



## Morphological and behavioral development of the piracanjuba larvae

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**ABSTRACT** - The objective of this work was to study the morphologic development and the swimming and feeding behaviors of piracanjuba larvae, *Brycon orbignyanus* Valenciennes (1849) (Characiformes, Characidae, Bryconinae), during the period from zero to 172 hours after hatching (standard length = 3.62 – 11.94 mm). The morphological analyses were accomplished by using a trinocular stereo microscope, while the behavioral analyses were performed through periodic observations. In 28 hours after hatching, the larvae (standard length =  $6.25 \pm 0.13$  mm) showed the following structural and behavioral characteristics that made them become active predators able to overcome a larval critical phase, the beginning of exogenous feeding: presence of pigmented eyes, terminal and wide mouth, developed oral dentition, developing digestive tube, yolk sac reduction, fins and swim bladder formation, horizontal swimming, cannibalism, and predation. Intense cannibalism among larvae was verified from 26 to 72 hours. At the end of the metamorphosis – 172 hours after hatching – the larvae measuring  $11.94 \pm 0.80$  mm in standard length presented a flexed notochord, caudal fin bifurcation, dorsal and anal fin formation, synchronized movements, and formation of shoals, characteristics that together allow enhanced perception and locomotion in exploration of the environment, determining the best moment for transferring to the fishponds. New studies can contribute to commercial fish farming by improving feeding management, performance, survival, and productivity of this species.

Key Words: behavior, *Brycon orbignyanus*, Characiformes, first feeding, morphology, ontogeny

## Desenvolvimento morfológico e comportamental de larvas de piracanjuba

**RESUMO** - Objetivou-se estudar o desenvolvimento morfológico e os comportamentos natatório e alimentar de larvas de piracanjuba, *Brycon orbignyanus* Valenciennes (1849) (Characiformes, Characidae, Bryconinae) no período de 0 a 172 horas após a eclosão (comprimento-padrão = 3,62 - 11,94 mm). As análises morfológicas foram realizadas com auxílio de um microscópio estereoscópico trinocular e as comportamentais, por meio de observações periódicas. Com 28 horas após a eclosão, as larvas ( $6,25 \pm 0,13$  mm) apresentaram características estruturais e comportamentais que as tornavam predadoras ativas, capazes de superar a etapa crítica da fase larval, o início da alimentação exógena: presença de olhos pigmentados, boca terminal e ampla, dentes desenvolvidos, tubo digestivo em desenvolvimento, saco vitelino em redução, nadadeiras e bexiga gasosa em formação, nado horizontal, canibalismo e predação. Canibalismo intenso entre as larvas foi verificado das 26 às 72 horas. No final da metamorfose, às 172 horas pós-eclosão, medindo  $11,94 \pm 0,80$  mm de comprimento padrão, as larvas apresentaram notocorda flexionada, nadadeira caudal em bifurcação e nadadeiras dorsal e anal em formação, movimentos sincronizados e formação de cardumes, características que, em conjunto, permitem melhor percepção e locomoção na exploração do ambiente e determinam o momento adequado para transferência para os viveiros de alevinagem. Novos estudos podem contribuir na atividade comercial para a melhoria do manejo alimentar, do desempenho, da sobrevivência e da produtividade desta espécie.

Palavras-chave: *Brycon orbignyanus*, Characiformes, comportamento, morfologia, ontogenia, primeira alimentação

## Introduction

Newly-hatched larvae of freshwater fish suffer significant changes in body shape, metabolism, swimming skills, and behavior during morphogenesis, which is a quick and complex process (Gisbert et al., 2002).

The determination of the first exogenous feeding is a decisive factor in the commercial rearing of fish larvae, because if the food is provided prior to this moment, it will increase production costs related to waste; on the other hand, if delayed, the lack of food may compromise the growth and survival of the larvae (Maciel, 2006).

The feeding behavior of larvae may be related to the perception and success of food consumption, to the distance covered to capture the food item, and their nutritional requirements. These variables are associated with feeding models, species, size, and distribution of the available food (Maciel, 2006).

According to Sakamura & Tsukamoto (2002), aggressive behavior, including cannibalism, has a significant impact in the early life of the fish, leading to a high mortality rate which generates economic damage to the producer.

Therefore, in order to establish rearing methods aimed at the production of high quality fingerlings for stocking in artificial fishponds and natural courses of water, the study of the biology of the initial stages of fish is necessary, particularly for native species, and this has been investigated in a few Brazilian institutions.

Among the native species, piracanjuba (*Brycon orbignyanus*) is appreciated in commercial and sport fishing because of its active and aggressive behavior and the organoleptic properties of its meat (Vaz et al., 2000). According to Murgas et al. (2003), the species accepts artificial diets, presents rapid growth, and it is easy to be reared. However, piracanjuba is one of the many species of the Brazilian fauna threatened by extinction (Buckup et al., 2007), and it has already disappeared from many parts of the Rio Grande because of elevated human interference in courses of water, such as reservoirs for hydroelectric dams, deforestation of riparian vegetation, water pollution, and indiscriminate predatory fishing. Thus, studies regarding to the zootechnical characteristics of this species may increase its economic value and, consequently, encourage its commercial rearing as well as the preservation of natural stocks (Freato et al., 2005).

