www.trjfas.org
ISSN 1303-2712
Turkish Journal of Fisheries and Aquatic Sciences 14: 863-874 (2014) DOI: 10.4194/1303-2712-v14_4_03

Larval Development of The Freshwater Angelfish *Pterophyllum scalare* (Teleostei: Cichlidae)

İhsan Çelik[1,*], Pınar Çelik[1], Mert Gürkan[2], Tolga Şahin[1]

[1] Çanakkale Onsekiz Mart University, Marine Sciences and Technology Faculty, Department of Aquaculture, 17100, Çanakkale, Turkey.
[2] Çanakkale Onsekiz Mart University, Faculty of Arts and Sciences, Department of Biology, 17100, Çanakkale, Turkey.

* Corresponding Author: Tel.: +90.286 2180018/1532; Fax: +90 286 2180543;
E-mail: ihsanceliktr@gmail.com

Received 31 October 2013
Accepted 21 October 2014

Abstract

In this study, the larval development of freshwater angelfish, *Pterophyllum scalare*, was described under controlled aquarium conditions. Major histomorphological changes and the allometric growth patterns during the larval development have been described. The larvae were obtained from three pairs of freshwater angelfish, which were maintained in glass tanks. The larvae were sampled for measurement and photography. They were observed under a stereomicroscope, photographed using a photomicrographic system. The larval development of angelfish were described from 1 days after hatching (DAH) to 24 DAH. Embryonic developmental stage was completed at day 3 after spawning at 24±1ºC. The newly hatched larvae had 4.24±0.28 mm total length (TL). The mouth opened at 3 DAH. The larvae started to swim actively within the next 3-4 days. Notochord flexion began at 3-4 DAH. The yolk sac has been totally absorbed at 6 DAH. The metamorphosis was completed and the larvae transformed into juveniles at 23-24 DAH.

Keywords: Ornamental fish, aquarium, larval development, allometric growth.

Tatlı Su Melek Balığının (*Pterophyllum scalare*) (Teleostei: Cichlidae) Larval Gelişimi

Özet

Bu çalışmada, tatlı su melek balığının (*Pterophyllum scalare*) kontrollü akvaryum koşullarında larval gelişimi incelenmiştir. Larvalar, cam tankta tutulan üç çift tatlısu melek balığından elde edilmiştir. Elde edilen larvalar ölçüm ve fotoğraflama için örneklenmiştir. Alınan örnekler stereomikroskop altında incelenmiş ve mikro fotoğraflama sistemi ile fotoğraflanmıştır. Melek balıklarının larval gelişimleri, yumurtadan çıktıktan sonraki 1 ile 24 gün arasında tanımlanmıştır. Larval gelişim boyunca gözlenen temel histomorfolojik değişimler ve allometrik büyüme modelleri tanımlanmıştır. Denemede, embriyonik gelişim safhası, 24±1ºC'de yumurtlamadan sonraki 3. günde sona ermiştir. Kuluçkadan yeni çıkmış larvaların 4,24±0,28 mm total boy (TL) uzunluğa sahip oldukları belirlenmiştir. Ağız açılımı, kuluçkadan çıktıktan sonraki 3.günde gerçekleşmiştir. Larva takip eden 3-4 gün içerisinde aktif olarak yüzmeye başlamıştır. Notokorda fleksiyonu açılımdan sonraki 3-4.günlerde başlamıştır. Besin kesesi, açılımı takip eden 6. günde tamamen tükenmiştir. Açılımdan sonraki 23-24. günlerde metamorfoz tamamlanmış ve larva juvenil formuna dönüşmüştür.

Anahtar Kelimeler: Süs balıkları, akvaryum, larval gelişim, allometrik büyüme.

Introduction

Cichlids are the most species-rich non-Ostariophysian fish family in fresh waters world-wide, and one of the major vertebrate families, with at least 1300 species and with estimates approaching 1900 species (Kullander, 1998). Cichlidae family includes freshwater and brackish water fish in Africa (900 valid, estimated more than 1300 species), North America and Central America (111 valid species), South America (291 valid species), Madagascar (17 valid species some of which live in brackish waters), Jordan Valley in Middle East (4 species), Cuba and Hispaniola (4 valid species some of which live in brackish waters) South India and Sri Lanka (3 valid species some of which live in brackish waters) and Iran (one species) (Kullander, 1998; Nico, 2010; Shukla, 2010).

Freshwater angelfish, *Pterophyllum scalare* which originate from Amazon River, Guyana and Orinoco, are one of the most important commercial cichlid species (García-Ulloa and Gómez-Romero

2005; Ortega-Salas *et al.,* 2009). Angelfish are quite popular around the world among aquarium hobbyist (Luna-Figueroa, 2003; Kasiri *et al.*, 2011). Angelfish grow to 15 cm total length and 25 cm total height at the depth of 1-2 m under natural conditions (Mills, 1993; Korzelecka-Orkisz *et al.,* 2012). They were characterized by specific reproductive behavior. The female of *P. scalare* generally lays up to 1000 eggs (Frank, 1984; Sieniawski, 2004; Cacho *et al.,* 2006).Since this species was generally used for behavioral studies, behavior types are described in detail (Saxby *et al.,* 2010). Previous studies on freshwater angelfish mainly concentrated on reproductive behavior (Degani *et al.,* 1997; Cacho *et al.,* 2006), spawning strategies (Cacho *et al.,* 2007; Ortega-Salas *et al.,* 2009), parental care (Degani *et al.,* 1997; Cacho *et al.,* 2006), growth performance (García-Ulloa and Gómez-Romero, 2005; Kasiri *et al.,* 2011a), species-specific diseases (Perlberg *et al.,* 2008; Murphy*et al.,* 2009) and morphology (Groppelli *et al.,* 2003).The literature on reproduction of freshwater angelfish is scare and limited to general descriptions.

Although there is a limited body of research on the first life stages of angelfish (Sakamoto *et al.,* 1999; Kasiri *et al.,* 2011b), there is deficient descriptive data on larval rearing. Larval stage, which is a critical stage in the lives of fish, is of great importance because this period involves functionaland morphological changes in internal organs, tissue systems and particularly in digestive systems (Govoni *et al.,* 1986; Bisbal and Bengston, 1995; Meijide and Guerrero 2000; Elbal *et al.,* 2004; Santamaría Marín de Mateo *et al.,* 2004; Çelik, 2010; Çelik *et al.,* 2011; Korzelecka-Orkisz *et al.,* 2012). This study aims to understand morphologic and histologic changes observed in freshwater angelfish (*Pterophyllum scalare*) during ontogenic stage which lasts for 30 days from hatching and to obtain better rearing data.

Materials and Methods

Broodstock Maintenance

The larvae were obtained from three pairs of angelfish broodstocks, which were kept in 40x40x40 cm aquarium at 50 L water volume. Each pair (one male and one female) spawned spontaneously. After spawning, the same male also spawned with the same female fifteen-thirty days after the first spawning. During the spawning period, one female generally spawned about 300-900 eggs. The broodstocks were fed three times a day with commercial ornamental fish feed (Tetramin Granulat, Tetra, Germany; Protein: 46%, Oil: 12%, Fibre: 3%, Ash: 11%, Moisture: 8%). Each of 50 L production tanks containing the broodstocks were cleaned with simple sponge. Water temperature was kept constant at 24±1ºC with 100 watt heaters placed in each aquaria. 10% water change was performed on daily basis. In broodstock aquariums, water parameters were kept at total general hardness of 6 dH (1 degrees general hardness (dH) = 1,78 mg/L $CaCO_3$), pH 6.5-7.5, conductivity 150-300 µS. Illumination was provided using fluorescent lights (900-1000 lumens) with 11 hours light/13 hours dark (11L/13D) photoperiod application (lights on; 07:00–18:00 hours).

Larval Rearing Protocol

The larvae fed from yolk sac in the first 3-5 DAH. The larvae started to swim actively within 3-4 days and began to feed exogenously. The larvae were kept together with their parents for the 10 DAH and parental care was allowed. The larvae were fed with *Artemia* until the end of the trial (25 DAH) once a day (400-500 µm) (INVE Aquaculture Inc., Dendermonde, Belgium). 10% water change was performed on daily basis in larvae development tank; water temperature was kept at 24±1ºC, pH 6.5-7.5, hardness was kept at 4-7 (ºD) and conductivity was kept at 300-600 µS.

