

## Morphological development of *Pellona flavipinnis* post-yolk-sac larvae and juveniles (Clupeiformes: Pristigasteridae)

WILLIAM SEVERI\* & NELSY F. VERANI\*\*

\*Laboratório de Ictiologia, Departamento de Pesca e Aqüicultura, Universidade Federal Rural de Pernambuco, Rua Dom Manoel de Medeiros, Dois Irmãos, 52171-900, Recife, PE, Brazil. wseveri@ufrpe.br

\*\* Departamento de Hidrobiologia, Universidade Federal de São Carlos, Rod. Washington Luiz, km 235, 13565-905, São Carlos, SP, Brazil. dnfverani@power.ufscar.br

### Abstract

Pristigasterids are predominantly coastal marine sardines of tropical and subtropical seas, with some species being found in fresh water. Two species of the genus *Pellona*, *P. castelnaeana* Valenciennes, 1847 and *P. flavipinnis* (Valenciennes, 1837), inhabit fresh waters of the neotropical region, with the latter occurring in the Pantanal of Mato Grosso, in the Paraguay River basin of central Brazil. The development of *P. flavipinnis* larvae and early juveniles was characterized from an ontogenic series of 27 specimens (6.3–35 mm SL), based on morphological and meristic characters. The larval and juvenile development of this freshwater sardine are typical of most clupeiforms. Exact hatching size is unknown, but it is less than 6 mm standard length (SL) and the yolk is absorbed under 10 mm. Transformation to the flexion stage occurs at about 11 mm, to the postflexion stage between 16 and 19 mm, and to the juvenile stage after 26 mm. The ventral scutes begin to form between 25 and 31 mm, with the full complement of scutes (32 to 37) formed between 32 and 35 mm. Total myomere count averages 42. As the dorsal fin and origin of the anal fin and vent migrate anteriorly, the preanal myomere count ranges from 29 in the flexion larvae to 24 in early juveniles up to 35 mm. Corresponding preanal lengths range from 85 to 61%, respectively.

**Key words:** Clupeiformes, Pristigasteridae, *Pellona flavipinnis*, larvae, development, Pantanal, Neotropical

### Resumo

Pristigasteridae é uma família de sardinhas de ambientes marinhos costeiros, predominantemente de mares tropicais e subtropicais, com algumas espécies de água doce. Duas espécies do gênero *Pellona*, *P. castelnaeana* Valenciennes, 1847 e *P. flavipinnis* (Valenciennes, 1837), habitam águas doces da região neotropical, sendo que a última ocorre no Pantanal de Mato Grosso, bacia do rio Paraguai, Brasil Central. O desenvolvimento de larvas e juvenis iniciais de *P. flavipinnis* foi caracterizado a partir de uma série ontogênica de 27 exemplares (6,3–35 mm CP), com base em

caracteres morfológicos e merísticos. O desenvolvimento de larvas e juvenis desta sardinha de água doce é típico da maioria dos Clupeiformes. O tamanho exato de eclosão não é conhecido, mas é inferior a 6mm de comprimento padrão (CP), e o saco vitelino é absorvido antes de 10mm CP. A transformação para a fase de flexão ocorre com cerca de 11 mm, para a fase de pós-flexão entre 12 e 15 mm e o período de transformação juvenil após 26 mm. Os escudos ventrais começam a ser formados entre 25 e 31 mm, atingindo o número final de 32 a 37, com tamanho entre 32 e 35 mm. O número médio de miômeros totais é 42. À medida que a nadadeira dorsal e a origem da anal e ânus migram anteriormente, o número de miômeros pré-anais varia de 29, na fase de flexão, a 24 em juvenis abaixo de 35 mm. A distância pré-anal correspondente varia, respectivamente, de 85 a 61%.

## Introduction

*Pellona* is one of eight genera included in the family Pristigasteridae, which comprises a group of predominantly coastal marine fish species inhabiting tropical and subtropical seas around Central and South America (Whitehead, 1985).

