Embryonic and larval development of *Brycon amazonicus* (SPIX & AGASSIZ, 1829)


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(With 2 figures)

Abstract

The objective of this study was to describe the embryonic and larval development of *Brycon amazonicus*, featuring the main events up to 50 hours after fertilization (AF). The material was provided by the Aquaculture Training, Technology and Production Center, Presidente Figueiredo (AM). The characterization was based on stereomicroscopic examination of the morphology of eggs, embryos and larvae and comparison with the literature. Matrinxã eggs are free, transparent, and spherical, with a perivitelline space of 0.56 ± 0.3 mm. The successive divisions give rise to cells with 64 blastomeres during the first hour AF. The gastrula stage, beginning 02 h 40 min AF, was characterized by progressive regression cells and the formation of the embryonic axis, leading to differentiation of the head and tail 05 h 30 min AF. From 06 to 09 h AF the somites, notochord, otic and optic vesicles and otoliths were observed, in addition to heart rate and the release of the tail. The larvae hatched at 10 h 30 min AF (29.9 °C), with a total length of 3.56 ± 0.46 mm. Between 19 and 30 h AF, we observed 1) pigmentation and gut formation, 2) branchial arches, 3) pectoral fins, 4) a mouth opening and 5) teeth. Cannibalism was initiated earlier (34 h AF) which was associated with rapid yolk absorption (more than 90% until 50 h AF), signaling the need for an exogenous nutritional source. The environmental conditions (especially temperature) influenced the time course of some events throughout the embryonic and larval development, suggesting the need for further studies on this subject.

Keywords: matrinxã, embryogenesis, oocytes.

Desenvolvimento embrionário e larval de *Brycon amazonicus* (SPIX & AGASSIZ, 1829)

Resumo

O objetivo deste trabalho foi descrever o desenvolvimento embrionário e larval do *Brycon amazonicus*, caracterizando os principais eventos ocorridos até 50 h Após Fertilização (AF). O material é proveniente do Centro de Treinamento, Tecnologia e Produção em Aquicultura em Presidente Figueiredo (AM). A caracterização foi feita com base na análise estereomicroscópica da morfologia dos ovos, embriões e larvas e comparação bibliográfica. Os ovos da matrinxã são livres, transparentes, esféricos com espaço perivitellínico de 0,56 ± 0,3 mm. As sucessivas clivagens originam células com 64 blastômeros na primeira hora AF. A gastrálula, iniciada 02 h e 40 min AF caracterizou-se por progressiva involução celular e formação do eixo embrionário, culminando com diferenciação de cabeça e cauda com 05 h 30 min AF. De 06 às 09h AF foi observada a formação de somitos, notocorda, vesículas óptica, ótica e otólitos, além de batimentos cardíacos e liberação da cauda. As larvas eclodiram com 10 h 30 min AF (29,9 °C), com um total de 3,56 ± 0,46 mm de comprimento total. Entre 19 e 30 h AF foram observadas: 1) pigmentação e formação do tubo digestivo 2) surgimento de arcos branquiais 3) nadadeira peitoral 4) abertura da boca e 5) surgimento dos dentes. O canibalismo iniciou mais precocemente (34 h AF), em relação aos trabalhos existentes com o gênero, o que associado à rápida absorção do vitelo (mais de 90% até 50 h AF), sinaliza a necessidade de ofertar recurso alimentar exógeno. As condições ambientais (especialmente temperatura e pH) influenciaram na abreviação de alguns eventos ao longo do desenvolvimento embrionário e larval, sugerindo a necessidade de estudos complementares a esse respeito.

Palavras-chave: matrinxã, embriogênese, ovócitos.
1. Introduction

The matrinxã *Brycon amazonicus* (Spix & Agassiz, 1829) is a rheophilic species native to the Amazon basin, which has great potential as a fish farming region, both in semi-intensive and intensive systems (Zaniboni-Filho et al., 2006). In the taxonomic revision of the genus Brycon, Lima (2003) concluded that the species *Brycon cephalus*, which lives in the Brazilian Amazon and is widely cultivated in Brazil, is in fact *Brycon amazonicus*, which is also synonymous with the species *Brycon siebenthalae*.

