Acta Scientiarum

http://www.uem.br/acta
ISSN printed: 1679-9283
ISSN on-line: 1807-863X
Doi: 10.4025/actascibiolsci.v35i3.12471

Early ontogeny of *Clarias gariepinus* (Siluriformes, Clariidae) and aspects of its invasion potential in natural freshwater environments

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ABSTRACT. This study aimed to describe the early development of *Clarias gariepinus*, a species that has been introduced into various watersheds worldwide, in order to help the identification of its eggs, larvae and juveniles in natural environments. The material used was obtained via induced spawning during 1999. After spawning, the periodicity of sampling varied according to ontogenic development. We analyzed 12 eggs, 146 larvae and 6 juveniles. Newly fertilized eggs are spherical, with a double membrane, the density of which varies. Initially the larvae have little pigmentation but this intensifies during development. They have four pairs of well-developed barbels, an elongated body, long dorsal and anal fins, no adipose fin and vesicles surrounding the finfold and barbels. The sequence of formation of the fins is: caudal, dorsal, anal, pectoral and pelvic. Growth pattern analysis revealed that metamorphosis usually occurs during the flexion stage. The reproductive performance of the species and its rapid early development favor aquaculture; however, they may also favor its invasion of natural environments, representing a threat for native populations.

Keywords: african catfish, invasive species, larvae, juvenile of fish.

Ontogenia inicial de *Clarias gariepinus* (Siluriformes, Clariidae) e aspectos de seu potencial de invasão em ambientes de água doce natural

RESUMO. Este trabalho teve como objetivo descrever o desenvolvimento inicial de *Clarias gariepinus*, uma espécie introduzida em várias bacias hidrográficas do mundo, a fim de auxiliar na identificação de seus ovos, larvas e juvenis em ambientes naturais. O material utilizado foi obtido por meio de desova induzida durante o ano de 1999. Após a desova, a periodicidade na obtenção das amostras variou de acordo com o desenvolvimento ontogênico. Foram analisados 12 ovos e 146 larvas e 6 juvenis. Os ovos recém-fecundados apresentam forma esférica, com membrana dupla, sendo que sua densidade difere ao longo da membrana. As larvas possuem inicialmente pigmentação escassa, que se intensifica ao longo do desenvolvimento; quatro pares de barbilhões bem desenvolvidos; corpo alongado; nadadeiras dorsal e anal longas; ausência de nadadeira adiposa e presença de vesículas circundando a membrana embrionária e os barbilhões. A sequência de formação das nadadeiras é: caudal, dorsal, anal, peitoral e pélvica. A análise do padrão de crescimento revelou que a maior parte da metamorfose ocorre no estágio de flexão. O desempenho reprodutivo da espécie e seu rápido desenvolvimento inicial se enquadram positivamente à aquicultura, porém, podem ser altamente favoráveis à invasão de ambientes naturais, representando um risco para as populações nativas.

Palavras-chave: bagre africano, espécie invasora, larvas, juvenis de peixes.

Introduction

Clarias gariepinus (Burchell, 1822), known as the African catfish, is widely distributed in Africa, as well as occurring in Israel, Lebanon and Turkey (TEUGELS, 1986). Among the main features of this species are the presence of a pseudo lung (arborescent organ), mucus production as an adaptation to living in adverse environments (DONNELLY, 1973), and the ability to leave water during the nocturnal period, using the pectoral fins, to search for food and breeding sites.

This species consumes a wide range of food, foraging during the night on a variety of prey, including arthropods, molluscs, fish, reptiles and amphibians, and when necessary, plants and plankton (YALÇIN et al., 2001).

Its rapid growth to high densities, its ability to capture atmospheric air, its resistance in low quality water and the great palatability of its meat make this species an exceptional candidate for aquaculture (APPELBAUM; KAMLER, 2000), and has led to its introduction in Europe, Asia and Latin America

(VERRETH et al., 1993). This species was introduced to Brazil in 1986 (AGOSTINHO et al., 2007) for cultivation in fish farms, but the meat was not accepted well commercially, leading to the search for other income options, such as fish-and-pay (VITULE et al., 2006). The resulting cultivation of fish in irregular ponds lacking protective barriers close to river beds led to the contamination of several rivers, endangering the native ichthyofauna. For example, approximately 656 thousand individuals of this species were estimated to have escaped from fish farming ponds during floods in the upper Paraná river watershed in 1996/1997 (ORSI; AGOSTINHO, 1999).

