



# International Journal of Fisheries and Aquatic Studies

ISSN: 2347-5129  
IJFAS 2014; 1(4): 01-07  
© 2013 IJFAS  
www.fisheriesjournal.com  
Received: 01-02-2014  
Accepted: 15-02-2014

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## Breeding and development of ornamental hill stream fish *Devario aequipinnatus* (McClelland) in captivity

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### ABSTRACT

*Devario aequipinnatus* is an active and brilliantly striped hill stream fish and has a great value as an ornamental fish in the global market. The aim of the present study was to breed this ornamental fish without the use of any hormones and study its development in captivity. Breeding of this ornamental fish was successfully carried out in July 2011 and in August 2012. The fishes were bred in captivity without the use of any hormones in a mature aquarium which simulated the natural habitat. Induction of spawning was successfully carried out by increasing the temperature and creating artificial rain. Embryonic and post embryonic development was recorded at intervals up to 65 days. The fertilized eggs measuring 0.9-1.2 mm in diameter were observed to be demersal, nonadhesive, almost spherical in shape, optically transparent and unpigmented. The embryos hatched 35-36 h post-fertilization from the chorion and measured  $2.8 \pm 0.1$  mm in total length. After 65 days of development it completely resembled the adult fish. The eggs and embryonic development of *D. aequipinnatus* resembled those of the closely related species *Danio rerio* and *Devario malabaricus*. The larvae and juveniles of *D. aequipinnatus* were also similar to those of the latter species in general morphology. Captive breeding of this ornamental fish can play a great role in the conservation and its habitat protection.

**Keywords:** Breeding, *Devario aequipinnatus*, Embryonic, Ornamental.

### 1. Introduction

Ornamental fish is often used as a generic term to describe aquatic animals kept in the aquarium hobby [1]. Ornamental fishes form an important commercial component of aquaculture, providing for aesthetic requirements and upkeep of the environment [2]. The growing interest in aquarium fishes has resulted in steady increase in aquarium fish trade globally. Together all countries of the European Union are the largest market for ornamental fish; however, United States is the single largest importer of ornamental fish in the world [3, 4]. The export of ornamental fishes from India is mainly confined to wild-caught species (85%) and few bred varieties of exotic species (15%). Among the wild-caught fishes exported from the country, West Bengal and north-eastern States are the main contributors [5]. Meghalaya situated in the north-eastern region of India, lies between 25° to 26° north latitude and 90° to 92° 45' east longitude is endowed with vast aquatic resources in the form of hill streams, rivers, reservoirs, ponds and paddy-cum-fish culture areas containing a rich variety of fish fauna. Many ornamental fishes like *D. aequipinnatus*, *D. dangila*, *D. rerio*, *B. bendelisis*, *B. rostrata*, *E. danricus*, *P. shalynius*, *L. guntea* etc. are exported out of the State as an aquarium fish and also consumed by the local populace. The ornamental fish trade has the potential to contribute to the economic growth of states concerned and the sustainable development of aquatic resources, but faces future challenges regarding environmental and social issues [6].

*D. aequipinnatus* belongs to family Cyprinidae, is an active and brilliantly striped ornamental hill stream fish and has a great value in the ornamental fish market. This fish is commonly called giant danio. It is widely distributed in Asia and native to India, Nepal and Sri Lanka [7]. It inhabits hill streams up to an elevation of 300 m and does not grow more than 15 cm [8]. It is found in shaded, mid-hill clear waters with pebble or gravel substrates [9] and occurs in schools at the surface in small high-gradient upland streams [9].

