SHAPE CHANGES IN THE EXTERNAL MORPHOLOGY DURING EARLY DEVELOPMENT OF THE OHRID TROUT (SALMO LETNICA KARAMAN, 1924)

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Abstract:

Early ontogenetic shape changes in Ohrid trout were studied, in order to reveal patterns of growth allometries that may reflect important early life history phases. Shifts in growth rates, and thus growth allometries are known to reflect transitions from the endogenous to exogenous feeding. This study relied on a landmark based, geometric morphometric analysis of nearly 120 specimens of Ohrid trout at different stages of ontogeny. Sampling was done from 25 days post hatching (when about 2/3 of the yolk sack had been resorbed) till 405 days post hatching, and specimens were grouped in 32 age groups. Twenty body landmarks were used as shape descriptors, whereas size was measured using centroid size. To estimate the shape changes during ontogeny, the mean Procrustes distance between all specimens of equal age, for all age groups, was calculated. A relative warp analysis showed that certain aspects of ontogenetic shape changes were not continuous, but indicated the existence of an inflection point during early ontogeny. The shape changes were mainly related to allometries in the head, caudal peduncle, and the ventral abdominal profile.

Key words: body shape; ontogeny; morphometrics; Salmonidae

Introduction

Fish larvae often go through very complex processes of morphogenesis and differentiation during growth. Since all systems grow at different rates, allometry is a common feature during larval development (Osse and van den Boogaart, 1995). Allometric processes are an important factor in defining the final shape variation in fishes (Loy et al., 1998; Reis et al., 1998). During their growth fishes experience a change in shape in relation to increasing their ability to perform vital biological roles, such as respiration, feeding and locomotion. Body structures develop according to their importance for primary functions (Osse and van den Boogaart, 1995; Simonovic et al., 1999; Russo et al., 2007). Knowledge about morphological development and growth patterns of young fish seem to be of importance as they may allow us to understand better the patterns underlying early life stages, their priorities during early growth, size related adaptations and provide insight into fish biology, behavior and ecology (Gisbert, 1999).

The study of body shape of organisms has been a subject of research for over a century. Only recently, geometric morphometric methods have been used to understand body shape changes and its associated allometry during ontogeny (Bookstein, 1991; Rohlf, 1998; Zelditch et al., 2004). The methods of geometric morphometrics combine the powerful and flexible tools of multivariate statistics with explicit consideration of spatial relations between parts and therefore make it possible to investigate morphological variation with direct reference to the anatomical context of the structure under study (Klingenberg et al., 2002). In the present paper we used geometric morphometrics to map quantitatively and qualitatively the

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ontogenetic shape changes in Ohrid trout during the transition from the endogenous to exogenous feeding period. The main goal is to see whether different rates of shape change exist and to what degree do they reflect distinct early life history phases.

**Material and Methods**

The samples of Ohrid trout were taken from the artificial spawning tanks located in the Hydrobiological Institute, Ohrid. The samples were raised in special incubators, with a constant water temperature of 10°, 5° C, corresponding to the lake environment. For this study, 122 specimens from different stages have been collected (about four samples for 32 different stages). The sampling was done from 25 days post hatching (when about 2/3 of the yolk sack had been resorbed and feeding up of the trout started) till 405 days post hatching. This time interval was chosen because in these stages the examined specimens share the same anatomical structures that could be used for the shape analysis. The specimens were anaesthetized, using an MS-222 (methanosulfate salt, 3-aminobenzoic acid ethyl ester) overdose, fixed in 10% neutral formalin and stored in 70% ethanol. Before fixation, the total length (TL) was measured in mm using calipers (accuracy of 0.05 mm). For this study, the TL for all examined specimens ranged between 22.2 mm and 94 mm TL.

For the morphometric analyses of the overall body shape, images were made using a HP Scan Jet 55-30 (resolution 2400 dpi). Twenty landmarks were digitized to follow and describe the body shape changes (Fig. 1): (1) anterior tip of the snout at the level of the upper jaw; (2) centre point of the eye; (3) posterior margin of the neurocranium; (4) dorsal point of the gill slit; (5) indentation where the opercular cover and sternohyoideus muscle cross; (6) antero-dorsal point of pectoral fin base; (7) postero-ventral point of pectoral fin base; (8) rostral point of the pelvic fin base; (9) rostral point of the anal fin base; (10) caudal point of the anal fin base; (11) rostral point of the ventral rim of the caudal peduncle; (12) middle of caudal peduncle; (13) rostral point of the dorsal rim of the caudal peduncle; (14) caudal point of the adipose fin base; (15) rostral point of the adipose fin base; (16) caudal point of the dorsal fin base; (17) rostral point of the dorsal fin base. As a result of the preservation, some of the specimens were flexed in a sagittal plane (flexion in a horizontal plane was removed by flattening the fish manually under the scanner). To avoid this flexion the "unbending landmarks" procedure was applied (TpsUtil, Rohlf, 2005). For that reason three unbending landmarks were digitized. The first one (landmark 18 in the Fig. 1) was the middle point of the line going from the caudal part of the head to the bottom of the head. The second landmark (landmark 19 on the Fig. 1) was the middle point of the line going from the rostral point of the dorsal fin base to the bottom of the body. The third landmark (landmark 20 on the Fig. 1) was the middle point of the line going from the rostral point of the adipose fin base to the bottom of the body. These lines were made in Corel Draw 11 (Corel Corporation), before digitizing of all landmarks. Based on the “unbending landmarks” and other morphological landmarks in tpsUtil (Rohlf, 2005) the new coordinates were calculated.

