Reproductive Biology of *Oligosarcus argenteus* (Gunther, 1864) Adult Males and Description of the Gonadal Maturation Stages

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**ABSTRACT**

*Oligosarcus argenteus* belongs to the Acestrorhynchinae subfamily, being restricted to South America, and found in several Brazilian hydrographic basins, in lotic and lentic environments, where they are able to reproduce. With the purpose of studying the reproductive biology of the males from this species, many morphological parameters were analyzed during a 24 month period, as well as characterizing the different testicular maturation stages. A maturity scale, with three stages (I – Initial Maturing, II – Intermediate Maturing, III – Final Maturing) was proposed for the adult males of *Oligosarcus argenteus*. The reproductive period was established by the bimonthly frequency of spermatogenesis and by the gonadal maturation stages.

**Key words**: Reproductive cycle, histology, testis, *Oligosarcus argenteus*

**INTRODUCTION**

The preservation, distribution, and population abundance of teleost fishes have been well studied in the past (Schulz and Martins-Junior, 2001; Uieda and Uieda, 2001; Schifino et al., 2004; Siqueira-Souza and Freitas, 2004). The reproductive cycle and the gametogenesis are important parameters used in the understanding of the native fish species reproduction processes, and in the establishment of conservation programs (Bazzoli and Godinho, 1991; Vazzoler, 1996). The annual cyclic activity of teleosts is arranged in a variable number of stages, considering different morphological and physiological criteria. Many studies have been made with the testicular cycle of teleost fishes (Ferrari, 1981; Andrade and Godinho, 1983; Silva, 1987; Azevedo et al., 1988; Patzner et al., 1991; Fraile et al., 1992; Modesto and Canário, 2002; Chaves-Pozo et al., 2005; Cruz-Landim et al., 2005), but there are still some questions about the reproductive cycle of many other species. The bocarra (*Oligosarcus argenteus*) is the species currently found in many hydrographic Brazilian bays, in lotic and lentic environments, where they are able to reproduce (Souza and Andrade, 1984). It belongs to the Order Characiformes, subfamily Acestrorhynchinae, which is restricted to the South

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American continent (Menezes, 1972). This species shows high reproductive rates, being used in biological control of proliferous fish as tilapia (Souza and Andrade, 1984). In the past, many studies have been done about the production (Silva, 1990), nutrition, ecology, reproductive cycle (Santos, 1993; Andrade et al., 1995, Santos et al., 1995), morphological (Matta et al., 1994), and histochemical characterization (Neves et al., 1991, 1995, 1996) of *O. argenteus*. The aim of this study was to describe the reproductive cycle and the testicular morphology of *O. argenteus*, giving additional information that could be used in later phylogenetic studies. These data aimed at improving the knowledge concerning the reproductive biology of the males in the Characiformes order.

**MATERIALS AND METHODS**

Male specimens of *O. argenteus* were monthly sampled during two years (from October 1990 to September 1992) from the water tanks of the Pisciculture and Hydrobiology Station of the Federal University of Viçosa, Minas Gerais, Brazil (20° 45’ S e 42° 51’ W). From an initial lot of 500 animals, eight specimens were collected per month and transported alive to the laboratory, and then denervated by spinal section. For each individual the total (TL) and the standard (SL) body length, (in centimeters), body weight (BW) (in grams), and the gonadal maturation stages, estimated by macroscopical observation of the gonadal volume and color were measured. The dissected gonads were weighed, fixed in Bouin liquid, and embedded in paraffin. The 5 µm slides were stained with hematoxilin and eosin. The PSP (Picrosirius-polarization) and Gomori reticulin techniques were used for the characterization of the distribution of collagen I and III in the testis. Three stages of gonadal maturation for the testicular cycle study, according to the frequency of testicular components: I – Initial Maturation, II – Intermediate Maturation and III – Final Maturation were considered. The bimonthly mean values of the Gonadosomatic Index (GSI) and of the testicular elements frequency were used to establish the maturation curve. The GSI was obtained dividing the gonadal weight by the body weight and multiplying the result by 100. The volumetric proportions of the testicular elements were calculated using a Zeiss KPL 10 ocular lens that had a Gahm grid (Sala et al., 1982). This grid was constituted by 5 lines and 25 points. For each animal, 20 histological fields were examined, which were analyzed horizontally. The incidence of points that were situated over primary and secondary spermatogonia, primary and secondary spermatocytes, spermatids, spermatooza, interstice and tubular wall, Sertoli cells, lumen and Leydig cells were considered.

