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Early development and allometric growth patterns of the grumatã (*Prochilodus vimboides* Kner, 1859)

Guilherme Souza[1], *Edésio J. T. Melo*[3], *Erica P. Caramaschi*[4], *Dalcio R. Andrade*[5] *and Leandro R. Monteiro*[2]

CBB, Universidade Estadual do Norte Fluminense, Campos dos Goytacazes, RJ; IB-CCS, Universidade Federal do Rio de Janeiro; and CCTA, Universidade Estadual do Norte Fluminense, Campos dos Goytacazes, RJ, Brazil

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Summary

The objective of this study was to characterize the early development and allometric growth of the grumatã (*Prochilodus vimboides*). We describe a sample of 266 eggs and larvae obtained through induced spawning. The eggs were spherical (mean 3.7 mm diameter), exhibited a yellow yolk and were non-adhesive and pelagic after fertilization and hydration. The time elapsed between the early cleavage and post-flexion stages was considered short (328 hours, 8054 hour-degrees) in regard to the development times of other Neotropical rheophilic species, but time to hatching was considerably longer than in other *Prochilodus* species. The most notable anatomical changes were observed between the end of the yolk larval stage and the beginning of the pre-flexion stage, when the larvae displayed directed swimming and the digestive system became functional, enabling the transition from endogenous to exogenous feeding. After hatching, the larvae grew from 6.04 to 15.15 mm in total length average. Two growth phases were observed at this stage: a non-linear asymptotic curve in yolk-sac larvae, and a linear constant-rate growth phase after exogenous feeding started. Allometric growth related to standard length was positive for head length, negative for eye diameter, and switched between phases from negative to positive in body depth and head height. Morphological development and allometric growth in different larval phases impose drastic anatomical and physiological changes that are synchronic with habitat changes and the flood cycles during the reproductive period.

Keywords: Allometry, Embryo development, Growth, Induced spawning, Larvae

Introduction

The *Prochilodus* genus comprises 13 described species (Reis *et al.*, 2003), occurring in nearly every major South American watershed (Castro & Vari, 2004). All species of the genus *Prochilodus* are iteroparous and exhibit a single seasonal spawning event, high fecundity, external fertilization and an absence of parental care (Lowe-McConnell, 1999). The grumatã (*Prochilodus vimboides* Kner 1859) occurs between the Jucuru and Paraíba do Sul Rivers, the Uruguai River and the headwaters of both the tributaries of the upper Paraná river and the São Francisco River (Castro & Vari, 2004). The conservation status of *P. vimboides* is vulnerable despite showing a wide geographic distribution. To date, there is no available ecological information regarding the

[1]All correspondence to: G. Souza. Laboratório de Ciências Ambientais, CBB, Universidade Estadual do Norte Fluminense, Av. Alberto Lamego 2000, Parque Califórnia, Campos dos Goytacazes, RJ cep 28013–602, Brazil. E-mail: guilhermesouza.bio@gmail.com.
[2]Laboratório de Ciências Ambientais, CBB, Universidade Estadual do Norte Fluminense, Campos dos Goytacazes, RJ, Brazil.
[3]Laboratório Biologia Celular e Tecidual, CBB, Universidade Estadual do Norte Fluminense, Campos dos Goytacazes, RJ, Brazil.
[4]Laboratório de Ecologia de Peixes, IB-CCS, Universidade Federal do Rio de Janeiro, Brazil.
[5]Laboratório de Zootecnia e Produção Animal, CCTA, Universidade Estadual do Norte Fluminense, Campos dos Goytacazes, RJ, Brazil.

initial and adult forms of the species. Studying early life stages is key for successful conservation programs, and morphological description is an important tool for distinguishing congeneric or sympatric species (Oliveira *et al.*, 2012). The great morphological similarity amongst congeneric species in early life stages (Snyder, 1981) and the lack of taxonomic keys are obstacles to the identification of material collected in the natural environment (Bialetzki *et al.*, 1998). Ahlstrom & Moser (1976) highlighted the importance of biological collections of ichthyoplankton because they enable the determination of life history characteristics, linking larval history to the adult phase. Such links are made mainly through meristic characters, also using a number of osteological characters, morphometric characters and pigmentation patterns. The species indicated above is an ecologically relevant species with a delicate population status. Thus, this study aims to describe the initial ontogeny of *P. vimboides* using morphological and meristic characters of the eggs and larvae and to determine allometric patterns of larval growth.

Material and methods

The breeding individuals of *P. vimboides* used to obtain egg and larvae samples were derived from the *exsitu* bank of endangered species from the Paraíba do Sul River watershed maintained by the non-governmental organisation the Piabanha Project, at Itaocara/Rio de Janeiro/Brazil (21°38′6.33″S 42°1′59.25″W). The breeding stock for this species was composed of individuals captured in the Imbé River basin. We used three females and six males at approximately 2 years of age. During the pre-experimental period, the fish were housed in a density of 1 individual/5 m^2. Reproduction was induced using pituitary extract, followed by scheduled egg and larvae sampling, which occurred in November 2011 in the incubators of the Piabanha Project. The observed fertilization rate was approximately 80%. The induced reproduction method followed the technique described by Woynarovich & Horváth (1983). The temperature, dissolved oxygen, pH and conductivity of the water were monitored at the time of the second dose until the pituitary time of extrusion of the oocytes, using, respectively, oximeter accuracy of 0.01 mg/l water (YSI 550), electronic pH meter accurate to two decimal places (YSI PH 100) and electronic conductivity with 1 mS accuracy YSI – EC300.

The egg and larvae samples in the incubators were obtained following the protocol used by Romagosa *et al.* (2001), which consisted of sampling eggs every hour after fecundation (HAF) until hatching. Beginning on the second day, the methodology was changed. Samples were collected every 2 h on the second day, every 3 h on the third day, and every 4 h on the fourth day. The samples ceased to be collected from the incubators when the beginning of exogenous feeding became clear. Thereafter, the larvae were transferred to a 1700 m^2 ground tank that was previously prepared and provided with abundant natural food, receiving daily commercial feed powder (50% crude protein). For the next 15 days, daily samples were collected from the tank using a net with a 500-micron mesh opening, all at the same time of day. The sampling procedures ceased on the 19th day, when the individuals started to exhibit a shape similar to the adults. The hour-degrees were recorded (sum of the water temperature values in degrees centigrade, measured during the incubation and nursery processes) using the thermometer of an oximeter (brand YSI 550) during the sampling procedures for early developmental stages. In total, 266 individuals, comprising embryos (108) and larvae (158), were sampled and measured.

