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Allometric growth pattern and morphological changes of green terror *Andinoacara rivulatus* (Günther, 1860) (Cichlidae) during early development: Comparison of geometric morphometric and traditional methods

Moshayedi F.[1]**; Eagderi S.**[1*]**; Rabbaniha M.**[2]

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Abstract

Allometric growth pattern and body shape changes of the Green terror (*Andinoacara rivulatus*)(Cichlidae) were studied using landmark-based geometric morphometric (GM) and traditional methods, from hatching up to 1266 Hours Post Hatching (HPH) under culture conditions. The left side of specimens were photographed using digital camera and morphometric characters, including total length, head length, tail length, trunk length, eye diameter, snout length and body depth were measured using ImageJ software. In GM method, ten landmark-points were digitized on 2D pictures. Allometric growth patterns were calculated as a power function of total length and described by the growth coefficient to reveal important steps in the species' early life history. The scores of relative warp analysis (RW) were used as descriptors for the variation in shape. The growth patterns obtained by both traditional morphometric (TM) and GM methods showed similar patterns, but GM showed effective results to interpret the morphological changes and revealing larval stages based on the body shape change. The results also showed higher growth rate of head and tail regions up to yolk sac absorption following by isometric patterns, after begin of exogenous feeding. Based on the findings, the early development of this species can be divided into five stages based on its morphology, including newly hatching larvae (up to 48 HPH), younger larvae (156 HPH), older larvae (426 HPH), younger juvenile (666 HPH) and juveniles. The results confirmed this fact that morphological development and growth patterns during early life stages in *A. rivulatus* closely match its immediate required function.

Keywords: Aquarium, Relative warp, Ontogeny, Morphometrics, *Andinoacara rivulatus.*

1-Department of Fisheries, Faculty of Natural Resources, University of Tehran, Karaj, P.O. Box 4111, Iran.
2-Iranian Fisheries Science Research Institute (IFSRI), Agricultural Research Education and Extension Organization (AREEO), Tehran, Iran.
*Corresponding author's Email: soheil.eagderi@ut.ac.ir

Introduction

Fish larvae often experience very complex processes of morphogenesis and differentiation during early developmental stages (Osse, 1990; Osse and Van den Boogaart, 1995, 1999; Gisbert, 1999; Loy *et al*., 2001; Hasanpour *et al*., 2015). Developments of organs and changes in morpho-anatomical characters occurred in a stepwise fashion, which is regulated by gene expression and influenced by the environmental parameters (Gilbert and Bolker, 2003). Differential relative growth is defined as allometry that describes how the morphological characteristics of an organism change in relation to each other and body size (Fuiman, 1983; Osse and Van den Boogaart, 2004). During the early development, alternations in body shape are related to the function of different organs such as respiration, feeding and swimming (Simonovic *et al*., 1999; Russo *et al.*, 2007).

Many works have investigated the change of body shape during early ontogenic stages in various fish species using traditional morphometric approaches but recently, geometric morphometric techniques have been applied (Bookstein, 1991; Rohlf, 1998; Zedditch *et al*., 2004). Geometric morphometric method is a useful tool in the field of developmental biology to extract shape data and analyze those data using multivariate statistical tests, showing how morphological structures are generated (Zelditch *et al*., 2004) by visualization techniques.

The Green terror, *Andinoacara rivulatus* (Günther, 1860), is a colorful freshwater fish of the family Cichlidae which its origin is from the Pacific side of South America in the coastal waters from the Tumbes River in Peru to the Esmeraldas River in Ecuador (Fishbase, 2016). This species is not listed on the IUCN Red List (Freyhof and Kottelat, 2008; CITES, 2013; IUCN, 2014). The green terror is a commercially valuable aquarium species in ornamental fish industry. Regarding a remarkable value of this species, its breeding and rearing are increasing (Lambert, 1998; Reis *et al*., 2003). Therefore, understanding of its growth pattern during early developmental stages can help producers by providing a guideline to optimize hatchery production (Koumoundouros *et al*., 1999; Van Maaren and Daniels, 2000). Hence, this study was conducted to investigate the changes of the body shape in Green terror during early developmental stage using geometric and traditional morphometric techniques. The results of this study can help to better understanding of shape change related to increasing size of this species as indicators of developmental priorities. In addition, this study can give an insight into the efficiency of these two methods to investigate the allometric growth patterns of fish species.

Material and methods

Specimens rearing and sampling

In 2014, two adult pairs of *A. rivulatus* were introduced into two 120 liter glass aquaria with proper aeration, equipped with the vase pots as spawning bed. Hatching occurred on female’s mouth and then larvae were removed and transferred to two separate 60 liter glass aquaria. The water parameters during raring period, including pH, hardness and temperature were kept 7.5, 5°KH (Carbonate Hardness) and 22°C, respectively. Larvae were fed with a mixture of *Artemia* nauplii and commercial food pellet (Biomar) twice aday.