Morphologic Observations

Random larvae specimens (n=5) were collected on daily basis in the first 10 DAH, while one specimen was collected every other day (n=5) from 10 DAH to juvenile period. Larvae specimens collected from the tank alive were anesthetized by clove oil (0.5 ml/L) and were analyzed under stereomicroscope (Tokyo, Japan). The specimens were photographed with the video camera connected to the microscope. Larvae measurements were conducted by image analysis of digital photographs recorded in computer environment. The same technique was used to record general morphologic and morphometric characteristics. Larval development periods were defined according to Kendall, *et al* (1984) and were categorized as I: yolk-sac larva, II: preflexion larva, III: flexion larva and IV: postflexion larva.

Histological Analyses

The larvae (n=5) were sampled on daily basis from hatching to 10 DAH and at every other day from 10 DAH to 30 DAH. These samples were first fixed in Bouin's solution and 70% alcohol; they were dehydrated in a series of alcohol concentration and were then removed from xylene and were treated with paraffin. Wax blocks were cut using 5 mm thick microtomes (Slee, Cut5062, Germany). Sagittal cross-sections were stained with Gill's hematoxylin/eosin (HE) according to general histology procedures. Histology samples, which were fixed to glass slides were analyzed under light microscope to define their larval development and were photographed using a color video camera.

Allometric Growth Measurements

Morphometric measurements during larval development were analyzed by allometric growth models. Allometric growth models were defined by linear regression formula determined by the association of a body part with total length (TL) (Fuiman, 1983; Gisbert *et al.*, 2002). Growth ratios of body characters such as BD (body depth), ED (eye diameter), HL (head length), PAL (pre-anal length), PrAM (pre-anal miomer length), PoAM (post-anal miomer length, SnL (nose length), tail length and body length according to total length were estimated according to, $Y = ax^b$ allometric equation (Figure 1). In this equation Y= dependent variable (measured character), X= independent variable (TL), a= intersection point and b= growth coefficient. As for growth coefficient; it was assumed that growth was isometric if b=1; positive allometric if b>1; negative allometric if b<1 (Çelik *et al.*, 2011).

Results

Morphologic Observations

Angelfish larvae had a yolk sac that was 50% of the total body length; notochord and primordial fin became distinguished. There were three pairs cement glands over the head. No eye, organ and fin differentiation was observed. Pigmentation was almost zero in body parts apart from the yolk sac (Figure 2a).

1 DAH: Mouth and anus are closed. 2 otoliths in otic capsule are clearly visible. (Figure 2b). The proportion of the yolk sac to total length is approximately 30%. Eyes began to differentiate. Notochord and primordial fin are clearly distinguished. Larvae have a semi-transparent look and pigmentation started on yolk sac (Figure 2b).

2 DAH: Mouth and anus are still closed. Pigmentation increased in caudal fin. Yolk sac continues to come smaller. Pigmentation in eyes increased (Figure 2c). Digestive system is clearly visible in the form of a linear tube. Heart is visible in the anterior of yolk sac. Cement glands over the head are still apparent (Figure 2c).

3 DAH: Mouth and anus are opened. Flexion occurred in notochord end. Pigmentation continued to increase. Yolk sac still remains and continues to get smaller. Cement glands on the head are still clearly visible (Figure 2d).

4 DAH: Pigmentation in the eyes is completed. Cement glands are still maintained. Caudal fin rays began to appear. Yolk sac continues to get smaller; pigmentation continues to increase (Figure 2e).

5 DAH: Swim bladder which formed today is clearly visible and extends to the posterior location. Cement glands on the head became smaller. Caudal fin rays continue to be more apparent. Dorsal and anal fin began to differentiate. Pigmentation continues to increase in the general of the larvae. Yolk sac is completely absorbed. Morphologic appearance of the larvae is like on the 6thday (Figure 2f).

7 DAH: Pigmentation continues to increase. Caudal fin rays became more apparent. Cement glands still remain. Swim bladder continues to grow posteriorly (Figure 2g).

8 DAH: Cement glands on the head disappeared on the 8 DAH. Consumed *Artemia* is clearly visible in the stomach. Dorsal and anal fin continued to develop. Swim bladder continues to develop by extending to the posterior of the body.

10 DAH: Dorsal and anal fin rays became apparent. Caudal fin development is about to be completed. Pigmentation continues to increase (Figure 2h).

11 DAH: Dorsal and anal fins continue to develop and extend. Larvae are still semi-transparent.

12 DAH: Pigmentation continues to increase. Undigested *Artemia* eggs in the stomach of semi-transparent larvae are clearly visible. Dorsal and anal fin continue to develop (Figure 2i).

14 DAH: Dorsal and anal fin continue to develop. Black pigmentation in the general of the body significantly increased (Figure 2j).

17 DAH: Dorsal and anal fin of the larvae began to look like fin form of adult individuals (Figure 2k).

18 DAH: Dorsal and anal fins extend and begin to take adult individual form. Pigmentation becomes apparent in the general of the body. In addition to dorsal and anal fins, body form of the larvae begins to look like adult individuals.

23 DAH: Larvae have a characteristic body structure and color specific to its species. Metamorphosis's completed and juvenile stage began (Figure 2l) in these days. The larvae could consume

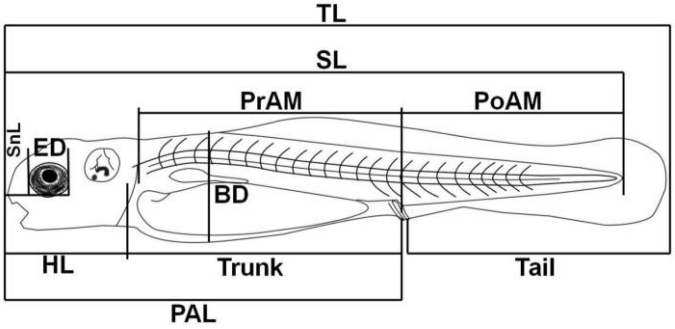

Figure 1. Morphometric characters measured in the freshwater angelfish larvae.

powder feed starting from 20th day. No difference is observed in larvae in terms of survival ratios when compared to the ones which are fed with alive food.

Histological Observations

1-2 DAH: Angelfish eggs were brown-orange colored, dark with a demersal and sticky structure. The larvae, which hatched from 0.9-1 mm eggs, had a yolk sac of approximately 30% of its total body length. The larvae, which had a linear long digestive system at 2nd day, attached to a surface by the cement glands over the head (Figure 3a). At 2nd day, head, body and tail are distinguished; the mouth is closed (Figure 3a). Fore brain and hindbrain sections can be distinguished. Optic vesicles are found in fore brain

