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Reproduction of Algerian Barbel *Luciobarbus callensis* (Cyprinidae) in the Agrioun River, Algeria[1]

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Abstract—The aim of the present study is to investigate the reproductive parameters in *Luciobarbus callensis*. Different indices related to the fish and to the sperm parameters are considered, including gonadosomatic index (GSI), hepatosomatic index (HSI), condition factor (K), sperm ejaculate volume and concentration, and sperm motility duration. Male and female GSI peak is observed in April-May. HSI expresses an inverse pattern compared to GSI in both sexes, and K index changes inversely compared to GSI. The lowest values of K index are observed during the spawning season revealing corporal reserve mobilization in the mating effort. The first sexual maturity of 50% of the fish is observed at 18 and 21 cm *TL* in the males and females, respectively. Maximum ejaculate volume (6500 μL on average) is observed in May and June. Positive correlations are observed between sperm ejaculate volume and fish length and weight.

Keywords: Luciobarbus callensis, gonadosomatic index, hepatosomatic index, condition factor, sperm parameters, Agrioun River, Algeria

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Barbels of the genus *Luciobarbus* (Cyprinidae) are the endemic freshwater species abundantly distributed throughout the Northern African region (Garcia et al., 2010; Kara, 2012). In fish, several indicators are recommended to study accurately reproduction dynamics including gonadosomatic index (GSI), hepaosomatic index (HSI), condition factor (K) (Lloret and Ratz, 2000; Mouneyrac et al., 2008), and sperm motility (Alavi and Cosson, 2005a). Different studies were dedicated to north African barbel (*Barbus callensis* = *Luciobarbus callensis*) including systematics (Almaça, 1970), salinity tolerance (Kraiem and Pattee, 1988), ecology (Bouhbouh, 2002), diet (Cherghou et al., 2002), intersex and histology (Djoudad-kadj et al., 2012), parasites (Hadou-Sanoun et al., 2012; Ould Rouis et al., 2016), reproductive biology (Ould Rouis et al., 2012; Mouaissia et al., 2017), biochemistry (Sila et al., 2012), age and growth (Mimeche et al., 2013; Morsi et al., 2015), genetics (Brahimi et al., 2017), and pollution (Habila et al., 2017; Khebbache et al., 2017). However, little information is available on the reproduction physiology of *L. callensis* in its natural condition, especially concerning the variations of semen parameters throughout the reproduction season. In this respect, sperm and egg qualities are considered as the most potent parameters revealing accurately the reproductive season in different species (Babiak et al., 2006). In fact, it is shown that ejaculate volume, gamete concentration and motility duration vary through the reproductive season both in freshwater (Billard et al., 1995; Alavi et al., 2008) and marine fish (Babiak et al., 2006; Rouxel et al., 2008). Similarly, it is shown that sperm quality could be significantly affected by fish biometry including fish length and weight (Alavi et al., 2009).

Thus, the aim of the present study was to investigate the reproduction activity in male and female *Luciobarbus callensis* throughout the spawning season by considering different indexes including gonadosomatic index (GSI), hepatosomatic index (HSI), condition factor (K), ejaculate volume, sperm concentration, and sperm motility duration. In addition, relationships between semen characteristics and fish length and weight, were explored.

MATERIALS AND METHODS

The barbel (*Luciobarbus callensis*, Cyprinidae family) was collected in the Agrioun River (Bejaia), located in Northeastern Algeria (36°36′54.25″ N and

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05°22′04.33″ E) between March and August 2011. The fish were captured using fishing rod connected to a lift net, 2 cm mesh size. Altogether, 130 specimens of *L. callensis* were captured (102 males and 28 females). The fish were brought to the laboratory alive, and total length (*TL*) of each fish was measured to the nearest 1 mm using a fish measuring board. Total wet weight (*W*) was recorded to the nearest 1 g using an electronic balance. The fish was cut open and the sexes and maturity stages were recorded. The gonads and liver were removed and weighed to the nearest 0.001 g.

