

EMBRYONIC AND LARVAL DEVELOPMENT OF THE “PIABANHA”, *Brycon insignis*, STEINDACHNER, 1876 (PISCES, CHARACIDAE)

[Desenvolvimento embrionário e larval da piabanha, *Brycon insignis*, Steindachner, 1876 (Pisces, Characidae)]

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ABSTRACT

This study presents preliminary observations on the embryonic and larval development until alevine stage of the “piabanha”, *Brycon insignis*. This is an indigenous and endemic species from Paraíba do Sul Basin, Brazil. The eggs were obtained after reproductive induction utilizing breeders kept in captivity, at “Estação Experimental de Paraibuna, São Paulo”, which belongs to CESP (Companhia Energética de São Paulo), during the period of January-February/96. Soon after artificial fertilization, the eggs were kept in incubators under constant temperature ($26 \pm 1^\circ\text{C}$). Samples were taken every 10 minutes during the first 3 hours, and after that, every 20 minutes, until hatching have occurred. After hatching, daily, observations took place until the attainment of the fingerling stage. The eggs presented coloration varying from dark to brownish green, rigid chorion and large perivitelline space. They were spherical, demersal, non-adhesive, and showed high hydration degree and their diameter varied from 3,750 to 4,100 μm . The stages of embryonic development observed and described began with cleavage, followed by morula, blastula, gastrula, neurula until hatching of non-pigmented larvae, which displayed total average length of $6.0 \text{ mm} \pm 0.22$, 14 hours after fertilization. On the second day of life, the larvae displayed total average length of $8.0 \text{ mm} \pm 0.21$, pectoral fins already formed, open mouth and intense cannibalism. On the fifth day of life, all fins were formed and the individuals were very similar to adult. On the 15th day of life, the alevine presented total length of $3.4 \text{ cm} \pm 0.39$.

Key words: embryonic development, larval development; *Brycon insignis*, fish, Brazil

RESUMO

O presente trabalho apresenta observações preliminares sobre o desenvolvimento embrionário e larval da piabanha, *Brycon insignis*, até a fase de alevino. *Brycon insignis* é uma espécie nativa e endêmica da bacia do Rio Paraíba do Sul, Brasil. Durante os meses de janeiro-fevereiro/96, os ovos foram obtidos através de reprodução induzida, utilizando-se reprodutores mantidos em cativeiro, na Estação Experimental de Paraibuna, pertencente à CESP (Companhia Energética de São Paulo). Logo após a fertilização, os ovos foram mantidos em incubadoras sob temperatura constante ($26 \pm 1^\circ\text{C}$). Amostras foram retiradas a cada 10 minutos durante as três primeiras horas e, depois, a cada 20 minutos, até a eclosão das larvas. Após a eclosão as observações foram feitas diariamente até a fase de alevino. Os ovos são esféricos e não adesivos, apresentando coloração variando do verde escuro ao verde acastanhado, córion rígido, grande espaço perivitelínico, elevado grau de hidratação e diâmetro variando entre 3750 e 4100 μm . As fases do desenvolvimento embrionário observadas tiveram início com a clivagem seguida pela mórula, blástula, gástrula e nêurula até a eclosão de uma larva despigmentada, medindo em média $6,0 \text{ mm} \pm 0,22$ de comprimento total, 14 horas após a fertilização. No segundo dia de vida, a larva apresentava, em média, $8,0 \text{ mm} \pm 0,21$ de comprimento total, nadadeiras peitorais formadas, boca aberta e intenso canibalismo. No 5º dia de vida, todas as nadadeiras estavam formadas e o indivíduo se assemelhava ao adulto. No 15º dia de vida, os alevinos apresentavam comprimento total médio de $3,4 \text{ cm} \pm 0,39$.

