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## Morphological development of larvae and juveniles of *Prochilodus argenteus*

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ABSTRACT: Prochilodus argenteus is an endemic fish species from the São Francisco River basin that is of high economic and environmental importance. The present study aimed to contribute with information to the taxonomic identification of larvae and juveniles of this species. Larvae, obtained from induced spawning of wild animals, were reared in ponds. Individuals were collected daily and classified into larval stages or juvenile phase. Morphological descriptions and morphometric measurements were performed, together with a piece wise linear regression analysis of the body proportions throughout the development process. Individuals in the preflexion stage had a standard length (SL) of 4.48 to 6.64mm, long to moderate body (BH/SL), small to moderate head (HL/SL), and a small to moderate eye (ED/HL). In the flexion stage, the SL varied from 6.60 to 11.00mm, long to moderate body, moderate head, and small to moderate eye. Larvae in the postflexion stage presented SL of 10.54-19.93mm, moderate body, moderate to big head and small eye. The juvenile phase included specimens with a SL of 18.27 to 42.21mm which presented a moderate to high body, big head and small to moderate eye. Regression analysis showed significant moments of change in rate of increase of the body proportions, presenting a change in the growth pattern from allometry to isometry during the early development.

Key words: curimatã, early development, morphometry, São Francisco River:

### Desenvolvimento morfológico de larvas e jovens de Prochilodus argenteus

RESUMO: Prochilodus argenteus é uma espécie de peixe endêmica da bacia do Rio São Francisco, com importância econômica e ecológica para a região. O objetivo deste trabalho foi contribuir com informações para a identificação taxonômica de larvas e jovens da espécie. As larvas foram obtidas através de reprodução induzida de animais selvagens e cultivadas em viveiros. Os peixes foram coletados diariamente e classificados nos estágios larvais e fase juvenil. Foi realizada a descrição morfológica e medidas morfométricas, além de uma análise de regressão linear segmentada das proporções corporais ao longo do desenvolvimento. O estágio de pré-flexão apresentou indivíduos com comprimento padrão (CP) de 4,48 a 6,64mm, corpo longo a moderado (AC/CP), cabeça pequena a moderada (CC/CP) e olho pequeno a moderado (DO/CC). Em flexão, o CP variou de 6,60 a 11,00mm com corpo longo a moderado, cabeça moderada e olho pequeno a moderado. No estágio de pós-flexão, as larvas apresentaram CP de 10,54 a 19,93mm, corpo moderado, cabeça moderada a grande e olho pequeno. A fase juvenil compreendeu indivíduos de 18,27 a 42,21mm CP, corpo moderado a alto, cabeça grande e olho pequeno a moderado. As análises de regressão evidenciaram os momentos significativos de mudança no ritmo de incremento das proporções corporais, demonstrando uma mudança no padrão de crescimento de alométrico para isométrico durante o desenvolvimento inicial. Palavras-chave: curimatã, desenvolvimento inicial, morfometria, rio São Francisco.

## INTRODUCTION

Fishes of *Prochilodus* genus, popularly known as curimatã, have a migratory behavior, are widely distributed in South American rivers, and represent an important fishing resource. This genus includes thirteen species of which ten are present in the Brazilian basins. For instance, the São Francisco River Basin is the natural habitat of the species *P. argenteus*, *P. costatus*, *P. vimboides*, and *P. brevis* (CASTRO & VARI, 2003). Although *Prochilodus argenteus* is considered an endemic species of the São Francisco River Basin it has been taken to other Brazilian basins (Northeast) through governmental fish stocking programs (CASTRO & VARI, 2004). In the Lower São

Francisco River region, this species has a substantial commercial value due to its participation in artisanal fisheries (SOARES et al., 2011) and subsistence aquaculture (RIBEIRO-NETO et al., 2016). On the face of its economic and ecological importance, the species was included in the recovery program of São Francisco River fish fauna implemented by government institutions. The identification process of *P. argenteus* in the ichthyoplankton fauna can reveal important information about breeding areas and feeding and growth routines essential for the management and conservation of species. In the case of the genus *Prochilodus*, it is relatively easy to find information on its larval development in published literature, from which were reported information about eight among

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the thirteen recorded species (CAVICCHIOLI & LEONHARDT, 1993; LEÓN et al., 1993; NAKATANI et al., 2001; ARIAS-GALLO et al., 2010; ABDO et al., 2015; SOUZA et al., 2015). However, the identification of *Prochilodus larvae* and a proper separation of their species are hampered by overlapping parameters (morphometric and meristic) due to the evolutionary proximity between species of this genus (CASTRO & VARI, 2004). Thus, the present study aimed to provide information for the taxonomic identification of larvae and juveniles of *P. argenteus* through the morphological and morphometric characterization of their early development in captivity. This could also enable differentiation from their congeners of the São Francisco River Basin.