Different aspects of larval biology and the embryological and larval development of *Brycon orbignyanus* have interested researchers and they have already been the object of study for many authors, such as Nakatani et al. (2001),

Ganeco et al. (2003), Saccol-Pereira & Nuñez (2003), and Reynalte-Tataje et al. (2002 e 2004), among others.

Thus, the objective of this study is to describe the ontogeny of piracanjuba larvae between zero and 172 hours after hatching, emphasizing their initial morphological development and swimming and feeding behaviors, with the answers serving as subsidies for higher efficiency in the commercial exploration of this species.

## Material and Methods

The piracanjuba larvae [*Brycon orbignyanus* Valenciennes (1849) (Characiformes, Characidae, Bryconinae)] were collected from the Estação de Pesquisa e Desenvolvimento Ambiental of Volta Grande – EDPA-VG based upstream to the Usina Hidrelétrica of Volta Grande – Companhia Energética de Minas Gerais (CEMIG) and located in the municipality of Conceição das Alagoas, Minas Gerais.

The specimens were obtained through induced spawning at 27°C and transferred to incubators in the Laboratório de Reprodução Induzida of the referred Station. Hatching occurred 18 hours after spawning, being considered as “time zero” the moment when 50% of the larvae had hatched.

Twelve hours after hatching, the piracanjuba larvae were fed with curimba (*Prochilodus lineatus*) larvae at 14 hours of age that were added to the incubators to minimize cannibalism.

The physical and chemical parameters of the incubator water, such as temperature (°C), pH, conductivity (µS/cm), dissolved oxygen (mg/L), and salinity (‰), were monitored every six hours by using a U10 Horiba multiparameter probe.

From the moment of hatching (hour 0) to 48 hours, 15 to 20 specimens were sampled every 30 minutes. After this period, the specimens were sampled every hour until 72 hours after hatching. From this moment on, the samplings were performed every 24 hours until completing 172 hours after hatching, totaling 113 samples. Immediately after the samplings, the specimens were fixed at room temperature in a neutral formol solution buffered to 4%, where they remained until the moment of analyses. For the morphological description, the material fixed in formol was transferred to distilled water for rehydration (Maciel, 1997). The larvae were measured according to Ricker (1968) by using a digital pachymeter.

The analyses consisted on mesoscopic verification of the following morphological characteristics, according to Godinho et al. (2003): the appearance and location of

pigmentation in the body (presence and types of chromatophores) and in the eyes (retina pigmentation), the movement of the oral cleft to a terminal position and the mouth opening (mouth differentiation), opening of the anus, the formation of branchial arches and operculum, the formation of teeth and denticles, the formation of fins, the outline of a swim bladder, the absorption of the yolk sac, and the flexion of the notochord. Furthermore, the number of myomeres (total, pre- and post-anal) were also observed, based on the work by Nakatani et al. (2001). The morphological description was performed with the aid of a trinocular stereo microscope with a maximum magnification of 70x, and the photographic records were made with a digital camera.

A sample of larvae was transferred from the incubators to three 2-L glass tanks every 12 hours in order to describe larval behavior, with observations lasting for at least 15 minutes. The events considered as relevant for the swimming behavior of larvae were, according to Maciel (2006), the following: (1) resting: position (lateral/vertical/oblique), location (surface/middle of column/bottom), isolated/grouped; (2) rectilinear movement: active ascent (vertical/oblique), reaches or does not reach the surface, ascent frequency (rare/frequent), active/passive descent (vertical/oblique), reaches or does not reach the bottom, trajectory (rectilinear/sinuuous), speed (slow/intermediate/fast), body position (horizontal/vertical/head down); (3) circular movement: left/right; (4) swimming: continuous or not, synchronized or not, dispersed or grouped.

For the analysis of feeding behavior, the moment of the first exogenous feeding and aggressive behavior, cannibalism, and predation among larvae were considered (Maciel, 2006).

## Results and Discussion

Based on the work by Boyd (1992), the physicochemical parameters of the incubator waters remained in acceptable levels for tropical species, with mean values of  $26.18 \pm 0.40^\circ\text{C}$ ,  $\text{pH } 6.62 \pm 0.17$ ,  $6.42 \pm 0.17 \text{ mg/L of O}_2$ ,  $50 \mu\text{S/cm}$ ,  $0.00\%$  of salinity.

