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RESEARCH

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Embryogenesis and early larval development in bocachico (*Prochilodus magdalenae*) in Colombia

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Abstract

Background: The bocachico (*Prochilodus magdalenae*) is a migratory freshwater fish and one of the main species of Colombian continental fishery. Interest in it is due to the fish's excellent reproductive characteristics and sociocultural value in its native region. As part of native fish species repopulation programs carried out by the GIPEN research group of the San Silvestre fish farm and ISAGEN, the present study aimed to characterize the embryogenesis and early larval development of bocachico from zygote phase to the exogenous feeding period, using morphological and histological landmarks.

Results: Embryos were obtained by fertilization from hormonally induced, wild-caught brood stock, and subsequent development was monitored at temperatures coinciding with native conditions. Embryonic development from fertilization to hatch lasted 13 h. This included the following stages: zygote, cleavage, blastula, gastrula, segmentation and organogenesis, pharyngula and hatching. Larvae emerged with unpigmented eyes; the cephalic area and the eye primordium were more developed at 1 day post-hatching. At 2–3 days post-hatching, the somites formed myotomes and pharyngeal arches were present as well as an undifferentiated digestive tract (endogenous feeding period). At 3–4 days post-hatching, the mouth was open, and the oral valve was developed; the mouth was continuous with the esophagus and the rest of the digestive system (beginning of the endo–exogenous feeding period). The yolk was observed for up to 9 days post-hatching (end of the endo–exogenous feeding period and beginning of the exogenous feeding period).

Conclusion: To the best of our knowledge, this is the most comprehensive analysis made of the development of the bocachico to date, from early embryonic development to the larval phase, and this research will contribute to improving the knowledge of the developmental intervals of this species, the critical stages in feeding phases, as well as a more concrete approximation of when the fish is suitable for repopulation based on its morphological development.

Keywords: Bocachico, Fish, Ontogeny, Embryonic development

Background

In Colombia, fishing and agriculture represent two important sectors of food production for national consumption and for exports, as well as two multipliers of the local economy that contribute to the reduction in poverty in rural zones. The country has substantial agricultural development potential due to a great quantity of water assets both of continental and of marine

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origin, optimal weather conditions for the cultivation of both tropical and subtropical species, and a wide range of aquatic organisms with aptitude for domestication. Despite aquacultural activity growing quickly in recent decades, the development and consolidation of the sector has progressed slowly both in relation to its potential and, in comparison, to other agricultural sectors. Its participation in wealth generation is quite low, and its contribution to Colombian food requirements, especially those of the poorest consumers, is not very significant (MADR, 2014).

In Colombia, fish farming has an increasing market and the potential for international expansion in the coming years. At present, 20% of the entire national fish production is reserved for the export market. According to the UPRA (Rural Agricultural Planning Unit), there are more than 18 million hectares available for the realization of this goal and to date; there is an estimated record of 2200 hectares in production, which is only 0.01%.

Fish farming is crucial for addressing human food demand in Colombia. Bocachico (*Prochilodus magdalenae*) farming is of great interest due to the fish's excellent reproductive characteristics, the fact that this species is a bottom feeder, and due to its sociocultural value in its native region.

The bocachico is a migratory freshwater fish and can be found in both swamps and rivers. It is one of the main species of the Colombian continental fishery. The bocachico is a medium-sized fish belonging to the order of Characiformes and the family of Prochilodontidae, which can reach up to 50 cm in length. It has a small, fleshy, prominent mouth with a series of tiny teeth and a prickly spine. Its color is uniformly silver with shades of red or yellow and has the peculiarity of having scales that are rough to the touch (Mojica, et al., 2012).

The bocachico is an endemic Colombian fish and is distributed in low areas of the Magdalena, Sinú and Atrato rivers and their tributaries. It can also be found in the marshes surrounding these rivers, in the Guájaro reservoir, in the Laguna de Luruaco and in the Canal del Dique. As for the state of exploitation and conservation of the bocachico in the Colombian industry, it is one of the most representative species of the Colombian ichthyofauna to the point of being considered an emblem in the Magdalena River fisheries. This species has been long known to Colombians and is considered an economic resource for many farmer households and a food source for thousands of riverside inhabitants (MADR—Ministerio de Agricultura y Desarrollo Rural 2008). However, the fish has been captured in juvenile or pre-adult states that do not exceed the legal minimum size of 25 cm and, since the species reaches its sexual maturity between 20 and 25 cm, a progressive decrease in its spawning biomass has been evidenced (Valderrama & Solano, 2004).

With the objective of repopulating *P. magdalenae* in the Magdalena River basin, numerous projects intending to characterize the development as well as zootechnical and productive characteristics of the species have been designed and implemented. In that sense, the characterization of ontogenic development was carried out and is shown below.

Methods

Location

The investigation was carried out at Piscícola San Silvestre S.A. (PSS), located in the municipality of Barrancabermeja (Santander), with geographical coordinates of 7° 06′31 North, 73° 51′23 West, at 75 MAMSL, with an average annual temperature of 28.4 °C.

Fish source

The broodstock (Fig. 1) was captured from the natural environment in the Magdalena river basin. They were moved to the PSS, where after a period of quarantine, they were transferred to ponds on land at a density of one fish every five square meters. Fertilizers were periodically provided to maintain adequate levels of primary productivity (phyto and zooplankton) in addition to balanced feed of 34% CP (1.5% of biomass) distributed in two daily rations.

For the management of experimental broodstock, they were desensitized with Eugenol at a dose of 40 mg/l for 2 min or until they observed relaxation and loss of mobility. Once the experimental phase was finished, the broodstock were left in ponds on land with good feeding until their recovery (Millán-Ocampo et al., 2012; Álvarez-Perdomo et al., 2016).

