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Description of the initial development and temporal distribution of Microphilypnus tapajosensis larvae and juveniles in a reservoir in the Eastern Amazon

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ABSTRACT

This study describes the initial development of *Microphilypnus tapajosensis* larvae and juveniles using meristic, morphological and morphometric data and verifies their temporal distribution in a reservoir in the Eastern Amazon, analyzing the hypothesis that this environment is ideal for the development and growth of the species. The larvae and juveniles of *M. tapajosensis* presented common characteristics of the Eleotridae group, with their standard length varying from 3.0 mm to 8.9 mm, presence of dendritic chromatophores distributed in the ventral region close to the caudal fin and 28 total myomeres. During the initial development of the larvae, the morphology is the character that best differentiates *M. tapajosensis* from its congeners, followed by the number of rays from the pectoral fins in juvenile individuals. The occurrence of specimens in larval and juvenile stages throughout the sampling period indicates that *M. tapajosensis* has uninterrupted reproductive activity, demonstrating itself as an opportunistic species. Finally, the presence of individuals throughout all months, in all larval and juvenile stages, supports the hypothesis that the reservoir has conditions for the growth and development of *M. tapajosensis*, indicating that this environment is conducive to the maintenance and species renewal.

Keywords: Ontogeny; River dammed; Fish larvae

RESUMO

Este estudo descreve o desenvolvimento inicial de larvas e juvenis de *Microphilypnus tapajosensis* por meio de dados merísticos, morfológicos e morfométricos e verifica sua distribuição temporal em um reservatório na Amazônia Oriental, analisando a hipótese de que este ambiente é ideal para o desenvolvimento e crescimento da espécie. As larvas e juvenis de *M. tapajosensis* apresentaram características comuns do grupo Eleotridae, com seu comprimento padrão variando de 3,0 mm à 8,9 mm, presença de cromatófaros dendríticos distribuídos na região ventral próximo a nadadeira caudal e 28 miômeros totais. Durante o desenvolvimento inicial das larvas a morfologia é o caractere que melhor diferencia M. tapajosensis de seus congeners, seguida pelo número de raios das nadadeiras peitorais em indivíduos juvenis. A ocorrência de espécimes em estágios larvais e juvenis ao longo de todo o período de amostragem indica que *M. tapajosensis* possui atividade reprodutiva ininterrupta, demonstrando-se como uma espécie oportunista. Por fim, a presença de indivíduos ao longo de todos os meses, em todos os estágios larvais e juvenis suporta a hipótese de que no reservatório possui condições para o crescimento e desenvolvimento de *M. tapajosensis*, indicando que este ambiente é propício para a manutenção e renovação da espécie.

Palavras-chave: Ontogenia; Rio barrado; Larvas de peixes

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1 INTRODUCTION

Studies on ecology of fish eggs and larvae have proven to be essential for the detection of periods and areas of spawning and nursery in natural environments (NAKATANI et al., 2001; ZACARDI & PONTE, 2016), which are pivotal for the implementation of management, monitoring and conservation actions for the sustainability of fishery resources (REYNALTE-TATAJE et al., 2013; BARBIERI & BONDIOLI; ZACARDI & BITTENCOURT, 2017). Nevertheless, these studies rely on previous knowledge about the identification of species (during larval phase) and their populations' characteristics, main challenges of taxonomists (BIALETZKI et al., 2008).

This limitation is due to the existence of great morphological similarity found in the early stages of development of the larvae and because they present differences in relation to adults, in addition to several species, usually of the same genus, having the habit of spawning in the same area and period, making it difficult to identification at specific levels (NAKATANI et al., 2001). The descriptions and illustrations of taxa are ways to achieve their identification, however the number of adequate descriptions of the development phases is reduced in specialized literature and generally dispersed with isolated or restricted access publications, imposing limitations on the knowledge of ecology of the ichthyoplankton (ZACARDI & BITTENCOURT, 2017).

Scientific works on freshwater fish larvae descriptions from different Brazilian drainage basins have been carried out (BIALETZKI et al., 1998, BIALETZKI et al., 2001, BIALETZKI et al., 2008, BIALETZKI et al., 2016; SANCHES et al., 1999; OLIVEIRA et al., 2012; ARAÚJO LIMA et al., 1983; ARAÚJO LIMA, 1991; SOUSA, 2002; OLIVEIRA, 2008; HONJI et al., 2012; SANTOS et al., 2017). These studies proved that morphometric analyzes combined with morphological and meristic characteristics allow the comparison of different stages of development, leading to the correct identification of species (SANCHES et al., 1999; ZACARDI & BITTENCOURT, 2017) and to the understanding of the ecological relationships of the early stages of the life cycle with the environment (OLIVEIRA et al., 2012). However, information about the early life stages of most fish in the Amazon basin is scarce and insufficient.