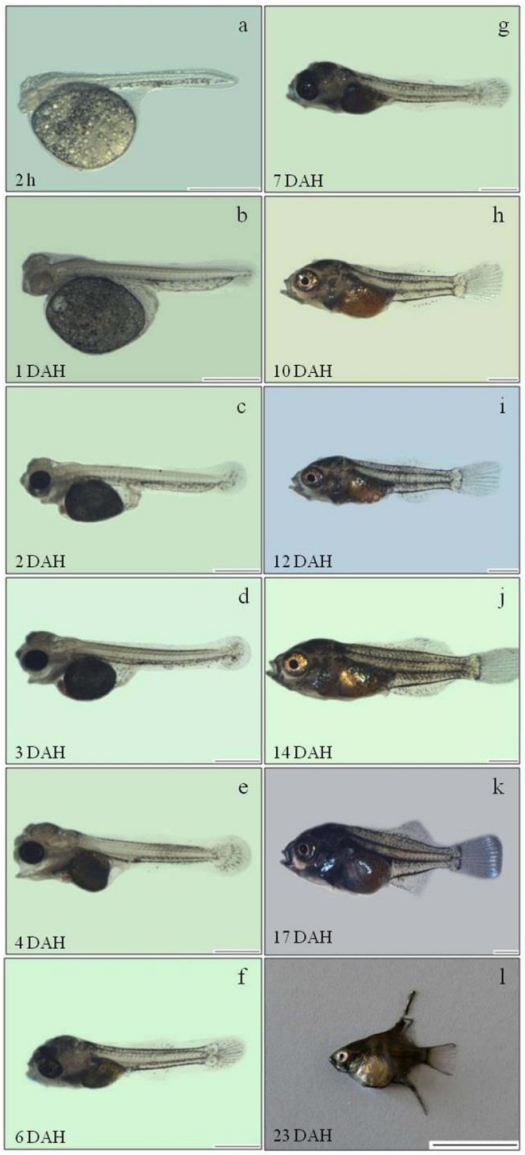

Figure 2. Larval development of freshwater angelfish (*Pterophyllum scalare*). (a) No eye, organ and fin differentiation was observed (2 h); (b) Mouth and anus are closed (1 DAH); (c) Digestive system is clearly visible in the form of a linear tube (2 DAH); (d) Mouth and anus are opened, flexion occurred in notochord end (3 DAH); (e) Pigmentation in the eyes is completed (4 DAH); (f) Swim bladder is clearly visible, yolk sac is completely absorbed (6 DAH); (g) Swim bladder continues to grow posteriorly (7 DAH); (h) Dorsal and anal fin rays became apparent (10 DAH); (i) Pigmentation continues to increase (12 DAH); (j) Black pigmentation in the general of the body significantly increased (14 DAH); (k) Dorsal and anal fin of the larvae began to look like fin form of adult individuals (17 DAH); (l) Metamorphosis is completed and juvenile stage began (23 DAH). Scale bars = 1 mm.

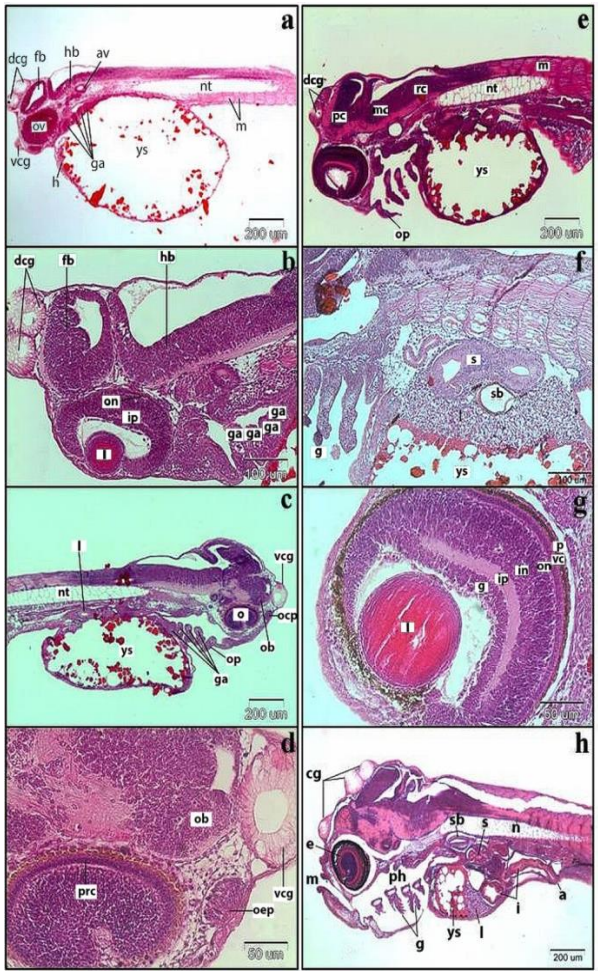

Figure 3. Sagital sections of freshwater angelfish larvae. (a) The mouth is closed (1-2 DAH); av: auditory vesicle, dcg: dorsal cement gland, fb: forebrain, ga: gill arch, h: heart, hb: hindbrain, nt: notochord, ov: optic vesicle, vcg: ventral cement gland, ys: yolk sac. (b) Lens is observed inside optic capsule (3 DAH); dcg: dorsal cement gland, fb: forebrain, ga: gill arch, hb: hindbrain, ip: iner plexiform layer, l: lens, on: outer nuclear layer. (c) The mouth is open (4 DAH); e; eye, i: intestine, nt: notochord, ob: olfactory bulb, oep: olfactory epithelium, op: operculum, vcg: ventral cement gland, ys: yolk sac (d) Olfactory epithelium is observed in anterior of the eye (4 DAH); ob: olfactory bulb, oep: olfactory epithelium, prc: photoreceptor cell, vcg: ventral cement gland.(e) Stomach and intestinal curves increased (5 DAH); dcg: dorsal cement gland, m: myomeres, mc: mesencephalon, nt: notochord, op: operculum, pc: prosencephalon, rc: rhombencephalon, ys: yolk sac. (f) Swim bladder is inflated (5 DAH); g: ganglion, s: stomach, sb: swim bladder, s: yolk sac. (g) The eye layers (5 DAH); g: ganglion, in: inner nuclear layer, ip: iner plexiform layer, l: lens, op: operculum, p: pigment epithelium, vc: visual cell layer. (h) Cement glands on the head are still visible and yolk sac remnants are visible in the anterior section of the liver (7 DAH); a; anus, cg; cement gland, e; eye, g; gill, i; intestine, l: liver m; mouth, n; notochord, ph: pharynx s; stomach, sb: swim bladder, ys; yolk sac.

laterals while audio vesicles are found in hindbrain line. Notochord extends from hindbrain lineto tail bud. Myomers are clearly apparent. Gill arches continue develop (4 pieces). Heart development continues within pericardial cavity. At the stage, erythrocytes are visible inside the heart which is in the form of a sac. In addition, peripheral blood circulation was observed in the larvae at this stage. Dorsal cement gland pair and ventral cement gland are present.

3 DAH: Yolk sac seems to be smaller than the previous stage. Lens is observed inside optic capsule which developed in lateral of forebrain (Figure 3b). Pigmentation occurs in the lens. Inner plexiform layer and outer nuclear layer can be distinguished in retina (Figure 3b). Operculum develops. Lengthwise extension is observed in gill arches. Dorsal cement glands are observed to be filled with mucus secretion. Digestive tube continues to develop, however mouth is not yet opened.

4 DAH: The mouth is open; yolk sac became smaller approximately 1/5 of the body (Figure 3c). Digestive system is observed to develop thanks to stomach formation and increased intestinal curve (Figure 3c). The larvae can feed exogenously at this stage and can perform short-term swimming movement. Olfactory epithelium is observed in anterior of the eye (Figure 3d). Olfactory budis observed to differentiate in anterior of fore brain. Photoreceptor cells in the eye are observed in a single row (Figure 3d). Development of median intestinal curves continues (Figure 3c). Yolk sac significantly became smaller and concentration of pigment yolk sac substance inside it significantly decreased (Figure 3c). Gill arches continue to develop. Opercular chamber is observed to increase in volume.

5 DAH: Stomach and intestinal curves increased, anterior intestine and posterior intestine can be distinguished from each other (Figure 3e). Cement glands are still visible over the head (Figure 3e), swim bladder is swollen; liver is developed (Figure 3f). Three regions in the brain can be distinguished (prosencephalon, mesencephalon and rhombencephalon). Lens and retina layers in the eye can be distinguished. These layers are ganglion cell layer, inner plexiform layer, inner nuclear layer, outer nuclear layer, visual layer and pigment epithelia layers from outer to inner respectively (Figure 3g). Cement glands were observed in dorsal of the head. Yolk sac significantly became smaller in anterior. Number of myomers increased. Heart formation in the form of sac continues to develop. Number of median intestinal curves increased. Renal tubules are observed to develop.