Gamete collection and in vitro fertilization

In the rainy season, evaluation of the different batches of broodstock was performed by slight abdominal pressure in the cranial–caudal direction, seeking to obtain semen from the males; in females, bulging and abdominal sagging were evaluated. The specimens that met these conditions were transferred to the management room where males and females were placed separately in

Fig. 1 *Prochilodus magdalenae* breeding female

circular pools, 2.1 m in diameter. An ovarian biopsy was taken from the females by the cannulation method and a sample of oocytes extracted, to which clarifying solution (Serra Liquid) was added, and the sample observed under stereoscope (Leica EZ4W). When the majority of oocytes presented the nucleus in migration, the female was considered fit for the application of the hormonal protocol. To the selected females, 5 mg/kg of EPC was applied intramuscularly in two doses, the first comprised 10% of the total dose, and 12 h later, a second dose (90%) was applied; at the time of application of the second dose in females, the total dose of EPC was applied to males consisting of 5.5 mg/kg of weight. Six hours later at approximately 28 °C, the females were removed from the water, dried thoroughly, and the oocytes, free of any contaminant, were removed and deposited in a clean container. Immediately afterwards one or two males were removed, dried, urine extracted and the semen, free of contaminants, was dropped onto the oocytes. With the help of a pen, the sexual products were mixed, and water was added to activate the sperm and start oocyte hydration.

Histology of the embryonic stages

Fertilized eggs from five female batches were spread onto 9-cm petri dishes at a density of ~150 eggs/petri dish. Early (zygote, cleavage, blastula and gastrula) and late (segmentation to hatching period) stage embryos were observed at 10-min and 30-min intervals in the late phases, respectively. Developmental stages were determined morphologically using a microscope (Leica DM750) equipped with a digital camera (Leica MC120 HD). Images of embryos were selected to categorize different developmental stages. To objectively describe *Prochilodus magdalenae* embryonic development, embryogenesis was divided into seven stages using well-known markers for freshwater fishes including zebrafish and longspine scraper, *Capoeta trutta* (Kimmel et al., 1995; Zadmajid et al., 2017). This included the following stages: zygote, cleavage, blastula, gastrula, segmentation and organogenesis, pharyngula and hatching.

Histology of the embryonic and larval stages

To characterize embryonic stages, 20 fertilized eggs from each stage were fixed in 10% buffered formalin, embedded in paraffin and sectioned at 5–6 µm with a rotatory microtome Leica RM2125 RTS. Sections were stained with hematoxylin and eosin (H&E) according to standard procedures (Wijayanti et al., 2017).

To characterize larval organogenesis, the fish were randomly sampled daily from three batches during the endogenous feeding period, endo–exogenous feeding period, and commencement of the exogenous feeding period. Sampled larvae were anesthetized with clove oil (40 ppm; Sigma-Aldrich, Inc., St. Louis, MO) and fixed in 10% buffered formalin, embedded in paraffin and sectioned at 5–6 µm with a rotatory microtome Leica RM2125 RTS. Sections were stained with hematoxylin and eosin (H&E) according to standard procedures, and slides were photographed with a Nikon Eclipse E600 microscope with a digital camera (NIKON DXM120).

For the management of the larvae, they were first anesthetized and later killed by thermal shock, that is, by immersion in a proportion of 60% ice and 40% water (average temperature of 4° C) for 5 min (Millán-Ocampo et al., 2012; Álvarez-Perdomo et al., 2016).

All procedures involving animal handling were performed in accordance with the standards for laboratory animal use described by the Committee on Care and Use of Laboratory Animal Resources of the National Research Council (National Academies, USA). National Research Council of the National Academies Eighth Edition (Albus, 2012).

Results

Zygote period (one cell)

The zygote period comprises the interval between fertilization and the occurrence of the first cleavage. It is characterized by the presence of a fertilized egg, which has a round, oval or polyhedral shape (Fig. 2). The longitudinal axis of the newly fertilized egg is longer than the transverse axis, and the animal pole is narrower than the vegetal pole (Kratochwil et al., 2015). The egg has a peripheral eosinophilic sheath called the vitelline membrane that covers the whole egg and is located next to the first egg membrane. The vitelline membrane is separated from the other structures of the egg by a clear space,

Fig. 2 *Prochilodus magdalenae*, zygote period, 1C: 1-cell

called the perivitelline space. The first egg membrane appears to increase in size over time. Inside, there is a space that occupies more than 95% of the volume and is filled with round, pleomorphic, eosinophilic particles in several sizes, some containing vacuoles that are separated from each other by small translucent spaces. Some eggs contain a basophilic, vitellus material with amorphous granules between the vitellus granules corresponding to non-yolky cytoplasm, which begins to stream toward the animal pole, segregating the blastodisc from the yolky, granule-rich, vegetal cytoplasm. This segregation continues during early cleavage stages.

Cleavage period

Two-cell stage (20 min PF)

In this stage, we were able to show the start of the cleavage 20 min post-fertilization (PF), which consisted of a meroblastic or partial cleavage of the blastodisc located in the animal pole. This division was presented in intervals and regular orientation, resulting in two equal, large cells that in the case of the bocachico (*Prochilodus magdalenae*) have a domed appearance or convex shape (Fig. 3A).

In the histological characterization of the H&E-stained sections of this phase, we observed oval-shaped, embryonated eggs containing an external, thin eosinophilic structure that encompasses the whole egg and corresponds to the vitelline membrane, separate from the other structures of the egg by a clear space; the perivitelline space. The central and most abundant material corresponded to vitellus, composed of circular and pleomorphic eosinophilic proteinaceous particles of different sizes, separated from each other by small, translucent spaces, and some containing vacuoles inside. Among the granules of vitellus, a basophilic material was observed.