Microphilypnus tapajosensis Caires, 2013, commonly known as “pacamãozinho”, is a forage species, belonging to the Eleotridae family, occurring in the Tapajós River basin and it is not economically relevant for regional fishing due to its small size (about 12.5mm long). This species possibly being important in the food chain in its habitat, serving as food for piscivores fishes, thus becoming a link of energy transfer in the aquatic system. The species of this genus are commonly found associated with leaves and/or bottom of channels, lakes and Rivers in the Amazon and Orinoco basins (KULLANDER, 2003; MENEZES et al., 2003). The absence of information on the biology and ecology of *M. tapajosensis*, may be directly related to the non-use of different sampling methodology in ichthyofaunistic studies, which are essential in reservoirs (Affonso et al., 2016). As this species is small, the conventional methodologies used (eg. cast nets, seine, gillnets) are limited to capture medium and large species, restricting the capture of miniature species.

In this context, the study aimed to (i) describe the initial development of larvae and juveniles of *M. tapajosensis* captured in a reservoir in the Amazon, (ii) analyze the meristic, morphological and morphometric data of this species by comparing them with the available information to its congeners and (iii) evaluate the hypothesis that a reservoir is a favorable place for the reproduction and development of *M. tapajosensis* through abundance data and temporal variation of the initial stages of this species.

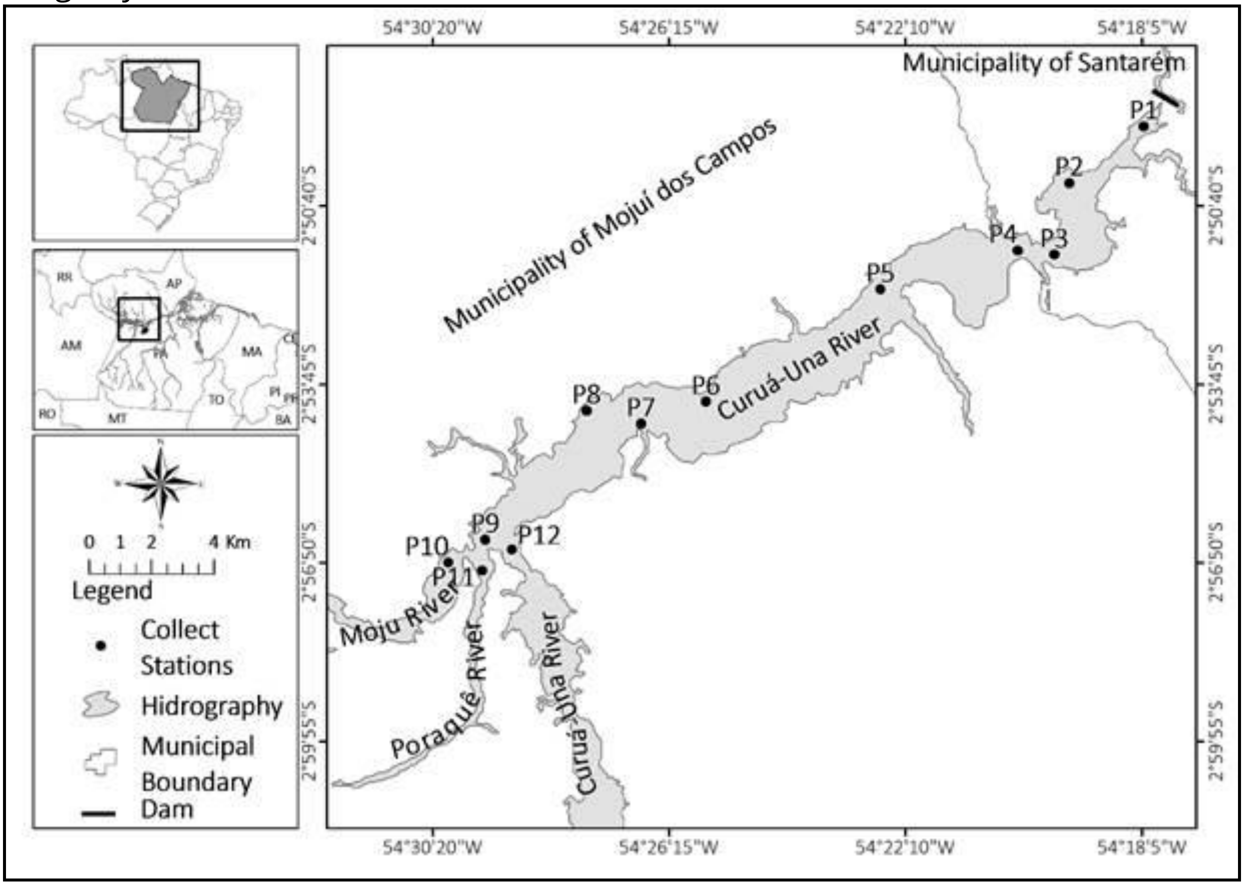
2 MATERIALS AND METHODS

2.1 Study area

The Curuá-Una River basin comprises an area of approximately 31,000 km^2 and it encompasses seven municipalities (Belterra, Uruará, Placas, Prainha, Medicilândia, Santarém and Mojuí dos Campos), where local human populations use it for fishing and electric energy generation. It is characterized by presenting clear waters, but the anthropogenic change caused by damming has marked the proximity of the reservoir with darker waters, due to the high concentration of decomposing organic matter

(JATI & SILVA, 2017). The Silvio Braga Hydroelectric Plant was the first hydropower plant to be commissioned in the Amazon and is placed at 70 km southwest of Santarém (main regional city), Pará, Brazil. Samplings were performed monthly from April 2016 to March 2017, in twelve collection stations distributed in stretches with distinct hydrologic features (lentic, semi-lotic and lotic) of the reservoir (Figure 1).

