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Embryogenesis and early larval development of the fish *Sorubim cuspicaudus* (Siluriformes: Pimelodidae)

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ABSTRACT

Introduction: *Sorubim cuspicaudus* is a migratory catfish listed as a vulnerable fish. The study of its embryonic and larval development allows the identification of morphological and chronological events necessary to establish adequate management practices.

Objective: To describe the main events of the embryonic development and early larval phase of the trans-Andean shovelnose catfish, *S. cuspicaudus*, under controlled conditions of incubation and larviculture.

Methods: Final maturing fish were induced for reproduction with a dose of 10 µg of GnRH/kg of live weight. The embryos were incubated at 28 ± 0.5 °C and were analyzed at early stages (zygote-gastrula) every 5 minutes and late stages (cleavage-hatching) every 15 minutes.

Results: Animal pole differentiation occurred at 0.5 hours post-fertilization (HPF), first cleavage at 0.58 HPF, 4, 8, 16, 32, 64 cells at 0.75, 0.92, 1.08, 1.17, 1.33 HPF respectively, blastula 1.5 to 4.37 HPF, gastrula 4.7 at 6.87 HPF, organogenesis 7.37 at 11.37 HPF, pharyngula 11.87 at 13.37 HPF, and hatching at 15.92 HPF. The opening of the mouth happened at 32 hours after hatching (HPH), food consumption at 43 HPH at 26.6 °C, yolk sac depletion 70.5 HPH, barbels at 35.7 HPH, fins at 6 days post hatching (DPH), swim bladder at 10.2 DPH, stomach with glands at 12 DPH, additionally with sensory and locomotion organs.

Conclusions: The fingerlings show complete development and escape instinct at 14 DPH. It is suggested that 14 DPH could be the minimum age to carry out restocking programs with this species.

Key words: histology; larvae; fingerling; growth; ontogeny; catfish.

RESUMEN

Embriogénesis y desarrollo larval temprano del pez *Sorubim cuspicaudus* (Siluriformes: Pimelodidae)

Introducción: *Sorubim cuspicaudus* es un bagre migratorio catalogado en grado de amenaza vulnerable. El conocimiento del desarrollo embrionario y larval permite identificar eventos morfológicos y cronológicos necesarios para establecer prácticas de manejo adecuadas.

Objetivo: Describir los principales eventos del desarrollo embrionario y fase larval temprana de *S. cuspicaudus*, bajo condiciones controladas de incubación y larvicultura.
Metodología: Peces en maduración final se indujeron para reproducción con una dosis de 10 µg de GnRH/kg de peso. Los embriones se incubaron a 28 ± 0.5 °C y, se analizaron en etapas tempranas (cigoto-gástrula) cada 5 minutos y tardías (segmentación-eclosión) cada 15 minutos.
Resultados: La diferenciación del polo animal ocurrió 0.5 horas post-fertilización (HPF), primer clivaje 0.58 HPF, 4, 8, 16, 32, 64 células 0.75, 0.92, 1.08, 1.17, 1.33 HPF respectivamente, blástula 1.5 a 4.37 HPF, gástrula 4.7 a 6.87 HPF, organogénesis 7.37 a 11.37 HPF, faríngula 11.87 a 13.37 HPF, eclosión 15.92 (HPF). Apertura bucal ocurrió 32 horas después de la eclosión (HPH), consumo de alimento a 43 HPH a 26.6 °C, agotamiento del saco vitelino a 70.5 HPH, barbicelos a 35.7 HPH, aletas a 6 días después de la eclosión (DPH), vejiga natatoria a 10.2 DPH, estómago con glándulas a 12 DPH, además de órganos sensoriales y de locomoción.
Conclusiones: Los alevinos presentan desarrollo completo e instinto de huida a 14 DPH. Se sugiere que 14 DPH podría ser la edad mínima para a llevar a cabo programas de repoblamiento con la especie.

Palabras clave: histología; larvas; alevinos; crecimiento; ontogenia; silúridos.

INTRODUCTION

The trans-Andean shovelnose catfish *Sorubim cuspicaudus* (Littmann et al., 2000), belongs to the Siluriform order, Suborder Siluroidei, and to the family Pimelodidae (Prieto et al., 2015). It is a fish characterized by its elongated body, capable of reaching lengths of up to one meter. Its dorsal side is dark, while the ventral side is entirely white. A distinctive feature is the black stripe that extends along the midsection of the body from the area around the eyes to the tip of the rays in the lower lobe of the caudal fin. The fish possesses a flat and broad head, with the upper jaw exceeding the length of the lower jaw. Its eyes are positioned laterally, and the maxillary barbels maintain a length below that of the dorsal fin. Furthermore, the adipose fin is notably shorter in comparison to the anal fin. This description is intended for potential inclusion in scientifically indexed journals (Galvis et al., 1997; Prieto et al., 2015).

S. cuspicaudus inhabits the basin of Lake Maracaibo in Venezuela and the basins and tributaries of the Catatumbo, Sinú, Atrato and Magdalena rivers in Colombia (Buendía-Lara et al., 2006). The fish has a scale-free exterior, absence of intramuscular bones, presents a favorable lipid profile, and readily adapts to captive conditions. Additionally, it displays a favorable receptiveness to induced reproduction techniques. Nocturnal in nature, it exhibits fast movements within moderately deep aquatic environments. It's a carnivorous fish, demonstrating a pronounced piscivorous inclination. Furthermore, the fish engages in reproductive migrations termed as "subienda," which involve upriver migration (Buendía-Lara et al., 2006). Therefore, it is considered a promising species with potential for commercial fish farming and food security for a growing human population. Nonetheless, this fish is not produced in enough production volumes due to technological constraints that hinder the steady and uninterrupted generation of fingerlings in the required quantities (Prieto et al., 2015).

In Colombia, there are approximately 150 000 artisanal fishermen in maritime and inland waters, representing more than 400 000 people, who directly depend on artisanal extractive fishing for income and food, reflecting the importance of the sector as a way of life (García-Benítez & Flores-Nava, 2016). In recent decades, the Magdalena River has undergone strong environmental transformations due to mining, oil extraction, and agriculture, among other anthropogenic activities (Lozano et al., 2017). Resulting in habitat loss and water quality degradation, affecting native fish (Lozano et al., 2017; Mojica et al., 2012). Due to the above, it is necessary to explore ex situ conservation strategies such as captive reproduction, with the purpose of producing fingerlings for restocking

programs, food security and promotion of the fish activity of the species (Prieto et al., 2015).

In the production of fingerlings of a native species, three stages are identified: broodstock management, induced reproduction and larviculture (Atencio-García et al., 2010). Significant progress has been made in the trans-andean shovelnose catfish regarding the first two production phases (Prieto et al., 2015). However, the greatest limitations are found in larviculture, particularly in the management of the first feeding. In this phase, the highest mortalities are recorded as a consequence of cannibalism and due to the change from live prays to the consumption of balanced diets (Atencio-García et al., 2010).

In *S. cuspicaudus*, an examination of embryonic development becomes imperative to establish a chronological sequence of the morphological and histological processes culminating in the genesis of a new organism. Such insights facilitate the refinement of incubation and larviculture methodologies tailored to the species' requirements (Valbuena-Villarreal et al., 2012). While numerous studies have studied the ontogenic development of fish, variations in the timing of differentiation, development, and functionality emerge across species during the initial stages of ontogeny. This diversity underscores the need for species-specific investigations and in-depth analyses to thoroughly comprehend these processes (Solovyev et al., 2016; Treviño et al., 2011; Valbuena-Villarreal et al., 2012). These studies are a valuable tool as a strategy to understand the physiology of the larvae and can be useful to improve culture (Gisbert et al., 2014; Lazo et al., 2011; Solovyev et al., 2016). Consequently, the objective of the present work was to describe the main events of the embryonic development and early larval phase of the trans-Andean shovelnose catfish, *Sorubim cuspicaudus*, under controlled conditions of incubation and larviculture.

