Stages of the embryonic development of the piavuçu *Leporinus macrocephalus* (Garavello & Britski, 1988)

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ABSTRACT. Understanding the embryogenesis of a species is a useful tool for locating areas for spawning and for the study of growth of the species in their natural environment. This study was conducted at the Panamá Fish Hatchery in Santa Catarina, Brazil, to characterize the embryonic development of the piavuçu *Leporinus macrocephalus* (Characiformes, Anostomidae). Description was based on the analysis of embryos collected at 10-minute intervals during the first three hours after fertilization, and later at 30-minute intervals until larva hatching. Piavuçu eggs are detached, transparent, small, spherical, with a large perivitelline space. Hatching takes place 11 hours and 30 minutes after fertilization at an average temperature of 28.2°C. Total length and weight of recently hatched larva were 2.39 ± 0.12 mm, and 0.5 ± 0.1mg respectively.

Key words: *Leporinus macrocephalus*, fish, embryonic development.

RESUMO. Estadios do desenvolvimento embrionário do piavuçu *Leporinus macrocephalus* (Garavello & Britski, 1988). O conhecimento da embriogênese de uma espécie é de grande importância, sendo uma ferramenta útil na localização de áreas de desova e no estudo do crescimento da espécie em ambiente natural. O presente estudo foi realizado na Piscicultura Panamá, Estado de Santa Catarina, e teve como objetivo caracterizar o desenvolvimento embrionário do piavuçu, *Leporinus macrocephalus* (Characiformes, Anostomidae). A descrição foi baseada na análise dos embriões coletados em intervalo de 10 min, durante as primeiras três horas após a fertilização, e posteriormente em intervalos de 30 min até a eclosão da larva. Os ovos do piavuçu se apresentaram livres, transparentes, pequenos, esféricos e com um grande espaço perivitelínico. Depois de 11 h e 30 min da fertilização, a uma temperatura média de 28,2°C, aconteceu a eclosão. O comprimento total das larvas recém eclodidas foi de 2,39 ± 0,12 mm com um peso de 0,5 ± 0,1 mg.

Palavras-chave: *Leporinus macrocephalus*, peixes, desenvolvimento embrionário.

Studies on the embryonic development of fishes, based on the monitoring of the evolution of eggs obtained from breeding in captivity, are a useful tool for the morphological and chronological characterization of events. Research in this area is very important to remove obstacles in egg and larvae identification collected from the environment (Snyder, 1981). Moreover, it may elucidate the ecological grouping of fishes, classifying them according to reproductive and ontogenetic characteristics (Sato, 1999).

Piavuçu, *Leporinus macrocephalus* (Characiformes Anostomidae), may be found in the Paraná and Paraguay river basins. It has a short and thick body, with three dark vertically elongated marks. The rear mark is sometimes diffuse and measures 600 mm (Britski *et al.* 1999). This species is commercially accepted and well know by commercial and sports fishermen and by collectors of ornamental fish. The fish is of excellent quality and when captured gives a good struggle when large. Fingerlings are very attractive. In spite of all these qualities, the piavuçu has been scantily studied, with no record on its embryonic development or on the species's larvae.

There is a current decline in the population of migratory fish owing to the construction of dams and deforestation along the riverbanks of Brazilian watersheds, transforming extensive stretches of rivers into lentic environments (Sato *et al.* 1996). The development of studies so that embryogenesis and other initial stages of life may be better understood are thus important to determine the distribution of eggs and larvae, the environmental conditions of spawning and growth areas. The embryonic development of the piavuçu *L. macrocephalus* will be provided in this paper.

Material and methods

This study was conducted during January 2000 at the Fish Hatchery, municipality of Paulo Lopes, Santa Catarina, Brazil.

Piavuçu reproducers, used for induced reproduction, were raised in 2.000 m^2 nurseries dug in the earth and maintained at a stocking density of 1 fish /8m^2. They were fed on balanced rations with approximately 28% gross protein.

A couple of *L. macrocephalus* were weighed and induced to reproduce through the application of Carp Pituitary Extract (CPE). Females received 0.5 mg/kg as first dose; a 5 mg/kg definitive dose was administered 9 hours later. Males first received a 0.4 mg/kg dose and a definitive dose of 1 mg/kg at 9-hour intervals too. The application was done by intraperitoneal injections. Immediately after the second application the fish were placed in a 1.000-liter plastic tank. The tank had an open water supply system, equivalent to 11 L/min. After 147 degree-hours (25°C) the final application, semi-natural hatching took place in the tank. It was noticed that the males emit sounds during the mating process.