Currently, this species is found in almost all Brazilian watersheds (ALVES et al., 1999; BRAUN et al., 2003; INGÊNITO et al., 2004; MILI; TEIXEIRA, 2006; VITULE et al., 2006; ROCHA, 2008). However, given its great invasive potential (VITULE et al., 2006), it is not clear whether it has actually become established. According to the definition given by Williamson and Fitter (1996), an introduced species is only considered established when there are one or more self-sustaining populations that are able to complete their life cycle within the new environment (reproduction and recruitment). Thus, studies on the eggs, larvae and juveniles of fish are of primary importance for detecting the establishment of a species in such environments, an essential aspect in decision-making for management purposes.

The present study investigated and described the early development of *Clarias gariepinus*, in order to allow its identification in natural environments and, therefore, contribute to the study of this invasive species in Brazilian watersheds. Traits of the embryology, development and morphology of larvae and juveniles and several aspects of its invasive potential in natural freshwater environments were also examined.

Material and methods

The material used in this study was obtained via induced spawning in the Estação de Aquicultura Aquafish, in Toledo Municipality, Paraná State, during 1999. After spawning, the periodicity of sampling varied according to the stage of development. The eggs were gathered soon after hydration at intervals of 2 hours until eclosion. The larvae were collected from eclosion until complete absorption of the yolk sac, at intervals of 2 to 6 hours; following this stage, larvae and juveniles were collected every 12 hours.

The eggs were classified into the following developmental stages: early cleavage, early embryo, tail-free and late embryo (NAKATANI et al., 2001). Morphometric characterization of the eggs was carried out by measuring the diameter of each egg (DE), the perivitelline space (PS) and yolk diameter (YD). The size of the perivitelline space was characterized according to its participation in the total volume of the egg (NAKATANI et al., 2001). After hatching, individuals were separated into larval (stages: yolk-sac larvae, pre-flexion, flexion, and post-flexion) and juvenile stages according to Ahlstrom and Ball (1954), modified by Nakatani et al. (2001). Besides describing each stage, the occurrence of the main morphological events was also recorded, and individuals that best represented the relevant traits were illustrated using a camera lucida coupled to a stereomicroscope.

In order to characterize early development, we measured, using a stereomicroscope with an ocular micrometer, the following body dimensions (mm) (AHLSTROM; MOSER, 1976): standard length (SL), snout length (SnL), body depth (BD), head depth (HD), head length (HL), eye diameter (ED) and the pre-pectoral (PPL), pre-pelvic (PVL), pre-dorsal (PDL) and pre-anal (PAL) lengths. For the analysis of body relationships (expressed as a percentage), the morphometric variables were related to the standard length (HD, BD, PPL, PVL, PDL and PAL) and head length (HD, SnL, ED) during development. The body relationships for body height (BD/SL), head length (HL/SL) and eye diameter (ED/HL) were established using the criteria suggested by Leis and Trnski (1989). For meristic characterization, we quantified, whenever possible, the number of pre- and post-anal myomeres, and the number of rays in the pectoral (P), pelvic (V), dorsal (D) and anal (A) fins.

To examine the growth pattern of the species, the morphometric variables (dependent variables) were plotted against the standard length and head length (independent variables), and their relationships were described by different models of growth representing different biological processes (KOVÁČ et al., 1999). Initially, the hypothesis that the development of the body relationships is isometric was tested using a simple linear regression. Together with the isometry hypothesis, we also tested the alternative hypotheses of gradually allometric (quadratic regression) or abrupt (piecewise linear regression) development, the latter being distinguished by breakpoints that reflect different growth rates. The selection of the best growth model for each morphometric variable in relation to body size was tested using the F test (SOKAL; ROHLF, 1981). A significance level of $p < 0.05$ was adopted.