MATERIALS AND METHODS

Location: The research was carried out at the Piscícola San Silvestre S.A. (PSS) fishery, located in the city of Barrancabermeja (Santander), with geographic coordinates: (7°06'31'-7°06'31" N & 73°51'23"-73°51'23" W), at 75 masl, with an annual average temperature of 28.4 °C.

Source of the fishes: Adult *S. cuspicaudus* fish (n = 100, mean weight = 700 ± 300 g) were collected from the middle Magdalena river basin (including the San Silvestre and Llanito swamps: 7°07'43" N & 73°54'57" W) through traditional fishing practice with cast nets, with the support of local fishermen in 2018 and 2019. The river water temperature was 25 °C. The broodstock were transported in plastic tanks with a capacity of 800 liters of water to the PSS. In the fish farm, the broodstock were subjected to a bath with salt (20 ppm) for 15 seconds and later they were transferred to rectangular pools with aeration and constant change of water (filtered from the Ciénega San Silvestre) where they remained for 24 hours, after this process, they were transferred to a pond of 1 200 m^2 with 1 meter depth. The fish were provided with juvenile red tilapia (*Oreochromis* sp.) as prey, allowing them to capture it freely, and were additionally supplied with commercial balanced feed containing 34 % crude protein (CP) at a daily rate of 1 % relative to the biomass. This feeding regimen was sustained until they reached the stage of full maturation.

Gamete collection and fertilization: Using ovarian biopsy, a mature female weighing 2 210 g and two males in the spermiation phase were selected for hormonal induction employing a dosage of 10 μg of GnRH analogue per kilogram of live weight. After 12 hours, gametes (seminal material and oocytes) were extracted for subsequent dry fertilization. Following fertilization and a one-hour hydration period, the oocytes (382.5 grams, equivalent to 1 093 oocytes/g) were introduced into two 60 l incubation tanks featuring an upward water flow (4 to 5 l/minute). Upon hatching, the resulting larvae were collected within a 200 l incubator before being transferred to circular tanks

offering continuous aeration, with a density of 80 larvae per liter.

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

Fertilized oocyte samples were deposited in 9 cm Petri plates at a density of ~150 oocytes/dish. Early-stage embryos (zygote, cleavage, blastula, and gastrula) were observed at 5-minute intervals and late stage (cleavage to hatch) at 15 and 30 minutes intervals. Developmental stages were determined morphologically using a microscope (Leica DM750, Germany) equipped with a digital camera (Leica MC120 HD, Germany). To objectively describe the embryonic development of *S. cuspicaudus*, embryogenesis was divided into seven stages using well-known markers for freshwater fish, such as *Danio rerio* (Kimmel et al., 1995) and *Capoeta trutta* (Zadmajid et al., 2017). This included the following stages: zygote, cleavage, blastula, gastrula, cleavage, and organogenesis, pharyngula, and hatching.

Histology of larval development: To characterize larval organogenesis, fish were randomly sampled daily, ten larvae per sample, from three batches during the endogenous, endoexogenous, and early exogenous feeding period. The sampled larvae were anesthetized with clove oil (40 ppm; Sigma-Aldrich, USA), their length and weight were measured to determine the specific growth rate (SGR) = 100x (Ln (final weight) - (Ln (initial weight)) / t; where t is the culture time expressed in days and Ln is the natural logarithm. The materials were fixed in 10 % buffered formalin, embedded in paraffin, and sectioned at 5-6 µm with a rotary microtome (Leica RM2125 RTS, Germany.) Sections were stained with hematoxylin and eosin (H&E) according to standard procedures and photographed under a light microscope (Nikon, eclipse 600, Japan) with a digital camera (Nikon, DXM120, Japan). The histochemical technique of Periodic Acid Schiff (PAS) staining was used through which they were characterized as the goblet cells of the esophagus, stomach, and intestine.

All procedures involving the handling of animals were performed in accordance with the standards for the use of laboratory animals outlined by the Committee on the 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). Additionally, this research has the research permit issued by the Colombian National Aquaculture and Fisheries Authority-AUNAP (Resolution 0955 of May 27, 2020).

RESULTS

S. cuspicaudus has slightly opaque yellowish oocytes; after one hour of hydration, they reach a diameter of 4 mm. After fertilization and hydration, they present a perivitelline space that easily doubles the diameter of the developing embryo; they are also of the telolecitic type, since the embryo is formed in the animal pole that corresponds to only one end of the cytoplasm and presents a large amount of yolk in the plant pole. *S. cuspicaudus* exhibits a partial or meroblastic division during the early stages of development as in most fishes (Kimmel et al., 1995; Ninhaus-Silveira et al., 2006). The embryonic development of *S. cuspicaudus* occurred at 28 ± 0.5 °C, dissolved oxygen of 6 ± 0.5 ppm and pH of 7.5 ± 0.2.

Zygote period (one cell): In the fertilized zygote, chorion initiates swelling, giving rise to the perivitelline space. The oocyte is spherical, characterized by dense cytoplasm comprising vacuolated and translucent materials, corresponding to protoplasmic residues. Approximately 0.5 hours post-fertilization (HPF), cytoplasmic segregation commences towards the periphery, leading to the initial differentiation of the vegetal and animal poles.

The latter is referred to as the blastodisc (1 cell), situated in a marginal zone primarily composed of primordial germ cells surrounded by a homogeneous vitelline envelope, where cellular divisions will commence.

Cleavage stage (cleavage) 2-64 cells:

Two-cell stage (at 0.58 HPF): During this phase, the cleavage stage can be observed, characterized by the formation of a two-cell arrangement in the blastodisc of the meroblastic type. Two blastomeres of similar size and dome-shaped structure are generated in the animal pole region. The area between the yolk and blastoderm curves smoothly without apparent differentiation between them.

Histological Sections Stained with Hematoxylin and Eosin (H&E): An eosinophilic structure is observed around the zygote, generating a translucent space corresponding to the perivitelline space, which is enveloped by the vitelline membrane. Within the yolk sac, circular eosinophilic structures of varying sizes are visible, separated by translucent eosinophilic material. In the animal pole region, blastomere division is evident. The blastomeres are of uniform size, dome-shaped, and have divided in the same plane. It can be noted that the more caudal region of the blastodisc, in contact with the yolk space, assumes a convex shape. Cytoplasm segregation towards the animal pole continues.

Four-cell stage (at 0.75 HPF): In the dorsal view of the animal pole, the division from two to four blastomeres is distinguished by a perpendicular cleavage plane relative to the previous division. The blastomeres adopt an ellipsoidal shape, exhibiting distinct differentiation. Additionally, the region in contact with the vitelline space maintains its convex morphology.

Histologically, the division of blastomeres is evident, signifying ongoing cellular activity. Simultaneously, the segregation of cytoplasm towards the animal pole persists, playing a role in the dynamic process of cellular differentiation and specialization.

Eight-cell stage (at 0.92 HPF): Horizontal division of the 4 blastomeres is observed in a cleavage of each one, forming 8 of these, which are intensely basophilic in color and darker than in their previous phase. In addition, the divisions have increased the size of the animal pole, the sac. yolk occupies approximately 75 % of the surface, its content is a particulate eosinophilic material of various sizes, among which translucent material is observed.

16-cell stage (at 1.08 HPF): Southern-shaped division, blastomeres with dome morphology, similar to previous phases, the cells of this stage have decreased in size, they are still located in parallel planes, with growth being along the two planes, producing a matrix composed of 4 x 4 cells. Abundant yolk is observed in the vegetal pole; cell activity can be observed.

Histologically, the blastomeres are seen to be intimately associated with one another, interconnected by translucent eosinophilic material resembling the substance that envelops the yolk sac.

