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Captive breeding, developmental biology and commercial production of *Dravidia fasciata*- An indigenous ornamental fish of the Western Ghats of India

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ABSTRACT

Ornamental fishes of the Western Ghats of India have great demand in the export market. At present these fishes are collected from the wild and exported. Hence many times, the demand could not be met due to short supply. The only remedial measure for a sustainable supply is to produce the fish in captive conditions. Unfortunately, the breeding technology for the ornamental fishes of the Western Ghats of India has not been attempted seriously till date. The present paper is almost a pioneering attempt to develop captive breeding technology for 12 prioritized species of the indigenous ornamental fishes of the Western Ghats of India. *Dravidia fasciata* is one of them. It is popularly known as Melon barb. It is a beautiful barb, growing to a maximum size of 80 mm. In the present paper the methodology of captive breeding of this fish is provided with the economics of its production. Melon barbs were collected from the wild and brought to the hatchery of College of Fisheries in oxygen filled plastic bags and gradually acclimatized to the captive conditions. Its size at first maturity, sexual dimorphism, and developmental biology were studied and described with photographs. The total length (TL) at first maturity for males was 50 mm (50-55 mm) and 40 mm for females (40-45 mm). A sexually mature male developed beautiful pinkish red tinge all over the body. The black bands over the body also became deeper in colour during this time. The intensity of the colour reached its maximum during the courtship activities. Male also possessed nuptial tubercles on the operculum which could be identified only by keen observation. But a sexually mature female did not develop any colour change by the onset of sexual maturity. The results of the study clearly demonstrated that *D. fasciata* could be successfully produced in captivity through scientific management of brooders, eggs, larvae and hatchlings. The successful development of captive breeding technology is likely to pave way towards commercialization of the technology thus leading to the sustainable export of the species.

INTRODUCTION

Kerala has rich sources of water bodies such as rivers, lakes, reservoirs, canals and ponds. The rivers of Kerala possess rich diversity of ornamental fishes, with over 155 species of indigenous species (Mercy et al., 2007). Some of the potential ornamental fish of Kerala namely loachs, barbs, danios, catfishes, perches and cichlids are in great demand in export market. At present, these fishes are collected from the wild and exported. Even though Kerala

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is a goldmine of indigenous ornamental fishes, the quantity of export is minimal. There are several reasons for the low export of fishes. The most important reason is that the exporters are not able to supply as per the demand. The demand usually, is for equal sized fishes in large quantities which cannot be supplied from wild collection alone. This can be achieved only through hatchery production. Hence, breeding in captivity is one of the desirable qualities of any ornamental fish. Unfortunately, the breeding technology for the ornamental fishes of the Western Ghats of India has not been attempted seriously till date, except for the work done by Mercy (2004) in which captive breeding technology was developed for 12 prioritized species of fishes. *D. fasciata* is one of them.

Dravidia fasciata is a beautiful indigenous ornamental fish found in the west flowing rivers of Goa, Karnataka, Kerala and up to Kanyakumari district in Tamil Nadu and also in the east flowing streams of River Cauvery basin in the foot of the Nallamala Hills (Jayaram, 1991). It is popularly known as melon barb. It is a small barb that grows to a maximum size of 8cm. It is omnivorous in diet and also is eaten by larger fish and crustaceans (Mercy et al., 2001). Captive studies on the behavior of the fish under aquarium conditions have shown that it occupies the mid water column in the tank.

Present paper describes the captive breeding technology of this beautiful ornamental fish. The development of captive breeding technology of this species clearly indicates that commercial production of *D. fasciata* is possible with this technology through scientific management of brooders, eggs, larvae and hatchlings. This success is likely to pave way towards commercial production of this species thus leading to its sustainable export.

MATERIALS AND METHODS

Specimens of *D. fasciata* were collected from River Pampa using cast net and small hand nets during the months of August-September (2002-05). They were brought to the hatchery of College of fisheries in oxygen filled plastic bags and gradually acclimatized to the captive conditions. Determination of size at first maturity and identification of male and female are two essential requirements for breeding a fish under captivity.