Four-cell stage (30 min PF)

The two blastomeres cleave incompletely. The partially ellipsoidal shape of the blastodisc was seen. The animal pole showed an incomplete division; the cells were dome-shaped, smaller than in the previous phase and becoming increasingly convex toward the vitelline membrane (Fig. 3B). Here, the first to second cleavage yielding four cells occurs (Fig. 3A). Histologically, the findings were similar to the previous phase; however, the cells are smaller and are characterized by their intense basophilic color and domed shape (convex). Abundant, circular and pleomorphic eosinophilic proteinaceous particles of different sizes corresponding to vitellus granules were observed.

Eight-cell stage (40 min)

In this stage, the blastodisc, located at the animal pole, was a darker shade than the rest of the egg. Histologically, in this phase the vitellus occupied 75% of the surface area and was mainly located at the center of the egg, composed of pleomorphic eosinophilic proteinaceous particles, with different sizes, separated from each other by small translucent spaces, and some containing vacuoles inside (Fig. 3B). The cleavage was characterized by the formation of two rows of four cells (blastomeres) clearly recognizable, smaller than in the previous phase and with an intense basophilic color (Fig. 3A).

16-cell stage (50 min)

The fourth set of cleavages occurs at 50 min (Fig. 3A). The cells presented a domed shape with a convex surface similar to the previous phases, but smaller. Histologically, it was striking that cells have more defined contours and some of them were observed in process of active cell division (Fig. 3B).

32-cell stage and 64-cell stage (60–70 min PF)

These stages occur between 60 and 70 min post-fertilization. In the 32-cell stage, cleavage was observed with a 4×8 arrangement vertical and parallel to the first cleavage, with smaller blastomeres than in previous stages and darker than the rest of the egg (fifth cleavage). In the 64-cell stage for the first time, two layers of blastomeres were observed, one on the top of the other with smaller cells than in the previous stages. This phase corresponds to the sixth cleavage (Fig. 3A).

Blastula period (80–220 min)

From 128-cell to 30% epiboly, in this phase, the blastodisc began to take the shape of a ball (Fig. 4A), with the cells arranged as a solid half ball with a high mound of cells

(See figure on next page.)

Fig. 3 **A** *Prochilodus magdalenae*, developmental cleavage period from (A) 2C: 2-cell; (B) 4C: 4-cell; (C) 8C: 8-cell; (D) 16C: 16-cell; (E) 32C: 32-cell to (F) 64C: 64-cell. **B** *Prochilodus magdalenae*, developmental cleavage period from (A) 4C: 4-cell with the animal pole showing an incomplete division; the cells are dome-shaped and become increasingly convex toward the vitelline membrane, H&E 4X; (B) 8C: 8-cell, the blastodisc is characterized to be a clear, yolk-free region located at the animal pole. The vitellus occupies 75% of the surface area composed of circular and pleomorphic eosinophilic proteinaceous particles with different sizes, H&E 4X; (C) 16C: 16-cell. The cells have a domed shape with a convex surface, some of them being in the process of active cell division, H&E 40X. Abbreviations: AP: animal pole; B: blastodisc; CD: active cell division; YG: yolk granules

