Journal of FISH BIOLOGY
Published for the Fisheries Society of the British Isles

Journal of Fish Biology (2010) **76**, 852–861
doi:10.1111/j.1095-8649.2010.02540.x, available online at www.interscience.wiley.com

Larval ontogeny and morphology of giant trahira *Hoplias lacerdae*

M. L. M. Gomes*†, S. L. P. Matta‡, V. A. Araújo§, G. M. F. Silva‡ and A. A. Zacaro‡

**Laboratório de Microscopia Eletrônica, Departamento de Biologia Celular, Instituto de Biologia, Universidade Estadual de Campinas, CP 6109 CEP 13084-971, Campinas, SP, Brazil, ‡Departamento de Biologia Geral, Universidade Federal de Viçosa, CEP 36570-000, Viçosa, MG, Brazil and §Departamento de Biologia Animal, Universidade Federal de Viçosa, CEP 36570-000, Viçosa, MG, Brazil*

(Received 22 October 2008, Accepted 10 November 2009)

In the present study, the morphology and behaviour of giant trahira *Hoplias lacerdae* larvae were investigated, from hatching to complete absorption of the yolk sac, under laboratory conditions. In the first day post-hatching (dph), the larvae presented a big ovoid-shaped yolk sac that underwent regression during larval ontogeny. The mouth opened 3 dph, when the pectoral fins were evident. From this day, the larvae were able to perform sudden bursts of activity and appear to be able to swim a few centimetres before sinking again. The branchial apparatus was defined at 5 dph, and by 6 dph the operculum was formed. The internal organs such as intestine, liver, kidney and external sensorial structures were present at 7 dph. The yolk sac remained until 7 dph.

Key words: Characiformes; developmental biology; neotropical fish; scanning electron microscopy.

INTRODUCTION

The early development of fishes is a dynamic process, with changes in ontogenetic state, coinciding with shifts in diet, microhabitat and behaviour (Pinder & Gozlan, 2004). The study of the initial ontogeny of fishes contributes to knowledge on bioenergetic growth, developmental biology, behaviour and systematics. Besides, it supplies data about larval morphology contributing to the conservation of endangered species (Gozlan *et al*., 1999; Green & McCormick, 2001).

The giant trahira *Hoplias lacerdae* Miranda Ribeiro belongs to the Erythrinidae family and this species is native from the Amazonic and mid-west regions of South America (Taphorn, 1992; Planquette *et al*., 1996). This species has a strong, dense and ossified head without a fontanel. The maxillary bones possess conical teeth. The body is elongated, presenting a conical shape with a round caudal fin (Oyakawa & Netto-Ferreira, 2007; Oyakawa & Mattox, 2009). In general, these fish are found

†Author to whom correspondence should be addressed. Tel.: +55 19 3521 6116; fax: +55 19 3521 6111; email: marcoslucca@yahoo.com.br

in the middle of aqueous vegetation habitats, where they have an exclusively carnivorous diet (small fishes, insects and crustaceans). This species is well adapted to handling when kept in captivity. It also shows significant resistance to temperature variations and dissolved oxygen levels (Ferreira *et al*., 1998).

The great morphologic similarity between different taxonomic groups is the main obstacle in the identification of most fish larvae, especially those collected in the natural environment. Recent morphometric studies using fish eggs and larva ontogeny can help to identify new taxonomic criteria, allowing comparisons between different species (Sanches *et al*., 1999).

The present study aimed to provide data on the larval development and behaviour of *H. lacerdae* during the 7 days of yolk-sac absorption.

MATERIALS AND METHODS

All larvae used in the present study were obtained from a natural spawning of *H. lacerdae* held at the Aquaculture Centre in the Federal University of Viçosa, Minas Gerais, Brazil. Eggs were collected and transferred to a rectangular incubation tank (40 l). The water temperature was 21–23° C during incubation and gentle aeration was provided using an air diffuser. All experimental and holding facilities were kept in an environmentally controlled room with the photoperiod set at 13L:11D.

Twenty specimens were sampled at random during seven consecutive days from the rearing tanks, and no exogenous feeding was supplied. In order to evaluate the external development of larvae, data on total length (L_T), standard length (L_S), body height (H_B) and yolk-sac length (L_{YS}) were obtained using callipers (0·01 mm) and a dissection microscope. All samples were preserved in glutaraldehyde 2·5% in sodium phosphate buffer, 0·1 mol l^{-1}, pH 7·4 fixative solution, just before analgesia treatment in cold water.