7 DAH: Yolk sac remnants are visible in the anterior section of the liver (Figure 3h). Larvae can actively swim and easily catch food. Cement glands on the head are still visible although they are no longer used (Figure 3h). Significant part of brain development is completed; eye pigmentation and lens formation color layers can be distinguished. Yolk sac is about to be absorbed cartilage of skull and jaw skeleton is observed to develop. Dorsal cementgl and and ventral cement gland are observed to be visible.9 DAH: Yolk sac is completely absorbed at 8-9DAH (Figure 4a). Digestive system consisting of stomach and intestines cover a considerable part of body cavity which includes organs (Figure 4a). A similar situation is clearly observed in 11-day larvae (Figure 4b). Goblet cells, which have the ability to secrete mucus, are visible among epithelium cells encircling pharyngeal cavity which form the anterior section of digestive tube. Intestinal curves and stomach can be distinguished. Liver is observed to be larger than previous stages. Dorsal cement gland pair and ventral cement glands disappeared.

17 DAH: Mouth, gill, eye and head structure of the larvae began to take juvenile body form (Figure 4c). Gill lamellae are clearly observed to develop (Figure 4c). Functionality of livers increased; adrenals are visible. Liver in ventral region of the body grew and extended to dorsal. Inclusion vacuoles are clearly visible in the intestine. The most important development in larvae at this stage is observed in eyes. Retina layers can be easily distinguished. Jaw and dorsal muscle arrangements are observed cartilage of jaw and skull skeleton continue to develop. Single cylindrical epithelium cells covering lumens in intestinal curves and microvillus are observed.

18- 30 DAH: Number of mucus cells in esophagus increased, vacuoles in the intestine become concentrated, digestive system developed to digest powder feed given after alive food (Figure 4d). Kidney observed in dorsal of swim bladder expanded by extending to posterior (Figure 4d). Vacuolization in liver in 23-25thdays indicates functionality of the liver (Figure 4e). Esophagus at the end of pharynx is in the form of a long thin tube. There is a high amount of goblet cells between epithelium cells covering esophagus lumen. Heart development continued. Glands and inclusion vacuoles in stomach and intestinal tissues indicate that development of digestive system is completed and larvae transformed to juvenile stage (Figure 4f).

Allometric Growth

Allometric growth models were defined by linear regression formulas determined by the association of certain body regions with total length (TL) (Fuiman, 1983; Gisbert *et al.*, 2002). Growth formula of angelfish which was calculated by exponential relation model during early larval period is $y = 4.36e^{0,0464x}$ ($R^2 = 0.96$, n = 77). Where, y represents total length (TL) and x represents day (Figure 5). Angelfish larvae had a mean length of 4.24 ± 0.28mm in 1 day. They reached 5.03 ± 0.24mm in the 5thday; 7.19 ± 0,38mm in the 5th day and 12.74 ± 0.6 mm in the24thday. As indicated in the graph, alive

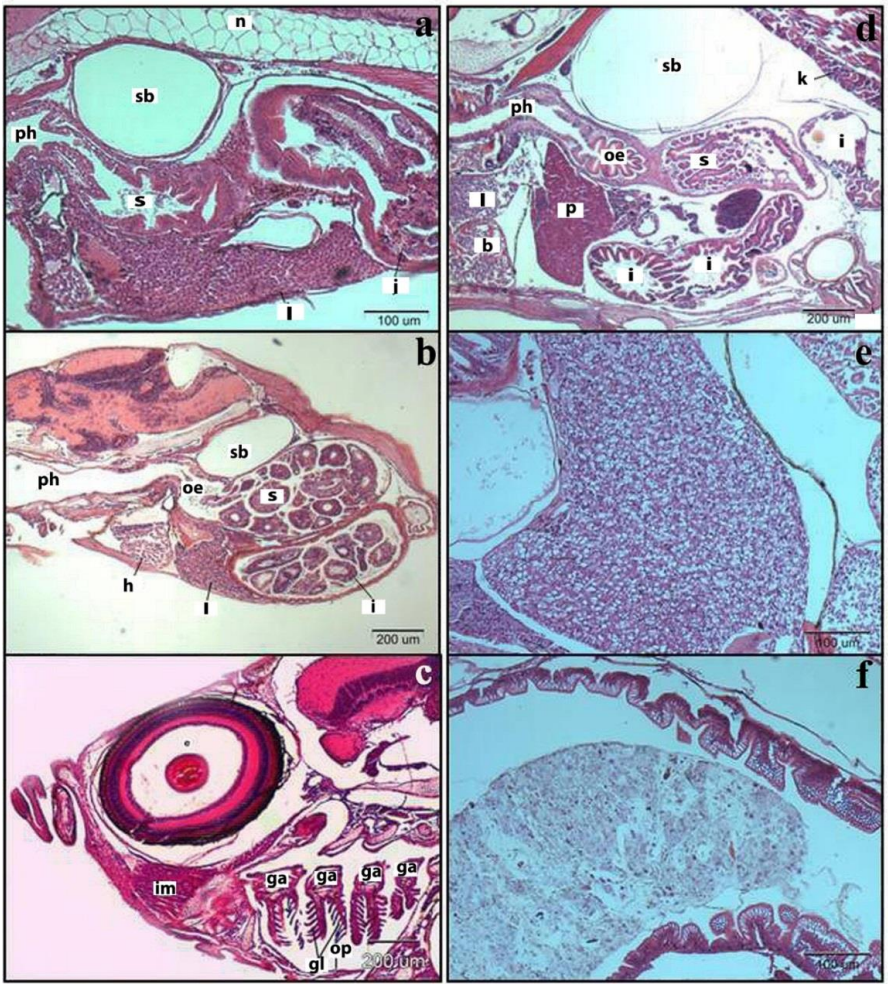

Figure 4. Sagital sections of freshwater angelfish larvae. (a) Yolk sac is completely absorbed (9 DAH); (b) Digestive system consisting of stomach and intestines cover a considerable part of body cavity which includes organs (11 DAH); (c) Mouth, gill, eye and head structure of the larvae began to take juvenile body form (17 DAH); (d) Kidney observed in dorsal of swim bladder (18 DAH); (e) Vacuolization in liver (23 – 25 DAH); (f) Glands and inclusion vacuoles in stomach and intestinal tissues (23 – 25 DAH). a; anus, e; eye, g; gill, ga: gill arch, gl: gill lamellae, h: heart, i; intestine, jm: jaw muscle, k; kidney,l:liver, n; notochord, oe: oesophagus, op: operculum, ph: pharynx s; stomach, sb: swim bladder.

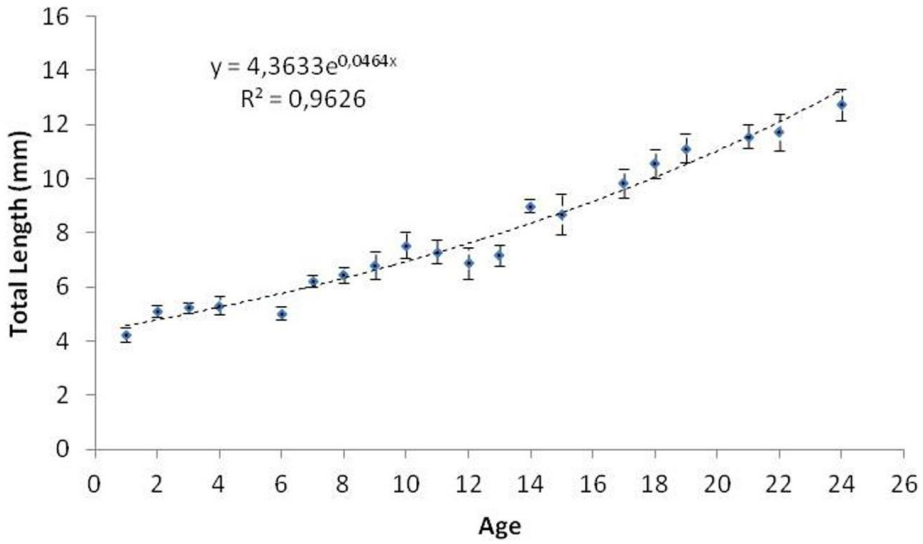

Figure 5. Growth of freshwater angelfish larvae from hatch to 25 DAH. Each point represents the mean total length ± std. dev.