A total number of 156 green terror larvae from hatching were collected as following: 18, 24, 48, 60, 72, 96, 120, 132, 156, 180, 204, 228, 258, 288, 330, 352, 378, 426, 498, 570, 666, 788, 906, 1026, 1146 and 1266 Hours Post Hatching (HPH). At each sampling phase, six specimens were removed and anaesthetized using 1% clove powder solution. Then, the left sides of specimens were photographed by a stereomicroscope equipped with a digital camera (Cannon) with 5 MP resolution. The larvae were fixed in 5% buffered formalin and then stored in 70% ethanol after 24 hours. For better observing and contrast, the specimens were stained using Toluidine blue.

Development of external morphology

The external morphological changes during early development were examined using a stereomicroscopy (Leica) with 40X magnification and the observed morphological changes were described up to 1266 HPH.

Allometric growth pattern (based on traditional morphometric method)

To study the allometric growth pattern using a traditional morphometric method, the total length (LT), head length (HL), tail length (TL), trunk length (TrL), eye diameter (ED), snout length (SnL) and body depth (BD) were measured from obtained images of specimens using Image J software (version 1.240) (Fig.1). The allometric growth patterns were calculated as a power function of total length using non-transformed data: $Y=\alpha X^b$, where Yis the independent variable, X is the dependent variable, α is the intercept and b the growth coefficient. Isometric growth, positive and negative allometric growths are indicated by $b=1$, $b>1$, $b<1$, respectively (Zelditch *et al*., 2004). The inflexion points of growth curves were determined according to Fuiman (1983) and Van snik *et al*. (1997). Drawing plates and data analysis were performed in MS Excel 2007 (Microsoft Corporation).

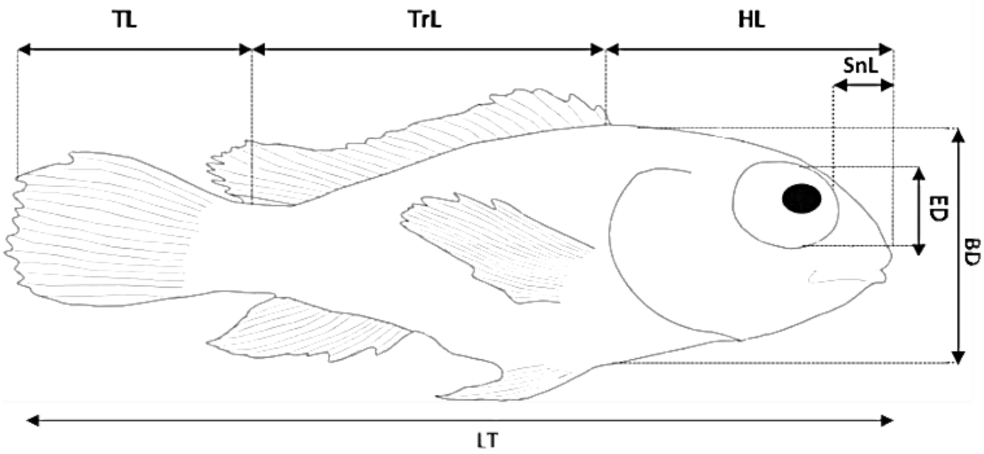

Figure 1: Measured morphometric characters in *Andinoacara rivulatus* from hatching up to 1266 HPH.

Body shape change (based on geometric morphometric (GM) technique)

To study the body shape change using GM approach, 10 landmark-points were defined and digitized on the specimens' pictures using tpsDig2 software (Rohlf, 2005) (Fig. 2) as follows: (1) anterior tip of the snout at the level of the upper jaw; (2) center point of the eye; (3) dorsal edge of the eye vertical to the eye center; (4) ventral edge of the eye vertical to the eye center; (5) dorsal edge of the head vertical to the eye center; (6) posterior of the caudal peduncle; (7) ventral point of the anus; (8) ventral edge of the head vertical to the eye center; (9) posterior end of the mouth slit; (10) dorsal margin of the trunk vertical to the anus. The selection of landmark-points was due to the deficiency of anatomical structure, especially during early developmental stage. The data was tested using tps Small software (Rohlf, 2005) to confirm suitability of the procrust distance instead of the tangent distance for further analysis. Procrust distance (PD) is a metrics for shape dissimilarity in GM technique (Bookstein, 1996). The landmark data were analyzed using Generalized Procrust Analysis (GPA), to remove non-shape related information. Then data were analyzed using relative warp analysis that is analogous to a principal component analysis (PCA) for the landmark-based shape data (Rohlf, 1993).

The relative warp scores (RW1 and RW2) were used as descriptors of the body shape variations (Russo *et al.*, 2007). Growth trajectory was computed by plotting RW1 and RW2 against TL. The correlation between shape descriptors and total length (TL) was tested with 1000 random permutations using tps Reg software.

