequency (SF as the number of strippings per female), weight of one egg (WE in mg), absolute weight of eggs (AWE in g) and absolute number of eggs (ANE as the number of eggs) as absolute female fecundity and relative weight of eggs (RWE in g/kg of female weight) and relative number of eggs (RNE as the number of eggs/kg of female weight) as relative female fecundity were recorded and calculated after each stripping of eggs.

AWE per each stripping was measured by the weighting of all eggs obtained from each female with a Kern and Sohn GmbH balance (Balingen, Germany) to the nearest 0.1 g. In total, 33 randomly collected eggs from each egg stripping were weighed with an Kern and Sohn GmbH electronic balance (Balingen, Germany) to the nearest 0.0001 g and WE was found in each egg stripping of each female. ANE per each stripping was calculated as follows:  $ANE = AWE/WE$ . Relative fecundity (RWE and RNE) was calculated when AWE and ANE was divided by the body weight of the female.

The same tagged males ( $n = 18$ ) were always used to record stripping frequency (SF) and fecundity (sperm production) monthly during the repro-

ductive season from March to May. Males were anesthetized before sperm stripping similarly like the females. Sperm was collected with a syringe fitted with a plastic needle. All efforts were made to avoid sperm contamination by urine, blood, water and mucus. Syringes were placed in an ice box and immediately transported to the laboratory for analyses.

The sperm volume (SV in ml) and density (SD in billions of spermatozoa/ml) were measured following the method of Alavi et al. (2010). To determine the sperm density, the sperm was diluted 10 000 times with 0.7% NaCl, a drop (10  $\mu$ l) was placed onto a haemocytometer (depth 0.1 mm) and covered by a coverslip. The sperm was then left for 10 min to allow sedimentation before counting 16 chambers. Absolute sperm production (ASP in billions of spermatozoa per sperm stripping) for each male was calculated as follows:  $ASP = SV \times SD$ . Relative sperm production (RSP in billions of spermatozoa/kg of male weight) was calculated when ASP was divided by the body weight of each tagged male.

Frequency of stripping, sperm volume, sperm density and absolute and relative sperm production were recorded, measured and calculated in each tagged male during the spawning period from March to May.

### Data analysis

All data on growth (TL, W, SGR and FC), survival (S), stripping frequency (SF), fecundity (ANE, RNE, AWE, RWE, ASP and RSP), sperm volume (SV) and density (SD) are presented as means ( $\pm$  SE) and statistical assessment was performed by Statistica software 6.1 (StatSoft, Inc., Czech Republic).

One-way analysis of variance ANOVA ( $P < 0.05$ ) was followed by Tukey's multiple comparison test (TL, W and FC) or non-parametric Kruskal-Wallis test. SGR and S were used for a comparison of growth and survival performances between captive females and males after three-year culture, respectively.

Two-way analysis of variance ANOVA ( $P < 0.05$ ) by Duncan's test were used for a comparison of all data on fecundity (ANE, RNE, AWE, RWE, ASP, RSP, SV and SD) in each month of the reproductive season. March, April and May were considered as the beginning, the middle and the end of the reproductive season.

Table 2. Growth and survival data in common barbel (*Barbus barbus*) under controlled conditions during three-year culture

Culture	Duration (days)	Initial			Final			SGR (%/day)	Cumulative survival (%)
		W (g)	TL (mm)	FC	W (g)	TL (mm)	FC		
26 May 03 –15 June 03	21	0.01 ± 0.002	11.4 ± 1.5	0.7 ± 0.05	0.2 ± 0.03	24.5 ± 3.5	1.2 ± 0.1	13.6 ± 1.1	76.0 ± 2.5
16 June 03 –26 May 04	345	0.2 ± 0.025	24.5 ± 3.5	1.2 ± 0.1	5.4 ± 0.5	85.2 ± 12.5	0.9 ± 0.1	1.0 ± 0.3	61.5 ± 3.1
27 May 04 –26 May 05	365	5.4 ± 0.5	85.2 ± 12.5	0.9 ± 0.1	29.2 ± 5.9	152.9 ± 23.5	0.8 ± 0.1	0.5 ± 0.04	58.9 ± 2.1
27 May 05 –26 May 06	365	29.2 ± 5.9	152.9 ± 23.5	0.8 ± 0.1	66.5 ± 9.8	205.6 ± 32.5	0.8 ± 0.1	0.2 ± 0.001	58.1 ± 1.2

W = body weight, TL = total length, FC = Fulton's condition coefficient, SGR = specific growth rate

## RESULTS

### Growth and survival of barbel in captivity from the larval period to the end of the first reproductive season

Growth and survival rates of barbel under controlled conditions during the three-year culture are presented in Table 2. During the larval period, larvae reached high SGR ( $13.6 \pm 1.1\%$ /day) and good survival rate ( $76.0 \pm 2.5\%$ ). Final larval body weight W ( $0.2 \pm 0.03$  mg), total length TL ( $24.5 \pm 3.5$  mm) and Fulton's condition coefficient FC ( $1.2 \pm 0.1$ ) were recorded at the end of the larval culture.