juvenile which started larval stage with 4.24 ± 0.28mm reached 12.74 ± 0.6 mm total length in transition to juvenile period (Figure 5). Pre-anal length showed positive allometric growth during larval development. Growth coefficients of morphologic characters were a = 0.45, b = 1.02, R^2 = 0.97, n = 77'dir (Figure 6a). In larval development, angelfish showed positive allometric growth in head

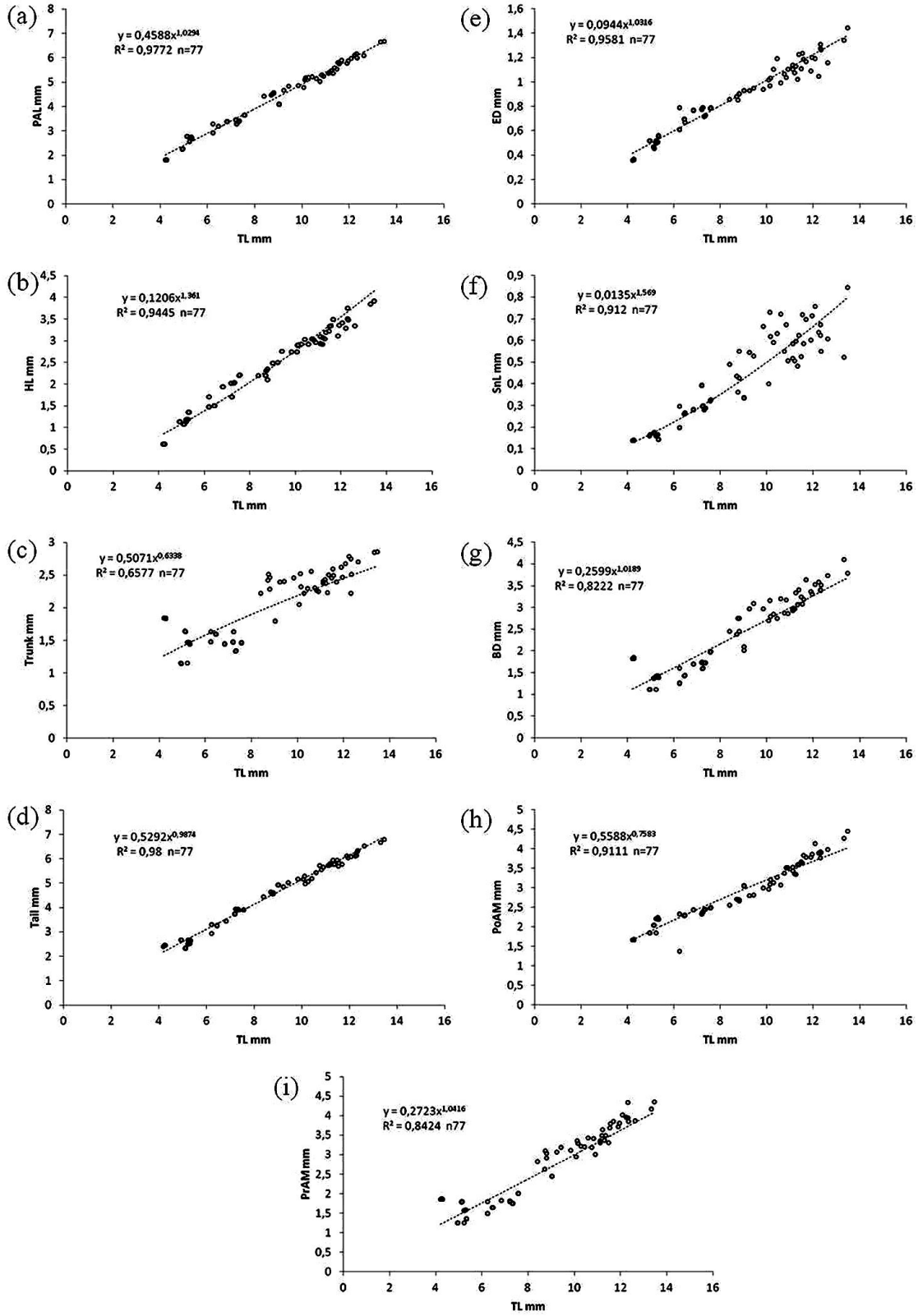

Figure 6. Allometric growth equations and relationship between nine body segments and total length in freshwater angelfish during larval development period (from hatch to 25 DAH). PAL, Pre-anal length; HL, Head length; ED, Eye diameter; SnL, snout length; BD, Body Depth; PoAM, Post-anal myomer length; PrAM, Pre-anal myomer length.

region and negative allometric growth in body region. Growth coefficients of morphologic characters were found to be tail (a = 0.52, b = 0.98 ,R^2 = 0.98, n = 77), ED (a = 0.09, b = 1.03, R^2 = 0.95, n = 77) (Figure 6d,6e). Growth coefficients of morphologic characters were found to be HL (a = 0.12, b = 1.3, R^2 = 0.94, n = 77) and body (a = 0.50, b = 0.63, R^2 = 0.65, n = 77) (Figure 6b, 6c). While caudal region showed negative allometric growth, eye diameter showed positive allometry. Growth coefficients of morphologic characters were found to be tail (a = 0.52, b = 0.98 , R^2 = 0.98, n = 77), ED (a = 0.09, b = 1.03, R^2 = 0.95, n = 77) (Figure 6d,6e). Nose length and body width: angelfish showed positive allometric growth during its larval development. Growth coefficients were calculated as SnL (a = 0.01, b = 1.5, R^2 = 0.91, n = 77), BD (a = 0.25, b = 1.01, R^2 = 0.82, n = 77) (Figure 6f, 6g). While pre-anal myomer length growth showed positive allometry, post-anal myomer length region showed negative allometric development during larval development of angelfish. Growth coefficients of these morphologic characters were calculated as PrAM (a = 0.27, b = 1.04, R^2 = 0.84, n = 77), PoAM (a = 0.55, b = 0.75, R^2 = 0.91, n = 77) (Figure 6h, 6i).

Discussion

In this study, the larval development of the laboratory-reared freshwater angelfish (*Pterophllum scalare*) are described (Figure 7). The full developmental sequence from hatching to juvenile incontrolled aquarium conditions is also stated. In addition, allometric growth of some body parts was studied. In some fish species, development of organs at early period occurs in embryonic stage prior to hatching (Falk-Peterson and Hansen, 2001). Hatched larvae had a developed organ system. These types of larvae can be fed with artificial food from the time when larvae mouths are opened and they absorb a considerable part (2/3) of their yolk sacs. The species which have these characteristics are called precocial larvae (Govoni *et al.,* 1986). On the other hand, in some species, organs differentiate after hatching, during or after metamorphosis. The larvae of these types of species are called altricial larvae (Falk-Peterson and Hansen, 2001).Histologic observations showed that newly hatched angelfish larvae which hatched from demersal and quite large eggs (0.9-1mm) attached on any substrate have a developed organ system. On the other hand, digestive tracts of the larvae which benefitted from quite large yolk sacs differentiated in 4thday. Thus, angelfish larvae are subject to intensive metamorphosis after hatching. In our previous studies, we observed that angelfish larvae were fed with rotifer and *Artemia* nauplii at 4and 5thday respectively. These observations indicate that angelfish larvae belong to altricial larvae group.

In a similar study, morpho-physiological changes was reported the early developmental stages of freshwater angelfish under different conditions (28°C; pH 6.8) (Korzelecka-Orkisz, 2012). Korzelecka-Orkisz *et al.,* (2012) described the egg membrane structures of angelfish (*Pterophyllum scalare*), morpho-physiological changes and the developing larvae and fry, but they didn't report histolological changes of angelfish larvae on the early developmental stages. The main differences between these previous studies and our study is that reported not only morphological findings but also histological findings and allometric growth data. On the other hand, some morphological findings such as exogenous feeding time, free swimming time and metamorphosis time are similar between these studies (Our study and Korzelecka-Orkisz, 2012). In our study, the morphological findings on larval development stage were reported more details and the morphologic findings were supported by histological observations.