Gonadosomatic index (GSI), hepatosomatic index (HSI) and condition factor (K) were calculated in both males and females, as GSI = $(GdW/GW) \times 100$, HSI = $(LW/GW) \times 100$, K = $(GW/L^3) \times 100$, where GdW = gonad weight (g), GW = gutted weight (g), LW = liver weight (g), and TL = total length (cm).To determine the length at first maturity (Lm), a logistic function was fitted to the fraction of mature fish against length interval using the nonlinear least square regression method. The logistic equation was: $P = 1/(1 + \exp(-r(L - Lm_{50})))$, where P = relative frequency of mature individuals per length class L, r = slope parameter of the maturity curve, and Lm_{50} = length at 50% maturity (King, 1995).

Sperm movement duration was assessed using a 1 stopwatch; sperm was considered as immotile when less than 5% of spermatozoa remained motile (Tuset et al., 2008). Fresh sperm was then diluted (1/1000) in fresh river water for motility activation. The parameters obtained by optical microscopy (10 × 40 magnifications) were as follows: spermatozoa concentration and sperm movement duration.

The ejaculate volume and sperm concentration were expressed as microliter and billions of spermatozoa per ml of semen, respectively. A Malassez cell counting chamber was used to measure spermatozoa concentration under a light microscope (×200). A droplet (15 µL) of diluted semen was placed on 2 Malassez cell (depth 0.1 mm) under a coverslip, and in 10 min, the number of spermatozoa was counted in 20 square cells.

Statistical analysis was performed using Statview 5.0 software (Abaccus). All experiments were repeated at least three times. Values are expressed as mean ± standard error. Coefficients of correlation were used to explore the existing relationships between semen parameters and somatic parameters. ANOVA analysis was used to compare values of each parameter during the reproduction season. Difference was considered as statistically significant at $p < 0.05$.

RESULTS

In Agrioun River, barbell body length in males and females varied from 7.8 to 29.2 cm (19.7 ± 0.4 cm, n = 102, mean ± SE) and from 16.4 to 33.3 cm (21.7 ± 0.9 cm, n = 28), respectively. Body weight varied from 13.3 to 210.0 g (77.0 ± 4.4 g, n = 102) and from 37.2 to 327.7 g (112.7 ± 14.4 g, n = 28), respectively. The sex ratio (males: females) of *L. callensis* was 3.6 : 1. During the sampling period females were captured occasionally, and the first individuals were observed from April 6.

Figure 1a, b represent GSI in the males and females during the study period, respectively. The male GSI increased from March to reach the highest values in April-May and decreased gradually to reach minimum values in August. In females, GSI presented the highest values in April-May and the lowest values in July-August. Female and male GSI ranged from 0.8 to 22.3% and from 0.1 to 15.0%, respectively. An opposite pattern was observed concerning GSI (Fig. 1a, b) and HSI (Fig. 1c, d), indicating the mobilization of hepatic reserves during gametogenesis. HSI decreased significantly in both sexes from April to show the lowest values in July. Males HSI (0.8 to 3.5%) values were slightly higher than those in the females (0.9 to 3.3%).

The condition factor (K) (Fig. 1e, f) showed a similar pattern as GSI and an inverse pattern compared to HSI. The condition factor varied from 0.8 to 1.1% in the males (Fig. 1e) and from 0.8 to 1.1% in the females (Fig. 1f). The K value was slightly higher in the females. The first sexual maturity of 50% of the fish (Lm_{50}) was observed at 18 and 21 cm TL in the males and females, respectively. (Fig. 2). On the basis of spermatozoa presence, the smallest mature male had 7.8 cm TL. Ejaculate volume (Fig. 3) increased from March to May-June and then decreased in July. Ejaculate volume and GSI peaked closer to each other, April-May for GSI (Fig. 1a) and May-June for ejaculate volume (Fig. 3). Sperm concentration varied from 01×10^9 to 13×10^9 spermatozoa/ml. The highest concentrations ($9.6 \pm 1.3 \times 10^9$ spermatozoa/ml) were observed between June and July, and the lowest concentrations ($2.6 \pm 0.6 \times 10^9$ spermatozoa/ml) were present at the end of the reproductive season (August). Sperm motility duration ranged between 15 s to 2 min 35 s. The rising values were observed in April, and the durations lower than 30 seconds were observed at the end of the reproduction season.