The swimming behaviour of the larvae was first recorded in the incubator soon after hatching and persisted until the pre-flexion stage. The records of swimming behaviour were subsequently correlated with the type of fish displacement and morphological changes observed in the larvae. Samples of embryos, larvae and juveniles were stored in numbered Eppendorf tubes with a buffered 4% formalin solution. The numbers represented the sequence of egg and larva sampling. Morphological descriptions and measurements were performed using a Bell Photonics stereomicroscope fitted with a digital camera (5 megapixel) and the software EUREKAM 10.0. The method proposed by Ahlstrom & Moser (1976), modified by Nakatani *et al.* (2001) was employed to describe the initial biometrics and ontogeny of the eggs and larvae originating from the artificial induction process. The following measurements (in millimetres; mm) were obtained for the eggs: egg area (EgA), egg perimeter (EgP), egg radius (ER), embryo area (EmA), embryo perimeter (EmP) and embryo radius (EmR).

The size of the perivitelline space was categorised in regard to its contribution to the total volume of the egg according to the recommendations made by Nakatani *et al.* (2001), as follows: restricted (0–9.9%), moderate (10–19.9%), wide (20–29.9%) and very wide (≥30.0%).

The following larval measurements were performed (mm): total length (TL), standard length (SL), yolk length (YL), yolk height (YH), head length (HL), head height (HH), rostrum length (RL), body height (BH), eye perimeter (EP), eye diameter (ED), snout–dorsal fin distance (SDFD) and snout–anal fin distance (SAFD).

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The embryonic stages were categorised into developmental stages following Ahlstrom & Ball (1954) and Kendall *et al.* (1984) and the modification made by Nakatani *et al.* (2001). The developmental stages were as follows: early cleavage (EC; when the first cells are formed); early embryo (EE; when the embryo is differentiated); free-tailed embryo (FT; when the tail is released from the yolk); and final embryo (FE); when the embryo is completely formed and ready for eclosion).

After eclosion, the larvae were classified into five categories according to notochord flexion and to the development of the caudal fin and its supporting elements (following Ahlstrom & Ball, 1954; Kendall, *et al.*, 1984; Nakatani *et al.*, 2001). The categories were as follows: yolk-sac larva (YL; stage between hatching and the first exogenous feeding); pre-flexion (PF; from the beginning of exogenous feeding until the beginning of notochord flexion, when supporting elements of the caudal fin appear); flexion (FL; from the beginning of notochord flexion, when supporting elements of the caudal fin appear, until complete flexion of the notochord, the appearance of the pelvic fin bud and the beginning of dorsal and anal fin ray segmentation); post-flexion (POF; from full notochord flexion, the appearance of the pelvic fin bud and the beginning of dorsal and anal fin ray segmentation until the full formation of the pectoral fin rays, absorption of the embryonic fin and the appearance of scales) and juvenile (JU; regarded as small adults, characterized by the complete formation of the fin rays and the appearance of scales, until the first indication of sexual maturity).

The following traits were emphasised in the larval ontogeny: the appearance of pigmentation, the appearance and regression of the embryonic fin, the appearance of branchial arches, the formation of the digestive tract, the emergence of gill slits and the operculum, the appearance and quantity of fin rays, the quantity of myomeres, the position of the mouth area, the appearance of the olfactory hole, the appearance of the gas bladder and the appearance of the operculum.

Growth patterns in different larval stages were assessed by fitting four growth models to the relationship between TL and hour-degrees (HD): linear, von Bertalanffy, Gompertz and Logistic (Katsanevakis, 2006; Katsanevakis & Maravelias, 2008), separating the curves for yolk-sac larvae and peri-flexion larvae (combining pre-flexion, flexion and post-flexion stages). The linear model presents the simplest growth pattern, with a constant growth rate, whereas the remaining models decelerate growth towards an asymptotic body size. Model fitting was assessed by information criteria (AIC), and the model with the smallest AIC value was selected as best fitting.

The allometric patterns during different larval stages were modelled based on the SL power function, and the allometric coefficients were calculated with the power function $Y = aX^b$, where Y is the dependent variable (measured characteristic); X the independent variable (SL – standard length); a is the intercept; and b is the allometric coefficient. Isometric growth occurred when $b = 1$, positive allometric growth when $b > 1$ and negative allometric growth when $b < 1$. The morphometric variables (response variables) were plotted against SL, and the relationships were described using regression models for log-transformed variables (Kováč *et al.*, 1999).

Results

The extrusion occurred 288.9 hour-degrees (HD) after hormonal induction. Under the following physicochemical average water temperature (°C), pH, dissolved oxygen (mg/l) and electrical conductivity (μS): 28.89 ± 0.44°C; pH 6.7 ± 0.43; 4.88 ± 144 mg/l; and 73.71 ± 0.65 μS. The number of hours elapsed after fecundation (per stage), the incubation water temperature and the recorded HD are described in Table 1.

The description of early development was divided into embryonic and larval stages. Six stages were observed within the embryonic development stage. The six stages lasted 24 h at an average temperature of 26.48 ± 1.41°C. The most relevant events in early development are shown in Table 2.

The eggs were spherical, exhibited a yellowish yolk and were non-adhesive and pelagic AF and hydration. There were no significant differences in the egg perimeter observed amongst the cleavage stages (CL) until the FE stage. The eggs presented a diameter range from 3.3–4.1 mm (mean ± standard deviation: 3.7 ± 0.19 mm) and an egg perimeter ranging from 10.21–12.84 mm (mean ± standard deviation: 11.52 ± 0.60 mm). The embryos exhibited an area of 1.88 ± 0.26 mm^2 (mean ± standard deviation) and occupied 17.30 ± 2.46% of the inner volume (a 12.98–23.07 range) of the egg (egg area was 10.95 ± 1.06 mm). Therefore, the perivitelline space was categorised as 'very wide' (Nakatani *et al.*, 2001).

Early cleavage

The reorganisation of the cytoplasm into the animal pole and vegetal pole occurred in the first hour, along with the early cleavages (Fig. 1*A*, *B*). Early cleavage ended in the second hour. The 'blastocyst' stage began in the third hour, when the blastoderm exhibited a domed shape (Fig. 1*C*). 'Gastrulation' occurred between the fourth and eighth hour, when epiboly

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Table 1 Environmental variables measured throughout the incubation of *Prochilodus vimboides* eggs and larvae

Stage	HAF	Temp ± sd	Temp range (°C)	HD range
EC	8	28.00 ± 1.19	26–29	29–224
EE	4	26	26	250–328
FTE	10	25.40 ± 0.51	25–26	354–582
FE	2	26.50 ± 0.70	26–27	608–635
YS	64	26.26 ± 1.32	24–29	693–2294
PF	48	–	24	2870–3446
FL	120	–	24	4022–6326
POF	72	–	24	6902–8054

EC: early cleavage, EE: Early embryo, FTE: free-tailed embryo, FE: final embryo, YS: Yolk-sac larva, PF: pre-flexion, FL: flexion, POF: post-flexion, HAF: hours after fecundation per stage; Temp ± sd: temperature ± standard deviation; Temp range °C: temperature range in degrees centigrade; HD range: hour-degree range.