Eighteen hours after spawning (hour zero), the piracanjuba larvae presented a standard length of  $3.62 \pm 0.17 \text{ mm}$  and an elongated and transparent body (Figure 1A). The hyaline embryonic fin was structured and it was possible to identify a total of 22 myomeres in a few specimens. In this phase, mouth and anus were closed and the optical vesicle was distinct and depigmented. The yolk sac presented an elliptic shape, with a caudal extension or tubular appendix (Figure 1A).

According to Nakatani et al. (2001), the larvae of *Brycon orbignyanus* hatch 14 hours after fecundation at a temperature of  $27.9^\circ\text{C}$ , measuring approximately  $3.50 \text{ mm}$  in standard length and with a relatively large yolk sac, slightly pigmented eyes, and no apparent pigmentation of the body.

According to Pavlov (1999), the degree of morphological development at the beginning of the larval period may vary among fish species.

After hatching, the piracanjuba larvae remained dispersed most of the time in a lateral decubitus position resting in the bottom of the tank. After a few minutes, they performed ascendant vertical movements without reaching the water surface and went back down to the bottom passively in rectilinear movements, with their heads tilted down. These observations are in conformity with those by Costa (2003) and Godinho et al. (2003) for other species. However, according to Woynarovich & Horvath (1989), the behavior of recently-hatched larvae may differ among species, because some of them swim vertically in the direction of the water surface and then move to the bottom, while others move occasionally or continuously.

Godinho et al. (2003) reported that the larvae of dourado (*Salminus brasiliensis*), piau-verdadeiro (*Leporinus obtusidens*), curimatá-pioa (*Prochilodus costatus*), and curimatá-pacu (*Prochilodus argenteus*) remained most of the observed time in lateral decubitus at the bottom immediately after hatching, possibly because of the weight of the yolk sac and the lack of fins and swim bladder. A similar behavior was reported by Baras (1999) in larvae of dourada (*Brycon moorei*), which were located in the lower portion of the tank and used the water flux to obtain greater propulsion.

One hour after hatching, the notochord and optical vesicle were differentiated and evident in the piracanjuba specimens (standard length =  $3.85 \pm 0.20 \text{ mm}$ ). The long and rectilinear digestive tube is visible and it curves to the ventral direction of the caudal region, but it is closed in both ends. A notch appeared ventrally in the cephalic region close to the yolk sac, defining the formation of the oral cleft. The number of total myomeres varied from 26 to 27. Two hours after hatching (standard length =  $4.00 \pm 0.11 \text{ mm}$ ), the eyes of the larvae became evident with pigmentation starting in the borders. The digestive tube became evident by ventrally curving in the posterior region, although it was still macroscopically confused with an extension of the yolk sac. The oral notch was evident in the ventral position of the cephalic region. In the larvae at four hours after hatching (standard length =  $4.34 \pm 0.06 \text{ mm}$  and total of 35 myomeres), the notochord

was well developed and the oral notch was more evident and structured. The increased pigmentation of the eyes evidenced the optical vesicle, but it became even more evident in the following hours. Six hours after hatching (standard length =  $4.47 \pm 0.12$  mm), it was observed the start of the corporal pigmentation in the craniodorsal region of the yolk. The absence of pigmentation in the recently-hatched larvae, which is also observed in other species, is considered a strategy to escape from predation in the most vulnerable period of their life (Bone et al., 1995; Maciel Jr., 1996).

Two hours after hatching, the frequency of ascendant vertical movements increased actively and the larvae reached the surface, which became constant. The specimens, dispersed throughout the case, presented sinuous and circular horizontal movements to the left until 14 hours after hatching; after this period, the movements became more active, rectilinear, and faster. Blaxter (1986) reported that soon after Teleostei larvae hatch, the simple system of segmentation (myomeres) becomes more

complex and the red muscle concentrates in the middle position of the flank.

From the ventral perspective, the developing oral cavity was wide in the piracanjuba larvae at 7 hours after hatching (standard length =  $4.51 \pm 0.17$  mm and 39 total myomeres), even though the mouth was still closed. Besides the pigmentation already described, these larvae showed pigmentation along the digestive tube and in the center of the eyes. The mouth of the larvae opened between eight and nine hours after hatching. For Pinder & Gozlan (2004), the opening of the mouth occurs at the same time that the individual is able to swim to the surface and inhale air to inflate the swim bladder, which was observed in a study with piracanjuba larvae. More prominent changes were observed in the larvae at 10 hours after hatching (standard length =  $4.79 \pm 0.13$  mm), such as the onset of pectoral fin formation above the yolk sac and a small reduction of the embryonic fin in the dorsal region close to the anal region, being distinguishable 29 pre-anal and 19 post-anal

