ORIGINAL RESEARCH

Metamorphosis of whitemouth croaker *Micropogonias furnieri* (Pisces, Sciaenidae)

Mara S. Braverman[1, *], Daniel Brown[1] and E. Marcelo Acha[1, 2]

[1]Instituto Nacional de Investigación y Desarrollo Pesquero (INIDEP), Paseo Victoria Ocampo Nº 1, Escollera Norte, B7602HSA - Mar del Plata, Argentina. [2]Instituto de Investigaciones Marinas y Costeras (IIMyC), Universidad Nacional de Mar del Plata (UNMdP), Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina

ABSTRACT. Fish metamorphosis is an important ontogenetic process with a key role on early stages survival and on successful recruitment to adult populations. The whitemouth croaker (*Micropogonias furnieri*) is an important commercial resource for the coastal fisheries of Argentina and Uruguay. Metamorphosis was studied using morphometric and morphological analysis during larval development. Changes in morpho-meristic characters before and after metamorphosis were employed to determine the length interval of this transition by employing Principal Component Analysis. Individuals (n = 430) from 4 to 41 mm standard length (SL) were collected in the Río de la Plata estuary (35.45° S, 56.35° W) in March 2006. Length ranges of individual's developmental stages were associated with the presence of key morphological characters. During early life stages, *M. furnieri* changes from a big-headed, robust shape larva to a slender and more elongated body form. Most of the morphometric variables showed an inflexion point at 15.2 mm SL, with a 95% confidence interval of 14.0-16.4 mm. The anterior part of the body grows faster during early stages, probably related to an intense feeding activity strategy. The completion of pectoral fin rays and the onset of squamation determine the beginning of metamorphosis at 11-12 mm SL. At around 18 mm SL, squamation ends, first barbels develop and the *sagittae* otoliths primordium is closed. The length-at-metamorphosis for *M. furnieri* was established between 9 to 18 mm SL, since all developmental characters studied highly overlapped at that interval. All those processes are indicative of the beginning of the juvenile period associated to the settlement and the start of a bottom-oriented life-style.

Key words: Sciaenidae, larvae transformation, ontogeny, morphology, morphometry.

*Correspondence:
mbraverman@inidep.edu.ar

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La metamorfosis de la corvina rubia *Micropogonias furnieri* (Pisces, Sciaenidae)

RESUMEN. La metamorfosis de los peces es un proceso ontogenético importante con un papel clave en la supervivencia de las primeras etapas y en el reclutamiento exitoso a las poblaciones adultas. La corvina rubia (*Micropogonias furnieri*) es un recurso comercial importante para las pesquerías costeras de la Argentina y Uruguay. Se estudió su metamorfosis mediante análisis morfométricos y morfológicos durante el desarrollo larvario. Se utilizaron los cambios en los caracteres morfomerísticos antes y después de la metamorfosis para determinar el intervalo de duración de esta transición mediante el Análisis de Componentes Principales. Se colectaron individuos (n = 430) de 4 a 41 mm de longitud estándar (LE) en el estuario del Río de la Plata (35,45° S, 56,35° W) en marzo de 2006. Los rangos de longitud de las etapas de desarrollo de los individuos se asociaron con la presencia de caracteres morfológicos clave. Durante las primeras etapas de vida, *M. furnieri* cambia de una larva de cabeza grande y forma robusta a una forma corporal más delgada y alargada. La mayoría de las variables morfométricas mostraron un punto de inflexión a los 15,2 mm LE, con un

intervalo de confianza del 95% de 14,0-16,4 mm. La parte anterior del cuerpo crece más rápidamente durante las primeras etapas, probablemente relacionada con una estrategia de actividad alimentaria intensa. La adquisición del número definitivo de radios de la aleta pectoral y el inicio de la escamación determinan el comienzo de la metamorfosis a los 11-12 mm LE. Alrededor de los 18 mm LE, termina la escamación, se desarrollan las primeras barbillas y se cierra el primordio de los otolitos *sagittae*. La longitud-de-metamorfosis para *M. furnieri* se estableció entre 9 y 18 mm LE, dado el alto grado de superposición que presentaron los caracteres de desarrollo estudiados. Todos esos procesos son indicativos del inicio del período juvenil asociado al asentamiento y al comienzo de un estilo de vida orientado hacia el fondo.

Palabras clave: Sciaenidae, transición larva-juvenil, ontogenia, morfología, morfometría.

INTRODUCTION

The way on that ontogeny of fishes proceed has been broadly discussed leading to two antagonistic visions. One states that ontogeny is a gradual process during which small and inconspicuous changes in form and structure accumulate continuously (Kováč and Copp 1999). The second view, represented by the 'theory of saltatory ontogeny' (Balon 1984), considers development as a sequence of longer stabilized steps (during which all the structures and organs develop gradually and continuously) alternating with thresholds. A threshold is a short interval where rapid changes occur from one steady state to the next. Nevertheless, early life stages of fish have been recognized by both 'gradualists' and 'saltationists' (see Kováč and Copp 1999) as being characterized by different fish capabilities, requirements, ecological interactions and growth/mortality events (e.g. Smith 1985; Koumoundouros et al. 2009; Nikolioudakis et al. 2010). The beginning of the larval period (Balon 1984) occurs when the individual is capable of feed orally, while the juvenile stage implies the disappearance of all larval characters and the appearance of nearly all the adult ones (Pavlov 1999). The transit between those periods consists on a remodeling process called 'metamorphosis' (Balon 1989), which represents an important ontogenetic event for fish with consequences for the survival of early stages and strong consequences for recruitment to adult populations (Govoni 2004).

To define the onset of the juvenile period, different approaches are traditionally used. From a functional and structural perspective, this event is associated with a change in allometric growth or shape (e.g. Copp and Kováč 1996; Sagnes et al. 1997; Kováč et al. 2006), coupled with a series of changes (abrupt or gradual) in morphological and meristic characters. Such changes are the acquisition of adult complement of fins spines and rays, adult pigmentation, onset or end of squamation, ossification of the axial skeleton and disappearance of larval characters (McCormick et al. 2002; Urho 2002; Ditty et al. 2003). From an ecological perspective, in demersal and benthic fish, metamorphosis is usually linked to settlement (Werner 2002) i.e., the ontogenetic shift by which pelagic larvae colonize benthic habitats (McCormick et al. 2002). Metamorphosis is a preparation for the colonization of a new habitat and the integration into a new trophic web, as larvae abandon the plankton to become part of the demersal-benthic communities (e.g. Secor 2015). That shift can be detected in otoliths of several fish species (Wilson and McCormick 1997, 1999) as a transition mark or 'check' (Campana and Neilson 1985). For temperate and cold water fishes, formation of accessory nuclei in their otoliths has been associated with metamorphosis and settlement processes (Sogard 1991; Morales-Nin and Aldebert 1997; Morioka et al. 2001; Buratti and Santos 2010).

Setting a threshold value as the 'length-at-metamorphosis' (L_{juv} from Fuiman's ontogenetic index) is highly complex due to individual variability and ontogenetic stage of individual characters. This is why in several studies a single

character (e.g., the onset or end of squamation, definitive fin radios acquisition, etc.) is considered, although this approach results inadequate for a precise definition of metamorphosis (Urho 2002; Ditty et al. 2003), where a series of morphological, morphometric, physiological and ethological events should converge. In that sense, Urho (2002) stated that 'the use of a single morphological trait to infer metamorphosis is as inadequate as using a single character for the identification of a species'. A clear determination of larvae-juvenile transition is particularly important to identify ontogenetic changes in the use of resources (Juanes and Conover 1994; Boglione et al. 2003). Most of the characters associated to metamorphosis undergo changes in a synchronized way (Ditty et al. 2003), suggesting the need to analyze the metamorphosis process as a holistic approach.