The material used in this study was placed in the collection of the Laboratório de Ictioplâncton of Nupélia (Núcleo de Pesquisas em Limnologia, Ictiologia e Aquicultura) of the State University of Maringá (UEM).

Results

Embryonic period

The development of the 12 eggs analyzed is illustrated in Figure 1a–d. Newly fertilized eggs are spherical and have a double membrane, the density of which varies spatially. Eggs have a medium diameter of 1.65 mm, a restricted perivitelline space with a medium size of 0.14 mm and a yolk sac with a medium diameter of 1.38 mm. At early cleavage (Figure 1a) after the morula stage, intense cell proliferation continues on the yolk surface. In the early embryo (Figure 1b) the process of cell migration involves the yolk, forming the germ ring; in the tail-free embryo (Figure 1c) somites become apparent along with the differentiation of the head and tail; the latter begins to be released from the yolk, but does not become totally free. Optic vesicles can also be visualized. In the late embryo (Figure 1d), the neural tube becomes prominent, along with the yolk sac, which is well defined, with a yellowish color, and is surrounded by the body; the tail is completely released.

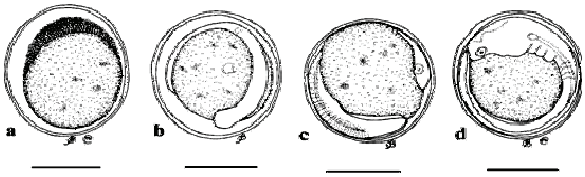

Figure 1. Embryonic development of *Clarias gariepinus*. a) early cleavage (1.73 mm DE); b) early embryo (1.71 mm DE); c) tail-free embryo (1.72 mm DE); d) late embryo (1.69 mm DE) (Scale = 1 mm).

Larval period

We analyzed 146 larvae (36 yolk-sac larvae, 11 pre-flexion, 57 flexion and 42 post-flexion stages) with a standard length increasing from 4.1 to 15.0 mm.

Yolk-sac larvae stage: Eclosion occurs after approximately 30 hours of incubation (25 °C), with the larvae measuring between 4.1 and 5.3 mm. The yolk sac is relatively large. A few pigmented areas may be observed on the head, around the eyes and on the dorsum (dendritic chromatophores). The eyes are slightly pigmented. Barbel buds are visible. An adhesive organ is present in the median region of the yolk sac, and at the end of this stage, this structure expands. The mouth is in the ventral position in less developed individuals. The mouth and the anus are closed; but at about 5 mm it is already possible to visualize the apertures of these structures. The finfold is turgid and tubular, extending from the post-cephalic to the ventral region, next to the end of the yolk sac. Pectoral fin buds can already be observed in some individuals. The notochord is bent and visible by transparency; however the support elements are not visible. The eyes are small (12.15 to 21.67% HL), the head varies from moderate to small (11.70 to 21.40% SL) and the body is moderately sized (24.42 to 31.20% SL) (Figure 2a; Table 1).