High cumulative survival S ( $58.1 \pm 1.2$ ) was found out at the end of three-year culture. It means that the highest mortality of fish was recorded during larval period and juvenile culture till the age of one year. During the subsequent culture, a high survival rate ( $94.4 \pm 2.5\%$ ) was recorded. Fulton's condition coefficient decreased during the entire culture period from final larval FC ( $1.2 \pm 0.1$ ) to FC of three years old fish ( $0.8 \pm 0.1$ ). One and two years old fish reached average body weight W ( $5.42 \pm 0.5$  g and  $29.2 \pm 5.9$  g, respectively) and total length TL ( $85.2 \pm 12.5$  mm and  $152.9 \pm 23.5$  mm, respectively). Final body weight and total length of three years old fish were W =  $66.5 \pm 9.8$  g and TL =  $205.6 \pm 32.5$  mm after their first reproductive season (Table 2).

### Effect of sex on growth rate

A significant difference was found out between body size and SGR of both sexes at the end of the

first reproductive season when the fish were three years old. Final body weight, total length and SGR of males were as follows: W =  $48.8 \pm 10.6$  g, TL =  $187.5 \pm 12.0$  mm and SGR =  $0.77 \pm 0.01\%$  per day at the end of the culture. In females, these parameters were: W =  $100.3 \pm 33.1$  g, TL =  $234.5 \pm 25.4$  mm and SGR =  $0.84 \pm 0.01\%$  per day (Table 3).

### Fish puberty

The first matured males released sperm at the end of October 2004 when they were 17 months old (W =  $24.6 \pm 10.2$  g and TL =  $145 \pm 17.2$  mm). Females (W =  $91.4 \pm 27.3$  g and TL =  $220 \pm 25$  mm) started to release eggs 15 months later compared to males (32 months old females).

### Reproductive performance and fecundity

All females were stripped 236 times in total when the average number of egg strippings per female was 2.65 during the reproductive season. In total, 20%, 25.8%, 30.3%, 14.6% and 9% of females were stripped once, twice and three, four and five times, respectively.

Stripping frequency and production (absolute and relative fecundity of females – ANE, RNE, AWE, RWE) during the reproductive season are documented in detail in Table 4. The highest and the lowest frequency of egg stripping was noted in April (119 strippings) and in March (38 strippings). The same results relevant to fecundity were observed

Table 3. Comparison of final W and TL and SGR between farmed females and males under controlled conditions at the end of three-year culture. Different letters indicate differences in W, TL, FC and SGR at the end of the culture ( $P < 0.05$ )

Sex	Duration (days)	Initial				Final				SGR (%/day)
		<i>n</i>	W (g)	TL (mm)	FC	<i>n</i>	W (g)	TL (mm)	FC	
Mixed population	1096	99	0.01 ± 0.002	11.4 ± 1.5	0.7 ± 0.05	178	66.5 ± 9.8	205.6 ± 32.5	0.8 ± 0.1	0.8 ± 0.02
Females						89	100.3 ± 33.1 <sup>a</sup>	234.5 ± 25.4 <sup>a</sup>	0.8 ± 0.1 <sup>a</sup>	0.8 ± 0.01 <sup>a</sup>
Males						89	48.8 ± 10.6 <sup>b</sup>	187.5 ± 12.0 <sup>b</sup>	0.7 ± 0.1 <sup>b</sup>	0.8 ± 0.01 <sup>b</sup>

W = body weight, TL = total length, FC = Fulton's condition coefficient, SGR = specific growth rate

during three months of the reproductive season. The highest average absolute fecundity (egg production) was observed in April when ANE = 2155 ± 925 eggs (27.4 g of eggs) were stripped from each female. The lowest egg production was found at the beginning of the reproductive season (March), when ANE = 1279 ± 298 eggs (14.8 ± 3.3 g of eggs) were obtained from each stripping of female. The same trend was noted in relative fecundity (RNE), which varied from 13 051 eggs/kg to 21 485 ± 9248 eggs/kg during the reproductive season.