#### MATERIALS AND METHODS

This study was conducted using individuals of *P. argenteus* that hatched in the laboratory by means of hormonal induction of wild broodstock from the Lower São Francisco river (Porto Real do Colégio-AL, 10°12′1.93"S and 036°47′30.32"W) and were kept in ponds in the Center of Reference for Aquaculture and Fisheries Resources of São Francisco (CERAQUA/ CODEVASF). Two days post-hatch (DPH) larvae, already presenting open mouths, were transferred from the nursery to a grow-out pond (2000m²) and were retained there for 44 days. Individuals were captured daily and fixed in 4% neutralized formalin. Each individual was morphologically examined and photographed with a digital camera (5.1 MP) attached to a light microscope. The Image J software was used for image capturing and morphological measuring (0.001mm accuracy) and the results were presented using an accuracy of 0.01mm. Fish were individually weighed on an analytical balance with an accuracy of 0.0001g.

Individuals examined were classified into larval stages of development as preflexion, flexion, and postflexion, and juvenile phase according to the flexion degree of the notochord and caudal fin development (KENDALL et al., 1984). In addition, the standard length (SL), body height (BH), head length (HL), predorsal (PDD), pre-pectoral (PPD), pre-pelvic (PPLD), and pre-anal (PAD) fin distances, as well as the eye diameter (ED) and upper maxillary length (UML), were measured.

Calculations of BH/SL, HL/SL, PDD/SL, PPD/SL, PPD/SL, PAD/SL, ED/HL, and UML/HL were performed. The ratio BH/SL, HL/SL, and ED/HL were respectively used to classify the body, head and eye sizes of the individuals according to LEIS & TRNSKI (1989). The body proportions

measurements were correlated with SL and HL and analyzed through the piecewise linear regression model  $Y=\beta 0+\beta 1X+\beta 2(X-bp)(X>bp)+\varepsilon$  in order to calculate the models break-points (bp) and estimate the shift instants  $(X_{bp})$  in the ratio increment on the morphometric measurements (body proportions) related to the initial development of the fish.

#### **RESULTS**

A total of 233 individuals were analyzed in this study, of which 46 were larvae in the preflexion stage, 31 in the flexion stage, 102 in the postflexion stage, and 49 in the juvenile phase. The mean water temperature was 29.3°C during the nursery period and 30.8°C during the grow-out period. The means and amplitudes of morphometric and meristic variables of each stage of development are presented in table 1.

Preflexion stage larvae (Figures 1 A and B) were between 2 and 5 DPH, with SL from 4.48 to 6.64mm and a weight of 0.0009-0.0028g. Their body was long to moderate (12.99-20.75% SL), with small to moderate head (19.51-27.61% SL) and small to moderate fully pigmented eye (21.82-29.07% HL). They presented open mouth in a terminal position and presence of exogenous food in the stomach and in the intestine. In this period, the embryonic finfold involves the body of the larva from the dorsal to the ventral region. Caudal, dorsal, anal, and pelvic fins are not visible. The gas bladder is pigmented and clearly visible in the upper portion of the abdominal cavity. At the beginning of the preflexion stage, dendritic chromatophores are present in the ventral region, from the posterior region of the head to the anus and in the posterior lateral and superior region of the head. During the development the pigmentation intensifies on the dorsal area and, later, on the body sides. At the end of the preflexion stage, chromatophores emerge in the area underneath the terminal portion of the notochord.