LIGHT MICROSCOPY

After fixation, the larvae were embedded in glycol methacrylate and histological sections (3 μm) were cut with a microtome using glass knives. The sections were stained in 1% toluidine blue–sodium borate solution.

SCANNING ELECTRON MICROSCOPY

After fixation, all larvae were washed with buffer solution (sodium phosphate buffer, 0·1 mol l^{-1}, pH 7·4) and dehydrated using ethanol (50, 70, 80, 95 and 100%). The specimens were critical point dried in a critic point dryer (CPD) using CO_2. They were mounted on scanning electron microscope (SEM) stubs and coated with sputtering gold, before being examined under a LEO VP 1430 scanning microscope (www.zeiss.com).

RESULTS

The mean values of L_T, L_S, H_B and L_{YS} of the *H. lacerdae* larvae during the experiment are shown in Table I. The L_T varied from 7·25 mm on the 1 day post-hatching (dph) to 9·81 mm on 7 dph; the L_S increased from 6·78 mm (1 dph) to 8·74 mm (7 dph); the H_B decreased from 1·88 mm (1 dph) to 1·61 mm (7dph) and the L_{YS} decreased from 2·45 mm (1 dph) to 1·88 mm (6 dph). The larval development of *H. lacerdae*, from the hatching to the yolk-sac absorption was completed after 7 dph (Fig. 1). This period was characterized by several morphological and physiological

TABLE I. Morphometry of *Hoplias lacerdae* larvae: mean ± S.E. values ($n = 20$) of total length (L_T), standard length (L_S), body height (H_B) and yolk-sac length (L_{YS}) during the 7 days post hatching (dph)

Time (dph)	L_T (mm)	L_S (mm)	H_B (mm)	L_{YS} (mm)
1	7·25 ± 0·31	6·78 ± 0·38	1·88 ± 0·14	2·45 ± 0·15
2	8·00 ± 0·18	7·46 ± 0·14	1·85 ± 0·11	2·42 ± 0·12
3	8·41 ± 0·15	7·82 ± 0·27	1·79 ± 0·14	2·27 ± 0·11
4	8·35 ± 0·29	7·92 ± 0·36	1·75 ± 0·14	2·15 ± 0·11
5	9·00 ± 0·18	8·35 ± 0·24	1·65 ± 0·08	1·98 ± 0·08
6	9·31 ± 0·22	8·63 ± 0·33	1·71 ± 0·14	1·88 ± 0·12
7	9·81 ± 0·57	8·74 ± 0·38	1·61 ± 0·15	*

*, Remnants of yolk sac (not measured).

changes, when the larvae underwent ontogenetic changes related to vision, capture and processing of food and differentiation of the gut and gill arches.

ONTOGENY

Day 1 post-hatching

The mouth opening and gill clefts were absent. Otic vesicles containing otoliths were visible in the upper posterior of the head region. The head was bent over the yolk sac, which was filled with individualized oil droplets [Fig. 2(a)]. The eyes did not contain pigmentation, and the mouth was not well formed. A wide primordial finfold, which bordered the notochord, was evident [Fig. 3(a)]. Notochord and miomeres, varying from 42 to 46, could be seen along the body.

Day 2 post-hatching

At this age, the otic vesicle region became more evident. Scattered chromatophores were located on the dorsal part of the head (Fig. 1), and the maxillary cartilages were in the beginning of the development. The mouth depression was well delimited, defining the region of the formation of the mouth. The pectoral fins, not functional yet, appeared above the yolk sac [Fig. 3(b)]. At this stage the gills arches could be identified.

Days 3 and 4 post-hatching

In the beginning of development, the chromatophores were localized on the dorsal body surface; nevertheless, the retina was completely and uniformly pigmented only at 3 dph [Figs 1 and 2(b)]. The gas bladder and the gill arches were in the final process of differentiation [Fig. 2(c)]. The mouth was also opened at this age [Figs 1 and 3(c)]. At 4 dph, the gut was already formed and the pectoral fins were well developed [Fig. 2(d), (e)].

