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Research Article

EMBRYONIC AND LARVAL DEVELOPMENT OF ANGEL FISH (*PTEROPHYLLUM SCALARE*) WITH REFERENCE TO FEEDING STRATEGY

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ABSTRACT

Ornamental fish industry in India is making good progress in breeding, farming and trade in the recent years. The trade of ornamental fishes which was earlier practiced as cottage is now well organized as a corporate industry for domestic and international markets. Considering the importance of angel fish (*Pterophyllum scalare*) in the domestic market, this fish was selected for present study to provide knowledge on their breeding, spawning, larval development and nutritional requirements. The eggs of this species measure 1.5 ± 0.03 mm. Fertilized eggs are grayish in colour, while unfertilized eggs are whitish in nature. Duration of embryonic development of angel fish is 48 hours. The newly hatched out larvae of angel fish settle at the bottom and exhibit demersal habit for 2 days and utilize nutrients from the reserved yolk. The larvae begin exogenous feeding from 3 dph onwards. The larval development of angel fish includes 1. Endogenous feeding stage, 2. Endo-exogenous feeding stage and 3. Exogenous feeding stage. The yolk sac stage falls within the Endogenous and Endo-exogenous feeding stage. The duration of the Endogenous, Endo-exogenous and Exogenous feeding stages in angel fish is 1–2, 3–5, 6–22 dph, respectively. At 22 dph the larvae undergo drastic changes in their morphological characters which transform them in to juvenile stages. Ontogenetic development of angel fish larvae indicate that there is synchronized development of sense organs, swimming structures, digestive tract and respiratory structures in accordance with the requirements of the different larval stages.

Keywords: Ornamental fish, *Pterophyllum scalare*, Breeding, Development.

INTRODUCTION

The developmental stages of fish are 1) Egg (fertilization to hatching) 2) larva (hatching to metamorphosis) and 3) juvenile (beyond metamorphosis). Embryonic stage is the stage that extends from spawning to hatching. Within the larval period, development is divided in to yolk sac, pre-flexion, flexion and post-flexion stages. The egg of different fishes show variation pertaining to their size, shape, colour, yolk diameter, pigmentation, size of oil globules and width of perivitelline space (Kendall, 1984). The larval period is characterized by the persistence of some embryonic organs and special larval organs like fin fold, external gills, spines, flaps and filamentous appendices that are later replaced by different definitive organs with the same function or that disappear with the loss of their functional need (Webb, 1999). According to (Balon, 1986, 1989a, 1989b) larvae represent temporary intervals inserted in the developmental sequences primarily in order to complete the nutrient provision needed for formation of the definitive phenotype. The larval period lasts up to the formation of axial skeleton or ossification of axial skeleton or differentiation of median fin fold or up to the disappearance of all larval characters and the onset of the development of the scaly integument. The attainment of full count of fin rays and beginning of squamation indicate end of larval period. From feeding strategy point of view, larval period could be categorized in to endogenous feeding, endo–exogenous feeding and exclusively exogenous feeding stages. There is marked variation in the duration of these feeding stages in different species of fishes. Likewise variation is also observed pertain to the duration of entire larval period of different fishes.

The loss of larval characters and attainment of juvenile characters denote the metamorphic stage of fishes.

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Most distinguishing feature of this stage is changes in body shape and pigmentation pattern; additional changes that may occur include fin migration, photophore formation, loss of specialized larval characters, eye migration and squamation. The onset of transformation stage shows variation in different species of fishes, in *Pagrus pagrus* it was at 40 dph (Machinandiarena *et al*., 2003), in *Paralichthys californicus* at 42 dph (Gisbert *et al*., 2002)while in *Acipenser baeri* it was between 20 and 40 dph (Gisbert, 1999). Transformed individuals that have reached the juvenile stage of development mostly resemble miniature adults. Rapid and complex morphological changes were reported in two hatchery-reared major carps catla (*Catla catla*) and rohu (*Labeo rohita*) from hatching to post-flexion stages (Sheriff & Altaff)2018. Considering the commercial importance as one of the popular aquarium fish, the angel fish (*Pterophyllum scalare*) is widely mass cultured in South India. In the present study early development and larval stages leading to juvenile stage was investigated and reported in order to understand their feeding strategy and to optimize juvenile production in hatcheries.