32-cell stage (at 1.17 HPF): Blastomeres form in parallel planes, some overlapping near the animal pole. Two distinct planes are evident, one of which is more elongated, resulting in a loss of uniformity compared to the previous stage. The division at this stage entails an arrangement of 4x8 blastomeres, now organized into two layers. The cells are smaller, with those closest to the yolk sac margin exhibiting slight flattening. An increase in the number of blastomeres and the height of the animal pole is apparent, and the histological section displays some overlapping cells.

Sixty-Four Cell Stage (at 1.33 HPF): The blastomeres are smaller in size, in comparison to the previous cycle; a second vertical growth phase occurs, and numerous overlapping blastomeres are observed. From a lateral view, a notably higher cellularity arranged in layers is

evident. The cells visible from the lateral layer are referred to as the blastodisc enveloping layer cells. Furthermore, histologically, while overlapping cells are observed, with eosinophilic material between the blastomere cells, the cells situated in the central region are smaller, better defined, and do not come into close contact with the cells at the same level.

Blastula period (1.5 a 4.37 HPF): Onset of the Blastula Period. Many blastomeres overlap with others, with some becoming indistinct, causing the dome-like shape to begin to transform into a semicircular form. Additionally, regarding the height aspect, cells of the enveloping layer can be observed in the lateral region of the animal pole. Histologically, an increased height at the animal pole is evident, with numerous closely adjacent blastomeres; the embryo begins to assume a spherical shape; the most outer layer of the blastomeres exhibits a scale-like appearance, and faint eosinophilic material can be seen among the blastomeres.

This stage represents a pivotal transition marked by changes in the embryo's morphology and cellular organization. The shift from a dome-like to a more semicircular form, coupled with the emerging cellular layers and the presence of distinct cell types, underscores the dynamic nature of early embryonic development.

In the lateral view a high layer can be observed, with irregular cellularity, the cells of the blastodisc envelope are considerably thinner than the rest of the cells. In the phase of 512 cells, an increase in the levels of blastomeres of the enveloping layer can be observed. There is reduced distinction between the cells of the enveloping layer and their connection with the yolk, which is particularly noticeable in the marginal zone adjacent to this region. In the dorsal view, identifying deep cells becomes challenging, with only enveloping cells being clearly apparent. Histologically, an increase in blastomere count is evident, where marginal cells adopt a flattened morphology. A nuclear eosinophilic structure is observed between the blastomeres and the yolk sac, known as the yolk syncytial layer. Additionally, it is noted that some adjacent blastomeres with this structure are more separated and lack connections with other blastomeres.

The height of the animal pole has decreased considerably, and it has begun to segregate along the perimeter of the yolk sac, taking on an elongated morphology, giving shape to the blastoderm.

The lateral view allows us to observe how the blastoderm has formed, which is characterized by being more uniform in terms of its longest axis and occupies approximately 30 % of the yolk. The front view allows us to show a slight asymmetry as far as thickness is concerned. Histologically it is observed that the syncytial layer of the yolk sac leaves its straight shape, begins to become curved and bends towards the curvature of the animal pole, the blastoderm has uniform thickness. The syncytial yolk layer begins to become thin.

Gastrula period (at 4.7 a 6.87 HPF): At 50 % epibolism (5.03 HPF), an expansion of the blastoderm becomes evident, encompassing half of the yolk sac's area. The edges of the blastoderm extend toward the vegetal pole, constituting what is referred to as the germinal ring. From a histological perspective, the outermost region of the blastoderm is characterized by flattened cells, corresponding to those on the outermost layer. Additionally, due to the condensation and arrangement of the blastomeres, the cells in proximity to the yolk sac are designated as the hypoblast, whereas those nearer to the enveloping layer are labeled as epiblast.

The blastoderm's growth continues, covering 75 % at 5.7 HPF and achieving 90 % coverage at 6.2 hours post-fertilization. Both extremities of the blastoderm experience a slight thickening. Within the yolk sac, a minor elevation forms at the vegetal pole; nevertheless, it disappears six hours after fertilization once the blastoderm has fully enveloped the entire yolk sac.

Segmentation period and organogenesis (7.37 at 11.37 HPF): The thickening of the

dorsal epiblast forms the neural layer in the anterior portion, which in histology allows us to observe the formation of a nerve primordium. This presents cells with cylindrical morphology and oval ends, compatible with somites. The yolk plug disappears. At the posterior end of the embryonic axis, a slight thickening of the tail is observed. The stage in which these phenomena occur is known as the "bud stage". Between 4-6 somites become visible in the neural tube. In addition, in the neural keel the appearance of the optic primordium can be observed, in the trunk, the neural plate remains present. At 10 hours, the appearance of the Kupffer vesicle is observed near the tail base, the yolk sac begins to retract and elongate caudally, near the tail. The neural plate and auditory vesicle have begun to show further development, as has the nervous tissue. At 12 hours, it has advanced to the point where distinguishable structures such as the tail, the primitive eye and the auditory vesicle begin to form, in addition the formation of encephalic nervous tissue begins to become visible, and the head continues to be attached to the yolk sac.

Pharyngula period (11.87 a 13.37 HPF): Bilateral and symmetrical distribution of somites, extending from head to tail. The elongation of the yolk sac towards the caudal area is more evident. The notochord becomes visible, histologically the formation of myotomes is observed, which are arranged as somites with eosinophilic muscle fibers located in the middle of the blastoderm and the tail. In addition, the notochord is distinguished by the presence of cells with a vacuolated cytoplasm with a nucleus towards the periphery. This structure, however, has not fully extended and is found in the blastoderm behind the yolk sac region, 11:00 to 1:00 in the region of the head the appearance of retinal tissue is observed for the first time, in the optic primordium.

Hatching period and early larval development - 15.92 hours post hatching (HPH): The post hatching period allowed to demonstrate the growth and primary development of several organs. These grow slowly and their appearance is mainly consistent with their rudimentary function. In the region of the yolk sac, the development of the primitive intestine was observed, characteristic for having an epithelium of simple cuboidal morphology, with some mucus cells arranged as a tube. In addition, advanced development of brain nervous tissue was observed; near it the growth of the primordial eye was observed; notochord has extended caudally but has not yet developed to tail; the somites of the middle and tail region of the blastoderm have developed myotomes, which histologically are oval-shaped divisions with muscle fibers inside. Fig. 1 shows the most outstanding morphological events in the stages of embryonic development with respect to time. The first changes were very fast, but from the blastula onwards it is evident that it takes longer to observe noticeable changes.

Day 1 DPH (Days Post Hatching): Head still attached to yolk sac, indicative of endogenous feeding; pigmented primordial eye which histologically shows closed retinal tissue; The digestive tract was characterized mainly by the presence of an oral cavity lined by a stratified squamous epithelium followed by the esophagus and later a tubular structure with a central lumen corresponding to the primitive intestine lined with a simple cylindrical epithelium supported by a thin layer of connective tissue. called the lamina propria, more externally the muscular tunic composed of smooth muscle and the serous tunic. Between the cranial region and the yolk sac, the heart was observed, made up of thin fibers with central nuclei, divided into two chambers. At this stage of development, the skeleton was made up mainly of hyaline cartilage.