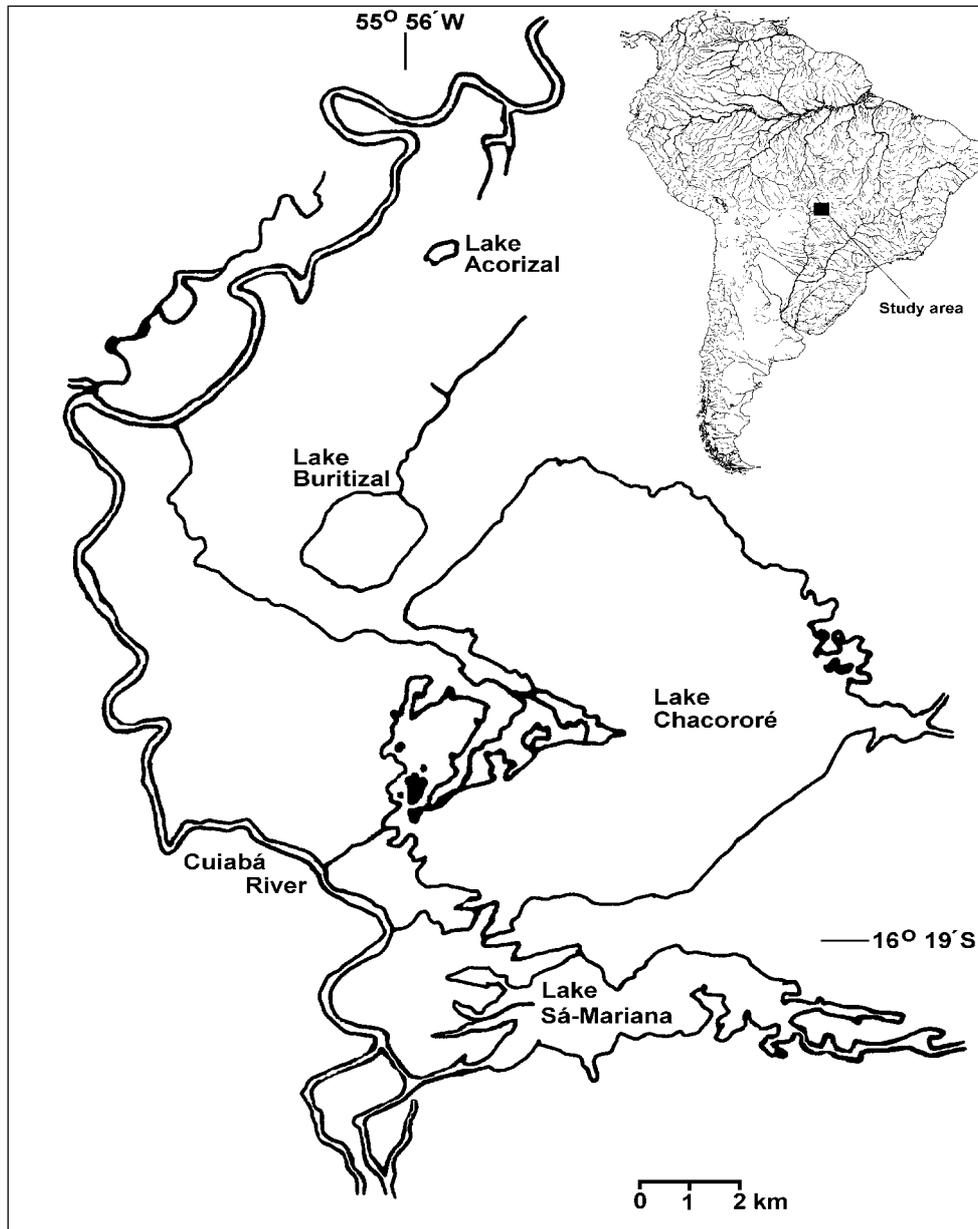
Four species of pristigasterids are known from fresh waters of South America (de Pinna & Di Dario, 2003): *Ilisha amazonica* (Miranda Ribeiro, 1920), *Pellona castelnaeana* Valenciennes, 1847, *Pellona flavipinnis* (Valenciennes, 1837), *Pristigaster cayana* Cuvier, 1829 and *Pristigaster whiteheadi* Menezes & de Pinna, 2000.

Whitehead (1985) noted the occurrence of the pristigasterids *Pellona castelnaeana* and *P. cayana* in continental South America, and suggested that the latter might actually comprise two species. This situation was addressed by Menezes & de Pinna (2000), who redescribed *P. cayana* and recognized a second species, which they described as *P. whiteheadi*. The existence of other undescribed species of *Pellona* and possibly *Ilisha* in the Neotropics has been suggested by de Pinna and Di Dario (2003).

*Pellona castelnaeana* and *P. flavipinnis*, which are sympatric in some South American drainages (e.g., Tocantins River), are superficially very similar, but show pronounced differences in number of lower gill rakers (23–31 vs. 12–14, respectively), as well as differences in number of ventral scutes ( $20-24+12-14 = 32$  to  $37$  vs.  $23-24+8-11 = 33$  or  $34$ , respectively) (Whitehead, 1967).