Palavras-chave: desenvolvimento embrionário, desenvolvimento larval, *Brycon insignis*, Brasil

Introduction

Species of *Brycon* genus have been the subject of interest in several research centers in Brazil, because they present fast growth and good resistance to handling and accept well artificial food (LOPES; SENHORINI; SOARES, 1995).

“Piabanha”, *Brycon insignis*, is a teleost species which belongs to Characidae family, Bryconinae sub-family, being indigenous and endemic from Paraíba do Sul River, Paraíba Valley region, São Paulo, Brazil (FOWLER, 1950). In the fifties, it was the 4th most captured species in commercial fishing, therefore displaying an important role in the region’s economy (MACHADO and ABREU, 1952). Currently, due to alterations in the natural environment caused by pollution, deforesting, and dam construction in the Paraíba River, the number the species has been reduced and so, they were considered practically extinct for fishing purposes.

Some aspects about induced reproduction of *Brycon insignis* have been reported by GIRARDI; FARIA; SANTOS (1993) and SALGADO *et al.* (1997). However, research with respect to biological behavior in captivity are still not completely developed. Therefore, this research aims to study the initial development of *Brycon insignis*, obtained in laboratory through hormonal induction and average lasting period of larval and embryonic and larval stages, until the 15th day of life.

Material and Methods

The experiment was conducted at “Seção de Hidrobiologia e Aquicultura, Estação Experimental de Paraibuna”, Brazil, which belongs to CESP (Companhia Energética de São Paulo), during the period of January-February/96. The breeders utilized were kept in 600 m² ponds, in a density of 1 fish/m², and fed with balanced diet containing 35% crude protein, twice a day. A total of 125 fishes, being 75 females and 50 males, were used. The females presenting swollen abdomen and red genital papilla, and males liberating a white milky semen with sperm motility equal to or higher than 80% were submitted to induced reproduction by means of hormone injections (hCG – human chorionic gonadotropin and crude carp pituitary extract). The eggs were obtained by extrusion and dry fertilization was carried out. Soon after hydration, the eggs were transferred to 250 L conic fiberglass incubators, with constant water renewal and flow rates of 50 L/min, and 26 ± 1° C temperature. Each incubator received 100 eggs/ L (eggs of a unique female in a 250 L incubator).

During the first three hours, egg samples were taken from the incubators at 10 minutes intervals and further on, at 20 minutes intervals until the larvae hatched. The samples were collected in Petri dishes, where they were examined under stereoscopic microscope, equipped with a micrometer in the eyepiece and micro-photographic camera. Initially, the eggs were measured, and, finally, alterations which occurred since the first cell division until the larvae hatching, were described. During 24 hours, egg and larvae development was continuously examined and the most important alterations were micro-photographed. A total of 200 eggs, 200 larvae and 50 alevins were measured. After hatching, the observations were made, daily, until the larvae reached alevin stage, that is, 15 days of age. The larvae remained in the incubators for 5 days. After mouth opening, newly-hatched larvae of “curimbatá” (*Prochilodus scrofa*) were offered as food. After the 5th day of life they were taken to a 200 m² pond, which had been previously prepared with Rotifera, Cladocera and Copepoda and enriched with “curimbatá” larvae.

Results and Discussion

In *Brycon insignis* during embryonic and larval development, until alevin stage, it was verified the sequence of events described in Table 1, divided in three stages: embryonic; larval and alevin.

After fertilization followed by hydration, the diameter varied between 3,750 and 4,100 µm. Authors, such as BERNARDINO *et al.* (1993) and ROMAGOSA *et al.* (1995), also observed high hydration in *Brycon cephalus* eggs. SATO *et al.* (1997) verified that hydration of *Brycon lundii* ovules promotes significant increase in the diameter after fertilization.