The digitization of the landmarks was done using tpsDig (Rohlf, 2005). All non-shape related information was removed from the dataset by applying the Generalised Procrustes Analysis (GPA), thus also removing size (Rohlf and Slice, 1990). Size was measured as an independent variable using centroid size. The data was tested using tpsSmall (Rohlf, 2005), which confirmed the suitability for further analysis. TpsRelw was used to perform a principal component analysis on the partial warp scores, yielding relative warp scores as descriptors for the variation in shape (Bookstein, 1991; Rohlf, 1993, 2005). The most important shape changes were visualised by generating deformation grids. The amount of shape variation being explained by size (centroid size, CS) was tested by performing a permutation test with 1000 random permutations (software tpsRegrw; Rohlf, 2005). The CS was log transformed because the allometric relationships are better described by a model that takes into account the progressive decrease of the rate of shape change during growth (Zelditch et al., 2004). Procrustes distance (PD), the generally accepted metric for shape dissimilarity in geometric morphometrics in Kendall shape space (Bookstein, 1996) was used as an univariate measure of shape (actually a shape difference between a specimen and the average consensus configuration), which provides a summary of underlying complex patterns of shape variation. Dynamics of quantitative shape changes during ontogeny are visualized by plots of PD (between each specimen and the shape calculated based on the four smallest specimens) on CS. The inflection points were calculated by the methods of Fuiman (1983) and Van Snik et al. (1997).
Results

The first five relative warps explained 72.49% of the body shape changes (RW1 39.50%, RW2 16.37%, RW3 7.24%, RW4 5.62% and RW5 3.76%). Figure 2 represents the morphospace defined by RW1 and RW2, which together explain 55.87% of the total body shape during ontogeny. In this morphospace the specimens are clearly spread along RW1 according to age (youngest specimens on left side of graph; older ones on right side) (Figs 3, 5). Other important trends in shape ontogeny of Ohrid trout are expressed by RW2 (Fig. 4, 6). Over the examined period, RW2 (going from - RW2 to + RW2) reflects: (1) elongation of the head (LM 1, 3, 4 and 5); (2) posterior shift of the eye with respect to an elongation of the head (LM 2) and (3) positive allometry of the caudal area especially evident by the posterior shift of landmark 12.

The regression models account for 34% of the external shape and the Goodall F test (p < 0.001) shows that there is a good relation between body shape variables and log of CS during the whole ontogeny. The RW1-scores also strongly correlated to the log of centroid size (p< 0.001; R² = 0.8094) (Fig. 5). In comparison with RW1, RW2 is not correlated with the log of CS (Fig. 6). However, in this case a clear inflection point is present (Fig. 7). This inflection point corresponds to a CS of 0.615, which corresponds to an age of 92 dph (29.7 mm SL). As such, it seems that ontogenetic shape changes in Ohrid trout follow a disrupt pattern with two phases: (1) pre-inflection shape changes, which include the formation of the adult adipose fin and elongation of the head and (2) post-inflection shape changes, which include elongation of the caudal region.

Throughout the examined period, RW1 (going from – RW1 to + RW1) (Fig. 3) explains: (1) a decrease of the primordial fin fold margin at the level of the adipose fin (LM 14 and 15); (2) decrease of the primordial finfold margin at the level of the dorsal and anal fin (LM 10, 11, 15 and 16) and (3) a relative increase in the body depth (LM 8 and 17).