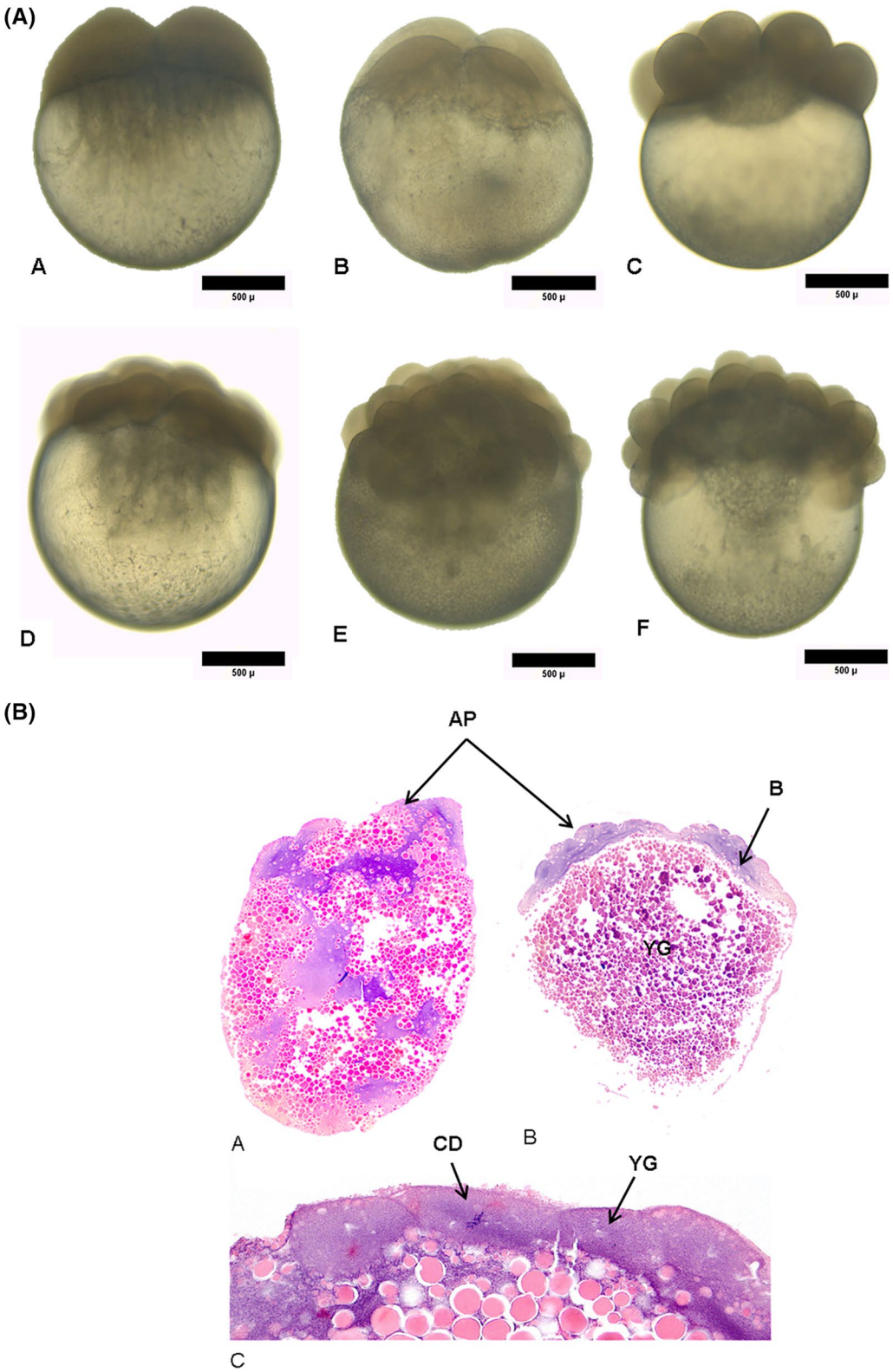

Fig. 3 (See legend on previous page.)

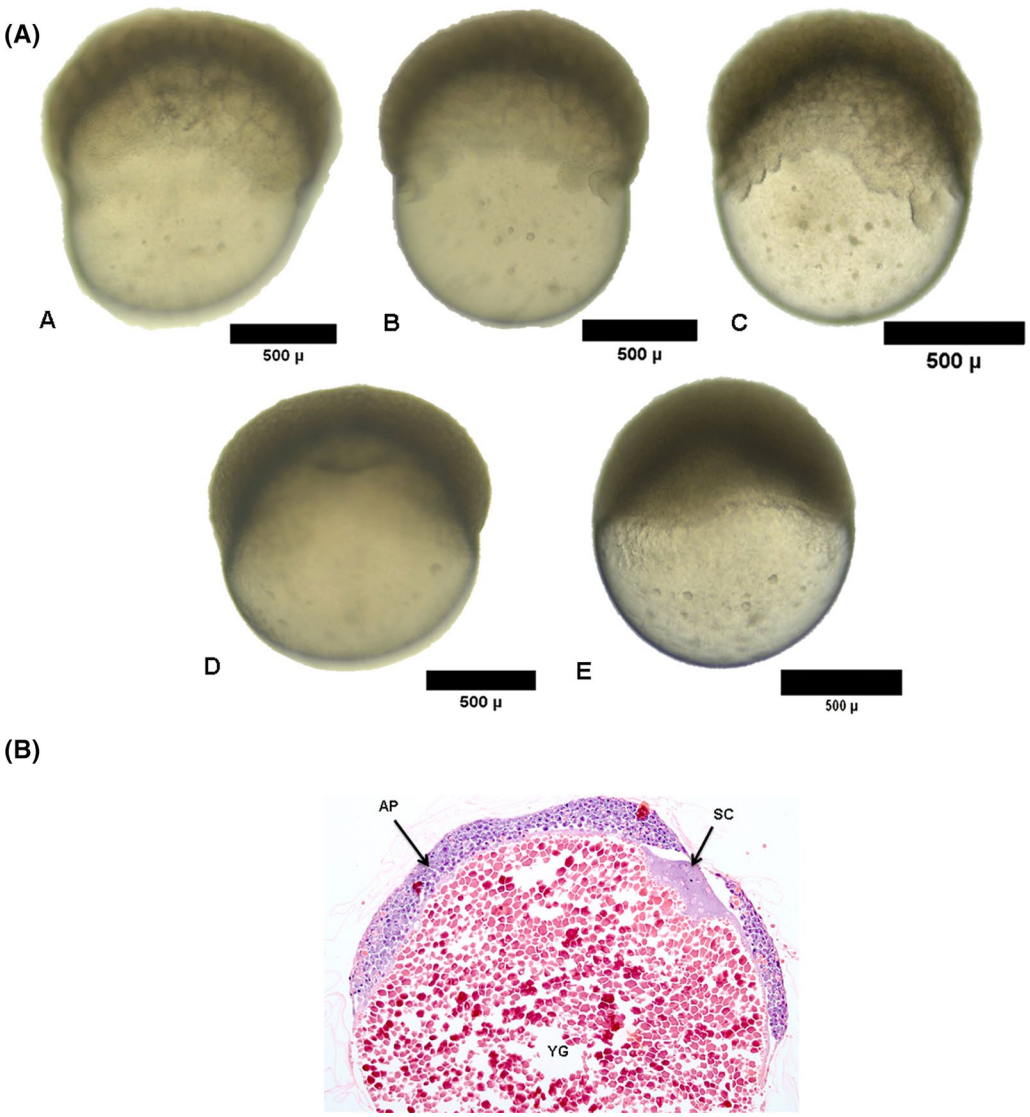

Fig. 4 **A** *Prochilodus magdalenae*, developmental blastula period from (A) 128C:128- cell; (B) 256C: 256-cell; (C) 512:512-cell; (D) > 1 K: High; to (E) 30% e.b.: 30% epiboly. **B** *Prochilodus magdalenae*, developmental blastula period. The cells are organized in more than 10 rows. A 10% epiboly stage is recognized, and a layer of elongated cells appears with slightly basophilic oval nuclei yolk syncytial layer (YSL) as part of the vitellus and support the animal pole, H&E 10X. Abbreviations: AP: animal pole; SC: syncytial cells; YG: yolk granules

on top of the yolk (Fig. 4B). These cells are smaller than in the previous stages, rounder and their edges and contours more defined. Translucent spaces were observed between the cells. The external layer of the blastomere showed a squamous appearance, forming the enveloping layer (EVL). In this phase, the cells were organized into more than 10 rows; the blastomeres at the ends lose their lower borders at yolk binding sites; the blastodisc flattens down upon the yolk cell and the animal–vegetal axis of the blastula shortens. The yolk syncytial layer (YSL) was seen as a layer of elongated cells with slightly basophilic, oval nuclei which are part of the vitellus and support the animal pole. This phase finished when epiboly reached a 30% (Fig. 4A). From this point, the blastoderm began a non-uniform thickening.