A cluster analysis was performed on the procrust distance computed from ten landmark configurations of the consensus shape of each growth phase, i.e. sampling group, using Ward's algorithm, to identify clusters between age or size groups.

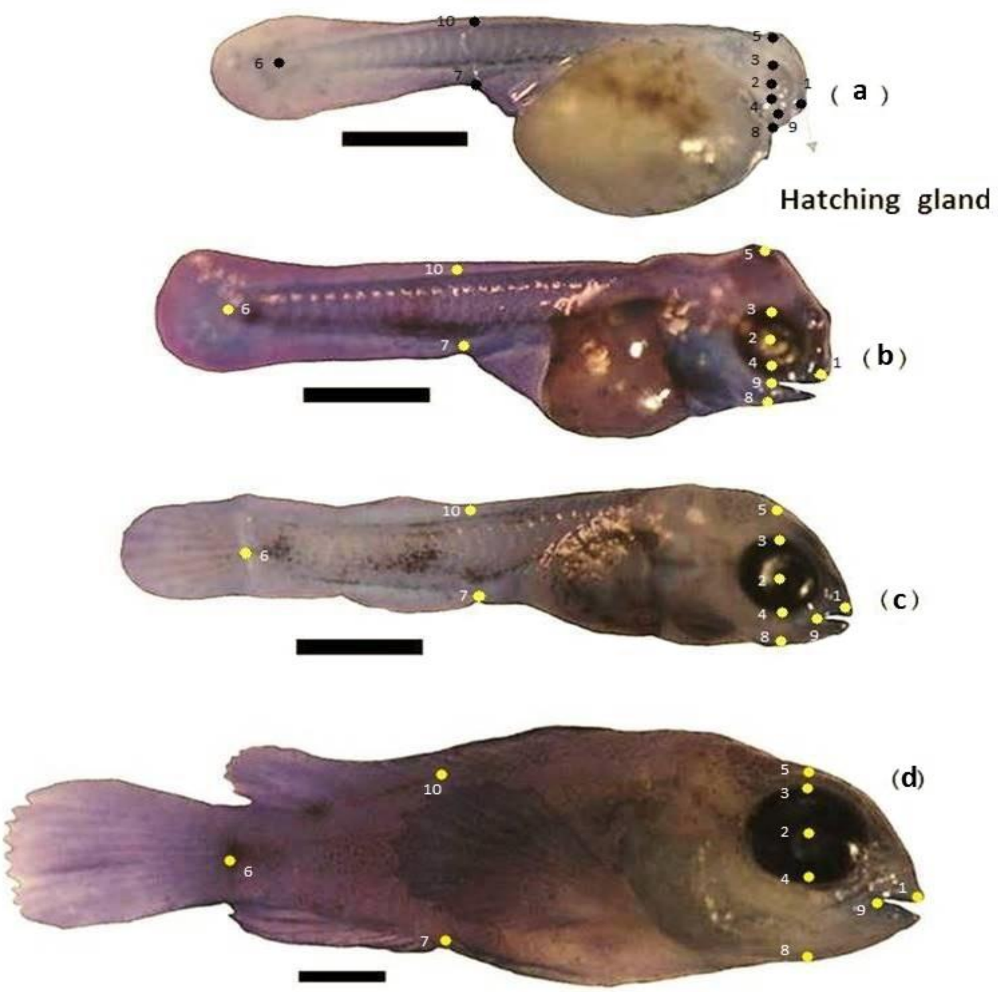

Figure 2: Early life stages and used landmark points in analysis of the body shape ontogeny of *Andinoacara rivulatus.* (a) 18 HPH, (b) 96 HPH, (c) 204 HPH and (d) 1266 HPH (scale bar= 1mm).

A Multi-Response Permutation Procedure (MRPP) was performed to test differences between development intervals. MRPP is a non-parametric procedure used to test the null hypothesis of no difference between two or more groups of entities that must be created a priori (Legendre and Legendre, 1998).

Results

Morphological development

During early life stages, at the hatching till 60 HPH, the mouth was not yet opened (Fig. 2 a). Larvae bear a large yolk sac and oil-droplet from hatching till 120 HPH. Although, between hatching and 120 HPH, most of the yolk sac was consumed, a relatively large oil-droplet was observed on the abdominal region up to 180 HPH.

The hatching gland was absorbed along with the absorption of yolk sac (Fig. 2a). The pigmentations on the external body surface were distinguished as dispread from hatching up to 108 HPH, especially on the abdominal region and head. Thus the pigmentations were increased particularly on the dorsal area of the body. The squamation in larvae occurred after 156 HPH as an important event during early developmental stage (Fig. 2c). At 352 HPH (TL=6.06 mm), dorsal and anal fins were observed.

The first observation of the pectoral fin formation was after 48 HPH. The primordial fin was observed till 330 HPH and then the formations of the dorsal and pelvic fins occurred. Pigmentation of the eye was observed after 48 HPH. Inflection of the notochord was occurred almost before formation of first caudal fin ray at 96 HPH. The complete formation of the caudal fin was occurred at 156 HPH.