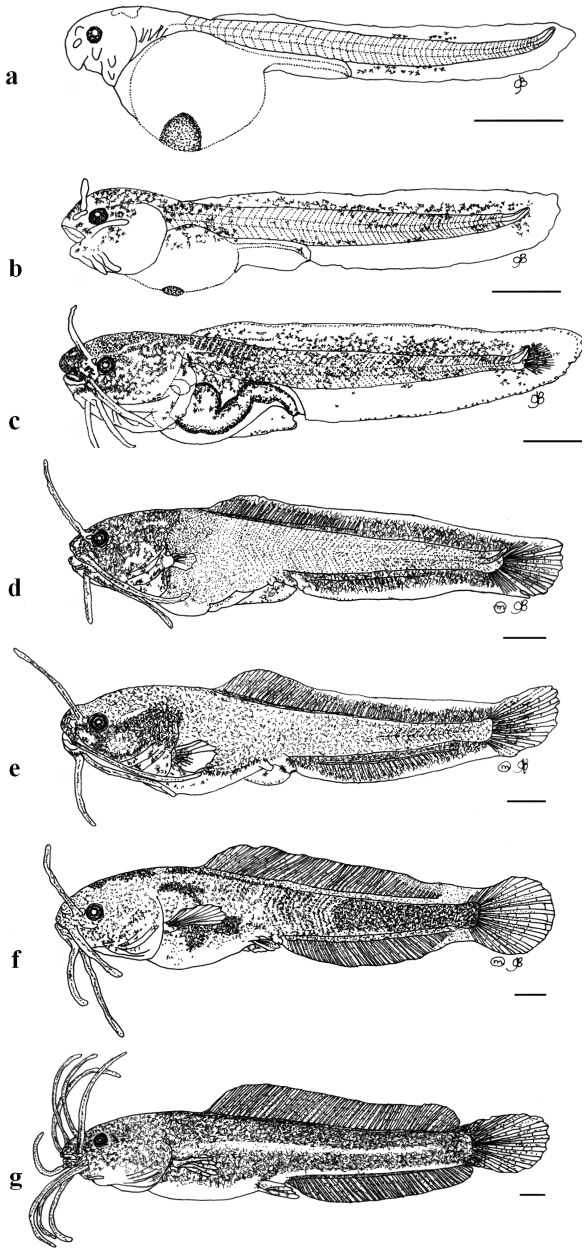

Figure 2. Early development of *Clarias gariepinus*, illustrating the individuals that best represented each period a) yolk-sac larvae (5.37 mm SL); b) pre-flexion (6.38 mm SL); c) early flexion (6.87 mm SL); d) late flexion (9.39 mm SL); e) early post-flexion (10.14 mm SL); f) late post-flexion (14.29 mm SL) and g) juvenile (29 mm SL) (Scale = 1 mm).

Table 1. Minimum and maximum values (Min; Max), means (X) and standard deviation (SD) (mm and %) of morphometric and meristic variables obtained from larvae and juveniles of *Clarias gariepinus* (LV = yolk-sac larvae: PF = pre-flexion; FL = flexion; FP = post-flexion; J = juvenile; n = number of individuals analyzed; af = absent fin; dv = difficult visualization; nf = unformed).