The whitemouth croaker, *Micropogonias furnieri* (Desmarest 1823), is the dominant species in terms of biomass in the Río de la Plata region and the main target of the coastal fisheries of Argentina and Uruguay, representing *ca.* 20% of coastal species landings in Argentina (Carozza et al. 2004). It is a demersal species with a long lifespan (up to 39 years in the region). In Argentina and Uruguay it reproduces from November to April, spawning several batches of pelagic eggs with an indeterminate annual fecundity. Main reproduction ground covers a narrow band across the inner Río de la Plata estuary between Montevideo (34° 50′ S-56° 10′ W) and Punta Piedras (35° 25′ S-57° 10′ W), at depths between 6 and 8 m (Acha et al. 1999; Carozza et al. 2004). The area is characterized by a bottom salinity front (Mianzan et al. 2001) in which whitemouth croaker eggs were only present below the halocline where salinity ranges from 9.7 to 27.3 (Acha et al. 1999). During warmest months (October through May), there is evidence of retention of whitemouth croaker larvae in the inner part of the Río de la Plata estuary. This area coincides with the location of the bottom salinity front and the maximum turbidity zone (Braverman et al. 2009). Larvae retention in this area would ensure closeness to the main nursery ground: Samborombón Bay (Mianzan et al. 2001). Early life stages of this species have been described for identification purposes with a classical approach (Sinque 1980; Weiss 1981), not intending to outline critical moments of the early life history, such as the larvae-juvenile transition. Thus, to define metamorphosis more accurately we employed a multivariate approach using morphometric and morphological analyses during larval development.

The multivariate approach consists of a morphometric analysis based on the principles of multivariate allometry described by Shea (1985) and a morpho-meristic analysis based on Ditty et al. (2003). The former studied the relationship between a group of morphometric measures and the individual's total length. The later consisted on the assignation of scores to individual characters, where the sum of them for each individual represents an index of morphological change associated to metamorphosis. Hence, the objective of this study was to investigate the larvae-juvenile transition (hereafter metamorphosis), looking for changes in morpho-meristic characters before and after the metamorphosis to determine the length interval for these changes.

MATERIALS AND METHODS

M. furnieri larvae and juveniles (less than a year-old) were collected during a scientific cruise to Río de la Plata spawning area in March 2006. A vertically stratified plankton sampling along transects perpendicular to bathymetry was performed during daylight. Samples were taken at stations separated 10-12 km at three depth levels. Water column was sampled above (or *c.a.* 2.5 m depth) and below (or *c.a.* 6.2 m depth) the halocline with a Motoda sampler equipped with a

mechanical opening-closing device and a 200 µm mesh span net. The third level was sampled by an epibenthic sledge with a 500 µm mesh span net (see Braverman et al., 2009).

A total of 249 *M. furnieri* individuals were identified on board and frozen for a later otoliths extraction; the rest of the samples were fixed in 4% formalin. Some specimens from the ichthyoplankton collection of the Instituto Nacional de Investigación y Desarrollo Pesquero (INIDEP) were also used to complete the length classes (n = 15). Those individuals from the collection come from the same study area. An alizarin dying technique (modified from Potthoff 1984 and Taylor and Van Dyke 1985) was performed onto the specimens for a better observation of structures.

Morphometric and morphological analyses were performed on an ontogenetic series from 4 to 41 mm standard length (SL). For morphometric analysis, images of each individual (n = 71) were taken using a digital camera mounted in a compound microscope. A series of measurements (Figure 1; Table 1) were taken from those images with the Axio Vision Software (Carl Zeiss). The shortening correction due to the fixation method was not considered assuming that it was similar in all measured individuals. For morphological analysis, a group of events related to the external morphology was analyzed on the ontogenetic series (n = 120). Development stage was determined following Moser (1996) and Fuiman and Werner (2002): larvae at pre-flexion, flexion, post-flexion, in transformation and early juveniles.

Distinctive characters of each individual were registered: fin rays appearance (pectoral, anal, dorsal and pelvic); scales coverage was classified into 4 categories: (a) on the caudal peduncle, (b) from the caudal peduncle to the anus, (c) from the caudal peduncle to the head, (d) totally scaled; and presence/absence of mentonian barbels. Finally, otoliths were extracted from frozen specimens (n = 85) using a NaClO solution for tissue disintegration. They were mounted in glass slides with a transparent mounting medium and polished when necessary with lapping film paper in a decreasing order of porosity (12, 9 or 3 µ) for a better visualization. Otolith development was studied by registering the occurrence of accessory nuclei and the primordium (polygonal area center-enclosed by the accessory nuclei).

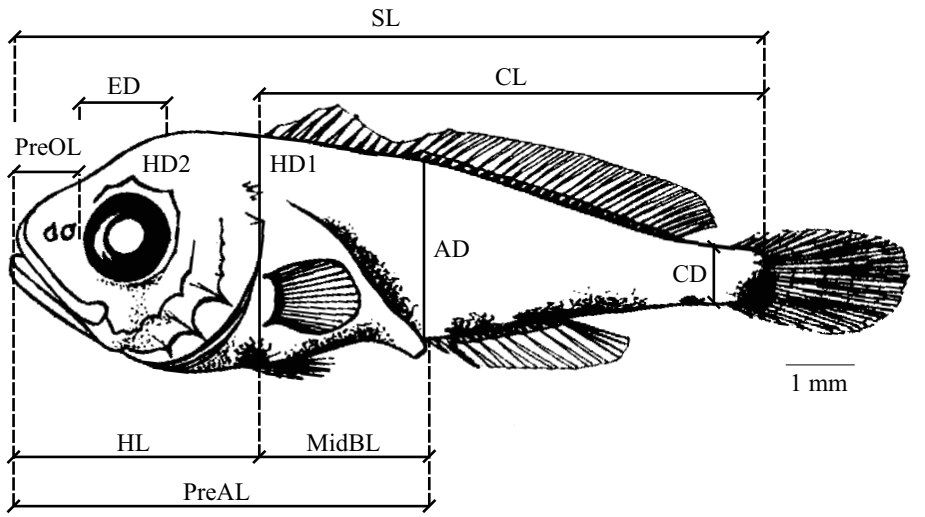

Figure 1. Morphometric measurements taken from *Micropogonias furnieri* individuals. SL: standard Length, ED: eye diameter, HL: head length, PreAL: pre-anal length, HD1: head depth 1, HD2: head depth 2, AD: anal depth, CD: caudal depth, Pre-OL: pre-orbital length, Mid-BL: mid-body length, CL: caudal length. Illustration taken from Weiss, 1981.

Table 1. Description of morphometric characters measured in *Micropogonias furnieri* and its abbreviations (adapted from Nikolioudakis et al. 2010).

