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Description of *Atherinella brasiliensis* (Quoy & Gaimard, 1825) (Atheriniformes: Atherinopsidae) larvae from the Jaguaribe River estuary, Itamaracá island, Northeastern Brazil

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The present study describes the external morphology and morphometry of the initial development of *Atherinella brasiliensis*, and contributes toward broadening knowledge on its biology. A total of 88 larvae and 14 juveniles were used to characterize the morphological development and analyze body proportions. Fish with standard lengths (SL) from 1.4 to 77 mm were used in the study. Larvae hatch at an average of 1.4 mm SL. In the preflexion stage, the larval body is enveloped by a finfold, which begins behind the head. Pectoral fins are the first to be formed and preflexion larvae have four characteristic dendritic chromatophores on the head. The flexion stage begins at an average of 4.4 mm SL; dorsal and anal fins already exhibit pterygiophores and a terminal, somewhat up-turned mouth. At 6.8 mm SL, the flexion stage ends. In the postflexion stage, larvae present greater ossification of the dorsal and anal fin rays, exhibit pelvic fin buds and a darkening of lateral pigmentation. At an average of 8.8 mm SL, head pigmentation intensifies and pelvic fins exhibit conspicuous ossifying rays. Larvae at 11.8 mm SL have all fins formed; the second dorsal fin is the last to be formed at an insertion point posterior to the anal fin. The juvenile period begins at approximately 12 mm SL. At this stage, *A. brasiliensis* has an anal fin located at the median portion of the body and the posterior end of pectorals surpasses the origin of pelvic fins, which are located at the midpoint between the pectoral and anal fins. Scales are present on the dorsal-lateral region behind the head. The morphological features of *A. brasiliensis* herein described allow an adequate identification of its larvae and differentiate them from hemiramphid and other atheriniform larvae, thus broadening knowledge on the larval biology of the species.

O presente trabalho descreve a morfologia externa e a morfometria do desenvolvimento inicial de *Atherinella brasiliensis*, contribuindo para a ampliação do conhecimento biológico da espécie. Um total de 88 larvas e 14 juvenis foram empregados para a caracterização do desenvolvimento morfológico e a análise das relações corporais. Peixes com comprimento padrão (CP) de 1,4 a 77 mm foram usados no estudo. As larvas eclodem com CP médio de 1,4 mm. No estágio de pré-flexão, as larvas apresentam o corpo envolvido por uma membrana embrionária, a qual inicia atrás da cabeça. As nadadeiras peitorais são as primeiras a se formarem e larvas em pré-flexão apresentam quatro cromatóforos dendríticos característicos na cabeça. O estágio de flexão inicia com um CP de aproximadamente 4,4 mm, as nadadeiras dorsal e anal já apresentam pterigióforos e uma boca terminal, ligeiramente inclinada para cima. Com um CP de 6,8 mm, o estágio de flexão termina. No estágio de pós-flexão, as larvas apresentam uma maior ossificação dos raios das nadadeiras dorsal e anal, juntamente com o surgimento dos botões das nadadeiras pélvicas e o escurecimento da pigmentação lateral. Com um CP médio de 8,8 mm, a pigmentação da cabeça se intensifica e as nadadeiras pélvicas apresentam raios conspícuos em ossificação. Com 11,8 mm a larva já apresenta todas as nadadeiras formadas, a segunda dorsal sendo a última a ser formada, num ponto de inserção posterior ao da anal. O estágio juvenil inicia-se com aproximadamente 12 mm CP. Neste estágio, *A. brasiliensis* apresenta a nadadeira anal localizada na porção mediana do corpo, e a extremidade posterior das peitorais ultrapassam a origem das pélvicas, as quais estão localizadas no ponto médio entre as peitorais e a anal. Escamas estão presentes na região dorso-lateral, atrás da cabeça. As características morfológicas das larvas de *A. brasiliensis* descritas permitem sua adequada identificação, bem como as diferenciam daquelas de hemiramphídeos e outros atheriniformes, assim ampliando o conhecimento sobre a biologia larval da espécie.

Key words: Silverside, Larval development, Early juvenile, Ontogeny.

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Introduction

Knowledge on the ecology of fish larvae is essential to a broader understanding of the biology and dynamics of species in the ecosystems they inhabit. However, the identification of these larvae is a serious problem for many taxonomists, as larvae and adults have different ecological demands and exhibit differences regarding the habitat, feeding and behavior (Leis & Trnski, 1989). These differences are attributed to ontogenic changes that occur during the larval stage, some of which are directly related to the development of the adult form and others are related to plankton specialization (Kendall *et al.,* 1984).