**RESULTS**

The testes of *O. argenteus* were paired, elongated, laterally flattened organs (Fig 1A), and were accommodated inside the body cavity, forming pronounced folds, one on the mean third and the other in the caudal third of the body. They showed similar lengths, and were separated along their trajectory and met next to the urogenital papilla forming the spermatic duct. The testicular surface was smooth, and the color and volume varied in accordance with the stage of gonadal maturation. The testis coloration varied from white to yellow in the majority of the animals collected in colder and dryer months and during the rainy and hot periods they showed a white-milky color. The testes were formed by the wound and anastomosed seminiferous tubules, which presented variable shapes and volumes. There was a connective tunica, the albuginea, with plenty of collagen (Fig. 1C) surrounding the testis, of which septs were originated responsible for the testis internal division in incomplete lobules. These lobules were responsible for the seminiferous tubules sustentation. The septs formed the interstice and were composed of a smooth connective tissue, with an abundant net of blood vessels, Leydig cells, and reticular fibers. (Fig 1B and C). Spermatogenic cell cysts, which were surrounded by Sertoli cells, formed the seminiferous tubule wall, and in each cyst, the germ cells were in the same maturation stage. The biometric measurements were made along the bimesters, presenting the following variations: total length (7.63-11.98cm), standard length (6.22-10.19cm), body weight (4.21-18.32g), gonadal weight (0.07-0.378g). The highest GSI mean value was observed between August/September 1992 and the lowest between February/March 1991 (Table 1).
Table 1 - Morphometric data bimonthly grouped for adult males of *O. argenteus*. TL = total length, SL = standard length, BW = body weight, GW = gonadal weight, and GSI = gonadosomatic index (mean ± standard error).

| Month/year | TL (cm) | SL (cm) | BW (g)   | GW (g)   | GSI (%)  |
|------------|---------|---------|----------|----------|----------|
| Oct/Nov –  | 10.47 ± 0.757 | 8.93 ± 0.724 | 10.42 ± 1.99 | 0.153 ± 0.032 | 1.468 ± 0.35 |
| Dec/Jan –  | 9.99 ± 1.2 | 8.44 ± 0.878 | 10.95 ± 2.869 | 0.152 ± 0.051 | 1.388 ± 0.65 |
| Feb/Mar –  | 10.55 ± 0.507 | 9.21 ± 0.675 | 12.74 ± 1.19 | 0.123 ± 0.002 | 0.965 ± 0.10 |
| Apr/May –  | 11.46 ± 1.24 | 9.94 ± 1.01 | 17.51 ± 4.72 | 0.184 ± 0.066 | 1.051 ± 0.13 |
| Jun/Jul –  | 11.58 ± 0.841 | 9.70 ± 0.694 | 16.22 ± 3.038 | 0.225 ± 0.073 | 1.387 ± 0.32 |
| Aug/Sep –  | 11.95 ± 2.06 | 10.09 ± 1.47 | 16.25 ± 6.415 | 0.312 ± 0.156 | 1.920 ± 0.59 |
| Oct/Nov –  | 11.61 ± 1.61 | 9.84 ± 2.195 | 15.71 ± 6.258 | 0.268 ± 0.079 | 1.706 ± 0.70 |
| Dec/Jan –  | 11.98 ± 0.817 | 10.19 ± 0.804 | 18.32 ± 2.682 | 0.378 ± 0.117 | 2.063 ± 0.72 |
| Feb/Mar –  | 7.63 ± 0.59 | 6.22 ± 0.536 | 4.21 ± 0.492 | 0.0713 ± 0.025 | 1.694 ± 0.59 |
| Apr/May –  | 9.00 ± 0.623 | 7.48 ± 0.526 | 7.76 ± 1.194 | 0.168 ± 0.041 | 2.165 ± 0.50 |
| Jun/Jul –  | 9.53 ± 0.99 | 8.05 ± 0.899 | 9.1 ± 2.403 | 0.147 ± 0.059 | 1.615 ± 0.51 |
| Aug/Sep –  | 9.29 ± 0.811 | 7.87 ± 0.727 | 9.81 ± 1.873 | 0.218 ± 0.061 | 2.222 ± 0.56 |

Testicular Maturation Stages

**Stage I - Initial**

During this phase, the testes were thin and clear, becoming pale and larger during their development. The seminiferous wall was thick, mainly formed by the primary spermatogonia cysts (Fig 1D). These were the biggest spermatogenic cells, presenting less condensed chromatin, an evident nucleolus and they were isolated within the cyst.