After the eggs had been laid, a sample with approximately 2.000 eggs was transferred to a 60-liter funnel-type incubator, maintained without water recycling. Incubator was placed in a 200L tank with a heater and a thermostat to avoid wide temperature variations. Aeration and movement of eggs were conducted through a porous stone placed at the bottom of the conical part of the incubator. A 500µm mesh was immediately installed above the porous stone to avoid sedimentation of the eggs.

So that the different stages of embryonic development could be observed and documented, 15-20 embryos were collected at 10-minute intervals, in the first 3 hours, and later every 30 minutes until the time of hatching. The collection of eggs and larva was done with a 50 mL beaker, and then immediately fixed in buffered formaldehyde 4%. Morphometric data obtained from eggs included: total egg diameter, the diameter of the vitellus and the perivitelline space, measured between the chorion and the yolk sac (Nakatani, 2001). Characterization of the recently hatched larva and its measurements were undertaken according to Ahlstrom *et al.* (1976) and Leis and Trnski (1989). For the morphological characterization of the larva at hatching, total pre- and post-anal myomeres were counted, while volume of yolk sac was calculated according to the method by Heming and Buddington (1988).

Observation and identification of the embryonic stages were undertaken at the Marine Fish Culture Laboratory of the Federal University of Santa Catarina, Brazil, utilizing a stereomicroscope coupled to a printer.

Temperature and dissolved oxygen were measured hourly by an oxymeter, while pH, ammonia and nitrite were estimated by the colorimetrical method and measured at the beginning and end of the experiment.

Results

Average temperature during the incubation phase was 28.2 ± 0,9°C, varying between 26.9 – 28.8°C. Average concentration of dissolved oxygen was 7.76 ± 0.24 mg/L, while pH remained between 7.5 and 8.0. Total ammonia and nitrite did not exceed 0.3 mg/l and 0.01 mg/l, respectively.

During incubation, the *L. macrocephalus* eggs remained free and transparent. After fertilization, their diameter increased 6.26 times, owing to hydration of the perivitelline space, which became broad and easily recognized. Once hydrated, the egg had a diameter of 2.2 ± 0.08 mm, a perivitelline space of 0.64 ± 0.08 mm and a vitellus diameter of 0.95 ± 0.04 mm. The moment of fertilization is considered as time zero for description of the events of this study. Polarization was observed after 30 minutes. In this stage the animal pole (blastodisk) had the form of a semispherical cavity. Eggs appeared to be telolecithal with an accumulation of vitellus in the vegetal pole (Figure 1 A). The first cleavage occurred at minute 40 (Figure 1 B). In the next 30 minutes the blastomere divisions 4, 8, 16 and 32 occurred; and after 100 minutes the morula stage was defined (Figure 1 C). The segmentation step was concluded in the blastula stage, in which the blastocoele and the embryonic shield were formed. These events were observed after 200 minutes (Figure 1 D).

Gastrulation is characterized as a phase in which the cells of the blastodisk shift and separate into epiblast and hypoblast. After 220 minutes, the epiblast and the syncytial perivitelline layer spread to cover, initially, half of the vitellus (Figure 1 E). This step also included the conformation of the embryo body, which occurred at minute 230. The closing of the blastopore took place between 275 and 305 minutes after fertilization (Figure 1 F). At this time, the neural line was also recognized, whereas the cephalic region became distinct from the caudal after 365 minutes (Figure 2-A).

At minute 425 the optical vesicles appeared at the level of the cephalic portion of the neural tube (Figure 2 B). The otic capsule was distinguished at minute 515 when presence of myomeres was clearly observed (Figure 2 C). The otoliths were distinguished just before hatching.

Within the chorion the embryo of the *L. macrocephalus* began to have strong contractions after 595 minutes, when the caudal portion was separated from the vitelline sac.