*Pellona flavipinnis*, known as apapá, sardinhão, or lacha, is the only recognized clupeiform species in the Pantanal of Mato Grosso (Britski *et al.*, 1999), but is generally found throughout South America, from Guyana and the Amazon River basin to Buenos Aires and Rosário, in Argentina (Fowler, 1948; Whitehead, 1970, 1973). The species is essentially a pelagic ichthyophagous fish, which attains a large size (e.g., over 1.5 kg and up to 50 cm standard length, SL), but is generally considered to be of little commercial value. Like most Clupeoidei, it is characterized by a moderately deep and compressed body, with a ventral keel of small scutes (modified scales) and a high and narrow head with a small, upturned, protractile terminal mouth, and projecting lower jaw (Santos *et al.*, 1984).

Matsuura (1974) described the larvae of two marine pristigasterids, *Pellona harroweri* (Fowler, 1917) and *Chirocentrodon bleekermanus* (Poey, 1867), but the freshwater species of *Pellona* and other clupeoids of continental South America have not been previously described. This paper partially fills that void by describing the post-yolk-sac and early juveniles of *P. flavipinnis* from the Pantanal of Mato Grosso.



**FIGURE 1.** Detailed map of the Pantanal of Barão de Melgaço, in the Cuiabá River basin, showing the lakes where *Pellona flavipinnis* early life stages were collected.

The ichthyofauna of the Pantanal of Mato Grosso comprises at least 263 species (Britski *et al.*, 1999), distributed among Myliobatiformes (3), Clupeiformes (1), Characiformes (109), Gymnotiformes (12), Siluriformes (105), Cyprinodontiformes (10), Beloniformes (2), Perciformes (18), Synbranchiformes (1), Pleuronectiformes (1) and Lepidosireniformes (1). Data on early life history of fish from the Paraguay river basin is scarce (e.g. Severi, 1997), and the description of larval stages provided in this paper for *P. flavipinnis* may be regarded as the first one for a freshwater clupeiform in the Neotropics.

### Materials and methods

Ichthyoplankton samples were taken monthly, between October 1994 and September 1995, in several biotopes in the Pantanal of Barão de Melgaço, Paraguay River basin, Mato Grosso state, central Brazil (Fig. 1). These were collected using a 0.5 mm mesh conical-cylindrical plankton net (0.39m diameter, 1.7m long) towed at the surface for 5 minutes, during daytime samplings.

The samples were fixed *in situ* in 5% buffered formalin, and sorted using a stereomicroscope for separation of eggs, larvae and juveniles. *P. flavipinnis* larval stages densities varied between 0.41 and 89.2 larvae per m<sup>-2</sup>, depending on sampling station and month of the year (Severi, 1997). Twenty-seven specimens were chosen for morphological characterization out of a total of ca. 80 clupeiforms collected. These were selected because of their better state of preservation, since most larvae were in initial developmental stages and were severely damaged or distorted. Measurements and drawings were done with aid of a micrometer eyepiece and a camera lucida. The material collected is deposited at the Fish Larvae Collection of the Laboratory of Ichthyology from the Federal Rural University of Pernambuco.

The development of *Pellona flavipinnis* larvae and early juveniles was characterized from an ontogenic series of 6.3 to 35 mm SL individuals. Their morphometry was based on measurements of standard length (SL), head length (HL), body depth (BD), pre-dorsal (PDD), preanal (PAD), pre-ventral (PVD) and pre-pectoral (PPD) distances, and eye diameter, according to Ahlstrom *et al.* (1976). Meristic characters included number of myomeres and fin rays. The larval development was divided into preflexion, flexion and postflexion larval stages, and juvenile stage, according to the degree of notochord flexion and caudal fin development (Kendall *et al.*, 1984). Corresponding numbers of 13, 4, 8 and 2 individuals were analyzed for each stage.

Body depth, head length, and preanal, pre-dorsal, pre-ventral and pre-pectoral distances of larvae and juveniles were correlated by linear regression. The relationships for body depth, head length and eye diameter were based on the categories proposed by Leis & Trnski (1989).

