

Embryonic and larvae development of piracanjuba, *Brycon orbignyanus* Valenciennes, 1849 (Pisces, Characidae)

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ABSTRACT. The knowledge of embryonic and larvae development of fishes is a fundamental key which enables a closer approach to their biology and taxonomy. The present study aims to characterize *piracanjuba* (*Brycon orbignyanus*) embryonic and larvae development. During the whole embryogenesis, 15 to 20 embryos were sampled and analyzed. Eggs of *B. orbignyanus* are semidense, transparent, spherical, and bear a large perivitelline space. Hatching takes place 18 hours and 30 minutes after fertilization at $25 \pm 0.8^\circ\text{C}$. Total length and weight of just hatched larvae were $4.46 \pm 0.39\text{mm}$ and $2.56 \pm 0.73\text{mg}$, respectively. Larvae presented entirely developed and pigmented eyes, as well as a vertical mouth opening of $15.2 \pm 1.9\%$ of body length, 36 hours after hatching, period from which intense cannibalism was observed.

Key words: *Brycon orbignyanus*, embryonic development, eggs, larvae.

RESUMO. Desenvolvimento embrionário e larval da piracanjuba, *Brycon orbignyanus*, Valenciennes, 1849 (Pisces, Characidae). O conhecimento do desenvolvimento embrionário e larval das espécies de peixes é de extrema importância por permitir um melhor estudo da biologia e sistemática da espécie. O presente estudo teve como objetivo caracterizar o desenvolvimento embrionário e larval da piracanjuba (*Brycon orbignyanus*). Foram realizadas amostragens contínuas de 15 a 20 embriões durante toda a embriogênese. Os ovos se apresentaram semidensos, transparentes, esféricos e com um grande espaço perivitelinico. Depois de 18 horas e 30 minutos da fertilização, mantidos a $25 \pm 0,8^\circ\text{C}$ aconteceu a eclosão. O comprimento total das larvas recém eclodidas foi de $4,46 \pm 0,39\text{mm}$ e o peso de $2,56 \pm 0,73\text{mg}$. As larvas de piracanjuba apresentaram forte canibalismo depois de 36 horas da eclosão, quando foi observada também a presença de olhos bem desenvolvidos e pigmentados, assim como uma abertura vertical da boca de $15,2 \pm 1,9\%$ do comprimento corporal.

Palavras-chave: *Brycon orbignyanus*, desenvolvimento embrionário, ovos, larvas.

Introduction

Popularly known as piracanjuba or pracanjuba, (*Brycon orbignyanus*), (Valenciennes, 1849) is a fish species broadly distributed in South America, which is found in the following rivers: Paraguay, Paraná, lower and medium Uruguay, and Prata (Gery *et al.*, 1987; Cavalcanti, 1998). *Piracanjuba* is a migratory species which reproduces between December and January and reaches up to 65cm and 10kg (Gery *et al.*, 1987). Due to its fast growth in captivity, omnivorous food habit, good acceptance of artificial feeds and low food conversion rates, it has been considered a promising species for fish farming (Cavalcanti, 1998). Nowadays, deforesting and presence of a great amount of dams, have

diminished *piracanjuba*'s landings, reducing and restricting its wild populations to small regions of the Uruguay River basin (Zaniboni-Filho and Schulz, forthcoming) and Paraná River (Gery *et al.*, 1987). Dams are the most common signs of human interference on the physiography of the region. There are more than 130 major reservoirs in Paraná river (dam > 10m high); among these, 20% are larger than 10,000 ha (Agostinho *et al.*, in press) reducing with this great part of the lotic area of the river and covering many of the places of growth of the larvae fish.

The description of embryonic stages of teleosts allows the identification of embryos in the wild, enabling precise evaluation of spawning sites. In

laboratories which massively produce fish juveniles, through induced fertilization, the previous knowledge of embryonic stages helps incubation management with regard to environmental variables which can lead to larvae malformation and low productivity in captivity (Alves and Moura, 1992).

Additionally, the knowledge of embryonic and larvae stages of fishes which stocks are reduced, as the *piracanjuba*, may be of great importance as a tool to identify its main reproductive grounds and thus, to guide environmental conservation planning and management.