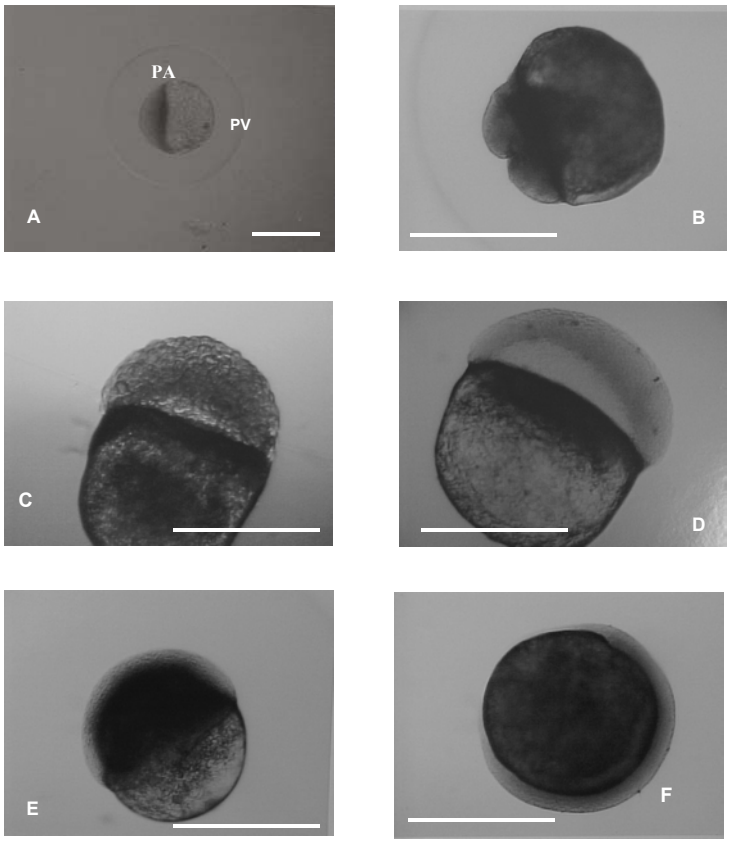

Figure 1. A- Phases of embryonic development of the piavuçu *Leporinus macrocephalus.* Formation of the blastodisk, distinguishing the animal pole (PA) and vegetal pole (PV). B- Segmentation. C- Morula. D- Blastula with formation of embryonic shield (EE). E- Gastrulation. F- Blastopore closure. (Bar: 1.0 mm)

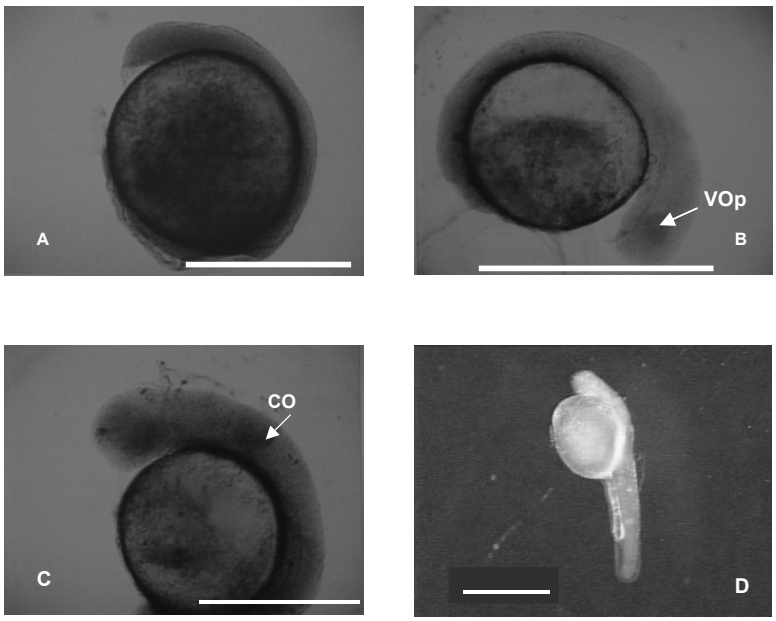

Figure 2. Phases of embryonic development of the piavuçu *Leporinus macrocephalus.* A - Distinction of head and caudal B - Appearance of optical vesicle (VOp) C – Formation of otic capsule (CO) D – Recently hatched larva (Bar: 1mm)

After 660 minutes, there was a thinning of the chorion. Hatching took place at minute 690 (11 hours and 30 minutes), when the first larva was observed (Figure 2-D). The hatching rate reached in this study was 99.25%. The larva at the time of hatching was long, with a standard length varying from 2.24 to 2.41 mm (2.39 ± 0.12 mm). The notochord was not flexed and was visible because of the transparency. Outline of the eyes was round with little pigment. The embryonic membrane ("finfold") was hyaline and without pigmentation. The airbladder was not inflated and had no pigmentation. The intestine was closed, relatively long and with pigmentation. Volume of vitelline sac was relatively large (0.25±0.03 mm^3). The myomeres were visible and totaled between 21 and 26 (between 17 and 20 in the pre-anal region and 4 and 6 in the post-anal). The recently hatched larva had a total length of 2.39 ± 0.12 mm and a weight of 0.5 ± 0.1mg, with vigorous vertical swimming.