![Figure 1](image1.png)

**Figure 1** - **A.** Lateral view of *O. argenteus* adult male, showing the testis inside the body cavity (arrow). **B-D.** Transversal sections of seminiferous tubules. **B.** General testes organization, showing the interstitial tissue (ti), and the seminiferous tubules (arrows). **C.** Reticular fibers in the intertubule tissue (arrow heads). **D.** First testicular maturation stage, where we see the seminiferous tubule wall constituted by primary spermatogonia cysts (arrows). ti = intertubule tissue. Bars: A = 1cm; B–D = 21µm
A variable number of secondary spermatogonia were observed inside the cysts (Fig. 2A), occurring together with the primary and secondary spermatocytes cysts and with spermatid cysts. Often empty tubules, adjacent to tubules full of spermatozoa were observed (Fig. 2B). In this stage, especially in the spermatogonia cysts, the presence of Sertoli cells was easily observed, which presented a triangular nucleus with an evident nucleolus (Fig. 2C). The GSI value was 1.87% (Table 2), and the mean spermatozoa volumetric proportion was 3.23%, against 10.18% for the spermatogonia, 27.24% for the primary spermatocytes, and 21.93% for the secondary spermatocytes/spermatids (Table 3).

Figure 2 - Light microscopy of *O. argenteus* testes in different maturation stages. The figures A-C shows the Stage I of testicular maturation. A. We note a variable number of secondary spermatogonia (sg) inside the cysts. In Fig. B empty seminiferous tubules (TS) were observed, as well as those with few spermatozoa (EZ) inside the lumen (L). C. Sertoli cells (S) and round spermatids cysts (C). The Fig. D shows the Stage II of maturation, with tubules presenting thinner walls and a great spermatozoon mass. In Fig E we see the Stage III of maturation, showing lumens full of spermatozoa (EZ) and tubular anastomoses (arrow). Bars: A and C = 15µm; B, D and E = 45 µm.

Table 2 - GSI (gonadosomatic indexes) per reproductive stage (RE), and number (n) of sampled adult males of *O. argenteus* (mean ± standard error).

| RE | n | GSI |
|----|---|-----|
| 1  | 93 | 1.87 ± 0.58 |
| 2  | 39 | 1.66 ± 0.61 |
| 3  | 63 | 1.67 ± 0.74 |

Table 3 – Volumetric proportions (%) of spermatogenetic cells per reproductive stages (RE) of males *O. argenteus*. SPTG = spermatogonias, SPTC I = primary spermatocyte, SPTC II/SPTD = secondary spermatocyte/spermatid, SPZ = spermatozoa (mean ± standard error).

| RE | SPTG       | SPTC I     | SPTC II/SPTD | SPZ       |
|----|------------|------------|--------------|-----------|
| 1  | 10.18 ± 4.06 | 27.24 ± 8.26 | 21.93 ± 7.91 | 3.23 ± 2.27 |
| 2  | 5.12 ± 2.08 | 20.34 ± 7.14 | 21.96 ± 6.63 | 19.63 ± 13.88 |
| 3  | 5.32 ± 2.13 | 15.81 ± 5.61 | 17.33 ± 5.08 | 36.88 ± 8.30 |
Stage II – Intermediate Maturation
The gonads became more voluminous and opaque, and presented a white-pale color. The spermatogenic activity increased and a large number of primary and secondary spermatocytes cysts and spermatids cysts were observed. The seminiferous tubules walls were thinner than in the Stage I, presenting spermatozoa accumulation (Fig. 2D).

During this stage, the GSI reached 1.66% (Table 2) and the mean spermatozoa volumetric proportion was 19.63%, 5.12% for spermatogonia, 20.34% for primary spermatocytes, and 21.96% for secondary spermatocytes/spermatids (Table 3).

Stage III – Final Maturation
In this phase, the gonads were opaque and pale, and there was a maximum of spermatozoa production. The seminiferous tubules walls tore, promoting intense anastomoses between them and causing large spermatozoa accumulation. (Fig. 2E). Within the seminiferous tubule walls, the cysts of all the spermatogenesis cell phases were observed. The GSI reached 1.67% (Table 2) and the mean spermatozoa volumetric proportion occupied by these cells was higher, reaching 36.88%, against 5.32% of spermatogonia, 15.81% of primary spermatocytes and 17.33% of secondary spermatocytes/spermatids (Table 3).