Day 5 post-hatching

At this age, a great reduction in the yolk-sac length was observed (Fig. 1 and Table I). The corporal pigmentation pattern was the same as the previous day, and

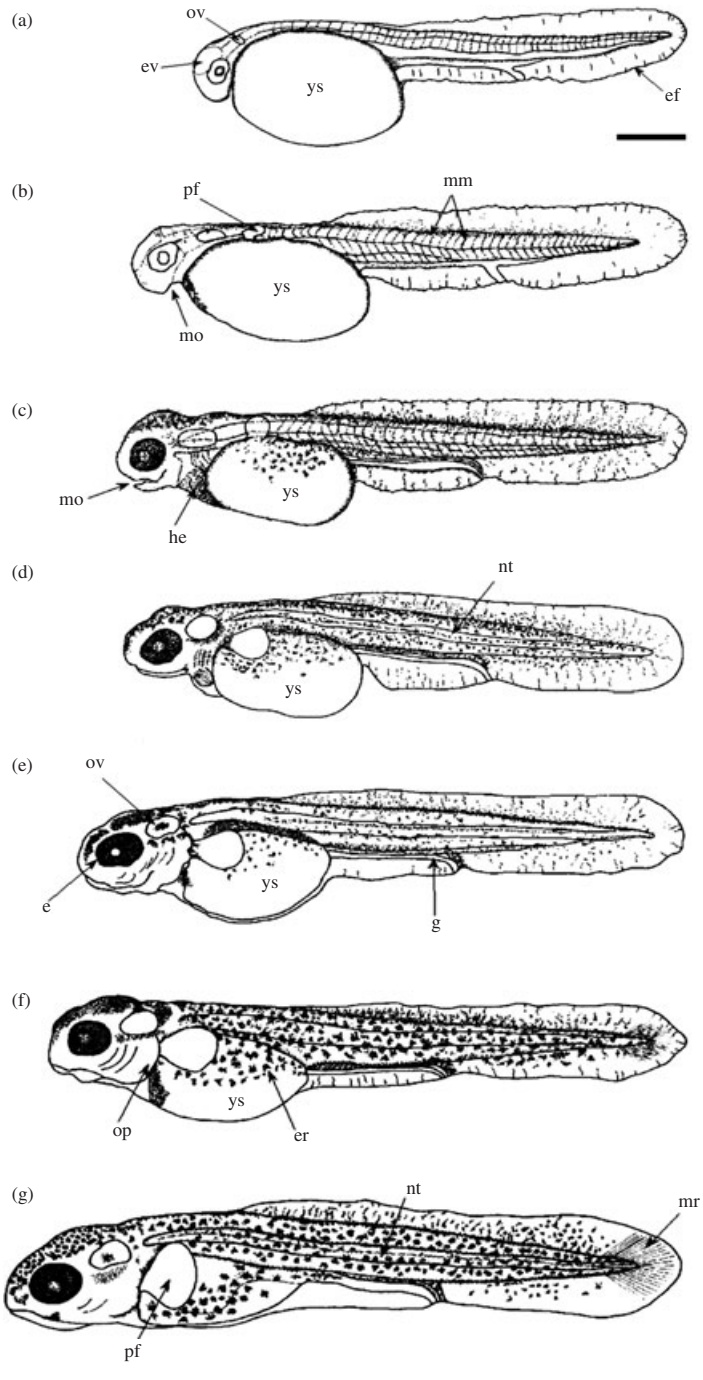

FIG. 1. *Hoplias lacerdae* larvae, from 1 to 7 day post hatching (dph). (a) 1 dph: otic vesicle (ov), encephalic vesicle (ev), yolk sac (ys) and embryonic fin (ef). (b) 2 dph: pectoral fin (pf), miomeres (mm) and future mouth place (mo). (c) 3 dph: heart (he) and opened mouth (mo). (d) 4 dph: notochord (nt). (e) 5 dph: eye (e) and gut (g). (f) dph: operculum (op) and chromatophores (cr). (g) 7 dph: mesenchymal rays (mr). All drawings are at the same magnification and the scale bar represents 1 mm.