Character	Abbreviation	Description
Standard length	SL	From the tip of the snout to the caudal rays insertion
Eye diameter	ED	Parallel to the longitudinal axis of the body
Head length	HL	From the tip of the snout to the margin of the gill cover
Pre-anal length	PreAL	From the tip of the snout to the anus
Head depth 1	HD1	From the dorsal to the ventral margin of the body at the operculum
Head depth 2	HD2	From the dorsal to the ventral margin of the body at the center of the eye
Anal depth	AD	From the dorsal to the ventral margin of the body at the anus
Caudal depth	CD	From the dorsal to the ventral margin of the body at the caudal peduncle
Pre-orbital length	PreOL	From the tip of the snout to the anterior margin of the eye
Mid-body length	MidBL	From the margin of the gill cover to the anus
Caudal length	CL	From the margin of the gill cover to the insertion of the caudal fin

Data analysis

Morphometry and bi-variate allometry

Firstly, morphometric relationships were studied using linear regression analysis between the logarithms of each variable (morphometric measurement) versus length (SL) (Table 1). We employed standard length for post-flexion individuals and notochord length for pre-flexion and flexion specimens. Allometric growth pattern of each variable in relation to SL was studied by using the logarithmic form of the allometric growth model (Huxley 1932): $log\ (Y) = log\ (a) + b\ log\ (SL)$, where Y is the variable examined and b is the allometric coefficient. Secondly, regressions' residuals (Y) were analyzed to identify possible allometric changes as follows: if allometric growth of the variables was constant (slope without changes) then residuals would show a random pattern; alternatively, if variables showed changes in their growth they would have a residual distribution (Y versus SL) with a specific form as it was shown by Sagnes et al. (1997), Gozlan et al. (1999) and Nikolioudakis et al. (2010).

Similarly, morphometric indexes (I_m) showing representative changes in the larvae-juvenile transition were analyzed. Indexes were calculated as the percentage of the morphometric variable (V_m) with respect to length (SL): $I_m = V_m/SL * 100$. The length at which the change in oblique orientation of I_m occurred (L_i) was estimated by using a piecewise linear regression fitted with a non-linear estimation procedure: $I_m = b_0 + b_1SL + b_2(SL - L_i)\ (SL \geq L_i)$; where b_0 is the y-intercept, b_1 is the slope of the relationship of the values $\leq L_i$, b_2 is the change in the slope (b_1) that results in the slope when $SL \geq L_i$, and L_i is the length at which slope changes (Nikolioudakis et al. 2010), considered here to be related to the transformation process (end of larval period).

Morphometry and multivariate approach

To determine the length-at-metamorphosis (L_m), log-transformed morphometric measurements with respect to SL using a Principal Component Analysis (PCA) with covariance matrices were studied (Jolicoeur 1963a, 1963b; Shea 1985). In a PCA of a logarithmic covariance matrix of groups of animals with different growth patterns, the first component (PC1) summarizes the variation in shape as a result of a common pattern of allometric growth; while the second component

(PC2) and the rest summarize the variations of shape as a result of divergent growth trajectories (Shea 1985). Then, the L_m at which a change in PC2 orientation occurs and the lengths of possible inflexion points (L_i) were statistically estimated using a quadratic function PC2 = a + b(PC1) + c(PC1)^2 of the Non-linear estimation Module of STATISTICA software v.7 2004 (StatSoft Inc.).

Morphology

Length ranges of developmental stages of *M. furnieri* were determined. These ranges were associated with the appearance/disappearance of several morphological characters (Table 2). Moreover, the pectoral fin ray's definitive number acquisition was analyzed with respect to length (pectoral fin tends to be the last one in acquiring the whole set of rays (Urho 2002)). Then, the squamation process was described. For morpho-meristic analysis, each specimen was scored for a suite of characters where each score represented a discrete ontogenetic event or a character stage of development (Table 2). Every character had equal weight, i.e. three possible states 0, 1 and 2. The sum of character scores of each individual as an index of overall change in morphology associated with metamorphosis was calculated and expressed as percentage of the maximum total score. Total scores for the character set were analyzed with respect to length classes performing a cluster analysis with complete linkage, and to the Manhattan (city-block) distances method (Ditty et al. 2003). Finally, the length at which 50% of morphological change occurs (L_{50}) was obtained from the relationship between the accumulative scores percentage (P) and SL described by the logistic function: P = 100/(1 + exp(-a*(SL-b))). Length at which P = 100% constitutes the juvenile stage length.

RESULTS

Developmental morphometry

Residual distribution of each log-transformed variable fitted against SL with an allometric function showed different responses (see Appendix). With few exceptions (ED and MidBL, Figure 1; Table 1), residuals revealed a non-random distribution indicating changes in the relative growth of morphometric variables evidenced by inflexion points in the distribution of morphometric indexes (Figure 2). Thus, ED grew isometrically while MidBL showed a positive allometry in the entire

Table 2. Scores assigned to each *Micropogonias furnieri* individual according to the developmental stage of considered characters. The scoring of rays was performed separately for each fin (pectoral, anal, dorsal and pelvic).

Character	Score	Observed pattern	Length ranges (mm SL)
Scales cover	0	No scales	4-10
	1	Scales do not cover the entire body	10.1-16.9
	2	Scales covering the entire body, including head	18-34
Fin rays	0	No rays	4-6.3
	1	Rays in formation	6.7-11.9
	2	Definitive rays	12-34
Otolith development	0	No accesory nuclei	4-10.9
	1	Accesory nuclei not closing the primordium	6.8-17
	2	Closed primodium	15-34.9