MATERIAL AND METHODS

Breeding of Angel fish

The brood stocks consisting of ten pairs of mature angel fish were maintained in cement cisterns and were fed chopped live food. At the commencement of breeding behavior by a pair, they were transferred to a separate breeding tank with ideal breeding conditions such as water temperature of 29 °C, DO of above 5 mg/l, pH of 7.2 and photoperiod of 12 h of light and 12 h of dark. Slates were placed in the tank on which female systematically attaches the eggs and the male subsequently fertilizes them. The fertilized eggs were sampled every 30 minutes to study the embryonic development and the changes were documented using Carl Zeis Stereoscopic Binocular Microscopic.

Larval rearing

The spawned and fertilized eggs of angel fish were hatched out after embryonic development and the hatchlings were then transferred to larval rearing tanks for about 4 days, during which period they need no exogenous food since they subsist on their own yolk. During the yolk sac period, the larvae alternate between period of activity and inactivity, and their movement is vertical. In this period the mouth of hatchlings is developed but not yet opened. The vertical movement of hatchling changed into horizontal swimming movement when most of the yolk sac gets absorbed. As soon as yolk sac was absorbed, the larvae were transferred to nursery tanks and were given exogenous live feed. Rotifers were given as starter feed to larvae. As supplementary feed, boiled and mashed chicken egg yolk was given to larvae. The hatchlings were kept in the hatchery on egg diet for 1 or 2 days with feeding every 2 h at optimum dissolved oxygen. The larvae were then transferred to the large larval rearing tanks and raised up to juvenile stage. From just hatched stage i.e. zero post hatched (0 dph) period to thirty day post hatch (30 dph) larvae were fixed in 5% neutral buffered formalin and their morphology was studied under a stereoscopic binocular microscope. Based on their feeding schedule angel fish larvae were categorized into endogenous feeding, endo-exogenous feeding, exclusively exogenous feeding and juvenile stages.

RESULTS AND DISCUSSION

The angelfish attains maturity at the end of first year and commences breeding. During spawning, male and female pair among themselves and make a characteristic movement and twist around each other. During the course of breeding, male darts at fish other than its mate in an attempt to establish their territory. The courtship behavior of this fish is smooth and gentle and swimming together side by side without giving indication of their breeding state. The gravid female angel fish has a bulged belly and shows aggressive behavior when it approaches the spawning period. Subsequently, the breeding pair selects a spawning site and thoroughly cleans it with their mouth. This activity continues for some time and when the cleanliness of the spawning site is established by both the parents, the female brooder makes a few attempt runs of spawning. She pulls her ventral fins close to the lower side of the abdomen so that her entire lower line is relatively straight to the substratum. In this state the ovipositor makes full contact with the spawning substratum. The male also makes a few practice runs prior to the actual spawning. During the process of spawning, the female passes over the substratum and deposit the eggs in straight lines, which adhere to the surface of the spawning site. The male moves in and runs over the strings of eggs just lay by the female and fertilize them by releasing the milt. In a spawning about 900 eggs are spawned depending on the size and the reproductive phase of the female. At the end of the spawning, the parents move over close to the spawns and fan continuously with their pectoral fins to create water circulation around the eggs. The fertilized eggs become grayish while unfertilized eggs turn white.

The grayish white fertilized eggs of angel fish are elongate in shape and their diameter ranges between 1.4 and 1.7 mm (Figure 1). Embryonic development of fertilized eggs of angel fish commences in about 1 h after fertilization. Two membranes are distinct in eggs after 4 hours of incubation. The mean diameter of the egg is 1.6 mm. This egg has a perivitelline space of 0.10 mm (Figure 2). Cleavage takes place at hourly interval and blastoderm becomes distinct in 12 h (Figure 3 and 4) after fertilization. The yolk material of this species is homogenous and light yellow in colour. In this species blastulation is completed after an incubation period of 18 hours (Figure 5). After 24 hours post fertilization, head, trunk and tail of the embryo are visible distinctly and myotomes are well formed (Figure 6 to 10). At this stage, the larva covers most of the peripheral part of the egg. Further development leads to organogenesis and completion of embryonic development. Dendritic melanophores are observed at 30 hours of incubation and they are mostly scattered on the ventral side of the developing embryo or on the dorsal side of the yolk. Embryonic development is completed after 48 hours of incubation and the yolk sac larva hatches out by rupturing the egg membranes. After hatching the yolk sac larvae settle at the bottom of the tank and show slow movement due to the contraction of tail muscle.