Start of exogenous feeding is a vital stage in developing larvae (Yúfera and Darias 2007).Like in many other fish species, (Tamaru *et al* 1994; Cahu *et al.,* 2003; Yúfera *et al.,* 2005; Çelik *et al.,* 2011) exogenous feeding starts before complete absorption of yolk sac in angelfish. Analyses on alive larvae under microscope showed that although yolk sac

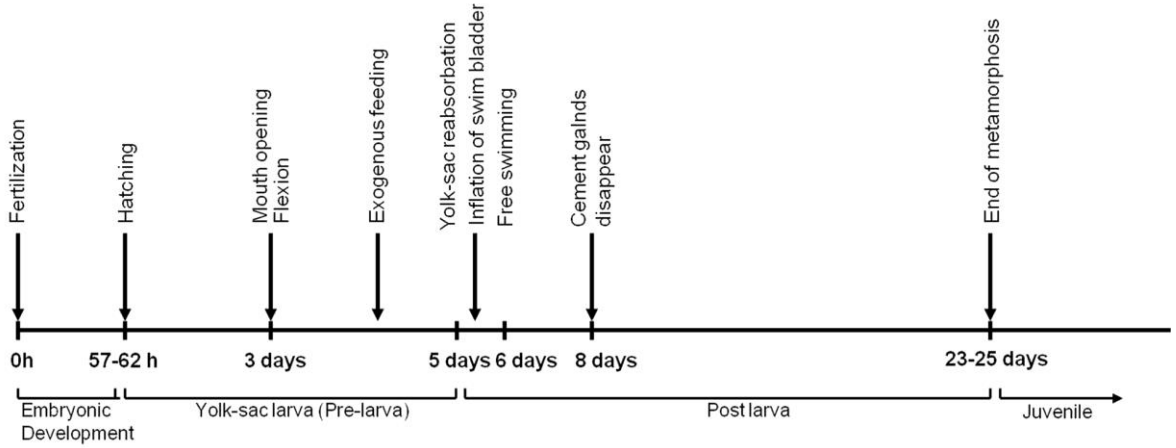

Figure 7. The main events of larval development in freshwater angelfish (*Pterophyllum scalare*).

could not be separated from other internal organs after 5. day, histologic findings showed that yolk sac became quite smaller in 6.day however remnants of yolk sac were observed in larvae until 7th day. On the other hand, it was concluded that angelfish larvae should be exogenously fed starting from 4.day. Digestive organs such as liver and pancreas also rapidly developed. The liver fully replaced shrinking yolk sac at 9thday and vacuolization which indicated lipid synthesis began to be observed from 11th day. Non-stained vacuoles which indicate lipid digestion in anterior and posterior intestines reported in previous studies (Govoni *et al.,* 1986; Kjørsvik *et al.,* 1991; Sarasquete, *et al.,* 1995; Hamlin *et al.,* 2000, Önal *et al.,* 2008) were also observed in the present study. These vakuoller observed in posterior intestine from 10thday were observed in the general of the intestine in the following days.

One of the most critical stages of larval development is considered to the first swelling of swim bladder (Blaxter 1992; Pelberg *et al.,* 2008). Majority of larvae swell their swim bladders in developmental stage corresponding to the time of absorption of yolk sacs and onset of exogenous feeding (Doroshev and Cornacchia 1979; Battaglene and Talbot 1990). In many analyzed species, swim bladder forms by a simple metamorphosis of alimentary canal (Boulhic and Gabaudan 1992; Govoni and Hoss 2001; Trotter *et al.,* 2004).

There are two types of swim bladders which are physostomous and physoclistous (Moyle and Cech 2000; Trotter *et al.,* 2004; Govoni and Forward 2008). In physostomoes, there is a connection between digestive tract consisting of the swim bladder and pneumatic tube. On the other hand there is no such connection inphysoclists (Steen 1970). Physostomous larvae are generally believed to swell their swim bladders by swallowing atmospheric air in water surface and sending to swim bladder through alimentary canal and pneumatic tube (Tait 1960; Steen 1970; Chapman and Hubert 1988; Battaglene and Talbot 1990; Chatain and Ounais-Guschemann 1990; Marty *et al.,* 1995; Govoni and Hoss 2001; Trotter *et al.,* 2005). Some of the studies reported that Characins including black skirt tetras, cyprinids, salmonids, pikes, catfish, mormyrids and eels consisted of species having physostomous type swim bladders (Moyle and Cech 2000; Çelik *et al.,* 2011, Çelik *et al.,* 2012). On the other hand, freshwater angelfish have physoclist swim bladders. In this study, swim bladder became apparent in 2th day. In this period, the larvae do not have the ability to swim actively and anus is not yet opened. According to morphologic and histologic findings, swim bladders were filled with low amount of atmospheric air.

In the present study, in addition to morphologic and histologic observations, allometric growth models of angelfish were also recorded. Allometric growth model is a commonly used method in analysis of relative growth in early larval period (Osse and van den Boogaart 2004; Peña and Dumas 2009). Larval development stages of teleostei were characterized by using high grade allometric growth models (Van Snik *et al.,* 1997; Geerinckx *et al.,* 2008). These models can be used by characterization of normal growth models in rearing and aquaculture management (Peña and Dumas 2009). The literature contains studies on allometric growth of different teleostei groups during their larval development (Osse and van den Boogaart 2004); however there is a limited body of research on allometric growth models of many ornamental fish. In this study, allometric growth models of freshwater angelfish larvae from hatching to 30.day were defined. Growth coefficients of body part were found to be different in three larval stages (Period I: Yolk sac larva, period II: Pre-larva, period III: Post-larva). These results are similar with other teleostei fish (Osse and van den Boogaart 1999; Geerinckx *et al* 2008; Huysentruyt *et al.,* 2009).

Success of larval production is a significant problem in rearing of many finfish. Larval death sometimes reaches to the level of 80% (Woolley and Qin, 2010). These mass deaths are thought be connected with factors such as broodstock quality, nutrition and environmental factors. Mass deaths often peak at the time of transition to exogenous feeding following the absorption of yolk sac (Woolley and Qin, 2010). Therefore, for a successful larval rearing, optimum culture conditions should be determined according to larval development stages.

Determining optimal rearing conditions requires conducting sophisticated scientific research on the larval development stages of fish species. The present study was carried out to address this need. In conclusion, our histo morphological findings revealed that underdeveloped organ systems of altricial angelfish larvae developed in a very short time after hatching. Angel fish larvae began to actively consume feed starting from 4th day. In addition, in the present study, artificial food were not used for 30 days and in accordance with the conventional protocol used in commercial angelfish rearing, the larvae were fed with *Artemia* from day 4. Angelfish larvae were successfully reared with this protocol. On the other hand, issues such as rearing larvae using different protocols and analysis of the effects of these protocols on the growth and survival of larvae by histologic studies and increasing the growth of larvae to sales lengths in a shorter time are among top priority issues in angelfish rearing. Results of the present study can contribute to better understanding of embryonic and larval development of angelfish and other commercial freshwater fish. These results can be used to explain the general outlook of first larval stages of the larvae under culture conditions and can help development of better larval rearing methods in production. Similarly, it will help to increase success rate in larval rearing of some freshwater ornamental fish. New approaches can be introduced to solve many problems in larval rearing of many other species.

Ackowledgement

This study was supported by Çanakkale Onsekiz Mart University, Commission of Scientific Research Projects (BAP), Project No: 2010/110, TURKEY.

References

Bisbal, G.A. and Bengston, D.A. 1995. Development of digestive tract in larval summer flounder. Journal Fish Biology, 47: 277-291. doi:10.1111/j.1095-8649.1995.tb01895.x

Battaglene, S.C. and Talbot,R.B. 1990. Initial swim bladder inflation in intensively rearedustralian bass larvae, *Macquaria novemaculeata* (Steindeachner) (Perciformes: Percichthyidae). Aquaculture, 86: 431–442. doi: 10.1016/0044-8486(90)90331-G

Blaxter, J.H.S. 1992. The effect of temperature on larval fishes. Netherlands Journal of Zoology, 42: 336–357. doi: 10.1163/156854291X00379

Boulhic, M. and Gabaudan, J. 1992. Histological study of the organogenesis of the digestivesystem and swim bladder of the Dover sole, Solea solea (Linnaeus 1758). Aquaculture, 102: 373–396. doi: 10.1016/0044-8486(92)90190-V

Celik,İ. 2010. Diskus Balıklarında (*Symphysodon spp.*) Larval ve Prejuvenil Gelişimin Mikrofotografi Metoduyla Tanımlanması. Journal of Fisheries Sciences, 4 (1): 99-111.

