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Reproductive biology and variations in the gonadal development of the fish Curimatã (*Prochilodus brevis* Steindachner, 1875) in captivity

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ABSTRACT: This study analyzed the reproductive biology of *Prochilodus brevis* held captivity during ontogenetic development in order to contribute to the conservation and improvements to induced breeding of this specie, which stands out for having commercial value attributed to the consumption of his "ova" and to be endemic in the Northeast. Sampling was fortnightly and 137 specimens were analyzed. Observations macroscopic and microscopic of the gonads allowed the identification of four stages of gonadal maturation: Immature, In Maturation, Mature and Regression. The age at maturity was predicted with a satisfactory degree of confidence, because the gonadal development was observed from fingerlings until adulthood. Hatching of fingerlings was accompanied at Estevão de Oliveira Fishery Station, in Caicó/RN/Brazil. Later the fingerlings were transferred to a tank in the Agricultural School of Jundiaí in Macaíba/RN/Brazil. Were verified juvenile males and females up to 225 days post-hatching (dph), in maturing stage from 247 dph, individuals with mature gonads from 274 dph and in regression stage from 359 dph for females and 410 dph for males, being corroborated by the variation in the curve of maturation based on the values of the Gonadosomatic Index. Thus, it was observed that even in captivity *P. brevis* reaches all stages of gonadal maturation, except for spawning, due to the absence of appropriate environmental conditions, since it is a species rheophilic. Therefore, it was found that the maturation of *P. brevis* occurs between 247 and 340 dph, corresponding to the period from October to January, similar to the natural environment.

Keywords: *Prochilodus*, gonadal ontogeny, gonadal histology, gonadosomatic index, aquaculture.

Biologia reprodutiva e variações no desenvolvimento gonadal da *Curimatã* (*Prochilodus brevis* Steindachner, 1875) em cativeiro

RESUMO: Este estudo analisou a biologia reprodutiva de *Prochilodus brevis* mantida em cativeiro durante o desenvolvimento ontogenético, a fim de contribuir com a conservação e o aprimoramento da reprodução induzida dessa espécie, a qual destaca-se por ter valor comercial atribuído ao consumo de sua "ova" e por ser endêmica da região Nordeste. A amostragem foi quinzenal, sendo analisados 137 exemplares. Observações macroscópicas e microscópicas das gônadas permitiu a identificação de quatro estágios de maturação gonadal: Imaturo, Em Maturação, Maduro e Regressão. A idade de maturação foi prevista com grau satisfatório de confiança, pois o desenvolvimento gonadal foi observado de alevinos até peixes na idade adulta. A eclosão dos alevinos foi acompanhada na Estação de Pesca Estevão de Oliveira, em Caicó/RN/Brasil. Posteriormente, os alevinos foram transferidos para um tanque na Escola Agrícola de Jundiaí, em Macaíba/RN/Brasil. Foram verificados machos e fêmeas juvenis até 225 dias após a eclosão (dae), em início de maturação com 247 dae, indivíduos com gônadas maduras a partir de 274 dae e em regressão a partir de 359 dae para fêmeas e 410 dae para machos, sendo corroborado pela curva de maturação com base nos valores do Índice Gônadossomático. Assim, observou-se que, mesmo em cativeiro, *P. brevis* atinge todos os estágios de maturação gonadal, com exceção da desova, devido ausência de condições ambientais adequadas, visto ser uma espécie reofílica. Portanto, constatou-se que a maturação gonadal de *P. brevis* ocorre entre 247 e 340 dae, correspondente ao período de outubro a janeiro, semelhante ao ambiente natural.

Palavras-chave: *Prochilodus*, ontogenia gonadal, histologia gonadal, índice gonadossomático, aquicultura.

1. Introduction

The worldwide decline of ocean fisheries stocks has provided impetus for rapid growth in fish and shellfish farming, or aquaculture. Between 1987 and 1997, global production of farmed fish and shellfish (collectively called 'fish') more than doubled in weight and value, as did its contribution to world fish supplies. Fish produced from farming activities currently accounts for over one-quarter of all fish directly consumed by humans. As the human population continues to expand beyond 6 billion, its reliance on farmed fish production as an important source of protein will also increase (ROSAMOND, 2000).

