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REPRODUCTIVE CYCLE OF MALE MATRINXÃ, *Brycon cephalus* (GÜNTHER, 1869) (TELEOSTEI: CHARACIDAE)

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ABSTRACT

The matrinxã (*Brycon cephalus*) is an abundant and economically important fish found in the Amazon river basin in Brazil. The seasonal morphological changes in the testes of this fish were studied. Captive-bred specimens were obtained from a breeding center between March 1994 and February 1996. The testes were classified as being of the tubular, unrestricted spermatogonial type, in which four phases of germ cells (spermatogonia, spermatocytes (primary and secondary), spermatids and spermatozoa) were identified. Based on the macro - and microscopic analyses of testes, and on the gonadosomatic index, four stages of germ cell development (resting, maturation, mature and regression) were identified.

Key words: *Brycon cephalus*, fish, gonadosomatic index, reproductive cycle, testes

INTRODUCTION

The matrinxã, *Brycon cephalus*, inhabits the Amazonian river basin where it is important in commercial fishing. This species has been considered a promising candidate for aquaculture because of its rapid growth rate and high market value. Matrinxã is becoming increasingly popular with pay-to-fish outlets because of its sporting characteristics, the taste of its flesh, and the ease with which it can be reared in captivity [8]. However, the development of reliable methods for spawning and fingerling production depends on a knowledge of aspects such as the morphological changes that occur in the testes during the reproductive cycle. In this study, we examined the histological changes in the testes of *B. cephalus* during the annual reproductive cycle in an attempt to elaborate a maturation scale for the testes of purpose-bred males.

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MATERIAL AND METHODS

Twenty-three adult male matrinxã were captured every two months from March 1994 to February 1996, at the Centro de Pesquisa em Aquicultura do Vale do Ribeira (CEPAR - Ribeira Valley Aquaculture Research Center), which belongs to the Fishery Institute in the city of Pariquera-Açu, São Paulo state, Brazil. The fish were raised in four 200 m^2 tanks at an initial density of 1.0 fish/m^2, and were fed twice a day with a balanced diet containing 28% crude protein.

In the summer, the fish were exposed to 18 h of light per day. The monthly water temperature ranged from 17ºC to 31ºC. The average rainfall was 200 mm in the summer and 20 mm in the winter.

Body weight (g) and total length (cm) were carefully recorded for fish that were gutted for inspection and classification of the testes. Both testes were removed and weighed. The macroscopic characteristics used in evaluation included size, color, superficial blood irrigation and the absence or presence of semen release. Small pieces from the cephalic, middle and caudal regions of the testes were fixed in Bouin's solution for 6 h. Dehydration, clearing and embedding of the material were done using standard techiques. Serial sections (5 µm) were cut and stained with Heidenhain's iron hematoxylin and eosin.

The scale for grading gonadal maturation in male matrinxã was elaborated based on macroscopic observations and on the analysis of histological sections of the testes. The classification of the developmental stages of the germ cells was based mainly on the occurrence and/or modifications of the germ cells and tissue structures.

Testicular development was analyzed quantitatively using the individual gonadosomatic index (GSI), expressed as (gonadal weight ÷ body weight) x 100. The mean GSI of each stage was also calculated.

RESULTS

The testes of *B. cephalus* formad a pair of elongated, symmetrical structures lying on either side of the air bladder, ventral to the kidneys. These organs were free from each other in their anterior and middle parts, but were fused in the posterior region. The spermatic ducts left the testes at the posterior end and joined to form a common sperm duct that opens to the outside by a urogenital aperture.

The testes of *B. cephalus* showed marked seasonal variations in size, shape, volume, texture and vascularization, especially during the reproductive stages (Fig. 1). The weight of these gonads varied from 0.001 g to 1.53 g. No pigmentation was observed in the testes during the annual cycle.