Figure 1 - Location of the study area, highlighting the collection stations in the Silvio Braga Hydroelectric Power Plant reservoir, Curuá-Una River basin, Pará



2.2 Sampling procedures

Larvae were captured on board of a local vessel by horizontal trawling (approximately 5 minutes), in open waters of the reservoir, with a conical plankton net with mouth opening of 60 cm and mesh of 300 µm, a mechanic flowmeter was coupled to the mouth to measure the volume of filtered water, in day and nighttime sampling cycles, a total of 288 samples.

After each trawling, the biologic material was submitted to euthanasia in eugenol solution according to the guidelines of the Brazilian National Council for

Animal Control and Experimentation (CONCEA, 2013). Afterwards, the material went through a process of 10% formalin fixation and, then, it was stored in 500 ml polyethylene recipients, properly labeled and transported to laboratory analysis.

2.3 Sample processing

In the laboratory, the biologic material was sorted, separating fish larvae from the suspended material, detritus and total plankton, using tweezers and Petri dishes under stereoscopic microscope. Subsequently, the *M. tapajosensis* larvae were identified, quantified and deposited in the Laboratory of Ichthyoplankton Ecology and Inland Waters Fishing, Institute of Water Sciences and Technology, Federal University of Western Pará, to constitute a reference collection.

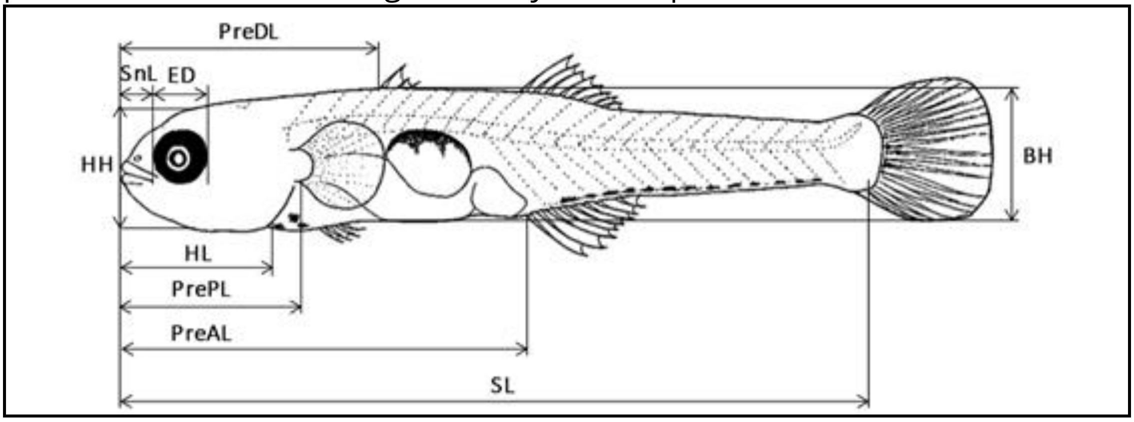
The larval identification was based on the regressive development sequence, as recommended by Ahlmstrom & Moser (1976). This technique consists of the identification regarding a sequence of individuals in different stages, since advanced stages larvae to newly hatched larva, supported by morphologic, meristic and morphometric characteristics. Among the features used for the identification, some were considered: body pigmentation pattern, body shape, fin formation sequence, relative position of the anal opening in relation to the body, shape of the digestive tract and the swim bladder, number of myomeres, rays and spines of the fins.

Subsequently, individuals were classified as to their larval and juvenile development phases, characterized by the complete formation of fin rays and the emergence of scales. There were captured 3,471 larvae and 855 juveniles of *M. tapajosensis*, from this total, 132 individuals were used for the morphological description (32 in preflexion, 36 in flexion, 30 in postflexion and 34 juveniles). The larvae were classified in three stages (preflexion, flexion and postflexion) according to the notochord flexion degree and caudal fin development and its support elements, in agreement with the terminology proposed by Ahlstrom & Moser (1976) and modified by Nakatani et al. (2001), individuals in yolk-sac stage were not captured.

Regarding the melanophores, shape (dendritic = ramified, punctiform and patch = several melanophores in shape of united points) number and position in relation to the larval body structures were considered.

Each stage description was based on the development degree and in the occurrence of the main morphological events, being illustrated those individuals which best represented these characteristics. For the initial development characterization, a stereoscopic microscope equipped with a linear scale micrometer ruler in the eyepiece reticle was used to obtain the following morphometric variables (mm): standard length (SL); snout length (SnL); eye diameter (ED); head length (HL); head height (HH); body height (BH); prepectoral length (PrePL); predorsal length (PreDL); preanal length (PreAL) (Figure 2).

Figure 2 - Ilustration of *M. tapajosensis* larva (Gobiiformes, Eleotridae) in the final phase of flexion, indicating the analyzed morphometric variables.



Meristic data were based on the counting, when possible, of the number of totals pre and postanal myomeres and the rays of the pectoral (P), pelvic (V), dorsal (D) and anal (A) fins. The biometric characteristics were determined according to the categories proposed by Leis & Trnski (1989) that related BH to SL, HL to SL and ED to HL.