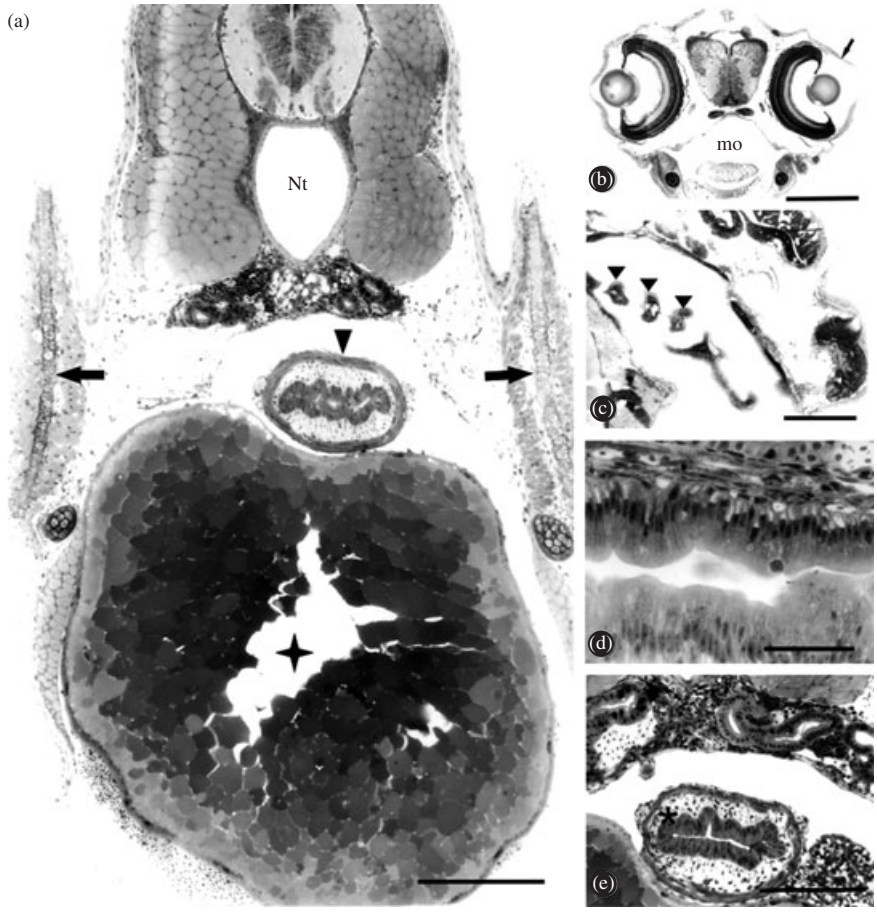

FIG. 2. Histological sections of *Hoplias lacerdae* larvae. (a) Section showing the notochord (Nt), pectoral fins (➡), gut (►) and the yolk sac (✦) at 5 days post hatching (dph). (b) Frontal section of the head showing the mouth cavity (mo) and the eyes (➡) at 5 dph. (c) Section showing the gill arches (►) at 4 dph. (d) Longitudinal section of the gut at 5 dph. (e) Transversal section of the gut (⁎) at 5 dph. Scale bars: (a), (b), (c), (e) 250 μm; (d) 10 μm.

the gut could be observed as a straight tube under the notochord (Fig. 1). The gas bladder appeared inflated. The operculum was evident and covered the branchial cavity [Fig. 3(d)].

Day 6 post-hatching

The beginning of mesenchymal ray formation was observed. The first rays were located at the posterior part of the embrionic fin. Some sensorial buds appeared immediately above the eyes [Fig. 4(a)–(c)].

Day 7 post-hatching

The larval ontogeny was almost finished and larval growth continued. Melanophores developed in the abdominal region, while those from the notochord and finfold