Material and methods

Piracanjuba livestock was kept for 3 years in 2.000m² earth ponds of a Panamá Fish Hatchery, located in Paulo Lopes municipality, state of Santa Catarina, Brazil. The selection of broodstock took place when animals presented gonad maturation characteristics described by Woynarovich and Horváth (1983). Selected animals were transported to laboratory, where males and females were kept separated in 500 liter tanks with water exchange rate of 4 to 5 liters per minute.

The conventional method for gametogenesis induction through injection of Carp Pituitary Extract (CPE) was applied. All broodstock received a preliminary dose of 0.25mgCPE/Kg (Zaniboni-Filho and Barbosa, 1996) and 24 hours afterwards, females received 0.5 and 5.0mgCPE/Kg with a 12 hour interval, while males received a single dose of 1.5mgCPE/Kg at the moment when the females had received the second dose.

After 143 degree-hour (24,2°C) from the last application, gametes were manually stripped and mixed for fertilization. A sample of 5g of eggs was placed into a 60L static hatching tank with a conical shaped bottom, provided with an airstone. To avoid variations of water temperature this tank was placed inside a bigger tank (200L) equipped with an electrical heater and thermostat.

In order to observe and register different embryonic developmental stages, 15 to 20 embryos were sampled every 10 minutes within the first 5 hours, and then every 30 minutes until hatching. During larvae development, 4 or 5 larvae were sampled every 2 hours. Samples were made using a 5mL pipette. Eggs and larvae were immediately fixed in buffered 4% formaldehyde solution (Nakatani *et al.*, 2001).

Eggs were characterized according to the following data: total diameter, yolk cell diameter and perivitelline space diameter (between the

chorion and the yolk cell) (Nakatani *et al.*, 2001). The criteria for measurement and characterization of just hatched larvae followed recommendations made by Ahlstrom *et al.* (1976) and Leis and Trnski (1989), while meristic characterization of just hatched larvae was based on the number of pre anal, post anal, and total myomeres. Yolk cell volume was calculated according to Heming and Buddington (1988).

The observation and identification of embryonic stages were carried out at the Marine Fish Laboratory of Federal University of Santa Catarina. For such, a microscope coupled and a printer were used.

Temperature and dissolved oxygen were measured hourly with a YSI-55 oxymeter, while pH, ammonia and nitrate concentrations were daily measured through the colorimetric method.

Results

The mean value of water temperature during incubation was $25.0 \pm 0.8^\circ\text{C}$, although it slightly decreased from the beginning of the experiment from 25.9 to 24.0°C in the end. Dissolved oxygen mean concentration and pH values were of $7.34 \pm 0.46\text{mg/L}$ and 7.4 to 8.1, respectively, while total ammonia and nitrite concentrations were below 0.4mg/L and 0.01mg/L, respectively.

During incubation, the eggs of *B. orbignyanus* were spherical, semidense, and transparent. The fertilization moment was taken as the time zero. After 10 minutes, the perivitelline space was observed. The mean diameter of eggs, after hydration, was of $3.46 \pm 0.29\text{mm}$. This egg presents a large perivitelline space ($0.71 \pm 0.05\text{mm}$) what it corresponds to the 20,52% of its participation of the total size of the egg. The main morphological events registered in each developmental stage of *piracanjuba* are briefed on Table 1 and pictured on Figures 1 and 2.

Hatching of larvae occurred 18 hours and 30 minutes after fertilization, which correspond to 451 degree-hour. The percentage of fertilization found in this work was 5%. Elongated on the anterior-posterior axis, just hatched larvae measured from 4.14 to 4.71mm ($4.46 \pm 0.39\text{mm}$) of total length (Lt). The notochord is straight and easily seen, as well as the large, rounded, fairly pigmented and undeveloped eyes. The embryonic membrane is hyaline and not pigmented, as well as the emptied air-bladder. The intestine is intermediate and closed. The yolk cell is relatively large ($0.62 \pm 0.09\text{mm}^3$). The number of visible myomeres ranged from 43 to 48 (27 to 29 pre anal

and 16 to 19 post anal). Just hatched larvae weighed $0.9 \pm 0.1\text{mg}$, and showed vigorous vertical swimming.