Flexion stage larvae (Figures 1 C and D) were between 5 and 6 DPH, with SL varying from 6.60 to 11.00mm and weight varying from 0.0025 to 0.0225g. Their body varied from long to moderate (16.68-25.71% SL), the head was moderate (24.75-32.03% SL) and the eye was small to moderate (20.63-28.51% HL). At this stage, the initial formation of rays (pterygiophores) occurs in the order of caudal, dorsal, and anal fins. The embryonic finfold is still present but it is shorter than in the preflexion stage and mainly restricted to the ventral region. Dendritic and punctate pigmented chromatophores are present on the body sides, including the lips. At the end of this stage,

Table 1 - Morphological variables of *Prochilodus argenteus* (Spix & Agassiz, 1829) at larval stages and initial juveniles reared in ponds from the  $2^{nd}$  to the  $44^{th}$  days post-hatch (n, number of individuals;  $\bar{X}$ , mean; SD, standard deviation; CF, caudal fin; DF, dorsal fin; AF, anal fin; PLF, pelvic fin and PF, pectoral fin).

Stage Phase	Larvae							Juvenile (n=49)		
	Preflexion (n=46)		Flexion (n=31)		Postflexion (n=102)		Juvenne (11–49)			
Measure (mm)	$\bar{X} \pm SD$	Range	$\bar{X} \pm SD$	Range	$\bar{X} \pm SD$	Range	$\bar{X} \pm SD$	Range		
SL	5.57±0.49	4.84-6.64	8.55±1.19	6.60-11.00	13.95±2.00	10.54-19.93	25.19±5.27	18.27-42.21		
HL	$1.31\pm0.20$	1.06-1.77	$2.38\pm0.43$	1.63-3.22	$5.13\pm1.00$	3.37-7.99	9.90±1.93	7.10-15.70		
BH	0.95±0.15	0.67-1.29	1.80±0.42	1.20-2.74	4.41±1.11	2.80-7.57	9.87±2.36	6.60-17.53		
PPD	$1.30\pm0.19$	1.06-1.76	$2.32\pm0.39$	1.65-3.12	$4.73\pm0.80$	3.18-6.76	8.39±1.66	6.17-13.44		
PDD	-	-	4.06±0.45	3.28-4.86	6.80±1.12	5.00-9.86	12.92±2.65	9.12-20.59		
PAD	-	-	6.38±0.99	4.71-8.53	11.03±1.69	8.42-15.94	20.74±4.42	14.78-35.06		
PPLD	-	-	-	-	7.73±1.36	5.64-11.47	14.85±3.00	10.86-24.51		
ED	$0.33\pm0.04$	0.26-0.41	0.54±0.11	0.39-0.74	1.24±0.33	0.76-2.21	2.69±0.50	1.78-3.70		
UML	0.35±0.06	0.25-0.50	0.60±0.09	0.44-0.76	1.13±0.31	0.67-2.02	2.28±0.57	1.39-4.08		
	Body Proportion (%)									
ED/HL	25.77±1.52	21.82-29.07	22.79±1.75	20.63-28.51	22.79±1.53	20.20-26.89	27.27±2.38	22.67-32.63		
UML/HL	26.77±2.64	20.85-34.63	25.69±3.04	18.81-30.78	21.37±1.81	17.93-26.14	22.85±2.27	17.17-27.83		
HL/SL	23.38±1.87	19.51-27.61	27.75±1.71	24.75-32.03	35.21±1.85	31.38-38.52	39.43±1.51	36.72-43.65		
BH/SL	16.97±1.69	12.99-20.75	20.79±2.30	16.68-25.71	29.21±2.15	25.36-36.32	38.98±1.45	35.99-41.97		
PPD/SL	23.33±1.82	19.51-27.61	27.06±1.45	24.53-30.45	33.13±1.83	29.27-36.84	33.39±1.62	30.46-38.62		
PDD/SL	-	-	47.11±2.03	43.00-50.84	$47.79\pm1.37$	45.14-52.35	51.32±1.34	48.35-54.66		
PAD/SL	-	-	74.51±2.31	69.32-78.80	78.83±1.81	75.88-84.19	82.27±1.06	79.23-84.64		
PPLD/SL	-	-	-	-	53.97±1.30	51.04-58.58	59.07±1.29	57.17-62.14		
				Number of Ra	ys					
CF	-	-	-	6 - 19	-	18 - 19	-	19		
DF	-	-	-	6 - 8	=	9 - 11	-	11		
AF	-	-	-	2 - 6	-	7 - 11	-	11		
PLF	-	-	-	-	-	0 - 9	-	8 - 9		
PF	-	-	-	-	-	0 - 14	-	15		

pigmentation is clearly observed between the rays of caudal, anal, and dorsal fins.