It mainly feeds on exogenous insects <sup>[10]</sup> and also on worms and crustaceans <sup>[11]</sup>. This fish is not found in local markets, but popular in aquarium trade <sup>[10, 12]</sup>. It has got 4 or 5 horizontal stripes, P, P+1, P-1, P-2 and P+2 present; four interstripes I+1, I-1, I+2 and I-2. P stripe broader and distinct than other stripes <sup>[13]</sup>. Many species that were formerly within the *Danio* genus, such as the giant danio, have now been reclassified as *Devario* <sup>[14]</sup>. There are currently approximately 44 danionin species <sup>[15]</sup>, distributed throughout South and Southeast Asia, their highest species diversity in north-eastern India, Bangladesh and Myanmar <sup>[16]</sup>.

Life in aquatic environment is largely governed by physico-chemical characteristics and their stability. Several physico-chemical or biological factors could act as stressors and adversely affect fish growth and reproduction <sup>[17]</sup>. The important physical and chemical parameters influencing the aquatic environment are temperature, rainfall, pH, salinity, dissolved oxygen and carbon dioxide and these parameters are the limiting factors for the survival of aquatic organisms <sup>[18]</sup>. However, fishes are more dependent on water temperature, pH, dissolved oxygen, free CO<sub>2</sub>, alkalinity and some salts for growth and development <sup>[19]</sup>. Productivity of a water body depends on its ecological conditions and productivity can be increased through constant monitoring of water quality <sup>[20]</sup>. In case of ornamental fishes, proper water quality maintenance is the primary protective measure as they are very sensitive to temperature and pH <sup>[21]</sup>. It has been reported that temperature induces gonadal maturation and affects electrophysiological sexual maturity indicators in some fish <sup>[22]</sup>. The importance of water temperature in influencing olfactory sensitivity in some male fish to pre-ovulatory and post-ovulatory ovarian pheromones has been reported by some authors <sup>[23]</sup>. Food is the basic prerequisite for growth, development, survival and existence of all organisms. It plays an important role in the migration, growth and spawning behaviour of the fish. Fecundity study, an important biological parameter depends upon the supply of food <sup>[24]</sup>. *D. aequipinnatus* was abundantly found in the State of Meghalaya until recent years. Unfortunately, the population of this important bio-resource of the State is gradually declining due to the destruction of habitat because of anthropogenic factors and overexploitation. Keeping this in view, breeding of this ornamental fish species was carried out as captive breeding can play a great role in the conservation of this fish species along with its habitat protection.

## 2. Materials and Methods

### 2.1. Collection of fishes

The fishes were collected from their natural habitat (the streams in Sohra in the East Khasi Hills in Meghalaya - 25°15'47.61" N, 91°42'45.72" E, Altitude - 1292 msl).

### 2.2. Domestication and maintenance of fishes

After collection of the fishes, were stocked in rearing tanks of size 1.5 m x 0.5 m x 1 m. Mature males and females were kept separately with the density of 6-7 in each tank. The rearing tanks were provided with dark substrate (gravel and rocks) and mild circulating water current with the help of electric motors for their adaptation. In general, they are very active swimmers and need a lot of swimming and hiding space in the aquarium. After proper acclimatization and maintenance, the domesticated brood stock was selected for the breeding experiments.

### 2.3. Spawning tank

Mature aquarium (1.5 m x 0.5 m x 0.5 m) with dark substrate (marbles) and natural day lighting provided with spawning mops made of green wool.

### 2.4. Water Parameters

The water parameters are very important for the rearing and breeding of this ornamental fish species. All the water parameters mentioned were maintained similar to the natural habitat or the place of collection. Fresh, dechlorinated and well aerated water was used for domestication of the fish in all the tanks. In all the tanks for rearing and breeding experiments pH was maintained in the range 6.7±0.2, nitrates, nitrites and ammonia were maintained at 0, hardness in the range of 60-70 mg L<sup>-1</sup>, conductivity in the range of 0.1±0.02 mS, total alkalinity in the range of 30-35 mg L<sup>-1</sup> and dissolved oxygen in the range of 8.0±0.5 mg L<sup>-1</sup>. In the rearing tanks temperature of 25±0.5 °C was maintained with the help of regulated water heaters. The water temperature was measured with the help of a centigrade thermometer. Alkalinity and total hardness were studied following standard procedures <sup>[25]</sup>. Conductivity, pH, and dissolved oxygen were analyzed using meters by Lutron Co. Test kits were used to check nitrates, nitrites and ammonia.