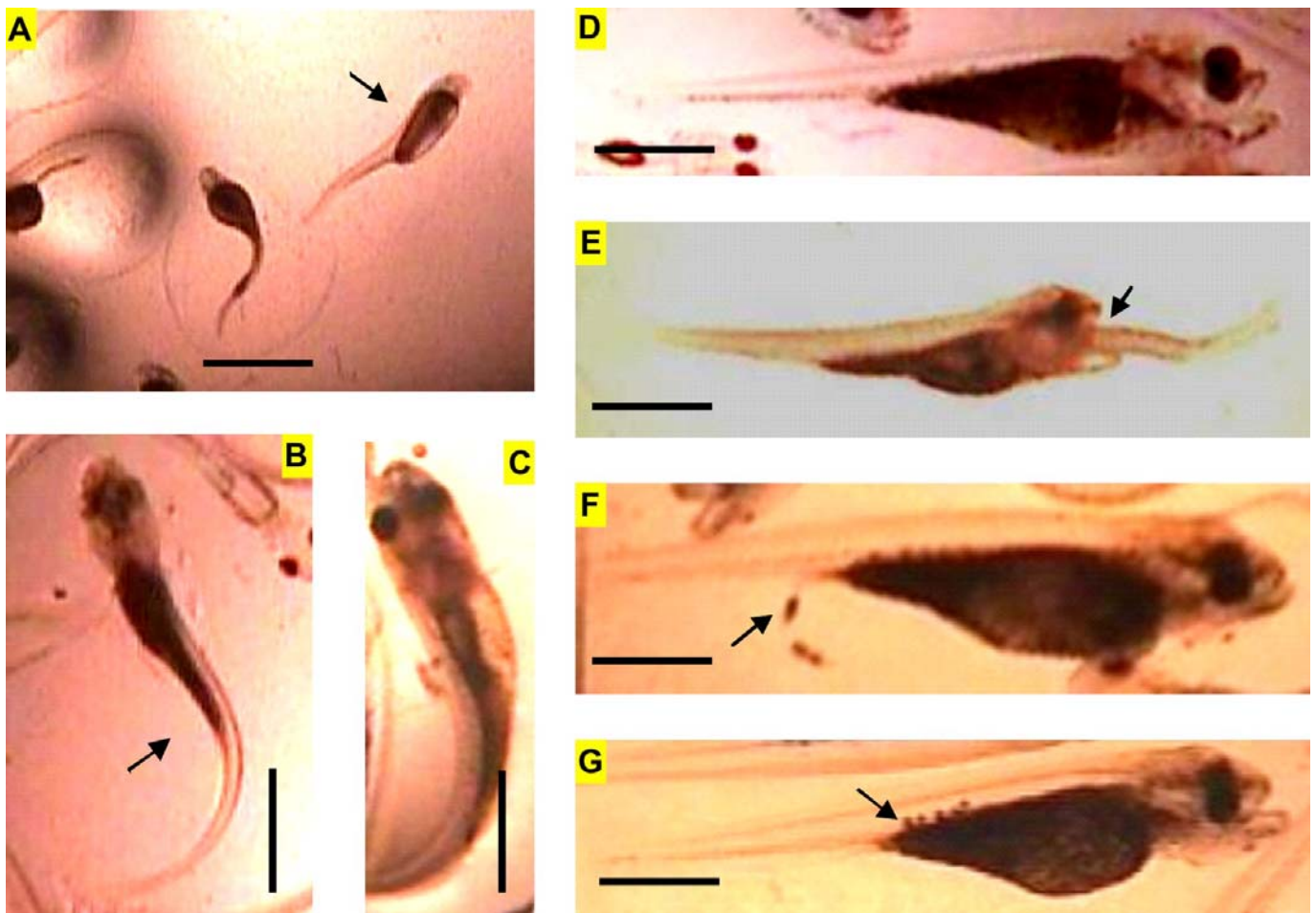


Figure 1 - Piracanjuba *Brycon orbignyianus* larvae. 1. A) Newly hatched (hour 0); B) 26 hours after hatching (arrow: body movement); C and D) 38 hours after hatching; E) 28 hours after hatching (arrow: cannibalism); F (arrow: feces) and G (arrow: body pigmentation) 32 hours after hatching. Bars: A = 2.00 mm; B = 1.40 mm; C and D = 1.25 mm; E = 1.50 mm; F and G = 1.10 mm.

myomeres. The developing branchial arches were visible, indicating the initial formation of the branchial chamber of the pharynx. According to Nakatani et al. (2001), the pectoral fin bud is present in *B. orbignyanus* specimens with a 4.80 mm standard length.