Postflexion stage larvae (Figures 1 E, F, and G) were between 7 and 40 DPH, with SL ranging from 10.54 to 19.93 mm and weight varying from 0.0178 to 0.2810g. The body was moderate (25.36-36.32% SL), with moderate to large head (31.38-38.52% SL) and small to moderate eye (20.20-26.89% HL). During this period, a vestigial finfold is present in the dorsal and ventral areas of the caudal peduncle, but it is well developed in the ventral region, between the anus and head. Appearance of the pelvic fin button followed by the development of its rays occur at the end of this stage, together with the appearance of the adipose fin, scales and discrete transverse stripes along the body sides because of agglomeration of chromatophores.

The juvenile phase (Figure 1 H) included individuals with 18.27-42.21mm SL, body weight from 0.1990 to 2.4380g, belonging to the period comprised between 20 and 44 DPH. The body varied from moderate to high (35.99-41.97% SL) and the

head was large (36.72-43.65% SL) with a small to moderate eye (22.67-32.63% HL). During this period, all fins are fully formed, with absence of the embryonic finfold. Transverse stripes are visible throughout the body, being more evident above the lateral line.

Models originated by piecewise linear regressions presented significant instants of change in the rate of increase considering the body proportions relative to SL and HL (Figure 2). The BH/SL proportion increased from the preflexion stage to the transition between the postflexion stage and the juvenile for individuals with SL<17.84mm, followed by a stabilization in larger individuals. The HL/SL proportion presented a similar pattern, differing only in the size at which the change occurs, this being at the end of the postflexion stage when the SL was 15.90mm.

All the proportions of pre-fin distances relative to SL had an initial increase during *P. argenteus* development, remaining constant afterwards. The PPD/SL proportion remained constant for individuals with SL>14.47mm and throughout the postflexion stage. The

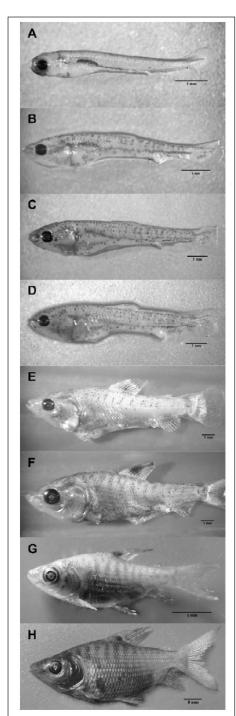


Figure 1 - Initial development of *Prochilodus*\*\*argenteus\* (Spix & Agassiz, 1829)

grown in experimental conditions from the 2nd to the 44th days post-hatch. 1A: initial preflexion stage, 1B: late preflexion stage, 1C: initial flexion stage, 1D: flexion stage, 1E: initial postflexion stage, 1F: postflexion stage, 1G: late postflexion stage and 1H: early juvenile phase.

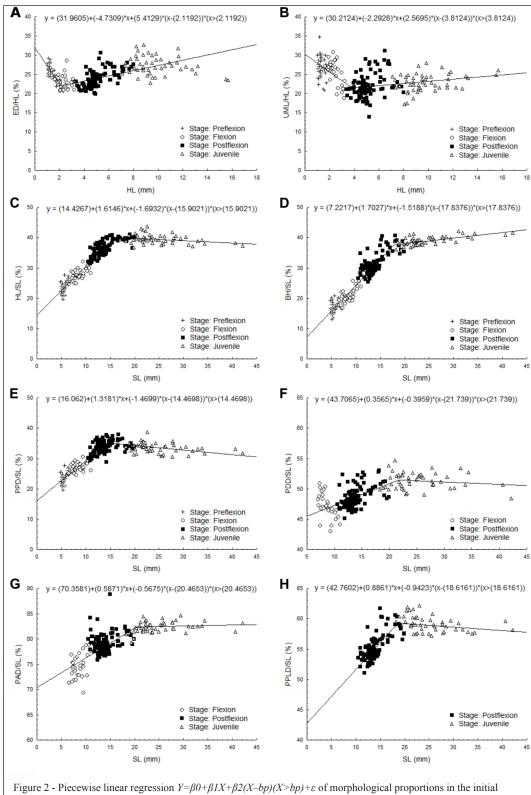
PPLD/SL proportion stabilized amid the postflexion stage and the juvenile phase in individuals with SL>18.62mm. The PAD/SL and PDD/SL proportions showed a tendency to the stabilization in the juvenile phase and in individuals measuring 20.47 and 21.74mm SL, respectively.