Table 2 Description of morphological events occurring after the fertilization of *Prochilodus vimboides* eggs, according to developmental stages.

Stage	HAF	HD	Description of development
EC	1	29	Cytoplasm reorganisation into the animal pole and vegetal pole
	2	58	End of early cleavages
	3	87	Blastoderm with a domed shape
	4	116	Animal pole covering 40% of the vegetal pole
	5	144	Animal pole covering 60% of the vegetal pole
	6	171	Animal pole covering 80% of the vegetal pole
	7	198	Animal pole covering 90% of the vegetal pole
	8	224	Blastopore closure
EE	9	250	Cranial region distinct from the tail of the embryo
	10	276	Evidence the of optic vesicle
	12	328	Occurrence of 18 somites
FTE	13	354	Tail of the embryo detached from the yolk sac
	14	379	Yolk sac expanded parallel to the embryo's body
	15	404	Occurrence of 20 somites
	16	429	Otolith formation
	19	504	Development of the embryonic membrane of the primordial fin
FE	24	635	Embryo body lengthening, increased frequency of tail beating, chorion rupture.

EC: early cleavage; EE: early embryo; FE: final embryo; FTE, free-tailed embryo; HAF: hours after fecundation; HD: hour-degrees.

increased, and the animal pole covered 40% (Fig. 1*D*), 60% (Fig. 1*E*), 80% (Fig. 1*F*) and 90% (Fig. 1*G*) of the animal pole during the fourth, fifth, sixth and seventh hour, respectively. The animal pole completely covered the vegetal pole in the eighth hour, corresponding to the blastopore closure stage (Fig. 1*H*). At this point, the embryo area ranged from 1.49–1.84 mm^2 (1.66 ± 0.14 mm^2).

Early embryo

Embryo differentiation began 9 h AF, when the cranial region and the tail of the embryo could be distinguished (Fig. 2*A*). The optic vesicle was clear in the 10th hour, and the embryo displayed 18 somites at the 12th hour.

Free-tailed embryo

The most relevant change in this stage occurred in the 13th hour, when the tail of the embryo was detached from the yolk sac. The yolk sac expanded parallel to the embryo's body at the 14th hour. Twenty somites were observed in the 15th hour, and the formation of otoliths began in the following hour. Another remarkable body change was observed only in the 19th hour, when the

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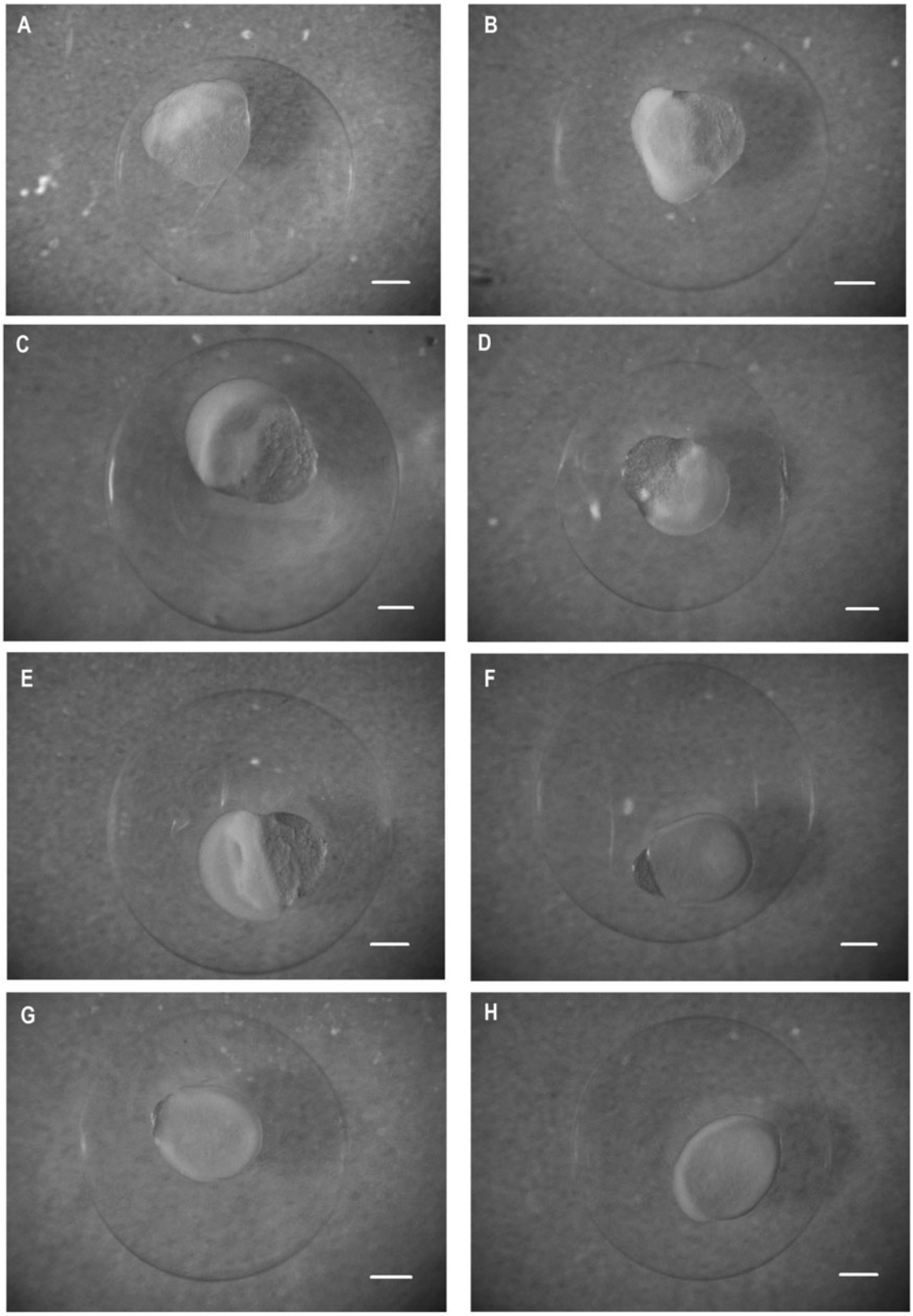

Figure 1 Early developmental stages of *Prochilodus vimboides.* (*A*, *B*) Cleavage, first and second hour after fecundation (HAF); (*C*) Blastula, third HAF; (*D*) Gastrula, fourth HAF (40% epiboly); (*E*) Gastrula, fifth HAF (60% epiboly); (*F*) Gastrula, sixth HAF (80% epiboly); (*G*) Gastrula, seventh HAF (90% epiboly); (*H*) Gastrula, eighth HAF (blastopore closure). Scale bars represent 0.28 mm.

