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Early larvae ontogeny of the Neotropical fishes: Prochilodus costatus and P. argenteus (Characiformes: Prochilodontidae)

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Abstract: Early development of fish larvae is a highly dynamic process and its study may provide important information about ontogenetic development, bioenergetic growth, behaviour, taxonomic characteristics for identification in natural environments, identification of spawning areas, and population monitoring. With the aim to provide knowledge about their growth and behaviour, to support larval rearing, and also taxonomic purposes, we studied the life history of the Prochilodus argenteus and P. costatus from hatching until the complete absorption of the yolk. Larvae were obtained through artificial reproduction at the Hydrobiology and Aquaculture Station of Três Marias, Minas Gerais, Brazil. Immediately after hatching, 100 larvae of each species were put in two plastic incubators for conditioning. On a daily basis, larval behavior was recorded and 14 larvae of each species were collected to analyse body morphology. On the first day after hatching, larvae of P. costatus and P. argenteus showed an elongated and transparent body; the yolk sac was filled with individualized yolk globules. In both species, the embryonic fin rounded the caudal region of the body, the retina was non-pigmented and the gut was obliterated. At the second day post-hatching, larvae of both species dendritic chromatophores had emerged, the mouth was obliterated and the pectoral fin was observed. The larvae showed 38-43 myomeres in P. costatus and 42-43 in P. argenteus. For both species, the gas bladder was inflated and the lumen of the gut was already open. On the third day post-hatching, the mouth of P. costatus and P. argenteus was already open in a sub-terminal position; the retina was pigmented; the gill arches had lamellar protrusions and were partially covered by the operculum. On the fourth day post-hatching, the pigmentation pattern was maintained with greater intensity; the mouth occupied a terminal position, the yolk sac was almost completely reabsorbed, and the pectoral and caudal fins showed mesenchymal rays in both species. The gut showed a broad lumen with folded mucosa and epithelium with striated border. The larvae of both species showed similar swimming behaviour. Our study provided understanding about the morphophysiological aspects, species identification, larval development and growth, and the ontogenic characteristics of two Neotropical fishes with importance for commercial and sports fishing.


Key words: larvae development, larval ontogenic, larval rearing, Prochilodontidae, swimming behaviour.
identifying fish eggs and larvae in their natural environments (Lopes, Oliveira, Bialetzki, & Agostinho, 2014). The problem is magnified in fish that perform reproductive migration, since they spawn in the main channel of the river during the rainy season, when floods take eggs and larvae to the floodplains (Santos & Godinho, 2002). For successful development in a hatchery, knowledge of the following ontogenetic events is essential: size and time at hatching, flexion of the notochord, duration of the yolk sac, the presence or absence of an adhesive organ, retinal pigmentation, opening of the mouth and intestinal lumen, development of fins, gas-bladder filling, and cutaneous pigmentation pattern (Santos & Godinho, 1994; 1996a; 2002). Right after hatching, the larvae usually remain resting in lateral recumbence, and this behaviour probably is due to the yolk sac weight and the absence of a functional gas-bladder and pectoral fins (Santos & Godinho, 2002).

Fish of the Prochilodontidae family are important in commercial and sports fishing, as well as being an essential source of food for human populations living around the rivers of South America (Sverlij, Ros, & Orti, 1993). *Prochilodus argenteus* (Spix & Agassiz, 1829) is the largest species among the Prochilodontidae, and it can attain a body weight of over 15 kg and is the main fish (biomass) for the commercial fisheries of the São Francisco River basin. *P. costatus* (Valenciennes, 1850) is smaller and less abundant than *P. argenteus* and can achieve a body weight of 6 kg. Furthermore, both species are endemic to the São Francisco River basin, perform reproductive migrations (Sato & Godinho, 2003), and have potential for aquaculture (Godinho, 2007).

The aim of this study was to focus on fish larvae and to draw needed attention to this critical life period that is so important for fish aquaculture and fisheries. Additionally, we sought to understand the anthropogenic impacts on the ecosystem of *P. costatus* and *P. argenteus*, which are useful for the improvement of hatchery practices in aquaculture and for the characterisation of the species.