Çelik, P., Çelik İ. and Cirik Ş. 2011. Siyah Neon Tetra (Hyphessobrycon herbertaxelrodi) Larvalarının Allometrik Gelişimi. Alınteri 19 (B), 25-32, ISSN: 1307-3311.

Celik,İ., Celik, P., Cirik, S., Gurkan, M. and Hayretdağ, S.2012. Embryonic and larvaldevelopment of black skirt tetra (*Gymnocorymbus ternetzi*, Boulenger, 1895) underlaboratory conditions. Aquaculture Research, 43 (9): 1260 – 1275. doi: 10.1111/j.1365-2109.2011.02930.x

Cacho, M.S.R.F., Yamamoto, M.E. and Chellappa, S. 2007. Mating system of the amazoniancichlid angel fish, *Pterophyllum scalare.* Brazilian Journal of Biology, 67 (1): 161-165. doi: 10.1590/S1519-69842007000100022

Cacho, M.S.R.F., Chellappa, S. and Yamamoto, M.E. 2006. Reproductive success and femalepreference in the amazonian cichlid angel fish, *Pterophyllum scalare* (Lichtenstein, 1823). Neotropical Ichthyology, 4 (1): 87-91. doi: 10.1590/S1679-62252006000100009

Cahu, C., Zambonino-Infante, J.L. and Barbosa, V. 2003. Effect of dietary phospholipid leveland phospholipid:neutral lipid value on the development of sea bass (*Dicentrarchus labrax*) larvae fed a compound diet. British Journal of Nutrition, 90: 21–28.PMID: 12844371

Chapman, D.C. and Hubert, W.A. 1988. Influence of access to air and of salinity on gasbladder inflation in striped bass. Progressive Fish Culturist 50, 23–27.

Chatain, B. and Ounais-Guschemann,N. 1990. Improved Rate of Initial Swim Bladder Inflationin Intensive Reared *Sparus auratus*. Aquaculture, 84: 345-353. doi: 10.1016/0044-8486(90)90099-9

Degani, G.A.D., Boker, R., Gal, E. and Jackson, K.1997. Oogenesis and steroid profiles duringthe reprodcutive cycle of female angelfish *Pterophyllum scalare* (Cichlidae). IndianJournal of Fisheries, 44(1): 1-10.

Doroshev, S.I. and Cornacchia,J.W. 1979. Initial swimbladder inflation in the larvae of Tilapiamossambica (Peters) and Morone saxatilis (Walbaum). Aquaculture, 16: 57–66. doi: 10.1016/0044-8486(79)90172-8

Elbal, M.T., Garcia, Hernandez, M.P., Lozano, M.T. andAgulleiro, B. 2004. Development ofthe digestive tract of gilthead seabream (*Sparus aurata* L.). Light and electronmicroscope studies. Aquaculture, 234: 215-238. doi: 10.1016/j.aquaculture.2003.11.028

Falk-Petersen, I.B. and Hansen,T.K. 2001. Organ differentiation in newly hatched common wolffish.Journal of Fish Biology, 59: 1465-1482. doi: 10.1111/j.1095-8649.2001.tb00212.x

Frank, S. 1984. Akvaristika. Pp. 196-199. Prace Praha, 342p.

Fuiman, L.A. 1983. Growth gradients in fish larvae. Journal of Fish Biology, 23: 117–123. doi: 10.1111/j.1095-8649.1983.tb02886.x

Garcia-Ulloa, M. and Gomez-Romero,H.J. 2005. Growth of angel fish *Pterophyllum scalare* [Gunther, 1862] juveniles fed inert diets. Rev. Avances de Investigación Agropecuaria, 9(3): 49-60.ISSN 0188-7890

Geerinckx, T., Verhaegen, Y. and Adriaens, D. 2008. Ontogenetic allometries and shapechanges in the suckermouth armoured catfish *Ancistrus cf. triradiatus* Eigenmann (Loricariidae, Siluriformes), related to suckermouth attachment and yolk-sac size.Journal of Fish Biology, 72: 803–814. doi:10.1111/j.1095-8649.2007.01755.x

Gisbert, E., Merino, G., Muguet, J. B., Bush, D., Piedrahita, R. H., Conklin, D. E., 2002. Morphological Development and Allometric Growth Patterns in Hatchery – reared California halibut larvae. Journal of Fish Biology 61: 1217-1229.

Govoni, J.J., Boehlert, G.W. and Watanabe,Y. 1986. The physiology of digestion in fish larvae.Environmental Biology of Fishes, 16: 59-77. doi: 10.1007/BF00005160

Govoni, J.J. and Forward,Jr.R.B. 2008. Buoyancy. In: Finn RN, Kapoor BG (eds) Fish Larval Physiology. Science Publishers, Enfield, NH, USA, pp 495-522.

Groppelli, S., Pennati, R., Sotgia, C. andDe Bernardi,F. 2003. Cement gland apparatus of theangelfish *Pterophyllum scalare* (Teleostei, Cichlidae): Functional morphology incomparison with adhesive organs of other Chordata. Italian Journal of Zoology, 70: 133-139. doi: 10.1080/11250000309356506

Govoni, J.J. and Hoss,D.E. 2001. Comparison of the development and function of theswimbladder of Brevoortia tyrannus (Clupeidae) and Leiostomus xanthurus (Sciaenidae). Copeia, 2: 430–442. ISSN: 00458511

Hamlin, H.J., Hunt VonHerbing, I. and Kling,L.J. 2000. Histological and morphologicalevaluations of the digestive tract and associated organs of haddock throughout post-hatching ontogeny. Journal of Fish Biology, 57: 716-732. doi: 10.1111/j.1095-8649.2000.tb00270.x

Huysentruyt, F., Moerkerke, B., Devaere, S. and Adriaens,D. 2009. Early development andallometric

growth in the armoured catfish *Corydoras aeneus* (Gill, 1858).Hydrobiologia, 627: 45–54. doi: 10.1007/s10750-009-9714-z

Kasiri, M., Farahi, A. and Sudagar, M. 2011a. Effects of Feeding Frequency on Growth Performance and Survival Rate of Angel Fish, *Pterophyllum scalare* (Perciformes: Cichlidae). Veterinary Research Forum.2: 97-102.

Kasiri, M., Sudagar, M. and Hosseini, S.A. 2011b. Effect of water hardness on egg hatchability and larval viability of angelfish (*Pterophyllum scalare* (Schultze, 1823).International Journal of Research in Fisheries and Aquaculture, 1(1): 6-10.

Kendall, A.W., Ahlstrom, E.H. and Moser,H.G. 1984. Early life history stages of fishes andtheir characters. In: H.G. Moser, W.J. Richards, D.M. Cohen, M.P. Fahay, A.W. Kendall, S.L. Richardson (Eds.) Ontogeny and systematics of fishes: American Society ofIchthyologists and Herpetologists, Special Publication,Allen Press Inc, Lawrence, Kansas, U.S.A. 11–22 pp.

Korzelecka-Orkisz, A., Szalast, Z., Pawlos, D., Smaruj, I., Tañski, A., Szulc, J. and Krzysztof, F. 2012. Early ontogenesis of the angelfish, *Pterophyllum scalare* Schultze, 1823 (Cichlidae). Neotropical Ichthyology, 10(3):567-576. doi: 10.1590/S1679-62252012005000017

Kjorsvik, E., Van der Meeren, T., Krvyi, H., Amfinnson, J. and Kvenseth, P.H. 1991. Early development of the digestive tract of cod larvae, *Gadus morhua*, during start-feeding and starvation. Journal of Fish Biology, 38: 1-15. doi: 10.1111/j.1095-8649.1991.tb03086.x

Kullander, S.O. 1998. A phylogeny and classification of the South American Cichlidae (Teleostei: Perciformes). In: L.R. Malabarba, R.E. Reis, R.P. Vari, Z.M.S. Lucena, C.A.S. Lucena (eds.) Phylogeny and classification of Neotropical fishes, Edipucrs, Porto Alegre. 461-498 pp.