2.4 Data analysis

To identify possible ontogenic variations during the larval periods, the analysis of body relationships and morphometric variables were related to the standard and head length. To verify the type of relationship between these variables, a calculation was performed using a potential equation, with untransformed data: $Y= ax^b$, where y = dependent variable; x = independent variable; a = intercept and b = growth coefficient (GALUCH et al., 2003). When b = 1, growth is isometric, when growth is positive, b> 1 and negative b <1. Regression analyzes were performed to verify the relationship of the independent variables analyzed (ED, SnL, HH, BH, PrePL, PreDL and PreAL) with the standard and head length (dependent) using the STATISTICA 7.0 software. The level of significance adopted was p <0.05.

The temporal distribution of M. tapajosensis was analyzed using the frequency of occurrence of the abundance of developmental stages calculated using the formula Fo = (Ta x 100) / TA, where: Ta is the number of samples in which the rate occurred and TA is the total number of samples. Then, the frequencies of occurrence of each stage of initial development (preflexion, flexion and post-flexion and juvenile) (dependent variables) were plotted against the months of sampling (independent variable).

3 RESULTS AND DISCUSSION

3.1 Initial development and body relations

3.1.1 Preflexion stage:

The smallest analyzed specimens were at the with SL varying from 2.3 to 3.8 mm (mean= 1.3 mm ± 0.45) (Figure 3A). The body is long and compressed, the notochord is visible due to transparency, not flexed. The head is small in relation to the SL (16.66%). At this stage, individuals are coated with hyaline and pigment less fin fold (embryonic membrane), which starts at the dorsal region behind the head, going through the caudal fin and stretching until the anus. The outline of the dorsal and anal fins is noticeable.

The yolk-sac is absorbed (absent) with the anal opening in the anterior region of the body, the intestine is functional and deviated only in the middle section, where it surrounds the inferior portion of the swim bladder, this, is found visible, inflated and transparent with its superior region pigmented. The pectoral fin buds are formed, however, without rays.

These characteristics are common to the other species of Eleotridae, such as those found in larvae of *Hypseleotris galii* and *Mogurnda adspersa* by Konagai & Rimmer (1985) and Close et al. (2005), and of another species of *Microphilypnus*.

The larvae in preflexion present subterminal mouth being modified according to its development, with large, spherical and well pigmented eyes, encompassing approximately 35.29% of the HL. Dendritic chromatophores are arranged linearly in the ventral and inferior regions of the head. The big eyes are a characteristic of *M. tapajosensis* larvae which differ from the *M. macrostoma* larvae and may be used as one of the morphological variables to distinguish both species, as well as the rounded snout, once *M. macrostoma* does not present such characteristic. However, the latter does not occur in the Curuá-Una River basin and is registered only in the Central Amazon.

3.1.2 Flexion stage:

The individuals present SL ranging from 4.0 to 4.9 mm (mean= 4.7 mm ± 0.25) (Figure 3B). The final section of the notochord is already flexed, and it is possible to observe the emergence of the hypural bones coupled with the first rays of the caudal fin. The head remain small in relation to the SL. The larvae no longer present the embryonic membrane and the second dorsal, anal (triangular) and caudal fins are in formation.

The anal fin starts its formation in the vertical direction of the base of the second dorsal fin's second ray. The eyes remain big amounting 39.28% of the HL. Pigmentation follows the same pattern found in the previous stage, but with increment and intensification of the chromatophores number in the inferior line of the ventral region, head and in the anal fin base. Myomeres with 28 evident segments (10 to 11 preanal and 18 to 17 postanal). This meristic data distinguishes the larvae of *M. tapajosensis* from *M. macrostoma*, which have a total of 27 myomeres, with only 14 postanal segments (Tab. II). Nevertheless, this feature must be associated to other primary features, such as pigmentation, size and stages of development, as overlap of the myomeres number may occur between similar species (BIALETZKI et al., 1998).

3.1.3 Postflexion stage:

The larvae vary between 5.3 and 7.0 mm of SL (mean= 6.1 mm ± 0.57) (Figure 3C) and presented ossification of the first dorsal fin rays (with 3 visible spines), and emergence of the pelvic and pectoral fins rays, the last is short and does not reach the half of the swim bladder. At this stage, the head (23.36% in relation to the SL) and the eyes (32.55% of the HL) are considered moderate (Table I).

The anal (8 rays), second dorsal (9 rays) and caudal (15 rays, short and convex) fins have their total number of rays formed and evident. The swim bladder and the notochord are still visible due to transparency, absent scales and the pigmentation is like the previous stage.

3.1.4 Juvenile:

Presented SL ranging from 7.3 to 8.3 mm (mean= 7.9 ± 0.27) (Figure 3D). The head is moderate in relation to the SL (29%). At this phase, they exhibit complete formation of the total number of fin rays: pectoral (9 rays), pelvic (4 rays), first dorsal (6 spines), second dorsal (9 rays) and anal (8 rays) (Table I). The pectoral fins are short, slender and diamond-shaped (reaching the median region of the swim bladder and covering almost all the digestive tract region).