One of the barriers to aquaculture growth of this species is related to low survival in the larval stage, where cannibalism has been recorded as the main cause of death (Bernardino et al., 1993; Faria, 1994; Lopes et al., 1995; Ceccarelli and Volpato, 2001; Leonardo et al., 2008). To conduct research on this native species, knowledge of the early stages of fish embryonic development is extremely important. The use of information on embryogenesis allows us to characterize morphological and chronological events that are important for studies on reproduction and growth (Reynalte-Tataje et al., 2001), taxonomy (Leite et al., 2006), and environmental quality assessment (Flores et al., 2002).

In fish culture, this information provides insight into the life cycle of the fish species (Ninhaus-Silveira et al., 2006) and may be a predictive tool to assess the viability of eggs after fertilization (Shields et al., 1997; Kjorsvik et al., 2003). In fish farms for juvenile production, the knowledge about embryonic development supports incubation management of water quality that could lead to abnormalities and low productivity in farming (Reynalte-Tataje et al., 2004). Thus, the identification and description of the main events during the embryonic development of *B. amazonicus* are justifiable, especially because of the scarcity of information that can be applied in regional studies, where differences in temperature could be significant for optimizing larval rearing in laboratory conditions. This study aims to describe and characterize the main events during the stages of embryonic and larval development of the matrinxã *B. amazonicus*.

2. Material and Methods

The experiment was conducted at the Fish Culture Station of the Center for Technology, Training and Production in Aquaculture (CTTPA), located in the village of Balbina, Presidente Figueiredo, Amazonas, in February 2010. For this work, we used *B. amazonicus* breeders acquired from the tributaries of the Black River and kept during the breeding season in a nursery of 600 m³ at a density of 300 g/m³. The selection of animals for induction was based on Woynarovich and Horvath (1983). The selected fish were induced according to the protocol described by Bernardino et al. (1993). After fertilization, the eggs were placed in 60-l conical glass fiber incubators at a density of 1 g/l until 50 h AF.

Samples that contained 10 eggs or larvae were collected during embryonic and larval development for observations *in vivo* in a stereomicroscope and then fixed in 4% formaldehyde for further confirmation. The collection started at the moment of fertilization, considered zero time. The samples were taken at intervals of 10 min up to 10 h after fertilization (AF). Between 10 and 17 h AF, the intervals were 30 min, and between 18 and 50 h AF, the samples were collected every 02 hours.

For morphometric characterization of dry fertilized eggs, perivitelline space (PS) and mouth opening and total length of newly hatched larvae were measured for 100 specimens with an ocular micrometer adapted on a stereomicroscope. To determine the size of the mouth opening, we referred to the protocol outlined by Shirota (1970).

3. Results

The physical-chemical water parameters were pH $5.20 \pm 0.21$, oxygen $6.70 \pm 0.16$ mg/l and temperature $29.97 \pm 0.19$ °C. The main events recorded at each stage of embryonic and larval development were presented in chronological order (Table 1), divided into two stages, the embryonic and larval stages, and the main characteristics were described based on external features.

After extrusion, the “matrinxã” oocytes had an olive green coloration, which remained until the end of embryonic development by staining of the yolk sac. The diameter of the dry fertilized egg was $1.28 \pm 0.19$ mm, and the animal pole was darker than the vegetative pole, which was light-colored and opaque. After 10 minutes of hydration, the eggs were $1.84 \pm 0.21$ mm in diameter, with the PS measuring $0.56 \pm 0.3$ mm.

The “matrinxã” eggs are meroblastic, telolecithal and discoidal (Wolpert et al., 2000). The first cleavages were observed 20 minutes after fertilization (AF), yielding cells with 02, 04, 08, 16, 32 and 64 blastomeres. As the targeting cell increases, the degree of compaction intensifies between blastomeres, hindering their individual observations. This event was observed 01 h 20 min AF.

The early gastrula was observed 02 h 40 min AF (Figure 1a), and the 100% epiboly stage was observed 05 h 10 min AF (Figure 1g). After somatic targeting, the differentiation of the head and tail began 5 h 30 min AF (Figure 1h), as did the appearance of somites in increasing numbers, reaching 32 somites 50 min before hatching (Figures 1i, 1j, 1k and 1l). Otic and optical vesicles were observed between 6 and 8 h AF (Figure 2b).