It seems to be a characteristic of the genus the occurrence of free and demersal eggs, with variation of the color among the various shades of green (*Brycon cephalus*: ROMAGOSA *et al.*, 1995; *Brycon lundii*: SATO *et al.*, 1997 and *Brycon cf erythropterus*: ECKMANN, 1984, and even gray (*Brycon cf reinhardti*). After fertilization *Brycon insignis* eggs are spherical, well hydrated, non-adhesive and present large perivitelline space. These characteristics are similar to those described for *Brycon cf. erythropterus* (ECKMANN, 1984); *Brycon cephalus* (LOPES; SENHORINI; SOARES, 1995); and *Brycon lundii* (SATO *et al.*, 1997). A large perivitelline space was described for some pelagic species (LAKE, 1967; MATSUURA, 1972; BALON, 1975). These authors believe that the large perivitelline space works

as a protection for the embryo against environmental damage and contributes to higher survival in lotic environments. We believe that the same happens to the eggs of *Brycon insignis*.

According to LAKE (1967), important ecological information originated from the knowledge of freshwater fish morphogenesis and ontogeny. The sequence of events that occur during embryonic development in *Brycon insignis* eggs, generally follows that of other fish species (GODOY, 1975; GODINHO; FENERICH; NARAHARA, 1978; TREJO and MARTINEZ, 1983; GASPAR, 1984; CUSSAC; MATKOVIC; MAGGESE, 1985; LOPES; SENHORINI; SOARES, 1995).

In *Brycon insignis* eggs, the presence of fat drops was never observed along the entire period of em-

bryonic development. On the other hand, LOPES; SENHORINI; SOARES (1995) observed the presence of a large drop of fat during embryonic development of *Brycon cephalus*.

MORAES and SCHUBART (1955) and GODOY (1975) reported that *Salminus maxillosus* eggs, during embryonic development, maintained the intensive green color observed since fertilization. According to LOPES; SENHORINI; SOARES (1995), other species such as *Prochilodus scrofa* and *Leporinus sp.* which have green eggs, soon after fertilization lose this color. As for *Brycon insignis* eggs, the dark or brownish green remained until the yolk sac had been entirely reabsorbed by the larvae. Similar observation was reported for *Brycon cephalus* eggs, in which the

Table 1. Development stages of piabanha, *Brycon insignis*

Stage	Phase	Time after fertilization	Description
Embryonic	Cleavage	40 min	-egg after the insemination (Figure 1A) - formation of perivitelline space, rigid chorion
		2 h	- cleavage stages followed, with successive cell divisions of 2, 4, 8, 16, 32 blastomeres (Figures 1B, 1C and 1D)
	Morula	2 h 30 min	- morula phase (Figure 1E) - multi-cellular layer expanding over the yolk surface, beginning with 1/3 epibolic followed by 2/3 (Figure 2A), until the closing of the blastopore forming the germination ring (end of gastrulation) (Figure 2B).
		4 h	- yolk acquires elongated shape showing the embryo shape (Figure 2C)
	Neurula	7 h	- differentiation between head and tail (Figure 2D)
		8h 30min – 9h 30min	- visualization of the first somites - appearance of the first optical vesicle (Figure 2E)
Embryo	Embryo	10-12 h	- reduction the yolk sac and the tail begins to separate (Figure 2E) - first tail movements, heartbeats with visible blood circulation, although blood is colorless (Figure 2E)
		13 h	- softening of chorion and accelerated embryo movements (Figure 2E)
	14 h	Hatching – non-pigmented body, draft of eye globe and cephalic mass, first swimming movements, average total length of 6.0 mm ± 0.22 (Figure 2F)	
	18 h	- larvae present pigmented eyes, traces of mouth and digestive tract; yolk keeps greenish color (Figure 3 A)	
	24 h	- quick reduction of yolk sac, presence of embryonic fin and horizontal swimming movements	
Larval	Larvae	38 h	- partially pigmented body, pectoral fins, traces of yolk sac, mouth opening, presence of tiny teeth in the maxillary and mandible; beginning of exogenous feeding (cannibalism) (Figure 3 C)
		94 h	- totally pigmented body, similar to adult, total length of 1.2 cm ± 0.24, formed fins, presence of scales and silver pigmentation (Figure 3 C)
Alevin		15 days	- total length of 3.4 cm ± 0.39 (Figure 3D)

olive-green color was kept until the embryo was formed (LOPES; SENHORINI; SOARES, 1995).