Size at first maturity

The length at which 50% of the fishes become mature is considered as the size at first maturity (Kagwade, 1975). Size at first maturity was computed with a total of 154 fishes of which 83 were females (ranging from 25 mm to 60 mm total length (TL)) and 71 were males (ranging from 30 mm to 65 mm TL). The total length of all the fishes collected was grouped according to different length groups. The percentage occurrences of mature fishes (early ripening, late ripening, ripe and partially spent) for both females and males were calculated. By plotting the percentage occurrence of mature fish (males and females) against respective length classes (5mm), the length at which 50% of the fishes become mature was demarcated.

Sexual Dimorphism

Different macroscopic and visual features were used for determining the sex of the individual. These includes 1) Overall body coloration (sexual dichromatism); 2) Bulginess of the stomach and 3) Behaviour in captive conditions

A total of 55 specimens were used for sex determination studies and they included fishes collected from their natural habitats and F1 and F2 generations of the hatchery reared fish. Different nuptial and breeding behavioral gestures like chasing; following, nubbing etc. also were used for distinguishing the sexes.

Development of brood stock

The brood stocks were raised on a mixed diet of artificial pelleted feed, live feeds like moina, mosquito larvae, blood worm and egg yolk. They were kept in glass tanks fitted with biological filter and in cement tanks devoid of biological filter. A daily water exchange at a rate of 1/3 was ensured in the cement tanks. They were continuously observed for their behavior in tanks. The maturity condition of the brooders was assessed based on the macroscopic characters such as body size, bulginess of belly and overall body colouration. Sometimes the behavioral patterns of the fishes were also considered. As the specimens became sexually mature they were separated sex-wise and kept in separate tanks of the same dimensions mentioned above.

Captive breeding

Breeding was conducted in small cement cisterns or round cement tanks as shown in the photographs (Plate). A breeding tank was set up providing the same water quality available in the natural habitat of the fish. The tank was cleaned properly and filled with water of desired quality up to three fourth of the tank. A separating net with small mesh mounted on a ring was kept 30cm above from bottom of the tank so that the eggs laid are fallen through the net to bottom of the tank This prevented the parents from devouring it. A pair of well-conditioned, fully mature melon barb (1:1 male: female) was introduced to the prepared breeding tank on an evening and were observed for their breeding. Next day morning eggs could be seen under the net trap in the tank. A total of 10 pair was used in each trial. The latency period was observed as the time duration between their introduction of conditioned pairs into the experimental tank and the start of spawning. Soon after the completion of laying eggs the fishes were removed from the breeding tank and the eggs were counted to find out the fecundity. The counting was conducted using the random sampling method.

Developmental biology

The fertilized eggs were collected soon after it was spawned. From each pair 5 to 10 eggs were collected and placed them in 2 liter capacity container. Developing eggs were observed with a trinocular microscope (Labomed) and photographs were taken with SLR camera (Nikon 90 X). The early developments up to hatching of the eggs were done in one hour interval. After hatching the developmental stages were photographed every 2 hours up to 24 hours and thereafter at every 24 hours up to the juvenile stage. All the measurements were taken under average room temperature of 26 to28 ˚C. The eggs were placed in cavity slides, immersed in water for observations and cavity blocks were used to observe larvae after hatching. The sampled eggs and larvae were fixed in 4% formalin for further observations.

In the present study, the developmental stages were divided into embryonic development, larval development and post larval development. The embryonic development started inside the

chorion and completed at hatching. The larval stage started from hatching and ended by the appearance of fin rays in all fins. After that, the larva was transformed into post larvae. The development of 25 individual embryos was documented right from fertilization. The water quality parameters were monitored weekly and daily exchange of 25% of water was done.

RESULTS AND DISCUSSION

Size at first maturity

The total length (TL) at first maturity was determined by analyzing the data relevant to all mature fishes (stage III and above examined). While the first mature male fishes appeared in 45-50 mm (TL) group (16.66 %), the first mature females appeared only in the group of 30-35 mm (16.66 %). All male fishes were matured on reaching a total length of 55 mm and all female fishes on reaching a length of 50 mm total length. The size at first maturity for males was 50 mm TL (50-55 mm) and for female it was 40 mm TL (40-45 mm) (Figure 1).