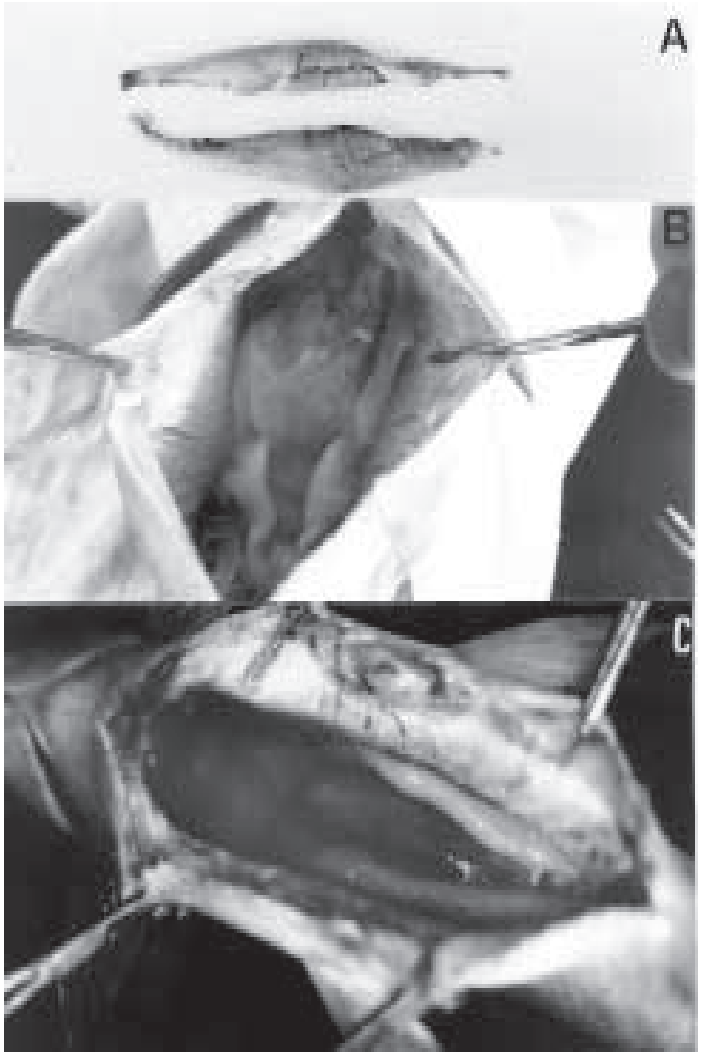

Figure 1. Testes of *B. cephalus* during maturation stage. **A -** resting phase, **B -** maturation phase, **C -** mature phase.

Structurally, the testes consisted of a large number of tubules bound externally by a peritoneal covering (Fig. 2 A,B). The seminiferous tubules, which were highly convoluted, varied in shape, size and arrangment, and contained spermatogonia, spermatocytes, spermatids, spermatozoa and cystic (Sertoli) cells. The germ cells within each cyst underwent synchronous development. The cystic cells varied in number and were distributed at the tubule periphery (intratubular compartment). The intertubular septum or stroma was formed by connective tissue containing fibrocytes, collagen fibers, myoid cells, and blood vessels. Interstitial Leydig cells were also present. Four stages of spermatogenesis were identified.