Fish belonging to the order Characiformes are currently distributed throughout the Neotropical region, with a high proportion of detritivorous fish species from the two families Prochilodontidae and Curimatidae (GURGEL et al., 2012). These families include stocks of important fish species which account for approximately fifty percent of the community biomass of some regions (BOWEN, 1983; FLECKER, 1996). Additionally, Maia *et al*. (1983) by analyzing the chemical composition of some species of the genus *Prochilodus* showed the low fat level and high protein level, and thus, highly important species for fish farming. Among the endemic freshwater fish,

potential for aquaculture, in Brazil the Prochilodontidae family is important, which *Prochilodus brevis, regionally known as curimatã, is part of* (SAINT-PAUL, 1986). It is a rheophilic fish which migrates during the rainy season for several kilometers to the headwaters of the river to spawn (CHELLAPPA et al., 2009). Although it has considerable economic importance in northeastern Brazil, its reproductive biology is scarcely known (ARAÚJO et al., 2003; GURGEL et al., 2012; NASCIMENTO et al., 2012).

The cultivating activity requires handling and creation measures that can be improved through studies about reproduction in fish. In this sense, the knowledge of reproductive tactics is fundamental for the comprehension of the cycle of life strategies as well as to guide measures of administration, handling and preservation in front of impacts, as the exhaustion of the natural resources (VAZZOLER, 1996). This way, the knowledge of the morpho-histological characteristics of the gonads´ structures is a basic and primordial stage for the comprehension of fish reproduction, especially when evaluated since the first stages of life.

Knowing about these reproductive characteristics helps to improve procedures which enable the gonadal maturation in captivity, so the induction of spawn can be performed (CONSTANTINOS et al., 2010). This becomes valid in front of reports about inadequate or insufficient results obtained with the reproduction induced with hormones, many times due to inadequate handling of the gametes in distinct stages of development, affecting their quality; the developmental stage of the gonads at the time the hormonal therapy is applied; the type of hormonal therapy; the possible stress induced by the manipulation necessary for the hormone administration and, in the case of artificial insemination, the latency period between hormonal stimulation and stripping for in vitro fertilization (CONSTANTINOS et al., 2010).

Therefore, this study aims to analyze the reproductive biology of *Prochilodus brevis* in captivity since hatching of the fingerlings, to identify the age of the fish in that each stage of gonadal maturation takes place. And thus contribute to the conservation and improvements in procedures for the maturation in captivity.

2. Material and methods

The fish larvae were obtained at Estevão de Oliveira Fishery Station, in Caicó/RN/Brazil. After ninety days of hatching of fingerlings, these were transferred to a tank measuring 9x3x1.5 meters, previously prepared with addition of land in the Agricultural School of Jundiaí in the city of Macaíba/RN/Brazil. The feeding, with industrial fish food, happened twice a day.

With the use of cast nets, with mesh 2 x 2 cm and length of 2.50 meters, were captured a total of 137 specimens of *P. brevis* since the early stages of life to adulthood. Sampling was fortnightlt, in the period of July 2010 to April 2011, between 8:00h am and 10:00h am.

Of each fish were registered data of total length and standard length, in centimeter, total weight (Wt) and weight of the gonads (Wg), in grams, sex and stage of gonadal maturation (VAZZOLER, 1996). The extraction of the gonads was performed through longitudinal abdominal incision, then fixed in Bouin's solution and submitted to histologic treatment by techniques of Hematoxylin-Eosin (MICHALANY, 1990).

Data analysis

The scale of gonadal maturation was based on macroscopic and microscopic characteristics (VAZZOLER, 1996) and average values of the Gonadosomatic Index (GSI). The age at maturity was predicted with a satisfactory degree of confidence, because the gonadal development was observed from fingerlings until adulthood.