Gastrula period (4–6 h PF)

This period ranged from 50% epiboly to bud stage (Fig. 5A); the first thing we observed in the bocachico embryos was the expansion of the blastoderm, reaching 50% of the yolk. Additionally, at this stage a thickening was observed on the edge of the expanding blastoderm toward the vegetal pole corresponding to the germinal ring, composed of a superficial layer (the epiblast) and

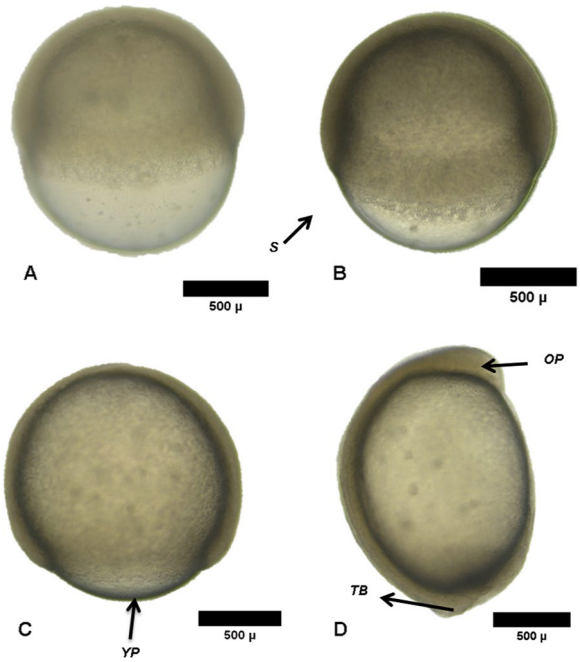

Fig. 5 *Prochilodus magdalenae*, developmental gastrula period from 50% e.b.: **A** 50% epiboly, **B** 75% epiboly, **C** 90% epiboly, and **D** bud stage. Abbreviations: OP: optic primordium; S: shield; TB: tail bud; YP: yolk plug

an internal layer (the hypoblast). A small widening corresponding to the embryonic shield was observed at one end of the blastoderm, and 60% coverage of the yolk by blastoderm was seen too. Approximately 5 h post-fertilization, 90% epiboly and the appearance of the yolk plug was observed. 100% epiboly was observed at 6 h PF, with the blastoderm completely covering the yolk plug, indicating the end of gastrulation. Additionally, near the region where the yolk plug closed, a small widening corresponding to the tail bud was observed (Fig. 5D).

Segmentation and organogenesis period (7–9 h PF)

During this period, we could observe the formation of the central nervous system CNS primordium (Fig. 6A), accompanied by the appearance of the first somites (4–6 somites on both sides) of the neural tube. Structures including the tail, the optic placodes and auditory vesicle begin to take shape, and the beginning of brain development was also evident in this period (Fig. 6B and C). Few foci of pigmentation on the yolk sac and later on the body axis were seen. At 8 h, the 12-somite stage was identified, the optic placodes begin to be discerned as solid masses, and the brain area was more evident (Fig. 6B). The 25 somite stage was observed at 9 h PF. Here, the head subdivision began to be seen more clearly; the tail bud is more prominent and protrudes away from the body. Kupffer's vesicle was seen at the base of the tail (Fig. 6B) and the optic and otic placodes showed further development.

Pharyngula period (9–12 h PF)

At the pharyngula stage, the embryo has developed a large number of somites with a symmetrical, bilateral distribution that extends to the end of a long post-anal tail (Fig. 7) and separated by septa. A well-developed notochord is visible. The nervous system is hollow and expanded anteriorly. At 10–11 h, extension of the tail rudiment barely begins to elongate the embryo. The eye primordium was seen, including the first stage of the retina, at 12–13 h.

Hatching period and early larval development (13 h PF to day 13 post-eclosion)

During the hatching period, the embryo continues to grow at about the same rate as before. Morphogenesis of many of the organ rudiments is now almost complete and slows down considerably (Fig. 8A), with some notable exceptions including the gut and its associated organs. The cephalic area and the eye primordium are more developed at 13–14 h and at 1 day post-hatching. At 2–3 days post-hatching, the somites take on an appearance that indicates the future division of the myotomes into the dorsal (epaxial) and ventral (hypaxial) muscle masses (Fig. 8B). Primordia of the pharyngeal arches are present at 2–3 days post-hatching, but at early timepoints they are difficult to distinguish individually. At 3–4 days post-hatching, the neural groove is observed and the nervous system is hollow and expanded anteriorly; also, the chondrocranium is recognizable; it is the initially cartilaginous region of the skull that eventually surrounds the brain and forms capsules over the sensory organs, including the olfactory organ, eye and ear (Fig. 8B). The white matter and the gray matter are well differentiated; in addition, the pons and spinal cord are recognized. The eyes have distinctive layers, including pigmented layers of the retina. Pigmentation is prominent in the eye but is still light enough so that one can readily visualize the unpigmented cell nuclei in this retinal epithelium. The lens is in an advanced stage of development. Some pharyngeal arches have small amounts of immature cartilage; the mandibular and hyoid arches form the jaws and the operculum.

The buccopharyngeal cavity was present, open and lined by a squamous epithelium surrounded by a thin layer of connective tissue. The oral valve was also present and the esophagus was visible with a cuboidal epithelium.