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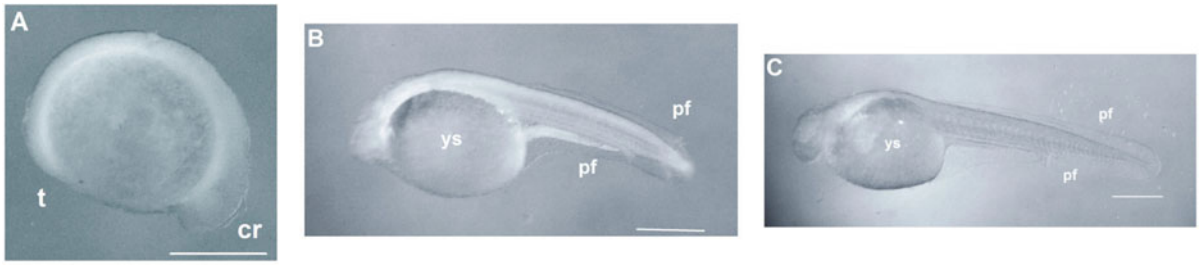

Figure 2 Early developmental stages of *Prochilodus vimboides*. (*A*) Early embryo, 9 hours after fecundation (HAF); (*B*) Free-tailed embryo, 19 HAF; (*C*) Final embryo, 24 HAF. Scale bars represent 1.0 mm. Abbreviations: cr – cranium; t – tail; ys – yolk sac; pf – primordial fin.

embryonic membrane of the primordial fin developed on the periphery of the caudal region of the larvae (Fig. 2*B*).

Final embryo

This stage occurred in 2 h (23rd and 24th) (Fig. 2*C*), during which no relevant body changes occurred. However, the embryo ruptured the chorion at the end of this stage.

Larval development

Larval development lasted 302 h, including 62 h in the yolk larval stage, 48 h in the pre-flexion stage, 120 h in the flexion stage and 160 h in the post-flexion stage. The most relevant events, morphometric data, meristic data and development times of the larval phases are shown in Tables 3 and 4.

Yolk-sac larva

This stage showed the greatest number of bodily changes. The larvae spent most of the time resting and clustered in the bottom of the incubator after hatching. The larvae performed vertical movements leading to the surface or near the surface in a short time span, then passively returned to the bottom with their head turned downward. The following structures developed in this stage: pectoral fins (without rays), the swimming bladder, and the optical and brain vesicles. In addition, the number of myomeres increased along the torso; the respiratory system (composed of the arches, gill filaments and opercula) developed; and the heart and nasal opening began to be formed.

These structural acquisitions occurred along with the initiation of horizontal swimming (72 h after fecundation). The digestive system developed; the mouth was terminal and open; the digestive tract was elongated, and the anus was open; and there was a reduction of the yolk reserve, which was nearly exhausted at this point. Pigments were present in several body parts (Fig. 3*A*).

Pre-flexion

The punctate and dendritic chromatophores increased progressively on the snout, the back of the head and the sides of the body, extending to the early portion of the anal region during the pre-flexion stage of the notochord. The number of myomeres along the torso increased, and the height of the hyaline fin was reduced along with the development of the caudal (with rays) and anal fins. In the pre-flexion stage, the larvae undergo more active movement in all directions in the water column. Food was observed in the digestive tract lumen based on transparency, along with the exhaustion of the yolk reserve. Notochord flexion began at the end of the pre-flexion stage (Fig. 3*B*). The numbers of pre-anal and post-anal myomeres (24–25 and 12–13 myomeres, respectively) were the same in the yolk-sac and pre-flexion stages (Table 3). In the flexion and post-flexion stages, it was not possible to quantify the number of myomeres throughout the body due to body growth and the occurrence of additional pigment.

Flexion

Flexion of the tip of the notochord began at the flexion stage. A high concentration of pigments was observed in the eyeballs, around the borders of the myomeres and on the dorsal fin, beginning the formation of a macula. Hours later, pigmentation intensified in the flanks forming transverse bands. The opercula were more developed. The caudal, anal and dorsal fins started to show rays, and the pelvic fin bud began to form (Fig. 3*C*).

Post-flexion

This was the longest larval development stage, lasting 160 h. The regression of the hyaline fin in the dorsal area, except for in the caudal peduncle region, and adipose fin formation occurred in this stage. Fin development, considering the presence of rays, occurred in the following order: pectoral, caudal,

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Table 3 Description of the main ontogenetic events in *Prochilodus vimboides* larvae

E	HAF	HD	Event description
YS	26	693	Invagination in the hyaline fin, delimiting the anus region
	32	859	Dorsal portion of the hyaline fin beginning at the 6th myomere; conspicuous optic vesicle and eyeball
	34	913	Pectoral fin bud; oral region ventrally located; regression of yolk tube expansion; appearance of appendices that make up the opercular region
	36	965	Differentiation and development of the brain vesicles; apparent heart
	38	1017	Differentiated branchial arches and filaments; disappearance of yolk tube expansion
	40	1071	Fourth ventricle and cerebellum apparent
	42	1125	Early jaw differentiation; early operculum development; developing the pectoral fin in the region of the third pre- anal myomere; well-pigmented retina
	46	1233	Visible olfactory openings; jaw extremity reaching the middle region of the eyeball from a perpendicular axis
	51	1368	Digestive tract located above the yolk sac; early swimming bladder formation
	57	1527	Subterminal mouth
	60	1605	Terminal mouth
	72	1906	Volume of the swimming bladder equivalent to the yolk sac volume; premaxillary bones
	76	2006	End of the pectoral fin, still without rays, without surpassing the fourth pre-anal myomere
	80	2102	Yolk sac with 1/3 of the original size
	84	2198	Swimming bladder occupying 50% of the abdominal cavity
PF	101	2629	Functional digestive tube and beginning of exogenous feeding; terminal mouth; yolk exhaustion
	136	3446	Transverse streaks along the digestive tract; beginning of dorsal, caudal (with rays) and anal fin formation; early flexion of the notochord
F	160	4022	Flexion of the tip of the notochord; well-developed operculum
	184	4598	Macula forming in the dorsal fin; all fins pigmented; gills visible through the operculum
	208	5174	Caudal fin is homocercal in shape; horizontal expansion of the swim bladder to the 13th myomere
	256	6326	Transverse bands along the flanks; dorsal fins with 11 rays, anal fin with 10 rays and caudal fin with 19 rays; formation of the pelvic fin bud
POF	304	7478	Early adipose fin formation
	328	8054	Appearance of scales

E: stages = YS: yolk-sac larva, PF: pre-flexion, FL: flexion, POF: post-flexion, HAF: hours after fecundation, HD: hour-degrees. Variables = BH: body height, ED: eye diameter, EP: eye perimeter, ER: eye radius, HL: head length, HH: head height, RL: rostrum length, SDFD, snout–dorsal fin distance, SAFD: snout–anal fin distance, TL: total length, SL: standard length, YH: yolk height, YL: yolk length.

dorsal, anal, pelvic and adipose. At the end of the post-flexion phase, the caudal, dorsal, anal and pelvic fins already exhibited rays. The quantification of pectoral fin rays was difficult because these rays were difficult to see. The adipose fin does not present rays at any life stage in this species. Finally, scales appeared, and complete regression of the hyaline fin occurred. The hyaline fin still exists in the caudal peduncle and in the early juvenile stage, when individuals begin to resemble adults in shape (Fig. 3*D*).