In the subsequent hours, the pigmentation increased in the eyes and in the flank along the digestive tube, reaching beyond the anal region, with dendritic chromatophores in the ventral region of the larvae. Thirteen hours after hatching (standard length =  $4.98 \pm 0.16$  mm), the nostrils were visible and open, the developing branchial arches were more structured, and the number of pre- and post-anal myomeres remained constant. The body pigmentation appeared in the craniodorsal region of the yolk sac and it was intense in the gular region, and the anal region was delimited. In the larvae at 14 hours after hatching, an initial migration from the mouth to the terminal position was observed. The body pigmentation continued to increase in the craniodorsal and ventral regions of the yolk sac, being more intense in the caudal region of this structure. A reduction was observed in the yolk sac. At 16 hours after hatching (standard length =  $5.51 \pm 0.13$  mm), the eyes of the larvae were more pigmented and visible by naked eyes. The mouth occupied the midventral region and kept migrating. The lips were thicker and the presence of oral, dental germs were observed in the upper jaw. The body pigmentation was intensified in the ventral region after the yolk sac and in the caudal region of the larva. Posterior to the yolk sac, a dilatation of the digestive tube was evident through transparency. The pectoral fins were more visible and open. The anal region was evident and presented an anal opening. The branchial arches were visible under the still developing operculum with no pigmentation and partially covering the branchial arches. This event marked the initial formation of the opercular chamber of the pharynx. At this moment, the larvae presented 50 myomeres, 30 pre-anal and 20 post-anal.

A prominent increase of body pigmentation in the cephalic region and yolk sac was observed in piracanjuba larvae at 17 hours after hatching (standard length =  $5.54 \pm 0.16$  mm). The mouth continued migrating and occupying a subterminal position and developed labial papillae were observed. After 20 hours, besides the eyes, the body pigmentation in the flank along the digestive tube and the end of the mouth migration were observed, the latter occupying a terminal position characteristic of the species, with a wide oral cleft. The branchial arches were visible in the opercular region with absence of branchial filaments. In the following hour, pigmentation was observed in the upper lip and it was enhanced in the

dorsal region of the head. In the specimens at 22 hours, apparently well-implanted canine oral teeth developed in the upper and lower jaws, and formed and pigmented eyes were developed. According to Souza (1999), the appearance of oral denticles enables the animal for a more efficient grip of food.

The total number of myomeres (total of 50, specifically 30 + 20) did not vary in the studied larvae at 23 hours after hatching (standard length =  $6.10 \pm 0.09$  mm), and the lower lip was more prominent than the upper. In the following hour, completing the first day (24 hours after hatching and standard length =  $6.14 \pm 0.12$  mm), the dorsal pigmentation increased significantly. By then, the structuring of the short, thin, branchial filaments was observed. The development of the branchial structure is important to allow the animal to swim, since gas exchange becomes more effective in comparison to the cutaneous respiration of early larval life (Bone et al., 1995; Maciel, 1997).

From 26 to 27 hours after hatching (Figure 1B), the specimens presented an aggressive behavior among themselves for the first time, defined by Sakakura & Tsukamoto (2002), as “nipping”, which consists of a fish voraciously attacking and biting the tail and body of another fish. From this moment on, the specimens started to swim in a horizontal, rectilinear direction. Similar ontogenetic changes were seen in the yellowtail (*Seriola quinqueradiata*) by Sakakura & Tsukamoto (1996).

According to Sakamura & Tsukamoto (2002), the development of the central nervous system is essential to start the aggressive behavior in fish larvae. For these authors, changes in swimming behavior, such as body undulations in W or J, are possibly precursors of aggressive behavior, which was also observed in piracanjuba (Figures 1B and 1C).

A very reduced and pigmented yolk sac was observed in the specimens at 27 hours after hatching (standard length =  $6.28 \pm 0.15$  mm; 30 + 22 = 52 myomeres), as well as better structured pectoral fins, nostrils positioned in the frontal region of the head. Furthermore, the head region and the flank along the digestive tube are more pigmented. The aggressive behavior of the piracanjuba larvae increased gradually until cannibalism and predation were seen frequently 28 hours after hatching, when larvae attacked and ingested other larvae (Figure 1E); this behavior evidenced the first exogenous feeding. In a few cases, one or more preys were kept immobilized in the oral cavity for some time, which suggests the functionality of the oral dentition. The voracity of the larvae was verified through the capture of others, regardless to the size class. However, since the curimbata (*Prochilodus lineatus*) larvae

introduced in the tanks were smaller and less developed than the piracanjuba larvae, predation was facilitated in the following hour. A similar voracious behavior has been seen in other *Brycon* species, such as matrinxã (*B. lundii*) (Woynarovich & Sato, 1990), matrinxã from the Amazon basin (*B. cephalus*) (Lopes et al., 1994; Senhorini et al., 1998), matrinxã (*B. moorei*) (Baras et al., 2000), piabanha (*B. insignis*) (Andrade-Talmelli et al., 2001), yamú (*B. siebenthalae*) (Atencio-García et al., 2003), among others.

For Reynalte-Tataje et al. (2002), cannibalism may be considered a common characteristic among fish and it is not restricted to only one life stage. Factors such as limited space or high density of stock, lack of food, and photoperiod may influence the rate of cannibalism (Hecht & Pienaar, 1993).