Variables (mm)	Larval Period								Juvenile Period	
	LV (n=36)		PF (n=11)		FL (n=57)		FP (n=42)		J (n=06)	
	Min/Max	X±SD	Min/Max	X±SD	Min/Max	X±SD	Min/Max	X±SD	Min/Max	X±DP
SL	4.1-5.3	4.8±0.3	5.8-6.6	6.2±0.3	6.3-9.8	7.7±0.9	9.8-15.0	11.9±1.4	22.9-29.0	24.9±2.5
HD	0.5-0.7	0.6±0.0	0.8-1.2	1.0±0.1	1.0-1.9	1.4±0.2	1.8-2.9	2.2±0.31	4.1-5.6	5.0±0.6
HL	0.6-1.1	0.7±0.1	1.2-1.4	1.3±0.1	1.6-2.6	2.0±0.2	2.3-3.6	2.8±0.3	5.6-6.7	5.9±0.5
SnL	0.3-0.3	0.3±0.0	0.3-0.4	0.4±0.0	0.3-0.6	0.5±0.1	0.5-1.1	0.8±0.2	1.4-1.9	1.7±0.2
ED	0.1-0.1	0.1±0.0	0.2-0.3	0.2±0.0	0.2-0.4	0.3±0.0	0.4-0.6	0.4±0.0	0.9-1.0	0.9±0.1
BD	1.2-1.4	1.3±0.1	1.3-1.7	1.4±0.1	1.2-2.5	1.6±0.4	2.1-3.7	2.8±0.4	4.6-6.3	5.4±0.7
PPL	0.7-0.9	0.8±0.1	1.1-1.4	1.3±0.1	1.4-2.6	1.8±0.3	2.3-3.7	2.9±0.4	5.6-6.9	6.0±0.6
PVL	af	af	af	af	af	af	5.7-7.1	6.3±0.4	10.7-13.6	11.8±1.2
PDL	af	af	af	af	af	af	3.1-6.7	4.5±0.7	7.6-9.9	8.8±1.0
PAL	af	af	af	af	af	af	6.4-8.0	7.1±0.5	12.7-15.4	13.8±1.2
Relations (%)										
HD/HL	62.6-100	90.2±9.2	66.4-93.6	77.5±7.6	54.6-88.9	69.1±8.1	68.6- 4.8	78.9±6.0	74.3-92.3	83.8±6.4
SnL/HL	25.2-45.2	38.5±5.6	23.2-31.6	27.2±2.4	17.2-27.3	22.7±2.2	20.0-36.9	26.6±3.3	25.5-30.7	28.2±2.4
ED/HL	12.2-21.7	17.5±2.6	12.0-18.8	13.7±1.9	8.9-16.7	12.9±2.0	12.0-19.0	14.7±1.2	14.9-15.6	15.4±0.2
HL/SL	11.7-21.4	14.3±1.8	19.2-24.4	21.1±1.3	21.7-29.3	25.7±1.6	20.0-26.7	23.9±1.3	23.1-24.4	23.7±0.5
BD/SL	24.4-31.2	27.6±2.1	19.0-28.2	22.5±2.7	16.2-27.0	20.9±2.9	19.5-27.1	23.9±1.4	19.6-22.9	21.5±1.1
PPL/SL	14.2-17.5	15.6±1.1	18.2-24.4	21.0±2.0	20.9-28.3	23.3±1.4	20.0-26.7	24.0±1.3	23.2-25.5	24.3±0.9
PVL/SL	af	af	af	af	af	af	41.9-54.3	47.9±2.7	45.3-50.5	47.6±1.8
PDL/SL	af	af	af	af	af	af	29.7-44.7	35.4±2.9	32.5-40.6	35.4±3.0
PAL/SL	af	af	af	af	af	af	46.9-57.0	54.0±2.1	52.2-57.4	55.2±1.8
Myomeres										
Total	dv	dv	dv	dv	58-63	60±1.1	56-62	59±1.9	56-60	58±1.4
Pre-anal	dv	dv	dv	dv	20-23	22±0.7	19-22	20±1.1	19-20	19±0.5
Post-anal	dv	dv	dv	dv	37-41	39±1.0	37-41	39±1.1	36-41	39±1.6
Rays										
P	nf	nf	nf	nf	nf	nf	nf	nf	8-8	8±0
V	nf	nf	nf	nf	nf	nf	nf	nf	6-6	6±0
D	nf	nf	nf	nf	nf	nf	nf	nf	67-69	69±0.8
A	nf	nf	nf	nf	nf	nf	nf	nf	51-55	53±1.4

Pre-flexion stage: At this stage, the larvae measure between 5.8 and 6.6 mm. The yolk is absorbed during development, but vestiges may still be present at the end of this stage. Pigmentation is unevenly distributed over the body, in the snout and in the upper part of the yolk sac. Dendritic chromatophores are also visible along the myomeres and in the dorsal region (finfold and in the own dorso). The pigmentation of the eyes is complete, and the eyes have a diameter of approximately 5 mm. The mouth is terminal and does not change further. The intestine is short and the anus opens in the median portion of the body. Four barbel pairs start forming: one nasal, one maxillary and two mentonian. The operculum is still not completely differentiated. The finfold becomes thinner, losing its tubular shape and presenting sinuosities. The pectoral fin bud can be seen by transparency, just below the opercular membrane. The eye is small (12.15 to 21.67% HL), the head varies from moderate to small (19.23 to 24.36% SL) and the body varies from long to moderate (19.00 to 28.21% SL) (Figure 2b; Table 1).

Flexion stage: The standard length of larvae in this stage varies between 6.3 and 9.8 mm. The yolk sac is totally absorbed by about 7.0 mm. The pigmentation intensifies throughout the body, in the embryonic membrane, the fin rays and the barbels. The body becomes elongated and subcylindrical in shape. The width of the mouth increases. The adhesive organ disappears completely. Parts of the digestive system and gills can be visualized by transparency. A number of vesicles are evident in the terminal region that surrounds the finfold and along the barbels. The rays of the caudal fin develop almost completely and those of the dorsal and anal fins also develop, reaching to the end or halfway along these fins by the end of this stage. At the end of this stage, the pectoral fins are already external to the operculum. During this stage, the myomeres can be quantified, varying from 58 to 63 (20 to 23 pre- and 37 to 41 post-anal). The eye is considered small (8.85 to 16.67% HL), while the head (21.69 to 29.28% SL) and the body (16.22 to 27.03% SL) are moderate (Figure. 2c and d; Table 1).