## Results

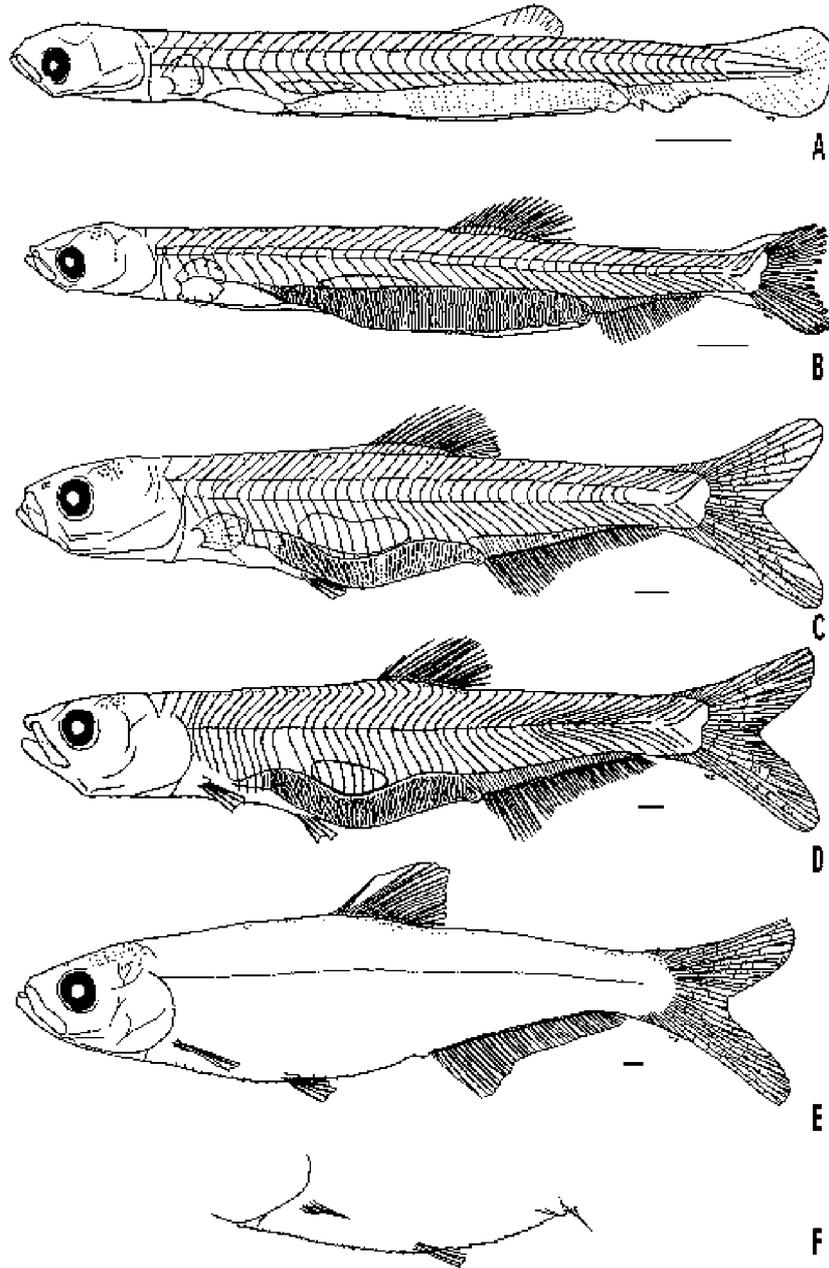
**Morphology:** Larvae 6.3 to 10.1 mm SL (Fig. 2A) were classified as preflexion; their straight notochord was easily visible when viewed through the translucent body. By 10 mm SL the outlines of the dorsal, anal, and caudal fin are well defined in the median finfold. The preanal finfold extends from the posterior portion of the yolk-sac or liver to the vent. The intestine is long, straight and vertically striated. No fin rays are evident in any fins. The swim bladder is small, slender, and located at about one-third of body length. Some chromatophores are sparsely distributed along the body. Total myomere number ranged from 39 to 43, including 28 to 30 preanal and 11 to 13 postanal. In this stage the body is elongate (10.0 to 12.5%), the head is small (11.9 to 17.5%), and the eye diameter varies from small to moderate (19.6 to 30.9%).

The flexion stage begins at about 11 mm SL. Larvae between 11.2 and 11.6 mm SL had slightly flexed notochords, indistinct pterygiophores, and no evidence of fin rays or ventral fin buds. A 15.3 mm SL larva (Fig. 2B) had well developed dorsal and caudal fins, with almost all fin elements present (15 and 19, respectively), and a developing but relatively short anal fin with most principal rays formed (35), but still no ventral fin buds. The dorsal finfold is reduced to a small strip above the caudal peduncle. The preanal finfold is also narrow, but divided beneath the swim bladder. The intestine is regressively shortening and thickening as the vent migrates anteriorly. The elongate swim bladder has moved posteriorly well behind the liver, but does not quite extend to under the origin of the dorsal fin. Pigmentation is dense along the back of the body and top of the head, but not evident above the gut or swim bladder. Myomeres are visible (39 to 44), with 28 to 29 preanal and 11 to 15 postanal. The body is elongate (11.2 to 15.5%), the head is small (13.5 to 16.5%), and the eye is small (19.2 to 31%).