Growth and allometry

Two larval growth stages, alternating with a stabilisation stage, were observed after the hatching of *P. vimboides* eggs. The first growth stage occurred throughout most of the yolk-sac larva phase (except the last hours), 88 HAF or 2.294 HD. In this first stage, the non-linear growth models were all equivalent according to AIC criteria (Gompertz AIC = 13.44, Logistic AIC = 14.61, von Bertalanffy AIC = 16.37) as the difference between the lowest AIC and the

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Table 4 Mean (x), standard deviation (sd) and range of variation (range) of the morphometric and meristic variables obtained from *Prochilodus vimboides* larvae (*n*: number of individuals evaluated, YS: yolk-sac larva, PF: pre-flexion, FL: flexion, POF: post-flexion)

Stage (*n*)	YS (125)		PF (4)		FL (30)		POF (10)	
	x ± sd	Range	x ± sd	Range	x ± sd	Range	x ± sd	Range
TL	6.04 ± 0.54	4.37–7.44	8.90 ± 0.62	8.28–10.08	11.84 ± 1.39	9.92–14.49	15.15 ± 1.01	12.79–16.76
SL	5.80 ± 0.52	4.26–6.99	7.68 ± 2.40	8.02–1.39	9.95 ± 1.05	8.43–11.93	12.45 ± 0.69	10.86–13.52
YL	1.23 ± 0.34	2.02–0.39	0.62 ± 0.09	0.55–0.68	na	na	na	na
YH	0.75 ± 0.21	1.41–0.36	0.58 ± 0.15	0.47–0.68	na	na	na	na
RL	0.34 ± 0.05	0.24–0.38	0.50 ± 0.07	0.42–0.59	0.83 ± 0.15	0.60–1.09	1.21 ± 0.11	1.02–1.43
ED	0.29 ± 0.05	0.18–0.38	0.52 ± 0.04	0.46–0.58	0.69 ± 0.08	0.56–0.86	0.94 ± 0.09	0.82–1.08
HL	0.94 ± 0.28	0.40–1.56	2.04 ± 0.23	1.54–2.25	2.85 ± 0.40	2.33–3.51	3.97 ± 0.46	3.01–4.74
HH	0.08 ± 0.10	0.54–1.04	1.50 ± .20	1.23–1.67	2.06 ± 0.371	1.67–2.81	3.09 ± 0.37	2.43–3.74
BH	1.09 ± 0.13	0.71–1.77	1.79 ± 0.17	1.62–2.08	2.49 ± 0.49	1.69–3.51	3.50 ± 0.33	2.74–3.99
SDFD	na	na	na	na	5.20 ± 0.96	4.39–8.51	6.13 ± 0.46	2.74–3.99
SAFD	na	na	na	na	7.70 ± 0.88	5.58–9.01	9.80 ± 0.62	5.25–6.86
Myom (*n*)	24		5		0		0	
		Range		Range		Range		Range
Pra		24–25		24–25	dv	dv	dv	dv
Poa		12–13		12–13	dv	dv	dv	dv
Rays								
P	na	na	na	na	na	na	na	dv
L	na	na	na	na	na	na	na	3–5
D	na	na	na	na	na	7–11	na	11–12
A	na	na	na	na	na	10–11	na	11–12
C	na	na	na	11	na	18–20	na	20–28

TL: total length; SL: standard length; YL: yolk length; YH: yolk height; RL: rostrum length; ED: eye diameter; HL: head length; HH: head height; BH: body height; SDFD: snout–dorsal fin distance; SAFD: snout–anal fin distance; BH: body height; P: pectoral fin; L: pelvic fin; D: dorsal fin; A: anal fin; C: caudal fin; dv: difficult visualization; na: not available; Myom: myomeres; Pra: pre-anal; Poa: post-anal. The absence of measurements is a consequence of the incomplete development of the larvae.

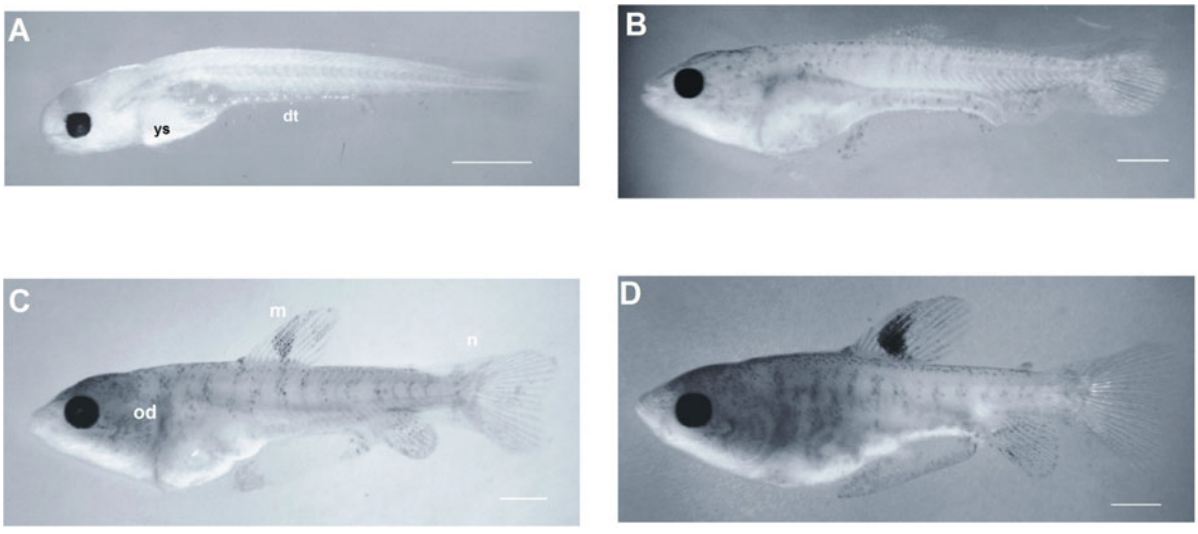

Figure 3 Early developmental stages of *Prochilodus vimboides*. (*A*) Yolk-sac larva, 42 HAF; (*B*) Pre-flexion, 136 HAF; (*C*) Flexion, 256 HAF; (*D*) Post-flexion, 304 HAF. Scale bars represent 1.0 mm. Abbreviations: dt – digestive tube; ys – yolk sac; m – macula; n – flexed notochord; od – developed operculum.