The beginning of the reproductive activity was determined using the distribution of percentage frequency of the individuals by stages of gonadal maturation. The curve of gonadal maturation was established based on average values of the Gonadosomatic Index (GSI) with regard to the age in days post-hatching of fishes, according to the expression: GSI = Wg/Wt x 100, where Wg is gonad weight and Wt is total weight (WOOTTON et al., 1978).

For statistical analysis it was used the program Statistica 10.0 to verify, through the methods of analysis of variance (one-way ANOVA) and Fisher, the existence of significant differences, at the level of 5%, among the average values of the variables GSI , with regard to the stages of gonadal maturation and age, in days post-hatching, of the fishes.

3. Results

Prochilodus brevis did not exhibit any secondary sexual characteristics during the reproductive period, and hence no sexual dimorphism was observed. As such, it was possible to identify the sex of each individual only after extraction of the gonads performed through a longitudinal abdominal incision. The following macroscopic stages of gonadal maturation were identified for both sexes: immature, maturing, mature and regression (Figure 1).

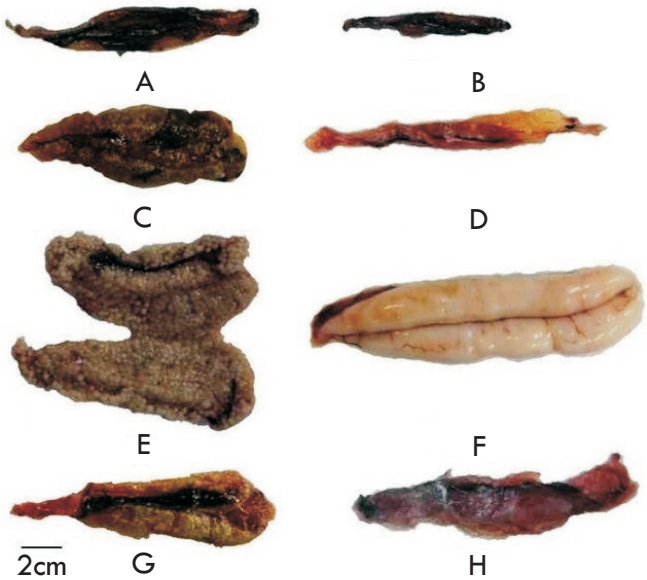

Figure 1. Female and male gonads of *Prochilodus brevis,* where (a) and (b) represent immature; (c) and (d) maturing; (e) and (f) mature; (g) and (h) regression. Scale bar= 2 cm.

Ovarian development

For *Prochilodus brevis* in captivity, the development of the ovaries was processed in four stages: Immature, In Maturation, Mature and Regression, described below:

Stage I: Immature - Immature ovaries are characterized macroscopically for showing translucent pink color, being flattened dorsoventrally and small, occupying a small portion of the abdominal cavity and showing little blood supply . Microscopically (Figure 2a), they reveal oocytes at stage I (chromatin-nucleoli) and at stage II (perinucleolar), with predominance of this last one, inserted in the ovigerous lamellae. At this stage are included females entering in gonadal maturation for the first time.

Stage II: In Maturation - The ovaries are bigger, of greyish-green color, with central blood vessel and intense peripheral vasculature. They occupy a greater portion of the abdominal cavity compared to the previous stage. Histological sections evidence oocytes at phases II, III, IV and V (Figure 2b), which abundance depends if the ovary is at phase of initial, intermediate or final maturation.

Stage III: Mature - Ovaries occupy the whole abdominal cavity, pressing the viscera and determining the animal´s distension of the abdomen. The color of the oocytes is light green. At this stage, ovaries are richly vascularized. Histological sections reveal total predomination of oocytes at phase V (Mature) and some oocytes at phase II (perinucleolar) (Figure 2c).

Stage IV: Regression - Ovaries with oocytes distributed randomly, white and pink. The vascularization becomes congested, showing evidences of the phase of follicular atresia. As the regression process advances, the amount of white and pink oocytes increases as well as the hemorrhagic aspect. Histologically, it is observed oocytes at the phases of initial (Figure 2d), intermediate and final atresia.