### 2.5. Feeding

The fishes were fed ad libitum with live food (mosquito larvae, chironomous larvae, white worms, chopped earthworms etc.) and with commercially available fish food.

### 2.6. Sexual differences in *D. aequipinnatus*

The mature females are larger when compared to males and have a rounded belly while the males are slender and streamlined and more colorful.

### 2.7. Selection of the brood pair

In the spawning tank, one gravid female was released along with two mature males and then their behaviour was observed. If a pair is interested then the male will constantly chase the female all around the tank and hit the female in its abdomen with its head for spawning. If no interest is seen between them then the male is replaced by another mature male to check for the interest between them for spawning. The male which shows interest in the female are kept in the spawning tank and observed till the spawning takes place.

### 2.8. Induction of spawning

(a) After releasing the brood pair in the spawning aquarium, temperature was gradually increased to 27±0.5 °C from 25±0.5 °C within 48 h.

(b) After 48 h, 3/4<sup>th</sup> of spawning aquarium water was removed and replaced by creating artificial rain with a sprinkler with water 4 °C cooler than the spawning tank of to 27±0.5 °C at night around 10 pm.

### 2.9. Embryonic and post-embryonic stages

The embryos were viewed with Olympus microscope (CH20i) in 10 X magnification and photographs were taken in with canon PowerShot SD960 IS. The eggs were removed from the spawning aquarium with a dropper to the hatching tank with the same water parameters as of the spawning aquarium with moderate aeration and the eggs were allowed to hatch without disturbance. The fry were fed with cultured infusoria and their

developmental stages were observed and recorded at intervals. Stages from fertilization to hatching were named following the *D. rerio* staging system Kimmel *et al.*<sup>[26]</sup> based on the landmark morphological features. The diameter of the eggs was measured by using a calibrated ocular micrometer. The post-embryonic stages were studied with the dissecting microscope and the growth in length of the larvae was taken at few days intervals to the 65<sup>th</sup> day using a measuring scale.

### 3. Results

Breeding experiments in captivity were conducted successfully for the ornamental fish *D. aequipinnatus* in July 2011 and August 2012 without the use any hormones. Mature female (Fig. 1a) can be easily recognized, having rounded and swollen belly while the mature male (Fig. 1b) are slimmer and more colourful. Brief descriptions of the embryonic and larval developmental stages of *D. aequipinnatus* are given in Table 1 and 2.