By measuring  $6.4 \pm 0.11$  mm in standard length and with a total of 53 myomeres, the piracanjuba larvae presented a full digestive tube 29 hours after hatching. In specimens fixed with their mouths open, a pigmented tongue was observed, besides a sharp reduction of the yolk sac. With the increased body pigmentation and the start of exogenous feeding, the visualization and delimitation of a few structures such as the yolk sac and the digestive tube became difficult. According to Gisbert et al. (2002), the development of feeding structures such as a functional jaw, is determinant for prey capture, which leads to larval growth and higher chances of survival. At this moment, the size of the yolk sac was still expressive and cannibalism was intense until approximately 72 hours after hatching (Figures 1 and 2). Cannibalism in piracanjuba larvae was also reported by Pedreira & Sipauba-Tavares (2002) and Reynalte-Tataje et al. (2004).

Only one piracanjuba specimen had an empty digestive tube 32 hours after hatching, and a reduction of the embryonic fin was observed (Figures 1F and 1G). Furthermore, excretion was observed in a few samples (Figure 1F) and cannibalism remained intense (Figure 3A).

In the following hour (standard length =  $6.86 \pm 0.15$  mm), all sampled specimens had a full and well-fed digestive tube, observing the preys inside the digestive tube, especially their eyes.

The initial pigmentation of the pectoral fin was observed in larvae at 38 hours (standard length =  $7.48 \pm 23$  mm and  $30 + 29 = 59$  total myomeres). Additionally, the still transparent operculum covered the branchial arches and cannibalism was present. At this moment, the larvae presented softer and slower movements because their digestive tube was still partially full (Figure 3D). At 42 hours after hatching, the larvae presented a thickening of the embryonic fin in the dorsal region, characterizing the initial formation of the dorsal fin, and the simple nostrils were pigmented. In the specimens with 45 hours (standard length =  $7.89 \pm 0.27$  mm), a tapering caudal portion of the digestive tube that delimited the middle and posterior intestines was observed through transparency. From then on, macroscopic quantification of myomeres is not possible anymore, because of the reduced transparency of the body wall.

A sharper reduction in the dorsal region of the embryonic fin was observed in larvae 48 hours after hatching, evidencing the formation of the dorsal fin. In one unfed specimen with an empty stomach, the distinction of the yolk sac was not possible. Fifty hours after hatching (standard length =  $8.02 \pm 0.06$  mm) (Figure 2A), the larvae presented regular variations in diameter along the posterior region of the middle intestine, which may indicate the formation of circular mucous folds. The tapering between the middle and posterior intestines became even clearer and the inflated swim bladder appeared as a refringent structure (Figure 2A).

Pigmentation on the dorsal region of the body with a few dispersed chromatophores was observed in larvae at 56 hours after hatching (standard length =  $8.05 \pm 0.50$  mm). In

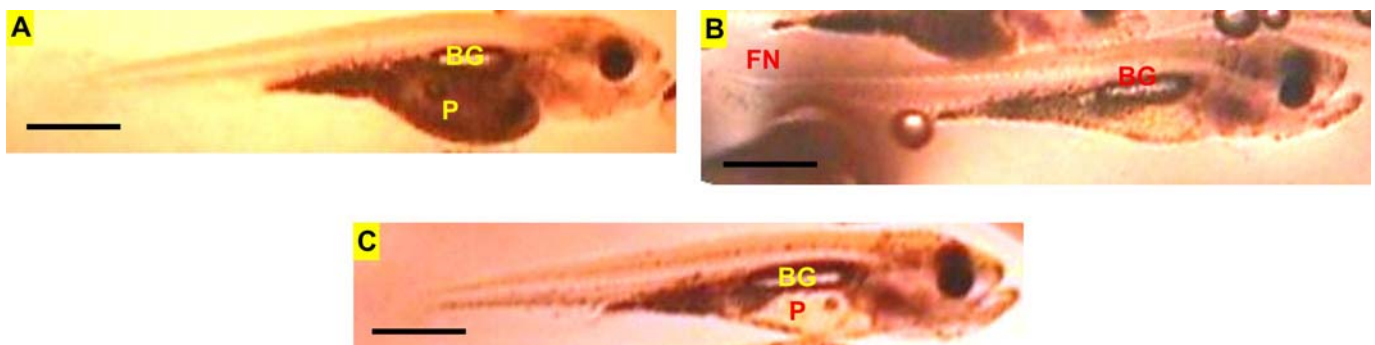


Figure 2 - Piracanjuba (*Brycon orbignyanus*) larvae 2. A) 50 hours after hatching; B) 58 hours after hatching; C) 64 hours after hatching. SB – swim bladder; FN – flexion of the notochord; P – digestive tube with prey. Bars: A and B = 1.10 mm; C = 1.14 mm.

a few specimens, the chromatophores formed a line along the flank in the future position of the lateral line. Even though the operculum has covered the branchial arches even more, it was flexible and transparent, allowing the visualization of the branchial arches. At 58 hours after hatching, the notochord deflected in the specimens with a standard length of  $8.09 \pm 0.9$  mm (Figure 3B), and consequently the formation of the caudal fin began, with the appearance of the hypurals and the first rays of this fin. The development of these structures is fundamental, because they increase the efficiency of swimming and capture of prey (Blaxter, 1988).

