Zygote **20** (August), pp. 269–276. © Cambridge University Press 2011
doi:10.1017/S0967199411000116 First Published Online 18 March 2011

Early development of *Astronotus ocellatus* under stereomicroscopy and scanning electron microscopy

Maria do Carmo Faria Paes[1], *Lilian Cristina Makino*[2], *Leonardo Avendaño Vasquez*[3], *João Batista Kochenborger Fernandes*[2] *and Laura Satiko Okada Nakaghi*[2]
Aquaculture Center of UNESP – CAUNESP – Jaboticabal, SP, Brazil; and Universidad Nacional da Colombia, Bogotá – D.C., Colombia

Date submitted: 01.09.10. Date accepted: 15.01.11

Summary

Astronotus ocellatus, popularly known as Oscar, is a cichlid fish from the Amazon basin (Brazil) with a great potential for fish farming. The aim of this research is to describe the morphology of eggs and larvae of *A. ocellatus* under stereomicroscopy and scanning electron microscopy. Eggs from natural spawnings were taken to hatcheries, collected at previously established time periods and then analysed. Oscar's eggs are demersal, adhesive and fragile to touch, with a slightly oval shape. The fertile eggs are yellowish in colour and when unfertilized are a white opaque colour. In the initial collection (IC), the majority of eggs were found to be at the gastrula phase with 30% epiboly. At 12 h after the IC, the formation of the embrionary axis and somites was observed, followed by differentiation of the tail and of the head. Fifteen hours after the IC, the emergence of the optic and otic vesicles, and of adhesive glands and the yolk pigmentation was observed. Larval hatching took place between 46 and 58 h after the first collection, at an average temperature of 27.45 ± 2.13 °C. The larval stage was characterized by the development of the heart, fins, branchial apparatus, neuromasts, taste buds and adhesive glands on the head. Larval development to yolk absorption took a period of 257 h. These results provide important information for reproduction, rearing and preservation of *A. ocellatus*.

Keywords: Embryo, Larvae, Morphology, Ontogeny, Oscar, Reproduction

Introduction

Astronotus ocellatus, popularly known as Oscar, is a cichlid fish from South America that lives in the Amazon river of Brazil and Peru. This species has great potential for fish farming, and is cultivated around the world as an ornamental fish (Machado, 1983), as well as being extremely intelligent, and suitable for sport fishing (Fury & Morelo, 1994) and also for human nutrition, because their meat is tasty, firm and there is absence of intramuscular bones (Fontenele e Nepomuceno, 1983). Adult specimens may reach 33 cm in length and weigh up to 1.5 kg (Machado, 1983). Their first gonadal maturation is reached at around 10 to 12 months of age, and an average about 1500 to 2000 eggs per clutch is obtained (Braga, 1962). It is a species of partitioned spawning that exhibits parental care behaviour and has three to four reproductive cycles per year (Silva *et al.*, 1993).

Despite the economic importance of this species, there are scarce data in the current literature, with information mainly about its reproductive biology and the early development of eggs and larvae.

Little is known about the early development of native species with partitioned spawning. Knowledge about embryos and larvae of any species is very important because it represents a useful tool for localization of spawning sites and studies on growth in a natural environment (Reynalte-Tataje *et al.*, 2001).

Data about the early developmental stages in native species are essential for research and fish farming (Ribeiro *et al.*, 1995). Most research with fish larvae has been focused on systematics and phylogeny, but lacks

[1]All correspondence to: Maria do Carmo Faria Paes. Aquaculture Center of UNESP – CAUNESP – Via de Acesso Prof. Paulo Donato Castellane, s/n, 14884–900, Jaboticabal, SP, Brazil. Tel:/Fax: +55 16 3209 2656. e-mail: mariacfpaes@yahoo.com.br.
[2]Aquaculture Center of UNESP – CAUNESP – Via de Acesso Prof. Paulo Donato Castellane, s/n, 14884–900, Jaboticabal, SP, Brazil.
[3]Universidad Nacional da Colombia, Carrera 45 N° 26–85 – Edificio Uriel Gutiénez, Bogotá – D.C., Colombia.

particular attention to morphological development in relation to function. The study of fish larvae is necessary to understand the biological role of larval characteristics in survival, mainly involving those functional mechanisms related to vital areas such as locomotion, feeding and breathing (Osse, 1990).

Studies using stereomicroscopy and scanning electron microscopy have provided three-dimensional images of the surface, very important for knowledge of external morphology and identification of eggs and larvae.