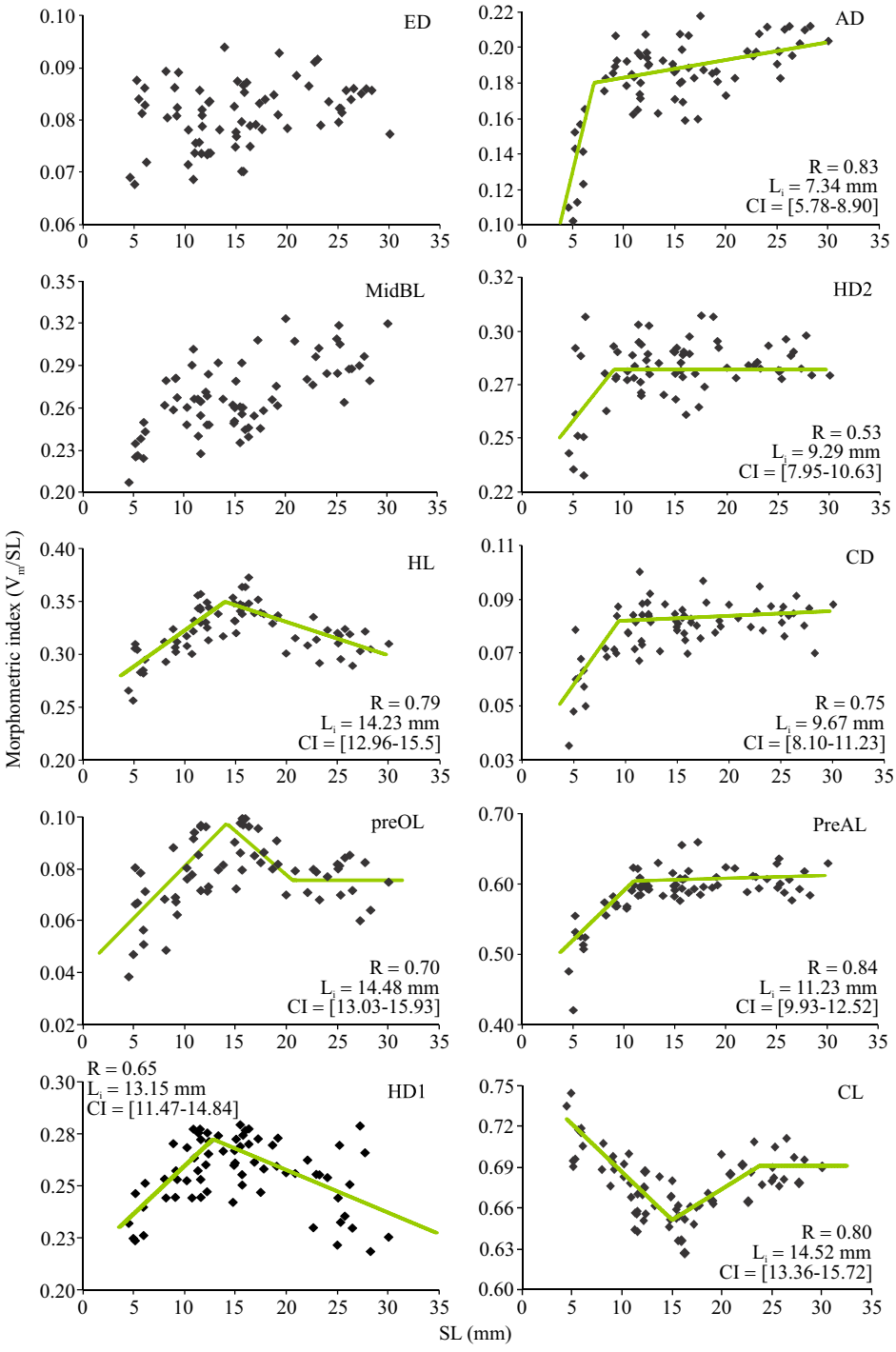

Figure 2. Relationships of morphometric indexes with standard length (SL) of individuals. Fitted lines came from non-linear regressions used to determine inflexion lengths (L_i). CI: 95% confidence interval for L_i, V_m: morphometric variable. Abbreviations as in Figure 1.

length range. In turn, HL, PreOL and HD1 grew faster than SL (positive allometry) to a maximum and then got slower (negative allometry), while CL showed an opposite pattern growing slower than SL until a minimum value when it started to grew faster. In addition, variables related with body depth (HD2, AD, CD) and PreAL grew faster than SL to a point where they remained constant (or slightly grew). Inflexion points (L_i) and their confidence intervals were determined by non-linear regressions of each variable (expressed as the I_m) with respect to SL (Figure 2).

Principal Components Analysis (Table 3) of log-transformed morphometric measurements using the covariance matrix revealed an important change in the oblique orientation of PC2 values of transformed variables when plotted against PC1 or SL (Figure 3). A highly significant quadratic relationship ($R = 0.62$, $p < 0.001$) allowed to determine an L_m = 15.18 mm SL [CI = 14.01-16.37]. When residuals of allometric regressions were separately examined for individuals < 15.18 mm SL and ≥ 15.18 mm SL, we observed they were randomly distributed. Because no pattern or structure were shown, it could be assumed that bi-variate allometric equations were appropriate to describe relative growth of individuals before ($SL < L_m$) or after ($SL \geq L_m$) the metamorphosis length.

Morphological events

The end of post-flexion stage and the beginning of the transition to juvenile (metamorphosis) was determined considering the completion of pectoral fin rays and the onset of squamation process, which occurred at 11 mm SL and 12 mm SL, respectively (Table 4; Figure 4 A: arrows 3 and 4). Meanwhile, at *ca.* 18 mm SL barbels' appearance and the end of squamation process established the end of metamorphosis according to morphological events (Figure 4 A: arrows 5 and 6).

First pectoral rays appeared between 5 to 7 mm SL and the fin rapidly completed its rays at 12 mm

Table 3. Principal Component results of the first (PC1) and second factor (PC2) for the log-transformed morphometric characters studied (abbreviations in Table 1).

	Eigenvectors coefficients		Factor score correlations		Factor-variable			
Morphometirc character	PC1	PC2	PC1	PC2	PC1	PC2	Eigenvalues	% total variance
SL	-0.275	0.163	-0.357	1.955	-0.996	0.063	0.595	97.872
ED	-0.284	0.284	-0.369	3.412	-0.986	0.106	0.007	1.137
PreAL	-0.305	0.048	-0.396	0.574	-0.998	0.017	0.002	0.385
HL	-0.292	-0.126	-0.379	-1.516	-0.995	-0.046	0.001	0.194
HD1	-0.291	0.075	-0.377	0.897	-0.997	0.028	0.001	0.155
AD	-0.339	0.063	-0.349	-1.474	-0.992	0.020	0.001	0.101
CD	-0.340	-0.056	-0.440	0.762	-0.990	-0.018	0.000	0.068
PreOL	-0.324	-0.821	-0.441	-0.674	-0.963	-0.264	0.000	0.058
HD2	-0.269	-0.123	-0.419	-9.876	-0.993	-0.049	0.000	0.030
MidBL	-0.317	0.289	-0.411	3.478	-0.990	0.097	0.000	0.001
CL	-0.268	0.298	-0.347	3.579	-0.990	0.119	0.000	0.000

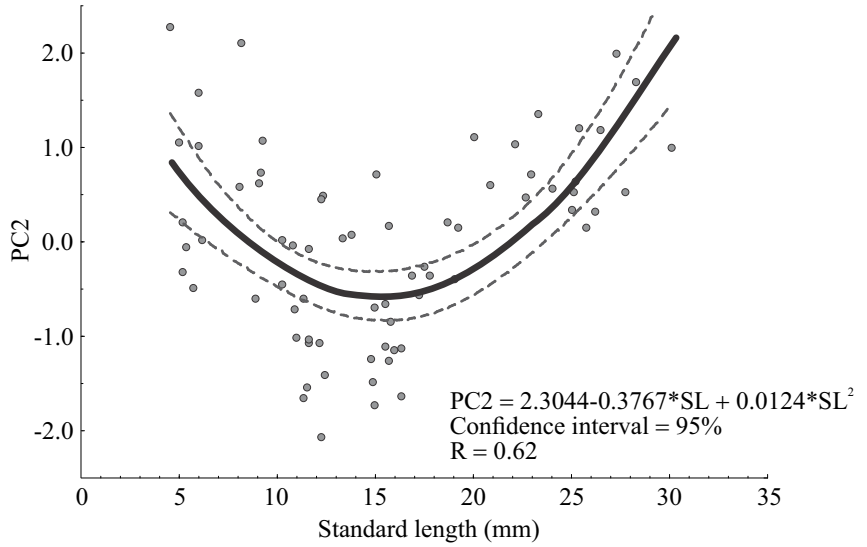

Figure 3. Multivariate analysis (PCA) showing the relation between SL (representing PC1) and PC2, and the quadratic fit (lines) assessed for the length-at-metamorphosis (L_m). Dashed lines represent 95% of confidence.

Table 4. Length intervals of developmental stages of *Micropogonias furnieri*. Transition length was calculated as the mean value between the highest value of a stage and the lowest value of the previous stage.