2 DPH: The cephalic region has undergone advanced development, with notable progress in the formation of the primordial eye and pigmented retina. Pigments are now becoming apparent in the pectoral and intestinal areas. An elongation of the mouth is evident, and histologically, clear demarcation of the diencephalon,

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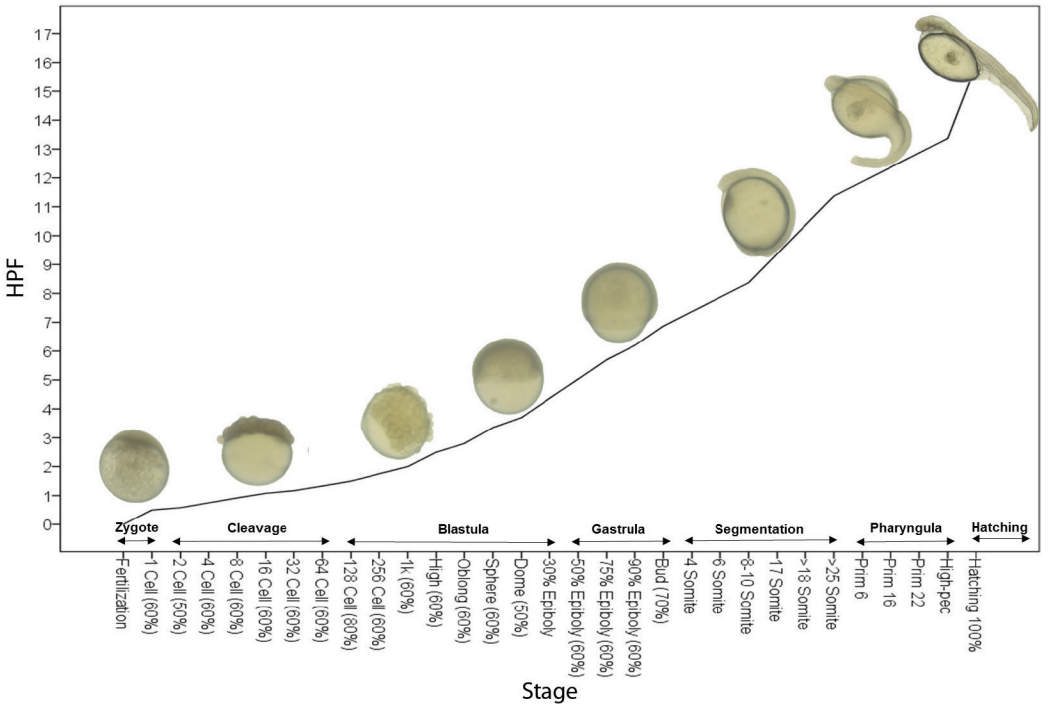

Fig. 1. Fundamental morphological events in embryo development regarding hours from fertilization to hatching.

telencephalon, and mesencephalon are observed within the head region. This differentiation is characterized by distinct gray and white matter separation, with the presence of neurons, neuropil, and some axons. Further histological analysis reveals differentiation of the granular layer, along with the presence of Purkinje cells and dendrites. Regarding the digestive tract, a similarity to day 1 report is observed, yet there is notable advancement in the development of the primitive intestine. Here, the mucosa, lined with simple columnar epithelium, exhibits a thin layer of connective tissue, giving rise to small folds projecting toward the lumen, forming the lamina propria (LP). Externally to the mucosa, a delicate layer of smooth muscle (tunica muscularis) is present, followed by the outermost layer, the tunica serosa.

While the skeletal structure remains cartilaginous, like to the prior stage, there is heightened prominence of the gill arches. In the caudal region, multiple myotomes are evident, along with a short yet distinguishable caudal fin. Notably, a significant portion of the larva continues to be comprised of the yolk sac, characterized by numerous globular structures that facilitate endogenous feeding. Observations reveal that the mouth and anus of *S. cuspicaudus* became apparent at 32 hours post-hatching, with the initiation of live food consumption observed at 43 hours post-hatching under a temperature of 26.6 °C.

3 DPH: A granular pigmentation pattern is evident spanning from head to tail, attributed to the presence of cardinal venules. Short barbicels extending from the mouth towards the caudal region were identified. Histologically, ganglion cells and pigmentary tissue were observed within the eye region. A connection between the central nervous system, spanning from the brain to the spinal cord, was evident. The skull structure remains cartilaginous, featuring the presence of gill arches and filaments.

Notably, there is an expanded mouth opening compared to prior stages, accompanied by a more developed esophagus. The mucosal tunic of the esophagus is lined with stratified cuboidal epithelium containing abundant goblet cells. External to the epithelium, a layer of loose connective tissue corresponds to the lamina propria. The muscular tunic consists of striated skeletal muscle, followed by the adventitia. Towards the esophagus's termination, a transition to a sizable saccular structure lined by simple columnar epithelium is observed. Below this layer, a lamina propria of loose connective tissue is visible, followed by a layer of smooth muscle known as the tunica muscularis, and finally, the tunica serosa, as depicted in (Fig. 2).

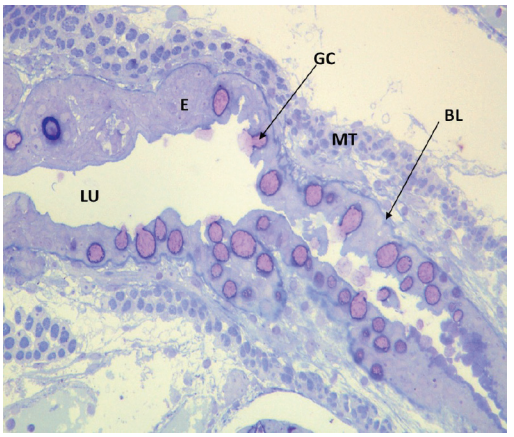

Fig. 2. Microscopic photograph of the esophagus of *S. cuspicaudus*, day 3 DPH, the mucosa is lined by a stratified cuboidal epithelium (E). The basal lamina (BL) on which the epithelium is supported can be seen, and more externally, the tunica muscularis (MT), composed of skeletal striated muscle. The lumen (LU) of the esophagus and goblet cells (GC) that produce mucus to facilitate food passage can also be identified.

This dilation of the digestive tract, marked by the presence of proteinaceous material within the lumen, is apparent. Adjacent to this structure, the more developed intestine features well-defined folds. Under a simple columnar epithelium, a lamina propria of loose connective tissue is observed, followed by a layer of smooth muscle (tunica muscularis), and an outermost layer, the serosal layer. The yolk sac has diminished in size compared to the previous phase. This reduction, coupled with the contents within the digestive tract, signifies an endoexogenous diet. Both the liver and pancreas are visibly distinct, with the former characterized by predominantly vacuolated hepatocytes, while the latter contains cells rich in birefringent eosinophilic granules (zymogen granules). At this stage, scant tubules, composed of a simple cuboidal epithelium corresponding to renal tubules, are discernible. The presence of the yolk sac was noted until 70.5 HPH.

4 DPH: Similar to the prior stage, notable advancements in head development and the differentiation of the fish's pectoral area were evident. It is remarkable the elongation of the heavily pigmented barbicels, which serve as sensory organs. The upper portion of the caudal fin initiates its development. Histologically, a cross-sectional view reveals the presence of both the dorsal and ventral medial fins, as well as the arrangement of the spinal cord in terms of white and gray matter. The notochord is also evident. The progression of the digestive tract closely resembles that of the preceding stage. However, more distinct intestinal folds and an increased number of goblet cells were observed. Notably, the yolk sac has nearly completely regressed at this point, marking the conclusion of the period of endoexogenous feeding.

5 DPH: At this stage the skull continues to be made of cartilaginous tissue similar to the previous stage. The gills, intestine and other organs showed further development. It is noteworthy that in some animals the appearance of a small crystalline lens in the eye begins to be differentiated.

6 DPH: Development similar to the previous stage with neurocranium composed of cartilage; well differentiated esophagus, branchial arches and barbicels more developed than in the previous phase. At this stage we can

highlight the organization of the hepatocytes in rows or cords, with central rounded nuclei; the pancreas attached to the liver with cells forming acini, spherical nucleus, basophilic cytoplasm and abundant intracytoplasmic eosinophilic granules (exocrine pancreas). Additionally, it is worth noting the greater development of intestinal folds and the presence of exogenous food in the intestinal lumen, (Fig. 3). A greater development of the eye and barbicels is also observed, the latter pigmented and with the presence of taste corpuscles, (Fig. 4). It is also more visible in this phase the kidney with its organization in tubules and lymphocytes in the interstitium.