Allometric growth pattern

Newly hatched larvae were 4.40±0.09 mm and 0.002±0.005 mg, in LT and BW, respectively, and increased to 10.72±2.65 mm and 0.1±0.08 mg at 1266 HPH. Analysis of the body characteristics showed that growth of all body segments can be divided into two phases (Fig. 3 and Table 1). The growth pattern of the cephalic region, including SnL, ED and HL showed a strongly positive allometric growth pattern in relation to TL prior to their inflection points at 5.54 (132 HPH), 6.22 (498HPH) and 6.7 (570 HPH) TL (mm), respectively, after the inflexion points, their relative growth pattern changed to be nearly isometric (Fig. 3 a, d, f and Table 1). The growth pattern of BD showed a relative isometric growth pattern (96 HPH) during inflexion stage at 5.33 mm LT and then had a positive pattern. Allometric growth of TL and TrL showed positive and negative patterns, respectively, during the flexion stage at 5.78 and 6.7 mm TL, respectively. They had negative patterns at the post-flexion period (Table 1).

Body shape change

The total length of examined specimens ranged between 4.4 mm and 10.71 mm. The first four relative warps explained 71.21% of the body shape (RW1=59.4%, RW2=11.81%, RW3=9.37% and RW4=7.15%). Fig. 5 displays the morpho space defined by RW1 and RW2 based on the consensus shape data of each sampling groups. The specimens are spread along RW1 according to age (youngest specimens on the left side of the graph, the older ones on the right side). RW1 (from +RW1 to -RW1) reflects (1) elongation of caudal length and (2) increasing the head depth and length, whereas RW2 (from -RW2 to +RW2) indicates (1) increasing body depth, (2) increasing eye diameter, (3) increasing head depth and length and (4) relative decrease of tail length.

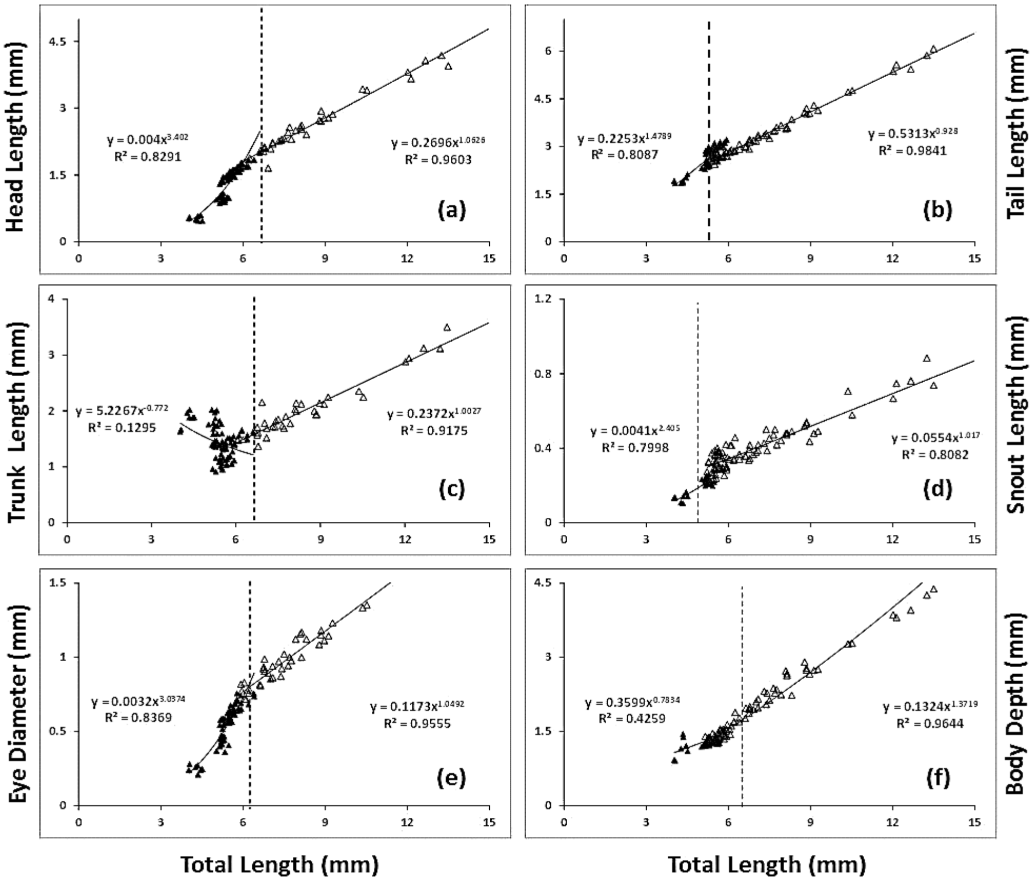

Figure 3: Growth allometries of the different body segments of *Andinoacara rivulatus* from hatching up to 1266 HPH. (R^2=correlated coefficient).