		Length (mm SL)		
Stage	N	Min	Max	Transition
Pre-flexion	19	-	6.2	〉 5.6
Flexion	26	4.9	8.4	〉 7.4
Post-flexion	26	6.4	11.3	〉 11.5
Transformation	32	11.7	19.2	〉 18.6
Juvenile	17	18	-	

SL (Figure 4 B). Squamation occurred in the transformation stage in a posterior-anterior direction (Figure 5). First scales appeared in the caudal peduncle separated from each other. Its morphology was rather simple: oval-shaped, with smooth edges and a few concentric rings (Figure 5.1). As squamation process progressed, scales morphology became more complex developing projections in its posterior margin (*ctenii*) and scallops in the anterior margin, and starting to overlap each other (Figure 5.3). The head was the last part to be covered (Figure 5.2).

Cluster analysis over the scores of morphological characters defined two main groups at a distance of 50% approximately (Figure 6 A); a group of small larvae from 4 to 9 mm SL, and a group of individuals larger than 9 mm SL. From the later, individuals bigger than 16 mm SL were grouped in an approximate distance of 15%, remaining an intermediate interval from 9-16 mm SL of non-homogeneous groups. On the other hand, cumulative scores showed a highly significant fit ($R = 0.9845$; $p < 0.001$) to a logistic function with L_{50} = 9.24 mm SL (Figure 6 B) and fully metamorphosed individuals larger than 18-20 mm SL.

DISCUSSION

Throughout the early life history, *M. furnieri* changed from a larval big-headed and robust shape to a slender and more elongated body

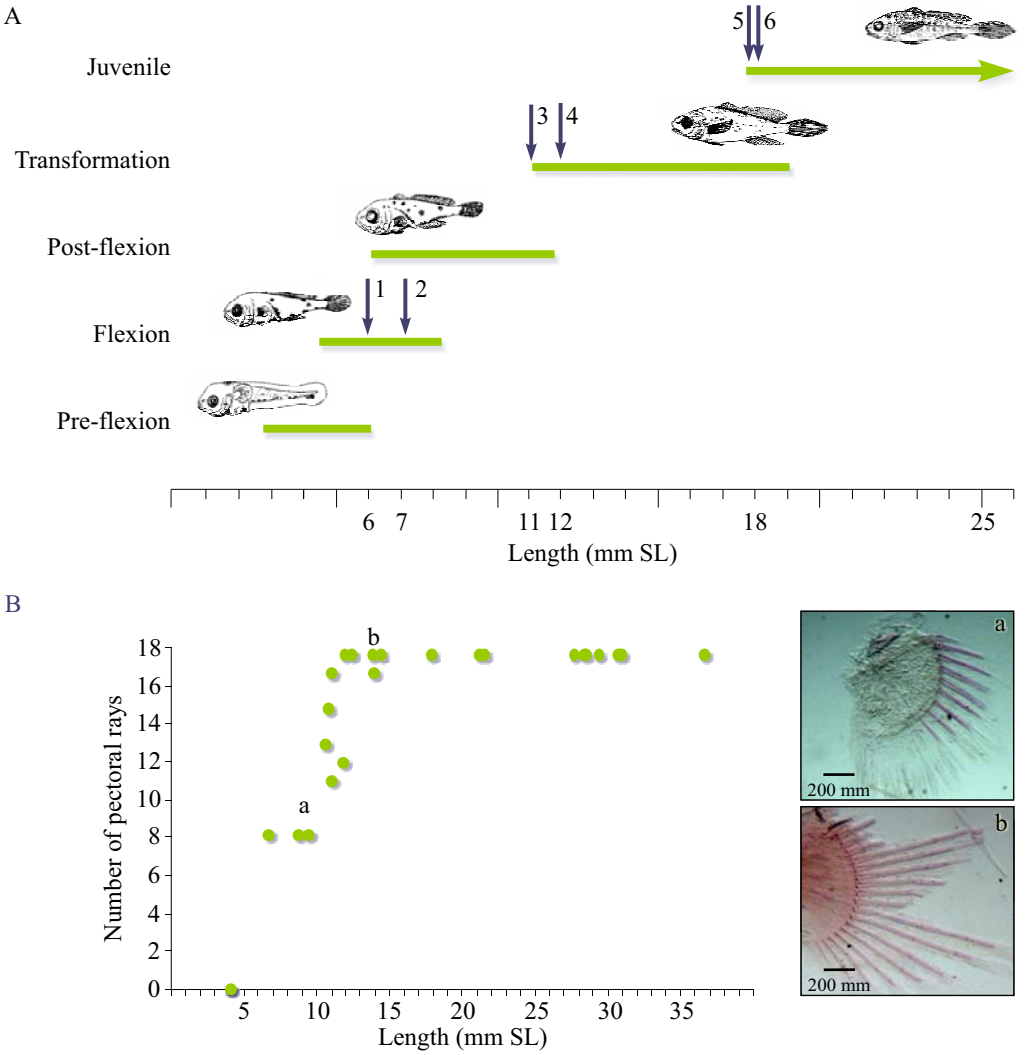

Figure 4. Summary of morphological events occurred throughout *Micropogonias furnieri* early ontogeny. A) Scheme of length intervals per stage. Arrows show important events: 1- caudal, dorsal and anal fins occurrence (rays in formation), 2- pelvic fin appearance, 3- onset of squamation, 4- pectoral fin definitive set of rays, 5- barbels occurrence, 6- ending of squamation process (scales in the head). B) Development of pectoral fins: a- rays in formation, b- definitive set of rays. Illustrations taken from Weiss, 1981.

shape. Most of the morphometric variables showed an inflexion point in their development and the compound analysis showed an inflexion length of 15.2 mm SL [CI = 14-16.4]. Multivariate morphometric analysis (PCA) indicated a change in the allometric growth of ontogenetic series evidenced by a change in the slope orientation of the PC2 versus PC1 (or SL) as stated by Shea (1985). Relative changes in the growth of whitemouth croaker were seen in the residuals of allometric relations as well as in the estimated L_m, resulting in a random distribution of residuals of the two size groups detected (i.e., $SL < L_m$ and $SL > L_m$). These results constitute a valid estimation of the length-at-metamorphosis from a morphometric point of view.