**Table 1:** Brief descriptions of the embryonic developmental stages of *D. aequipinnatus*.

| Stage Name                                   | Time after fertilization | Characteristics   | Figure Number |
|--|--------------------------|---|---------------|
| <b>Zygote period</b>                         |                          |   |               |
| 1 cell                                       | 0000 h                   | Yolk-free cytoplasm begins to stream toward the animal pole gradually segregating the blastodisc from the vegetal cytoplasm.      | Fig. 2a       |
| <b>Cleavage period</b>                       |                          |   |               |
| 2-cell                                       | 45 min                   | Two blastomeres are rounded just after first cleavage.  | Fig. 2b       |
| <b>Blastula period</b>                       |                          |   |               |
| 256-cell                                     | 2 h 45 min               | Blastodermal cells are smaller than those of the previous stage and the number of marginal cells increased.                       | Fig. 3a       |
| 512-cell                                     | 3 h                      | Loss synchronicity of cell divisions.   | Fig. 3b       |
| Oblong                                       | 3 h 55 min               | Blastula acquires a smoothly outlined ellipsoidal shape.  |               |
| Sphere                                       | 4 h 15 min               | Border between the blastodisc and the yolk was considerably flattened.  | Fig. 3d       |
| Dome   | 4 h 40 min               | Blastoderm formed a dome-like shape because of the bulging up of the yolk cell towards the animal pole and the epiboly began.     | Fig. 3e       |
| <b>Gastrula period</b>                       |                          |   |               |
| 50% epiboly                                  | 5 h 35 min               | Gastrulation began.   | Fig. 4a       |
| Shield                                       | 6 h 30 min               | AP view reveals the embryonic shield, as well as the germ ring.   | Fig. 4b       |
| 75% epiboly                                  | 8 h 25 min               | Embryonic shield becomes less distinctive.  | Fig. 4c       |
| 90% epiboly                                  | 9 h 30 min               | Yolk plug is clearly seen in the vegetal pole.  | Fig. 4d       |
| Bud  | 10 h 40 min              | Epiboly comes to a close as the blastoderm completely covers the yolk plug. Early polster seen. Tail bud appeared.                | Fig. 4e       |
| <b>Segmentation period</b>                   |                          |   |               |
| 1 – somite                                   | 11 h                     | First somitic furrow forms.   | Fig. 5a       |
| 2 – somite                                   | 11 h 15 min              | Two somites are seen.   | Fig. 5b       |
| 4 – somite                                   | 11 h 40 min              | Optic primordium begins to show.  | Fig. 5c       |
| 5 – somite                                   | 12 h 05 min              | Brain primordium is distinctively thickened into the neural keel and the optic primordium is seen to develop a horizontal crease. | Fig. 5d       |
| 8 – somite                                   | 13 h 05 min              | Optic primordium has a prominent horizontal crease.   | Fig. 5e       |
| 12 – somite                                  | 15 h 30 min              | Tail bud becomes more prominent.  | Fig. 5f       |
| 15 – somite                                  | 17 h 40 min              | Somites are of chevron shape.   | Fig. 5g       |
| 17 – somite                                  | 18 h 50 min              | Otic placode is seen.   | Fig. 5h       |
| 20 – somite                                  | 22 h                     | Tail extension is seen.   | Fig. 5i       |
| 23 – somite                                  | 23 h 10 min              | Otic vesicle is clearly seen.   | Fig. 5j       |
| <b>Pharyngula period</b>                     | 28 h                     | Eyes are clearly visible with lens and retina.  | Fig. 6a       |
| ''   | 33 h                     | Embryo continues to exhibit spontaneous side-to-side contractions involving the trunk and tail.                                   | Fig. 6b       |
| ''   | 34 h 55 min              | Embryo breaks the chorion slowly to hatch.  | Fig. 6c       |
| <b>Larva after hatching from the chorion</b> | 36 h                     | Head slightly bent on the yolk, the eyes were large and yolk sac was present. Responsive to stimulus.                             | Fig. 7        |

**Table 2:** Brief descriptions of the larval development of *D. aequipinnatus*.

| Age of larvae (days) | Length     | Characteristics  | Figure Number |
|----------------------|------------|--|---------------|
| 4                    | 3.1-3.3 mm | Head straightened; eyes were prominent and pigmented black in colour. A dark lateral band was seen forming between anteriormost trunk and caudal fin base. | Fig. 8a       |
| 6                    | 4.5-4.8 mm | Mouth appeared to be open and slit like. Complete resorption of the yolk sac and minute pectoral fins were observed.                                       | Fig. 8b       |
| 10                   | 8.2-8.3 mm | Mouth parts appeared to be well developed. Larva is seen to take the shape of a fry.   | Fig. 8c       |
| 20                   | 1.1-1.2 cm | Pigmentation was clearly seen in the anterior part of the body and the caudal fin. The caudal fin, dorsal fin and anal fin were seen to be developing.     | Fig. 8d       |
| 25                   | 1.3-1.4 cm | Pigmentation all around the body. The anterior portion was thicker and tapered towards the tail dorsal fin and anal fin appeared very prominent.           | Fig. 8e       |
| 35                   | 1.9±0.1 cm | The body organs were darker.   | Fig. 8f       |
| 45                   | 2.4±0.2 cm | Horizontal stripes were seen to be developing and the fins were attaining colour.  | Fig. 8g       |
| 65                   | 3-3.2 cm   | Completely resembled the adult fish in all the features and could be easily identified.  | Fig. 8h       |