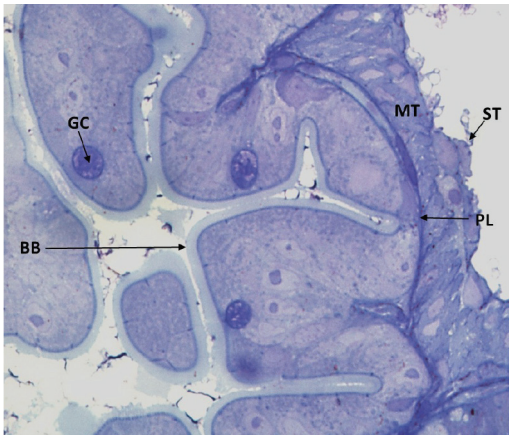

Fig. 3. Microscopic photograph showing the mucosa forming folds, lined by a simple cylindrical epithelium with goblet cells (GC); posterior to the tunica mucosa we find the tunica muscularis (MT) composed of smooth muscle and more externally a thin layer corresponding to the serosa (ST). The lamina propria (PL) can be observed beneath the epithelium.

7 DPH: The morphological findings are similar to the previous stage, however, a greater differentiation of the retinal layers is striking, as well as a larger crystalline lens. Gill arches and filaments are more developed. Larger intestine with several segments and abundant folds. The presence of zooplankton and various proteinaceous fragments apparently parts of (insects) in the light becomes more visible. On dilation caudal to the esophagus the muscular coat thickens. Some melanomacrophages begin to appear in the liver and pigments are also evident in the kidney. Brain shows optic and olfactory lobe development.

8 DPH: complete formation of the dorsal and caudal fin, it is possible to differentiate a double layer of smooth muscle in the dilation of the digestive tract posterior to the esophagus. Formation of the foregut is observed, with tall, simple cylindrical epithelial cells, with nuclei towards the basal domain; some folds in this region appear quite long to the point that they come into contact with folds on the

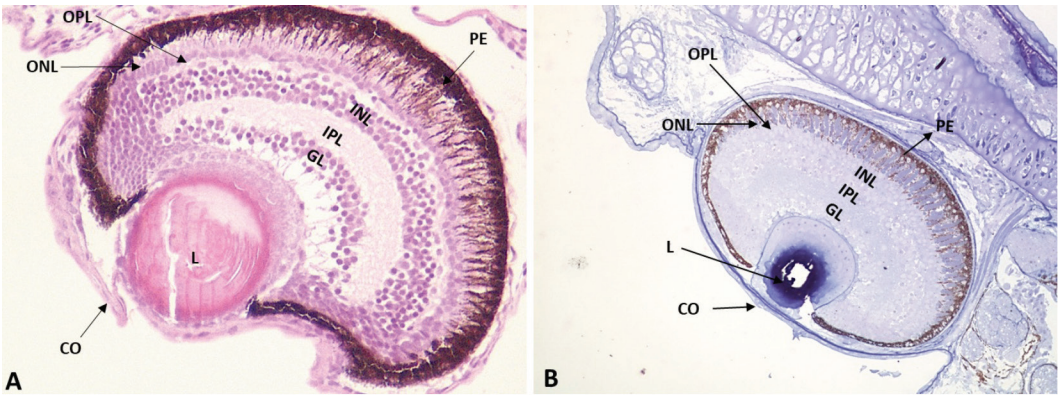

Fig. 4. Microscopic photograph of the eye of *S. cuspicaudus*, showing the cornea (CO), inner nuclear layer-nuclei of bipolar cells (INL), inner plexiform layer (IPL), lens (L), ganglion cell layer (GL), outer nuclear layer-nuclei of rods and cones (ONL), outer plexiform layer (OPL), and pigment epithelium (PE). The image displays the layered structure of the developing retina, which is essential for vision development in this catfish species.

front face. These folds also showed ripples and ramifications.

9 DPH: The morphological findings are similar to the previous stage. It is noteworthy the greater development of gill filaments with already distinguishable lamellae; the esophageal muscular tunic presents a greater thickness, being observed in some areas composed of 3 layers of striated skeletal muscle. Intestine is more developed and differentiable between segments with posterior segment cells with abundant cytoplasmic vacuoles.

12 DPH: At this stage, a well-formed fingerling is observed with well-developed gills, filaments and well-distinguishable lamellae. Eye well developed. In the digestive tract, it is noteworthy that in the dilation posterior to the esophagus, the mucosa has begun to form crypts and glands can be seen in some areas; The entire structure is covered by simple columnar epithelium and the layer of connective tissue between the glands (LP) and external to this other layer of connective tissue corresponding to the tunica submucosa is evident. External to this, the muscular tunic was observed, made up of two layers of smooth muscle: the circular layer, the longitudinal layer, and the serous tunic. In this phase, for the first time, it can already be defined that this dilation of the digestive tube corresponds to the stomach. The liver presented greater development than in the previous stages and in the pancreas not only acinar cells but also ducts can be observed. In the gills, the chlorine cells, the pillar cells of the lamellae and the marginal canal are distinguishable. Intestinal folds appear long and wavy. Abundant zooplankton fragments are evident in the stomach and intestinal lumen.

14 and 15 DPH: fingerling with developed digestive tract characterized by an oropharyngeal cavity lined by a stratified squamous epithelium and posterior to this an esophagus with a stratified cuboidal epithelium with abundant goblet cells, lamina propria of connective tissue, muscular tunic composed of 2 to 3 layers of striated skeletal muscle and the tunica adventitia, (Fig. 5); posterior to the esophagus, stomach, (Fig. 6); with simple columnar epithelium, abundant glands, folds in the mucosa, LP clearly visible; tunica submucosa, tunica muscularis, and more externally the tunica serosa. In the light, there are

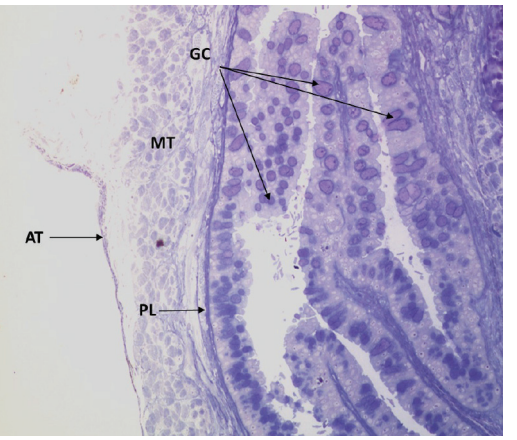

Fig. 5. Microscopic photograph of the caudal part of the esophagus, highlighting the presence of folds projected cranially, a mucosa lined by cuboidal cells, and abundant goblet cells (GC). A transition zone between the esophagus and the stomach is observed. The lamina propria (PL) and very thick muscular tunica (MT) are more developed. The adventitial tunica (AT), the outermost layer of the esophagus, can also be identified in this transition zone.

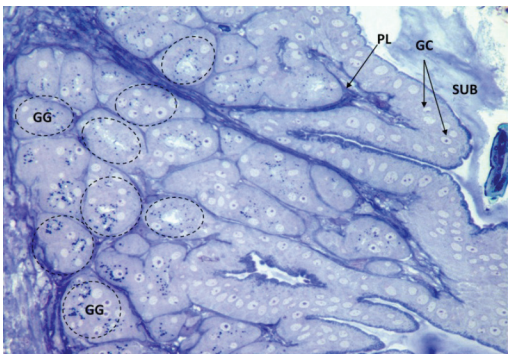

Fig. 6. Microscopic photograph of the stomach with the presence of abundant gastric glands (GG) with a simple columnar epithelium, goblet cells (GC); some of these cells have glandular contents in their cytoplasm (oxyntic or parietal cells). Connective tissue fibers corresponding to the lamina propria (PL) are observed between the glands. The submucosa (SUB), a layer of connective tissue beneath the lamina propria, can also be identified in this histological section.