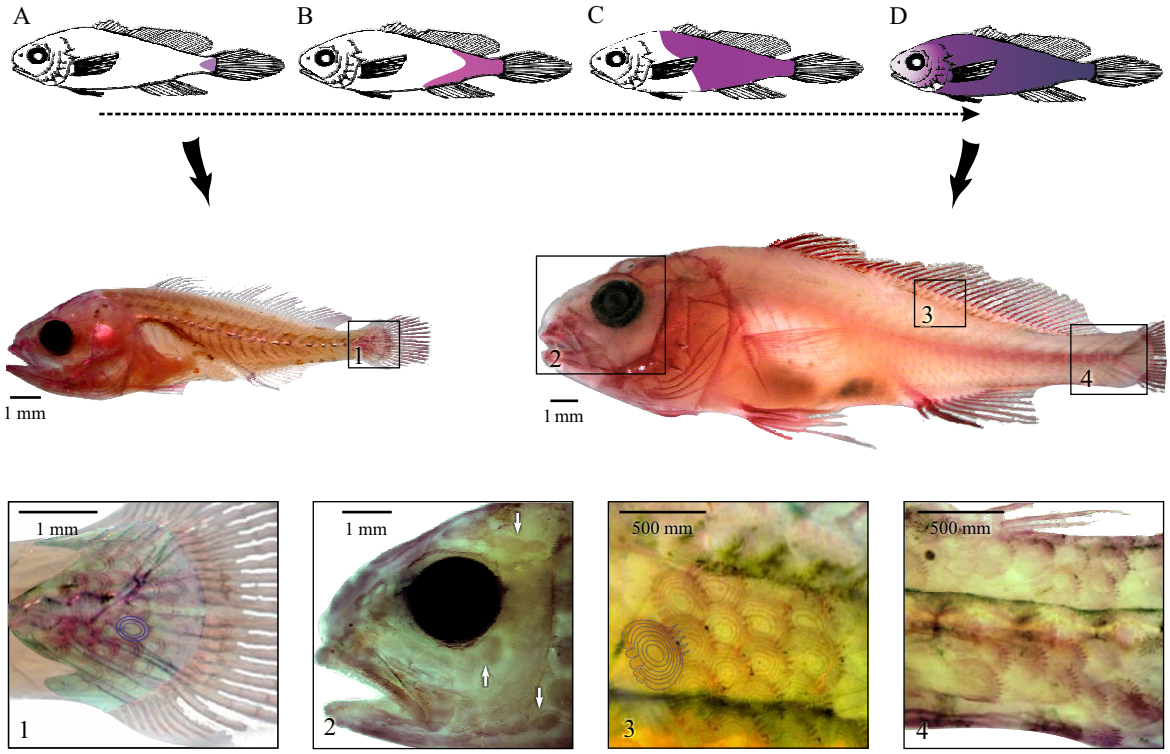

Figure 5. Sequence of squamation (A-D) observed in *Micropogonias furnieri*. Different colors indicate degree of squamation, from lesser (A) to greater (D) complexity. Boxes 1-4: details of coverage and complexity of scales throughout the body. White arrows indicate scales position. Box 3: scheme of a scale.

Two well-defined groups were found in the scoring analysis (morphology): one with individuals < 9 mm SL and other with individuals > 16 mm SL, matching the length at which 50% and 100% of the body shape change occurs (L_{50} = 9.2 mm SL and L_{100} = 17 mm SL, respectively). Individuals between 9 to 16 mm SL did not form a homogeneous group probably due to they were undergoing a shape transition. In accordance to Weiss (1981), morpho-meristic analysis during this length interval showed the definitive pectoral ray's acquisition and the onset of squamation at 11 mm and 12 mm SL, respectively. These events occurred close to the appearance of accessory nuclei in the otoliths (Braverman et al. 2015), coincidently with the change in body proportions. Finally, squamation ended at 18 mm SL approximately, when first barbels appeared and the otolith primordium was closed (*c.a.* 16 mm SL, Braverman et al. 2015).

Early in larval development, variables related to the head showed a higher relative growth rate than the rest of the body. A higher growth of the anterior part of the body during the first ontogenetic stages has been related to feeding (Fuiman 1983; Osse et al. 1997), because an early development and differentiation of nervous (brain), sensorial (neuromasts, photoreceptors and olfactory receptors) and digestive (mandible structures) systems would contribute to improve prey detection and capture. Fin formation would increase swimming efficiency and the appearance of barbel during metamorphosis would constitute an important character helping juveniles to colonize a new habitat (settlement) and start a bottom-oriented lifestyle.

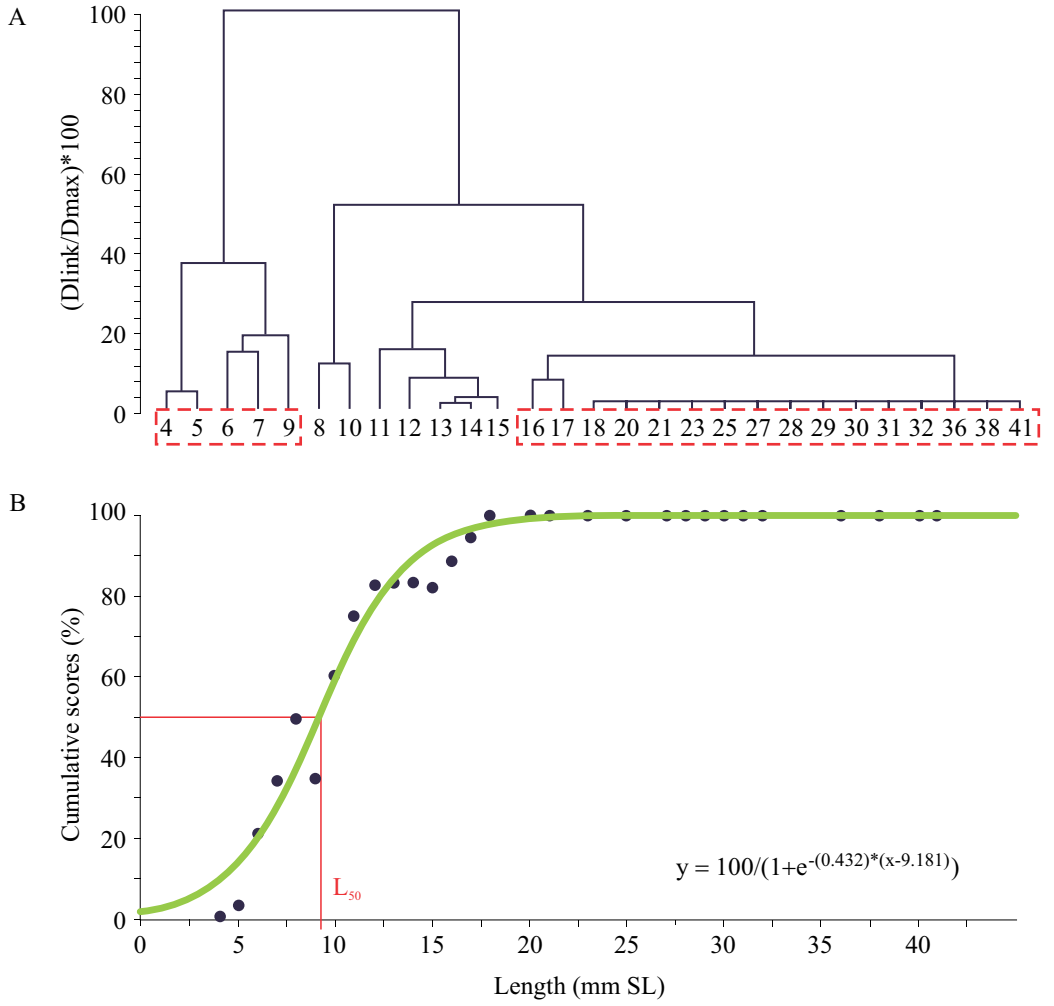

Figure 6. Morphological analysis of *Micropogonias furnieri* ontogenetic development. A) Cluster analysis of morphological characters based on Manhattan distances' method (City-Block) with complete linkage. B) Logistic graph and function with cumulative scores of characters with respect to length.

In terms of body height and pre-anal length, larvae changed their growth rate at smaller lengths (9-11 mm SL) while the head and the rest of the body showed an opposite pattern changing their growth rates at larger lengths (13-15 mm SL). We suggest that as the head reduces its relative growth the rest of the body has to grow faster to maintain proportions. Our findings could indicate a sequence of growth for these stages of development, giving priority first to the feeding function and then to locomotion and predators escape instead of digestive system development (Osse and van den Boogaart 1995). This could be possible because of the high digestibility of croaker larvae's prey (tintinnids, copepod nauplii and small copepods, Rodríguez-Graña et al. 2018) and the powerful digestive enzymes of fish larvae as shown in herring *Clupea harengus* (Pedersen et al. 1987). Moreover, it has been suggested that fish larvae can utilize exogenous enzymes from their prey to improve digestion (Lauff and Hoffer 1984; Kolkovski et al. 1993).