When studying relationships between fish biometric parameters and semen characteristics, significant correlations were observed between ejaculate volume and fish length ($r = +0.48$, $p < 0.05$, $n = 102$) and weight ($r = +0.45$, $p < 0.05$, $n = 102$). Lower correlations were observed between sperm concentration and fish length ($r = +0.35$, $p < 0.05$, $n = 102$) and weight ($r = +0.21$, $p < 0.05$, $n = 102$). Sperm motility duration showed significant negative correlation with fish length ($r = -0.047$, $p < 0.05$, $n = 102$) and no significant correlation with fish weight ($r = -0.002$, $p < 0.05$, $n = 102$).

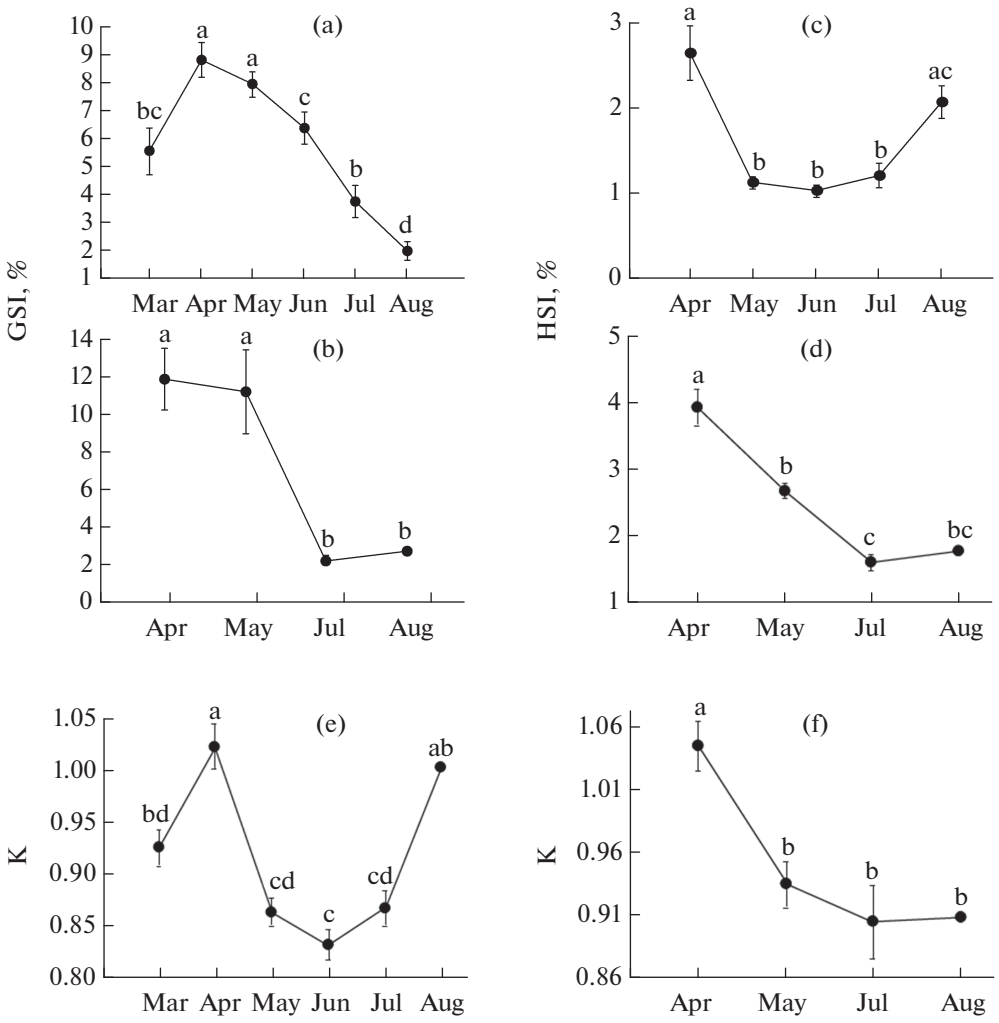

Fig. 1. Monthly variation of mean: gonadosomatic index (GSI) of the (a) males and (b) females, hepatosomatic index (HSI) of the (c) males, and (d) females, and condition factor (K) of the (e) males, and (f) females of *Luciobarbus callensis* throughout the reproductive season. Values are expressed as mean ± standard error (n = 102 and 28 for the males and females, respectively). Values with different letters are statistically different at $p < 0.05$.