Given the biological and economical importance of *A. ocellatus* for fish farming, the aim of this present research was to analyse its embryonic and larval development using stereomicroscopy and scanning electron microscopy.

Material and methods

Two sets of 150 specimens of *A. ocellatus* from 1 to 2 years old were kept in separated 50 m^3 masonry tanks and fed twice a day with commercial food containing 40% raw crude protein. Two plastic cylinders and ceramic bricks were placed in the tanks to provide both shelter and an area for spawning. Natural spawn were then observed from October 2007 to February 2008.

As soon as the spawn in the tanks was observed, they were removed and transported to plastic incubators that had a continuous water flow. Sample collection was performed according to the following procedure: initial collection at the moment the spawn in the tank was observed, every 6 h up to larval mouth opening, then every 12 h up to 161 h post-hatching (hPH) and every 24 h up to 383 hPH.

Samples were fixed in Karnovsky's solution for 24 h, washed up in phosphate-buffered saline, pH 7.4 and stored in 70% ethanol in a refrigerator. Afterwards, the samples were observed and the images were captured under a stereomicroscope, model MZ8 equipped with LEICA DFC 280 automatic photomicrography. For scanning electronic microscopy analysis, the samples were post-fixed in 1% osmium tetroxide for 2 h and washed again in the same buffer. Then, the samples were dehydrated in a graded series of ethanol at 30, 50, 70, 80, 90 and 95% concentrations plus three washes at 100% (15 min each) and dried to the critical point in a liquid CO_2 drier, mounted in a copper grid, coated with gold–palladium ions and observed and photographed under a scanning electron microscope (JEOL-JSM 5410).

The average physico-chemical water parameters monitored in the incubators were as follows: temperature 27.45 °C; pH 4.83; dissolved oxygen 5.52 mg/l; alkalinity 52 mg/l; ammonia 117.45 μg/l and conductivity 35.51 μS/cm.

Results

Astronotus ocellatus exhibits external fertilization and parental care. The eggs adhere to a substrate and spread in a single layer. They are demersal, adherent and fragile to touch, with a slight ovoid shape, a large yolk sphere and a small perivitelline space. The fertilized eggs have a yellowish colouration and, when unfertilized, they are opaque white.

The first egg collection was performed immediately after their removal from the tank (initial collection – IC). At this moment, most of eggs were in the initial gastrula stage, with about 30% epiboly (Fig. 1*A*) and covered with a thick jelly that made the eggs adherent. After 6 h, the eggs presented 50% epiboly, and at 12 h after IC, the formation of the embryo had already begun.

The beginning of embryo organogenesis is characterized by the formation of the embryonic axis and the appearance of somites followed by tail and head differentiation (Fig. 1*B*). From 15 h after IC we observed the tail extension, the beginning of yolk pigmentation (melanophores) (Fig. 1*C*) and the formation of adhesive glands on the head (Fig. 1*D*), and optic and otic vesicles. Prior to hatching, the prelarvae (Fig. 1*E*) showed strong and continuous tail movements to break the chorion.

Hatching

Hatching took place between 46 to 58 h after the initial collection. The recently hatched larvae were transparent, with pigmentation restricted to the yolk (Fig. 1*E*). The finfold was very large, the mouth was closed and the adhesive glands were highly developed, comprising two pairs in the upper part and one pair in the lower part of the head (Fig. 2*A*).

6 hPH

A primordial heart started beating in the inner part of the embryo. By scanning electronic microscopy, it was possible to observe the cephalic groove, the conspicuous adhesive glands and both otic vesicle and nasal orifices (Figs. 4*A* and 5*A*). Due to the absence of fins and swim bladder and the large amount of yolk, the larvae remained grouped in the bottom of incubators, moving with rapid tail movements.