Otoliths initially in two pairs were observed 08 h 20 min AF within the optic vesicle and became larger and more distinct as the larvae grew. After 15 h 30 min AF, otoliths were clearly observed in three pairs (Figure 2d). The first heartbeat and circulation were observed 09 h 10 min AF, coinciding with the observation of 32 somites on the axis of the notochord, more easily seen at this stage. The tail partially detached from the yolk, the yolk sac was elongated, and light muscle contractions were observed and spaced, which intensified the proximity of hatching. Notochord and myomeres were easily observed 09 h 10 min AF.

As the embryo movement flow intensified, there was a progressive weakening and rupture of the chorion, an event...
recognized as hatching, which occurred asynchronously from 10 h AF; 50% of individuals hatched at 10 h 30 min AF. The size of newly hatched larvae was 3.56 ± 0.46 mm, without retinal pigmentation. The yolk sac was the main visible structure, which highlights the presence of the calf, with intense olive coloration. Chromatophores distributed on the retina could only be observed 18 h 30 min AF (Figure 2c).

Pectoral fins and first gill arches (Figure 2d) were observed at 26 h AF, which was when a deepening of the epithelium of the inlet mouth was also observed, resulting in the oral cavity opening at 28 h AF. Denticles were observed starting at 30 h AF and were fully developed at 34 h AF, which was when cannibalism started. The denticles were presented as well-developed, conical shapes that turned back. The newly hatched larvae exhibited erratic vertical swimming characterized by upward movement, followed by constant sinking in the water column.

At 33 h AF, swimming larvae presented intense and consecutive jaw movements followed by frequent attacks 34 h AF. At this phase, the retina was partly pigmented, the diameter of the eyes was 0.30 ± 0.13 mm, the yolk sac was remarkably reduced, and the mouth opening was 1.42 ± 0.18 mm, 23.01% of the standard length (6.17 ± 0.38 mm).

On the matrinxã, dendritic chromatophores were clearly observed on the cranial region and yolk sac 36 h AF; then, there was total retinal pigmentation 38 h AF. At this time, the body pigmentation extended over the pectoral region, with greater intensity in the cranial and lateral regions. The distribution of pigment in the body permits better survival of the larvae, related to masking, as at this stage the larvae are susceptible to predation, and represents
specific features used in taxonomy. Larvae 38 h AF present calves with an apparent reduction of 80% over the initial volume (newly hatched).

Between 40 h and 44 h AF, it was observed that the larvae mouth was completely opened with sharp teeth, a differentiated and slightly yellowish digestive tract and dark pigmentation on the intestine. From 46 to 48 h AF, it was possible to distinguish blood circulation through reddish flow, and the yolk was almost totally absorbed. At 50 h AF, larvae swam horizontally, and several cannibalism episodes were confirmed by observation of partially ingested larvae.

4. Discussion

The morphological and morphometric characteristics of dry fertilized eggs and hydrated eggs were similar to those reported in other studies using *Brycon* (Bernardino et al., 1993; Lopes et al., 1995; Romagosa et al., 2001; Clavijo-Ayala and Arias Castellanos 2004; Nakaghi et al., 2013). The matrinxã’s pattern of development is similar to that reported by Kimmel et al. (1995) for zebra fish, in which the blastoderm is formed by a single outer layer of flattened cells, known as the involucral outer layer, and a more rounded deep layer of cells. The increase of

**Figure 1.** Embryonic development of *B. amazonicus* (29.9 °C). (a) early gastrula (50% epiboly, 2 h 40 min AF), advanced gastrula in (b) 3 h 30 min AF; (c) 4 h 10 min; (d) 4 h 40 min; and (e) 5 h AF; (f) and (g): end of gastrula (100% epiboly to 5 h 10 min AF); (h) early differentiation of head and tail (5 h 30 min AF), (i, j, k and l) emergence of somites 5, 7, 11 and 15, respectively; (*) highlights the somites.
cell segmentation enhances the degree of compaction between blastomeres and marks the end of the ball stage (Wolpert et al., 2000), also referred to as morula and early blastula (Andrade-Talmelli et al., 2001; Reynalte-Tataje et al., 2001; Romagosa et al., 2001; Anjos and Anjos, 2006; Ninhaus-Silveira et al., 2006; Faustino et al., 2007; Paes, 2008; Nakaghi et al., 2013).