The number of pectoral fin rays, in most developed specimens, may be a meristic characteristic used to differentiate the larvae of *M. tapajosensis* from *M. acangaquara*, that present a larger number of rays (13 to 14) and are registered in the region, being common in the Tapajós River basin (Tab. II).

The individuals present similar body shape in relation to the adults, the intestine reaches the median body region, the mouth is subterminal oblique, with a prognathic maxilla fused medially in the mandibular symphysis, narrow interorbital width, double nostrils and presence of scales. The eyes are moderate and encompass 26.31% of the HL.

The linear pigmentation pattern remains in the inferior line of the ventral region, but punctiform chromatophores emerge, irregularly distributed in the head. Moreover, the total number of myomeres remains with 28 segments (12 to 13 preanal and 15 to 16 postanal). No changes were observed in BH in relation to SL at any of the initial development stages.

Adult individuals do not present visible swim bladder and notochord because they are covered by the epidermis. Pores are absent in the head. They exhibit dark brown chromatophores pigmentation far more intense than previous stages and irregularly distributed through the body (scales), head and mouth. Punctiform chromatophores between the pelvic and caudal fins rays form small patches, in addition to the presence of pigmented bands in the dorsal and anal fins. The head profile is little depressed, nostrils are double, and scales are big and ctenoid (Figure 3E), features already described by Caires (2013).

Figure 3 - Initial development of *Microphilypnus tapajosensis* captured in the Silvio Braga Hydroelectric Power Plant reservoir, Curuá-Una River basin, Pará: (A) larvae in preflexion (3.8 mm); (B) flexion (4.8 mm); (C) postflexion (6.8 mm); (D) juvenile (8.3 mm) e (E) adult (12.5 mm) (scale = 1 mm)