The postflexion stage probably begins between 16 mm and 19 mm SL. Individuals in this stage ranged from 19.5 to 25.2mm SL. Their body is moderate (19.5 to 25.2%), the head is moderate (20.8 to 23.8%), and the eye is small (14.6 to 20%). By 22.8 mm SL (Fig. 2C) the dorsal fin has moved anteriorly to about mid-body, and the anal fin is longer than at previous stages (Fig. 2B), with a more anterior origin. The number of fin rays present in the anal (36) and pelvic (6) fins is almost complete, but not in the pectoral fins, although there is evidence of some differentiation. The intestine is shorter, and the anus is now located below the posterior margin of the dorsal fin. More concentrated pigmentation is evident on the head, along the back of the body, on the supporting elements of the dorsal and anal fin rays, and along the base of the caudal fin rays. The liver is greatly reduced. The enlarged swim bladder results in depression of the gut and deepens the body, contributing to a convex profile.

A 25.5 mm SL individual (Fig. 2D) had all fins with definite rays, and may be regarded as a larva at the end of postflexion stage. The swim bladder has assumed an oval shape and is located below the beginning of the dorsal fin. More intense pigmentation is

evident on the head. The number of myomeres varied between 41 and 43, with 25 to 27 preanal and 15 to 18 postanal.



**FIGURE 2.** Larvae of *P. flavipinnis*: A) 10.1 mm SL (preflexion); B) 15.3 mm SL (flexion); C) 22.8 mm SL (postflexion); D) 25.5 mm SL (postflexion); E) 31.7 mm SL (juvenile) and F) 35.17 mm SL (juvenile ventral profile). Each bar equals 1 mm.

The juvenile of 31.7 mm SL (Fig. 2E) has clearly formed fins, a completely scaled body, and has assumed an adult shape, but the ventral scutes do not yet reach the anus. By 35.2 mm (Fig 1F) all ventral scutes are present, with about 20 to 24 anterior to the pelvic fins and 12 to 14 from there to the vent. For the size range of juveniles analyzed, myomere number varies from 42 to 45, with 24 preanal and 18 to 21 postanal. The body is moderate (32.2 to 35%), the head is moderate (21.2 to 23.3%), and the eye is small (24.4 to 24.5%).

The number of total myomeres observed among *P. flavipinnis* larvae ranged from 39 to 45, with a mode of 42 in 40% of the fish examined. The order of fin formation is dorsal (D) + anal (A), caudal (C), ventral (P2) and pectoral (P1).

**Morphometrics:** The morphometric development of the larvae presented significant Pearson's linear correlation coefficients (r) between 0.96 and 0.99 (p<0.001), for the pre-fin distances (Fig. 3A), body height (Fig. 3B) and head length (Fig. 3C) relative to SL, as well as for the eye diameter in relation to head length (Fig. 3D). Size ranges for the different measurements at developmental stages are detailed in Table 1A.

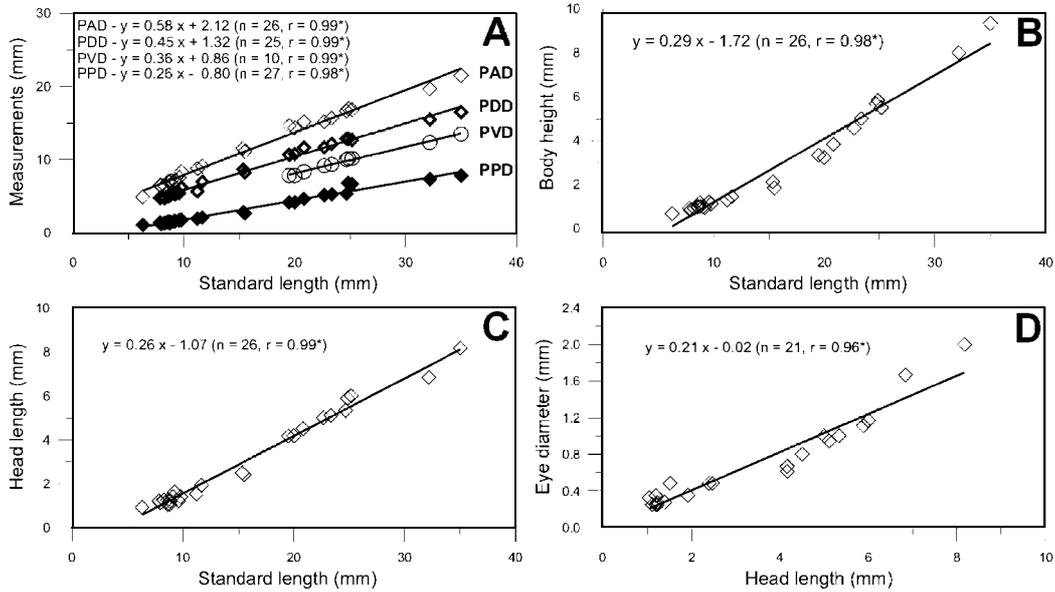
**TABLE 1.** Body measurements (A) and proportions (B) of *Pellona flavipinnis* larvae and juveniles. Body proportions are expressed as % standard length (SL), except for eye diameter (ED) in relation to head length (HL). Dashes indicate no fin development.