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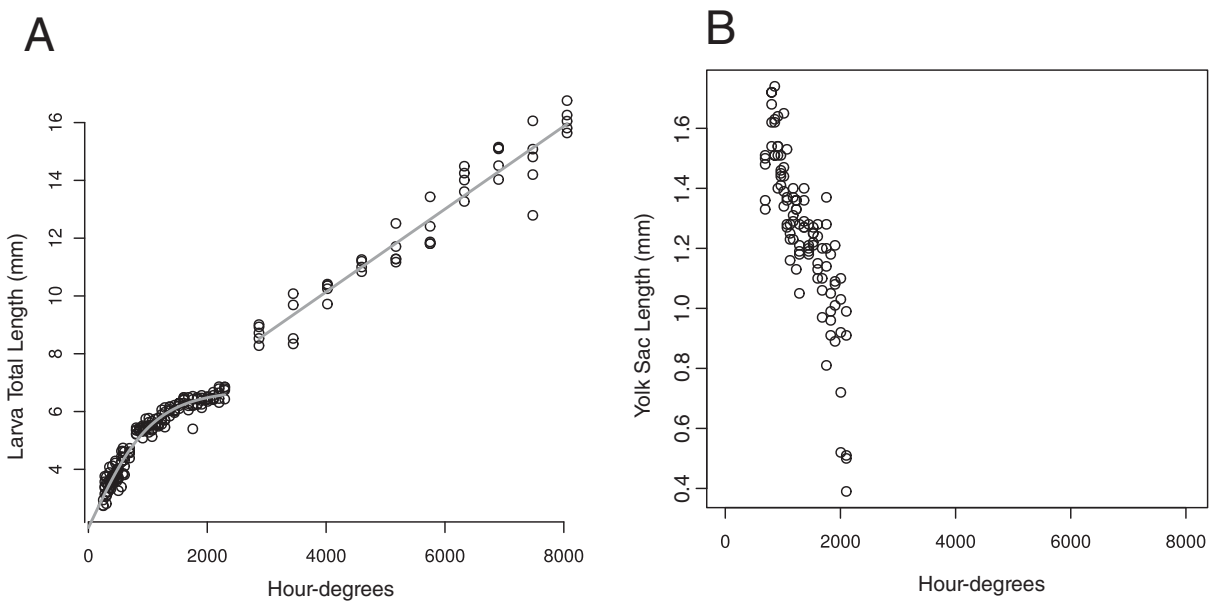

Figure 4 Larval growth and development of *Prochilodus vimboides*. (*A*) Growth curves for the different developmental stages, indicating the models with the best fit. The Gompertz model (curved line) showed the best fit (AIC criterion) in the yolk-sac larval stage, and the linear model (straight line) showed the best fit in the flexion, pre- and post-flexion (peri-flexion) stages. (*B*) Scatter plot showing the decrease in yolk length with hour-degrees in *Prochilodus vimboides* during the yolk-sac larva phase.

remaining ones is <3. The linear model performed significantly worse (linear AIC = 208.68), suggesting a deceleration of growth towards the asymptotic length of 6.73 mm (sd = 0.0693 mm) (Fig. 4*A*). There was no significant growth once the yolk was nearly consumed (Fig. 4*B*). The second growth phase combined the period before and after notochord flexion, and the fit of the models were all equivalent (Linear AIC = 99.60, Logistic AIC = 101.21, Gompertz AIC = 101.35, von Bertalanffy AIC = 105.84). In this case, we parsimoniously selected the linear model as the best fit and assume a constant growth rate of 0.00144 mm/hour-degree (Fig. 4*A*) throughout the flexion stages, all the way to the juvenile stage. Because of the biphasic growth, allometric coefficients were calculated for each larval stage separately (Table 5 and Fig. 5).

Among the yolk-sac larva stage variables, only HL exhibited positive allometry relative to SL (Table 5, Fig. 5*B*). During the yolk-sac larva stage, HL proportion represented on average 16.37% of the total size of the larvae (Table 6). The positive allometric growth in HL persisted throughout the latter stages (Table 5 and Fig. 5*B*) and the HL proportion reached approximately 30% of body length (Table 6). Head height switched from negative to positive allometry between phases (Table 5 and Fig. 5*A*), also reached approximately 30% BP in the post-flexion stage (Table 6). Rostrum length switched between no growth to isometric growth between phases (Table 5 and Fig. 5*D*). Given that HL was partially determined by RL, its positive allometry must be due to the growth of the braincase. Eye diameter (ED) exhibited negative allometry in all phases (Table 5 and Fig. 5*C*), decreasing its relative proportion relative to the head (Table 6). The allometric coefficient of BH was not significant (actually a negative relation) in the larval yolk phase (Table 5 and Fig. 5*E*). This negative association is due to the reduction of the yolk sac, causing a 'decrease' in BH as the length increases. In later stages, BH displayed a positive allometric coefficient, causing an increase in the body proportion for this variable (Table 6). The distances from the snout to the dorsal and anal fins were both isometric (Table 5 and Fig. 5*F*), but could only be measured in the peri-flexion stages.

Discussion

Both the duration of the embryonic period and the early development pattern vary considerably among different fish species (Balon, 1981). Water temperature is the environmental factor that most strongly influences development in these organisms (Chambers & Leggett, 1987; Souza, 2004; Ninhaus-Silveira *et al.*, 2006) and higher temperatures speed the early development process. Longer photoperiods during incubation also accelerates the initial development and egg hatching (Hernández Cuadrado, 2013). *P. vimboides* showed a slightly longer hatching time

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Table 5 Allometric coefficients (regression on standard length) for the different larval growth stages of *Prochilodus vimboides*

	Variable				
Yolk-sac larva stage	Coefficient	Standard error	LL	UL	Allometry
HL	1.739	0.077	1.587	1.891	Positive
HH	0.382	0.037	0.309	0.454	Negative
RL	0.259	0.355	− 0.522	1.040	NS
BH	− 0.141	0.048	− 0.235	− 0.046	NS
ED	0.406	0.029	0.348	0.464	Negative
Peri-flexion stage	Coefficient	Standard error	LL	UL	Allometry
HL	1.305	0.054	1.198	1.413	Positive
HH	1.318	0.054	1.210	1.426	Positive
RL	0.977	0.036	0.905	1.049	Isometry
BH	1.328	0.037	1.255	1.402	Positive
ED	0.604	0.032	0.539	0.669	Negative
SAFD	1.042	0.024	0.994	1.091	Isometry
SDFD	0.922	0.042	0.837	1.006	Isometry

The 95% lower (LL) and upper (UL) confidence limits based on a *t* distribution. The peri-flexion stage is the sum of the pre-flexion, flexion and post-flexion stages. HL: head length; HH: head height; RL: rostrum length; BH: body height; ED: eye diameter; SAFD: snout–anal fin distance; SDFD: snout–dorsal fin distance; NS: not significant.