Table1: Growth coefficients slope (*b*) and intercept (α) and R^2 values of metric variables in 156 specimens of *Andinoacara rivulatus* before and after inflection point.

	Before inflection point				**After inflection point**			
	B	α	R^2	***p*- value**	***b***	α	R^2	***p*- value**
Head length	3.402	0.004	0.829	*	1.062	0.269	0.960	*
Trunk length	0.772	5.226	0.129	*	1.002	0.237	0.917	*
Eye diameter	3.037	0.003	0.836	*	1.049	0.117	0.955	*
Tail length	1.478	0.225	0.808	*	0.928	0.531	0.984	*
Snout length	2.405	0.004	0.799	*	1.017	0.055	0.808	*
Body depth	0.783	0.359	0.425	*	1.371	0.132	0.96	*

*significantly different ($p<0.05$)

The regression models account 70.95% of the external shape, and the Goodal F test (p<0.0001) showed a good relationship between body shape variables and total length during early development. The RW1-scores strongly correlated to the total length (R^2=0.717 and p<0.0001) (Fig. 5).

The inflection point corresponds to an age of 132 HPH (5.54 mm TL) (Fig. 6). Based on the inflection point, the body shape change of *A. rivulatus* during early developmental stage can be divided into two phases, including (1) pre-inflection body shape change occurs mostly along RW2 axis showing relative increasing of the caudal length and head depth and (2) post- inflection body shape change along RW1 that shows decreasing of the tail length and increasing of the head and body depth and length. As shown in Fig. 5, the body shape of the *A. rivulatus* changes to a deeper body and head, from early developmental stages up to 1266 HPH. In addition, upper shift of the eye was observed.

The cluster analysis applied to landmark data of the consensus shape of each sampling group discriminates five groups (Fig. 7), which are recognized and named as following based on their important morphological characteristics: (1) newly hatched larvae consisting specimens with yolk sac, (2) younger larvae characterized by opened mouth and lacking yolk sac, (3) older larvae, characterized by squamation and formation of the fin rays, (4) younger juvenile distinguished by distinction of all fins and (5) juvenile referred to specimens approaching miniature form of the adults.

The comparisons of allometric growth patterns of the head, trunk and tail in different larval stages were depicted in Fig. 8, showing the differential growth rates in relation to the body size. During the newly hatched larval stage, the growth rate of the head was higher whereas, that of the trunk was the lowest. During the next stages, i.e. younger larval stage, the allometric pattern of the head was positive and began to decrease becoming almost isometric in the juvenile stage. At the younger larval stage, the growth rate of the trunk began to increases, becoming positive and reaching its maximum development at older larvae stage. During all growth stages, the tail shows an almost isometric allometric rate with the exception of older larval and juvenile stage, showing negative allometric growth patterns. During the younger juvenile and juvenile stages, the main growing region of the body was trunk showing a deeper body shape.

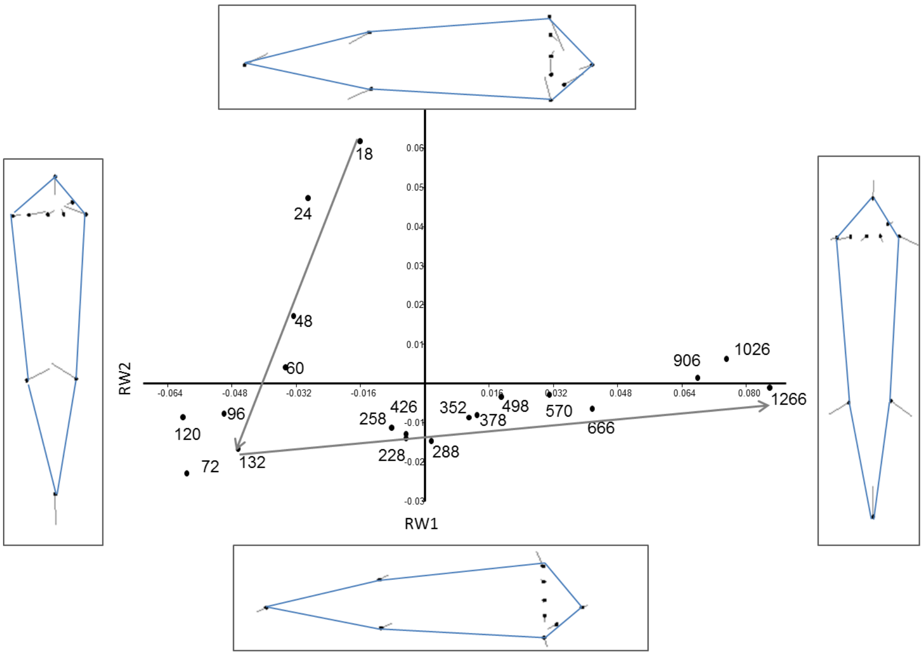

Figure 4: Scatterplot depicting the ordination of *Andinoacara rivulatus* specimens on RW1 and RW2 from 18 HPH up to 1266 HPH (vectors show directions of the body shape change along the axis of the ordination plot).