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abundant remains of zooplanktonic organisms. The liver is well developed with hepatocytes forming cords and with vacuolated cytoplasm. The pancreas is well-developed with abundant exocrine cells and presence of ducts. The intestine also presented a well-marked development with abundant elongated folds and undulations; covered by a simple columnar epithelium and presence of connective tissue under the epithelium corresponding to the lamina propria (LP). Behind this is a layer of smooth muscle, the tunica muscularis. In the light abundant remains of zooplanktonic organisms (Fig. 7). The fins are observed with greater development than in the previous stages with ossified parts and the presence of spines on the pectoral fins.

Fig. 8 describes the main morpho-anatomical events that occurred over time, from the moment of hatching to day seven.

Fig. 9 presents a summary of the critical moments, such as mouth opening, first feeding from 43 hours to day 5, and feeding with wild plankton in land-based ponds from 5 days to 20 days post-hatch. when the study ended. Day 1 the eyes, central nervous system, and very bulging yolk sac are observed. On day 2, the mouth opening and the start of live food consumption are observed. On day 3, a more developed intestine is observed, with more defined folds and

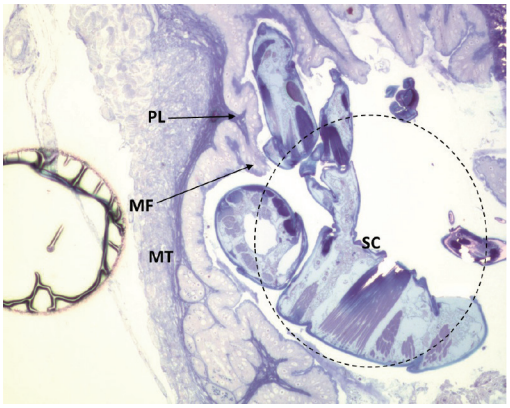

Fig. 7. Microscopic photograph of the stomach with plankton, well-developed stomach with a mucous tunic lined by a simple cylindrical epithelium and a lamina propria (PL) of lax connective tissue; abundant mucosal folds (MF) forming the mucosa are observed. Additionally, the presence of glands and the muscular tunic (MT) composed of smooth muscle is also evident. The stomach content (SC) consisting of plankton and other food remnants can be seen in the gastric lumen.

the presence of intestinal content. On day 4, the branchial arches, the more developed oral cavity, the more developed eyes, the gray and white matter are observed. On day 5, the eye identifies the pigment epithelium, the external plexiform layer, the external nuclear layer, cornea, lens, internal nuclear layer, internal plexiform

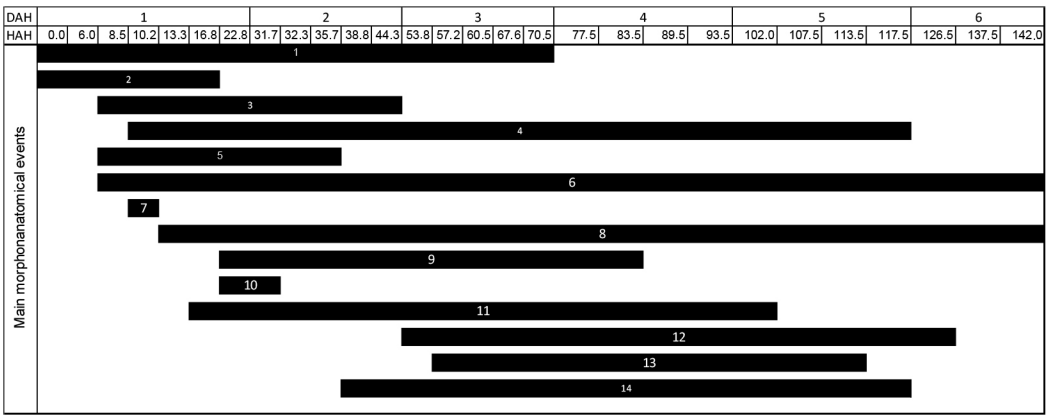

Fig. 8. Main morpho-anatomical events during the ontogeny of the Trans-andean shovelnose catfish *S. cuspicaudus*. The numbers in the lines and the black lines represent the event and and the competition time as follows: 1. yolk sac decrease, 2. development of cephalic area, 3. development of eye and retinal pigmentation, 4. pectoral fins, 5. development of barbicels, 6. caudal fin, 7. filling of swim bladder, 8. gill arches, 9. body pigmentation, 10. elongation and position of the mouth, 11. mouth and anal opening, 12. more developed dental plates and nasal passages (nostrils), 13. first curvatures of the digestive system (separation of stomach and intestine), 14. ossification (rays) and caudal fin elongation.

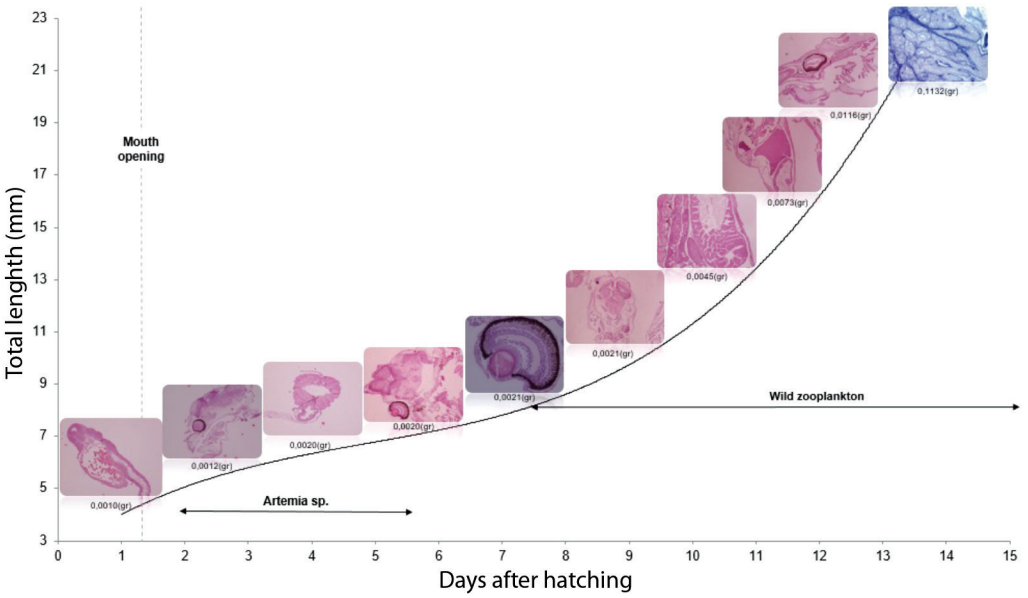

Fig. 9. Main histological changes and growth curve of *S. Cuspicaudus* larvae and/or young fish, from hatching to day 14.

plate, ganglionic layer. Between days 6 and 7, a well-differentiated esophagus is observed, as well as elongation of the branchial arches. Day 8 you can differentiate the stomach and foregut. Day 9 and following, greater development of gill filaments with lamellae is observed. On day 14, the fingerling presents physical and physiological characteristics similar to those of an adult specimen.

DISCUSSION

Fish oocytes are classified as telolecitos, because they present a large amount of yolk (Zadmajid et al., 2018). *S. cuspicaudus* oocytes conform to this classification, presenting meroblastic divisions, limited to the animal pole; the average diameter of fully hydrated *S. cuspicaudus* oocytes was 4 mm and the larvae were 3.96 mm long at hatching, similar to that reported for *Pimelodus grosskopfii* (Valbuena-Villarreal et al., 2012). The diameter of the oocytes influences the incubation period, with larger ones having a longer incubation time than small oocytes (Valbuena-Villarreal et al., 2012), but greater than *Zungaro jahu*, 2.4 mm in hydrated oocytes and 4.3 ± 0.2 mm TL at hatching (Nogueira et al., 2012). Fish species that generate larger oocytes have a longer incubation period compared to species with small oocytes (Valbuena-Villarreal et al., 2012). *S. cuspicaudus* produced oocytes considered small (0.91 mm) and presented a short incubation period. Temperature is another element that plays an important role in incubation. In the temperature range of a species, at higher temperatures, the incubation time is shortened while at lower temperatures, this time is increased (Portella et al., 2014).