**Fig 1:** (a) Mature female (7.8 cm), (b) Mature male (8.1 cm)

### 3.1. Spawning behaviour

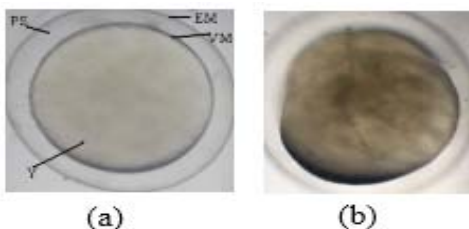
Marked spawning pattern was observed in both the male and the female fish during the dawn. The male was observed constantly hitting the female on the abdomen with its head while chasing her all around the aquarium. After this activity of 20-25 min they started spawning after the sunrise. The female released eggs in batches of 15-20 and the eggs were immediately fertilized by the male sideways and they continue spawning frequently up to 6-7 days till the female released all the ripe eggs. The female in the first spawning batch released 50-60 eggs all around the aquarium. The percentage of fertilization in this case was seen to be 75-80.

### 3.2. Embryonic development

The recorded embryonic development of *D. aequipinnatus* is described as below:

#### 3.2.1. Zygote period

The newly fertilized eggs were demersal and seen to scatter and settle at the bottom of the tank. The unfertilized eggs were opaque and yellowish in colour. The diameter of the zygote was 0.9 to 1.2 mm (Fig. 2a). The fertilized eggs were non-adhesive, whitish in colour and optically transparent. Fertilization activated the cytoplasmic movements. The yolk-free cytoplasm begins to stream toward the animal pole gradually segregating the blastodisc from the vegetal cytoplasm.



**Fig 2:** (a) Fertilized egg, (b) 2-cell stage (EM-Egg membrane, VM-Vitelline membrane, PS-Perivitelline space, Y-Yolk)

#### 3.2.2. Cleavage period

The first cleavage occurred at 45 min after fertilization. The two blastomeres are rounded just after first cleavage (Fig. 2b). After the first cleavage, the blastomeres divided synchronously at intervals of 15-20 min.

#### 3.2.3. Blastula period

The blastula period began with the 128-cell stage and ended with the beginning of the gastrulation. The cell divided more or less synchronously during the early stages of the blastula period. The blastoderm cells in the 256-cell stage (Fig. 3a) and 512-cell stage (Fig. 3b) are smaller than those of the previous stage and the number of marginal cells increased. The important processes that occur during the blastula period are that the embryo enters midblastula transition, the yolk

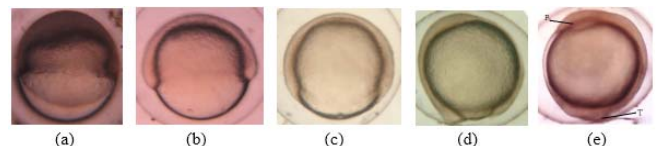
syncytial layer forms and epiboly begins. During the oblong stage (Fig. 3c) the blastula acquires a smoothly outlined ellipsoidal shape, as viewed from the side. At the sphere stage (Fig. 3d), the border between the blastodisc and the yolk was considerably flattened. At the dome stage (Fig. 3e), the blastoderm formed a dome-like shape because of the bulging up of the yolk cell towards the animal pole and the epiboly began. Epiboly continues during the gastrulation period.