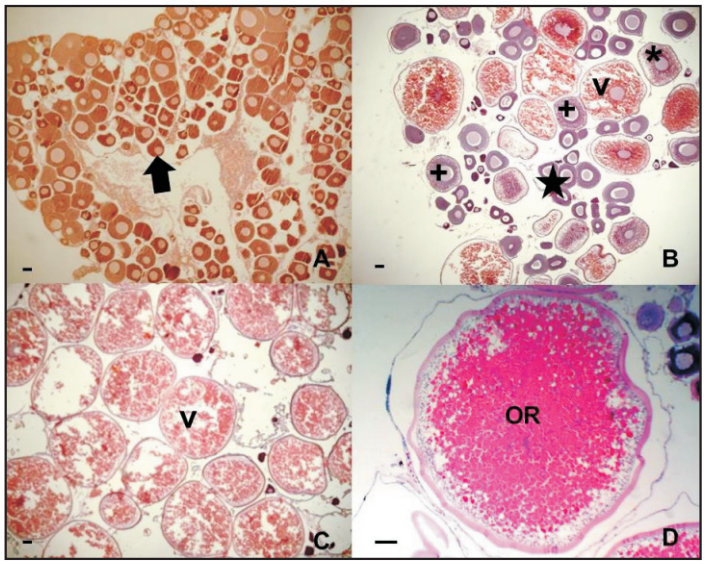

Figure 2. Ovarian tissue of *Prochilodus brevis* in different stages of maturation (A) Immature. (B) Maturation. (C) Mature. (D) Regression. (star) oocyte in perinucleolar phase (stage II); (+) oocyte in phase of formation of the vitelline vesicle (stage III); (*) oocyte in vitellogenic phase (stage IV); (V) mature oocyte (stage V); (OR) oocyte in regression. Coloration HE. Scale bar= 100µm.

Testicular development

For males, it was verified that the development of the testicles of *P. brevis* in captivity is processed at four stages: Immature, In Maturation, Mature, and Regression.

Stage I: Immature - Testicles are very thin, filiform and translucent macroscopically. When observed in light microscope, show abundant interstitial stroma, few spermatogonias distributed randomly and absence of apparent seminiferous tubules (Fig. 3a).

Stage II: In Maturation - Testicles are broader, assuming pinkish white color. In the light microscope, the seminiferous tubules show cysts of different phases of the spermatogenic lineage cells and lumen with low quantity of spermatozoids (Fig. 3b).

Stage III: Mature - Testicles reach their maximum development, with shape of tumescent strings and dense, with pink or milky white color, able to perform the spermiation. Histologically, they show high amount of spermatozoids occupying the lumen of the seminiferous tubules and spermatic duct (Fig. 3c).

Stage IV: Regression - The germinal epithelium, discontinuous, shows reminiscent vacuolated cysts and the interstice is thick. The tubular light appears, irregularly, with spermatozoids (Fig. 3d).

These results show that *P. brevis* in captivity reaches all stages of gonadal maturation naturally until the mature stage. In mature stage, oocytes in the final stages of development (V oocytes) initiate follicular atresia, interrupting spawning. However, as follicular atresia was not observed in oocytes reserve (II oocytes), then the next reproductive cycle occurs the same way.