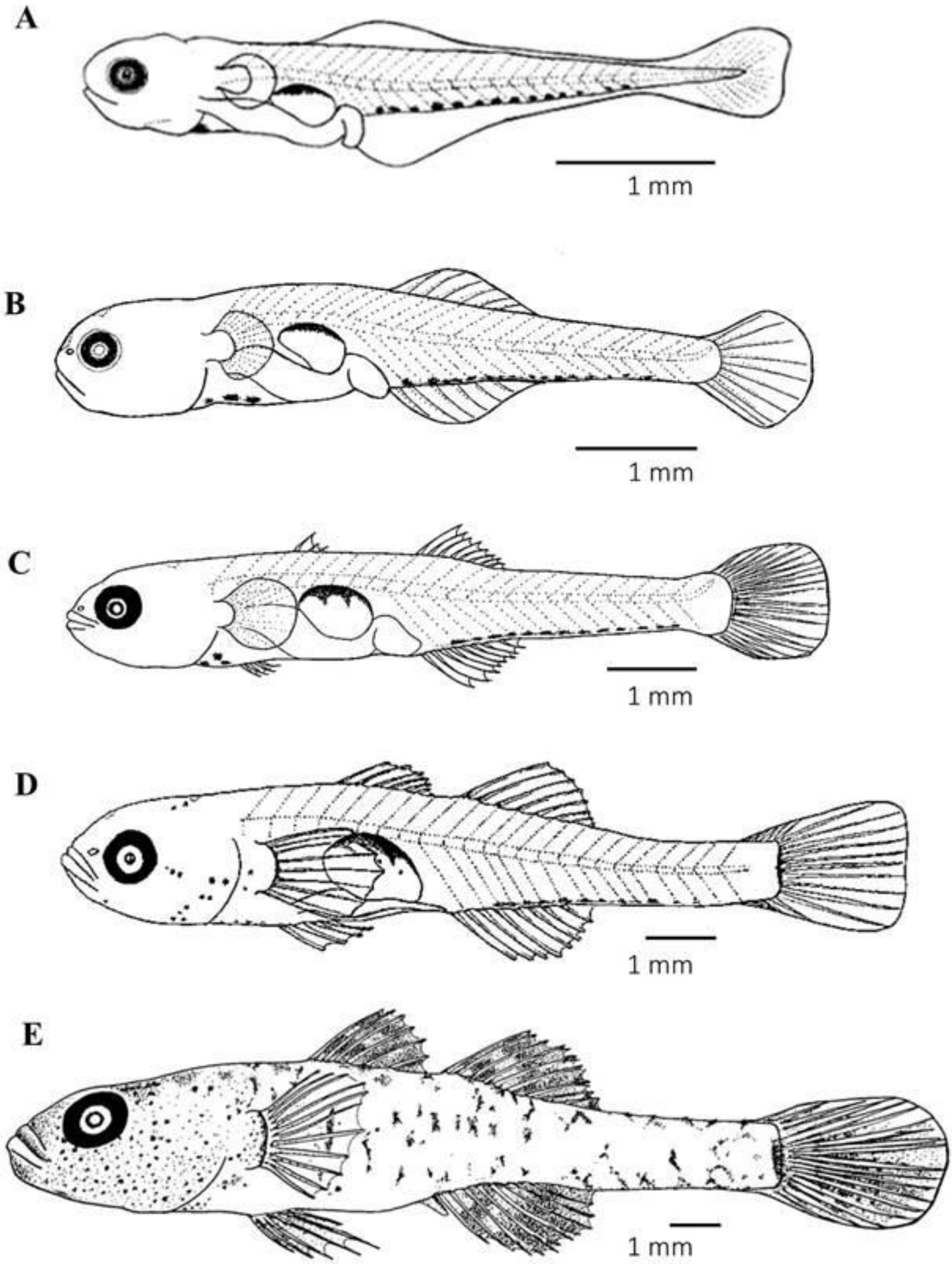


Table I - Minimum (Min.) and maximum (Max.) values, mean (X) and standard deviation (SD) for the morphometric and meristic variables in Microphilypnus *tapajosensis* larvae and juveniles. AF= absent fin; DV = difficult viewing; n = number of analyzed individuals; SL = standard length; HL = head length; SnL= snout length; ED = eye diameter; HH = head height; BH = body height; PreDL = predorsal length; PreAL = preanal length; PrePL = prepectoral length; PR = pectoral fin ray; VR = pelvic fin rays; FDR = first dorsal fin spines; SDR = second dorsal fin rays; AR = anal fin rays and CR = caudal fin rays.

	Development stages							
	Preflexion (n= 32)		**Flexion (n= 36)**		**Postflexion (n= 30)**		**Juvenile (n= 34)**	
Variables	**Min-Max**	**X±SD**	**Min-Max**	**X±SD**	**Min-Max**	**X±SD**	**Min-Max**	**X±SD**
SL	2.30-4.00	3.10±0.45	4.00-4.90	4.7±0.2	5.00-7.00	6.15±0.57	7.00-8.00	7.80±0.28
HL	0.40-0.60	0.50±0.06	0.10-1.00	0.8±0.20	1.10-1.90	1.45±0.16	2.00-2.50	2.25±0.14
SnL	0.10-0.20	0.10±0.01	0.20-0.30	0.2±0.04	0.30-0.50	0.40±0.04	0.50-0.50	0.50±0.00
ED	0.10-0.30	0.20±0.04	0.30-0.30	0.3±1.13	0.40-0.50	0.50±0.05	0.60-0.60	0.60±0.00
HH	0.40-0.60	0.50±0.04	0.60-0.80	0.7±0.07	0.80-1.10	1.0±0.07	1.40-1.50	1.40±0.05
BH	0.0-0.60	0.50±0.06	0.50-0.80	0.6±0.08	0.80-1.10	1.00±0.08	1.30-1.50	1.40±0.06
PreDL	AF	AF	AF	AF	2.30-2.30	1.70±0.00	2.80-3.40	3.00±0.12
PreAL	AF	AF	3-3	0.00±3.00	3.10-4.30	3.60±0.33	4.00-4.80	4.45±0.18
PrePL	DV	DV	DV	DV	1.40-2.00	1.70±0.18	2.00-2.70	2.40±0.16
	Number of myomeres							
Preanal	10-11		10-11		12-13		12-13	
Postanal	18-17		18-17		15-16		15-16	
Total	28		28		28		28	
	Number of spines/rays							
Pectoral	AF		AF		DV		9	
Pelvic	AF		AF		4		4	
First dorsal	AF		AF		III		VI	
Second dorsal	AF		7		8		8	
Anal	AF		6		8		8	

Table II - Comparative data of the meristic values found in the literature among similar species of *Microphilypnus*

Meristic data	**Species of the genus *Microphilypnus***				
	M. tapajosensis	***M. mascrostoma***	***M. acanguaquara***	***M. ternetzi***	***M. hypolyrasimeion***
Myomeres	26-27	26	26-27	27-29	26
Number of spines/rays					
First dorsal	VI	VI	VI	VI	VI
Second dorsal	8	8	8	8	8
Anal	8	8	8	78	8
Pectoral	13	13	14	13	13
Pelvic	-	-	-	-	-

*The meristic data used for comparison with *Microphilypnus tapajosensis* were taken from Caires (2013); Caires & Figueiredo (2011) and Caires & Toledo-Piza (2018).

3.2 Morphometric relationships

Linear regressions performed between SnL, ED and HH in relation to the HL (Figure 4) and BH, the HL and PrePL in relation to the SL suggest negative allometric growth between them (b<1) and demonstrates a proportionately lower growth of these variables in relation to HL and SL (Figure 5).

Figure 4 - Morphometric relations from *M. tapajosensis* larvae as a function of head length (HL)