Hatchling of angel fish has an elongate body with a rectangular head, tapering tail with a fin-fold (Figure 11). The yolk sac is bulbous at its proximal end and narrows towards the distal end. The eye is prominent and colourless. The body is transparent and devoid of pigmentation. It measures 3.6 ± 0.2 mm in length. Mouth and anus are not opened. The presumptive mouth is covered by a membrane. It is observed that the hatchling exhibits slow swimming for most of the time and remains at the bottom of the container passively. At this stage, the dorsal side of the body is light yellowish in colour, while the ventral part is pale yellow. The caudal region of the larva is bordered by primordial fin fold which is round in shape.

The exclusively endogenous feeding larval stage lasts for 2 days in angel fish (Figure 12 and 13).The total length of this stage larva measures 3.7± 0.2 mm. At this stage, the eyes on the head are bent downwards and its ventral surface is still attached to the yolk sac. Olfactory lobes and cement glands are observed on the dorsal surface of the cephalic region. The digestive tract appears as a straight tube lying below the notochord. The yolk sac is conical in shape and projects posteriorly. Heart is distinct and dendritic melanophores are spread on the ventral margin of the notochord caudal region of the fin fold and the yolk sac. During later part of this stage, the terminal region of the notochord is parallel to the longitudinal axis of the body. In this stage, pectoral fin buds appear laterally at the posterior region of the head. During the course of endogenous feeding stage head is lightly lifted up from the yolk sac. In addition to the ventral margin, dendritic melanophores spread towards the dorsal margin of the notochord. The distal region of the notochord is also pigmented. They are mostly seen densely aggregated during the endogenous mode of feeding.

Endoexogenous feeding stage commences from end of 2 dph and lasts up to end of 5 dph (Figure 14 and 15). During this period in addition to utilizing the yolk material larva begins to feed on external food. The total length of this stage larva measures 5.2 ± 0.2 mm. At 2 dph, there is flexion of terminal region of the notochord which constitutes the beginning of flexion stage. During this stage, opening of the mouth is established and the larva feeds on exogenous feed such as algal material. The posterior region of the digestive system communicates to the exterior through the anal opening. During this period yolk is utilized fully and the yolk sac is absorbed. The larva learns to subsist on exogenous feed. The heart is tubular in form. Development of pectoral fin lobes is observed. The fin fold in the caudal region gets pigmented. Melanophore appears on the dorsal side of the cephalic region and extends into the trunk and tail regions. They are completely absent on the dorsal finfold. The rudimentary gill cover could be distinguished as a deep cleft and pre anal finfold is distinct. Once these larvae reach the endoexogenous stage (2 to 5 dph) they slowly show a swim up behavior and enter in to the exogenous type of feeding. At this stage they exhibit a remarkable schooling behavior.

At 3 dph, larval eyes are darkly pigmented and head becomes slightly elongated. In this stage substantial utilization of the yolk is observed and the volume of the yolk sac is reduced. Heart and gill arches show progress in their development. The ventral fin fold is devoid of melanophores. Dorsally elevated caudal fin fold gets linear. The melanophores are dendritic and further development of maxilla and Meckel's cartilage was observed. The swim bladder gets inflated at 3 dph. During 4 dph, the larval fin fold shows protrusions of dorsal and ventral primordial fin folds from where the future dorsal and anal fins develop. The concentration of dendritic melanophores increases in the hind brain region. Melanophores are also observed on either sides of the upper and lower lip and they are also scattered on the notochord. Folding of the digestive tract is also observed in this stage. In 5 dph larva, complete flexion of terminal region of the notochord takes place and the endoexogenous period comes to an end. The larva at the end of 5 dph acquires characteristics for sustenance with external food (Figure 16).

This larval period of exogenous feeding stage of angel fish can be divided in to two phases' viz. micro zooplankton feeding phase and large zooplankton feeding phase. Micro zooplankton feeding phase extends from 6 to 11 dph. This stage larva measures 9.2 ± 0.2 mm in total length (Figure 17). During this stage post flexion take places. Commencement of post flexion stage is characterized by the occurrence of dense melanophores on the body. Between 6 dph and 11 dph, variation in the morphometrics rather than differentiation in ontogeny are observed and formations of few melanophores on the dorsal and ventral fin fold at 9 dph are observed. Caudal fin rays commence their development in this stage. At 9 dph, the dorsal and ventral fin fold gets separated from the caudal fin. Development of rudimentary finrays in dorsal and anal fins is observed at 11 dph larva of this stage. Melanophores are highly concentrated on the myosepta. The dorsal and ventral regions of the finfold, where the rays originate get pigmented intensively.