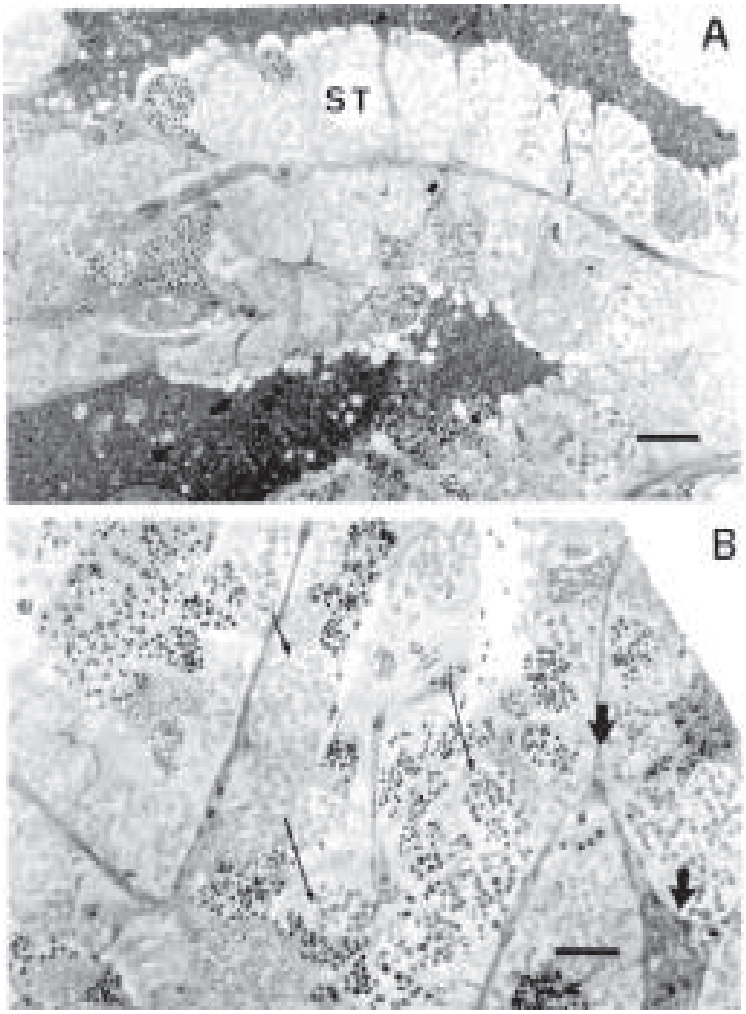

Figure 2. Sections through the testes of *B. cephalus* during maturation. **A** - Seminiferous tubules (ST) with germs cells in different stages of development. **B** - Seminiferous (thin arrows) and interstitial (thick arrows) components. Bar = 263 μm.

*Cell stage I - spermatogonia***:**

These large spherical cells occurred in all stages of the reproductive cycle. The nucleus was located at the center of the cell, and the chromatin threads radiated out from the nucleolus. The abundant cytoplasm had little affinity for

stains. The spermatogonia frequently occurred in isolation near the internal wall of the seminiferous tubules and were completely surrounded by the cytoplasm of cystic cells

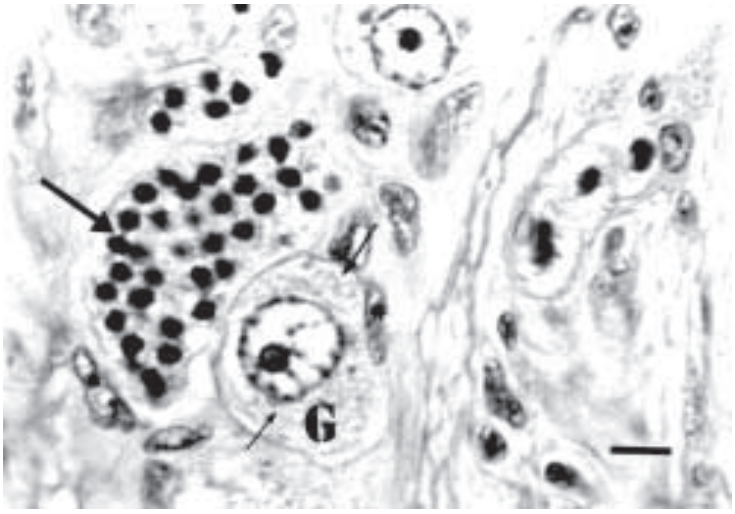

Figure 3. Section through the testes of *B. cephalus* in resting phase. G - spermatogonia (thin arrows), ∗ - cystic cells, thick arrows - spermatids. Bar = 26 μm.

which showed elongated or triangular nuclei (Fig. 3).

Cell stage II – primary and secondary spermatocytes

The primary spermatocytes were spherical, occurred in cysts, and were smaller than the spermatogonia. These cells originated from one spermatogonium, and were surrounded by a thin covering which formed a cyst. The nucleus was large compared to the size of the cytoplasm and showed granular chromatin. There was no nucleolus and the chromatin underwent the meiotic stages. The borders of the cytoplasm were not observed, and there was low affinity for the stains used (Fig. 4 A,B). Primary spermatocytes divided to produce smaller secondary spermatocytes with a shorter life span. The chromosomal material of the secondary spermatocytes was either pushed towards one pole of the nucleus to form a cup-shaped structure, or condensed further into a deeply stained mass with a small clear space at the center.