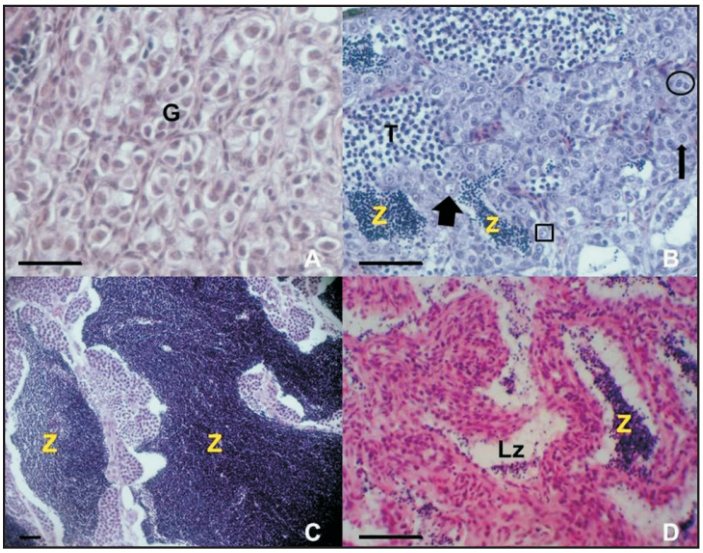

Figure 3. Testicle of *Prochilodus brevis* in different stages of maturation (A) Immature. (B) Maturation. (C) Mature. (D) Regression. (G) spermatogonias; (thick arrow) primary spermatogonia; (square) secondary spermatogonia; (thin arrow) primary spermatocyte; (circle) secondary spermatocytes; (T) spermatids (Z) spermatozoids; (Lz) Light of the seminiferous tubules. Coloration HE. Scale bar= 100µm.

Variation of the Gonadosomatic Index

The average values of gonad weight (Wg), gonadosomatic index (GSI), with the respective standard deviations (s.d.), for females and males are shown on Table 1.

Table 1. Average values of gonad weight (Wg), gonadosomatic Index (GSI), with the respective standard deviations, for females and males of *Prochilodus brevis*.

Stage	Females		Males	
	Wg (s.d.)	GSI (s.d.)	Wg (s.d.)	GSI (s.d.)
I-Immature	0.1257 g (0.0748 g)	0.7797 g (0.3568 g)	0.1583 g (0.1574 g)	1.0630 g (1.0547 g)
II-In Maturation	2.9601 g (1.2986 g)	13.0396 g (6.3061 g)	0.5343 g (0.4003 g)	2.0630 g (1.3557 g)
III-Mature	3.2551 g (2.0577 g)	14.1201 g (5.5916 g)	0.9004 g (0.4737 g)	3.3831 g (1.5289 g)
IV-Regression	2.1455 g (1.2530 g)	10.0963 g (4.819 g)	0.3000g (0.1681 g)	1.2641 g (0.6712 g)

Both for females and males, the highest values of gonadosomatic index (Figure 4) happened at the stages "in maturation" and "mature", significantly differing from the stages "immature" and "regression" (p= 0,000). The variation of the maturation curve based on the average values of the gonadosomatic index was consistent with the macroscopic and microscopic observations of the gonads.

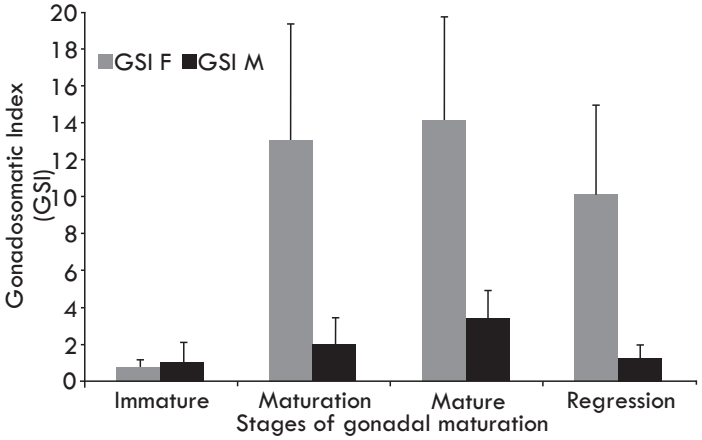

Figure 4. Gonadosomatic index (GSI) by stage of gonadal maturation of *Prochilodus brevis*, where (F) represent females and (M) males.

Stages of maturation

The distribution of average values of length and weight (Figure 5) by age in days post hatching (dph) of fishes showed higher averages in the period from 247 dph to 359 dph, befitting the period of maturation of the gonads (Figures 5, 6).