The heart is identified with differentiation between the atrium and the ventricle; the liver has undeveloped hepatocytes with cytoplasmic vacuoles. In the kidney,

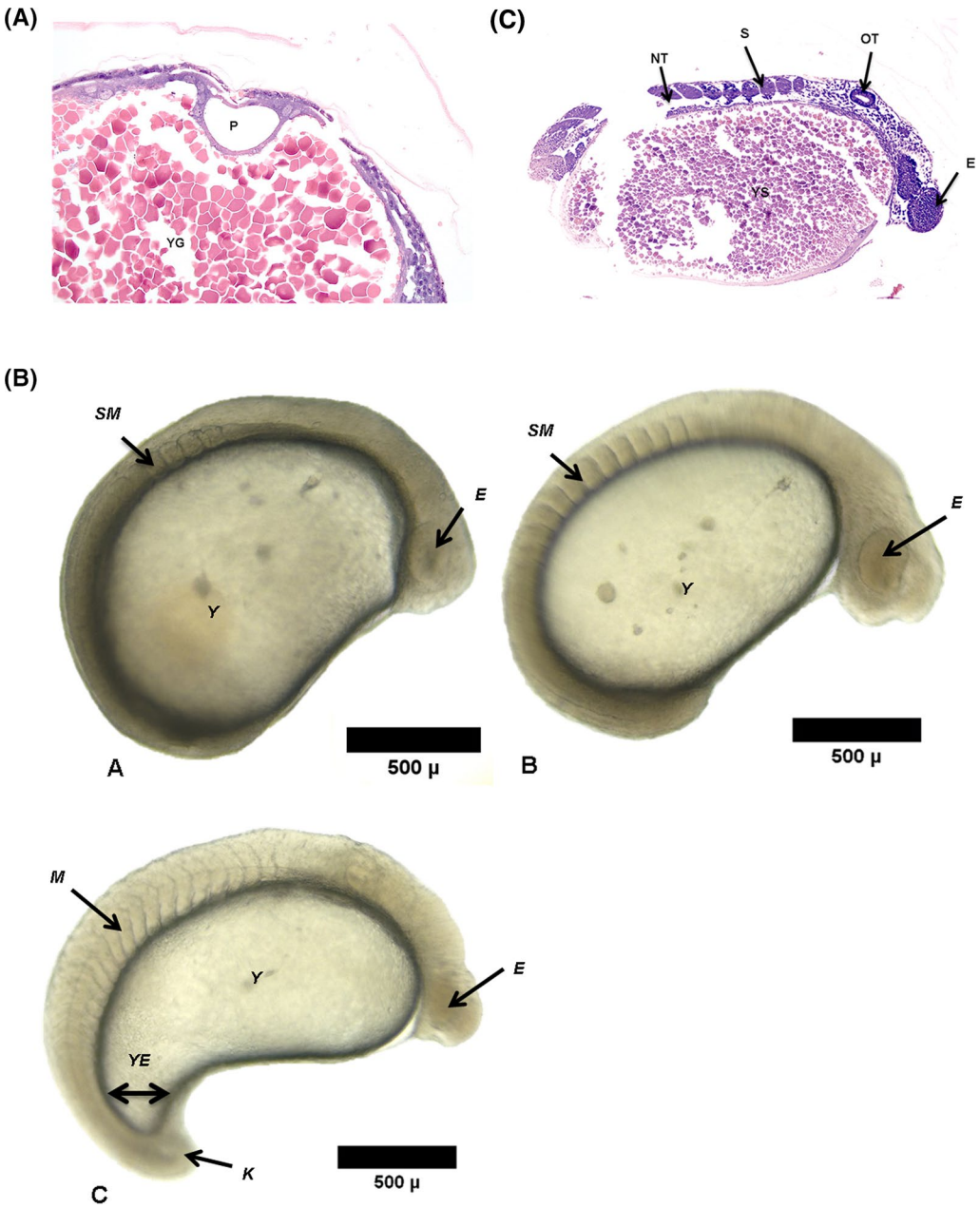

Fig. 6 **A** *Prochilodus magdalenae*, developmental segmentation and organogenesis period, the anlage organ or primordium of the CNS is recognized, H&E 20X. Abbreviations: P: primordium of the CNS; YG: yolk granules. **B** *Prochilodus magdalenae* segmentation period. (A) Life embryos with: 4–6 somites; (B) 18 somites; (C) 25 somites. The optic placodes become discernible as solid masses, the head subdivision is more apparent, and the tail bud is more prominent. Abbreviations: E: eye; K: Kupffer's vesicle; M: myomere; S: shield; SM: somites; Y: yolk; YE: yolk extension. **C** *Prochilodus magdalenae* segmentation period. The neural tube is recognized. Further, the somites, tail, and eye can be observed, H&E 4x. Abbreviations: NT: neural tube; E: eye; S: somite; YS: yolk sac

few renal tubules with cuboidal epithelium are now recognizable, and there is also hematopoietic tissue. The swim bladder appears ventral to the kidney and has a thin layer of squamous cells with an elongated nucleus. The primordium of the intestine is located ventral to the bladder, one or more layers of cylindrical epithelial cells are observed, and the intestinal folds are not yet recognized. The red muscle is recognized although it is not striated. The skin has scales and a simple epithelial layer.