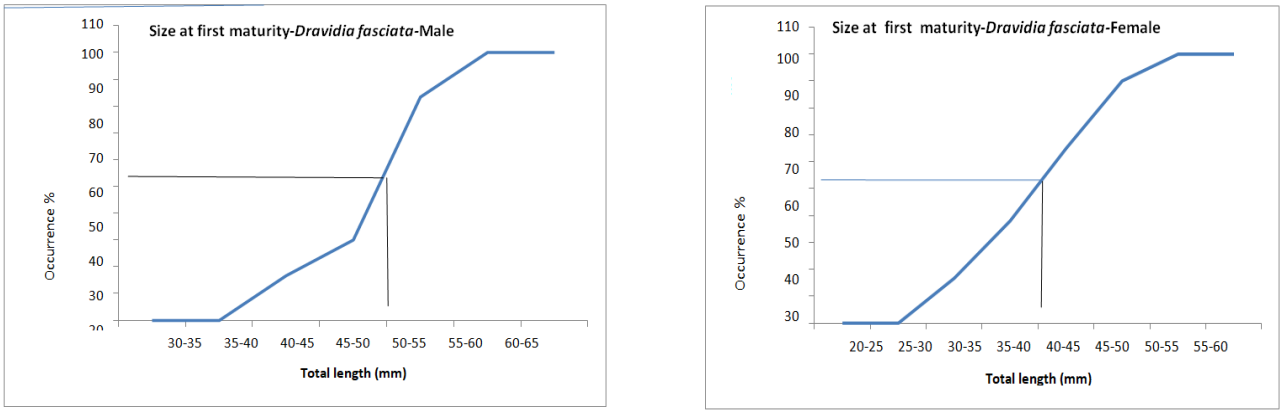

Figure 1. Size or total length of Dravidia fasciata at first maturity

The smallest mature male is within 40-45 mm length group. If the length at which 50% of the fishes are mature can be considered as the minimum length at first maturity (Kagwade, 1975), the specimens below 40 mm TL for males and 30 mm TL for females were not mature. The present study showed that the smallest mature male is bigger than that of the mature female in *D. fasciata*. Mercy et al. (2005) reported the size at first maturity of *Puntius melanostigma* as 50 mm for males and 55 mm for females. In the case of the African minnow, *Enteromius paludinosus,* sexual maturity was reached within a year at 50.0 mm TL (Cambray and Burton, 1985). In the case of European minnow, *Phoxinus phoxinus* the short lived populations of river Frome in England contained two spawning age groups and the largest fish caught was only 78.0 mm long. The size at first maturity ranged from 50-55 mm as two year olds (Mills, 1987). Six *Barbus* species studied in Sri Lanka had maximum total length of between 42.0 and 101.0 mm and a short life span (De Silva et al.,1985). In the freshwaters of South Africa out of the 52 *Barbus* species studied 43 attained maximum fork lengths of less than 150.0 mm (Cambray and Burton, 1985). The information on initial sexual maturity gives the ornamental fish producers the idea on the age at which the fish become mature

so that they could provide appropriate environment for the fish to spawn and obtain the maximum number of fry.

Sexual dimorphism

The male and female *Dravidia fasciata* showed clear differences in body coloration which could be termed as sexual dichromatism. The colour differences become prominent at the onset of sexual maturity. All the immature fish appeared in a dull grayish silvery colour. A sexually mature male developed beautiful pinkish red tinge all over the body (Figure 2). The black bands over the body also became deeper in colour during this time. The intensity of the colour reached its maximum during the courtship activities. But a sexually mature female did not develop any colour change by the onset of sexual maturity. It remained in the same colour pattern as that of a juvenile. Male also possessed nuptial tubercles on the operculum which could be identified only by keen observation. These types of nuptial tubercles are distinguishable in other cyprinids like gold fish, *Carassius auratus* and Indian major carps which have tubercles on pectoral fin rays also.

Another distinguishing character was the bulginess of stomach. A sexually mature female *Dravidia fasciata* exhibited a more swollen and deeper stomach than that of the males. The reproductive behavioural patterns exhibited during the onset of maturity were also used to distinguish sexes.

***Dravidia fasciata* male**	***Dravidia fasciata* female**
	

Figure 2. Sexual dichromatism of Dravidia fasciata

In cyprinids, sexual dimorphism in morphological characteristics other than colouration or presence of nuptial tubercles is uncommon (Scott and Crossman, 1973). Sexual dimorphism is a widespread phenomenon in fishes and may occur for a variety of reasons including, mate selection, male to male competition for mates, differences in sexual roles, predator avoidance, territoriality and ecological processes (Hubbs et al., 1974; Fernandes, 1998). Sexual dimorphism in body size, coloration, fin length, nuptial tubercles, and intromittent organs has been observed in many fish families (Scott and Crossman, 1973). Sex identification has practical applications in captive propagation processes.