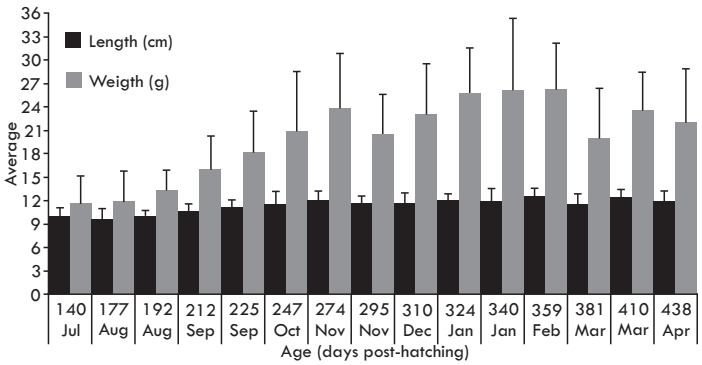

Figure 5. Average values of total length and total weight, with the respective standard deviations, by days post-hatching of *curimatã* fish, *Prochilodus brevis*.

The frequencies of the different stages of maturation on the sampled individuals differed between the sexes. For the females, was presented higher frequency of "in maturation" stage at 247 days post-hatching (dph), with presence of individuals in "mature" stage from 274 dph, reaching totality at 295 dph. The "regression" stage began from 359 dph (Figure 6).

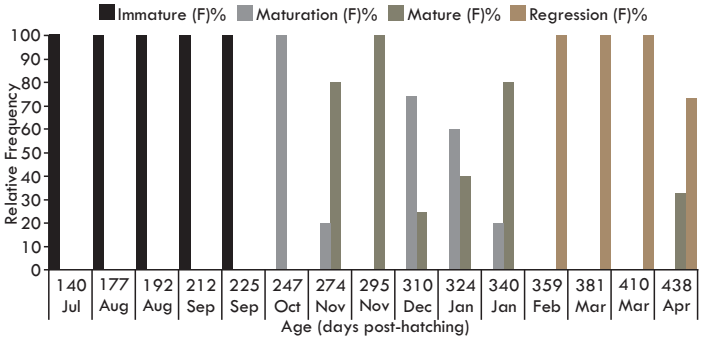

Figure 6. Relative frequency of the stages of gonadal maturation by days post-hatching of *curimatã* fish, *Prochilodus brevis*, for females.

For the males, "in maturation" stage began at 247 dph, and the appearance of mature individuals at 274 dph reaching the totality at 359 dph. About the "regression" stage, it was found from the age of 410 dph (Figure 7).

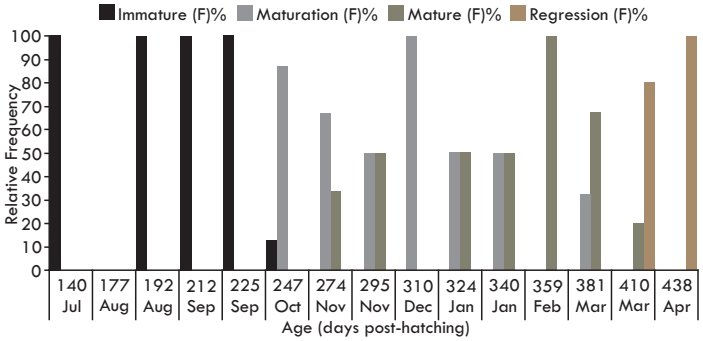

Figura 7. *Relative* frequency of the stages of gonadal maturation by days post-hatching of *curimatã* fish, *Prochilodus brevis*, for males.

The distribution of the average values of the Gonadosomatic Index by days post-hatching of the females (Figure 7) showed elevation from 247 dph, with maximum values at 340 dph. About the distribution for the males, it was noticed more elevated values between 324 and 359 dph (Figure 8).