The piecewise linear model of the ED/HL proportion showed a reduction in the body proportion from the preflexion until the beginning of the flexion stage and in individuals with HL<2.12mm, as well as an increase in the subsequent developmental stages. The UML/HL proportion decreased until the early postflexion stage, the larvae having reached 3.81mm HL, remaining constant thereafter.

### DISCUSSION

Morphometric and meristic variables of larvae and juveniles of P. argenteus obtained from wild broodstock were similar to those reported for other species of *Prochilodus* (NAKATANI et al., 2001; ARIAS-GALO et al, 2010; SOUZA et al, 2015), which can be explained by the evolutionary proximity between species (CASTRO & VARI, 2004). Furthermore, the body proportions of P. argenteus reported in this study were quite similar to those described in the literature for its congeners from the São Francisco River Basin, namely P. costatus, P. vimboides, and P. brevis (Table 2). However, the maximum and minimum values of ED/HL, HL/SL, and BH/SL recorded for each larval stage varied between species. Morphometric data showed differences in the average ED/HL proportion in the preflexion and postflexion stages and in the BH/SL proportion in the flexion stage between P. argenteus and P. brevis. The average HL/SL proportion in the postflexion stage was greater in *P. argenteus* than in *P. vimboides*. Formation of fin rays in *P. argenteus* seemed to begin later than in **P. vimboides** since the latter presents rays in the caudal fin already in the preflexion stage. Moreover, in P. vimboides, dorsal and anal fins are practically formed at the end of the flexion stage, which was not observed in P. argenteus at the same larval stage. Although the paucity of morphological data for larvae of these species hampers an accurate taxonomic identification of all larval stages, the information herein provided serves as discriminators that contribute for the larvae identification process.

The observed morphometric variables of larvae and juveniles of *P. argenteus* from the Lower São Francisco River differed from those recorded by NAKATANI et al. (2001) for this species based on cultured individuals originating from the Upper São Francisco River (Três Marias-MG, 18°11′59.80″S



development of Prochilodus argenteus.

Table 2 - Body proportions and meristic data of larvae and juveniles of **Prochilodus** species occurring in the São Francisco River Basin ( $\bar{X}$ , mean; SD, standard deviation; CF, caudal fin; DF, dorsal fin; AF, anal fin; PLF, pelvic fin and PF, pectoral fin).

Stage/Phase	Prochilodus argenteus		Prochilodus costatus <sup>1</sup>		Prochilodus vimboides <sup>2</sup>		Prochilodus brevis¹			
	$\bar{X} \pm SD$	Range	$\bar{X} \pm SD$	Range	$\bar{X}$	Range	$\bar{X} \pm SD$	Range		
	ED/HL									
Preflexion	25.77±1.52	21.82-29.07	29.73±2.95	25.98-33.60	25.47±2.79	22.22-32.46	32.24±3.10	25.15-37.38		
Flexion	22.79±1.75	20.63-28.51	26.44±1.59	23.81-28.57	24.81±2.82	17.91-33.86	$25.99\pm2.05$	23.81-28.63		
Postflexion	22.79±1.53	20.20-26.89	26.16±1.93	18.96-28.22	23.70±2.44	17.29-27.90	29.03±2.01	25.06-33.27		
Juvenile	27.27±2.38	22.67-32.63	-	-	-	-	-	-		
HL/SL										
Preflexion	23.38±1.87	19.51-27.61	19.93±0.94	18.02-22.12	24.90±2.55	17.94-27.30	19.72±1.93	17.45-25.38		
Flexion	27.75±1.71	24.75-32.03	25.89±0.95	24.36-27.71	28.41±2.04	20.52-30.88	27.73±2.89	24.42-31.62		
Postflexion	35.21±1.85	31.38-38.52	33.84±2.69	29.71-43.04	27.71±3.68	-	33.72±1.70	30.03-36.61		
Juvenile	39.43±1.51	36.72-43.65	-	-	-	-	-	-		
BH/SL										
Preflexion	16.97±1.69	12.99-20.75	15.36±0.84	14.03-17.10	21.18±1.33	19.28-23.31	19.47±1.28	16.03-21.90		
Flexion	20.79±2.30	16.68-25.71	19.15±1.36	17.24-21.74	24.77±2.33	18.59-29.42	26.11±6.29	19.14-37.31		
Postflexion	29.21±2.15	25.36-36.32	30.36±3.54	21.70-34.46	28.10±1.33	25.23-30.02	31.4±2.27	26.11-35.59		
Juvenile	38.98±1.45	35.99-41.97	-	-	-	-	-	-		
			]	Number of rays	(fins)					
Preflexion	-		-		CF 11		-			
Flexion	CF 6-19; DF 6-8; AF 2-6		-		CF 18-20; DF 7-11; AF		-			
riexion					10-11					
Postflexion	CF 18-19; DF 9-11; AF 7-		-		CF. 20-28; DF 11-12; AF		-			
1 ostilexion	11; PLF 0-9; PF 0-14				11-12; PLF 3-5					
Juvenile 3	CF 19; DF 11; AF 11; PLF		CF 19; DF 13; AF 10-11;		CF 19; DF 12-13; AF 10-		CF 19; DF 12-14; AF 10-12;			
	8-9; PF. 15		PLF 8-9; PF 14-17		12; PLF 9-10; PF. 13-16		PLF 9; PF 14-16			