DISCUSSION

The results showed that in the females, GSI was consistently higher than in the males (mean ± SE: 8.7 ± 1.2 and 7.0 ± 0.3, respectively); this is in accordance with values reported for *Barbus callensis* (= *Luciobarbus callensis*) in Tunisia (Kraiem, 1997), Morocco (Bouhbouh, 2002), and Algeria (Ould Rouis et al., 2012; Morsi et al., 2015; Mouaissia et al., 2017). This difference could be related to the composition of ovarian tissue containing more energy as reported by Encina and Granado-Lorencio (1997). On the basis of GSI, *L. callensis* females mature slightly earlier than the males in the Agrioun River, similarly as reported in Beni Haroun dam in Algeria (Mouaissia et al., 2017). Fish in the Agrioun River, showed appreciate condition with K factor values near to 1. This could be due to abundance of food resources and to the water quality. K values are similar to those observed in Hamiz dam, Algeria (Ould Rouis et al., 2012), but are slightly higher and lower than those reported in K'sob dam (M'sila, Algeria) and Beni Haroun dam (Mimeche et al., 2013; Mouaissia et al., 2017), respectively.

The results showed that GSI and HSI changed inversely both in the males and females, and this reveals the mobilization of hepatic reserves in the reproduction effort, particularly during GSI rising when intense germ cells multiplication is observed (Lloret et al., 2002). Interestingly, K factor expressed a similar patter as GSI, indicating consequently that body stored energy is not involved in gametogenesis. However, K index decreased significantly during the spawning season revealing mobilization of corporal reserves during the mating effort. Mean size at first sexual maturity of *L.s callensis* was estimated as 18 cm in the males and 21 cm in the females. These values are lower to those reported in Hamiz dam where the mean

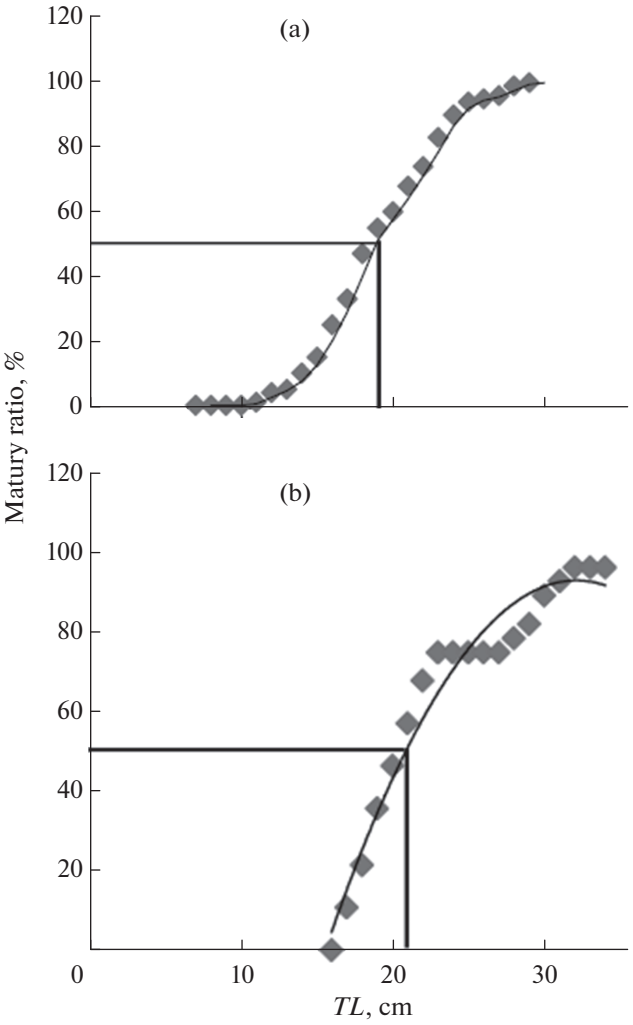

Fig. 2. Size at first maturity in *Luciobarbus callensis* ((a) males and (b) females) adjusted to a logistic model (n = 102 and 29 for the males and females, respectively). The horizontal line indicates the maturity ratio (%) and the vertical line indicate the total length (cm) of fish.

sizes at first sexual maturity are 19.6 and 27.7 cm in the males and females, respectively (Ould Rouis et al., 2012). However, the current values are higher than those observed in Morocco (15.4 cm for males and 16.8 for females) (Bouhbouh, 2002).