12–30 hPH

The body pigmentation of larvae increased and the pectoral fin bud appeared; the head was already detached from the yolk; the heart was pumping blood throughout the entire larval extension; the yolk was highly vascularized and the eyes were clearly pigmented. At this stage, the larvae tried

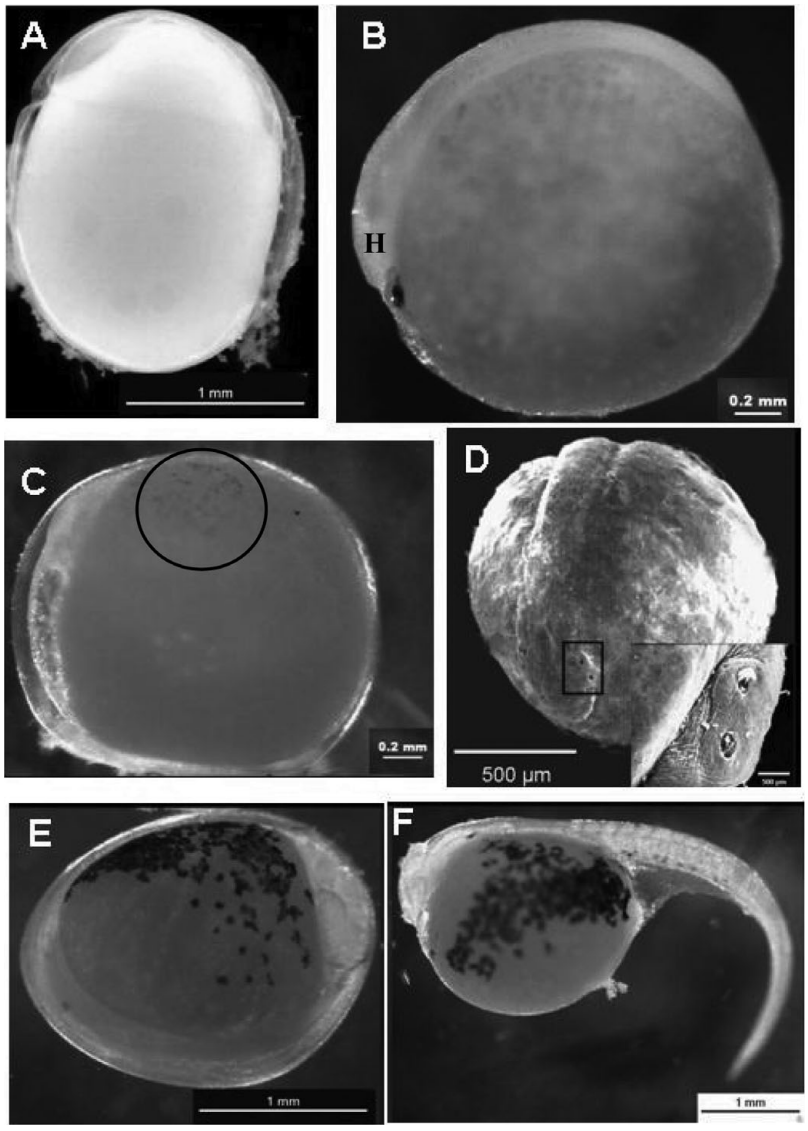

Figure 1 Photomicrographs and electron micrographs of *Astronotus ocellatus* (*A*) Stage gastrula. (*B*) Differentiation of the head (H) and tail. (*C*) Yolk pigmentation initial (circle). (*D*) Formation of the cephalic gland. (*E*) Prelarva shortly before the outbreak. (*F*) Newly hatched larva.

to escape the pipette aspiration and proved to be negative photosensitively when exposed to the stereomicroscope light.

41–77 hPH

There was a deep fold in the epithelium covering the mouth entrance and the formation of branchial arches had started (Fig. 4*B*). At 53 hPH, the mouth was open and occupied the anterior head portion, below the eye line. As well as the head pigmentation, the pectoral fin was already noticeable; the gill lamellae were formed and the opercle was starting to cover them (Figs. 2*C* and 4*C*). At 77 hPH, the larvae presented free pectoral fins, the embryonic finfold was reduced, the opercle covered nearly the entire well developed gills and the lower jaw was completely formed (Figs. 2*D* and 4*D*) and constantly moving.

89–113 hPH

The larvae presented a formed branchial apparatus. There was evidence of the main lamellar of the gill arches and of the intense blood vascularization in the yolk and head (Fig. 3*A*). The adhesive glands were now not as evident, the pectoral fins moved strongly and the opercle covered the gills slit at 101 hPH (Fig. 2*E*, 3*B* and 4*E*), with the annus opened (Fig. 3*C*).