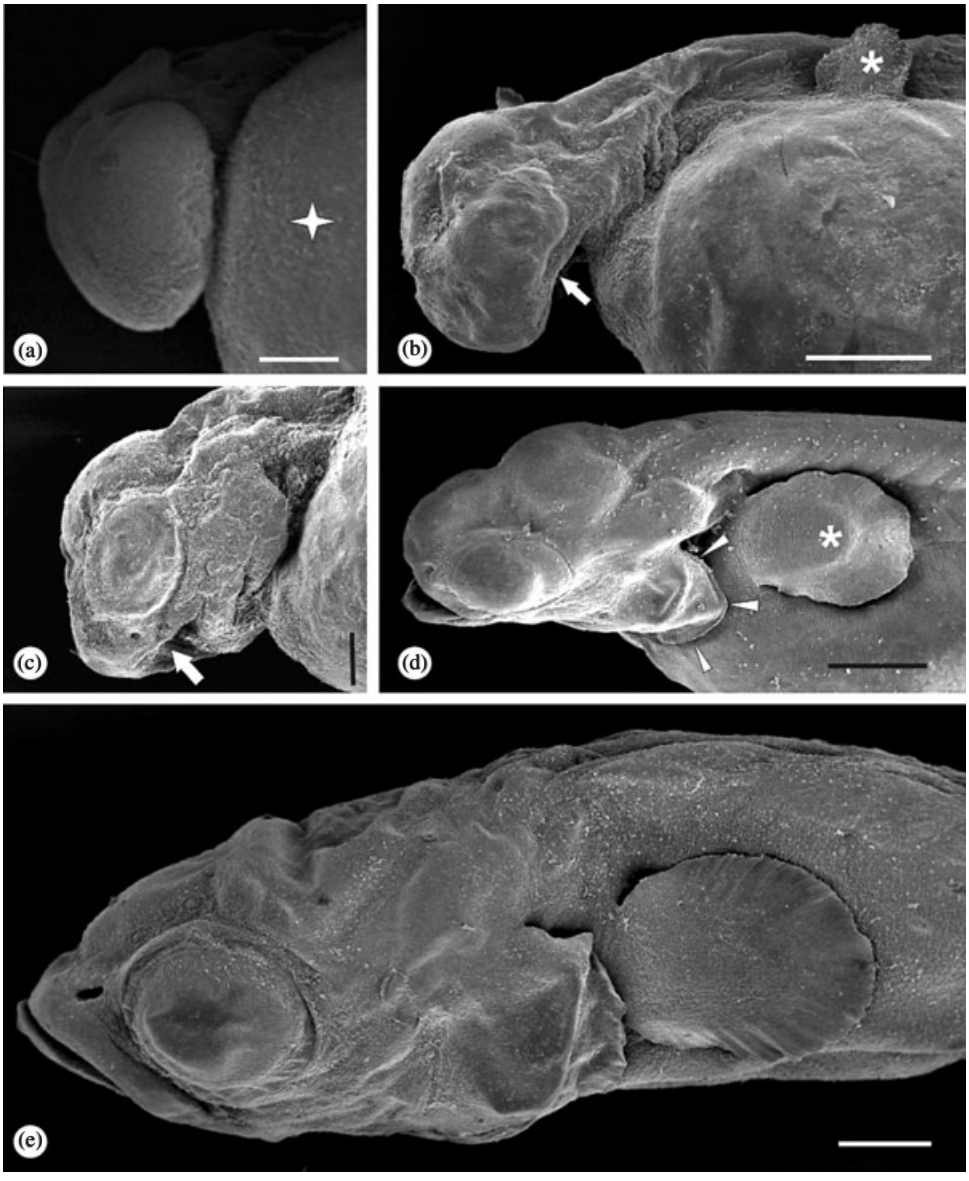

FIG. 3. Scanning electron micrographs showing different developmental stages of *Hoplias lacerdae* larvae: (a) 1 day post hatching (dph) showing a prominent yolk sac (✧), (b) 2 dph showing the pectoral fin (⁎) and the region of future mouth formation (⇨), (c) 3 dph showing the opened mouth (⇨), (d) 5 dph showing the pectoral fin (⁎) and the operculum (⊳) and (e) 7 dph with any yolk sac and with all the structures formed already. Scale bars: (a), (b), (c) 150 μm; (d), (e) 10 μm.

increased in size and number (Fig. 1). The yolk sac was almost absorbed and no new structure was seen [Fig. 3(e)]. The external sensory structures were more developed during this stage, and similar sensory structures on the lateral portions of the larval body could also be noticed [Fig. 4(d)].