**Fig 3:** Blastula stages. (a) 256-cell stage, (b) 512-cell stage, (c) oblong stage, (d) Sphere stage, (e) Dome stage.

#### 3.2.4. Gastrula period

In the gastrula period, extensive cell movements were observed, including involution, convergence and extension, producing the three primary germ layers and the embryonic axis. Gastrulation began with cell involution at around 50% epiboly (Fig. 4a). The blastoderm thickened at the leading edge all around the blastoderm rim giving rise to the germ ring. At the shield stage (Fig. 4b), animal pole view most easily reveals the embryonic shield, as well as the germ ring. At 75% epiboly stage (Fig. 4c), the embryonic shield becomes less distinctive, as compared to shield stage and its cells repack to elongate the shield along the AP axis. At 90% epiboly stage (Fig. 4d), the yolk plug is clearly seen in the vegetal pole. In the bud stage (Fig. 4e) epiboly comes to a close as the blastoderm completely covers the yolk plug. The tail bud then appeared as a distinct thickening at the caudal end of the embryo, near the site yolk plug closure. Early polster is seen.



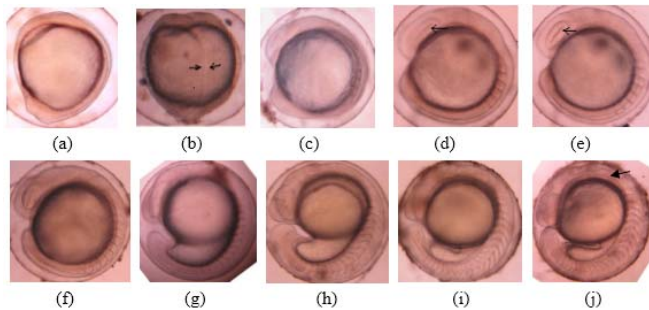
**Fig 4:** Gastrula stages. (a) 50% epiboly stage, (b) Shield stage, (c) 75% epiboly stage, (d) 90% epiboly stage, (e) Bud stage. (P- Polster, T-Tail bud)

#### 3.2.5. Segmentation period

The segmentation period was characterized by the sequential formation of the somites, and this period lasted to just prior to hatching. During this period, the embryo elongated along the AP axis, the tail bud developed longer and rudiments of the primary organs became visible. Somites formed in bilateral pairs, as the developing embryo extends posteriorly. During the one-somite stage (Fig. 5a), the first somitic furrow forms. Dorsal view of the two-somite stage is shown in (Fig. 5b). The notochord rudiment is shown between the arrows. In four-



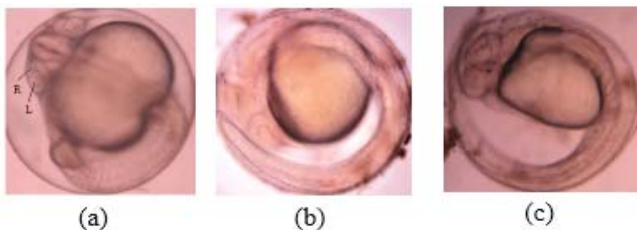
somite stage (Fig. 5c), the optic primordium begins to show. At the five-somite stage (Fig. 5d), the brain primordium is distinctively thickened into the neural keel and the optic primordium is seen to develop a horizontal crease (arrow). In the eight-somite stage, the optic primordium has a prominent horizontal crease shown by an arrow (Fig. 5e). The midbrain rudiment lies just dorsal and posterior to optic primordium. In the twelve-somite stage, the tail bud becomes more prominent (Fig. 5f). In the fifteen-somite stage, somites are of chevron shape (Fig. 5g). The otic placode is seen at the seventeen-somite stage (Fig. 5h). The yolk extension is now clearly delimited from the yolk ball as the tail straightens out. Tail extension is seen in twenty-somite stage (Fig. 5i). The arrow indicates the otic vesicle in the twenty three-somite stage (Fig. 5j).



**Fig 5:** Segmentation stages. (a) 1-somite stage, (b) 2-somite stage, (c) 4-somite stage, (d) 5-somite stage, (e) 8-somite, (f) 12-somite, (g) 15-somite, (h) 17-somite, (i) 20-somite, (j) 23-somite.