Cell stage III - spermatids

Secondary spermatocytes divided further to produce spermatids which are relatively small cells with extremely condensed chromatin and a barely visible cytoplasmic border (Fig. 3, 4B). The spermatids were characterized by structural changes, especially in the appearance of their nuclear content. Initially, the chromatin showed coarse granules distributed homogeneously within the cell. As maturation progressed, the chromatin condensed to form coarse clots and the cells became relatively smaller.

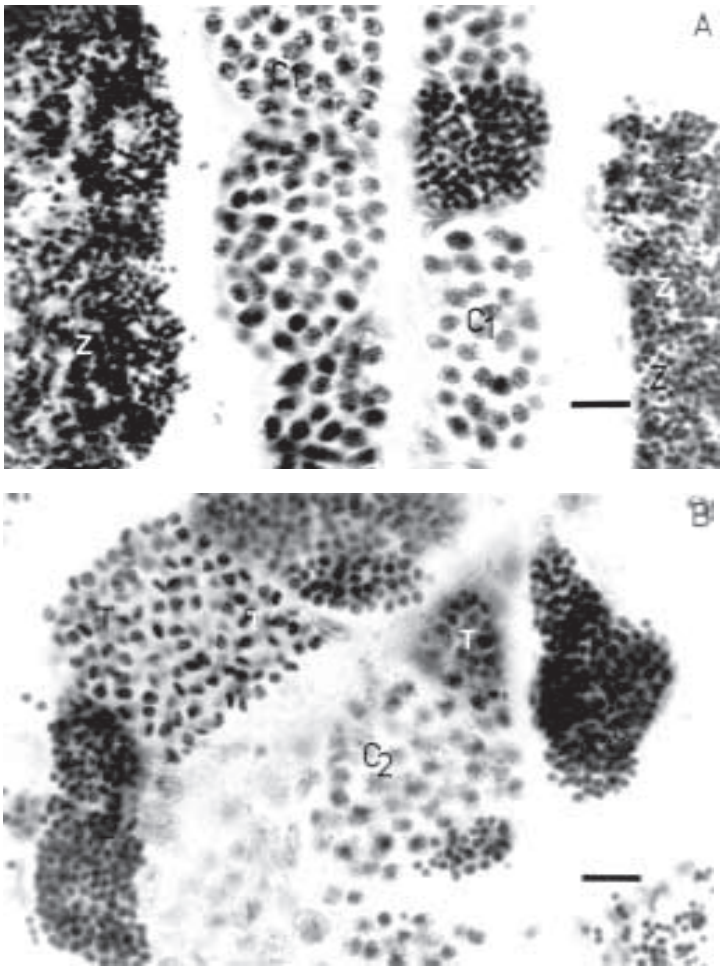

Figure 4. Sections through testes of *B. cephalus* in advanced maturation. **A** - Primary spermatocytes (C1) and spermatozoa (Z). **B** - Secondary spermatocytes (C2) and spermatids (T). Bar = 26 μm.

Cell stage IV - spermatozoa

The spermatids were ultimately transformed into spermatozoa, the smallest cells in the testes. The spermatozoa stained strongly and occurred in the lumen of seminiferous tubules. The head of the spermatozoon was a round structure that stained deeply with hematoxylin whereas the tail was not stained (Fig. 4A).

Cystic cells

These cells formed the somatic envelope of the sperm cells, and were distributed at the periphery of the cysts or around the spermatogonia. The cystic cells had an elongated nucleus with an irregular shape and a single nucleolus. The cell borders were difficult to distinguish (Fig. 3).

Interstitial or intertubular compartment

This triangular-shaped connective tissue compartment filled the space among three or four seminiferous tubules (Fig. 2B). Blood vessels, cells and fibers were present.

Based on macroscopic and microscopic observations of the testes during seasonal changes and on the gonadosomatic index (GSI), the annual reproductive cycle of male *B. cephalus* was divided into four stages.