The beginning of gastrula marks the end of the blastula stage, and it is followed by epiboly, defined as the movement of the yolk syncytial layer and blastodisc around the yolk cell. Epiboly starts with the deep layer cells radially intercalating and the yolk cell “doming,” which was recorded by Andrade-Talmelli et al. (2001) and Paes (2008) when eggs presented with 30% epiboly and by Kimmel et al. (1995) and Sampaio (2006) when it was in the 50% epiboly stage. With B. amazonicus, Clavijo-Ayala and Arias Castellanos (2004) observed the early gastrula (10% epiboly) at 5 h AF (27.5 ± 0.2 °C), and Nakaghi et al. (2013) observed the beginning of the epiboly and gastrula stage when cell proliferation covered approximately 30% of the yolk, at 3 h AF (28.08 °C). We observed the same event 1 h 20 min AF (29.9 ± 0.18 °C), and the difference could be related to temperature.

Clavijo-Ayala and Arias Castellanos (2004) indicated that high incubation temperatures (30 °C) lead to exogastrulation (protusion of yolk mass) in B. amazonicus oocytes due to enzymes and proteins that participate in the formation and performance of microtubules and the direction of epibolic movements.

The gastrulation stage ends when the blastoderm completely covers the yolk, characterizing the 100% epiboly stage, which is marked by the presence of primary germ layers (endoderm, mesoderm and ectoderm) and the embryonic axis, giving rise to the subsequent stage characterized by organogenesis (Wolpert et al., 2000). Some authors consider the 100% epiboly stage equivalent to the closure of the blastopore and attribute this moment the opening of the primitive gut in larvae.

In this work, the temperature most likely influenced gastrulation, observed earlier (5 h 10 min AF, 29.9 ± 0.18 °C) than what was recorded by Neumann (2008) (6 h AF, 27 °C) and Clavijo-Ayala and Arias Castellanos (2004) (6.5 h AF, 27.5 °C) for B. amazonicus eggs and later compared with B. insignis (“piabanha”) (4 h AF, 26.1 °C) (Andrade-Talmelli et al., 2001).

During gastrulation, the mesoderm that has moved into the embryo is positioned in the block side of the notochord, a structure that supports the spinal cord and extends from head to tail in a central position (Paes, 2008). Blocks of mesoderm positioned beside the notochord give rise to the muscles, limbs, spine and dermis, called somites, which segment, forming myomeres widely used in taxonomic studies of fishes (Araújo-Lima and Donald, 1988; Wolpert et al., 2000).

The emergence of ocular and auditory capsules marks the beginning of the development of sensory structures in fish larvae and is an event that often follows the emergence of myomeres in different species (Reynalte-Tataje et al., 2001;
Sanches et al., 2001; Ninhaus-Silveira et al., 2006; Pereira et al., 2006; Clavijo-Ayala, 2008). As observed by Falk-Petersen (2005), at the stage at which these capsules emerge, larvae have no stability in water, coordination of movements, or visual acuity. In B. amazonicus, optics and optical capsules were observed between 6 and 8 h AF, coinciding with the results for B. cephalus, as observed by Romagosa et al. (2001) (26 °C), Lopes et al. (1995) (30 °C) and Alexandre et al. (2010) (26 °C).

The embryonic development of B. amazonicus has been reported in the literature, ranging from 10 h 30 min AF (in this work and Lopes et al., 1995), to 11 h AF (Romagosa et al., 2001- 26 °C), 13 h AF (Nakaghi et al., 2013- 28.08 °C) and 13 h 30 min AF (Mira-López et al., 2007-28.02 °C). For B. orbignyanus, hatching was recorded at 18 h 30 min AF (Reynalted-Tataje et al., 2001- 25°C) and 13 h AF (Landines-Parra, 2003; Landines-Parra et al., 2003-28.01 °C). Temperature can speed up or slow down larvae hatching (Trejo and Martinez, 1983), mainly for tropical fishes, where there is a direct correlation between water temperature and embryonic development (Johnston and Vieira, 1996).

For B. amazonicus, Clavijo Ayala and Arias Castellanos (2004) observed that the increase of incubation temperature decreases the duration of embryonic development but also increases the occurrence of deformed embryos. All oocytes incubated at 32 °C shattered by 3 h Kamler and Keckes (1988) associated deformations and death in the eggs and embryos of fish at different stages of development with the proteins denaturing or the inactivation of enzymes when subjected to temperatures too low or too high.