It is known that environmental factors act upon the embryonic development. Corroborates this KUO; SHEHADEH; NASH (1973) reported that water temperature and turbulence are important during egg incubation. TREJO and MARTINEZ (1983) observed that temperature can speed up or slow down larvae hatching. JOHNSTON and VIEIRA (1996) reported that for tropical fishes there is a direct correlation between water temperature and duration of the embryonic period. ECKMANN (1984), when following embryonic development of *Brycon cf erythropterus*, reported that hatching occurred approximately 16 hours after fertilization, at 26°C temperature. LOPES; SENHORINI; SOARES (1995) report, that for tropical species, such as *Piaractus*

mesopotamicus and *Colossoma macropomum*, that larvae hatching occurred from 12 to 14 hours after fertilization, at 28 to 30°C average temperature, but when water temperature was changed to 25-26°C, the embryonic development period lasted 16-18 hours. The same authors observed that at 30°C average temperature, the embryonic development of *Brycon cephalus* eggs lasted 10 hours and 30 minutes. For *Brycon insignis*, larvae hatched in 14 hours, at 26 ± 1°C average temperature. Similar results were obtained by OTERO (1988) for *Brycon moorei sunesis*. Therefore, it is possible to conclude that for species of *Brycon* genus the time needed for larvae hatching is shorter when compared to those of other tropical species, and this might indicate an advantage in the production process.

Figure 1. 1A – Egg of *Brycon insignis*, 40 minutes after fertilization (4.6 x); 1B, 1C and 1D – cleavage stage, 2, 4, 8, 16 and 32 blastomeres (3.6x) and 1E – morula phase (3.6x)