125 hPH

Exogenous feeding was initiated. Taste buds could be noticed only on the lip surface (Fig. 5*D*). The olfactory

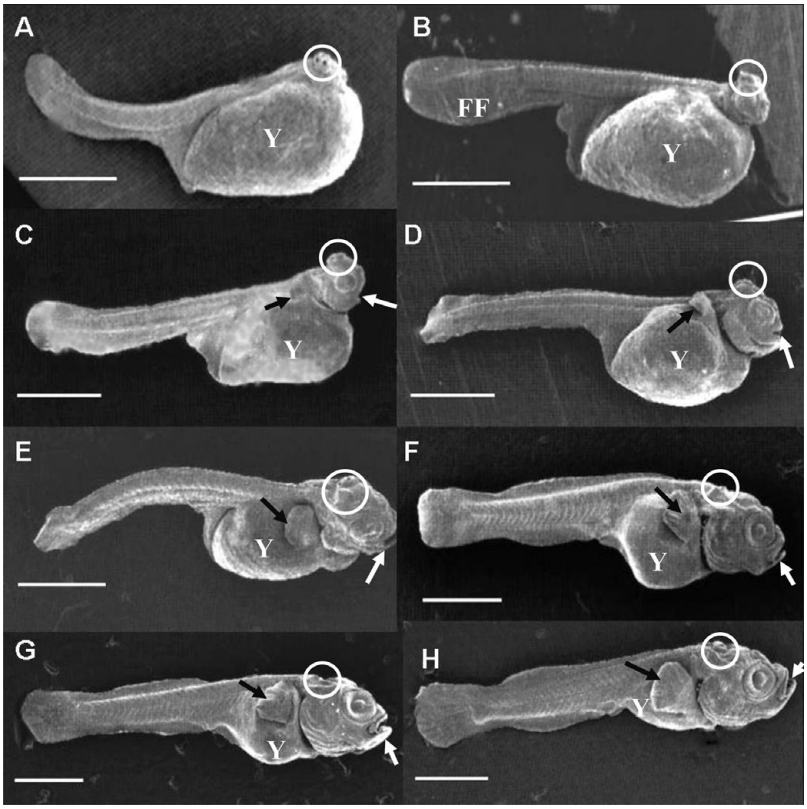

Figure 2 Electron micrographs of *Astronotus ocellatus* showing their development. (*A*) Newly hatched larva; (*B*) 30 hPH; (*C*) 65 hPH; (D) 77 hPH; (*E*) 101 hPH; (*F*) 149 hPH; (*G*) 185 hPH; (*H*) 209 hPH. hPH, hours post-hatching. FF, finfold; Y, yolk; white arrow, mouth; circle, adhesiveness glands; black arrow, pectoral fin.

epithelium had many cilia; larvae already presented vertical swimming and chased brine shrimp napuli.

137–383 hPH

The first rays of the caudal fin appeared (Fig. 3*D*); only one pair of adhesive glands was present in the upper region of the head and the other were absorbed. The heart was already located between the bones of the opercle. At 149 hPH, the formation of dorsal and anal fins was initiated; the opercle was completely formed and rays appeared in the pectoral fins, the mouth assumed a terminal position (Fig. 2*F*) and the neuromasts appeared around the eyes (Figs. 2*G* and 5*B*) and bile excretion through the anus could also be observed. With 209 hPH the larva is well formed but still has a yolk (Fig. 2*H*). At 257 hPH, the yolk was completely absorbed. From 383 hPH on, six branchial arches with mucus-secreting cells were detected (Fig. 5C), similar to those found in the skin. The pectoral fins were completely formed; the dorsal and anal fins were starting to develop; the adhesive glands were degenerated; the eyes were completely formed, with intense pigmentation and definitive coloration of retina (Fig. 3*E*).

Discussion

Oscar's eggs are ovoid shaped, demersal, adhesive, fragile to the touch, with a large yolk sphere and a small perivitelline space. The characteristics of Oscar's eggs confirmed the description presented by Perini *et al.* (2009) who asserted that eggs of non-migratory or sedentary species usually exhibit some degree of adhesiveness, a small perivitelline space, are less numerous and may be subject to parental care.

The first stage observed in the studied spawnings was the gastrula phase. Chacon (1959) analysed the development of eggs of *A. ocellatus* by stereomicroscopy and reported that the gastrula phase occurs approximately 5 h after the first cleavage. However the environmental conditions of each experiment must be taken into account, as several factors might influence development time, in particular temperature (Osman *et al.*, 2008).

In this present research, somitogenesis in *A. ocellatus* occurred before the end of epiboly, similar results were obtained by Morrison *et al.* (2001) for Nile tilapia. This occurrence is due to the large size of the eggs, a common characteristic of species with parental care (Suzuki, 1992).