<sup>&</sup>lt;sup>1</sup>Morphometric data from NAKATANI et al. (2001).

and 045°15′7.89"W) and Sub-middle São Francisco River (Paulo Afonso-BA, 09°22'49.20"S 038°13'0.18"W). This probably occurred due to differences between the broodstock populations used, environmental characteristics, and physical separation of the river by hydroelectric dams. In addition, water quality parameters such as temperature, pH, and dissolved oxygen of the nursery and ponds where larvae and juveniles develop can influence their morphology. Therefore, these intraspecific morphometric differences may occur due to environmental and biological pressures to which populations are exposed during their development (LEARY et al., 1992; WIMBERGER, 1992; MATTHEWS, 1998; SOUZA JÚNIOR et al., 2002; SHIBATTA & OHARA, 2007; MACHADO & FORESTI, 2012). In addition, the latitudinal difference in the environmental temperature can influence the threshold size and the duration of each developmental stage among geographically isolated population of a single fish species (TAKAHASHI et al., 2012; McLEOD et al., 2015).

The changes in the body proportions over the initial development of *P. argenteus* until stabilization depended on the morphometric characteristic considered. The increase or reduction of the body proportions occurred due to allometric differences between measurements. The initial difference in the increasing ratio of morphometric measurements is a common feature in Teleostei and is associated with evolutionary strategies such as the prioritization of the development of the body structures for feeding and swimming (FUIMAN, 1983; OSSE et al., 1997; KOVÁČ et al., 1999).

Ontogenetic shifts in the habitat use are usually associated to changes in the morphometric proportions of structures related to feeding, swimming speed and movement maneuverability such as mouth position and gap size, the position of unpaired and paired fins and its development degree (ABELHA et al., 2001; MACHADO-EVANGELISTA et al. 2016). This seems to be the case of *P. argenteus* and possibly of other *Prochilodus* species that change

<sup>&</sup>lt;sup>2</sup>Morphometric data from SOUZA et al. (2015).

<sup>&</sup>lt;sup>3</sup>Number of fin rays of juvenile stage of *P. costatus*, *P. vimboides*, and *P. brevis* obtained from CASTRO & VARI (2004).

from a zooplanktophagous to an iliophagous feeding habit with the concomitant change from a pelagic to a benthonic behavior. In the experimental ponds preflexion and flexion *P. argenteus* larvae fed mainly on pelagic zooplankton (rotifers and cladocerans) even though from the postflexion stage onwards algae and particulate organic matter were the predominant items reported in the digestive tract (unpublished data).

# BIOETHICS AND BIOSSECURITY COMMITTEE APPROVAL

The authors of the article entitled "Morphological development of *larvae* and juveniles of *Prochilodus argenteus*" declare, for all due purposes, that the project that gave rise to the present data has not been submitted for evaluation to the Ethics Committee of the Companhia de Desenvolvimento dos Vales do São Francisco e Parnaíba. However, they are aware of the content of the Brazilian resolutions of the Conselho Nacional de Controle de Experimentação Animal (CONCEA) <a href="http://www.mct.gov.br/index.php/content/view/310553">httml>involving the use of animals in experimentation. Thus, the authors assume full responsibility for the presented data and are available for possible questions, if required by the competent authorities.

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