(24 hours after fecundation – HAF) at an average temperature of 26.5 ± 1.41°C when compared with the hatching times of other species belonging to the same family (Characidae) at similar or lower temperatures, such as *Leporinus frederici* 13 HAF at 27°C (Sanches *et al.*, 2001), *L. piau* 21 HAF at 24°C (Borçato *et al.*, 2004), *Prochilodus magdalenae* 14 HAF at 24°C (Arias-Gallo *et al.*, 2010), and *P. lineatus* 18 HAF at 25°C (Botta *et al.*, 2010; Hernández Cuadrado, 2013). Although egg size in *P. vimboides* lies well within the range of other species in the genus (2.23–4.25 mm) (Nakatani *et al.*, 2001; Arias-Gallo *et al.*, 2010), the yolk-sac larvae are larger (mean = 5.8 mm) than in other *Prochilodus* species (Arias-Gallo *et al.*, 2010). Hatching times can be adaptive as part of life history strategies, as they can be related to environmental particularities and species-specific developmental rates (Fuiman, 2002). The delayed hatching in *P. vimboides* might be adaptive, as their larvae hatch with larger size than other *Prochilodus* species. It is, however, difficult to assess the relative influence of environmental factors other than temperature (light, pH, oxygen) in this comparison.

P. vimboides larvae hatch with a large yolk sac in relation to their body length (~30%), becoming quite heavy and spending much of the time at the bottom of the incubator. A change in swimming patterns from vertical to horizontal was observed during the larval development. Initially, the larvae performed only vertical ascending movements until reaching the water surface and then returned passively to their original position. After the development of sense organs and pectoral fins (though without rays), the individuals became capable of swimming both vertically and horizontally. Mouth opening and filling of the swimming bladders occurred simultaneously, as larvae were able to swallow air to inflate the gas bladder (Pinder & Gozlan, 2004). The development of fins facilitates balance and direction in the water column, being also responsible for the swimming behaviour changes in other species (Santos, 1992; Santos & Godinho, 2002; Beerli *et al.*, 2004; Mukai *et al.*, 2010).

In the pre-flexion stage (101 HAF), a transition occurred from endogenous to exogenous feeding. The mouth, digestive tract and anus became functional, enabling the larvae to capture, digest and excrete food. The same pattern of anatomical development is observed in the pre-flexion stage of the congeneric species *P. magdalenae*, 106 HAF, when the yolk reserve becomes depleted (Arias-Gallo *et al.*, 2010). Similar transitions have been described for *Engraulis mordax* (Lasker *et al.*, 1970); *Cyprinus carpio*, *Clarias gariepinus* (Van Snik *et al.*, 1997); *Danio rerio* (Jardine & Litvak, 2003); *Leporinus piau* (Borçato *et al.*, 2004) and *Steindachneridion parahybae* (Honji *et al.*, 2012).

Yolk depletion and the food transition proved to be the time of stabilisation in the growth of *P. vimboides* larvae. Yolk consumption in teleost fish occurs in three distinct stages, the first of which is the FE stage, while the other two occur during the yolk-sac larva stage (Heming & Buddington, 1988; Peña

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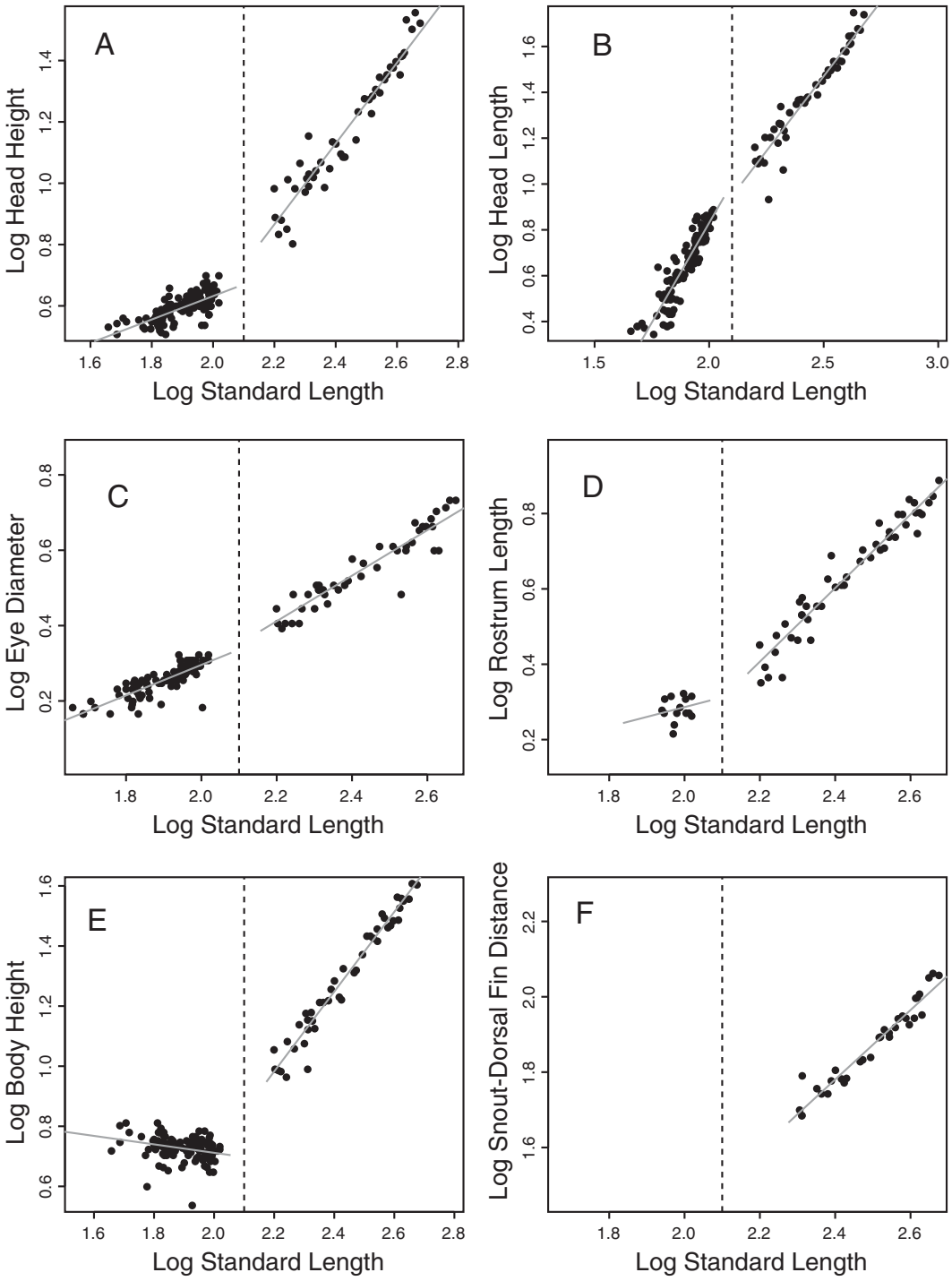

Figure 5 Allometric regression lines between log-transformed variables. (*A*) Head height, (*B*) head length, (*C*) eye diameter, (*D*) rostrum length, (*E*) body height and (*F*) snout–dorsal fin distance relative to standard length of *Prochilodus vimboides* during the yolk-sac larva stage (to the left of the vertical dotted line) and pre-flexion, flexion and post-flexion stages (peri-flexion, to the right of the dotted line), a total of 19 days after fecundation.