A

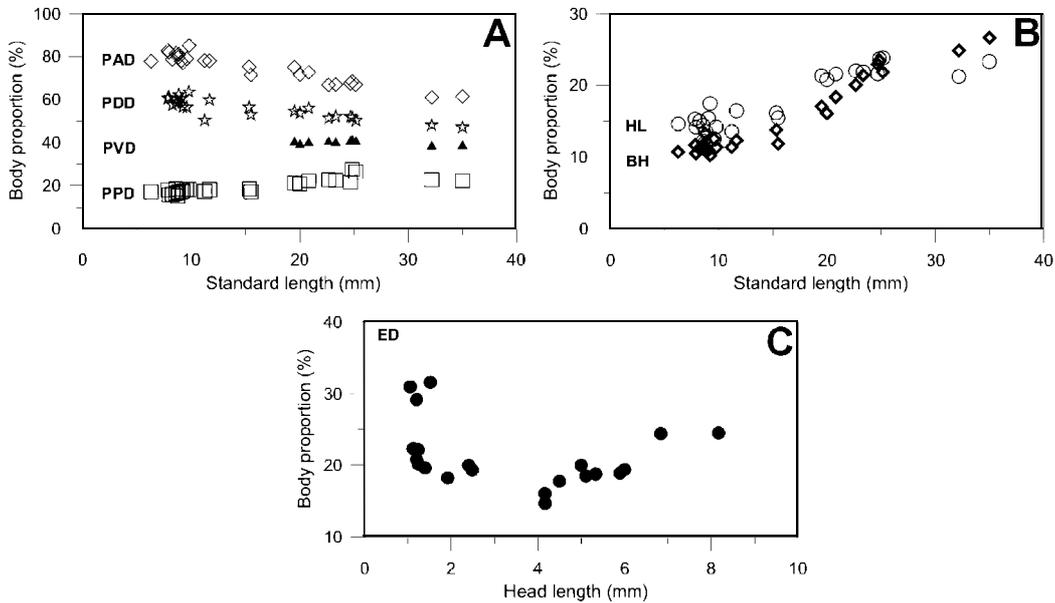
Stage	Measurements							
	SL	BH	HL	ED	PDD	PAD	PPD	PVD
preflexion	6.28–9.77	0.68–	0.92–	0.25–	4.74–	4.89–	1.08–	-
		1.16	1.39	0.35	6.22	8.33	1.78	
flexion	11.22–	1.28–	1.52–	0.35–	5.67–	8.78–	1.95–	-
	15.50	2.12	2.48	0.48	8.67	11.55	2.83	
postflexion	19.50–	3.22–	4.17–	0.61–	10.66–	14.67–	4.16–	7.83–
	25.17	5.83	6.00	1.16	12.67	16.84	6.83	10.17
juvenile	32.17–	8.00–	6.83–	1.67–	15.50–	19.67–	7.33–	12.34–
	35.00	9.34	8.17	2.00	16.50	21.50	7.83	13.50

B

Stage	Proportions							
	BH	HL	ED	PDD	PAD	PPD	PVD	
preflexion	10.2–	11.9–	19.6–	56.5–	77.1–	15.2–	-	
	12.5	17.5	30.9	63.6	85.2	18.4		
flexion	11.4–	13.5–	18.2–	50.5–	71.7–	17.2–	-	
	13.8	16.4	31.6	60.0	78.2	18.5		
postflexion	16.1–	20.8–	14.7–	50.3–	66.9–	20.8–	39.2–	
	23.5	23.8	20.0	56.0	72.8	27.5	40.9	
juvenile	24.9–	21.2–	24.4–	47.1–	61.1–	22.4–	38.3–	
	26.7	23.3	24.5	48.2	61.4	22.8	38.6	



**FIGURE 3.** Linear regressions of: A) preanal (PAD), pre-dorsal (PDD), pre-ventral (PVD) and pre-pectoral (PPD) distances; B) body height; C) head length on standard length, and D) eye diameter on head length, of *P. flavipinnis* (SL = 6.28–35.01 mm and HL = 0.92–8.17 mm).