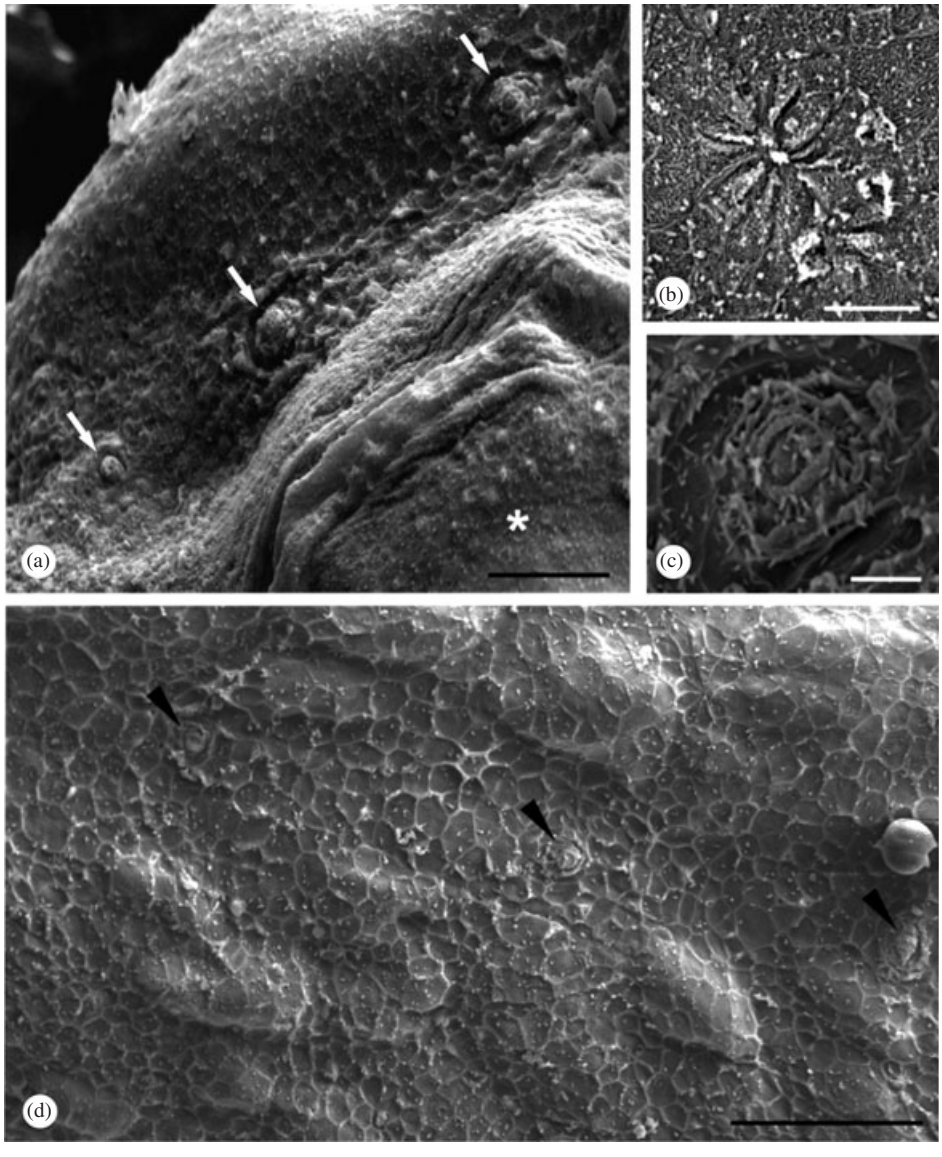

FIG. 4. Scanning electron micrographs of *Hoplias lacerdae* larvae. (a), (b), (c) Cephalic region during 7 days post hatching (dph) showing the formation of sensorial structures (⇨) above the eyes (*), and details of the (b) early and (c) late stages of formation. (d) 7 dph showing the sensorial structures along the body lateral surface (►). Scale bars: (a), (d) 70 μm; (b), (c) 15 μm.

SWIMMING BEHAVIOUR

Right after hatching, the larvae were immobile and lay on the bottom of the tank. Their body was partially enveloped by an embryonic fin, although they did not show any visible pectoral fin. After 3 dph, some vertical and oblique movements towards the water surface were observed, possibly because of the swim-bladder inflation,

diminution of the yolk sac and development of functional pectoral fins. At 7 dph, the larvae underwent active horizontal movements, coinciding with the complete absorption of the yolk sac and development of the fin rays in the tail.

DISCUSSION

Fish growth is the result of a complex combination of factors interacting dynamically according to age and functional requirements. Major morphological and physiological changes take place during the early life of fishes. This is a period of rapid development and growth marked by substantial changes in fish size, shape, structure, physiology and behaviour (Fuiman & Higgs, 1997).

There was some similarity in the larval pattern of body pigmentation between *H. lacerdae* larvae and other species belonging to the Labridae (Kimura *et al*., 1998), as well as to *Chondrostoma toxostoma* (Vallot) (Gozlan *et al*., 1999). The dark colouration observed in larvae of *H. lacerdae* may be related to hiding strategy, which is in agreement with the description of the dark pigmentation of the dorsal part of the larval body of the pelagic fish *Leucaspius delineatus* (Heckel) made by Pinder & Gozlan (2004). A description of the chromatophores is important as a taxonomic character for larvae identification (Faber & Gadd, 1983; Santos & Godinho, 1996; Gozlan *et al*., 1999).