All males produced sperm during the entire reproductive season. Sperm volume showed a significant decrease towards the end of the reproductive season. The highest (SV = 0.42 ± 0.08 ml)

and lowest (SV = 0.15 ± 0.04 ml) sperm volumes were recorded in March and May, respectively. In general, sperm density also decreased towards the end of the reproductive season. The highest sperm density was found in March (SD = 18.8 ± 1.0 × 10<sup>9</sup> sperm/ml) and the lowest in April (SD = 11.8 ± 0.9 × 10<sup>9</sup> sperm/ml) and May (SD = 12.4 ± 1.4 × 10<sup>9</sup> sperm/ml) without significant difference. The same trend was observed in absolute and relative sperm production when the average sperm production decreased from March (ASP = 7.9 ± 0.08 × 10<sup>9</sup> spermatozoa and RSP = 171.7 ± 8.2 × 10<sup>9</sup> sperm/kg) to May (ASP = 1.9 ± 0.06 × 10<sup>9</sup> spermatozoa and RSP = 38.1 ± 2.0 × 10<sup>9</sup> sperm/kg) (Table 4).

Table 4. Stripping frequency, egg and sperm production in captive females and males of common barbel (*Barbus barbus*) during the first reproductive season. Different letters within a column indicate differences in all parameters among the months (phases) of reproductive season ( $P < 0.05$ )

Time of stripping	Female						Male					
	<i>n</i>	stripping frequency (stripping/month)	ANE (eggs)	RNE (eggs/kg)	AWE (g)	RWE (g/kg)	<i>n</i>	stripping frequency (stripping/month)	SV (ml)	SD (billions sperms/ml)	ASP (billions sperms)	RSP (billions sperms/ml)
March	90	38	1279 ± 298 <sup>c</sup>	13051 ± 3040 <sup>c</sup>	14.8 ± 3.3 <sup>c</sup>	151 ± 28.9 <sup>c</sup>	90	90	0.42 ± 0.08 <sup>a</sup>	18.8 ± 1.0 <sup>a</sup>	7.9 ± 0.08 <sup>a</sup>	171.7 ± 8.2 <sup>a</sup>
April	89	119	2155 ± 925 <sup>a</sup>	21485 ± 9248 <sup>a</sup>	27.4 ± 11.2 <sup>a</sup>	243.3 ± 89.6 <sup>a</sup>	89	89	0.28 ± 0.05 <sup>ab</sup>	11.8 ± 0.9 <sup>b</sup>	3.3 ± 0.05 <sup>b</sup>	67.6 ± 3.9 <sup>b</sup>
May	89	79	1526 ± 459 <sup>b</sup>	15214 ± 4371 <sup>b</sup>	19.6 ± 6.2 <sup>b</sup>	195.4 ± 69.2 <sup>b</sup>	89	89	0.15 ± 0.04 <sup>b</sup>	12.4 ± 1.4 <sup>b</sup>	1.9 ± 0.06 <sup>c</sup>	38.1 ± 2.0 <sup>c</sup>

ANE = absolute number of eggs, RNE = relative number of eggs, AWE = absolute weight of eggs, RWE = relative weight of eggs, SV = sperm volume, SD = sperm density, ASP = absolute sperm production, RSP = relative sperm production

## DISCUSSION

### Growth and survival of barbel in captivity from the larval period to the end of the first reproductive season

Our study confirmed the previous observations that live feed is not an essential diet for larvae in common barbel at the start of exogenous feeding (Wolnicki and Górny, 1995; Policar et al., 2007). This fact is caused by relatively advanced ontogenic development of barbel larvae at the beginning of exogenous nutrition compared to larvae of other cyprinids (Peňáz, 1971, 1973; Fiala and Spurný, 2001; Policar et al., 2007; Wolnicki et al., 2009). Fast growth and high survival rate could be achieved under controlled conditions if the used artificial feed met the nutrient requirements of barbel larvae (Wolnicki and Górny, 1995; Fiala and Spurný, 2001; Policar et al., 2007). In the present study constant water temperature about 21°C was maintained during the larval period. Previous studies also showed high growth and survival rate at a water temperature between 21°C and 26°C under controlled culture (Philippart et al., 1989; Fiala and Spurný, 2001). Wolnicki and Górny (1995), Fiala and Spurný (2001) and Policar et al. (2007) reported specific growth rate (SGR) of about 6.0–14.5%/day and survival rate of about 73–95% in larvae under controlled and optimum conditions. The present study confirmed this information and allowed to keep a juvenile culture under controlled conditions. Only a few studies have reported the juvenile culture of common barbel (Labatzki and Fuhrmann, 1992; Philippart et al., 1989; Policar et al., 2007). No detailed study of juvenile culture under controlled conditions has been published yet. However, the conception of captive broodstock culture and controlled mass production of eggs in common barbel were described by Philippart (1982), Poncin et al. (1987) and Philippart et al. (1989).