Post-flexion stage: During this stage, the standard length increases from 9.8 to 15.0 mm. In most individuals the finfold is completely absorbed. The pelvic fin bud arises with development of its rays at 14.6 mm. The caudal, dorsal, anal and pectoral fins present segmented rays. As the fins are delimited, the total number of vesicles declines

considerably; they only remain visible in the barbels. The total number of myomeres varies from 59 to 62 (20 to 22 pre- and 38 to 41 post-anal). The eye is considered small (12.04 to 19.00% HL), the head varies from small to moderate (19.97 to 26.73% SL) and the body from long to moderate (19.50 to 27.06% SL) (Figure. 2e and f; Table 1).

Juvenile period

We analyzed 6 juveniles with a standard length between 22.9 and 29.0 mm. These have a wide and terminal mouth in which the maxilla is longer than the mandible. The pigmentation is similar to the adult, with the delimitation of a white strip in the ventral region, which does not exceed the height of the pectoral fins, plus a longitudinal strip starting close to the operculum and ending in the region of the caudal peduncle. The sequence of the formation of fin rays is caudal, dorsal, anal, pectoral and pelvic. The total number of rays varies between 67 and 69 in the dorsal fin, and between 51 and 55 in the anal fin; the pelvic fins have 8 rays and the pectoral fins have 6. The total number of myomeres varies from 56 to 60 (19 to 20 pre- and 36 to 41 post-anal). The eyes are considered small and are located more dorsally (14.90 to 12.58% HL), the head is moderate (23.14 to 24.38% SL) and the body varies from long to moderate (19.61 to 22.87% SL) (Figure 2g; Table 1).

Body relationships

As indicated in Table 2, the relationships among the head length were best represented by the piecewise model (discontinuous growth). The variables snout length, eye diameter and head depth, initially, the model showed more rapid growth, reducing in 0.58, 0.32 and 1.54 mm, respectively. Among the variables related to the standard length, four of the six tested were better represented by piecewise regression. The body depth (with a breakpoint of 2.02 mm) showed a decrease in growth velocity from the breakpoint. The head length (with a breakpoint of 2.01 mm), pre-pectoral (with a breakpoint of 2.31 mm), pre-dorsal (with a breakpoint of 5.39 mm) the model showed an increase in the velocity of the growth after the breakpoint. The pre-pelvic and pre-anal distance presented the isometric growth (linear regression).

Discussion

The morphological events recorded during the embryonic development of *C. gariepinus* were similar to those of other freshwater Siluriformes (GODINHO et al., 1978; CUSSAC et al., 1985; MATKOVIC et al., 1985; CARDOSO et al., 1995). The spherical shape of the eggs was like that generally found in the Clariidae family (RIEHL, 1996). The egg diameter decreased during development, as reported by Zaki and Abdula (1983), who also noted an increase in the diameter of the double membrane. According to the latter authors such observations may be related to metabolic processes in the embryo during development.

Clarias gariepinus spawns in shallow and flooded areas of rivers, lakes and streams (DE GRAAF; JENSEN, 1996) and the eggs are dispersed on the vegetation or benthic objects (ZAKI; ABDULA, 1983), attaching to these substrates via a specific adhesive apparatus on the outer membrane (RIEHL; APPELBAUM, 1991). This spawning behavior explains the restricted perivitelline space recorded in the eggs, which is characteristic of species without reproductive migration (NAKATANI et al., 2001).

Success during ontogeny is mainly due to the rapid development of structures related to feeding and avoidance of predation (BLAXTER, 1988). In *C. gariepinus* development of the mouth and anal apertures, eye pigmentation, the four barbel pairs and changes in the finfold occur simultaneously with yolk absorption and are related to foraging ability, indicating the beginning of exogenous feeding.