In early development of most fishes, the yolk is the main source of energy and nutrients for the developing embryo and newly hatched larva (Rønnestad *et al*., 1994). It is the main energy source for fishes during the initial development stages (Kamler, 1992). The transition from the endogenous to the exogenous feeding suggests that this is the most critical period in the life of the larvae, once that several morphophysiological changes have occurred. As well as other species that show a large yolk sac, *H. lacerdae* also shows a longer time for larval development (Luz & Portella, 2002, 2005). In *C. toxostoma* (Gozlan *et al*., 1999), the yolk sac is reduced and the larvae of this species have a rapid absorption of all the yolk stored in it.

The larval head length and width increase rapidly, allowing the uptake of larger and larger food particles because these are energetically more favourable. In addition, the fast growth of the head is related also to the fact that as the yolk sac becomes depleted, the larvae must switch to exogenous feeding, thus needing a functional food intake apparatus (Brooks & Dodson, 1965; Mathias & Li, 1982). Like larvae of other species of fishes, *H. lacerdae* larvae show a better larval swimming capacity during the transition from the endogenous to exogenous feeding.

As observed in other families, the mouth opening is a critical ontogenetic event in larval life due to competition for food and predation (Balon, 1985; Coughlin, 1991). The gut of *H. lacerdae* larvae, as well as that observed in the majority of teleosts, developed slowly after hatching (Neves, 1996). Even if the mouth is open, this fact does not imply that the larvae have an exogenous feeding (Boglione *et al*., 2003). The mouth of *H. lacerdae* larvae was opened during 3 dph. This event only occurred before the complete formation of the stomach and gut, probably not allowing the digestion and absorption of food in this phase.

The mouth aperture allows the inflation of the swim-bladder, which is one of the most important steps in the development of larvae swimming behaviour (Santos & Godinho, 1994, 1996, 2002; Gozlan *et al*., 1999; Pinder & Gozlan, 2004). The

inflation of the gas bladder is an event that initiates during the transition between the reabsorption of the vitelinic sources and the exogenous feeding, with an increase in the specific gravity (Santos, 1991). The present study showed that *H. lacerdae* larvae had an inflated gas bladder on 5 dph, coinciding with the period when the larvae began to demonstrate a better swimming capacity. Also, the pectoral and caudal fins, important acquisitions during the organogenesis of fishes, facilitate the balance of the larvae in the water column. The *H. lacerdae* larvae showed active horizontal and vertical movements during 7 dph, mainly due to the presence of well-developed fins.

Boglione *et al*. (2003), working with *Diplodus puntazzo* (Cetti) larvae, associated the age with the development of sensorial structures and observed that in larvae they are not completely formed. In *Dicentrarchus labrax* (L.) larvae (Santos, 1991), however, these sensorial buds were developed right after hatching. In *H. lacerdae* larvae, the sensorial buds can be observed externally on the head and on the lateral surface of the body during 6 dph.

The authors wish to thank the Departamento de Biologia Animal (DBA, Universidade Federal de Viçosa, UFV), for the pisciculture laboratory facilities. The authors also thank the Núcleo de Microscopia e Microanálise (NMM, UFV) for the scanning electron microscopy facilities.

References

Balon, E. K. (1985). The theory of saltatory ontogeny and life history models revisited. In *Early Life History of Fish* (Balon, E. K., ed.), pp. 13–30. Dordrecht: D. W. Junk.

Boglione, C., Giganti, M., Selmo, C. & Cataudella, S. (2003). Morphoecology in larval finfish: a new candidate species for aquaculture, *Diplodus puntazzo* (Sparidae). *Aquaculture International* **11,** 17–41.

Brooks, J. L. & Dodson, S. I. (1965). Predation, body size and composition of plankton. *Science* **50,** 28–35.

Coughlin, D. J. (1991). Ontogeny of feeding behaviour of first-feeding Atlantic salmon (*Salmo salar*). *Canadian Journal of Fisheries and Aquatic Sciences* **48,** 1896–1904.

Faber, D. J. & Gadd, S. (1983). Several drawing techniques to illustrate larval fishes. *Transactions of the American Fisheries Society* **112,** 349–408.

Ferreira, E. J. G., Zuanon, J. A. S. & Santos, G. M. (1998). *Peixes Comerciais do Médio Amazonas: Região de Santarém, Pará*. Brasília: Imprensa Nacional.

Fuiman, L. A. & Higgs, D. M. (1997). Ontogeny, growth and the recruitment process. In *Early Life History and Recruitment in Fish Populations* (Chambers, R. C. & Trippel, E. A., eds), pp. 225–249. London: Chapman & Hall.