**MATERIAL AND METHODS**

The specimens of *P. costatus* and *P. argenteus* were captured by professional fishermen from the São Francisco River, in the Três Marias region, Minas Gerais, Brazil. Following capture they were confined in 1.5 x 1.5 x 1.5 m aquaculture cages, which were located inside large earthen tanks of 200 m² with a mean depth of 1 m at the Três Marias Integrated Centre for Fisheries and Aquaculture (CODEVASF). Fish were fed on commerce al feed containing 36 % crude protein at an amount equal to 1.5-2.0 % of their live weight per day, five days per week. For the experiments, fish selected were those in advanced gonadal maturation stages. For fertilisation procedures, females were chosen by external morphological characteristics that indicated that they were ready for spawning induction procedures; and males those that released sperm with gentle pressure on the coelomic cavity.

Specimens were induced to reproduce through hypophysation: a single dose of crude carp pituitary extract at a ratio of 2.5-3.0 mg/kg of body weight was given to the females (Woynarovich & Horváth, 1980). Fish were kept in reproduction tanks, with constant recirculating tap water at a temperature of 26 °C. A dry fertilisation procedure was conducted and eggs were then transferred to funnel-shaped incubators (20 litres), which were maintained at 25.5 °C. Every 10 minutes, fresh eggs samples were collected for analyses. After hatching, larvae of *P. costatus* and *P. argenteus* were maintained for four days, at a water temperature of 25.5 °C, with a photoperiod of 13h light and 11h dark. The water conductivity (106.5 ± 1.4 µS/cm) and pH (7.2 ± 1.1) were similar in both experiments. On a daily basis, larvae behavior and the number of dead larvae were recorded, and 14 larvae of each species were analysed for morphological changes.

For histology procedures, four larvae were fixed in Bouin’s (eight hours) and ten others in 2 % formalin (six hours) before both being transferred to 70 % alcohol. The larvae fixed in Bouin’s were then embedded in glycol
methacrylate and submitted to routine histological techniques following Pearse (1985), and stained with Toluidine Blue acid 1%. For those fixed in formalin, the standard lengths (SL, mm) were measured using an ocular micrometer attached to an Olympus SZ-11 stereoscopic microscope. Data were expressed as mean ± standard deviation (SD).

Handling of the specimens followed the guidelines and standards of CONCEA (Brasil, 2013).

Controversy exists regarding the nomenclature used to describe the different stages of early fish development. In the present study, we used the term larvae to indicate the ontogenetic period that begins at hatching and ends with the absorption of the yolk sac, as used by Santos and Godinho (2002) and Sado and Kimura (2006).

RESULTS

During the experiment, larval survival rate of *P. costatus* was 100 % and for *P. argenteus* it was 96.6 %. Fig. 1A-H showed details of the corporal morphology of these two species from immediately after hatching until four days post-hatching.

**Ontogenesis:** Daily changes are described in the next paragraphs.

Day one - SL (mm) *P. costatus* = 3.1 ± 0.34 and *P. argenteus* = 3.2 ± 0.04.

After hatching, the larvae had an elongated and transparent body with olfactory pits, encephalic vesicles, and otic vesicles with two otoliths. The yolk sac was filled with individualized yolk globules. Pronephric kidneys and the notochord were evident. The embryonic fin rounded the caudal region of the body. In both species, the retina was non-pigmented (Fig. 2A), the kidney ducts were evident and the intestine was obliterated (Fig. 2B). For both species, no adhesive organs were observed.

Day two - SL (mm) *P. costatus* = 3.8 ± 0.31 and *P. argenteus* = 4.2 ± 0.13.

Dendritic chromatophores appeared in the integument region of the gut and yolk sac, the mouth was obliterated, and the pectoral fin was observed in both species. The *P. costatus* larvae had 38-43 myomeres, whereas *P. argenteus* had 42-43, and button of arches became evident (Fig. 2C). The gas bladder was inflated and the lumen of the intestine was open (Fig. 2D).

Day three - SL (mm) *P. costatus* = 5.9 ± 0.26 and *P. argenteus* = 5.9 ± 0.22.

Dendritic chromatophores appeared in the tegument of the optic vesicles and the cardiac region. The mouth was open and in a sub-terminal position and the retina was pigmented. The arches showed lamellar protrusions and were partially covered by operculum (Fig. 2E).