The large zooplankton feeding phase extends from 12 to 22 dph and mostly feed on cladocerans and copepods (Figure 18). The body of 14 dph larva is fully covered by melanophores. At this stage, three types of melanophores are observed viz., dendritic, stellate and punctuate. The punctuate and stellate melanophores are observed on the dorsal, ventral and lateral sides of the gut, while dendritic melanophores are distributed throughout the body. The caudal fin which was round at the beginning of the development under goes slight changes in its shape. Segmentation in the fin rays of caudal fin is observed from 14 dph and gut coiling is evident. During the developmental period of 15 to 20 dph, post flexion stage larva of angel fish shows less morphological changes except the dense formation of melanophores on the body surface and elongation of this fin rays in the middle of the dorsal fin. However, substantial increase in the dimension of the larva is recorded. At the end of this phase (22 dph), except the ventral region of the gut and head, all other body parts of the larvae are covered by dendritic melanophores. Higher concentration of the melanophores is observed on the anterior region of the dorsal and anal fins than other regions. This stage undergoes drastic changes in the body form to get transformed into juvenile stage (Figure 19).

On completion of 24 dph, the larva characters of angel fish get disappeared and it also marks the commencement of juvenile stage (Figure 20). This stage is characterized by shifting of pelvic fin from the abdominal to thoracic region, the caudal fin becomes truncated and the pigmentation pattern changes from aggregated dendritic to distinct punctuate form. Disappearance of preanal fin fold and coiling of digestive tract are observed. Angel fish in the juvenile stage, although smaller and sexually immature (Figure 21), are already similar in form, behavior and physiology to adults (Figure 22).

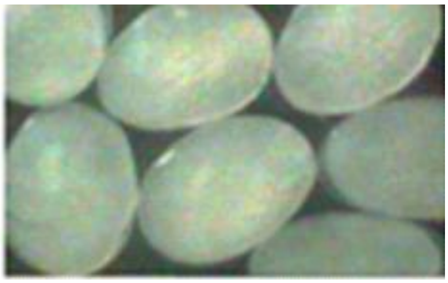

Figure 1. Fertilized eggs.

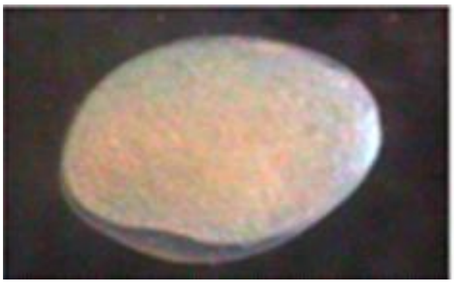

Figure 2. Egg with previtiline space.

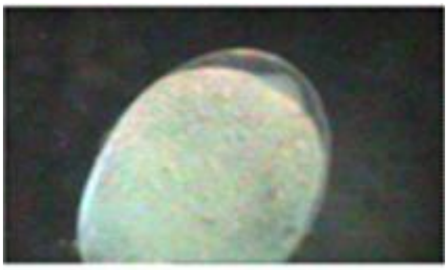

Figure 3. Cleavage and blastulation.

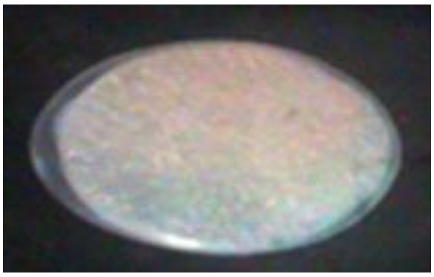

Figure 4. Cleavage and blastulation.

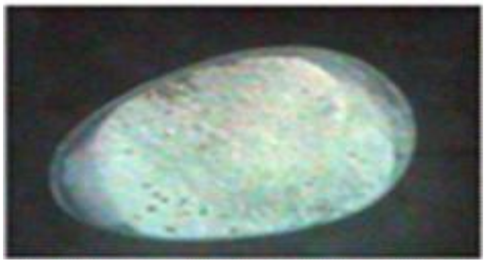

Figure 5. Cleavage and Blastulation.