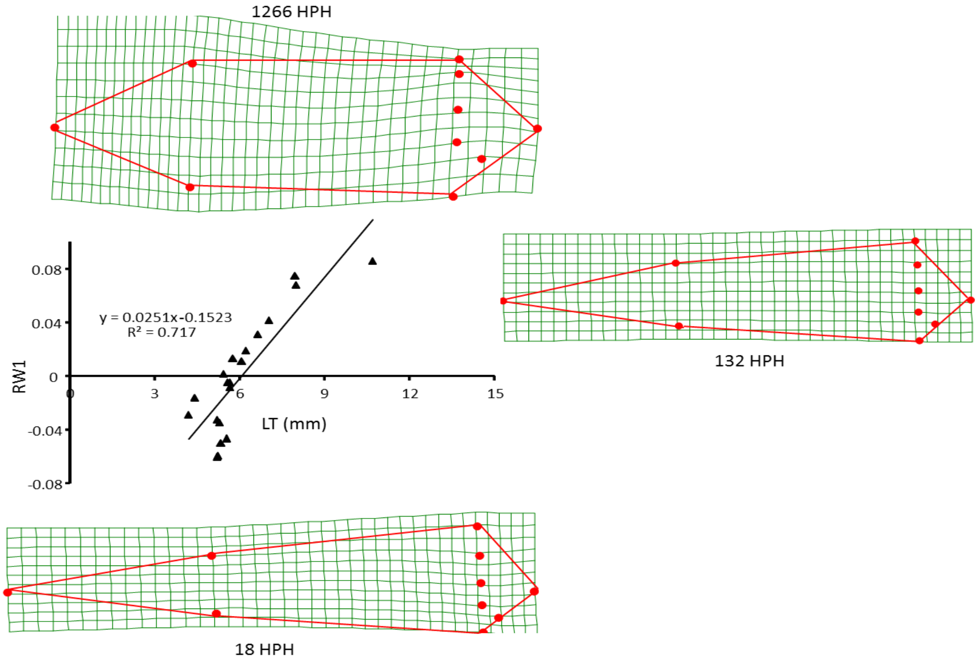

Figure 5: Growth trajectory from hatching up to 1266 HPH in *Andinoacara rivulatus*: Rw1 and LT. The bottom, upper and the right deformation grids are the shape of specimens at the beginning (18 HPH), end (1266 HPH), and inflection point (132 HPH), of developmental stages, respectively.

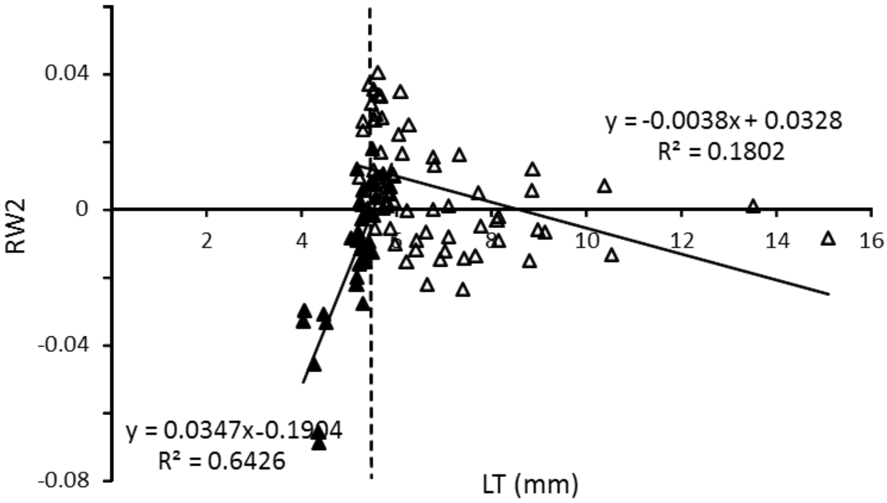

Figure 6: Relative warp 2 (RW2) versus total length (TL) after calculation of the inflection point in *Andinoacara rivulatus.*