The squamation process in demersal species has been considered an indicator of transformation between larval and juvenile stages (Miller et al. 2003), along with the process of primordium formation in otoliths, with drastic changes in early life history of fish as observed in *Trachurus japonicus* (Xie et al. 2005) and *Merluccius hubbsi* (Buratti and Santos 2010). Similar results were reported in blennids (Ditty et al. 2003), considering a group of morphological characters in which the ontogenetic development of different structures seemed to overlap during metamorphosis (see also Kanou et al. 2004; Nikolioudakis et al. 2010). Those findings are consistent with the high level of synchronization achieved in certain ontogenetic thresholds (Balon 1984) to get to the next step in development (e.g., from larval to juvenile period) (Kováč 2002). Differences in timing and developmental rates of individual characters make difficult the recognition of thresholds that could be in the root of the 'saltationist-gradualist' debate (Kováč and Copp 1999). Hence, multivariate approach performed in our study helps to a better identification of thresholds based on the degree of coincidence of changing variables.

Multivariate approach showed that developmental characters studied on the whitemouth croaker highly overlapped, even though morphological or morphometric characters could begin the transformation at different ontogenetic times (lengths, *sensu* Fuiman et al. 1998) and at different speeds. Although not all the inflexion points were detected at the same size, 10 morphometric measurements demonstrated significant changes in relative growth within a length interval similar to that found in the study of morphological events. Considering all the events together, it is strongly suggested a developmental interval of 9 to 18 mm LS as the moment of greatest change in body shape, i.e., metamorphosis.

Most marine fishes tend to spawn at specific times and places within predictable and distinctive circulation features. The most common life history of fishes that use estuaries involves spawning of planktonic eggs at sea and the subsequent recruitment to estuaries as post-larvae or juveniles. Due to the net seaward movement of estuarine waters, the export of early life-history stages from estuaries has been argued to be a major problem for estuarine spawners (Boehlert and Mundy 1988), and the lack of retention mechanisms has been proposed to explain why fishes do not typically spawn inside estuaries (e.g., Dando 1984; Haedrich 1992). Unlike most estuaries, spawning activity is rather common in the Río de la Plata, in which the existence of retention mechanisms has been proposed (Acha et al. 1999; Simionato et al. 2008; Braverman et al. 2009). This estuary is very shallow and essentially dominated by the wind. Retention process is a consequence of the estuarine response to natural wind variability at the scale of 3-5 days acting over bathymetric features (Simionato et al. 2008). Moreover, river discharge fluctuations modulate retention variability at interannual scales (Acha et al. 2008). Whitemouth croakers spawn well inside the estuary at the bottom salinity/turbidity front (Macchi and Christiansen 1996; Acha *et al.* 1999), where their larvae remain retained (Braverman *et al.* 2009). Retention is by no manner a 100% effective mechanism and recruitment success of *M. furnieri* shows strong fluctuations that would be linked to the dynamics of the estuarine waters (Acha *et al.* 2008). For many marine fishes habitat shifts often occur at metamorphosis when larvae undergoes a striking change from a pelagic, planktonic organism, to a demersal one (Werner 2002). This seems to be the case with *M. furnieri*, whose smaller larvae (< 10 mm SL) appear at the whole water column but larger sizes (> 10 mm SL) probably undergo the settlement process inhabiting near the bottom (Braverman *et al.* 2009).

During settlement, larvae and young juveniles move from pelagic to demersal food webs with attendant changes to their foraging success and predation risk. However, settlement means not only the encounter of a new array of prey and

predators, it also means a shift from a pelagic and more dispersive stage to a demersal and more sedentary one (Secor 2015). Retention would improve when larvae or early juveniles remain in the bottom boundary layer (Mann and Lazier 1996), in this way settled croakers would diminish their chances of being exported from the estuary. A fast metamorphosis and successful settlement could enhance survival, and consequently improving recruitment. So identifying the processes that allow juveniles to attain retention inside adequate habitats is important to effectively understand marine species population dynamics.

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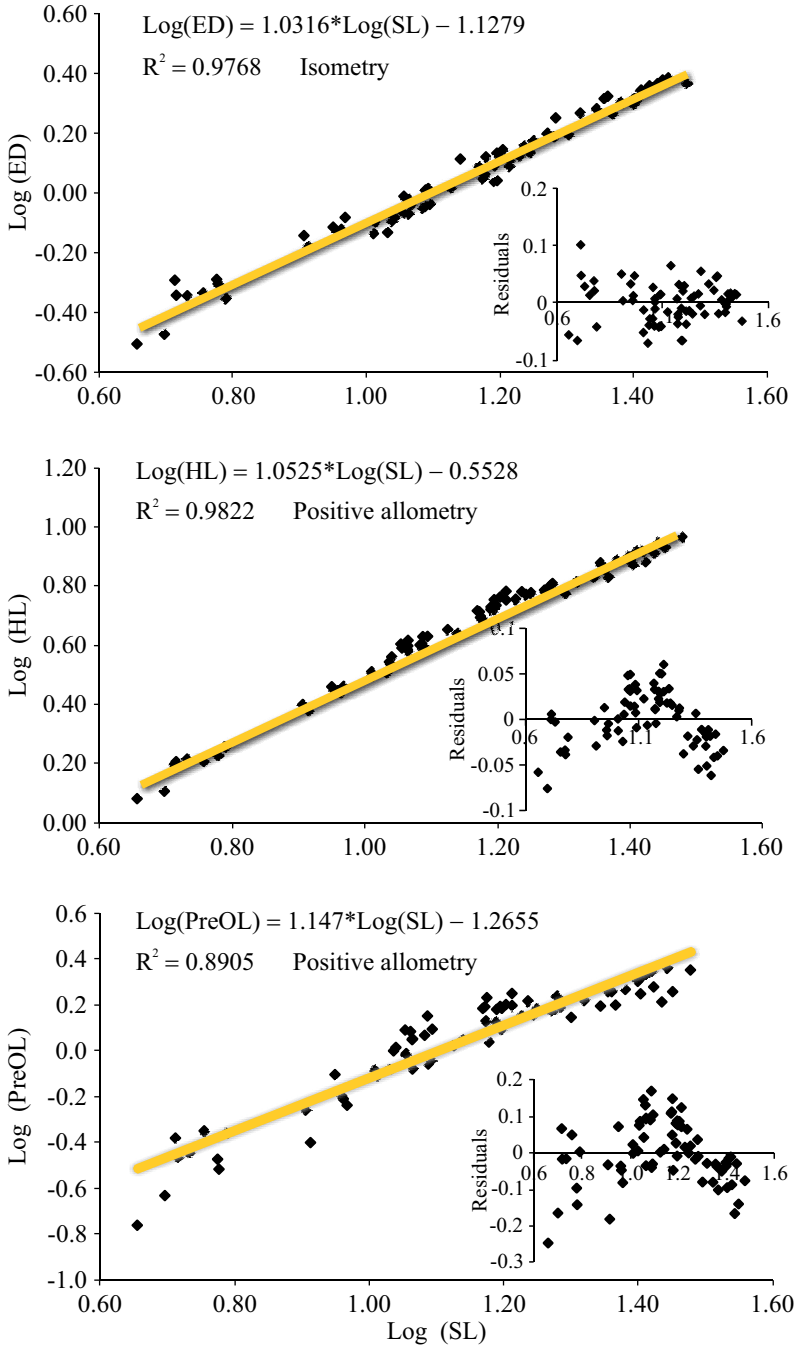
APPENDIX

Linear regressions of log-transformed morphometric variables and residuals' charts used to identify changes in variables meaning changes in individual's shape.