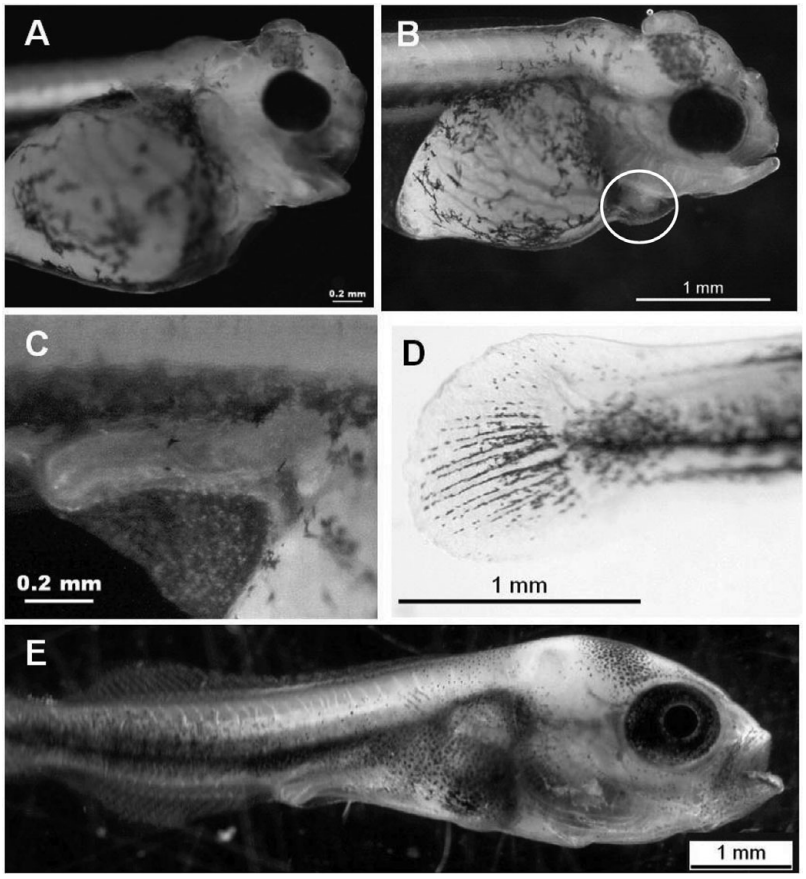

Figure 3 Photomicrographs of *Astronotus ocellatus*. (*A*) 89 hPH larva beginning on the pigmentation of the head, yolk sac is richly vascularized, training and irrigation of the branchial apparatus. (*B*) 101 hPH larva with pectoral fins free, heart (circle) and vascularization of the yolk. (*C*) 113 hPH opened anus. (*D*) 137 hPH Pigmentation of caudal fin rays. (*E*) 383 hPH larva with yolk completely absorbed, well trained eyes, heart, staying under the cap, body pigmentation, digestive tract formed, inflated swim bladder and early onset ray of the dorsal and anal. hPH: hours post-hatching.

The larvae hached with closed mouth and anus, were transparent, with a large yolk sac and less eye pigmentation, similar to most of the larvae of freshwater fish (Nakatani *et al.* 2001). However, *A. ocellatus* had glands used for adhesion present in the head that appeared even before hatching and developed mainly in the newly hatched larvae. These glands have been described in some freshwater species (*Hoplias malabaricus*, Araújo-Lima & Bittencourt, 2002; *Symphysodon* spp., Morais, 2005; *Paracheirodon axelrodi*, Anjos & Anjos, 2006). The adhesive glands in fish are composed of a group of mucous-producing cells and serve to prevent the larvae dispersing from the nest, which would reduce the efficiency of parental care (Araújo-Lima & Bittencourt, 2002).

The development of many structures and organs, such as, eyes, taste buds, fins, neuromasts, heart, opening and position of the mouth, branchial apparatus, etc., is closely related to survival and adaptation of the larvae. The eyes of Oscars develop very quickly, even before the mouth opening, representing the first sensitive organ to be effectively functional.

The larvae of *A. ocellatus*, which had constituted and pigmented eyes, escaped easily from pipette suction during sample collection. These data reveal typical characteristics of a species whose escape from predators and feeding is mainly dependent on their sight (Osman *et al.*, 2008).