The high number of aggregated species with great morphological similarities and the lack of taxonomic keys, guides, adequate descriptions and comparative literature are factors that aggravate the identification of fish larvae at the species level (Fuiman *et al*., 1983). Identification is quite complex and constitutes a comparative elimination process, where the use of larval development series at different stages is essential (Powles & Markle, 1984). The diagnostic characteristics for larvae can be meristic, morphometric, structural and pigmentary. It may also involve the stages of organ development relative to the size of the larvae (Blaxter, 1984).

According to Dyer (1998), the Atheriniformes order includes 49 genera distributed among six families, of which Atherinopsidae has thirteen genera and 104 species (Nelson, 1994), commonly known as New World silversides. From those of the *Atherinella* genus, only *A. brasiliensis* (Quoy & Gaimard, 1824) – previously referred to as *Xenomelaniris brasiliensis* – and *A. blackburni* (Schultz, 1949) have been recorded in Brazil (Dyer, 2003).

Atherinopsidae comprises the subfamilies Menidiinae and Atherinopsinae, proposed as a separate family by Saeed *et al.* (1994), the status of which was further confirmed by Dyer & Chernoff (1996). These animals live mainly in coastal, estuarine or freshwater environments. Adults of most species have a small, slender, elongate body with a silvery lateral line and can be found in shoals. Atherinopsid larvae have an elongate, laterally slim body, a wide, short head and are easily recognized by a line of melanophores along the dorsal and lateral midline from the head to the caudal region. They can be confounded with Atherinidae, Clupeidae, Engraulidae, Hemiramphidae and Mugilidae, but are generally recognizable by their extreme slenderness and the forward position of the anus (Vásques-Yeomans, 2006). Among atherinopsid larvae, however, such differentiation is difficult due to morphometric and pigmentation similarities. Early life history (ELH) stages are known for only three species occurring in the Western Central North Atlantic: *Menidia beryllina* (Cope, 1867), *M. menidia* (Linnaeus, 1766) and *Membras martinica* (Valenciennes, 1835) (Richards, 2006).

The aim of the present study was to characterize the initial development of *A. brasiliensis* through a description of their external morphology and morphometric aspects, thereby contributing toward the identification and definition of its initial phases as well as broadening knowledge on Atherinopsidae larvae and juveniles in the Western Atlantic.

Material and Methods

Monthly ichthyoplankton samples were obtained from April 2001 to April 2002 at different stations along the Jaguaribe River estuary. The estuary is located on Itamaracá Island, on northern coast of the State of Pernambuco at a distance of 50km from the state capital, between 07º43'08" and 07º45'32"S and 034º50'14" and 034º51'05"W. Collections were performed through surface horizontal tows with a conical-cylindrical 500 µm mesh plankton net. All material was fixed in 5% buffered formaldehyde solution and sorted in the laboratory under a stereomicroscope. Preliminary identification of the *Atherinella brasiliensis* larvae was carried out based on the investigations of Lippson & Moran (1974) and Able & Fahay (1998). Classification in the development stages – yolk-sac larvae, preflexion, flexion and postflexion – was determined through the development sequence of the caudal fin and its supporting elements (Nakatani *et al.,* 2001).

Eighty-eight larvae and 14 juveniles were used to characterize the morphological development and analyze body proportions of *A. brasiliensis.* Larval standard lengths ranged from 1.4 to 11.8 mm, whereas juvenile standard lengths ranged from 11.6 to 77 mm.

Individuals were illustrated and morphologically characterized with the help of a stereomicroscope, equipped with a micrometric scale and a camera lucida. The following measurements were taken according to the procedures described by Leis & Carson-Ewart (2000): standard length (SL), pre dorsal-fin length (PDL), preanal length (PAL), pre pelvic-fin length (PP_2L), head length (HL), eye diameter (ED) and body depth (BD). The pre pectoral-fin length (PP_1L) was measured from snout tip to insertion of pectoral fin. Abbreviations for fins are as follows: pectoral (P_1), first dorsal (D_1), second dorsal (D_2), pelvic (P_2), anal (A) and caudal (C). Variation in body proportions throughout development was analyzed regarding SL for BD, HL, PDL, PAL, PP_1L and PP_2L, whereas ED was expressed in relation to HL (Leis & Carson-Ewart, 2000). The relationships for BD, HL, and ED followed the categories proposed by Leis & Trnski (1989). Selected specimens were cleared and stained (Dingerkus & Uhler, 1977) for meristic counts.