**FIGURE 4.** Relations between: A) preanal (PAD), pre-dorsal (PDD), pre-ventral (PVD) and pre-pectoral (PPD) distances; B) head length (HL) and body height (BH) and standard length, and C) eye diameter (ED) and head length, of *P. flavipinnis* (SL = 6.28–35.01 mm and HL = 0.92–8.17 mm).

The range of body proportions for measurements at the different developmental stages is indicated in Table 1B. The pre-fin distances varied between 47 and 63% for the dorsal fin, 15 and 28% for the pectoral, 38 and 41% for the ventral, and 61 and 85% for the anal fins (Fig. 4A). A marked difference in dorsal and anal fins insertion was evident during development, with an anterior migration for both fins, while pectoral and ventral fins remained within a similar position along the body since their formation.

The length of the head varied between 12 and 24% (Fig. 4B), with values above 20% observed in larvae over 20.0 mm SL, coinciding with the development of the intestine, advance of the dorsal fin and increase of body height. The height of the body varied between 10 and 27% (Fig. 4B), with the main differentiation occurring around 20 mm SL, owing to the increase of the intestine curvature and modification of the swimbladder from an elongate to a more lobulate shape. The diameter of the eye varied between 14 and 32% of the head length (HL) (Fig. 4C).

## Discussion

Apart from their relevance for evaluating recruitment, population dynamics, trophic structure and fishery potential (Fuiman, 2002), understanding the early life history of fishes provides useful information on species life strategies, taxonomy and diversity. Studies on Neotropical freshwater clupeiform biology and ecology require urgent attention, since data on feeding, reproduction, population dynamics and environmental demands are insufficient to address several questions regarding the relative importance of most species in river, lake, and reservoir fisheries, as well as their trophic role in food webs.

Although the eggs and larvae of most clupeids that occur in waters along the U.S. Atlantic and Gulf of Mexico have been described, the larvae of many Central and South American clupeid, engraulid and pristigasterid species remain undescribed (Ditty *et al.* 2006). The paucity of data regarding larval and juvenile taxonomy stages of freshwater pristigasterids in Central and South America has also been emphasized by de Pinna & Di Dario (2003).

The main difficulty in discriminating and identifying clupeiform life stages relates to their similar external morphology and the lack of appropriate descriptions of larval development and identification keys for congeners or closely related taxa (Ditty *et al.*, 2006; Farooqi *et al.*, 2006). Such taxonomic approaches have been undertaken for some marine clupeiforms, e.g. *Sardinella* species in the Northern Gulf of Mexico (Ditty *et al.*, 1994), but none for their freshwater counterparts, at least in the Neotropics.

In spite of recent contributions on larval development of Neotropical species, mainly in the orders Characiformes, Siluriformes and Perciformes (e.g. Nakatani *et al.*, 2001), there is still little information on several other freshwater groups, including species of the orders Clupeiformes, Gymnotiformes, Beloniformes, and Pleuronectiformes.

Larvae of *Pellona flavipinnis* could be easily diagnosed among other fish larvae collected in the Pantanal wetlands for their long and slender body, and long gastrointestinal tract (McGowan & Berry, 1984).

Head length, body height, eye diameter and pre-ventral distance in *P. flavipinnis* increase more markedly with increasing body size than in its marine congener *P. harroweri* (Matsuura, 1974). The head of *P. flavipinnis* is proportionally smaller in the larval stage (less than 20% SL) than in *P. harroweri*, with a similar relationship in the juvenile stage for both species. *P. flavipinnis* juveniles present a flatter profile than *P. harroweri*, so that the range for the height/length relation in the former (10–27%) is smaller than in the latter (approximately 10 to 40%). The ventral fin insertion corresponds to approximately 40% SL in both species.

The relative position of the dorsal and anal fins insertions, as well as the length of the gut, can be used to separate larval stages of clupeids and pristigasterids from engraulids (McGowan & Berry, 1984). The range of variation for the pre-dorsal and preanal distance to standard length does not show such great differences between larval and juvenile stages in *P. flavipinnis*, as compared to *P. harroweri* Matsuura (1974). These relations indicate a more anterior insertion of the dorsal fin and a more posterior insertion of the anal fin in *P. flavipinnis*. In adults of the species, these proportions tend to vary around 51% and 64% SL, respectively, for the dorsal and anal fins (personal observation).