Taste buds are also important structures involved in the capture of food. According to Matsuoka (2001) the olfactory organs and taste buds act as chemoreceptors, whose function is to capture the smell and the taste of saturated substances present in the water. The number of taste buds would also be related in the ability of fish to effectively locate a food source (Caprio, 1988). Species that inhabit deep waters and/or turbid water, for example, *Pseudoplatystoma corruscans*, have large numbers of chemical receptors, not only around the eyes, but also in the barbels and on the body surface and have poorly developed eyes. In contrast, the *A. ocellatus*, which inhabits clear, calm and shallow water (http://fishbase.org, 2010) was noted to have few taste buds, free neuromasts were located in the head and big eyes developed.

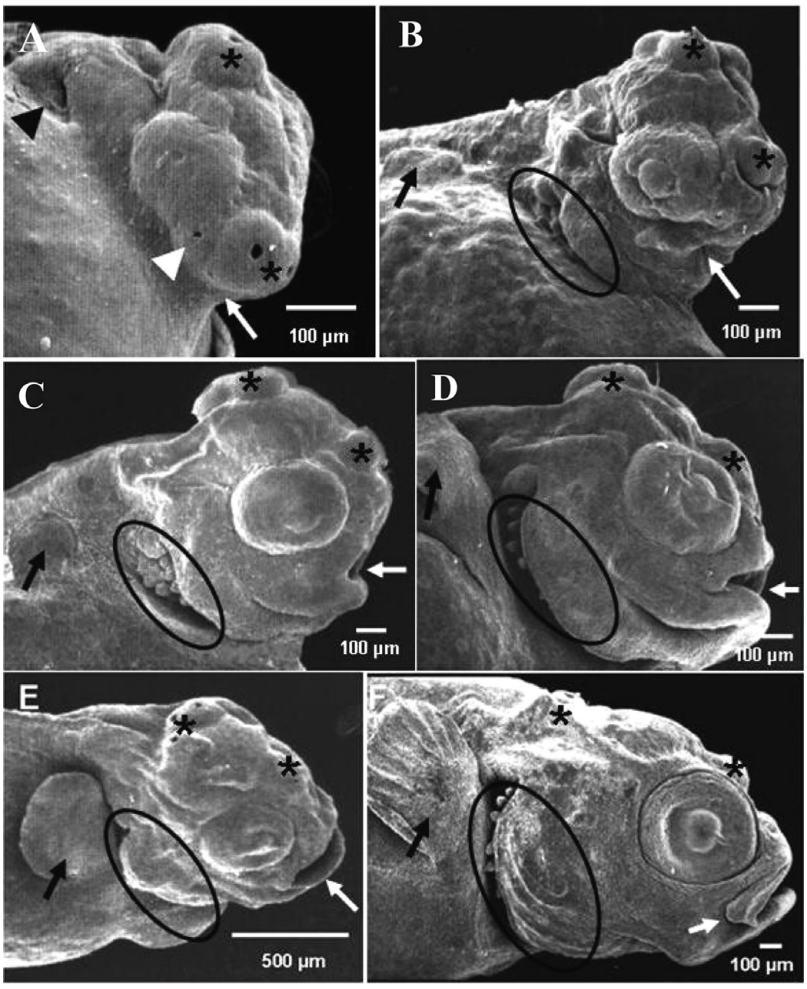

Figure 4 Electron micrographs of *Astronotus ocellatus* showing the development head structures. (*A*) 6 hPH; (*B*) 41 hPH; (*C*) 65 hPH; (*D*) 77 hPH; (*E*) 101 hPH; (*F*) 149 hPH. White arrowhead: nasal orifice; black asterisks: adhesive glands; black arrowhead: otic vesicle; black arrow: pectoral fin; black circle: gill formation; white arrow: mouth. hPH: hours post-hatching.

The development of the circulatory system begins with the appearance of the heart in the embryo during somitogenesis, and it continues during larval development (Hu *et al.*, 2000). The heart is a structure that develops rapidly, being the first functional organ in the larvae. Osman *et al.* (2008) argued that in *Clarias gariepinus*, the rapid formation of the heart indicates an adaptation of native species to warm waters, the increased metabolic rate being a result of oxygen demand and nutrients under elevated temperatures.

The Oscar has a large yolk sac that within 257 h of hatching (HPH) was completely absorbed. According to Nakatani (2001) species with larger yolk sacs are better prepared to survive for longer periods without relying on exogenous feeding. Tengjaroenkul *et al.* (2002) argued that to prevent larvae mortality and feed waste, exogenous feeding must begin when the yolk is completely absorbed. However, in this research exogenous feeding was offered at 125 hPH and the larval survival index from then until the end of the experiment was of an order of 100%. The size of the yolk and its absorption time are also species specific. Larvae with higher amounts of endogenous nutrition have a longer period to adapt to capturing external foods items during the period that they are maintained by yolk sac reserves (Woynarovich & Horváth, 1983; Bonislawska *et al.*, 2000; Gisbert *et al.*, 2000).