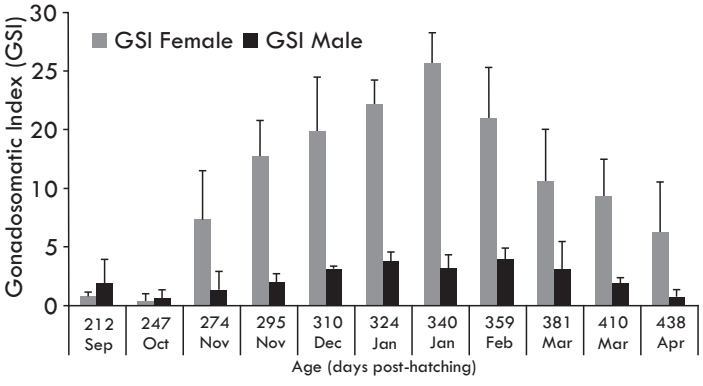

Figure 8. Distribution of the average levels of Gonadosomatic Index by days post-hatching of *curimatã* fish, *Prochilodus brevis*, for females and males.

4. Discussion

Structural changes along the reproductive cycle enable inferring about the functional state of the gonads.

The description of gonadal development, based on macro and microscopic observations, is being largely used in the determination of the reproductive period of the species (BARBOSA et al., 2012).

According to Bazzoli (2003), in Characiformes the morphological characteristics observed in the ovaries like volume, color and thickness suffer seasonal modifications, according to the stages of the reproductive cycle, just like it was verified for the gonads of *Prochilodus brevis*. The referred author affirms that the color of mature ovaries depends on the color of their vitelogenic oocytes. For the fishes of River São Francisco hydrographic basin are considered three predominant categories of colors: a) yellow, in Tetragonopterinae, Erythrinidae, Curimatidae, Doradidae, Pimelodidae and Loricarridae; b) gray, in Anostomidae and Prochilodontidae and, c) green, in Bryconinae, Salminae and *Pseudopimelodus*. In the present study, it was observed mature ovaries of greyish green color.

In most Characiformes, testicles are paired organs, stretched and fusiform, located dorsally-laterally to the swim bladder and coelomic cavity and dorsally to the digestive tube. They are attached to the swim bladder through the mesorchium (BAZZOLI, 2003).

The testicles of *curimatã* are paired organs, free in the anterior and middle portions, linked in the third posterior forming a unique spermatic duct; they have smooth external surface and suffer modifications in volume, shape and color during the gonadal development, like Nascimento et al. (2012) demonstrated, such observations corroborate the findings in the present study for *Prochilodus brevis*. The morphological variations observed in the testicles of *P. brevis*, during the reproductive process are related to the stages of gonadal maturation, being limited the changes in color (from transparent in immature phase to milky white at the reproductive period), shape, volume and vascularization.

According to Agostinho et al. (2003), *piracema* (Brazilian phenomenon of migration) fish, known as rheophilic, need to migrate at the reproductive period, to promote the final maturation of the gonads. According to the authors, the period of floods, the increase of the photoperiod, the amount of dissolved ions (conductivity) and the water´s temperature are the factors that induce the finalization of this process. Fishes which show this behavior only release their gametes under these starting signals; otherwise, the spawn does not happen even if the gonads are already developed. In this context, as *P. brevis* is a rheophilic species, it was observed the process of follicular atresia under captivity situation. According to Filho and Weingartner (2007), the fact of a species not performing reproduction in captivity, during the fattening phase, can be considered an advantage, because it enables that the energy supplied in food can be canalized to the growth of the body, instead of directed for the gonadal development and reproductive behavior.

The histologic analysis of ovaries of *P. brevis*, when kept in confinement, showed that the processes of follicular atresia, or oocyte degeneration, happen in specimens ready for the spawn that are not submitted to any hormonal treatment, suffering normal ovarian regression. In this study, it should be pointed out that the presence of the atretic follicle was observed only in mature ovaries, in the final stages of oocyte maturation (mature oocyte), never in previous phases of maturation, therefore differing from the observations performed by Rizzo and Bazzoli (1995), which in females of *Prochilodus affinis*, kept in tanks, the degenerative phenomena can happen at any stage of oocyte maturation.