Day four - SL (mm) *P. costatus* = 6.1 ± 0.29 and *P. argenteus* = 6.0 ± 0.18.

The pigmentation pattern remained the same as day three for both species, however, with greater intensity. The mouth occupied a terminal position, the yolk sac was reabsorbed, and the pectoral and caudal fins showed mesenchymal rays. The gill arches were entirely covered by operculum (Fig. 2F) and the intestine had a large lumen with pleated mucosa and an epithelium with striated border (Fig. 2G).

Swimming behaviour: Larvae of both species presented similar swimming behaviour during the larval development. During days one and two, they exhibited active movement in the vertical direction towards the surface of the water, but sometimes not reaching it before descending passively to the bottom. When in rest, larvae remained dispersed in the bottom of the incubator and in lateral recumbence. On day three, they also began to swim in the horizontal direction and when resting, they exhibited the same behaviour as the first two days. By day four, they were swimming at all levels and directions of the water column, and most of larvae when in rest remained in ventral decubitus.
DISCUSSION

Several factors have shown the importance to investigate fish larval biology, including the possibility of investigating natural larval responses under controlled laboratory conditions. Moreover, our ecosystems are currently threatened by climate change and pollution, and a deep understanding of fish larval biology is required to deal with these impacts (Helvik et al., 2009). In Brazil, at least 40 species of freshwater fish have potential for aquaculture (Godinho, 2007), and in order to improve the performance of native fish aquaculture, the early life history of fish larvae has to have known. This can provide basic information to improve the efficiency of a hatchery and reduce the larval high mortality rates (due to transition to exogenous feeding). Furthermore, this knowledge of the early life history describes characteristics that allow identification of species in the natural environment, as Ziober, Bialetzki and Mateus (2012).

Fig. 1. (A-D) Larvae of Prochilodus costatus, and (E-H) larvae of Prochilodus argenteus (A) Day one: 1=olfactory pit, 2=eye, 3=encephalic vesicles, 4=otic vesicle, 5=myomeres, 6=notochord, 7=embryonic fin, 8=yolk sac, 9=heart – standard length 3.1 mm; (B) Day two: 10=mouth depression, 11=dendritic chromatophores, 12=digestive tract; (C) Day three: 13=sub-terminal mouth, 14=eye with pigmented retina, 15=pectoral fin, 16=gas-bladder, 17=caudal fin, 18=gill arches; (D) Day four: 19=terminal mouth, 20=mesenchymal rays of the pectoral fin, 21=operculum. (E) Day one: 1=olfactory pit, 2=eye, 3=encephalic vesicles, 4=otic vesicle, 5=myomeres, 6=notochord, 7=embryonic fin, 8=yolk sac, 9=heart; (F) Day two: 10=mouth depression, 11=pectoral fin, 12=caudal fin, 13=dendritic chromatophores, 14=digestive tract, 15=gill arches; (G) Day three: 16=sub-terminal mouth, 17=eye with pigmented retina, 18=gas bladder, 19=operculum; (H) Day four: 20=terminal mouth, 21=mesenchymal rays of the pectoral fin. Scale bar = 1mm.
Fig. 2. Histological sections of the larvae: Day one - *P. argenteus*: (A) transversal section of the head showing brain (*) and non-pigmented retina (arrowhead); (B) transversal section of the body showing notochord (N), kidney ducts (arrowhead), and intestine (arrow). Day two - (C) *P. costatus*: sagittal section of the branchial region showing button of the gill arches (B). Day three - (D) *P. costatus*: sagittal section of a larva showing myomeres (M), notochord (N), kidney duct (arrowhead), gas bladder wall (G), intestine (I), and yolk sac (Y). Day three - (E) *P. argenteus*: sagittal section of the head and body, showing pigmented retina (arrowhead), encephalic vesicle (*), otic vesicle with two otoliths (O), gill arches (arrow), operculum (P). Day four - (F) *P. costatus*: frontal section of the head and body with pigmented retina, (arrowhead), branchial apparatus (*), operculum (P), heart (H), and pectoral fins (F). Day four - (G) *P. costatus*: sagittal section of the body highlighting caudal portion of the gas bladder (G), myomeres (M), and the intestine with striated border (I). Scale bar = 100 µm.
A detailed description of the chromatophores of fish larvae is important, as each species has a distinct pattern and location of chromatophore pigmentation that can be used in identification (Kendall, Ahlstrom, & Moser, 1984). Biologically, larval body pigmentation contributes to camouflage them in aquatic substrates (Sanches, Nakatani, & Bialetzki, 1999). In this study, the species presented only dendritic chromatophores, which is similar to what has been recorded in other neotropical species (Cavicchioli & Leonhardt, 1993; Sanches et al., 1999; Bialetzki et al., 2001; Santos & Godinho, 2002; Gomes, Matta, Araujo, Silva, & Zacaro, 2010; Nogueira et al., 2012a). The larvae of Prochilodus corruscans (Santos & Godinho, 1994), Brycon hilarii (Oliveira, Bialetzki, Gomes, Santin, & Taguti, 2012), and Clarias macrocephalus (Morioka, Vongvichith, Phommachanh, & Chantasone, 2013) showed only punctate chromatophores, while Trichogaster pectoralis (Morioka, Ito, & Kitamura, 2010) and Zungaro jau (Nogueira et al., 2012a) exhibited punctate and dendritic chromatophores. Moreover, Nakatani et al. (2001) stated that the intensity of corporal pigmentation of larvae could vary according to the habitat in which they live, whilst Oliveira et al. (2012) emphasized that changes in pigmentation patterns are a genetic characteristic of the species, and are thus useful in taxonomic differentiation.