A. ocellatus development is considered slow when compared with species that migrate, but it is in line with the development of the most lentic species. The occurrence of morphological structures that allow the capture of food even before the exhaustion of endogenous energy reserves provides for an increased chance of survival during the larval stage.

The simultaneous differentiation of structures such as pectoral fins, heart and gills was remarkable, as well as the rapid formation of the eyes and reveals that this fish is a typical tropical species and can be characterized as a visual predator.

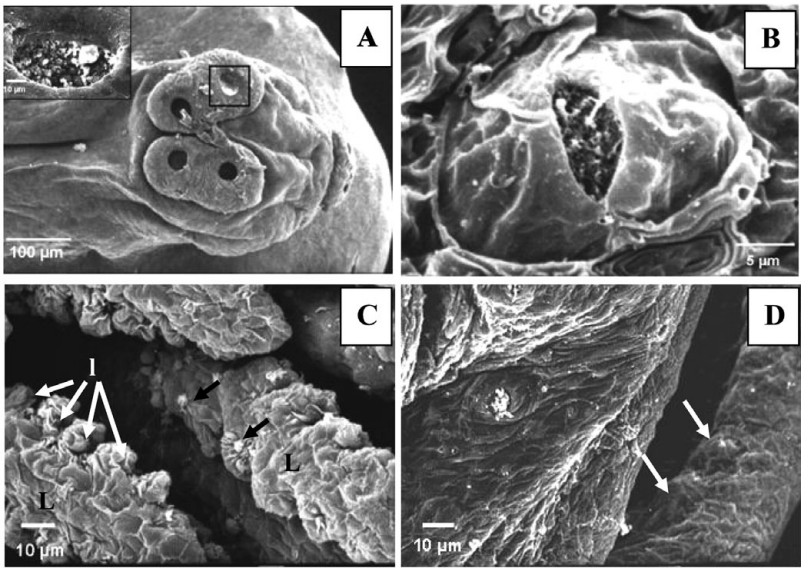

Figure 5 Electron micrographs of *Astronotus ocellatus*. (*A*) 6 hPH adhesive glands present on the head. (*B*) 185 hPH neuromast around the eyes. (*C*) 383 hPH gill filaments showing the main (L) and secondary (l) lamellae that compose the gill with the presence of mucus released by the secretory cells (black arrows). (*D*) 383 hPH taste buds in the mouth (arrows). hPH: hours post-hatching.

The results presented here provide information that will be useful for the biology, reproduction and management of rearing *A. ocellatus*.

Acknowledgements

The authors are grateful to FAPESP (process 2006/54048–0) and CNPq (process 134265/2006–8) for financial support.

References

Anjos, H.D.B. & Anjos, C. R. (2006). Biologia reprodutiva e desenvolvimento embrionário e larval do cardinal tetra, *Paracheirodon axelrodi*, Schultz, 1956 (Characiformes: Characidae), em laboratório. *Bol. Inst. Pesca* **32**, 151–66.

Araujo-Lima, C.A.R.M. & Bittencourt, M.M. (2002). A reprodução e o início da vida de *Hoplias malabaricus* (Erythinidae; Characiformes) na Amazônia Central. *Acta Amaz.* **32**, 693–7.

Bonislawska, M., Formicki, K. & Winnicki, A. (2000). Size of eggs and duration of embryogenesis in fishes. *Acta Ichthyol. Piscat.* **30**, 61–71.

Braga, R.A. (1982). Freqüência de desova de reprodutores de apaiari, *Astronotus ocellatus ocellatus* Spix (Pisces, Cichlidae), mantidos em cativeiro. *DNOCS* **1**, 351–7.

Caprio, J. (1988). Peripheral filters and chemoreceptor cells in fishes. In: *Sensory Biology of Aquatic Animals* (eds Atema J., Fay R.R., Popper A.N. & Tavolga W.N.). pp. 313–38. New York: Springer-Verlag.

Cestarolli, M. A. (2005). Larvicultura de pintado *Pseudoplatystoma coruscans* (Agassiz, 1829): aspectos da alimentação inicial e do desenvolvimento de estruturas sensoriais. Thesis, Jaboticabal, Centro de Aqüicultura, Universidade Estadual Paulista.