Gozlan, R. E., Copp, G. H. & Tourenq, J. N. (1999). Early development of the sofie, *Chondrostoma toxostoma*. *Environmental Biology of Fishes* **56,** 67–77.

Green, B. S. & McCormick, M. I. (2001). Ontogeny of the digestive and feeding systems in the anemofish *Amphiprion melanopus*. *Environmental Biology of Fishes* **61,** 73–83.

Kamler, E. (1992). *Early Life History of Fish – An Energetics Approach*. London: Chapman & Hall.

Kimura, S., Nakayama, Y. & Kiriyama, T. (1998). Comparison of laboratory-reared eggs, embryos and larvae of five labrid fishes. *Environmental Biology of Fishes* **52,** 187–201.

Luz, R. K. & Portella, M. C. (2002). Larvicultura de trairão (*Hoplias lacerdae*) em água doce e água salinizada. *Revista Brasileira de Zootecnia* **31,** 829–834.

Luz, R. K. & Portella, M. C. (2005). Freqüência alimentar na larvicultura do trairão (*Hoplias lacerdae*). *Revista Brasileira de Zootecnia* **34,** 1442–1448.

Mathias, J. A. & Li, S. (1982). Feeding habits of walleye larvae and juveniles; comparative laboratory and field studies. *Transactions of the American Fisheries Society* **111,** 722–735.

Neves, C. A. (1996). Estudo morfológico e histoenzimático do desenvolvimento ontogenético do trato digestivo de larvas e alevinos de trairão (*Hoplias* cf. *lacerdae*) e de pacamã (*Lophiosilurus alexandri*). PhD Thesis, Federal University of Minas Gerais, Brazil.

Oyakawa, O. T. & Mattox, G. M. T. (2009). Revision of the Neotropical trahiras of the *Hoplias lacerdae* species-group (Ostariophysi: Characiformes: Erythrinidae) with descriptions of two new species. *Neotropical Ichthyology* **7,** 117–140.

Oyakawa, O. T. & Netto-Ferreira, A. L. (2007). Família Erythrinidae. In *Catálogo das espécies de peixes de água doce do Brasil* (Buckup, P. A., Menezes, N. A. & Ghazzi, M. S., eds), pp. 63–64. Rio de Janeiro: Museu National.

Pinder, A. C. & Gozlan, R. E. (2004). Early ontogeny of sunbleak. *Journal of Fish Biology* **64,** 762–775.

Planquette, P., Keith, P. & Le Bail, P. Y. (1996). *Atlas des poissons d'eau douce de Guyane*. Tome I. Paris: Muséum Nationale d'Histoire Naturelle.

Rønnestad, I., Koven, W. M., Tandler, A., Harel, M. & Fyhn, H. J. (1994). Energy metabolism during development of eggs and larvae of gilthead sea bream (*Sparus aurata*). *Marine Biology* **120,** 187–196.

Sanches, P. V., Nakatani, K. & Bialetzki, A. (1999). Morphological description of tha developmental stages of *Parauchenipterus galeatus* (Linnaeus, 1766) (Siluriformes, Auchenipteridae) on the floodplain of the upper Paraná River. *Revista Brasileira de Biologia* **59,** 429–438.

Santos, A. M. P. (1991). Morfogênese dos estados larvares de *Solea senegalensis* Kaup e *Dicentrarchus labrax*. *L. Revista de Biologia da Universidade de Aveiro* **4,** 301–311.

Santos, J. E. & Godinho, H. P. (1994). Morfogênese e comportamento larvais do surubim (*Pseudoplatystoma coruscans*). *Arquivos Brasileiros de Medicina Veterinária e Zootecnia* **40,** 139–147.

Santos, J. E. & Godinho, H. P. (1996). Larval ontogeny and swimming behaviour of the leporin fish *Leporinus elongatus* (Valenciennes, 1874) under experimental conditions. *Arquivos Brasileiros de Medicina Veterinária e Zootecnia* **48,** 109–116.

Santos, J. E. & Godinho, H. P. (2002). Ontogenic events and swimming behaviour of larvae of the characid fish *Salminus brasiliensis* (Cuvier) (Characiformes, Characidae) under laboratory conditions. *Revista Brasileira de Biologia* **19,** 163–171.

Taphorn, D. C. (1992). *The Characiform Fishes of the Apure River Drainage*. Guanare, Venezuela: Biollania.