Table 2. Statistics from linear, quadratic and 'piecewise' regressions for the morphometric variables obtained in relation to the head length and standard length in larvae and juveniles of *Clarias gariepinus*. R^2 = coefficient of determination, L = linear regression, Q = quadratic regression, S = piecewise regression, BM = best model, BP = breakpoint, a and b = regression parameters and n = number of analyzed individuals. ⋆: $p < 0.05$.

Relations	R^2			F-test			BM	BP	a1	b1	a2	b2	n
	L	Q	S	Q/L	S/Q	S/L							
SnL/HL	0.92	0.93	0.96	11.82⋆	116.84⋆	69.89⋆	*S*	0.58	0.14	0.17	0.29	-0.05	127
ED/HL	0.94	0.94	0.96	10.60⋆	50.91⋆	32.91⋆	*S*	0.32	0.09	0.06	0.15	-0.01	127
HD/HL	0.96	0.96	0.98	31.35⋆	77.34⋆	62.85⋆	*S*	1.54	0.53	0.26	0.87	-0.22	152
HL/SL	0.95	0.94	0.98	-20.02⋆	235.89⋆	92.67⋆	*S*	2.01	0.43	-1.34	0.23	0.15	152
BD/SL	0.94	0.94	0.97	0.83	137.09⋆	69.81⋆	*S*	2.02	0.88	0.88	0.41	0.20	152
PPL/SL	0.98	0.98	0.98	-14.98⋆	56.13⋆	16.88⋆	*S*	2.31	0.31	-0.60	0.24	0.05	116
PVL/SL	0.98	0.98	0.98	-0.00	0.98	0.48	*L*	-----	0.34	1.86	-----	-----	21
PDL/SL	0.94	0.94	0.96	0.08	15.34⋆	7.73⋆	*S*	5.39	0.43	-1.02	0.23	3.13	30
PAL/SL	0.99	0.99	0.99	-0.23	4.81⋆	2.26⋆	*L*	-----	0.44	1.32	-----	-----	24

However, the stomach only becomes physiologically functional after this (ADRIAENS; VANDEWALLE, 2003). This reduced functioning of the stomach may increase the pressure for efficient foraging, to balance the sub-optimal digestive process.

The scarce pigmentation in the less developed larvae may be beneficial for the species. According to Nakatani et al. (1997), larvae with little pigmentation are common in pelagic environments, and changes may occur when they explore other environments. Thus, during the dispersal of early larvae of *C. gariepinus,* the lack of pigmentation provides camouflage against visual predators. The increase in pigmentation observed during development is indispensable for this camouflage and suggests behavioral changes in the fish.

The lack of pigmentation in the eyes of the larvae at the yolk-sac stage is also observed in most fish species (NAKATANI et al., 2001) and is characteristic of species with indirect development, i.e., those in which the larvae hatch at an early developmental stage (BLAXTER, 1988). Complete pigmentation of the eyes is observed during pre-flexion, when theoretically individuals may move actively and capture food. The small size of the eyes suggests that they are less important for prey capture (HECHT; APPELBAUM, 1988), whereas the circumoral barbels are fundamental for this purpose (SCHWASSMANN, 1971), and at pre-flexion are already well developed.

The adhesive organ at the base of the yolk sac allows the attachment of the larvae to some types of substrate (LEGENDRE; TEUGELS, 1991) that may be displaced in currents, playing an important role in larval dispersal. The increase in the size of this organ during the yolk-sac stage indicates the requirement for this structure in order to successfully complete this stage. This organ retracts from the moment the larvae start to move actively in the water. Movement may also be associated with changes in turgidity recorded in the finfold (yolk-sac larvae to pre-flexion), since the pair fins have not yet developed at this stage.

According to Dunn (1983) the number, structure, position and sequence of development of the fins are decisive for the identification of fish larvae. The first fin developed by *C. gariepinus* is the finfold; this fin is important for locomotion and orientation (WEBB; WEIHS, 1986), besides supporting the formation of unpaired fins, and remains until the post-flexion stage. Adriaens and Vandewalle (2003) reported that, in the families Clariidae and Bagridae, the finfold is modified into the caudal, dorsal, adipose and anal fins (the adipose fin is absent in *C. gariepinus*). This may be associated with the type of embryonic development, where the yolk reserve is small and the incubation period is short, impeding the full development of the fins.