Chacon, J. O. (1959). Evolução de ovo, larva e alevino de apaiari, *Astronotus ocellatus ocellatus. DNOCS* **28**, 31–47.

Fishbase (2010). In: www.fishbase.org, accessed on August 28, 2010.

Fontenele, O. & Nepomuceno, F.H. (1983). Exame dos resultados da introdução do *Astronotus ocellatus ocellatus* (Agassiz, 1849), em açudes do Nordeste do Brasil. *DNOCS* **41**, 85–99.

Fury, J.R. & Morello, F.A. (1994). The contribution of an exotic fish, the oscar, to the sport fishery of the Everglades water conservation areas. In: *Annual Conference of the Southeastern Association of Fish and Wildlife Agencie*s **48**. Biloxi. *Proceedings.* 474–81.

Gisbert, E., Williot, P. & Castelló-Orvay, F. (2000). Influence of egg size on growth and survival to early stages of Siberian sturgeon (*Acipenser baeri*) under small scale hatchery conditions. *Aquaculture* **183**, 83–94.

Hu, N., Sedmera, D., Yost, H.J. & Clark, E.B. (2000). Structure and function of the developing zebrafish heart. *Anat Record* **260**, 148–57.

Machado, C.E.M. (1983). Criação de apaiari. São Paulo: Nobel.

Matsuoka, M. (2001). Development of sense organs in the Japanese sardine *Sardinops melanostictus*. *Fish. Sci*. **67**, 1036–45.

Morais, B.F. (2005). Sistema intensivo de criação e manejo de cria do acará-disco, *Symphysodon* spp. Masters degree, Universidade Federal Rural de Pernambuco, Recife.

Morrison, C.M., Miyake, T. & Wright, J.R. (2001). Histological study of the development of the embryo and early larva of *Oreochromis niloticus* (Pisces: Cichlidae). *J. Morphol*. **247**, 172–95.

Nakatani, K., Agostinho, A.A., Baumgartner, G., Bialetzki, A., Sanches, P.V. & Cavicchioli, M. (2001). Ovos e larvas de peixes de água doce: desenvolvimento e manual de identificação. Maringá: Eduem/Nupélia.

Osman, G.M., Wuertz, S., Mekkawy, I.A., Verreth, J. & Kirschbaum, F. (2008). Early development of the African catfish *Clarias gariepinus* (Burchell, 1822), focusing on the ontogeny of selected organs. *J. Applied Ichthyol*. **24**, 187–95.

Osse, J.W.M. (1990). Form changes in fish larvae in relation to changing demands of function. *J. Zool*. **40**, 362–85.

Reynalte-Tataje, D.R., Zaniboni-Filho, E. & Muelbert, B. (2001) Stages of the embryonic development of the piavuçu *Leporinus macrocephalus* (Garavello e Britski, 1988). *Act. Sci.* **23**, 823–7.

Ribeiro, C.R., Leme dos Santos, H.S. & Bolsan, A.A. (1995). Estudo comparativo da embriogênese de peixes ósseos (PACU, *Piaractus mesopotamicus*; tambaqui, *Colossoma macropomum* e híbrido tambacu). *Rev. Bras. Biol.* **55**, 65–78.

Perini, V.R., Sato, Y., Rizzo, E. & Bazzoli, N. (2009). Biology of eggs, embryos and larvae of *Rhinelepis aspera* (Spix & Agassiz, 1829) (Pisces; Siluriformes). *Zygote* **18**, 159–171.

Silva, J.W.B., Regis, R.C. & Bezerra, A.T. (1993). Produção de alevinos de apaiari, *Astronotus ocellatus ocellatus* (Cuvier, 1829) Swainson, 1839, em viveiros. *Ciên. Agron.* **24**, 22–6.

Suzuki, H.I. (1992). Variações na morfologia ovariana e no desenvolvimento do folículo de peixes teleósteos da bacia do rio Paraná no trecho entre a foz do rio Paranapanema e a do rio Iguaçu. Masters degree, Universidade Federal do Paraná, Curitiba.

Tengjaroenkul, B., Smith, B.J., Smith, S.A. & Chatreewogsin, U. (2002). Ontogenic development of the intestinal enzymes of cultured Nile tilapia, *Oreochromis niloticus L. Aquaculture* **211**, 241–51.