After 10.5 hours from hatching, larvae opened their mouth, while their digestive tract was clearly delineated. Such larvae tract has entirely pigmented eyes.

Piracanjuba larvae presented strong cannibalism 36 hours after hatching, even showed some yolk. At this stage, otoliths were verified, eyes were well developed and mouth had a vertical opening of $15.2 \pm 1.9\%$ of body length. Furthermore, larvae presented conical teeth, complete digestive tract and greenish ventral portion.

Table 1. Description and timing of *Brycon orbignyanus* main embryonic morphological events. Incubation temperature of $25.0 \pm 0.8^\circ\text{C}$.

Stage	Time	Description	Figure
1. Formation of blastodisk	0.25 HAF ¹	Formation of animal and vegetal poles. Telolecithal eggs. The first cleavage occurs after 30 minutes from fertilization. Until the next hour, the 32 blastomeres are already formed.	1A
2. Lower blastula	2.0 HAF ¹	Complete formation of blastula. Blastodisk still in cleavage.	
3. Higher blastula	4.0 HAF ¹	Flattening of blastomeres.	1B
4. Gastrulation	5.0 HAF ¹	Blastoderm begins to surround the yolk. Invagination of endoderm and ectoderm. Formation of blastopore.	
5. Closure of blastopore	6.5 HAF ¹	Fusion of blastodisk lips. Delimitation of the embryo body and the yolk cell.	1C
6. Beginning of organogenesis	7.5 HAF ¹	Beginning of head and tail differentiation.	
7. Eye vesicle	9.5 HAF ¹	Total differentiation between cephalic and caudal zones. Presence of eye vesicles.	1D
8. Otoliths and olfactory orifices	15.5 HAF ¹	Presence of olfactory orifices and otoliths in the auditory vesicle.	2A
9. Hatching	18.5 HAF ¹	Vigorous embryo movements and hatching. Larvae present eye ball traces. Total length (Lt) of 4.46mm.	2B
10. Larvae with yolk cell		Larvae bear straight ended notochord.	
10.1. Mouthopening	10.5 HAH ²	Larva lengths (Lt) $5.65 \pm 0.12\text{mm}$ when its mouth opens.	2C
10.2 Teeth	22.0 HAH ²	Appearance of a little conical teeth	
11.1 Beginning of cannibalism	36.0 HAH ²	Post-larva lengths (Lt) $5.65 \pm 0.12\text{mm}$.	2D

¹HAF = hours after fertilization; ²HAH = hours after hatching.

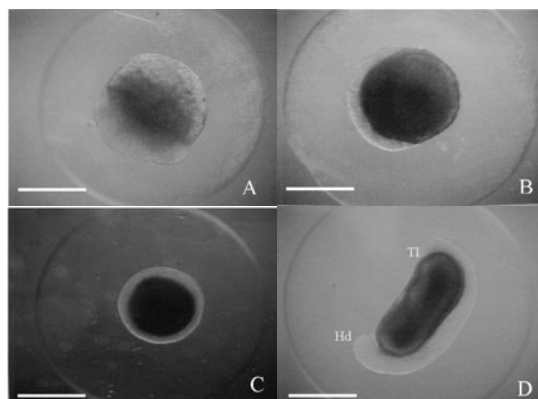


Figure 1. Phases of embryonic development of *piracanjuba* (*Brycon orbignyanus*). A. Cleavage of blastodisk (8 blastomeres); B. Lower blastula, flattening of blastomeres; C. Closure of blastopore; D. Head (Hd) and tail (Tl) differentiation. (Bar = 1.0mm)