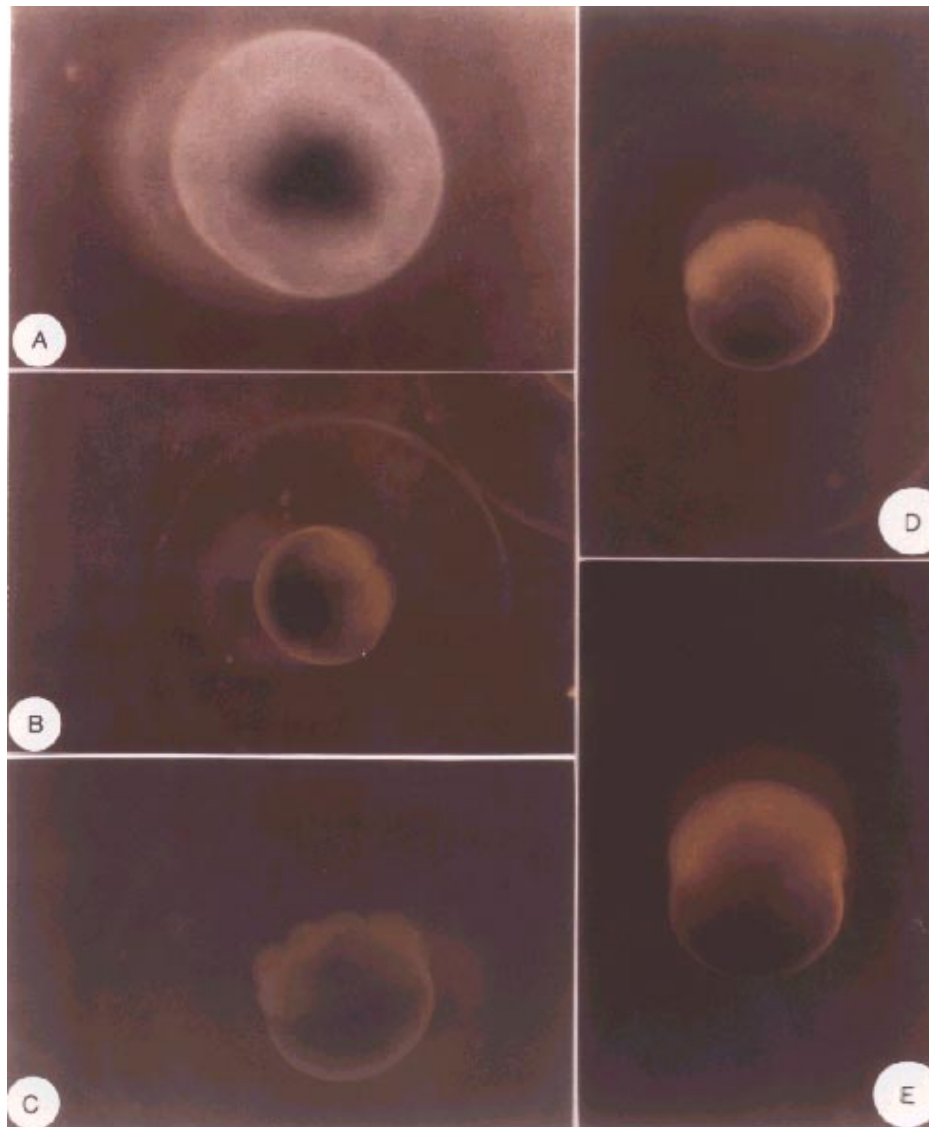
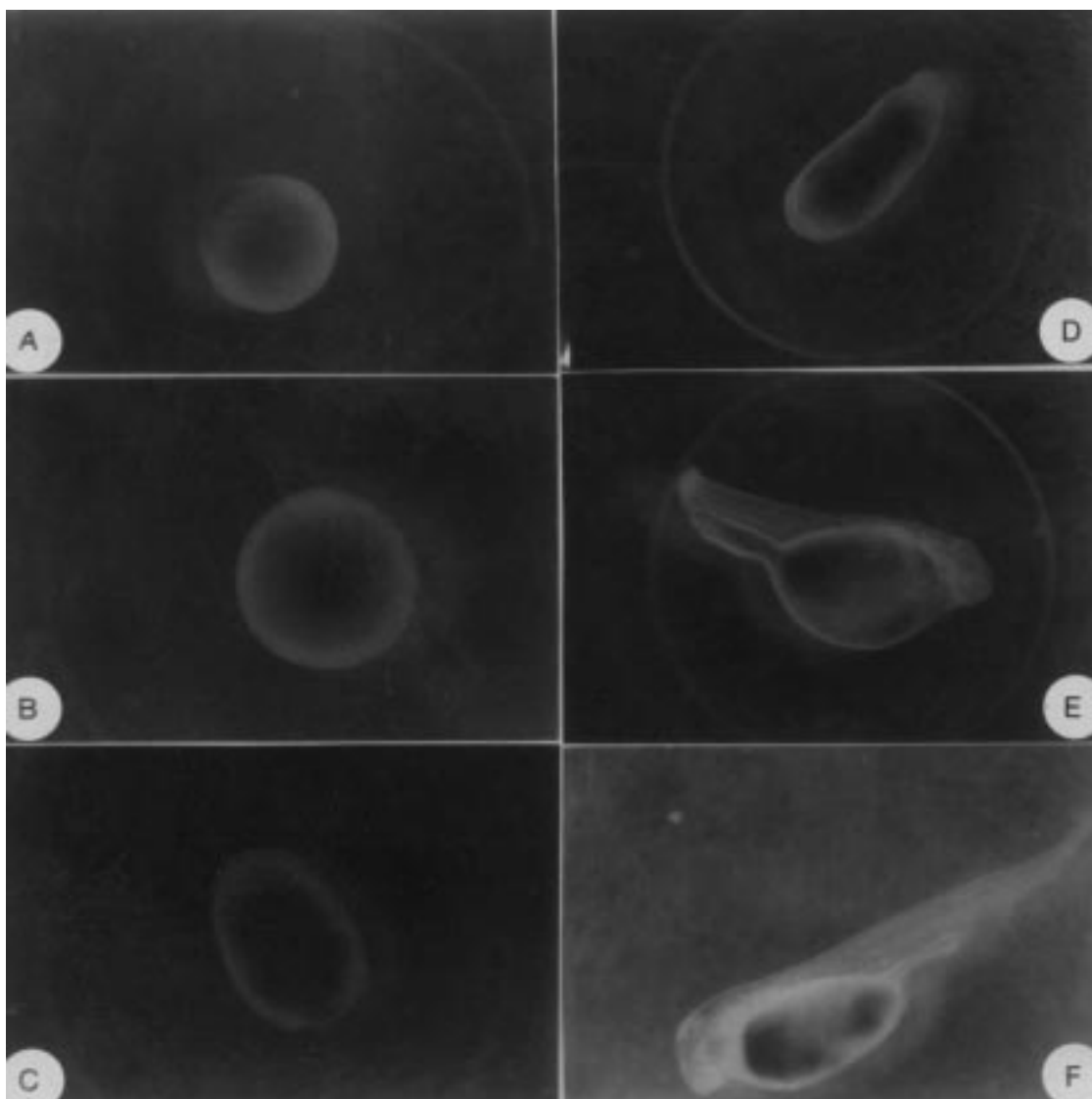