Results

Table 1 displays the amplitude and size ranges of body proportions of *Atherinella brasiliensis* larvae and juveniles. Table 2 displays the meristic counts for selected specimens. *A. brasiliensis* larvae hatch at sizes smaller than 1.4 mm SL. The only specimen analyzed at this size was in the preflexion stage. The specimen could not be depicted or measured due to its distorted state, but its taxonomical status was determined by the typical pattern of head pigmentation (see below). Thus, the exact size of yolk-sac larvae could not be determined.

The preflexion stage, which lasts until the beginning of notochord flexion and formation of the hypural bones, varied from 2.3 to 4.7 mm SL (n=62, mean=3.5±0.59 mm). At this stage, larvae are nearly completely enveloped by an embryonic membrane (finfold) along the midline of the body, which begins precisely behind the head. The pectoral fin buds (P_1) are the first to form and are located in a high lateral position on the body just behind the head (Fig. 1a). The larva has four characteristic dendritic chromatophores on the head – two placed anterolaterally, a third more posteriorly along the midline, forming a triangle, followed by a fourth placed further posteriorly – and pigmentation along the whole median portion of the ventral region (Figs. 1b and c). The number of chromatophores along the midventral line varies between 22 and 28. The caudal fin (C) supporting elements are first noticed at this stage and it is also possible to identify the position where the second dorsal (D_2) and anal fins will form. The total number of myomeres is 34, but pre and postanal myomeres are not clearly distinguished due to early development of rays. The body is elongate (14.2 to 16.1%), the head is small (18.2 to 19.9%), and the eye is large (42.2 to 53.0%).

The flexion stage ranges from 4.4 to 6.8 mm SL (n=14, mean=5.7±0.71 mm) and is characterized by the flexion of the notochord tip. With an average size of 4.7 mm SL (Fig. 1d), the larva exhibits a finfold enveloping the second dorsal and anal fins, where pterygiophores can be seen. However, the rays are almost completely formed in both fins only at 6.4 mm SL (Fig. 1e), when larvae no longer have an evident embryonic membrane. At this size, the mouth assumes a slightly upwardly inclined terminal position. The chromatophore count varies from 29 to 30 on the midventral line. The total number of myomeres is 34 (16-17 preanal, 17-18 postanal). At this stage, the body varies from very elongate to elongate (9.8 to 15.1%), the head is moderate (20.0 to 22.9%), and the eye is large (34.9 to 50.0%).

In the postflexion stage, larval size varied from 7.4 to 11.8 mm SL (n=12, mean=9.0±1.7). The larva exhibits ossification of the second dorsal and anal fin rays. It also has pelvic fin (P_2) buds (Fig. 1f) located anterior to the anus. Intensification of head (Fig. 1g) and lateral body pigmentation is observed and is probably related to the formation of the silvery line. At approximately 12.0 mm SL, all fins are completely formed and the first dorsal (D_1), which is the last to ossify, is located in a position slightly posterior to the anal fin insertion (Fig. 1h). The number of chromatophores along the midventral line varies from 30 to 33, but these may be fused into a single line in some individuals at this stage. The myomeres are still visible and vary from 33 to 35, with 16 to 17 preanal and 17 to 18 postanal. The body varies from very elongate to elongate (9.6 to 16.1%), the head is moderate (23.4 to 25.4%), and the eye is large (33.3 to 40.3%).

The juvenile period in *Atherinella brasiliensis* may begin at approximately 12 mm SL, but sizes ranged from 11.6 to 77.0 mm (n=14, mean=36.1±24.3). The anal fin is located at the median portion of the body, slightly behind the first dorsal fin. The posterior margin of the pectorals surpasses the origin of the pelvic fins, which are located at the midpoint between the insertion of the pectoral and anal fins. Scales can be seen in the dorsal-lateral region near the head in individuals above 13.00 mm SL. Lateral pigmentation is more intense