On the other hand, the presence of a wide perivitelline space in *S. cuspicaudus* is related to the incorporation of water (hydration); a wide perivitelline space protects the embryo against environmental attacks, contributing to its survival and giving it buoyancy in the water column, which allows it to obtain a greater supply of oxygen, as well as greater dispersion in the aquatic environment (Cerdà, 2002).

The zygote period lasted 0.5 hours, similar to that reported for *Cyprinodon variegatus*

(Lencer & McCune, 2018), longer than the time reported for *P. grosskopfii*; 0.3 hours at 27 ± 1 °C (Valbuena-Villarreal et al., 2012); 0.15 hours for *Ompok bimaculatus* at 27 ± 1 °C (Arambam et al., 2020); but it was less than in *Z. jahu*, 0.75 hours at 27.3 ± 0.4 °C (Nogueira et al., 2012). In this period, the cytoplasm moves towards the animal pole to form the blastodisc, which is where future cell divisions will take place (Zadmajid et al., 2017).

The cleavage period in *S. cuspicaudus* was identified from 0.58 hours post fertilization (HPF) when more than 50 % of the oocytes with two cells were observed, to 1.33 HPF when blastomeres of 64 cells were observed; which was longer in *Z. jahu* from 0.8 to 1.67 HPF (Nogueira et al., 2012). It is defined as the period composed of six stages, from two to 64 cells (Zadmajid et al., 2017).

The blastula period, in which the embryo changes from spherical to oblong in shape and begins epibolism, in *S. cuspicaudus* occurred from 1.5 to 4.37 HPF; slower than *P. grosskopfii* from 1.4 to 3.3 HPF (Valbuena-Villarreal et al., 2012), and *Pseudoplatystoma fasciatum* from 1.15 to 3 HPF at 27.3 ± 0.6 °C (Zapata-Berruecos et al., 2007), but faster than in *Eremophilus mutisii*, from 9 to 11-12 HPF (Moncaleano-Gómez et al., 2018). At the animal pole, a ball shape, called the blastula, becomes visible and the embryo changes from spherical to oblong in shape (Zadmajid et al., 2018).

The gastrula period in *S. cuspicaudus* ranges from 4.7 to 6.87 HPF; similar to *Z. jahu* 4.67 to 7.5 (Nogueira et al., 2012) and, *P. fasciatum*, 4 to 6 HPF (Zapata-Berruecos et al., 2007) time longer than in *P. grosskopfii*, from 3.6 to 5.7 HPF (Valbuena-Villarreal et al., 2012), and lower than in *E. mutisii*, from 12 to 30 HPF (Moncaleano-Gómez et al., 2018). Cell migrations are generated to ensure that each germ layer (mesoderm, ectoderm) is in the right place, so that the organs and body tissues can be formed in the correct location, the embryonic shield is formed marking the dorsal side of the embryo, in addition the tail bud is formed indicating the end of the epibolia (Zadmajid et al., 2018).

The segmentation and organogenesis period in *S. cuspicaudus* goes from 7:35 to 11:37 HPF, "at 11 HPF the first voluntary movements are observed", similar to *P. fasciatum*, 7-11 hours (Zapata-Berruecos et al., 2007) and to *P. grosskopfii*, from 7 to 11 HPF when the first autonomic movements and blood circulation with increasingly strong heart movements are observed. The development of the Kupffer vesicle and the formation of the subdivisions of the brain are observed, the straightening of the hind trunk occurs, the bulges along the dorsal neural tube are observed, indicating the formation of the hindbrain rhombomeres; divided segments of the neural tube within the hindbrain (Zadmajid et al., 2018).

The pharyngeal period in *S. cuspicaudus* begins at 11:87 and ends at 13:37 HPF; and in *D. rerio* from 24 to 48 HPF (Kimmel et al., 1995). Finally, hatching in *S. cuspicaudus* occurs at 15.92 at 28 °C, which is faster than in other catfishes; 20-23 h for *Heterobranchus longifilis* at 29 °C (Nwosu & Holzlöhner, 2000), 24 to 36 h for *Pangasius sutchi* at 20 °C-30°C (Islam, 2005), 25.5 h for *Rhamdia quelen* at 26 °C (de Amorim et al., 2009), 40 h for *Clarias gariepinus* at 24 °C (Osman et al., 2008), 23 ± 1 h for *O. bimaculatus* at 27.0 ± 1.1 °C (Pradhan et al., 2013), 18 HPF at 27 ± 1 °C (Arambam et al., 2020), 4 to 6 days for *Ictalurus punctatus* at 25-27 °C (Chapman, 2000), same 15-17 h to *Hemisorubim platyrhynchos* at 29 °C (Faccioli et al., 2016) and, slower than *P. grosskopfii*, 12 h at 27 ± 1 °C (Valbuena-Villarreal et al., 2012). Hatching is triggered by environmental signals such as low oxygen tension, light intensity, release of hatching enzymes as a signal to adjacent eggs at different times during embryo development (Nogueira et al., 2012).

Limited biological and productive performance data is available for *S. cuspicaudus*, mainly in the first stages of development that are critical for the species. Hence, we document early development, from oocyte fertilization, up to 20 DPH, when the fingerling exceeds 4 cm TL. Morphological and histological reference points were employed, which can be utilized by researchers and the aquaculture industry

in forthcoming studies. Detailed information on embryogenesis and larval development is essential for captive reproduction of the species, taking into account that high mortalities occur in these early stages (Prieto et al., 2015). In this regard, relevant information on eye, mouth and gut development will help optimize early handling and feeding protocols, which is essential for survival during this critical period (Portella et al., 2014; Zadmajid et al., 2018).

In *S. cuspicaudus*, the development of the eye begins from the first DPH where a pigmented primordial eye is observed which histologically shows closed retinal tissue, at the second DPH the pigmented retina is observed, at the third DPH histologically in the region of the eye ganglion cells were observed and pigment tissue, the connection of the central nervous system between brain and spinal cord was evidenced. At the fifth DPH the appearance of a small lens in the eye begins to differentiate, at the seventh DPH a greater differentiation of the retinal layers is observed, as well as a larger lens, 12 DPH, a well differentiated retina is observed and the fully developed eye. In this sense, (Papadakis et al., 2018) in *Argyrosomus regius* they found that at the first DPH the differentiation of the retina began in layers, at 3 DPH cone cells and the differentiation of cells in each layer of the central part of the retina was visible, at 6 DPH it was visible. They observed the rods and increased their density up to 17 DPH.

The mouth and anal opening in *S. cuspicaudus* occurred 32 hours after hatching at 26.6 °C, in the catfishes *Ompok bimaculatus* and *H. platyrhynchos* it occurs at 2 DPH (Faccioli et al., 2016; Pradhan et al., 2013). In *Cichlasoma uruphthalmos*, the mouth opening is detected at 2 DPE and the larvae begin to feed exogenously between 5 and 6 DPE 28.1 ± 1.1 °C (Portella et al., 2014).

Mouth opening time varies between species and is influenced by temperature (Faccioli et al., 2016; Portella et al., 2014; Zadmajid et al., 2018). In addition, the opening of the mouth and the depletion of the yolk sac are events considered to be markers of the beginning of feeding in larvae of fish (Faccioli et al., 2016; Kimmel et al., 1995). In the trans-Andean shovelnose catfish, *S. cuspicaudus* the yolk sac was observed up to 70.5 HPH, which is a shorter period than that of other catfish, such as *H. platyrhynchos* at 4 DPH (Faccioli et al., 2016), *C. gariepinus* (Osman et al., 2008), *Silurus glanis* (Kozarić et al., 2008), *R. quelen* (de Amorim et al., 2009) and *O. bimaculatus* whose yolk sacs last up to 5 DPH (Pradhan et al., 2013). In this sense, *S. cuspicaudus* has a short endotrophic feeding period of approximately 27.5 hours. The initiation of feeding and the transition from endogenous to exogenous food is a critical period in the development of fish larvae, because they need to generate the ability to survive on exclusively exogenous food and has been associated with mass mortalities (Faccioli et al., 2016; Gisbert et al., 2018; Portella et al., 2014; Prieto et al., 2015). At 4 DPH, a greater number of goblet cells was observed in the intestine; this characteristic was also observed in *H. platyrhynchos* larvae during days 3-4 post hatching (Faccioli et al., 2014).