Testis stage I - resting

At the beginning of the study (March 94), all the samples collected were in the resting stage or stage I (March to August). The total length of the fish was 35 cm. The testes, which were thin, slender and whitish in color, adhered to the body wall and extended to the anterior region of the abdominal cavity (Fig. 1A). Microscopically, the tubules were full of numerous spermatogonia (Fig. 3). The mean GSI at this stage was 0.019.

Testis stage II - maturation

The testes showed a more pronounced whitish coloration than in the previous stage. At the beginning of this stage (mid September), the testes were sharper, and by the end of this stage (November), had become thicker (Fig. 1B). Light abdominal pressure caused the fish to eject fluid and transparent semen. The seminiferous tubules were larger and contained cysts with spermatocytes. The cysts generally showed a higher number of secondary spermatocytes, spermatids and spermatozoa in the lumen of the tubules (Fig. 3, 4A). The mean GSI at this stage was 0.62.

Testis stage III – mature

In this stage, which lasted from November to the beginning of January, the testes showed an increase in volume and weight, and were more vascularized and opaque than in stage II (Fig. 1C). The sperm were released in a large quantity of transparent fluid after slight pressure to the abdomen. The testes showed intense spermatogenesis in this phase and spermatozoa filled all of the tubules. The highest mean GSI recorded was 1.90.

Testis stage IV - regression

This stage (end of January to February) was seen in the gonadal development of specimens not induced with hormones for reproduction. The spermatozoa that were not eliminated underwent reabsorption. The testes decreased in size and became similar to those in stage I and assumed a flaccid hemorrhagic aspect with blood clots. Light microscopy of the testes revealed total disorganization of the tubular structure, with a large number of dispersed spermatozoa. The reabsorption of germ cells was very fast. Primary spermatogonia were also observed. The mean GSI value at this stage was very low (0.02).

DISCUSSION

The testes in *B. cephalus* are paired organs that show seasonal variations in shape, volume and weight, depending on the degree of maturation. The same pattern has been reported in male *B. cephalus* from the Amazon basin [10]. The germ cell organization in *B. cephalus* testes corresponded to the "irrestricted spermatogonial testis" described by Grier [3] and Grier *et al.* [4]. According to these authors, this type of testis is typical of most teleosts [3] and was described for matrinxã by Romagosa *et al.* [6].

During the reproductive cycle of matrinxã, the spermatogonia are located outside the cysts. This situation is similar to that for *Oreochromis niloticus, Piaractus mesopotamicus* and *Hoplias malabaricus* [7]. The term "cystic cells" as used here is based on a previous recommendation for teleost fish [7]. These cells have an irregularly shaped nucleus, a prominent nucleolus and an elongated cytoplasm.

The morphological variation in the testicular activity of *B. cephalus* allowed division of the reproductive cycle into four stages, as in *Piaractus mesopotamicus* [7]. Thus, the spermatogonial proliferation characteristic of the resting stage occurred when the daylight hours increased. Spawning is probably associated with the maturation stage when the seminiferous tubules and the spermatic duct are packed with spermatozoa. During this period (November-January), the hours of daylight, water temperature and rainfall are maximal [6]

During the year, *B. cephalus* testes went through four stages of maturation, namely, resting, maturation, mature and regression. Intensification of the spermatogenic activity in the mature stage (end of October-November), increased the number of spermatocytes and spermatids. However, the nuclear volume decreased during spermiogenesis. This process has also been described in bluegill, *Lepomis macrochirus* [9]. In this stage, spermatozoa were first observed inside the cysts, and then in the lumen of the seminiferous tubules after the cysts had broken. Similar features were observed by Billard [2] in *Salmo gairdneri.* In captivity, the testes of matrinxã do not complete the maturation cycle, and the mature stage is followed by testicular regression, as defined by Andrade-Talmelli *et al.* [1] and Romagosa *et al*. [6].

The control of teleost spermatogenesis by external factors, particulary temperature and photoperiod, suggests that there may be marked variations among species [7]. The annual testicular cycle in *B. cephalus* is regulated by temperature and photoperiod. Matrinxã spermatozoa are classified as primitive [2, 5-7], and their morphological features correspond to those found in teleost fish with external fecundation.

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