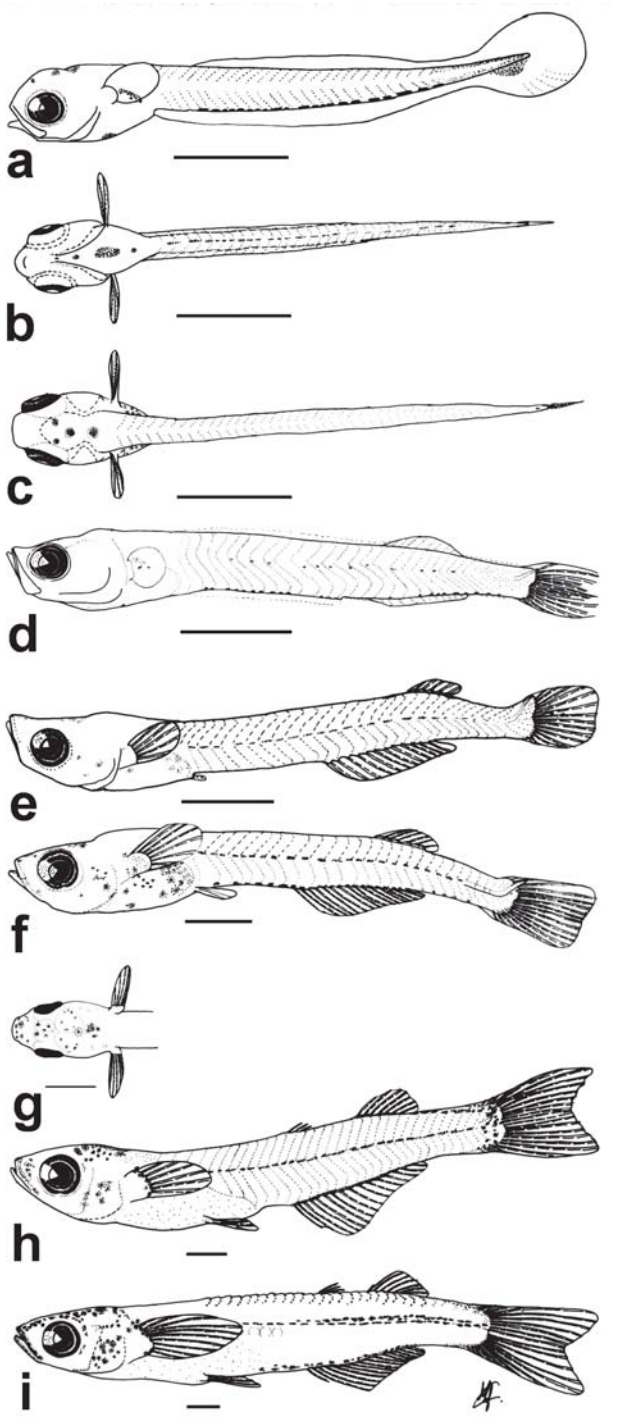

Fig. 1. Larval, transforming larval, and juvenile *Atherinella brasiliensis:* 3.5mm preflexion larva in lateral (**a**), ventral (**b**) and dorsal (**c**) views; (**d**) 4.7 mm larva at the beginning of flexion stage; (**e**) 6.4 mm at the end of flexion larva; (**f**) 8.8 mm postflexion larva; (**g**) head view of a 12 mm transforming larva; (**h**) 12 mm transforming larva; and (**i**) 15 mm juvenile. Scale bar = 1 mm.

Table 1. Morphometrics of preflexion, flexion, postflexion stages and juveniles of *Atherinella brasiliensis* (expressed as percent of SL, except for ED, expressed as percent of HL). Mean ± SD is given when sample size n>1. Dashed lines differentiate preflexion (1), flexion (2), postflexion (3) stages and juveniles (4). Abbreviations: HL = head length; ED= eye diameter; BD = body depth; PAL = preanal length; PDL = predorsal length, nv = not visible; nd = not defined.