Figure 2. 2A – Egg of *Brycon insignis* showing multi-cellular layer expanding over the yolk surface (3.6 x); 2B – closing of the blastopore, germination ring (3.6 x); 2C – neurula phase (3.6x); 2D – embryo shape (4.6 x); 2E – yolk sac reduction and the tail begins to separate (4.6 x) 2F – just-hatched larvae with non pigmented body (4.6 x)



The larvae of *Brycon insignis* hatch when they have approximately $6.0 \text{ mm} \pm 0.22$ total length, in agreement with data obtained by ECKMANN (1984), that observed 5.0 to 6.0 mm total length newly hatched larvae of *Brycon cf. erythropterus*.

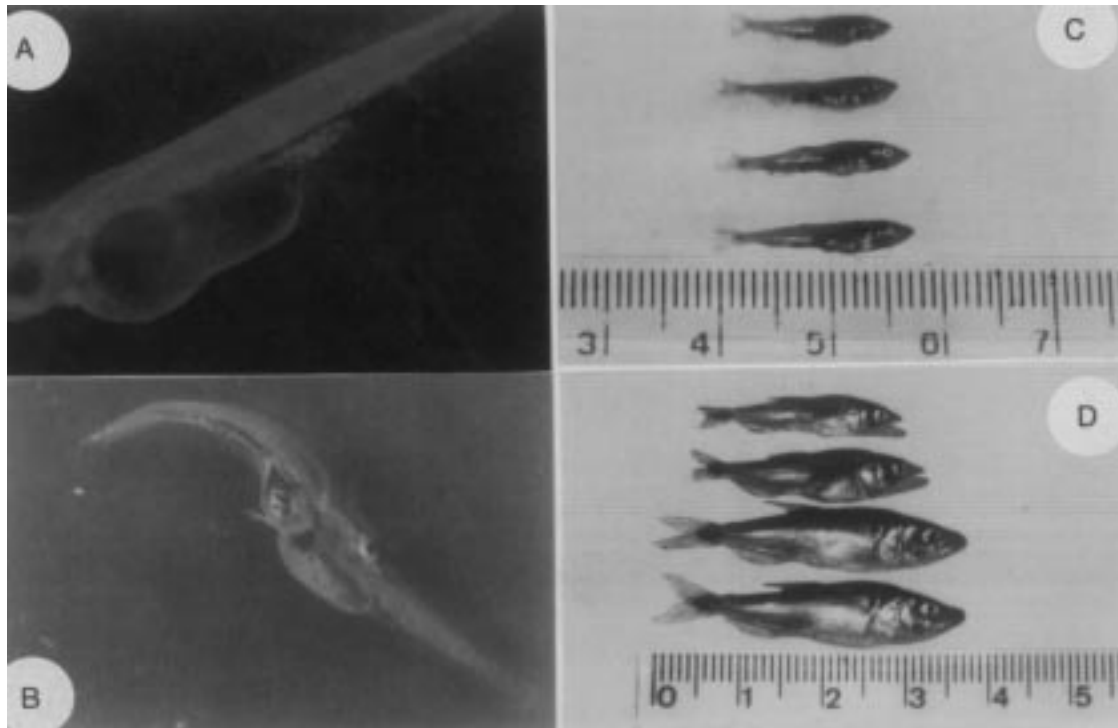
After hatching, *Brycon insignis* larvae absorbed the yolk sac quickly, and approximately 40 hours later (2nd day of life) they had the mouth opened and exhibited intense cannibalism. Similar results were observed for other species with carnivore habit during at least one period in their lives, such as *Salminus maxillosus* (GODOY, 1975); *Brycon orbignyanus* (BELMONT, 1994); *Brycon cephalus* (LOPES; SENHORINI; SOARES, 1995) and *Brycon lundii* (SATO *et al.*, 1997).