Males and females usually differ not only in

reproductive organs, but also in external structures that are not directly related to reproduction. Information about sexual dimorphism is required for understanding the ecology, behavior and life history of a species. In addition, knowledge of sexual dimorphism and its appearance during ontogeny is indispensable when making morphological comparisons between populations. Although sexual differences in a variety of external structures have been noted in many species, studies on the sexual difference in fresh water fishes of India are less. A comprehensive study was done by Inasu (2008) in which sexual dimorphism of 26 species of Indian fishes was compiled. Mercy (2004) and Mercy et al. (2001, 2002, 2007, 2013) have described the sexual dimorphism of *Danio malabaricus, Pristolepis marginata*, *Puntius melanostigma*, *Garra mullya, Puntius pookodensis, Nemacheilus triangularis* and *Puntius denisonii*, which are important freshwater ornamental fishes of the Western Ghats of India.

Dravidia fasciata exhibited sexual dichromatism rather than sexual dimorphism. Breeding adults of male *D. fasciata* had marked sexual dichromatism. Males became pinkish when they became sexually mature. Reproductive females did not have pink colour. The colour became intense after the fish started breeding. It gradually faded after the courtship. This suggests that sexual dichromatism in the body is a secondary sexual character that may be regulated by reproductive hormones. Although sex in *D. fasciata* is genetically determined, it is currently unknown what genes or hormones might regulate secondary sexual dichromatism of body in this fish. Further analysis of the genetic and developmental mechanisms that underlie sexual dimorphism in *D. fasciata* will be possible by using the genomic tools established by Peichel et al. (2001) and Peichel (2005) and will provide a complement to ecological studies to discern the functional significance of sexual dichromtism in *D. fasciata.* Similar type of secondary sexual characters were also observedin *P. melanostigma* (Mercy et al., 2004) and *P. pookodensis* (Eapen, 2013).

Captive Breeding

The fully mature and well-conditioned male and female fishes were introduced in the prepared experimental tank (Plate) at a sex ratio of 1:1 (Female: Male). Soon after introduction, the fishes did not show any indication of breeding behavior. After half an hour, the fishes started its breeding behavioral signs like chasing, nubbing, following etc.

Breeding Behaviour

Parental care of the eggs and hatchlings either by male or by female parent was not observed in *D. fasciata.* It agrees with general behaviour of the cyprinid fishes. In the case of *D. fasciata,* it not only showed any signs of parental care but it also showed the tendency to deavour the eggs and hatchlings. So an appropriate breeding trap was needed in the captive breeding set up to protect them from the hungry parents. All cyprinids spawn using egg scattering methods and do not usually exercise parental care. But an exception is reported in fathead minnow *Pimephales promelas* (Sargent, 1989). The number of eggs spawned was at a range of 180 to 415 with a mean 264±86.6. The survival rate was at the range of 49 to 68%, with a mean 55.16±2.7.

Developmental biology

Embryonic development

Immediately after fertilization the eggs were swollen up considerably by absorbing water and within five minutes they attained a spherical, transparent and slightly adhesive structure. A streaming movement of the egg protoplasm took place, which resulted in the formation of blastodisc. The fertilized eggs of *D. fasciata* were amber coloured *en mass*, yolked, glossy, translucent and spherical with an average diameter of 0.85 mm (0.85 ± 0.02 mm). Like most other cyprinids the eggs of *D. fasciata* were free and demersal but not adhesive. The location of the micropylar region was distinct as a small depression in the animal pole, while it was absent in unfertilized eggs. The yolk which often had a yellowish tinge was coarsely granulated. The eggs were easily collected and transferred for incubation in hatching tanks with continuous oxygenation. The observations revealed that the hatching of eggs was accomplished 23 to 27 hrs in the ambient temperature of 26 ˚C (26 ± 2˚C). Neutral pH was maintained for the medium throughout the studies. After observations, some of the eggs were preserved in 5 % formalin for future studies. All measurements were made on fresh specimens using a calibrated ocular micrometer. Photographs of the developmental stages are provided in Plate (Annex).