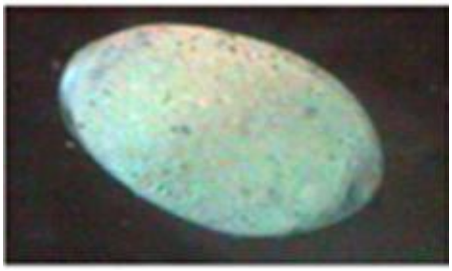

Figure 6. Gastrulation.

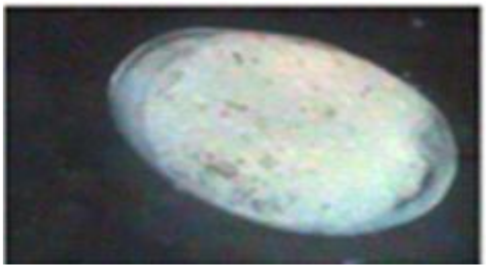

Figure 7. Gastrulation.

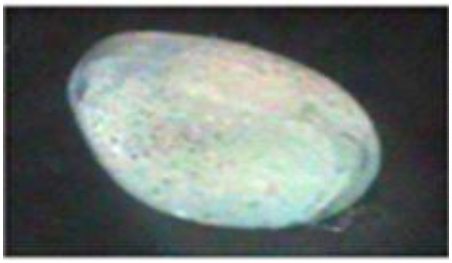

Figure 8. Organogenesis.

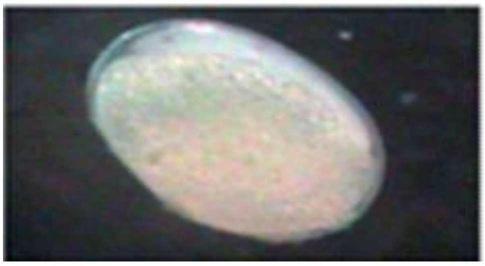

Figure 9. Organogenesis.

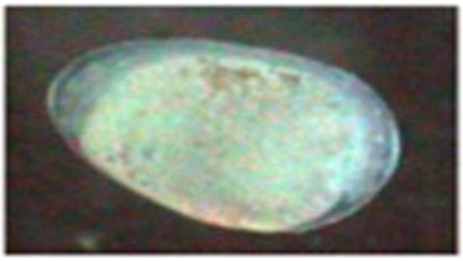

Figure 10. Organogenesis.

Figure 1-10. Angel fish embryonic development.

Figure 11. Hatchling.

Figure 12. Endogenous feeding stages.

Figure 13. Endogenous feeding stages.

Figure 14. Endoexogenous feeding stage.

Figure 15. Endoexogenous feeding stage.

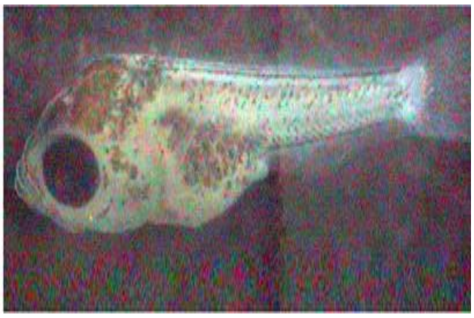

Figure 16. Exogenous feeding stage.

Figure 17. Exogenous feeding stage.

Figure 18. Exogenous feeding stage.

Figure 11-18. Angel Fish Larval Development.

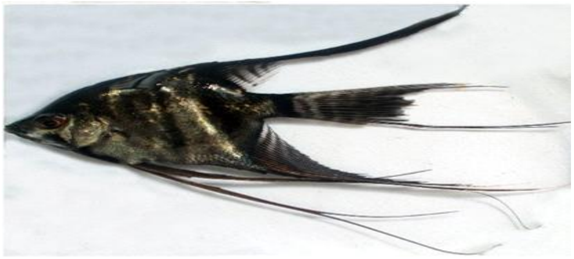

Figure 19. Metamorphic stage.

Figure 20. Juvenile stage.

Figure 21. Immature stage.

Figure 22. Adult.

Figures 19-22. Angel fish juvenile and adult stages.