Luna-Figueroa, J. 2003.*Pterophyllum scalare* (Pisces: Cichlidae): Influencia de alimentovivo en la reproducion y el crecimiento. II Congreso Iberoamericano Virtual deAcuicultura, CIVA 2003 (http://www.civa2003.org), 55-65 pp. (20 de julio de 2005).

Meijide, F.J. and Guerrero, G.A. 2000. Embryonic and larval development of a substrate-brooding cichlid *Cichlasoma dimerus* (Heckel, 1840) under laboratory conditions.Journal of Zoology, 252(4): 481–493. doi: 10.1111/j.1469-7998.2000.tb01231.x

Marty, G.D., Hinton, D.E. andSummerfelt, R.C. 1995. Histopathology of swimbladdernoninflation in walleye (*Stizostedion vitreum*) larvae: role of development and inflammation. Aquaculture, 138: 35– 48. doi: 10.1016/0044-8486(95)01129-3

Mills, D. 1993. Angelfish. Pp. 123-125. In: Mills, D. Aquarium Fish. , London, a Dorling Kindersley Book, 304p.

Moyle, P.B. and Cech,J.J. 2000. Fishes, an Introduction to Ichthyology. Prentice Hall UpperSaddle River. NJ. p. 610.

Murphy, B.G., Bradway, D., Walsh, T., Sanders, G.E. and Snekvik, K. 2009. Gastriccryptosporidiosis in freshwater angelfish (*Pterophyllum scalare*). Journal of Veterinary Diagnostic Investigation, 21: 722-727.PMID: 19737774

Nico, L. 2010. *Pterophyllum scalare*. In: *USGS* Nonindigenous Aquatic Species Database. Florida, USA. http://nas.er.usgs.gov/queries/FactSheet.aspx?speciesID=475. [Visited Feb. 2012].

Ortega-Salas, A.A., Cortes, G.I. and Reyes-Bustamante, H.2009. Fecundity, growth, and survival of the angelfish *Pterophyllum scalare* (Perciformes: Cichlidae) underlaboratory conditions. Revista de Biología Tropical, International Journal of TrophicalBiology and Conservation, 57(3): 741-747.PMID: 19928467

Osse, J.W.M. andvan den Boogaart,J.G.M. 1999. Dynamic morphology of fish larvae, structural implications of friction forces in swimming, feeding and ventilation. Journalof Fish Biology, 55 (Supplement A): 156–174. doi: 10.1111/j.1095-8649.1999.tb01053.x

Osse, J.W.M. andvan den Boogaart,J.G.M. 2004. Allometric growth in Fish Larvae: Timing and Function. In: J.J.Govoni (ed.) The development of form and function in fishes and the question of larval adaptation, American Fisheries Society, Symposium40, Bethesda, Maryland.167-194 pp.

Onal, U., Langdon, C. and Celik, I.2008. Ontogeny of the digestive tract of larval perculaclownfish, *Amphiprion percula* (Lacepede 1802): a histological perspective.Aquaculture Research, 39: 1077-1086. doi: 10.1111/j.1365-2109.2008.01968.x

Perlberg, S.T., Diamant, A., Ofir, R. and Zilberg,D. 2008. Characterization of swim bladdernon-inflation (SBN) in angelfish, *Pterophyllum scalare* (Schultz), and the effect ofexposure to methylene blue. Journal of Fish Diseases, 31: 215-228. doi: 10.1111/j.1365-2761.2007.00895.x

Pena, R. andDumas,S. 2009. Development and allometric growth patterns during early larvalstages of the spotted sand bass *Paralabrax maculatofasciatus* (Percoidei: Serranidae). Scientia Marina, 73: 183-189. doi: 10.3989/scimar.2009.73s1183

Pelberg, S.T., Diamant, A., Ofir, R. andZilberg,D. 2008. Characterization of swim bladder non-inflation (SBN) in angelfish, *Pterophyllum scalare* (Schultz), and the effect of exposureto methylene blue. Journal of Fish Disease, 31: 215-228. doi: 10.1111/j.1365-2761.2007.00895.x

Sakamoto, H., Yoshida, M., Sakamoto, T. and Uematsu, K. 1999. Development of the Myotomal Neuromuscular System in Embryonic and Larval Angelfish, *Pterophyllum scalare*. Zoological Science, 16: 775-784. doi: 10.2108/zsj.16.775

Santamaria, C.A., Marin, de Mateo D., Traveset, R., Sala, R., Grau, A., Pastor, E., Sarasquete, C. andCrespo,S. 2004. Larval organogenesis in common dentex *Dentex dentex* (L.Sparidae): histological and histochemical aspects. Aquaculture, 237: 207-228. doi: 10.1016/j.aquaculture.2004.03.020

Saxby, A., Adams, L., Snellgrove, D., Wilson, R.W. and Sloman,K.A. 2010. The effect of group size on the behaviour and welfare of four fish species commonly kept in home aquaria.Applied Animal Behaviour Science, 125: 195–205. doi: 10.1016/j.applanim.2010.04.008

Shukla, J.P. 2010. Freshwater aquarium: preparation and maintenance. In: K. Pandey, J.P. Shukla (eds.) Fish and fishery Rostagi, Meerut, India. 461–473 pp.

Sarasquete, M.C., Polo, A. and Yufera,M. 1995. Histology and histochemistry of the development of the

digestive system of larval gilthead seabream, *Sparus aurata*. Aquaculture, 130: 79-92. doi: 10.1016/0044-8486(94)00175-N

Sieniawski, A. 2004. Skalary. [Angelfish]. Tarnów, Wydawnictwo AMBRA, 138p.

Steen, J. B. 1970. The swim bladder as a hydrostatic organ. InFish Physiology, Vol. IV (Hoar, W. S. & Randall, D. J., eds), pp. 413–443. New York: Academic Press.

Tait, J.S. 1960. The first filling of the swim bladder in Salmonids - Can. J. Zool. 38: 179-186.

Tamaru, C.S., Murashige, R. and Lee,C.S. 1994. The paradox of using background phytoplankton during the larval culture of striped mullet, *Mugil cephalus*, L. Aquaculture, 119: 167–174. doi: 10.1016/0044-8486(94)90173-2

Trotter, A.J., Pankhurst, P.M. and Battaglene, S.C. 2004. Morphological development of theswim bladder in hatcheryreared striped trumpeter Latris lineata. Journal of Applied Ichthyology, 20: 395–401. doi: 10.1111/j.1439-0426.2004.00564.x

Trotter A.J., Pankhurst P.M. & Battaglene S.C. (2005) a finite interval of initial swimbladder inflation in Latris lineata revealed by sequential removal of water-surface films. Journal of Fish Biology 67, 730–741.

Van Snik, G.M.J., van Den Boogaart, J.G.M. and Osse, J.W.M. 1997. Larval growth patterns in *Cyprinus carpio* and *Clarias gariepinus* with attention to the finfold. Journal of Fish Biology, 50: 1339–1352. doi: 10.1111/j.1095-8649.1997.tb01657.x

Woolley, L.D. and Qin,J.G. 2010. Swimbladder inflation and its implication to the culture ofmarine finfish larvae. Reviews in Aquaculture, 2: 181-190. doi: 10.1111/j.1753-5131.2010.01035.x

Yufera, M., Fernandez-Diaz, C. andPascual,E. 2005. Food microparticles for larval fishprepared by internal gelation. Aquaculture, 245: 253–262. doi: 10.1016/j.aquaculture.2005.04.026

Yufera, M. andDarias,M.J. 2007. The onset of exogenous feeding in marine fish larvae.Aquaculture, 268: 53-63. doi: 10.1016/j.aquaculture.2007.04.050