The fertilized egg was telolecithal and cleavage was meroblastic. The blastoderm formed was restricted to animal pole at the point of entrance of sperm at the level of the micropyle, leaving large yolk mass at the vegetal pole. The first cleavage was meridional and incomplete. The second division was at perpendicular to the first and the third division resulted in the formation of 8 cells. The 4^{th} cleavage resulted in the formation of sixteen celled stage at 1.3 hrs and formation 32 celled stage occurred at about 1.45 hrs. A clear blastocoel began to appear at about 3.3 hrs and the blastula at this stage appeared as a cap of cells over the yolk. By 5 hrs it started to roll over the cytoplasm. After 5.5 hr, the blastoderm covered more than half of the yolk surface. At about 6.5 hours the early gastrula stage was reached and an embryonic shield was appeared. Gradually, epibolic germ layers were spread to the equator of the spherical yolk surface and at 6 hrs, the germ ring invaded $3/4^{th}$ of the yolk surface. At 7.5 hrs, the neural plate was formed and gradually almost $5/6^{th}$ of the yolk surfaces become invaded. As the blastopore got closed, yolk plug was projected and the head rudiment was seen lifted up. By 10 hrs, the optic rudiment appeared and gradually by 10.5hrs it became differentiated into a vesicle. At this stage, the head and tail got differentiated and the myotomes also became clearly visible. At 12 hrs the tail bud was formed and the embryo appeared very much elongated and was seen encircling over the yolk, reaching nearly $3/4^{th}$ of its circumference. At 15 hrs, caudal fin fold rudiment was drawn out from the yolk and the head region became more and more differentiated. Yolk sac got stretched and assumed a characteristic beaked appearance. Tail bud was projected out from the beak like yolk mass distally at around 16 hrs. Paired somites also became distinct at this point of time. At 18 hrs, optic vesicle became

conspicuous and the head region got separated; the caudal fin fold became very much elongated and the embryo appeared 'C' shaped encircling the yolk. At this period, the muscular somites were seen twitching at intervals. At 21 hrs the heart and optic capsule became conspicuous and embryonic movement became rapid. The heart began to pulsate at 21.5 h. At 22 hrs, tail got free and encircled almost 90 percent of the yolk mass. Gradually the heart pulsation became more rhythmic. The embryo began to roll within the egg case. As the development advanced, the embryo appeared more and more elongated and the tail overlapped the head. The myotomes and auditory vesicle became more prominent and the twitching of embryo started within the cytoplasm. As time passed the twitching movement of the embryo became faster.

Close to hatching, twitching and lashing of embryo inside the egg capsule became rapid. The egg shell was broken up and the tail emerged out first, followed by the head region. Hatching occurred at around 23.5 hrs. Egg hatching was protracted and the incubation period fluctuated between 23and 27 hrs post fertilization at the ambient temperature.

Newly hatched larva

The newly hatched larva appeared to be sluggish and remained at the bottom. It was transparent without any pigmentation. A continuous fin fold was present starting from the start of the dorsal fin, surrounding the tail and ended in the insertion of the ventral fin. Oral and anal orifices were completely absent. The length of the hatchling at this stage was 2.3 mm. In 24 hours it attained a mean length of 16 mm. Melanophores started to appear on the opticrim and myotomes.

At 48 hours, the larva appeared slender and elongated. The yolk was very much reduced, though not completely exhausted. The hatchlings gradually began to swim up towards the water surface and sometimes found hung up from the water surface. Melanophores became conspicuous on the body surface. Fin rays were started to appear in the pelvic fin and stomach was visible through the transparent body. Above the stomach the gas bladder appeared as a glittering droplet. A small depression was started to appear on the fin fold at the portion of anus.

Juvenile Phase: One month old juvenile (average 25.80 ±1.39 mm TL) of *D. fasciata* (Originally *Puntius)* showed vertical body banding pattern. Many species of *Puntius* possessed black body markings at their juvenile phase which were quite different in size and shape. The juveniles of *D. fasciata* possessed three vertical black cross bands at nuchal, pre dorsal and caudal positions. Details of embryonic developmental stages are given in Table 1