In angel fish breeding, spawning and fertilization of eggs is highly systematic activity. Usually about 900 eggs are spawned by a breeding pair and 80% of the eggs are fertilized. The fertilized eggs are elongate and grayish white in colour. They measure 1.5 ± 0.2 mm in diameter. In the case of ornamental fish, average diameter of eggs of most of the species are about 0.8 mm, However, a wide range in the egg diameter is reported in many species such as 0.4 mm for *Gobies* (Watson & Chapman, 2002), 1.0–1.6 mm for *Symphysodon* sp. (Celik *et al*., 2012), 4.0 mm for *Tropheus moori* (Coleman & Galvani, 1998), 2.9 mm for *Cyathopharynx fucifer* (Coleman & Galvani, 1998), 0.75 mm for *Puntius conchonius* (Bhattacharya *et al*., 2005), 1.5-2.0 mm for ornamental catfish (Watson & Chapman, 2002) and 0.98 mm for *Gymnocorymbus terretzi* (Celik *et al*., 2012). The egg size and fecundity depends on several factors such as brood stock age, brood stock size, feed and water quality. The variety of reproductive strategies of fish can cause great differences amongst species in the number of eggs and egg size. However, the eggs of a fish species are in a common size range.

In the present study it is observed that the egg diameter increases after fertilization which is due to the formation of fertilization membrane and previtelline space. Sheriff & Altaff, (2018) reported hydration of eggs of catla and rohu after fertilization which led to a considerable increase of its volume. In the case of *Catla*, there is an increase in egg volume by 3 to 5 times, while in the case of rohu it increases by 2 to 4 times. As a result of imbibing water, in the fertilized eggs of both the species, egg hardening as well as variation in colouration was observed. Increase in the volume of the egg after fertilization was also reported in many fishes. Like many typical teleosts angel fish is externally fertilizing and the entirety of embryonic development occur outside the mother's body. The eggs are supplied with large yolk volume to sustain early development in the absence of direct maturation. Following sperm entry and extrusion of second polar body, the cytoplasm and yolk begin to separate, forming a single blastomere at the animal pole occupying top of a continuous acellular yolk mass. Streaming of cytoplasmic constituents to the animal pole is observed in the fertilized egg of the angel fish. The angel fish shows a discoidal meroblastic cleavage pattern, where the large yolk volume restricts cell division to a small area at the animal pole. The early embryo forms as a small disc sitting on top of the large yolk. The first divisions are vertical and there is no cytoplasmic growth during this period. The second cleavage is horizontal and forms two tires of blastomeres. During 16 to 32 cell stage, the mass of cells from the blastodisc becomes stratified into more than one layer. Similar to most species, the blastomeres of angel fish are regular in size and shape. However, existence of inter and intra-specific variation on the general pattern is reported (Hall *et al.*, 2004). During later cleavage it is only the marginal blastomeres, which are positioned on the periphery of the blastodisc maintaining their cytoplasmic bridges with the yolk

The major developmental events of *D. dentex* and staging of larval form after hatching larvae (Santamaria, 2004) resembles with those recorded for angel fish. Observation of angel fish development indicates that major primordial organs and organ systems are formed during the embryonic development constituting first stage of development. The freshly hatched larvae of angel fish are transparent with pigmentation scarcely distributed over their dorsal surface. Notochord and myotomes are clearly visible. The primordial fin fold is well developed. Heart functioning commences prior to hatching. The yolksac is relatively large. Digestive tract is a straight tube and respiratory structures are poorly developed, while kidney is pro-nephric in nature. Such characteristics for freshly hatched teleost larvae have been reported by Hoar *et al.*, (1983). The hatched out larva undergoes intensive organogenesis during the second, third and fourth stages during which complete differentiation of the organ systems to their final stage is achieved. The fifth stage larva shows most of the juvenile characters, indicating that during this stage there are no new structural appearances but the already formed structures undergo growth and expansion. Such a development pattern is also reported for *D. dentex* by Santamaria, (2004). The metamorphosis of larvae of fishes to the adult form might require minor changes as in the case of salmonids and eels, or marked changes as in the case of flatfish. The most obvious changes are related to the formation of scales, pigmentation, swim bladder and lateral line system. In flatfish there is rotation of the optic region of the skull and change in the normal orientation of the body so that they are shifted to one side (Pandey *et al*., 1992). Though the development and metamorphosis of angel fish in general resembles other teleosts there are many features which are specific to *Pterophyllum scalare*.

CONCLUSION

The angel fish embryonic and larval development takes 24 days to attain juvenile stage. Compared to other teleost variation is observed with regard to the exclusively endogenous, endo-exogenous and exclusively exogenous larval feeding strategy.

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