Considering the spermatogenic cell volumetric proportions and the gonadal maturation stages the reproductive period of O. argenteus occurred between October and March (Table 4).

| Month/Year    | SPTG | SPTC I | SPTC II/SPTD | SPZ |
|---------------|------|--------|--------------|-----|
| Oct/Nov – 1990 | 9.2  | 15.3   | 23.2         | 26.0|
| Dec/Jan – 1991 | 6.2  | 12.5   | 18.3         | 34.9|
| Feb/Mar – 1991 | 6.5  | 17.9   | 19.6         | 29.4|
| Apr/May – 1991 | 8.7  | 14.3   | 21.1         | 28.3|
| Jun/Jul – 1991 | 8.3  | 24.0   | 24.6         | 12.1|
| Aug/Sep – 1991 | 10.8 | 21.1   | 30.8         | 2.5 |
| Oct/Nov - 1991 | 4.9  | 12.9   | 26.4         | 24.2|
| Dec/Jan – 1992 | 3.6  | 20.3   | 17.5         | 25.2|
| Feb/Mar – 1992 | 4.4  | 22.4   | 14.8         | 35.4|
| Apr/May – 1992 | 9.8  | 28.9   | 17.7         | 2.3 |
| Jun/Jul – 1992 | 7.9  | 32.4   | 13.7         | 4.8 |
| Aug/Sep – 1992 | 11.9 | 29.5   | 19.0         | 6.2 |

DISCUSSION
The testicular morphology of O. argenteus was similar to the one described for the majority of the teleosts (Andrade, 1980; Ferrari, 1981; Bazzoli, 1985; Silva, 1987; Andrade, 1990; Cruz and Santos, 2004; Hojo et al., 2004, Santos et al., 2004), with no accessory organs as occurs in other fish species (Chacon and Mendes-Filho, 1972; Van den Hurk et al., 1987; Patzner, 1989; Lahnsteiner et al., 1993; Lau and Sadovy, 2001). Grier et al. (1980) described two testicular patterns based on the spermatogonia distribution along the seminiferous tubules. In Atheriniformes, the spermatogonia are restricted to the distal portion of the seminiferous tubules. However, in Salmoniforms, Perciforms and Cypriniforms, these cells are distributed along all the tubular structure. This last type, called spermatogonial unrestricted is also found in O. argenteus. Apparently the presence of spermatogonia inside the seminiferous tubules throughout the year acted as a spermatogenic cell reposition source, as suggested by other authors (Ferrari, 1981; Andrade and Godinho, 1983; Bazzoli, 1985; Tavares, 1986; Azevedo et al., 1988; Buxton 1990; Ferreira and Godinho, 1990; Pecio and Rafinski, 1994; Burns et al. 1995). The spermatogenic cells frequently
indicated that the reproductive period of *O. argenteus* extended from October to March, as occurred with several tropical fish species in the southern hemisphere. Apparently the males of this species are able to reproduce at any time of the year, because of the presence of spermatogonia and spermatozoa inside the seminiferous tubules during this period. However, the reproductive phase can be associated with the female ovarian maturation period, according to Santos et al. (1995).

A spermatic duct surrounded by Sertoli cells, beginning in the mean third of the gonad and advancing until the final third was observed, as described by Andrade (1980) for *Leporinus silvestrii*. The Gonadosomatic Index (GSI) is an index used for the reproductive period determination in a large amount of animal species, including the fishes. The variation of their values is directly related to the periods of spermatozoa production, extrusion and absorption and provides data for the reproductive effort of the species (Le Cren, 1951; Mazzoni et al., 2002).

Barbieri (1981) and De Vlaming et al. (1982) accepted that the GSI should be the best reproductive period indicator, presenting peaks in specific months. In *O. argenteus* this index was not a good indicator because it did not present variation during the sampled months. This could be explained by the resource availability, wide photoperiod, and high temperatures along the year (Mazzoni et al., 2002). Nevertheless, other teleost species as *Characidium* sp. (Mazzoni et al., 2002) and *Moenkhausia intermedia* (Hojo et al., 2004) presented indexes that varied in accordance with the reproductive cycle period, with higher values during the hottest months.

The utilization of spermatogenic cell morphometry as a reproductive stage indicator allowed to determine the three stages of the *O. argenteus* reproductive cycle. These results differed from other authors who found four or five stages (Modesto and Canário, 2002; Cruz-Landim et al., 2005). Results didn’t show a gonadal rest period and consequently a gonadal recrudescence period, as found in other teleost species (Chaves-Pozo et al., 2005).

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