By their 5th free life day, *Brycon insignis* presented $1.2 \text{ cm} \pm 0.24$ average total length, similar to adults, and

by the 15th day, they presented $3.4 \text{ cm} \pm 0.39$ average total length. The survival rate for *Brycon insignis* was low ($\pm 2\%$), similar to that mentioned by SALGADO *et al.* (1997).

It is known that there is much to be learned in larviculture, mainly for species with this kind of feeding behavior. As it has been said previously, dam construction in the Paraíba River together with other factors caused changes in the aquatic environment, which by its turn reduced markedly the number of *Brycon insignis*. This species presents reproductive migration during certain period of the year, spawning completely during this period, producing free eggs which hatch quickly and are not assisted by the parents. Thus, fish species that complete their life cycle in stable environments, present better capacity to

Figure 3. 3A – Larval of *Brycon insignis* showing pigmented eyes and traces of mouth and digestive tract (4.6 x); 3B – larval present pectoral fins; mouth opening and intense cannibalism; – alevine present totally pigmented body, similar to adult, total length of 1.2 ± 0.24 cm; 3D – juvenile present 3.4 ± 0.19 cm average total length



adapt to conditions imposed by the transformation of the environment. The requirements of habitats for spawning, together with other characteristics of the species biological behavior, should be considered, predicting any type of modification of the hydrological system, and so, keeping the biological diversity.

Conclusion

The eggs of *Brycon insignis* are spherical, demersal, non-adhesive and have average diameter varying from 3,750 to 4,100 μm , with large perivitelline space and resistant chorion. It has been noticed, by observing the sequence of events of the embryonic development, that the stages of cleavage occurred during a 2 hours period. The first signs of embryo movement were observed 11 hours after fertilization, and the eclosion of the non-pigmented body larvae ($6.0 \text{ mm} \pm 0.22$) occurred 14 hours after fertilization. Four hours after eclosion, the larvae presented intense vertical swimming movements, pigmented eyes and a primary digestive system. On the 2nd day of life, the larvae were pigmented and showed open mouth and the yolk sac was almost completely reabsorbed. During this period, intense cannibalism was observed. On 5th day of life, all fins were developed and the larvae were

similar to the adults. On the 15th day of life, they presented an average total length of $3.4 \text{ cm} \pm 0.39$.

Acknowledgements

We are grateful to CESP (Companhia Energética de São Paulo), Paraibuna, São Paulo, for providing the fish utilized in this study.

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