Sperm parameters varied significantly during the reproductive season as previously reported in common barbel *Barbus barbus* (Linnaeus, 1758) (Alavi et al., 2008). Ejaculate volume particularly, expressed visible variations with the highest values in May-June (2179 ± 208 μL) and the lowest values in the beginning and the end of the reproductive season (589 ± 83 μL). This is previously reported in common barbel (Alavi et al., 2008) and common carp (*Cyprinus carpio*) (Christ et al., 1996; Cejko et al., 2018). On the basis of ejaculate volume, the intense reproduction activity in *L. callensis* appears to take place from May to June.

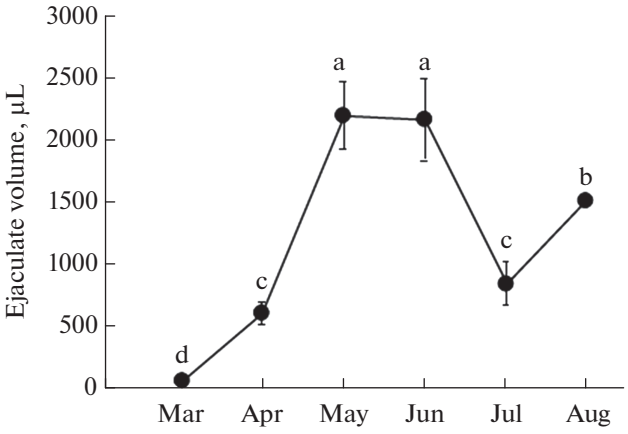

Fig. 3. Ejaculate volume in *Luciobarbus callensis* throughout the reproductive season. Values are expressed as mean ± standard error (n = 102). Bars with different superscripts indicate statistically significant differences. Values with different letters are statistically different at $p < 0.05$.

It has already been claimed that the spermatozoa concentration and duration of spermatozoa motility express a regular decrease from the beginning to the end of the reproductive season (Alavi et al., 2008). In the current results, such findings were not observed; however, two levels of gamete concentration were distinguished, a mean of $5.949 \times 10^9 \pm 2.852$ spermatozoa/ml from April to June, and a mean of $7.258 \pm 4.591 \times 10^9$ spermatozoa/ml from June to August. This is probably not related to spermatogenesis activity but driven by the reduction of ejaculate volume (Fig. 3). Sperm motility duration showed a regular decrease from the beginning to the end of the reproductive season. Such findings were previously reported in common carp (Christ et al., 1996) and Caspian roach (*Rutilus rutilus caspicus*) (Golpour et al., 2013). In freshwater fish, commonly the duration of sperm movement short (up to few minutes) (Alavi and Cosson, 2005a). In *L. callensis*, this duration was on average 86 ± 0.7 s (mean ± SD), values similar to those reported in common carp (Billard and Cosson, 1992; Billard et al., 1995) and Caspian roach (Golpour et al., 2013), with 80–90 s and 90 s, respectively.

When exploring, relationships between fish length and weight on one hand and sperm parameters on the other hand, a significant correlations were observed between ejaculate volume and fish length (r = + 0.48) and between ejaculate volume and fish weight (r = + 0.45). No significant correlations were observed regarding sperm concentration and motility duration (r = 0.05). In common barbel, Alavi et al. (2009) reported significant correlation between spermatozoa concentration and fish length and between spermatozoa concentration and fish weight.

In conclusion, the current results showed that spawning activity in *L. callensis* lasts from March to August with an intense activity in April and May. In

the males, the hepatic reserves are mobilized during the spermatogenesis activity, and body reserves are mostly exhausted during the mating activity.

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COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interests. The authors declare that they have no conflict of interest.

Statement on the welfare of animals. All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

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SPELL: 1. immotile, 2. coverslip