Discussion

The variety of reproductive strategies of fish cause great differences among species in the number of eggs, egg size, quantity of reserves accumulated during oogenesis, rhythm and duration of the embryonic development. This is due to the fact that the reproductive options selected by the species have strategic ecological importance. According to Blaxter (1988), the influence of the initial size of the egg on survival and development has important ecological implications. Large eggs generally produce larger larvae with higher reserves of yolk sac. However, this characteristic may provoke future problems in their movement and the prolongation of the period of endogenous feeding.

Most migratory commercially important characids have eggs with rather big diameters: 1.80 ± 0.15 mm for the *Colossoma macropomum* (Araujo and Goulding, 1998), 2.90 to 4.25 mm for the *Brycon* (Lopes *et al.,* 1995; Sato, 1999), 3.20 to 3.80 mm for the *Salminus* (Morais Filho and Schubart, 1955; Sato, 1999) and 3.00 to 4.70 mm for the *Prochilodus* (Vazzoler and Menezes, 1992; Sato, 1999). Smaller sizes may be found in species of the genus *Leporinus*. Sizes may reach 2.30 ± 0.09 mm for *L. elongatus*, 2.20 ± 0.10 mm for *L. reinhardti* and 3.02 ± 0.11 mm for *L. taeniatus* (Sato, 1999). In this study, the egg of the piavuçu *L. macrocephalus* had an average diameter of 2.2 ± 0.08 mm during incubation.

After hydration, the piavuçu eggs increased in volume by 6.26 times. Sato (1999) also found a high degree of hydration in the eggs of other species of *Leporinus*; an increase of 6.37 times the volume for *L. elongatus*, 11.1 times the volume for *L. taeniatus* and 11.9 times the volume for *L. piau*. Lake (1967) mentions that a large perivitelline space protects the embryo against damage in flowing water, an environment in which most migratory fish lay their eggs (Baumgartner *et al.,* 1997). These eggs are rather different from the eggs of demersal fish which have a small perivitelline space (Ihering and Azevedo, 1936; Godinho *et al.* 1978). A large perivitelline space (0.64 ± 0.08 mm), such as found in the eggs of *L. macrocephalus*, indicates that its initial stages should occur in lotic environments as occurs with other migratory characids.

The importance of physical-chemical parameters such as temperature, ionic concentration, pH and luminosity, is evident in the aquatic environment, since brusque changes or insufficient size may alter the characteristics of the embryo and the resulting larva. Among these parameters temperature has the greatest influence in the hatching of larva. Working with eggs and larva of *Prochilodus scrofa* at different temperatures, Curiacos (1999) observed that between the fertilization of the eggs and the beginning of exogenous feeding, 53.7 or 114.5 hours are needed when the eggs are maintained at 32 or 23°C, respectively. It has also been found that the embryonic development was not complete when the incubation was conducted at a temperature of 20°C. According to Lopes *et al.* (1995), the larvae of the pacu, *Piaractus mesopotamicus*, and of the tambaqui, *Colossoma macropomum*, hatch 13 hours after fertilization when the temperature varies between 28 and 30°C. Embryonic development increases to 18 hours when the temperature is 25°C. According to Landínez (1995), at a temperature of 27°C, the larva of yamu *Brycon siebenthalae* hatch 12 hours after fertilization. According to Lopes *et al.* (1995), the larva of matrinxã, *Brycon cephalus*, hatch 10.5 hours after fertilization at 30°C. According to Sato (1999), the larva of *L. elongatus* incubated at a temperature between 23 and 24°C hatch 477 ± 11 degree-hours, while the evolution of the egg of *L. taeniatus* until hatching took 512 ± 13 degree-hours at the same temperature range. Length of the embryonic period of the piavuçu *L. macrocephalus* analyzed in this study, was 11 hours and 30 minutes, at an average temperature of 28.2 ± 0.9°C, equivalent to 324 degree-hours.

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