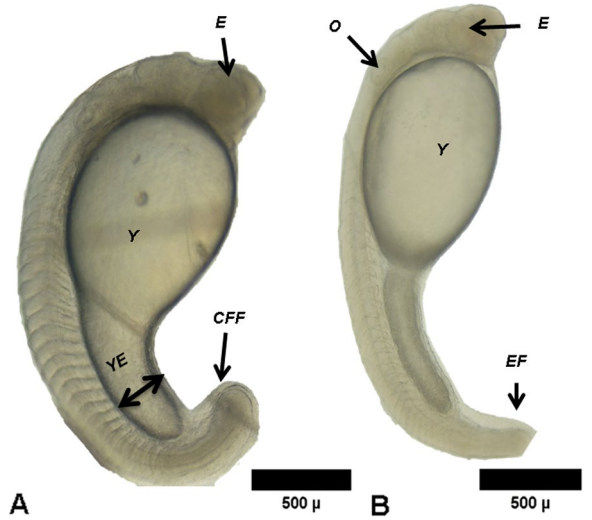

Fig. 7 *Prochilodus magdalenae* pharyngula period. **A** A completed set of somites that extend to the end of a long post-anal tail is seen. The nervous system is hollow and expanded anteriorly; **B** extension and elongation of the tail were seen and the eye primordium was seen too. Abbreviations: CFF: caudal fin fold; E: eye; EF: embryonic fin; O: otic cup; Y: yolk; YE: yolk extension

At 6 days post-hatching, the neurocranium is observed; it is composed of cartilage. The brain has lobe differentiation. The esophagus can be seen with epithelial cells, submucosal glands and a muscle layer.

At 7–8 days post-hatching, the gill arches have larger filaments and lamellae. The gastric glands start their development and differentiation and the gastric muscle tunic is recognizable. At 8–9 days post-hatching, the gill arches have larger filaments and lamellae and a larger number of scales on the skin. Finally, a better differentiation of the optic lobe and olfactory lobe is observed in the brain (Fig. 8B).

At 10–12 days post-hatching, the neurocranium is observed; it is composed of cartilage and bone (Fig. 8B). The optical lobes and olfactory lobes are distinctive, and meninges, pons and spinal cord are observed. The gill arches are developed with filaments and developed lamellae. In the digestive system, the esophagus can be seen with epithelial cells, glands and a muscle layer; the stomach has developed muscle layers. The liver has hepatocytes with basophilic cytoplasm in gradual maturation. In the kidney, the cranial kidney with hematopoietic tissue is distinguished from the caudal kidney with tubules (Fig. 8B).

Discussion

In the present study, we characterized the different phases of the ontogenic development of bocachico (*Prochilodus magdalenae*), a native species in some regions of Colombia. This was done in order to establish both its reproductive development and the best intervals at which to orchestrate its release and thus contribute to a repopulation strategy of native fauna to the local water environments.

The organogenesis of the digestive system of *P. magdalenae* was studied. At 3–4 days post-hatching, the mouth was open, and the oral valve was developed, the mouth was continuous with the esophagus and the rest of the digestive system, which has a recognizable epithelium at the intestinal level with cuboidal or columnar morphology. The time of mouth opening varies among species and is influenced by temperature (Gisbert et al., 2004; Zaiss et al., 2006) because temperature increases metabolism (Qu et al., 2012). Moreover, mouth opening and yolk depletion are generally events that are considered markers for the beginning of feeding in fish larvae (Gisbert & Williot, 2002). In this studied species, the yolk was observed for up to 9 days post-hatching.

In larval rearing, the onset of feeding and the transition of endogenous to exogenous feeding is a crucial moment in development and fish larvae survival (Yúfera & Darias, 2007; Yúfera et al., 2014). Previous studies (Chen et al., 2006; Govoni et al., 1986; Segner et al., 1993) have described the transition of endo–exogenous to exogenous feeding as a critical period, because the fish need to develop the ability to survive solely through exogenous feeding.

On the other hand, at the moment of mouth opening *P. magdalenae* still did not have a functional stomach or esophagus. If exogenous feeding starts at this time, it can result in slower growth than in the period with endogenous resources.

The submucosal glands and the muscle of the esophagus developed at 6 days post-hatching, and the glands and muscle of the stomach developed at 7–8 days post-hatching, demonstrating that this may indicate the beginning of exogenous feeding.

Conclusion

In this study, we were able to characterize the ontogenic development of the bocachico including not only the early embryonic development but also the larval phase using conventional microscopic observation and