& Dumas, 2009). In contrast, *P. vimboides* undergoes two separate growth stages, before and after the beginning of the pre-flexion stage. The first at the expense of yolk consumption, with a decelerating growth rate. The second, based on exogenous feeding, with a constant growth rate (at least for the period measured). This switching of growth phases is critical from a nutritional point of view and may kill the larvae if they do not find exogenous food (Bailey & Houde, 1989). According to Blaxter (1988), the

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Table 6 Body proportions (%), means (x), standard deviations (sd) and range of variation (range) of the morphometric and meristic variables obtained from *Prochilodus vimboides* larvae

Stage (*n*) BP (%)	YS (78)		PF (5)		FL (21)		POF (15)	
	x ± sd	Range	x ± sd	Range	x ± sd	Range	x ± sd	Range
YL/SL	21.40 ± 6.65	7.40–32.63	na	na	na	na	na	na
YH/SL	13.11 ± 13.97	5.95–24.60	na	na	na	na	na	na
RL/SP	na	na	5.97 ± 0.68	5.13–7.11	0.07 ± 0.02	0.04–0.12	9.86 ± 0.46	9.30–10.57
ED/HL	32.68 ± 7.62	23.53–59.57	25.47 ± 2.79	22.22–32.46	24.81 ± 2.82	17.91–33.86	23.70 ± 2.44	17.29–27.90
HL/SL	16.37 ± 3.67	8.64–23.32	24.9 ± 2.55	17.94–27.30	28.41 ± 2.04	2052–30.88	27.71 ± 36.80	31.83–32.60
HH/SL	14.26 ± 2.07	9.71–18.67	17.75 ± 2.14	14.33–20.83	20.65 ± 1.85	18.86–23.87	28.10 ± 1.33	25.23–30.02
BH/SL	19.19 ± 2.94	12.09–29.55	21.18 ± 1.33	19.28–23.31	24.77 ± 2.33	18.59–29.42	28.10 ± 1.33	25.23–30.02
SAFD/SL	na	na	na	na	75.79 ± 6.55	49.42–79.59	78.68 ± 1.05	76.51–79.83
SDFD/SL	na	na	na	na	49.23 ± 1.65	47.42–50.65	49.24 ± 1.49	46.89–51.61

n: number of individuals evaluated; BP: body proportions; YS: yolk-sac larva; PF: pre-flexion; FL: Flexion; POF: post-flexion; SL: standard length; YL: yolk length; YH: yolk height; RL: rostrum length; HL: head length; HH: head height; SDFD: snout–dorsal fin distance; SAFD: snout–anal fin distance; ED: eye diameter; na: measurement not available. The absence of measurements is a consequence of the incomplete development of the larvae.

larvae must have a functional mouth and intestine and developed eyes after absorbing the yolk to be able to seek exogenous dietary sources (e.g., plankton and/or organic particles). The allometric positive growth of head dimensions is associated with changes in feeding habits, with brain mass development and with the development of bones in the oral region (Kováč *et al.*, 1999), indicating that many relevant morphological changes with ecological importance are accomplished even before they are functionally needed (Gisbert, 1999). The mouth must be open, and all organs related to prey capture (eyes, sensory organs, fins) and food intake (absorption, digestion and assimilation) must be formed for development to continue (Yúfera & Darias, 2007).

Changes in the oral region began in *P. vimboides* at the yolk-sac larva stage. In this period, the oral region shifted from a ventral to a terminal position, which is apparently synchronised with the food transition phase. Head allometry was positive throughout all development phases, however, the eye showed negative allometry and progressively decreased its proportion relative to HL after exogenous feeding began. Eye development with positive allometry is usually related to visual acuity (Fuiman, 2002) and its relevance to visualize prey and rheotactic behaviour (Gisbert, 1999; Gisbert *et al.*, 2002), and allometric positive growth of ED during the yolk-sac larva phase is common (Gisbert, 1999, 2002; Fuiman, 2002; Kupren *et al.*, 2014). This pattern suggests that the ability of *P. vimboides* larvae to capture prey may not depend on visual development.

In the post-flexion stage, body length and depth increased, fin development occurred, and the larvae moved rapidly in the water column. Better performance in propulsion leads to increased efficiency in prey capture, in both quality and quantity, and enables greater agility in escaping predators (Blaxter, 1988; Bailey & Houde, 1989; Fuiman, 2002). Such abilities should positively influence the survival of *P. vimboides* larvae, as observed for *P. magdalenae* and *P. lineatus*, due to the similarities in the early development of these species.

The external morphology of *P. vimboides* resembled that of an adult at the end of the post-flexion stage, when the specimens exhibited a fusiform and tall body, long pectoral fins and a developed caudal fin. These morphological characteristics are typical of species inhabiting regions with strong river currents (Alexandre *et al.*, 2014). It may be inferred that the larvae of *P. vimboides* inhabit marginal lakes that are abundant in natural food, at least in the pre- and post-food transition stages. Cunico *et al.* (2002) indicated that marginal lakes represent genuine natural nurseries that meet the feeding needs of growing fish larvae. Therefore, a relationship between species morphology and ecology may occur in the early stage of ontogeny, as the chronology of the early development of the eggs and larvae of *P. vimboides* appear to be in line with the transition stages from lotic (river channel) to lentic (flooded marginal areas) and again to lotic environments (when the juveniles return to the river channel). The description of larval development presented here allows for the identification of specimens in ecological field studies, where some species breed at the same time. The synchrony of the reproductive period, larval drift and floods ensure maximum food availability in the early stages of development, enabling rapid growth through the early larval stages, which are vulnerable to more intense predation (Fuiman, 2002). The connection between fish reproduction and flood cycles through

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larval development enables the maintenance of viable populations and is relevant for management and decision taking by environmental agencies.

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Conflicts of interest

None.

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