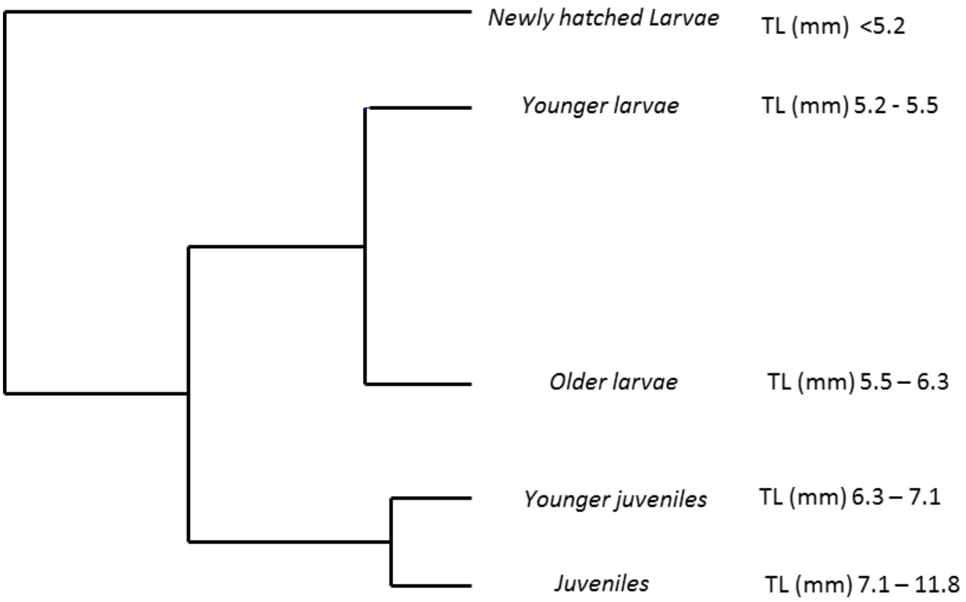

Figure 7: Dendrogram of the cluster analysis performed on ten landmark configurations of the consensus shape of each sampling group in *Andinoacara rivulatus*.

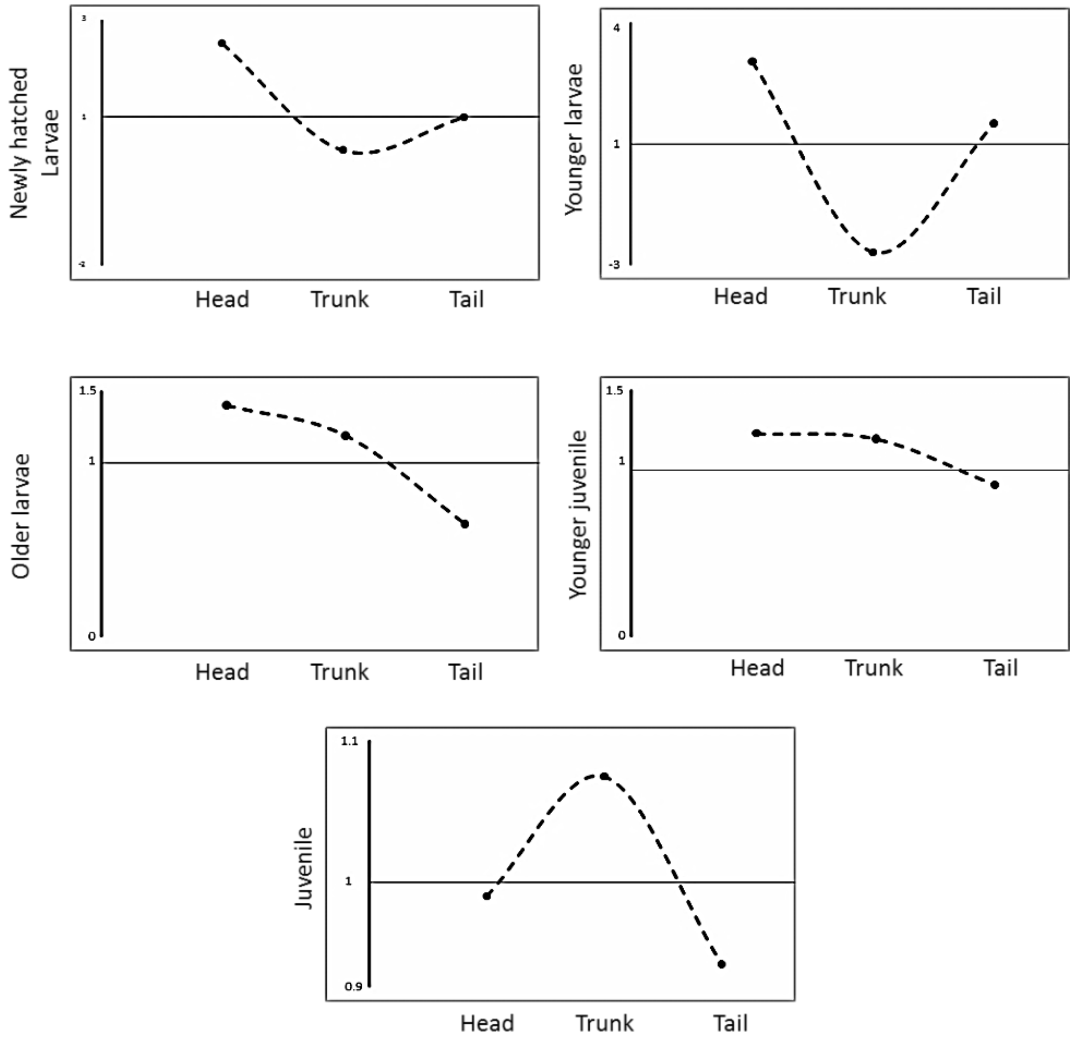

Figure 8: Changing growth coefficients (slope *b*) of head, trunk and tail region of in *Andinoacara rivulatus* in relation to total length (TL) in the different larval stages.