Stage/period	SL (mm)	n	HL	ED	BD	PAL	PDL
	2.3 – 3.0	16	18.52 ± 1.89	53.02 ± 4.84	16.09 ± 0.82 (n=5)	nv	nv
1	3.2 – 3.5	16	18.15 ± 1.60	49.49 ± 3.77	15.42 ± 0.92	nv	nv
	3.6 – 3.9	13	19.87 ± 2.42	42.23 ± 5.25	15.71 ± 2.16	nv	nv
	4.0 – 4.7	17	18.25 ± 2.39	47.87 ± 5.58	14.20 ± 2.12	nv	nv
	4.4 – 4.6	2	20.01 ± 0.63	50.00 ± 7.86	nd	nd	58.89 ± 0.28
2	5.1 – 5.7	5	22.92 ± 2.29	36.27 ± 1.95	14.18 ± 4.13	54.81 ± 1.54	69.40 ± 2.33
	6.0 – 6.5	6	20.68 ± 0.60	34.92 ± 4.80	15.12 ± 1.67	54.75 ± 3.63	69.94 ± 2.35
	6.8	1	22.79	38.71	9.82	55.88	58.82
	7.4 – 8	4	24.47 ± 2.66	39.58 ± 3.58	9.64 ± 3.10	nd	nd
	8.2 – 9	5	25.30 ± 3.08	40.30 ± 7.07	11.22 ± 1.04	60.34 ± 0.86	72.22 ± 1.20
3	9.4	1	23.40	36.36	11.70	57.45	69.15
	11.2	1	25	35.71	16.06	57.14	71.43
	11.8	1	25.42	33.33	15.25	58.47	71.19
	11.6	1	24.14	35.71	17.24	55.17	68.97
	12	3	23.89	33.12	13.33	55.28	69.72
	13	1	23.08	30.00	11.54	53.85	66.92
	14.20	1	23.94	41.18	12.68	56.34	53.52
	34 – 39	2	24.59 ± 1.49	30.63 ± 0.88	16.13 ± 0.76	57.30 ± 4.91	73.54 ± 3.34
4	41 – 48	2	24.82 ± 0.26	31.49 ± 4.47	16.87 ± 0.29	58.71 ± 3.00	76.54 ± 2.67
	55	1	24.36	29.10	18.00	60.91	76.73
	65	1	23.54	29.41	18.62	61.85	76.62
	72	1	23.89	27.33	18.75	65.83	76.81
	77	1	21.69	27.54	16.49	61.04	77.53

Table 2. Meristic counts of preflexion, flexion, postflexion stages and juveniles of *Atherinella brasiliensis*. Numbers in bold indicate the SL at which a full complement of rays and total vertebrae number is first attained. Dashed lines differentiate preflexion (1), flexion (2), postflexion (3) stages and juveniles (4). Abbreviations: D = dorsal; A = anal; P_1 = pectoral; P_2 = pelvic; C = caudal, nv = not visible.

Stage/ Period	SL (mm)	Fin rays						Vertebrae		
		D		A	P_1	P_2	C	Precaudal	Caudal	Preurals+urostyle
		1st	2nd							
	4.2	nv	nv	nv	base	nv	nv	nv	nv	nv
1	4.5	nv	nv	nv	base	nv	nv	nv	nv	nv
	4.7	nv	nv	nv	base	nv	nv	nv	nv	nv
	5.3	nv	nv	nv	base	nv	base	nv	nv	nv
2	7.0	nv	7	14	6	base	3+9+8+3	**17**	**18**	**2+1**
	8.2	nv	8	15	7	4	5+9+8+5	17	18	2+1
	9.5	nv	8	17	7	4	5+9+8+5	17	18	2+1
	10	nv	**9**	19	10	**6**	7+9+8+8	17	18	2+1
	11	2	9	19	12	6	7+9+8+8	17	18	2+1
3	11.3	2	9	19	12	6	7+9+8+8	17	18	2+1
	11.5	2	9	19	12	6	**8+9+8+9**	17	18	2+1
	11.8	2	9	19	12	6	8+9+8+9	17	18	2+1
	11.6	2	9	19	12	6	8+9+8+9	17	18	2+1
4	12	**3**	9	19	**13**	6	8+9+8+9	17	18	2+1
	12.4	3	9	19	13	6	8+9+8+9	17	18	2+1
	13.5	3	9	19	13	6	8+9+8+9	17	18	2+1
	15.8	3	9	**20**	13	6	8+9+8+9	17	18	2+1

and the formation of two longitudinal rows of chromatophores can be seen. The two rows begin precisely behind the insertion of the anal fin and extend to the caudal peduncle (Fig. 1i). Midventral line chromatophores are fused into a single line.

All larval stages and juveniles exhibit 25 to 30 chromatophores along the midlateral line. The first appearance of fin rays in *Atherinella brasiliensis* follows the sequence $P_1 \Rightarrow C \Rightarrow D_2$, $A \Rightarrow P_2 \Rightarrow D_1$. A definitive number of vertebrae is attained at 7.0 mm SL, at the end of the flexion stage (Table 2). Total number of fin elements is first recorded as follows: D_2 and P_2 at 10 mm (post-flexion), C at 11.5 mm (post-flexion), D_1 and P_1 at 12 mm, and A at 15.8 mm (juvenile) (Table 2).