In a way to follow the dynamics of the shape changes during the ontogeny, the mean Procrustes distance (PD) (between all specimens in one stage, as well as, PD between all specimens during ontogeny) against the age was calculated (Fig. 8) and PD (between specimens and consensus) against log of CS was calculated (Fig. 9). In figure 8 it can be verified that mean PD did not seem to change according to the age (R²= 0.0739). In this case it must be concluded that variation in shape is quite constant over the whole size range. On the contrary, there is a correlation between PD and log of centroid size (CS) (p< 0.001; R²= 0.7778) (Fig. 9).
Figure 2. Scatterplot depicting the ordination of the 122 Ohrid trout specimens on RW1 and RW2. The two relative warps explained 55.87% of the total variance in shape of the samples. Youngest specimens labeled from 1, older have a higher number.
**Figure 3.** Deformation grid representing body shape variation in Ohrid trout associated with the lowest relative warp 1 scores (-RW1) and with the highest relative warp 1 (+RW1) scores (with respect to the consensus configuration).

**Figure 4.** Deformation grid representing body shape variation in Ohrid trout associated with the lowest relative warp 2 scores (-RW2) and with the highest relative warp 2 (+RW2) scores (with respect to the consensus configuration).
Figure 5. Relative warp 1 (RW1) versus log of centroid size (Log CS)

Figure 6. Relative warp 2 (RW2) versus log of centroid size (Log CS)

Figure 7. Relative warp 2 (RW2) versus log of centroid size (Log CS) after calculation of the inflection point
Discussion

This study showed that most of the shape changes of the Ohrid trout primarily involve the elongation of head, of the caudal area, positive allometry of the body depth and shape changes at the level of the adipose fin (Fig. 3 and 4). During the examined period, the unpaired fins (dorsal, anal and adipose fin) showed a negative allometry. Previous studies allowed us to link the here observed changes in the external morphology to changes of the skeletal structures (Ristovska et al., 2004; 2006 a,b). Internal changes are mainly linked to chondrification and ossification of the axial skeleton and fins. Our previous investigations show that the first structures that develop during the early stages are the structures from the head, pectoral girdle and caudal fin, followed by the formation of the unpaired fins. Present results are in
agreement with those of other studies (Fuiman, 1983; Osse, 1990; Osse and Boogart, 1995; van Snik et al., 1997; Gisbert, 1999; Russo et al., 2007), confirming the hypothesis that morphological development and growth patterns during early life stages closely match primary functions. Predation and starvation are considered to be main agents causing larval mortality, whereas food uptake and early development of swimming organs must be first priority (Osse et al., 1997).

Ontogenetic shape changes in Ohrid trout indicate the existence of an inflection point at 92 dph (Fig. 7), allowing the distinction of two phases. The first phase includes the formation of the adult adipose fin and elongation of the head after which follows the elongation of the caudal region. Gosline (1971) suggested that the adipose fin may be important during the juvenile phase for generating a dorsal thrust vector symmetrical to that of the ventral anal fin during swimming. This could be one of the reasons why in the first stages of the development of the Ohrid trout the adipose fin changes its shape. The elongation of the caudal region after 92 dph could be related to the function of the caudal fin in the subcarangiform swimming mode. According to Lindsey (1978), salmonids achieve propulsion by lateral undulation of the posterior half of the body (subcarangiform swimming mode).

The reported positive allometric growth of anterior and posterior parts of the Ohrid trout body, prior to the development of the trunk region, during the first stages of its development was not surprising, as it is common for most fish species. This ensures that the essential organs for primary functions (feeding, respiration and locomotion) are developed first (Osse and van den Boogart, 1995; Gisbert et al., 2002). Osse (1990) showed that the intestine length only starts to grow positively allometrically when the swimming motion of the larvae has changed from anguilliform to subcarangiform.

It has been suggested that escape performance improves as true fins form (Hale, 1999, Gibb et al, 2006). Approximately around 92 dph most of the structures of the unpaired and paired fins from Ohrid trout have developed and ossified. It has been suggested that escape performance improves as true fins form (Hale, 1999) presumably because true fins stiffened by bony rays create a deeper, thinner, low-mass fin that will improve the transfer of momentum between the body and the surrounding fluid (Weihs, 1973). The 92 dph is a stage when all lateral line canal bones are present (Ristovska et al., 2004). The neuromasts are thought to play an important role in providing sensory feedback during feeding behavior and escaping from predators (Omori et al., 1996). According to Kawamura (1991), sensory canals on the head and trunk begin to differentiate during the larval period and are completely formed at the beginning of the juvenile stage.

In conclusion, the results of this study showed that important morphological and morphometric modifications occur during Ohrid trout early life stages, as the feeding, swimming, sensorial and respiratory systems developed. Based on the degree of skeletal development, it seems that approximately at 92 dph, the trout has attained the juvenile phase. This is a stage when the Ohrid trout possesses most of the skeletal structures and it is accompanied by discrete morphological changes of the body parts. However, to confirm this hypothesis, further investigations in which development of the other systems of the trout, such as the sensory or digestive are included, are required.

References:


