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ADVANCES IN CONSERVATION BIOLOGY: PIONEERING EFFORTS IN CAPTIVE BREEDING AND LARVAL REARING OF THE ENDEMIC ORNAMENTAL FISH, MOUSTACHED DANIO (DANIO DANGILA)

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Abstract

Moustached danio, Danio dangila, is a potential ornamental fish of India, but the stock of this endemic Cyprinid is declining in the wild due to environmental variation and anthropogenic activities. Therefore, the present work on captive breeding and larval rearing was undertaken to conserve this species. During the investigation, live fishes were brought from Assam (North-East Indian region) to ARTP, Chennai, Tamil Nadu. Initially, the species were tried to breed through environmental manipulations, but the species did not respond in captivity. In experiment 1, trials to breed the species using the synthetic hormone WOVA-FHwere undertaken and revealed the highest spawning (1002.50±52.6), fertilization (84.0±2.72%) and hatching rate (86.5±2.9%) after intraperitoneal injection at 0.5 ml/kg for females and 0.3 ml/kg for males (T3). In experiment 2, larval rearing in nursery raceways and FRP tanks at different stocking densities for 45 days showed that the RAS-based nursery raceways produced the highest survival (91.79±1.67%) and specific growth rate (0.13±0.001%/day) at 30 numbers/L stocking density. This investigation standardized the breeding and larval rearing protocol in advanced aquaculture systems and has significant implications for the conservation, nutritional security and seed production of the species.

Key words: Breeding, Moustached danio, Danio dangila, Raceway culture, WOVA-FH.

INTRODUCTION

In 2022, the global market for ornamental fish reached a value of USD 5.88 billion. Forecasts indicate a steady growth trajectory at an anticipated 1compound annual growth rate (CAGR) of 8.5% from 2023 through 2030(Grand View Research, 2023). The surge in demand for vibrant aquarium ornamental fish, notably among the millennial demographic, reflects a cultural inclination towards a sophisticated lifestyle, thereby propelling the industry forward. Furthermore, the market from a wave of inventive advancements in aquarium technology, introducing sophisticated designs that cater to consumers seeking distinctive centerpieces for their living spaces. Alongside these developments, enduring trends such as aquascaping and planted tanks continue to gain traction, further enriching the market landscape. Worldwide, around 2500 ornamental fish species are traded by 125 countries, of which was 65 % of fresh water fish(King, 2019; Sneddon et al., 2016).

Northeast India (21o57'N to 29o30'N Longitude and 89o46'E to 97o30'E Latitude) is endowed with a vast expanse of freshwater habitats, mainly flood plains (1.59 lakh ha), rivers (20,875km), reservoirs (0.33 lakh ha), tanks and ponds (3.71 lakh ha), including the mighty Brahmaputra and adjoining wetlands.Out of the 600 species found in India, 296 species (49.33%) are considered potential ornamental fish species, contributing to an 11.6% share of the world freshwater ornamental fish species trade by value (Dhar & Ghosh, 2015; Sandilyan, 2016).

Danio dangila, a cherished member of the Danionin group in the Cyprinidae family under Cypriniformes order(Rainboth, 1994), is a favored ornamental fish. Recognizable by its olive back and silvery sides adorned with a mottled pattern, this species has captivated hobbyists both locally and globally(Banerjee et al., 2017). Its petite size allows for lifelong habitation within aquariums, earning it the classification of a prized ornamental fish. Widely distributed across India, Bangladesh, Nepal, Bhutan, and Myanmar, these fish exhibit hardiness and remarkable compatibility within community tanks(Kullander, 2015). Designated as Least Concern due to its extensive distribution, D.dangila faces a decline in population owing to exploitation for the ornamental trade. Presently, collectors sourced by intermediaries harvest these ornamental fish from their natural habitats, supplying them to traders or exporters. Morphometric and meristic traits serve as valuable tools for accurate species identification in both laboratory settings and natural environments. Despite its Least Concern status, the population of D. dangila (Hamilton, 1822) is steadily dwindling due to habitat loss and the relentless exploitation of its natural habitats(Banerjee et al., 2017).

Therefore, standardization of captive breeding and larval rearing techniques of D. dangila species could play a major role in increasing the population of this incredible species. Hence, for conservation, nutritional security and meet the demand of the aquarium trade, the breeding and larval rearing protocol for the endemic fish D.dangilahas been standardized in captivity.

MATERIALS AND METHODS

Experimental Design and Setup

Live fishes were procured from the Brahmaputra stretch near Rajabari Grant in Assam (Lat. 26o28'24.44" N,Long. 92o32'02.3" E), packed in oxygenated polythene bags, and brought to the hatchery situated at the ICAR Research Complex for the Northeast Hilly region, Manipur, for acclimatization. Thereafter, the fishes were re-packed and brought to Aquatic Rainbow Technology Park (ARTP), Dr. MGR Fisheries College and Research Institute, TNJFU Madhavaram campus, Chennai (Lat. 13° 9'42.81"N, Long. 80°15'1.86"E) through air following the methods of live fish transportation(Rosten & Kristensen, 2010). The fishes were stocked at 100 numbers/tank in circular fiberglass reinforced plastic (FRP) tanks (10000 L) attached to a recirculatory aquaculture system facility (RAS). The fishes were fed with live food organisms (Thermocyclopshyalinus, Tubifextubifex and Eudrilussp.) and an artificial diet (35% protein) at 4-5% of body weight. Fishes were observed periodically for morphological indicators of maturity and health issues. Reverse Osmosis (R.O.) and U.V. filtered water were used for domestication, breeding, and larval rearing of the species because the ground water hardness was above 800 mg/L.