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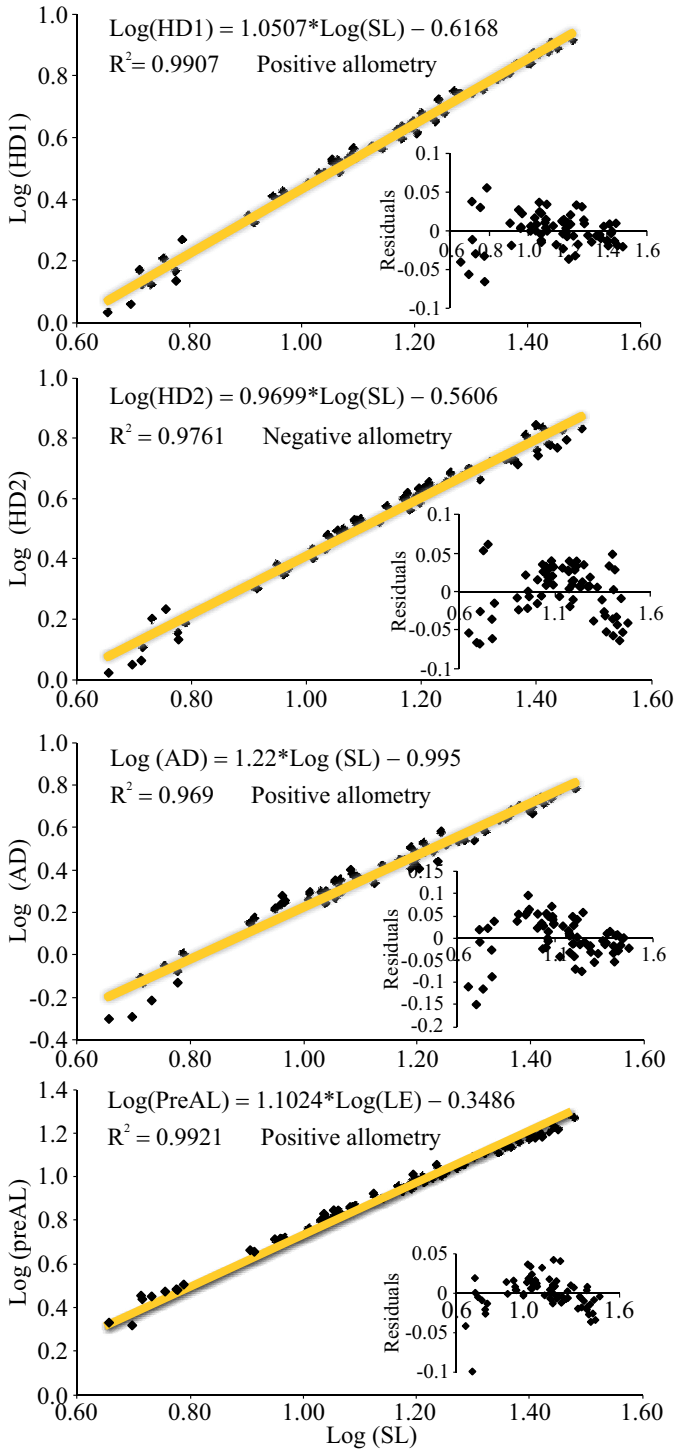
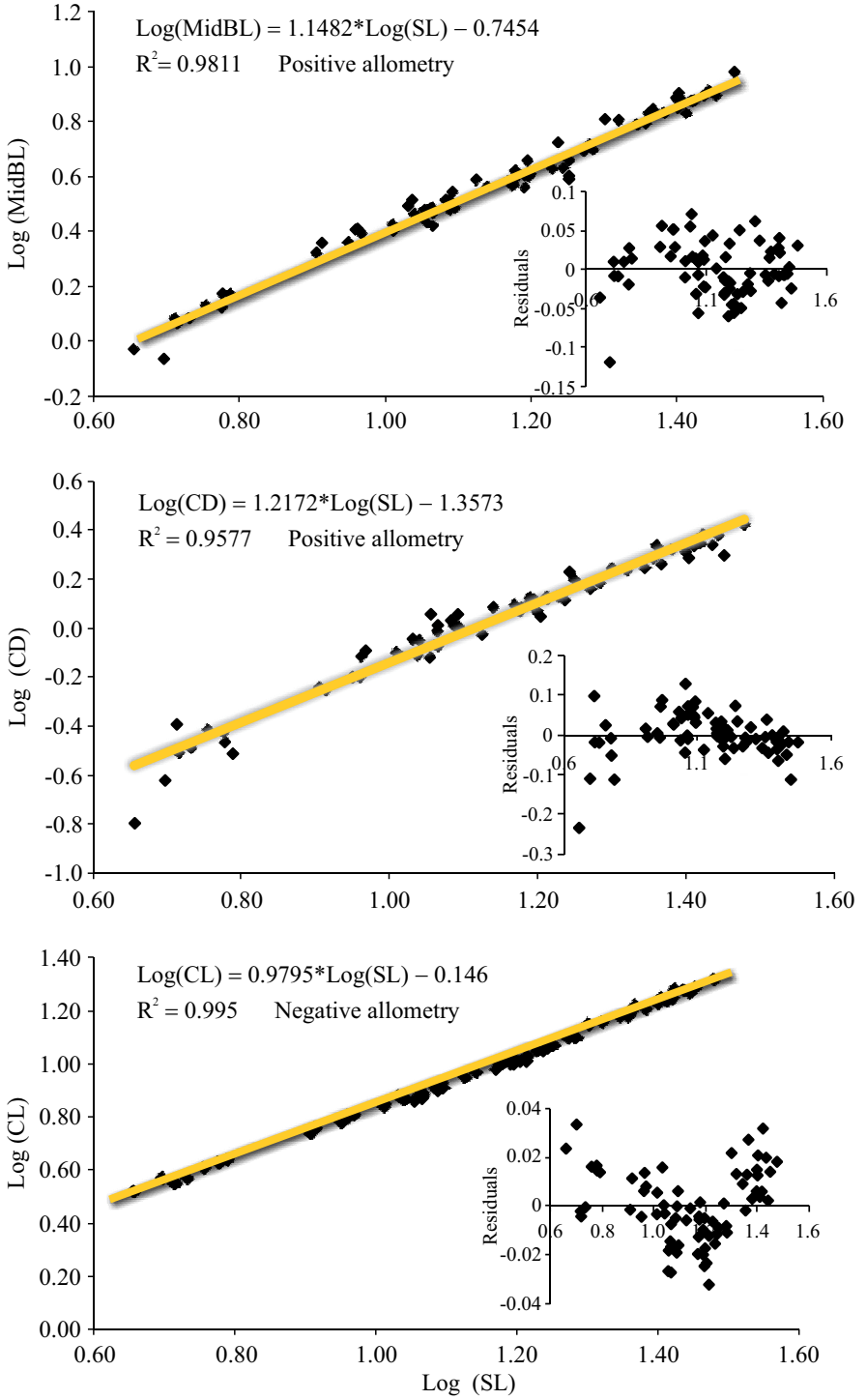
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