The most useful character for identifying clupeiform larvae is the total number of myomeres and vertebrae (McGowan & Berry, 1984). These seem to overlap in clupeiforms, as observed for cypriniforms and characiforms (Araújo-Lima & Donald, 1988). For instance, *Sardinella brasiliensis* larvae and juveniles have 46 vertebrae and 45 to 47 (usually 46 or 47) myomeres (Matsuura, 1975). For adult *P. flavipinnis*, the typical number of vertebrae (i.e., 42) (Whitehead, 1973) is similar to the average number of myomeres in larvae.

The order of fin formation in *P. flavipinnis* is similar to the usual sequence of D & A, C, P2 and P1 found in most clupeiforms (Ditty *et al.*, 2006; Farooqi *et al.*, 2006).

The positions of the dorsal and anal fins can be defined by the number of pre-dorsal and preanal myomeres. During larval metamorphosis, the position of the dorsal fin, vent, and origin of the anal fin move anteriorly in relation to both proportional length and myomere number. This migration takes place approximately at the same time as the number of anal-fin rays stabilizes. This aspect was considered by Matsuura (1975) to be important for larvae identification of this group. The origin of the anal fin in *P. flavipinnis* larvae of 11.2 (flexion stage) and 35 mm SL (early juvenile) coincides with myomeres 29 and 24 respectively, representing an advance of five myomeres. For *Sardinella brasiliensis* (Steindachner, 1879) there is a corresponding advance of four myomeres (Matsuura, 1975).

With the exception of the genus *Chirocentrus*, all Clupeoidei possess an obvious pelvic scute with ascending arms, located just in front of the pelvic fins (Whitehead 1985),

and a series of abdominal (or ventral) scutes. Their numbers vary from 1 to 30 among clupeids and pellonids, and engraulids (McGowan & Berry, 1984). In most species, these scutes continue forward to the branchial region, forming a prepelvic series (Whitehead, 1985). *Pellona flavipinnis* has 20–24 (prepelvic) + 12–14 (postpelvic) = 32–38 ventral scutes (Whitehead 1985). The scute formation in juveniles of this species probably occurs between 25 and 30 mm SL, based on the largest specimen examined without scutes and the smallest specimen with scutes. A 32 mm larva had 11 + 5 scutes and a 35 mm larva had 21 + 12 scutes.

**TABLE 2.** Meristic characters of Neotropical freshwater pristigasterids.

Species (author)	Fin elements				Vertebrae	Scutes
	Dorsal	Pectoral	Ventral	Anal		
<i>Pellona flavipinnis</i> <sup>1,3</sup>	iii–iv 14–17	i 14–15	i 6	iii–iv 35–41	42 (40–45)	32–38
<i>Pellona castelnaeana</i> <sup>1,3</sup>	iii 15	i 15	i 6	iii 31–35	45–46	33–34
<i>Pristigaster cayana</i> <sup>2</sup>	iii 12–13	i 10–12	absent	ii–iii 44–53	43(42–44)	30–35
<i>Prsitigaster whiteheadi</i> <sup>2</sup>	iii 11–13	i 10–11	i 3 i	ii–iii 41–47	43–44	29–34
<i>Ilisha amazonica</i> <sup>3,4</sup>	20	NA	6	47–52	NA	25–26

Author: <sup>1</sup>Whitehead (1973); <sup>2</sup>Menezes & de Pinna (2000); <sup>3</sup>Whitehead (1985); <sup>4</sup>McGowan & Berry (1984)

NA: information not available

For the other freshwater pristigasterids sympatric with *P. flavipinnis* in some Amazon rivers (Santos *et al.*, 1984), *P. castelnaeana* has a ventral series comprised of 33–34 scutes; *P. cayana* 30–35, and *P. whiteheadi* 29–34 (Table 2). Both *Pristigaster* species may also be distinguished from *Pellona* species by their deeper body, which is greater than over 50% standard length (Menezes & de Pinna, 2000). *Ilisha amazonica*, another pristigasterid occurring in the Amazon region, may be separated by the number of ventral scutes (25–26) and a greater number of anal-fin rays (47–52) than either *P. flavipinnis* (38–46) or *P. castelnaeana* (34–38) (Whitehead, 1985).

In *Pellona harroweri*, the definitive number of ventral scutes was observed in juveniles over 25 mm SL (Matsuura, 1974). As is true for *Sardinella brasiliensis* (Matsuura, 1975) and *Chirocentron bleekermanus* (Matsuura, 1974), formation of prepelvic scutes in *P. flavipinnis* occurs before formation of the postpelvic scutes, and is completed after formation of the fin rays.

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