The development of the dorsal and caudal fins is related to the hydrodynamic regime under which the larva moves. Spontaneous and feeding activities, vertical migration and diving reach were intensified as the larvae get older (Blaxter, 1986).

At 64 hours after hatching, the larvae was  $8.47 \pm 0.37$  mm in standard length and the intestinal mucous folds and swim bladder were much more evident (Figure 3E). At 66 hours, the dorsal fin was delimited in the larvae with

$8.54 \pm 0.2$  mm in standard length, being able to count four rays in formation. A thickening and reduction of the embryonic fin was observed in the region where the anal fin will be formed. The operculum, with the posterior margin delimited by pigments, completely covered the branchial arches, though they are still visible through transparency; also, the pelvic fins were formed. In the specimens at 72 hours after hatching (standard length =  $9.13 \pm 0.18$  mm), the swim bladder was more visible and refringent, and the branchial filaments already with the branchial lamellae could be visualized. The anal, pelvic, and dorsal fins were in formation, with the dorsal fin still presenting four rays. Without the yolk sac, these larvae moved synchronously in the water column. According to Diaz et al. (2003) and Pinder & Gozlan (2004), the larvae improve their swimming and food capture skills with age.

On the fourth day (96 hours after hatching), the larvae measured  $9.67 \pm 0.26$  mm in standard length and ten dorsal fin rays were formed. The already flexed notochord continued to be visible through transparency. The pigmentation formed a line along the flank and it was