The relatively fast embryonic development is a common characteristic among species with reproductive strategies that involve migration and spawning in lotic environments (Sanches et al., 2001), and for the genus Brycon, it can be considered an advantage in the reproduction process (Andrade-Talmelli et al., 2001; Romagosa et al., 2001). In relation to the size of newly hatched larvae, B. orbignyanus ("piracanjuba") (Andrade Talmelli et al., 2001) and B. insignis ("piabanha") (Reynalted-Tataje et al., 2004) were bigger than B. amazonicus ("matrixâá") (this work; Mira-López et al., 2007; Romagosa et al., 2001; Lopes et al., 1995). In addition to species-specific characteristics, the water temperature of incubation influences the size of newly hatched larvae and early development (Curiacos, 1999).

The pigmentation of the retina is related to the functionality of the eyes (Falk-Petersen, 2005), and this event was completed in B. amazonicus between 32 and 36 h after hatching (25 °C) (Arias Castellanos et al., 2003) and between 21 and 24 h after hatching (27 °C) (Mira-López et al., 2007). The absence of pigmentation in the body from the moment of hatching possibly implies delayed development of the visual system, which in turn delays the capture of prey but does not delay the development of additional sensory systems (Roo et al., 1999; Clavijo-Ayala, 2008).

In this work, chromatophores distributed on the retina could only be observed in B. amazonicus at 18 h 30 min AF, and it was totally pigmented approximately 36 h AF.

Pectoral fins and first Gill arches were observed at 26 h AF, a similar period to that reported by Mira-López et al. (2007) for B. amazonicus (between 28 h 30 min and 31 h 30 min AF). According to Paes (2008), the appearance of the pectoral fin is an important event in the organogenesis of fish larvae because these structures facilitate the balance and direction in the water column. This structure, emerging together with the opening of the oral cavity 28 h AF, signals the larvae’s ability to search for exogenous food. Silva et al. (2007), however, verified through histological analysis the presence of an oropharyngeal membrane despite the presence of denticles, as described by Ceccarelli (1997) and Romagosa et al. (2001). Neumann (2008), observed denticles in B. amazonicus only 35 h AF (27.98 ± 0.81 °C).

The erratic vertical swimming is associated with the process of filling the bladder (Maciel et al., 2010). This behavior, associated with consecutive mandibular movements followed by frequent attacks at 34 h AF, could be associated with the mechanism for developing muscular and skeletal structures involved in the capture and arrest of the first food (Herbing, 2001).

Cannibalism, observed 34 h AF, presents earlier compared with other studies on B. amazonicus (Bernardino et al., 1993 and Lopes et al., 1995), 46 h AF (30 °C) and Romagosa et al. (2001), 36 h AF (26 °C). Ceccarelli (1997) describes a sequence of events that characterizes the observed cannibalism: fixation of the prey, approach, boat, bite and apprehension. At 38 h AF, the larvae present considerable calf reduction (Neumann, 2008), indicating an apparent ability to begin an important stage in the transition from endogenous (yolk) to exogenous (live or artificial food) nutrition.

At 48 h AF, the juveniles are able to perform the first feeding because the yolk sac is now almost non-existent, and stomach and intestine are well-differentiated (Mira-López et al., 2007). They present similar structures to those of adult specimen, with a completely pigmented body, well-formed caudal and pectoral fins, and silvery scales. Matrixá starts feeding at the end of the first day (Romagosa et al., 2001). Despite variation in the register of cannibalism occurrence (34 to 36 h AF), the intensification of this behavior at 50 h AF signals the need to intervene in the management practices through the provision of exogenous food, aiming to minimize the effect of cannibalism on larval mortality.

5. Conclusions

The “matrixá” B. amazonicus has fast embryonic and larval development, thus requiring differentiated management during the initial phase. Their ability to hunt and capture is evidenced by cannibalistic behavior, as aforementioned in relation to other fish with the genus Brycon.

The fast depletion of yolk signals the need for supplementary feeding, allowing minimization of the loss due to cannibalism, which optimizes growth. Future studies
are needed to further evaluate the effect of temperature and pH on the embryonic and larval development, aiming to standardize the hatching protocol for the species.

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