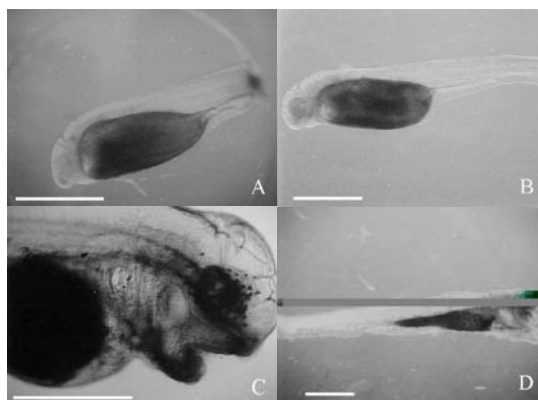


Figure 2. Phases of embryonic development of *piracanjuba* (*Brycon orbignyanus*). A. Embryo just before hatching; B. Just hatched larvae; C. Mouth opening; D. Larvae 36 hours after hatching, beginning of cannibalistic behavior. (Bar = 1.0mm)

Discussion

Piracanjuba (*Brycon orbignyanus*) eggs present similar characteristics to those found in other species (*Brycon cephalus*, *Brycon insignis*) belonging to the same genus, such as the total diameter and the large perivitelline space (Bernardino *et al.*, 1993; Andrade-Talmelli, 1997; Andrade-Talmelli *et al.*, 2001). Further on genus *Brycon* this characteristic is common for migratory species, such as *curimbata* (*Prochilodus lineatus*) (Curiacos, 1999), *Salminus brasiliensis* (Morais Filho and Schubart, 1955) and *Leporinus obtusidens* (Nakatani *et al.*, 2001).

According to Nakatani *et al.* (2001), the perivitelline space is considered large, when occupied between 20 and 29.9% of the total volume of the egg. A large perivitelline space is believed to enhance the embryo survival, protecting it from mechanical injuries (Andrade-Talmelli, 1997).

It has been observed in the *Brycon* genus free and semidense eggs, with slight difference in colors, varying from green (*B. insignis* and *B. lundii*), pale purple (*B. lundii*) to gray or wine (*B. cf. reinhardti*) (Andrade-Talmelli, 1997). In this work, (*Brycon orbignyanus*) egg was greenish, being this color dominant in the yolk cell until it was fully consumed. Similar observations were described for other species of the *Brycon* genus (Lopes et al., 1995; Andrade-Talmelli, 1997), as well as for *Salminus maxillosus* larvae (Morais Filho and Schubart, 1955).

In vitro reproduction of *B. orbignyanus* has led to low fertilization rates, usually inferior to 50% (Belmont, 1994) and 12.8% (Zaniboni-Filho and Barbosa, 1996). In the present study, a rather lower rate of 5% was achieved.

The morphological events registered during piracanjuba's embryogenesis are similar to those found in other neotropical freshwater fishes (Godinho et al., 1978; Sato 1999). The relatively short embryogenesis period and the single spawning of free eggs are also common characteristics in neotropical migrant fishes which migrate to reproduce. According to Lopes et al. (1995), eggs of *B. cephalus* hatch after 10.5 hours, when incubated at 30°C. Morais Filho and Schubart (1955) observed the embryogenesis of *S. maxillosus*, registering an incubation period of 23 hours for temperatures between 23 and 24°C and a mean Lt of 4.8mm.

Regarding incubation temperatures, Curiacos (1999) found for *Prochilodus scrofa* = *P. lineatus* that the time required for hatching at 23°C is almost twice the time needed at 29°C. Sato (1999), working with *Prochilodus affinis*, registered an embryonic development time of 20 hours at a mean temperature of 23.5°C.

In the present work, hatching began after 18.5 hours after the fertilization of eggs, however the last larvae hatched 3 hours later. For a temperature of 25.0±0.8°C, piracanjuba's larvae assumed a cannibalistic behavior, 36 hours after hatching, even bearing some yolk. This behavior, as well as the morphological features described, at this time, matches with what Ceccarelli (1997) described for *B. cephalus* 38 hours after hatching. According to some authors, the cannibalism during larvae stages observed in the genus *Brycon* is the main problem found in its larviculture management (Woynarovich and Sato, 1989; Bernardino et al., 1993).

Fox (1975) has posted that cannibalism is a common behavior in the animal kingdom. For fishes, cannibalism usually occurs within a heterogeneous-sized population, in situations of scarcity of feed, high stocking densities, short of

shelter and light and dark conditions (Hecht and Pienaar, 1993).

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