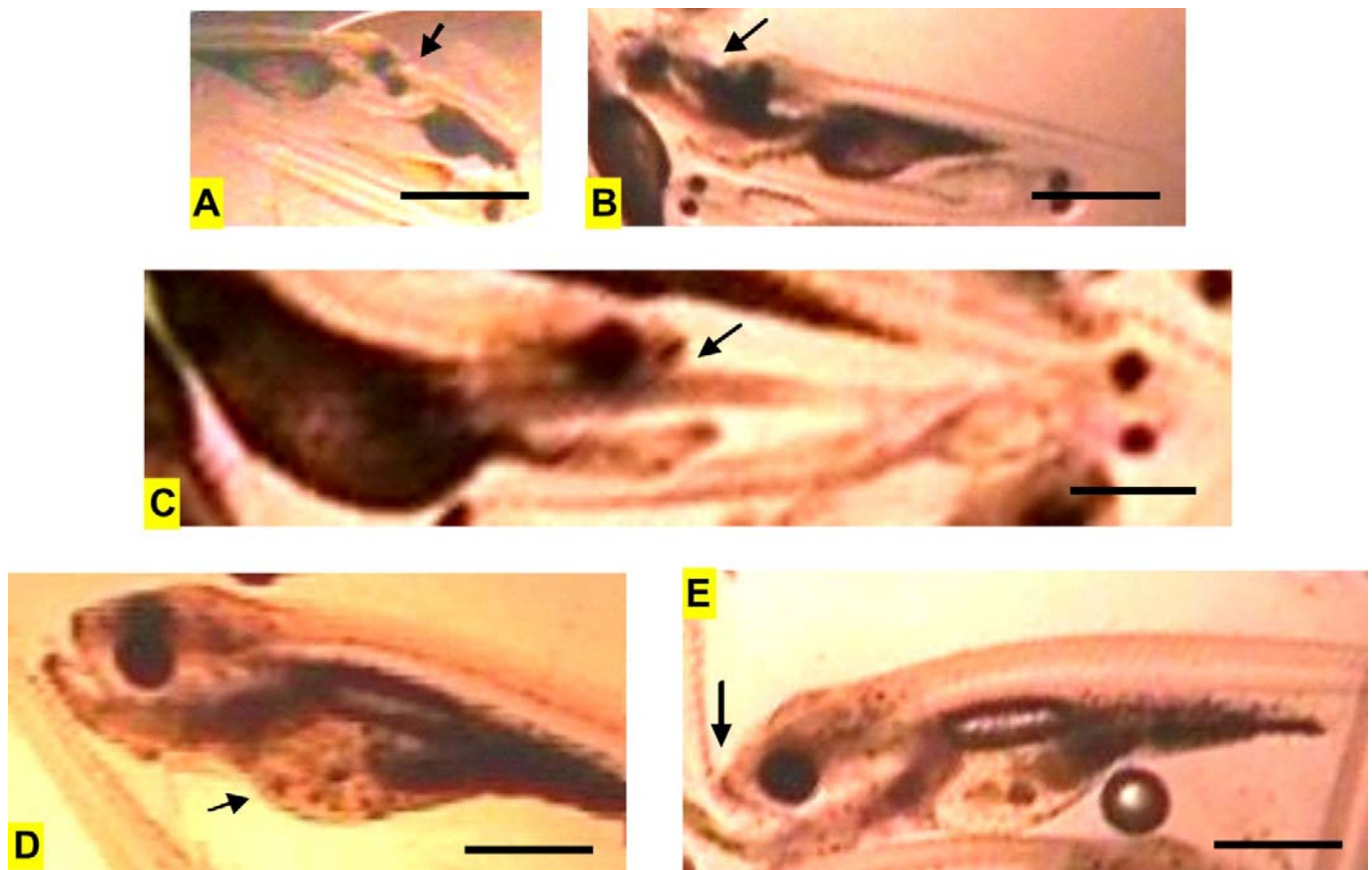


Figure 3 - Cannibalism (A and C) and predation (B) in piracanjuba (*Brycon orbignyanus*) larvae (arrows). A), B) and C) 32 hours after hatching; D) 38 hours after hatching; E) 64 hours after hatching. Bars: A = 2.70 mm; B = 1.50 mm; C = 0.75 mm; D = 0.85 mm; E = 1.10 mm.

dispersed in the caudal fin. On the fifth day, the anal fin rays appeared in the specimens at 124 hours after hatching (standard length =  $10.33 \pm 0.33$  mm). The contour of the lips was pigmented and the operculum remained little pigmented.

On the sixth day at 148 hours after hatching, the piracanjuba larvae (standard length =  $11.11 \pm 0.33$  mm) presented a significant increase in pigmentation throughout the entire body and a structured anal fin with formed rays. In the specimens at 7 days (or 172 hours) after hatching and measuring  $11.94 \pm 0.8$  mm in standard length, the pigmentation was more intense in the cephalic region and contoured each myomere; nevertheless, the general pigmentation was still little developed. While the embryonic fin was almost totally absorbed, the caudal fin undergoing bifurcation and a slight tapering of the body initiated the formation of the caudal peduncle. In this phase, the larvae still did not present the morphological characteristics of fingerlings.

The scarce pigmentation in larvae may be associated with behavioral characteristics, because according to Galuch et al. (2003), fish larvae from tropical freshwaters with pelagic behavior are generally little pigmented, but they potentially change their pigmentation patterns when exploring other regions.

The development of fin rays in piracanjuba larvae presented a pattern similar to other Characiformes, starting with the caudal and followed by the dorsal, anal, pelvic, and pectoral fins. A similar sequence was observed in cascudinha (*Psectrogaster amazônica*) and branquinha de cabeça lisa (*Potamorhina altamazonica*) by Nascimento & Araújo-Lima (1993), peixe canivete (*Apareiodon affinis*) by Bialecki et al. (1998), piau-três-pintas (*Leporinus friderici*) by Sanches et al. (2001), and pequirá (*Bryconamericus atramineus*) by Galuch et al. (2003).

According to Dasilao Jr. et al. (2002), the heterocercal caudal fin gives a degree of self-stability to larvae, particularly during swimming. The same authors reported that many morphological changes during ontogeny improve caudal propulsion, such as the caudal region that became relatively longer and narrower and thus more functional and agile.

The piracanjuba larvae started to present gregarious behavior at the end of the larval period at 172 hours after hatching, and groups of individuals formed small schools. Vaz et al. (2000) states that piracanjuba exhibits a gregarious behavior between individuals and this would be a way of protection against predators. The same observation in Atlantic herring (*Clupea harengus*) and sea bass (*Dicentrarchus labrax*), led Blaxter et al. (1983) and Diaz et al. (2003) to suggest that canal neuromasts are involved in

this shoal formation. Kawamura et al. (1990) showed that by cutting the nerves of the lateral line of adult red sea bream (*Pagrus major*) and bluegill (*Lepomis macrochirus*), the mean distance between neighbor fishes increased within the school without altering the gregarious behavior. Nevertheless, the lateral line system is not the only one involved in this process. The early eye development observed in piracanjuba was also seen in perch (*Perca fluviatilis*) by Ahlbert (1973) and in sea bass (*Dicentrarchus labrax*) by Mani-Ponset et al. (1993). Diaz et al. (2003) reported that groups of sea bass (*Dicentrarchus labrax*) dispersed in the dark, consequently evidencing the essential role of vision in this type of behavior.

## Conclusions

The piracanjuba larvae at 28 hours after hatching develop a set of morphological and behavioral characteristics such as pigmented eyes, developed oral teeth, mouth size and functionality, developing digestive tube, and swimming skills that make them active predators capable of overcoming one of the critical stages of the larval phase, the onset of exogenous feeding. At this moment, the exogenous food must be available to avoid or minimize cannibalism. At the end of metamorphosis (172 hours after hatching), the pigmentation of the eyes and the dorsum combined with the swimming ability of the larvae and their gregarious behavior indicates the adequate time to transfer them to the fingerling fishponds. These observations may contribute to the improvement of management, performance, survival, and productivity of the species.

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