### 3.2.6. Pharyngula period

During this period the embryo is a bilaterally organized, with a well-developed notochord and a newly completed set of somites that extend to the end of a long post-anal tail. Body axis straightens from its early curvature about the yolk sac, circulation, pigmentation, and fins begin development. The nervous system is hollow. The head straightens out and lifts dorsal side. The brain is prominently sculptured. The blood flow is visible. Pigment formation begins in cells of the pigmented retinal epithelium. The embryo continues to exhibit spontaneous side-to-side contractions involving the trunk and tail and the rate of contractions increases in bursts till the embryo hatches out of the chorion. The embryos in the pharyngula period are shown in Figs. 6a and 6b. The embryo just before hatching from the chorion at 34 h and 55 min is shown in Fig. 6c.



**Fig 6(a-c):** Pharyngula stages.  
(R-Retina, L-Lens)

### 3.2.7. Larva after hatching from the chorion

Just after hatching from the chorion the larva at 36 h measured  $2.8 \pm 0.1$  mm (Fig. 7). Head slightly bent on the yolk, the eyes were large, yolk sac was present on the anterior ventral side of

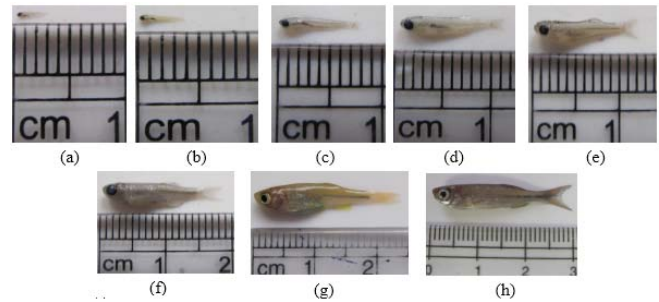
the body and the heart and the optic vesicle were seen. They were responsive to stimulus and settled in the substrate.



**Fig 7:** Larva after hatching from the chorion  
(YS- Yolk sac, S-Somites, CFB-Caudal fin bud)

### 3.3. Larval development

The freshly hatched larva was measured and the subsequent development of its growth was recorded as follows:



**Fig 8:** Shows the post-embryonic development of *D. aequipinnatus*. (a) 4 days old, (b) 6 days old, (c) 10 days old, (d) 20 days old, (e) 25 days old, (f) 35 days old, (g) 45 days old, (h) 65 days old.

#### 3.3.1. 4 days old

The length of the larva was 3.1-3.3 mm (Fig. 8a). The head straightened, eyes were prominent and pigmented black in colour. The heart increased in size and the alimentary canal was differentiated above the yolk sac. A dark lateral band was seen forming between anteriormost trunk and caudal fin base. The gill arches were seen. The larvae were sticking to the substrate and aquarium glass.

#### 3.3.2. 6 days old

The length of the larvae increased to 4.5-4.8 mm (Fig. 8b). The mouth appeared to be open and slit like. The larvae started swimming and feeding. There was complete resorption of the yolk sac and minute pectoral fins were observed. The eyes were well set in the optic sockets. The dark lateral band was more prominent seen between the operculum and the caudal fin base.

#### 3.3.3. 10 days old

The body length increased to 8.2-8.3 mm (Fig. 8c). The mouth parts appeared to be well developed. Development of more prominent pectoral fins was observed. The larva is seen to take the shape of a fry.

#### 3.3.4. 20 days old

The fry measured 1.1-1.2 cm (Fig. 8d). Pigmentation was clearly seen in the anterior part of the body and the caudal fin. The caudal fin, dorsal fin and anal fin were seen to be developing. The eyes and other organs of the body further developed.