According to Lasker, Feder, Theilacher and May (1970), the mouth opening and retinal pigmentation of the larval Sardinops caerulea occur almost simultaneously, since these two events are closely related to the start of fish feeding, which is in agreement with the larvae of this study that showed these events from day three. On the other hand, retinal pigmentation does not have a defined pattern across Neotropical fish species and starts at different developmental stages among species. The retina of Piabucina pleurotaenia (Allison, 1974), Helostoma temminckii (Souza & Severi, 2000), Auchenipterus osteomystax (Bialetzki et al., 2001), and Mogurnda adspersa (Close, Pusey, & Arthington, 2005) were pigmented at hatching. However, Araújo-Lima (1991), Economou, Daoulas and Psarras (1991), Santos and Godinho (2002), Gomes et al. (2010), and Nogueira, Godinho and Godinho (2012) reported for the species that they studied that pigmentation occurs after hatching, as in the species studied here. The reason for these different patterns of retinal pigmentation in Neotropical teleosts still remains unclear (Nogueira et al., 2012). Godinho, Santos and Sato (2003) suggested that for piscivorous species (Salminus brasiliensis and Pseudoplatystoma corruscans), mouth opening occurs earlier (day two) than in other species.

The development of the gas bladder is an important acquisition in the organogenesis of teleost larvae, because it facilitates their stability in the water column. In the present study, the gas bladders inflated on day two, as also recorded by Santos and Godinho (2002) for S. brasiliensis, another migratory species. The lumen of the pneumatic duct in the larval of this work was open and connected the dorsal wall of the oesophagus to the gas bladder; possibly, in these larvae, bladder inflation occurs when a larva reaches the surface of the water. The larvae of this study showed a button of pectoral fins on day three, similar to the findings of Araújo-Lima (1985) and Santos and Godinho (1996a) for other fish species. However, the larvae of Hoplias malabaricus (Matrovic & Pisano, 1989) and Inlecypris auropurpureus (Sado & Kimura, 2005) have already this structure immediately after hatching. For the two species in this work, as in other laboratory studies (Santos & Godinho, 1994; 1996a; 1996b; 2002 and Guimarães-Cruz, Santos, Sato, and Veloso-Junior, 2008), the emergence of pectoral fin buttons occurred before all other fins, which helped to the larvae movement in the water column.

The period of yolk sac absorption is vital to the larvae because it is the period when the digestive system is still in differentiation. The yolk sacs of P. argenteus and P. costatus larvae remained until day four; this result is in agreement with previous reports, which state that for Neotropical larvae this period may vary between two days in Zungaro jau (Nogueira...
et al., 2012a) to 12 days in *Hoplias lacerdae* (Gomes et al., 2007). Therefore, Guimarães-Cruz et al. (2008) affirmed that it is crucial to know the precise age of yolk exhaustion for artificial cultivation systems, since this facilitates the provision of food according to specific species development and thus reducing mortality rates during these delicate stages.