Discussion

At the hatching, the majority of structures and functional systems of Green terror, including mouth, gills, fins and scales had not yet fully differentiated. Development of the functional systems in the recently hatched larvae are occurred in a relatively short period, suggesting that growth functionally optimizes the survival as a common feature among teleost fish to interact with environmental conditions (Gisbert, 1999).

The growth patterns obtained by both traditional morphometric (TM) and GM methods showed similar pattern of the body change during early developmental stage. TM data are measurements of lengths, depths and widths. A problem of TM data sets in this study was that information about body shape change during early development can only derived from separate body segments based on their growth coefficient (*b*) in relation to total length. Consequently, complex aspects of shape change are not addressable. In contrast, GM approach was highly effective to address more complex aspects of the body shape change by visualization. The potential to visualize shape changes by deformation grids showed that more morphological alternations in compare to TM approach. In addition, GM provided only one inflection point showing main stage of the body shape change that can indicate important stage of larval development as a guideline to optimize hatchery production (Koumoundouros *et al*., 1999; Van Maaren and Daniels, 2000). Furthermore, GM technique could reveal different larval stages based on the body shape change of larvae corresponding to various morphological changes.

Based on the results, both the growth trajectories obtained by GM and TM reflect the rate of shape changes are very intensive during ontogeny similar to many teleost fishes (Osse and Van den Boogaart, 1995; Klingenberg, 1996; Loy *et al*., 1998) and decrease with growth until an apparently stable relationship between shape and size. Based on the larval stages obtained using GM method, the first two developmental stages i.e. newly hatched and younger larvae show the U-growth profile. This profile shows positively, negatively and isometrically allometric growth in head, trunk and tail regions, respectively, revealing the initial efforts to complete the most essential priorities during early ontogenic stages. The allometric growth pattern of the head was very high and positive during earlier developmental stages i.e. at first four stages up to younger juvenile with a decreasing trend. Higher growth rate of the head region is due to its vital structures such as brain, gill, mouth and eye, related to important function such as sense, feeding and respiration (Osse and van den Boogaart, 1995, 2004). During first stage, mouth opening was also occurred, and the yolk sac was depleted at the end of this stage. Yolk sac is an endogenous food reserve for larvae that is used for development and energetic purposes (Koumoundouros *et al.*, 1999; Russo *et al.*, 2007). Moreover, along with the absorption of yolk sac, the larvae must switch to exogenous feeding, and thus they need a functional food intake apparatus (Van Snik *et al*., 1997), which in this species, the exogenous feeding was started on 3 dph. The positively allometric growth of tail in the two first stages of *A. rivulatus* can reflect its priority after head development that is related to vital functions such as feeding, improving swimming ability and predator avoidance (Osse and Van den Boogaart, 2004) and has been explained as an adaptation to reduce and optimize the energetic costs of larval transport as well (Osse and Van den Boogaart, 1995, 2004). Therefore, the results showed higher growth rate of head and tail region up to yolk sac absorption following by an isometric patterns, after begin of exogenous feeding.

Body depth showed a relative isometric growth pattern during newly hatched and younger larvae and then a positive pattern despite the negative allometric pattern of trunk length reflecting to obtain a deeper body shape from a fusiform one. This can be associated with development of internal organs such as digestive tract and related glands to increase enzymatic activities (Pena and Dumas, 2009).In addition, a deep body can help a rapid turning and maneuvering in tight quarters as rocks and eelgrass bed and reduction of costs of locomotion (Ontario, 2011; Van Snik *et al*., 1997). In nature, *A. rivulatus* inhabits tropical rivers, mostly still waters of both turbid and clear flowing stream basins bearing aquatic weeds where they feed on worms, crustaceans, and insects (Lamber, 2001), therefore, obtaining such a body shape can be an adaptation to its life style suggesting that growth functionally optimizes the survival (Osse *et al*., 1997) and adaptation to the environmental conditions (Dettlaff *et al*., 1993)

The strong positive allometric growth in eye diameter could be indicated the growth of brain and enhancement of vision (Wainwright and Richard, 1995). Moreover, visual development was considered an indicator of development and differentiation of neural and sensorial structures, which would allow the larvae to react to light stimuli, detect prey and potential predators in water column (Gisbert *et al*., 2002; Gisbert and Doroshov, 2006).

In conclusion, the present study showed the importance of morphological modifications occurred during *A. rivulatus* early life stages, showing that these changes are in agreements with functional demands throughout ontogeny and its adaptation to adult life style. In addition, morphological analyses of this study provided valuable information appropriating to improve rearing of this ornamental species.

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