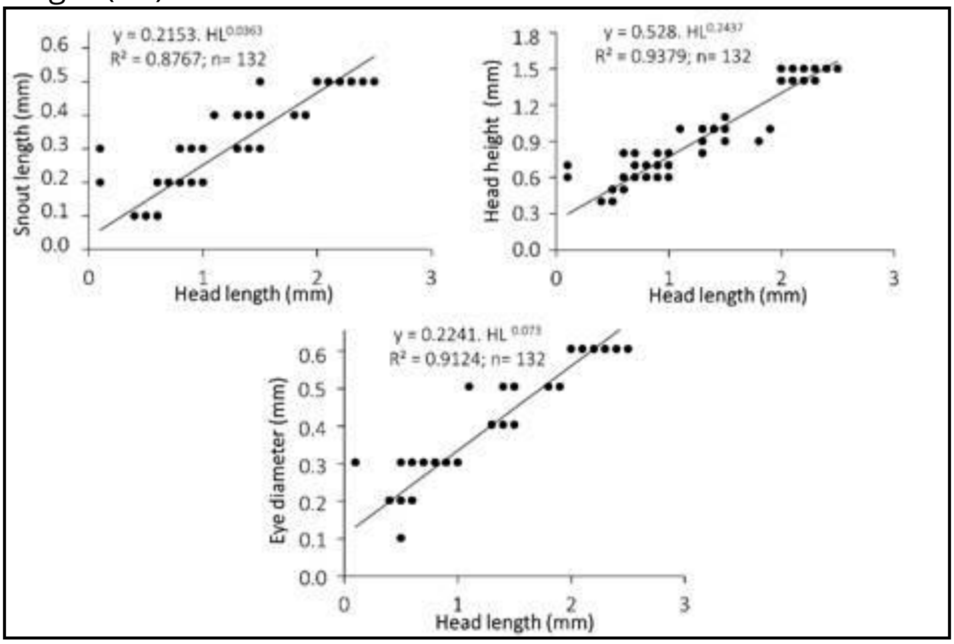
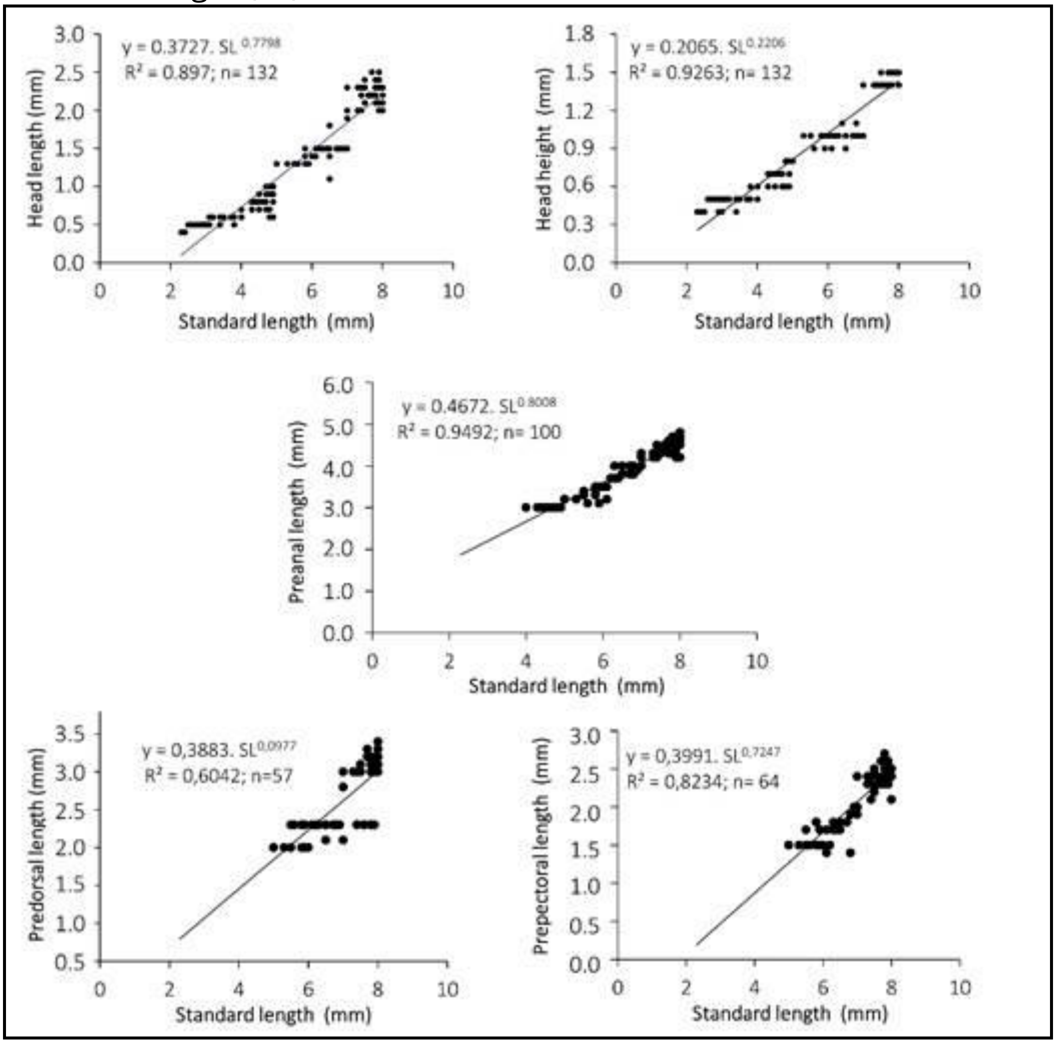


Figure 5 - Morphometric relations from *M. tapajosensis* larvae as a function of standard length (SL)



These results are similar to the observed by Bialetzki et al. (1998), Oliveira et al. (2012), Zacardi et al. (2014), for various species of Brazilian fish, but it differs from other studies such as Sanches et al. (2001) and Galuch et al. (2003). The found growth pattern may be related to a higher morphophysiological demand, regarding competition and food resource capture, hence, accelerating body length to the detriment of other structures. Nonetheless, it is possible that this result may have suffered interference from the low number of analyzed individuals, once most structures emerge only during the most advanced larval stages.