Sagnes et al. (1997) proposed that larval development is characterized by morphological 'leaps', during which one or more parts of the body grow rapidly. In *C. gariepinus*, seven of nine measurable traits demonstrated isometric by parts development, i.e., there was early growth similar to the independent variable with a sudden change at a certain size (breakpoint), six of which were within the flexion stage and one at the beginning of the post-flexion stage. According to Kováč et al. (1999) such breakpoints may represent the threshold between the larval and juvenile periods or the threshold between the stages within a period, and may be related to morphological, physiological and/or ecological and behavioral changes. During this interval in the life history of this species, there is much external metamorphosis, with the head and the fins developing rapidly, with little variation in the head and body length. The decrease in growth rate of the variables associated with the head of larvae of *C. gariepinus* indicates that there are large changes in this structure during the flexion stage. These changes are probably due to the formation of the brain, leading to the diversification of motor and sensory skills (BIALETZKI et al., 1998).

Changes in the fins associated with the elongated and subcylindrical shape of the body during the flexion stage permit anguiliform locomotion and, in more developed individuals, even movement into terrestrial environments, which besides aiding in prey capture, allow the accomplishment of great displacements. Another feature recorded at the flexion stage was the increase in vesicle size, allowing their visualization. If these structures act in environmental perception, as suggested by Bruton (1979), displacement and capture of prey may depend upon them.

Clarias gariepinus has several characteristics that allow its invasion into new environments, including adaptations to living out of water, an omnivorous diet, and the ability to move between environments and bury itself during drought (CAMBRAY, 2003). Among the early characteristics considered advantageous for its dispersal and establishment we highlight its rapid embryonic development (allowing rapid hatching) (ADRIAENS; VANDEWALLE, 2003), the presence of many small eggs (LEGENDRE; TEUGELS, 1991) and its rapid larval development (KAMLER et al., 1994).

Mili and Teixeira (2006), Vitule et al. (2006) and Rocha (2008) performed studies in different Brazilian watersheds and found individuals of *C. gariepinus* whose total length exceeded the size of first maturation (230 mm) reported by Yalçin et al. (2001); furthermore, Vitule et al. (2006) and Rocha (2008) captured reproductively mature individuals, inferring species establishment based on these data; however, the authors did not demonstrate the entire reproductive cycle in the sampled environments, since there was no record of eggs and larvae. Four juveniles of this species (total length around 30 mm) were reported in Aguapeí river, a tributary of the Paraná river, and fishermen have also caught adult individuals in recent years (Makrakis M. C., pers. comm.).

The lack of literature about the occurrence of eggs, larvae and juveniles of this species in natural environments (except those from which the species originated) probably reflects its lack of establishment, caused by interaction with ecological factors that interfere negatively with reproduction, as well as early development. Confirmation of establishment will require further detailed studies in different environments in order to affirm the existence of self-sustaining populations with the resulting entry of new individuals into the population. It is worth noting that, in the case of introduced species, the absence of evidence is not evidence of absence (CASAL, 2006).

Conclusion

Based on the results obtained in the present study, the most important traits for the identification of larvae of *C. gariepinus* in natural environments are the pigmentation pattern, coupled with the presence of four pairs of well developed barbels (uncommon trait in native Siluriformes in Brazilian watersheds), and elongated body, long dorsal and anal fins, the lack of an adipose fin and the presence of vesicles surrounding the finfold and the barbels. Features of its reproduction and early development make it suitable for aquaculture; on the other hand they also favor the invasion of natural environments. Hence this species may have a significant negative impact on the native ichthyofauna and its establishment would undoubtedly represent a great threat for biodiversity conservation. Further detailed studies, including those on reproduction and the distribution of eggs, larvae and juveniles, will be vital to explain the establishment of this species in Brazilian watersheds.

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Received on February 10, 2011.
Accepted on June 16, 2011.