Although goblet cells are observed in the esophagus of *S. cuspicaudus* from day 3, this organ is well differentiated at 6 DPE and, by day 9, it is observed to be thicker and have three layers of skeletal striated muscle in some areas, which it allows the distension of the organ and the apprehension of the prey; the oropharynx and esophagus had a stratified epithelium with goblet cells. According to (Galvão et al., 1997) the appearance of goblet cells indicates that the oropharynx and esophagus are ready to receive exogenous nourishment as their secretions protect the epithelium from damage caused by the passage of food.

According Prieto & Atencio-García (2008), *S. cuspicaudus* larvae are considered altricial with rapid yolk sac depletion. The trans-Andean shovelnose catfish larvae began their first feeding (newly hatched brine shrimp nauplii, instar I) at 43 HPH, at an average temperature of 26.6 °C, with a weight of 1.2 ± 0.1 mg, total length of 5.0 ± 0.3 mm. The body was observed to be translucent, with the presence of the formed digestive tract, without differentiation of annexed glands, whitish barbels

in formation and defined gill lamellae, mouth in terminal position and fully pigmented eyes, similar to that reported by (Prieto et al., 2015).

Also, at the beginning of feeding, the intestine showed a wide lumen that formed in the anterior region with a sac-like shape. Some authors have reported larval extracellular proteolytic digestion in the foregut, where the pH is alkaline and trypsin-like enzymes promote proteolytic activity (Faccioli et al., 2016; Portella et al., 2014). At 6 DPH the organization of the hepatocytes in rows is observed, with central rounded nuclei, the exocrine pancreas attached to the liver with cells forming acini, spherical nuclei and at 12 DPH the liver presented greater development and, in the pancreas, as well as acinar cells ducts are also seen. At 12 DPH gastric glands were found in the stomach of *S. cuspicaudus*. According to (Yang et al., 2010), gastric glands in catfish appear earlier than in other orders. However, the appearance of gastric glands in *S. cuspicaudus* can be considered late as it occurs after other catfish, including *Pelteobagrus fulvidraco* at 3 DPH (Yang et al., 2010), *O. bimaculatus* in 8 DPH (Pradhan et al., 2012) and *P. sutchi* in 9 DPH (Islam, 2005). The appearance of these glands is an important event in larviculture and, together with the secretion of pepsin, indicates that the stomach has become functional and marks the transition from larvae to juveniles (Ma et al., 2014).

Tank color had a significant effect ($p < 0.05$) on the overall performance of *C. gariepinus* fingerlings, (Okomoda et al., 2017) who found that rearing in black tanks resulted in higher daily feed intake and better growth performance compared to other tank colors. In the same study they found that the fat and protein content of the carcass at 8 weeks revealed a trend similar to that observed for growth; it is probable that the culture techniques used in the present study favor the growth conditions of the species. In the same way, and as in other catfish, it was observed that the tilefish has a preference for darkness, similar to what was reported for *C. gariepinus*, (Almazán-Rueda, 2004; Britz & Pienaar, 1992).

Compared to temperate-water fish, tropical fish develop faster due to the effect of temperature on metabolic rate (Gillooly et al., 2002). The larvae and fingerlings of *S. cuspicaudus* had a growth, with some variations to those reported for *Pseudoplatystoma punctifer* (Darias et al., 2015; Fernández-Méndez et al., 2015; Gisbert et al., 2014). The specific growth rate (SGR) was low (13.56) up to 6 DPH (7.16 ± 0.7 mm TL) corresponding to the stage called first feeding, when the larvae were fed with brine shrimp and kept in circular pools with artificial aeration at a temperature of the water of 26.6 °C, followed by a medium growth (SGR 22.38) until 11 DPH at 27.8 °C coinciding with the development of the stomach and its attached glands and, finally, a high growth (SGR 34.88) until 20 DPE at 27.8 °C moment where the growth evaluation ended. In this sense, the growth pattern of *P. punctifer* larvae and early juveniles in terms of weight showed an initial phase of slow growth (SGR 0.19 ± 0.00) up to 12 dpf (12.02 ± 0.18mm TL), corresponding to the larval stage, followed by an exponential growth rate (SGR 0.53 ± 0.10) from 12 DPH onwards, coinciding with the start of the juvenile stage (Castro-Ruiz et al., 2019). Similar growth patterns have also been reported in tropical and freshwater fish species, such as *P. fulvidraco* (Yang et al., 2010), *Mystus nemurus* (Srichanun et al., 2012), *Centropomus undecimalis* (Jimenez-Martinez et al., 2012), *Petenia splendida* (Uscanga-Martínez et al., 2011) and *Lutjanus guttatus* (Moguel-Hernández et al., 2014).

The low and medium growth rate observed from hatching to 12 DPH in *S. cuspicaudus* can be interpreted as an evolutionary strategy to consume available energy from yolk sac and prey reserves to promote larval physiological changes (gastrointestinal and body system development) rather than somatic growth, as also reported in *O. bimaculatu* (Pradhan et al., 2013), *Pangasianodon hypophthalmus* (Rangsin et al., 2012) and *Atractosteus tropicus* (Frías-Quintana et al., 2015).

In this study, three important physiological events were evidenced that favored the growth and development of *S. cuspicaudus* larvae as

follows: 1. From hatching to 6 DPE (days post-emergence), a marked development of organs was observed, including digestive organs, probably due to the type of live food supplied, *Artemia salina*, favors the development of aquatic organisms. 2. From 7 to 11 DPE, a greater development of gastric glands was observed, which favored the digestion of nutrients and, therefore, growth. 3. From 12 to 20 DPE, a higher specific growth rate was observed, probably due to the variety of live food (primary productivity), present in the ponds. It is known that live food is important for larval development, thanks to its contributions to fatty acids and essential amino acids.

The changes in the digestive system during the first days are sufficient to allow the ingestion and digestion of food through the formation of goblet cells in the esophagus (day 3), development of the eye (day 6), development of the stomach, foregut, liver, pancreas, and gastric glands (day 12). The onset of exogenous food consumption in *S. cuspicaudus* occurs at 43 HPH, but full development of the stomach together with the gastric glands was only observed up to 14 HPH, at which time full development of other organs was evidenced, including sensory and locomotion organs.

Based on these findings, it is indicated that a minimum of 14 days post-emergence (DPE) is necessary to procure fingerlings of trans-Andean shovelnose catfish exhibiting commendable quality, resilience, and a morphology akin to that of adult specimens. This includes characteristics such as ossified fin rays and the manifestation of innate behaviors like the escape instinct. These attributes render these fingerlings suitable for restocking initiatives.

Ethical approval: All applicable international, national, and institutional guidelines for the care and use of animals were strictly followed. All animal sample collection protocols complied with the current laws of Colombia. All procedures involving the handling of the animals were performed according to the Guide for the Care and Use of Laboratory Animals (Albus, 2012). A permit was granted by the National Aquaculture and Fisheries Authority—AUNAP of Colombia under Resolution 0955 (27 May 2020).

Ethical statement: The authors declare that they all agree with this publication and made significant contributions; that there is no conflict of interest of any kind; and that we followed all pertinent ethical and legal procedures and requirements. All financial sources are fully and clearly stated in the acknowledgments section. A signed document has been filed in the journal archives.

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