**3.3.5. 25 days old:** The fry attained the size of 1.3-1.4 cm (Fig. 8e). At this stage the body length did not increase much as compared to the previous stage but this stage was mainly characterized by pigmentation all around the body, the anterior portion was thicker and tapered towards the tail dorsal fin and anal fin appeared very prominent.

### 3.3.6. 35 days old

The body length was 1.9±0.1 cm (Fig. 8f). Pigmentation was prominent and darker all around the body. The body organs were darker.

### 3.3.7. 45 days old

The length of the fish was 2.4±0.2 cm (Fig. 8g). Horizontal pigmentation was prominent and the fish resembled the adult fish. The horizontal stripes were seen to be developing and the fins were attaining colour.

### 3.3.8. 65 days old

At this stage the fish measured 3-3.2 cm (Fig. 8h) and completely resembled the adult fish in all the features and could be easily identified. The horizontal stripes matched the adult fish and attained the colourful pigmentation, which characterizes it as an ornamental fish.

## 4. Discussion

The present study demonstrates the successful breeding of *D. aequipinnatus* in captivity without the use of any hormones along with its development till it resembles the adult fish. The main stimulus for the induction of spawning is seen to be due to the sudden cooling of the spawning aquarium by the artificial rain created with water 4 °C cooler than water in the spawning tank and then gradual increase of temperature in the spawning aquarium from 25±0.5 °C to 27±0.5 °C after which the pair started spawning as it was noted by Breder and Rosen<sup>[27]</sup> that adding a dash of cold water to aquaria could encourage spawning in zebrafish and Kayaba *et al.*<sup>[28]</sup> demonstrated the effect of an increased temperature stimulus on the induction of spontaneous spawning of cultured barfin flounder. The development of this species is found to be similar to closely related species such as *D. rerio* (see: Hisaoka and Battle<sup>[29]</sup>, Kimmel *et al.*<sup>[26]</sup> and *D. malabaricus* (see: Jones<sup>[30]</sup>). As reported by Kimmel *et al.*<sup>[26]</sup> in *D. rerio* that the embryos within a single developing clutch develop and hatch sporadically from the chorion, this fish also showed the same pattern. The embryos started hatching after 35-36 h and the hatchlings started swimming after 5-6 days. According to Andrews<sup>[31]</sup> the parents eat their own eggs; therefore, eggs had to be removed to the hatching tank after each batch of spawning. *Devario* species typically inhabits faster flowing water unlike zebrafish, which inhabits the margins of streams and rivers<sup>[32]</sup>. Therefore, maintaining moderate water current in the aquarium creates natural habitat like condition for this fish. The growth of post-embryonic stages was observed to be slower in the rearing tanks compared to the natural habitat as the availability of natural food and feeding frequency was less in the captive condition compared to the natural habitat and also the growth rates of fish vary with factors such as temperature, food availability and exploitation<sup>[33]</sup>. A clear observation was that the 45 days old juvenile fish of 2.4±0.2 cm in the present study did not develop the lateral bands and coloration as compared to the fishes of the same length found in the natural habitat. Although the majority of freshwater fish

involved in the aquarium fish trade are from captive-bred sources, significant numbers are still removed from the wild<sup>[34]</sup>. Therefore, captive breeding can play a great role in increasing the population of this species which can be used by the aquarium keepers so that this species is not exploited from the wild which will help in the conservation of this fish species along with its habitat protection.

## 5. Conclusion

This study documents the breeding of ornamental fish *D. aequipinnatus* in captivity without use of any hormones and embryonic and post embryonic development up to 65 days till it completely resembles the adult fish. The subject matter in this paper is useful for fish breeders, aquarium keepers and those involved with or interested in the study of fish larval and fry development.

## 6. Acknowledgements

The authors are thankful to Ministry of Environment and Forest, New Delhi and UPE scheme of UGC to Life Sciences, North Eastern Hill University, Shillong for financial support. The encouragement and the facilities provided by the Head of Department of Zoology for carrying out the work are highly acknowledged.

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