According to Woynarovich and Horváth (1980), the behavior of newly hatched larvae may differ between species. Some larvae swim vertically towards the water surface and then go to the bottom, and others move continually or occasionally. Shortly after hatching, the larvae of *P. argenteus* and *P. costatus* remained in lateral decubitus for most of the observation period. Possibly, this behaviour is due to the weight of the yolk sac and the absence of fins and gas-bladder. Occasionally, they had active vertical movements towards the water surface before descending passively. These observations are in accordance with Azevedo and Vieira (1938), Azevedo, Vianna-Dias and Vieira (1938), and Azevedo and Gomes (1942) for larvae of other Neotropical species. In addition, with the gradual reduction of the yolk sac and the emergence and development of the gas bladder and pectoral fins, the larvae began to move horizontally at different levels of water column.

Many of the problems related to juvenile fish production in aquaculture, as well as understanding the natural variation in fish populations, are linked to the larval stage (Helvik et al., 2009). In this sense, our study provides understanding about the morphophysiological aspects, species identification, larval development and growth, behaviour, and the ontogenic characteristics of *P. argenteus* and *P. costatus*.

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**RESUMEN**

Ontogenia temprana de larvas de peces neotropicales: *Prochilodus costatus* y *P. argenteus* (Characiformes: *Prochilodontidae*). El desarrollo temprano de las larvas de peces es un proceso altamente dinámico y estudiarlo proporciona información importante sobre su desarrollo ontogénico, su crecimiento bioenergético, su comportamiento, las características taxonómicas para la identificación en entornos naturales, la identificación de las zonas de desove y la vigilancia de la población. Los primeros años de vida de *Prochilodus argenteus* y *Prochilodus costatus* fueron estudiados desde la eclosión hasta la completa absorción de la yema, con el fin de proporcionar conocimiento sobre los caracteres taxonómicos y el crecimiento y comportamiento, lo cual puede ser aplicado al desarrollo de la cría de larvas. Las larvas fueron obtenidas junto a la Estación de Hidrobiología y Acuicultura de Três Marias, Minas Gerais, donde fueron reproducidas artificialmente. Inmediatamente después de la eclosión, aproximadamente 100 larvas de cada especie fueron acondicionadas en dos incubadoras de plástico. El comportamiento de las larvas fue registrado diariamente y fueron recolectadas 14 larvas de cada especie para análisis morfológico del cuerpo. En el primer día después de la eclosión las larvas de *P. costatus* y *P. argenteus* presentaban cuerpos alargados y transparentes. El saco vitelino se llenó de glóbulos de vitelo individualizados. En las dos especies, la aleta embrionaria circundaba la región caudal del cuerpo, la retina no presentaba pigmentos y el intestino estaba obliterado. En el segundo día, en las larvas de ambas especies habían surgido cromatóforos dendríticos, la boca estaba obliterada y fue registrada una aleta pectoral. Las larvas presentaban 38-43 miómeros en *P. costatus* y 42-43 en *P. argenteus*. En las dos especies la vejiga de gas estaba inflada y el lumen del intestino ya estaba abierto. En el tercer día, la boca tanto del *P. costatus* como del *P. argenteus* ya estaba abierta en una posición subterminal. La retina estaba pigmentada, los arcos branquiales tenían protuberancias laminares y estaban parcialmente cubiertos por el opérculo. En el cuarto día, el patrón de pigmentación se presentó con mayor intensidad; la boca ocupó una posición terminal, el saco vitelino fue casi completamente reabsorbido y las aletas pectorales y caudales presentaban rayos mesenquimales en ambas especies. El intestino mostró un amplio lumen, con la mucosa plegada y el epitelio con borde estriado. Las larvas de ambas especies mostraron un comportamiento similar de natación durante el periodo de prueba. Nuestro estudio provee conocimiento sobre aspectos morfofisiológicos, identificación de especies, desarrollo y crecimiento larval y características ontogénicas de dos peces subtropicales de importancia para la pesca comercial y deportiva.
**Palavras clave:** desenvolvimento da larva, ontogenia larval, cria de larvas, Prochilodontidae, comportamento de natação.

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