Table 1- The embryonic developmental stages of *Dravidia fasciata*

Time after fertilization (Hours)	Developmental event
01.30	16 celled stage
01.45	32 celled stage
02.00	Early morula
03.30	Blastulation; Blastodisc formation
06.50	Gastrulation; early gastrula
07.00	Late gastrula
07.50	Neurula stage
08.00	Closure of blastopore/ Yolk plug
10.00	Optic rudiment appears
12.00	Formation of head and tail, Appearance of myotomes
16.00	Tail region detaches from the yolk
21.00	Twitching
22.30	Heart beat and blood flow starts
23.00 – 24.00	Hatching

The knowledge on different embryonic stages and its timings of developmental events has importance in developing hatchery techniques of a species. As far as *D. fasciata* is concerned, because of its importance in the ornamental fish trade, defining an effective hatchery technique is tremendously valued. In general, developmental biology of a species consisted of embryonic, larval and juvenile stages. The morphology of larval and juvenile *D. fasciata viz.,* overall appearance, fin ray formation, and pigmentation patterns were similar to that of many other cyprinids such as *Puntius pookodensis* (Jacob, 2013) and also as reported by Jones (1938); Balinisky **(**1948) and McClure (1999).

It is also to be noted that *D. fasciata, Puntius filamentosus and Puntius denisonii* co-exist and breed in the same habitat at the same season and the larvae of all the three species look alike with dark bands across the body. It is quite difficult to distinguish between the larvae unless experienced. In the case of *P. filamentosus* and *P. denisonii* the cross-bands are retained for about one month. They gradually fade to a single spot at the caudal peduncle in the case of *P. filamentosus* whereas they are completely vanished in *P.denisonii* (Mercy et al., 2013). Dry-season spawning of *D. fasciata* has already been observed and published by Harikumar et al. (1994). In the present study it has been observed that the peak period of breeding of *D. fasciata* is during the months from November to March/April. De Silva et al. (1985) have reported that among *Puntius* species in SriLanka seasonal and perennial breeders share the same macrohabitat.

Survival rate

Number of eggs produced at a time ranged from 30-40 eggs per gram bodyweight. Average weight of the female fish was 10-12gms. About

90 % of the eggs were fertilized and at the end of one month survival rate was 60-65%. On an average from a pair of brood 180-200 young ones can be obtained if properly maintained.

The economics of production: the cost of production is summarized in the following table.

Fixed cost	In US$
Cement Tanks: 12 x $ 30	$ 360
Net + Accessories	$ 15
Operational cost	
Brood fish, 20 pair :20x$ 2	$ 40
Feed+ accessories	$ 50
Packing and sale	$ 15
Total expenditure	$ 480
Income : From a single brood minimum number of 150 fishes can be obtained after three months. So from 20 pairs 3000 melon barbs can be obtained on an average	
Sale of fish 3000x$0.5/	$1500

Reproductive strategy

Studies on different aspects of captive breeding revealed that *D. fasciata* is an asynchronous spawner *i.e*., continuous development and release of gametes in the gonads are evident. The fish did not show any affinity towards aquatic plants or the presence of plants did not have any stimulating effect to start the spawning activities. In general, the reproductive strategies of *D. fasciata* showed that it is an iteroparous species, *i.e*., they spawn more than once during their lives and gonochoristic*, i.e.,* their sexes were separate and exhibited external fertilization without parental care. The fish possessed an asynchronous type ovary *i.e.,* oocytes of all stages of development are present without dominant populations. But the peak breeding time of the fish is during the period from November to March. *D. fasciata* can be bred continuously if proper conditions are provided, but the peak season is during the months of November–March/April. The species could be categorized as a batch spawner *i.e.,* eggs are recruited and ovulated from the population of yolked oocytes in several batches over a protracted period during spawning season. A summary of reproductive strategies shown by *D. fasciata* is shown in table 2.

Table- 2: Summary of Reproductive strategies based on different components of breeding systems in *Dravidia fasciata*

Sl. No.	Component of breeding system	Reproductive strategy
1	Number of breeding opportunities	Iteroparous (Multiple breeding)
2	Type of spawning	Batch spawner
3	Mating system	Promiscuous (both sexes with multiple partners during breeding season)
4	Gender system	Gonochoristic
5	Secondary sexual characteristics	Sexually dichromatic
6	Spawning site preparation	No preparation
7	Place of fertilization	External
8	Embryonic development	Oviparity
9	Parental care	No parental care
10	Ecological group	Pelagophils
11	Reproductive guild (Balon1975)	Ecological classification: Non-Guarders Ethological classification: Open substratum spawners Morphotype :Pelagophils

CONCLUSION

The results of the study clearly demonstrated that *D. fasciata* could be successfully produced in captivity through scientific management of brooders, eggs, larvae and hatchlings. The successful development of captive breeding technology is likely to pave way towards commercialization of the technology thus leading to the sustainable export of the species as well as its conservation.