### Fish puberty

Under optimal water temperature (20–24°C) and using the trout feed in captive culture, barbel females attained their first sexual puberty at 18–25 months of age (1.5–2.08 years) when the fish reached TL from 230 mm to 320 mm (Philippart et al., 1989; Poncin, 1989). The present study showed slower growth and later puberty in females that might correspond to the use of carp feed (Karpico

with the lower protein and fat level (33% protein and 6% fat) compared to trout feed with the protein and fat level of about 46% and 15%, respectively, used in the study published by Philippart et al. (1989) and Poncin (1989). However, we can confirm that the controlled culture allows to reduce the age of the first puberty. Wild barbel females attained the first puberty at 4–6 years of age (48 to 72 months), it means 2–4.5 years later compared to the broodstock from captive culture (Philippart et al., 1989; Baras and Philippart, 1999).

### Reproductive activity and fecundity

The multi-reproductive performance of females in common barbel shows asynchronous oocyte maturation (Poncin et al., 1996; Lefler et al., 2008). Philippart et al. (1989) and Poncin (1989) described multi-reproductive performance in females during the reproductive season from January to July. Each female of these studies was stripped in 15-day intervals (10 strippings per female during the reproductive season, which was equal to 1.7 stripping per female and month of the reproductive season). The average absolute egg production per female of TL between 180 mm and 500 mm was reported to amount to 8000 eggs per stripping, depending upon the fish size. It means that one female produced about 80 000 eggs per reproductive season. Our results showed lower reproductive activity (2.65 strippings per female during the three-month season equal to 0.88 stripping per female and month of the reproductive season) and lower egg production when the average egg production from all strippings was 1240 eggs. The lower egg production determined in the present study might be due to two reasons: the first reason is the size of our females. Smaller females ( $W = 100.3 \pm 33.1$  g and  $TL = 234.5 \pm 25.4$  mm) were used in present study. The second reason is the lower reproductive activity caused probably by a lower frequency of female stripping (weekly) when anaesthesia was used. We found that the more frequent fish manipulation and use of anaesthesia in barbel broodstock decreased their reproductive activity. According to our experience, the weekly interval of fish manipulation with the use of anaesthesia is a maximal frequency which did not negatively affect the reproductive activity of broodstock. Fish manipulation during the reproductive season has not been described in captive barbel broodstock by any authors yet.

Besides total egg production after the reproductive season, the recording of changes in egg production during the season was the most important part in this study. No study on egg production and fecundity in captive barbel broodstock has been carried out yet. This information is very important for fish producers to illustrate the optimum period of the reproductive season. There are a few studies showing the effect of the season on the reproductive performance of females in different fish species such as perch *Perca fluviatilis* (Kestemont et al., 1999; Migaud et al., 2001, 2004), common carp *Cyprinus carpio* (Kucharczyk et al., 2008) and walleye *Stizostedion vitreum* (Malison et al., 1998). The present results showed that the highest reproductive performance of females was in the middle of the reproductive season and the lowest at the beginning and end of the season. In general, the reproductive activity of non-hormonally treated broodstock seems to be higher in the middle of the reproductive season (Policar et al., 2008).

The present study showed no interruption of reproductive performance in males during the reproductive season. All males released sperm spontaneously; however, the quality and quantity of sperm varied during the reproductive season. The present study confirms the previous reports of our studies that the highest sperm production occurs at the beginning of reproductive season and decreases toward to the end of reproductive season (Alavi et al., 2008a,b,c). Alavi et al. (2008a,b,c) also observed the highest sperm velocity in the middle of the reproductive season and the lowest at the beginning and end of the season. The highest sperm quality in the middle of the spawning period was also confirmed in other fish species, e.g. *Salmo trutta caspius* (Hajirezaee et al., 2010).

## CONCLUSION

We observed high and acceptable specific growth and survival rates in common barbel cultured in an intensive culture system under controlled conditions from the larval stage to the first reproductive season.

A faster growth rate was found in females compared to that of males. Early puberty was observed in 17 months old males and 32 months old females.

Multi-egg and sperm stripping was observed in barbel broodstock during the three-month reproduc-

tive season, representing asynchronous maturation of gametes. The highest egg production was noted in the middle of the season (April) and the lowest at the beginning of the season (March). The highest sperm production was found out at the beginning of the season and the lowest at the end of the season.

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