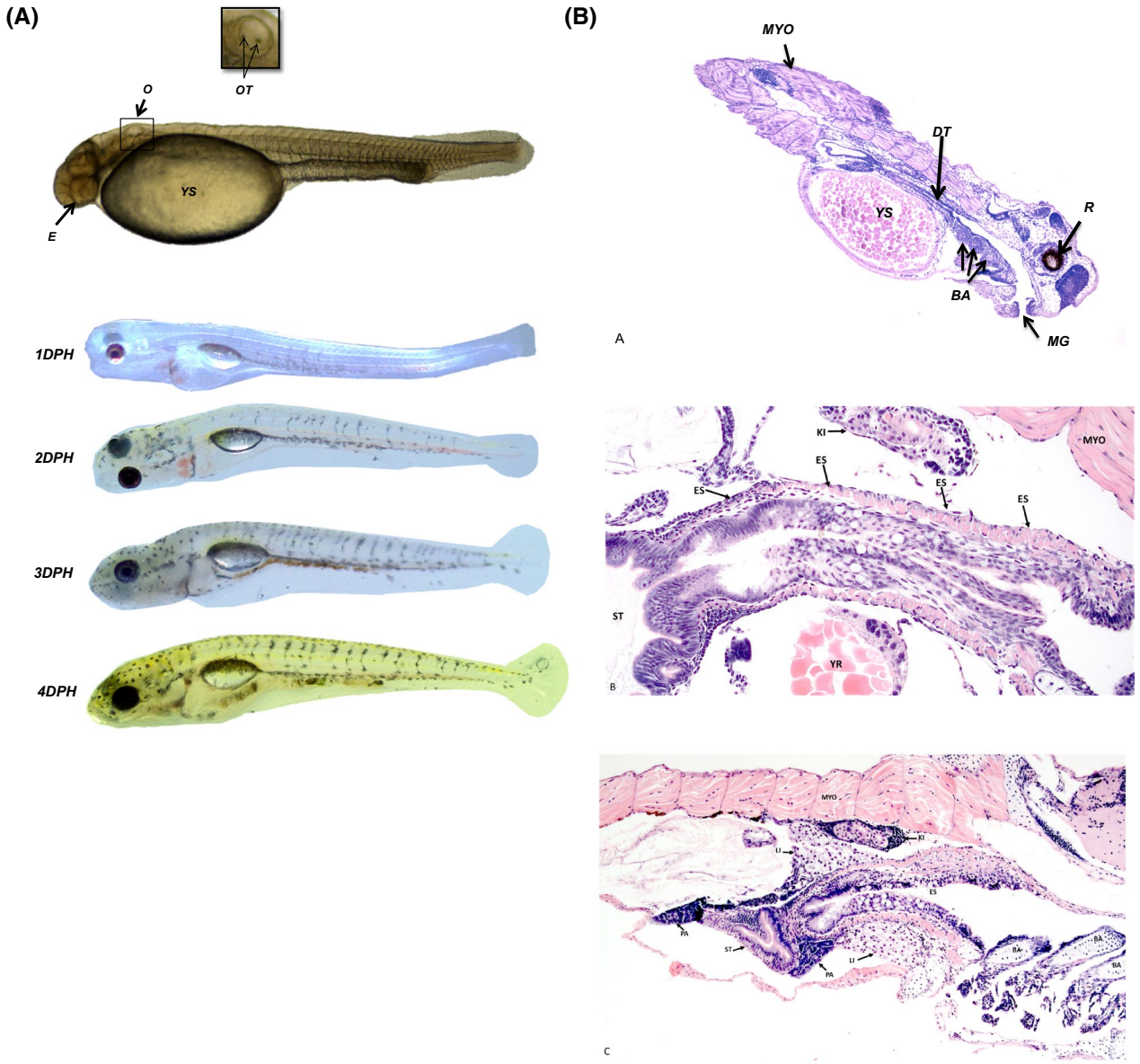

Fig. 8 **A** *Prochilodus magdalenae* hatching period and early larval development. Morphological development of larvae from 1 day post-hatch (DPH) to 4 DPH. Abbreviations: E: eye; O: otic cup; YS: yolk sac; OT: otolith. **B** *Prochilodus magdalenae*. (A) Endogenous feeding period: The yolk sac is seen; the digestive tract is a straight tube; retinal pigmentation starts; retina become multilayer structure; gill anlage visible; mouth gap increases and the mouth arrives at the subterminal position; and jaw structures are well defined, H&E 4X. (B) Endo–exogenous feeding period: remains of yolk sac are present; esophagus, stomach and kidney are evident, H&E 20X. (C) Exogenous feeding period: The yolk sac fully depletes; myotomes are W-shape. Esophagus, stomach, liver, pancreas and kidney are evident H&E 10X. Abbreviations: BA: branchial arches; GF: gill filaments; ES: esophagus; E: eye; J: jaw; LI: liver; M: mouth; MG: mouth gap; MYO: myotome; NA: narines; PA: pancreas; R: retina; RY: yolk remains; ST: stomach; YS: yolk sac; KI: kidney

histology which allowed us to have a more detailed view of the changes that occur in each phase. We managed to characterize 7 stages of embryonic development: zygote, cleavage, blastula, gastrula, segmentation and organogenesis, pharyngula and hatching, which took place over a period of 13 h. In addition, we determined the periods of endogenous, endogenous–exogenous and exogenous feeding based on the histological changes observed in the digestive tract. Together with the histomorphological findings of cranial, branchial, sense organ and fin development observed in the field, these findings indicate that bocachico fry should be released into the natural environment 12 days after hatching and not before. This research will contribute to improving the knowledge of

this species' developmental intervals, the critical stages in feeding phases, as well as a more concrete approximation of when the fish is suitable for repopulation based on its morphological development. According to our knowledge, this is the most comprehensive analysis made of the development of the bocachico in Colombia.

Abbreviations

AP: Animal pole; AUNAP: National Aquaculture and Fisheries Authority; B: Blastodisc; BA: Branchial arches; CD: Active cell division; CFF: Caudal fin fold; E: Eye; EF: Embryonic fin; ES: Esophagus; EVL: Enveloping layer; GF: Gill filaments; H&E: Hematoxylin and eosin; J: Jaw; K: Kupffer's vesicle; M: Mouth; M: Myomere; MADR: Ministry of Agriculture and Rural Development; MG: Mouth gap; MYO: Myotome; NA: Narines; NT: Neural tube; O: Otic cup; OP: Optic primordium; P: Primordium of the CNS; PF: Post-fertilization; PSS: Piscícola San Silvestre S.A.; R: Retina; RY: Yolk remains; S: Shield; SC: Syncytial cells; SM: Somites; ST: Stomach; TB: Tail bud; UPRA: Rural Agricultural Planning Unit; Y: Yolk; YE: Yolk extension; YG: Yolk granules; YP: Yolk plug; YS: Yolk sac; YSL: Yolk syncytial layer.

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Author contributions

JYB standardized and applied the protocols of induced reproduction of the study species, to carry out the collection and registration of the samples, to contribute in the analysis of the results and in the writing and redaction of the manuscript. JAC and GVM performed the application of histology techniques, reading and analysis of the histology sections and contributed to the analysis of the results and the writing of the manuscript. YOA collaborated in the collection of samples and taking photographic records and contributed to the analysis of the results. AEP collaborated in the analysis of the results and advised the study from his extensive field experience in this species. All authors have read and agreed to the final manuscript.

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Availability of data and materials

The datasets generated and analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

All procedures involving animal handling were performed in accordance with the standards for the use of laboratory animals described by the Committee on Care and Use of Laboratory Animal Resources of the National Research Council (National Academies, USA). In addition, this research has the research permit issued by the National Aquaculture and Fisheries Authority-AUNAP (Resolution 0955 of May 27, 2020).

Consent for publication

Not applicable.

Competing interests

The authors declare that they do not have any conflicts of interest.

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