Discussion

Atherinidae and Atherinopsidae larvae can be identified by their long and slender body, with pigment on the dorsal midline, a wide and round head, short intestine and the ab-

sence of spines on the head (Leis & Carson-Ewart, 2000; Richards, 2006). The *Atherinella brasiliensis* larvae herein described belong to Atherinopsidae (Menidiinae), sensu Dyer & Chernoff (1996). Information on the early life history of the subfamily species has been thus far restricted to *Membras martinica, Menidia beryllina* and *M. menidia,* as presented by Lippson & Moran (1974), later compiled by Martin & Drewry (1978) and recently summarized by Vásquez-Yeomans (2006). The head pigmentation pattern may be useful for distinguishing among larvae, as *M. martinica* possess one large and a few small melanophores, whereas *M. beryllina* has three small melanophores, and *M. menidia* has four large melanophores (Lippson & Moran, 1974). The pigmentation pattern of *A. brasiliensis* differs from that of *M. menidia* by exhibiting a single row of melanophores, whereas the latter exhibits another pigmentation line in the ventral region. Separating *A. brasiliensis* larvae from other atherinopsids that occur in the Western Central North Atlantic is not an easy task. Most meristic counts overlap, especially fin rays and total number of vertebrae, (see Vásquez-Yeomans, 2006 for a summary of meristic characters), and their definitive number is only attained in transforming larvae and early juveniles.

In the Western South Atlantic, *Atherinella brasiliensis* may be found together with some other atherinopsids, different from those found in northern waters, such as *Odontesthes* and *Basillichthys* species, which can be distinguished by their greater number of pre-caudal vertebrae – 25 to 27 in the former and 30 to 34 in the latter (Dyer, 2000) – against 17 in *A. brasiliensis*. According to Carpenter (2002), Atherinopsidae larvae in the Western Atlantic exhibit similarity with those from the families Hemiramphidae and Atherinidae. This similarity can be differentiated in preflexion-stage larvae through the pigmentation of the dorsal region, as Hemiramphidae larvae have two parallel lines of pigment (Castro, 2005), whereas *A. brasiliensis* has only one line of pigment in this region. This characteristic is considered a derived condition for Atheriniformes (Dyer & Chernoff, 1996).

The proportion of body depth in relation to SL shows an increment in late larvae and early juveniles above 12.0 mm SL, which initially corresponds to 12% and extends to 17% in adult individuals. This differs from the Hemiramphidae larvae described by Castro (2005), in which the body depth/SL ratio was greater in early development phases due to the presence of the yolk-sac, which diminishes as endogenous food is consumed.

Dyer & Chernoff (1996) considered a relation of 33% between PAL and SL, as a characteristic of Atheriniformes, while external groups, such as Beloniformes, Cyprinodontiformes and Mugilidae, present values above 40% for this relation. Nevertheless, such proportions differ from those registered for Atheriniformes in this and other studies (e.g. Leis & Carson-Ewart, 2000), although values above 60% were recorded for the beloniform species they analyzed. Castro (2005) recorded a relation between 56 and 79% for the pre-anal distance in *Hyporhamphus* spp. larvae (Hemiramphidae, Beloniformes).

Lima-Dominguez & Kurtz (2000) carried out a study on the age and growth of *Atherinella brasiliensis* larvae in the state of Rio de Janeiro, southeastern Brazil, and estimated SL at hatching as 1.71 mm, with 2 mm at the end of the yolk-sac stage and 8 mm at the end of the preflexion stage. In the present study, the smallest larva captured has a SL of 1.4 mm and the preflexion stage was observed in larvae with up to 4.7 mm SL. The authors cited also estimated the instantaneous rate of larval growth, which initially varied from 1.2 mm day^{-1} to a maximum of 1.54 mm day^{-1} for four-day-old larvae. Houde (1989) pointed out that larvae that develop at low latitudes have high mortality rates, rapid growth and, consequently, a larval stage of short duration, requiring a high rate of food ingestion to maintain an accelerated metabolic rhythm. At low latitudes, where higher temperatures prevail for longer periods of time, a faster larval development is expected. An average temperature of 28.5ºC was recorded in the area where the larvae described here were collected (França, 2005).

The morphological characteristics of *Atherinella brasiliensis* described herein allow an adequate identification of its larvae as well as a differentiation from those of Hemiramphidae and other Atheriniformes.

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