According to Romagosa et al. (1985), the oocytes of *Piaractus mesopotamicus* at stage of ovarian regression are similar to the ones of *curimbatá*, when opaque and white oocytes are observed. In the present study were also identified opaque and white oocytes of *P. brevis* at the stage of ovarian regression.

In contrast to the regression process in captivity, the technique of "hipofization" is being widely used to induce reproduction in migratory fish through the application of natural hormones present in the hypophysis of mature fish. However, the regression period can affect the artificial reproduction of the species since degenerative processes in the ovaries reduce the rate of fertilization (RIZZO; BAZZOLI, 1995). Therefore, knowledge about the process of gonadal regression as well as the age at which the species begins the process of follicular atresia in captivity helps the new methods of artificial reproduction.

It is also possible to stimulate the reproduction of migratory fish through environmental induction, triggering the entire process in captivity by maintaining environmental conditions suitable to stimulate reproduction. However the complexity of the environmental mechanisms that control gonadal development and reproductive behavior makes it very difficult to simulate in a situation of captivity. Results that prove the success of the environmental induction for the final maturation and spawn of the Brazilian migratory fish are very rare (FILHO; WEINGARTNER, 2007).

In this study, the average values of the Gonadosomatic Index (GSI) were higher for female when compared to male´s values. These results indicate that the increase in volume of the ovaries during the maturation process happens in a more accentuated way than for the testicles, which is caused by the accumulation of yolk vesicles by those ones. The distribution about the stages of gonadal maturation showed the highest values for stages "in maturation" and "mature" and the lowest values for specimens in "regression", considering fishes in reproductive activity. Araújo et al. (2003) found similar results for *Prochilodus cearensis*. These results demonstrate that Gonadosomatic Index is good indicator of reproductive activity of fish.

From the results it was possible to evaluate that the *curimatãs* held in captivity showed the average values of body measurements (length and weight) consistent with the growth and maturation of gonads. This can be explained because during the maturation gonadal, the fish require larger body proportions to accommodate the gonads and thus ensure greater fecundity (VAZZOLER, 1996).

Mature individuals found from November to March (274 to 381 days post-hatching "dph") and at the stage of regression (410 dph) verified in February, March and April, match with the results found for the same specie at Itans weir (7° 30' 20" South, 38° 55' 48" East)/Brazil (ARAÚJO et al., 2003).

In the present study, females and males reached each stage of gonadal maturation at the same ages, but the males reached higher values for the frequencies of the stages "in maturation", "mature" and "regression" in later periods than the females. However, these data do not match the ones obtained through studies in natural environment with *Prochilodus brevis* (ARAÚJO et al., 2003; GURGEL et al., 2012). In such studies, it was noticed higher frequency of mature males in months previous to the period of higher frequency of mature females.

The increase in values of the Gonadosomatic Index (GSI) next to the period of spawn suggests that the gonads growth is proportional to the fish´s growth and that the changes observed in its percentage, in relation to the weight of the samples, are essentially originated from the seasonal variations related to the reproductive cycle (ROSCH, 2000). In this context, it was observed crescent values of GSI from 247 dph to 340 dph, corresponding from October to January, which was also observed by Boncompagni-Júnior et al. (2013) for females and males of *curimbatá* (*Prochilodus argenteus*). From 340 dph it is observed a decrease in the average values of GSI due to the stage of "regression" exhibit higher percentage in this stage of the fish´s development.

5. Conclusions

Given the activity of overfishing in the semiarid in the reproductive period of this specie, which is an important ecological component in South American rivers, the age of the fish in that each stage of gonadal maturation takes place is relevant due to the need for conservation of endemic species through the development of fish farming. So, the study provides important information, helping to improve procedures that enable the completion of gonadal maturation in captivity. The use of four methods (macroscopic appearance of gonad, GSI, histological examination and ontogenetic variations in the gonadal development) have proved useful in monitoring individual characteristics of the gonads over a complete reproductive cycle of *P. brevis* in captivity.

6. Acknowledgments

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