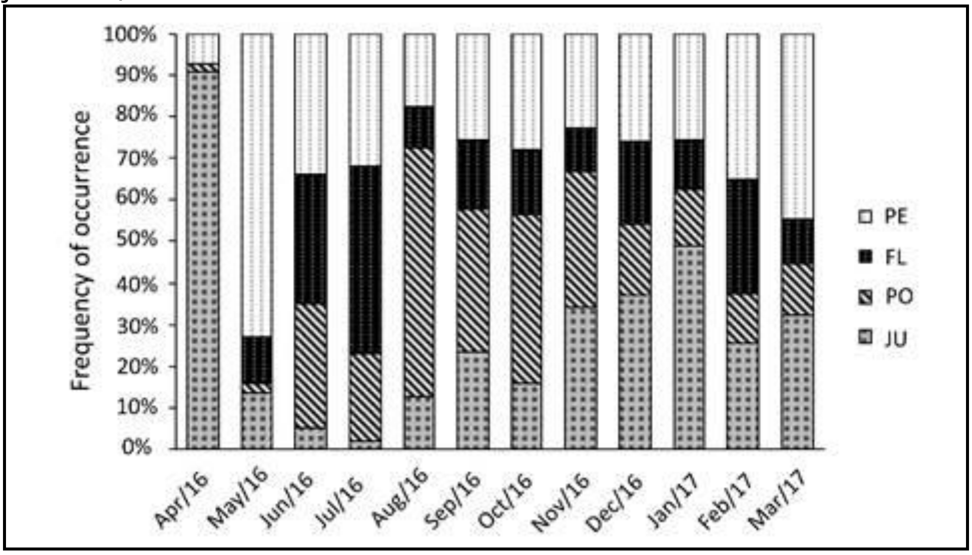
Ontogeny is not just a sequence of stages, it is a continuous process with temporal accelerations (KAMLER, 2002), this ontogenetic variation is instigated by larval size, resource use ability and the capacity of interaction with other individuals

(WERNER & GILLIAM, 1984). It is important to highlight that alterations in larval morphological features, normally end at the final larval period (FOSTNER et al., 1983).

3.3 Temporal distribution

The presence of *M. tapajosensis* larvae in diverse development phases, in all months throughout the year, suggests an extensive reproductive period and indicates the uninterrupted spawning strategy type used by this species. Furthermore, it shows the relevance of the reservoir of Curuá-Una Hydropower Plant as a breeding, development and growth area (Figure 6).

Figure 6 - Monthly frequency of occurrence by stage of *M. tapajosensis* in the reservoir of the Silvio Braga Hydroelectric Power Plant, in the Curuá-Una River basin, Pará, between April 2016 and March 2017. (PF= preflexion, FL= flexion, PO= postflexion, JU= juvenile)



The capture absence of individuals in the larval yolk-sac stage is justified by the fact that it occurs shortly after larvae hatching, succeeding the spawning period, and being considered by Ré (1984) the shortest stage of the larval period, in tropical fish, this process only lasts a few hours, depending on the species.

However, the more advanced stages register, such as postflexion and juveniles suggests that, at least, part of the larvae can develop in this environment, due to its semi-lotic and lentic characteristics, being used as reproduction sites, larval drift and nursery areas. Thus, *M. tapajosensis* is a small-sized species, sedentary, resident and with high productive potential in the reservoir area.

Commonly, reservoirs' ichthyofauna is mainly composed of opportunistic, sedentary, small- and medium-sized and of low or noneconomic value species (HOEINHAUS et al., 2009; AGOSTINHO et al., 2016).

According to Arrington & Winemiller (2006), habitat occupation patterns by some fish species or, yet, a preference for a specific aquatic environment may be related to the site's own characteristics, such as ideal conditions to species reproduction and to entirely complete their initial development. This is reflected in the adequacy of an area for offspring survival in terms of food resources availability and refuge against predation.

4 CONCLUSIONS

The analyzed larvae of *M. tapajosensis* showed common characteristics from the group Eleotridae. That allow an adequate identification of the genus and expanding the knowledge on this species' larval biology. The larval development supports a better comprehension of the initial phase of fish life cycle, as well as increasing the understanding of their behavior in the environment and contributing for the creation of environmental management plans to the protection of species and sustainable exploitation of fishery resources.

As the larvae of *Microphilypnus* are morphologically similar among them and have some meristic data overlaps, it can be difficult to correctly distinguish species. However, *M. tapajosensis* larvae differ from *M. macrostoma* by presenting big eyes and rounded snout, and from *M. acangaquara* by the higher number of pectoral fin rays (13-14). The corporal relations indicated an allometric growth between them ($b<1$) during their development, i.e. there was a proportionately higher increment of these variables in relation to SL and HL.

The study also broadens the species' area of occurrence, which was limited only to the Tapajós River basin and now is also registered in the Curuá-Una River, part of the interbasin of Tapajós-Xingu River, Amazon basin.

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