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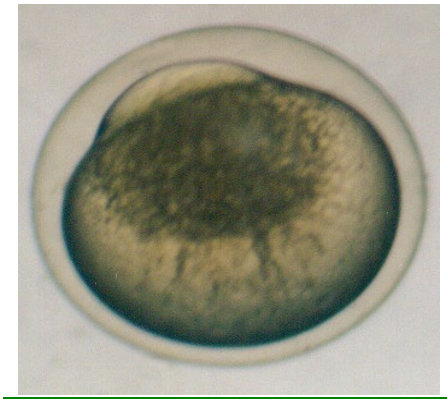
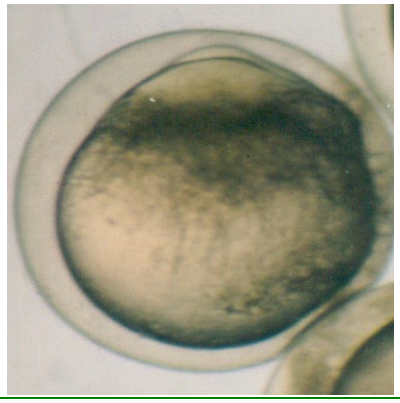
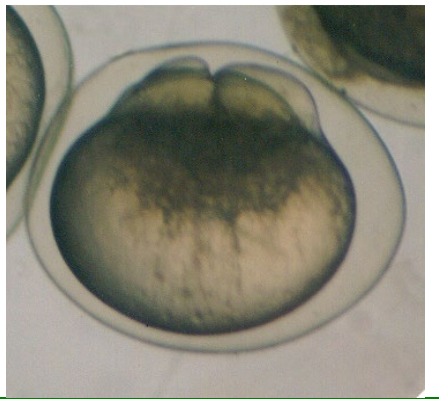
Plate: Developmental stages of *Dravidia fasciata*



Single celled stage

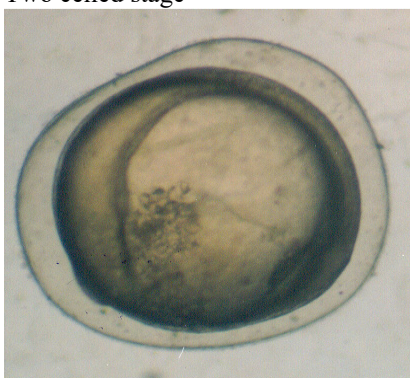


Two celled stage

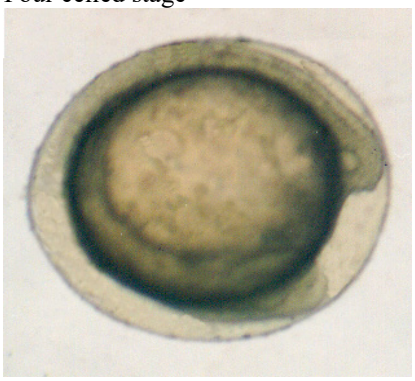


Four celled stage

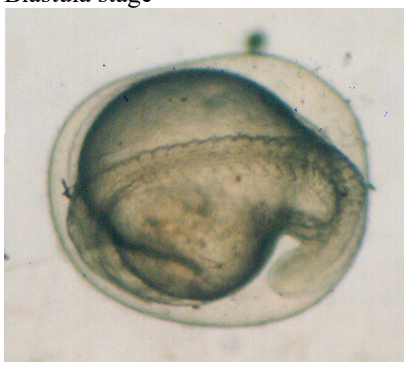
Blastula stage



After four hours



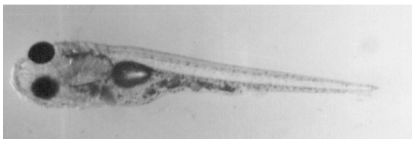
After 10 hours



After 12 hours

Just hatched larva

24 hours after hatching



48 hours after hatching

Juveniles

Breeding tank

Breeding tank