In the experimental tanks, water quality parameters were maintained at pH - 6.9-7.2, total dissolved solids - 30-50 mg/L, dissolved oxygen - 5.5-6.5 mg/L, free CO2 - <1.0 mg/L, ammonia - <0.05 mg/L, alkalinity - 45-50 mg/L and were determined periodically by standard methods. The water temperature was maintained at 20-25oC using regulated water heaters (Thermostat).

Selection of the Brood Pair

Velon screens (10mm mesh) made hapas (30cm×30cm×30cm) were fixed in 20 aquarium tanks (60cm×30cm×45cm; L×B×H) and filled with water up to 15 cm. Male brooders with a ventrally straight belly, oval anal opening, and slightly rough anal fin with red linings were selected, whereas female brooders with a bulged and ventrally curved belly, round anal opening, and whitish yellow lining on anal fins were segregated(Lekang, 2013). To confirm maturity, oocyte diameter was staged by in vivo biopsy using a polyethylene cannula and observed under a trinocular microscope (NLCD-120E, Lawrence and Mayo).

Hormonal Induction

The synthetic hormone WOVA-FH was obtained from Biostadt India Ltd., Worli, Mumbai, India. Hormone was injected twice to the females, while males were injected once during the resolving dose of the female (6 h) intraperitoneally using an insulated syringe (1.0mL, Terumo U40) after dilution (Table 1). While injecting, care was taken to reduce the chance of cardiac puncture by the needle. This experiment followed a completely randomized design (CRD) using four treatments in triplicate with control for each hormone.

Treatments	Hormonal Dosage for Females	Hormonal Dosage for Males
T_0	No Inducement	No Inducement
T_1	0.3	0.1
T_2	0.4	0.2
T_3	0.5	0.3
T_4	0.6	0.4

Table 1. Treatments based on the hormonal dose applied (ml/kg)

Breeding Performance and Embryonic Development

The breeding performance of an individual female was evaluated based on breeding behavior, latency period (h), number of spawned eggs, fertilization rate (%), and hatching rate (%). The latency period was defined as the time gap between hormonal inducement to both the parents and the first appearance of the spawned eggs(Kumar et al., 2018). The number of spawned eggs from each female was calculated by collecting the spawned eggs in a graduated measuring cylinder and counting them in unit volume(Behera et al., 2007). The fertilization rate was determined by

counting eggs with intact nucleus from the total number of eggs(Das et al., 2016). Hatching rate was calculated by dividing the number of hatchlings by the total number of fertilized eggs multiplied by hundred(Bhosale, et al., 2017). Three-days old hatchlings were transferred in 200 L capacity circular FRP tanks connected with RAS and 0.2 m/sec water flow facilities arranged by fixing ducklet- like structures.

After spawning, brooders from the aquarium tanks (hapa) were transferred to FRP tanks. Eggs were collected using a dropper for embryonic development observations under a trinocular microscope, while post-embryonic development was observed under a stereozoom microscope attached with a camera (EM 33 MEIJI).

Experimental Setup for Larval Rearing

The experiment was conducted following a 2×4 factorial design for 45 days at 10, 30, 50, and 70 numbers/L stocking densities in triplicate (Table 2). Spawns (4 DPH) were stocked in two rearing systems viz. nursery raceways (10000 L) (6×2×1.15 m) and FRP tanks (1000 L) attached to RAS and 0.4-0.5 m/s water flow. Spawns were fed to satiation initially with rotifers, Brachionuscalyciflorus, followed by moina (Moinamicrura), cyclops (Thermocyclophyalinus), and an artificial diet (52% protein) at 5-8% of body weight. The results were obtained by analyzing the final weight gain, average daily gain (ADG), specific growth rate (SGR), and survival rate (%).

Rearing systems		Treatments	T_1	T_2	T_3	T_4
1.	Nursery Raceways	Stocking density (numbers/L)	10	30	50	70
2.	FRP tanks					

Table 2. Experimental setup based on the rearing system and stocking density

Statistical Analysis

Initially, the experimental data were tested for normality of distribution using Shapiro– Wilk's W-statistic (PAST, Version 16.0). For experiment 1, one-way ANOVA was performed to compare significant differences in hormonal treatments using Duncan's multiple range test ($p<0.05$). For experiment 2, nursery rearing data were analysed using two-way ANOVA in SPSS (version 20.0, SPSS Inc., Michigan Avenue, Chicago, Illinois, USA). The results are presented as mean ± standard error (SE).

RESULTS AND DISCUSSION

The breeding performance of D. dangila induced with cumulative doses of hormone and nursery rearing in two systems with different stocking densities showed significant variation ($p<0.5$) and are described in Tables 3 and 4.

Treatments	Weight (g)		Latency Period (h)	Number of eggs spawned	Fertilization rate (%)	Hatching Rate (%)	Remarks
	Male	Female					

T_0	5.25±0.95	6.57±0.20	0^a	0^a	0^a	0^a	No spawning
T_1	4.75±0.30	6.50±0.14	0^a	0^a	0^a	0^a	No spawning
T_2	5.02±0.34	6.30±0.19	$8.82±0.56^c$	$675.5±52.61^b$	$72.0±3.39^{bc}$	$75.0±3.0^b$	Partial spawning
T_3	4.8±0.23	6.60±0.23	$7.87±0.12^b$	$1002.50±52.6^c$	$84.0±2.72^c$	$86.5±2.9^{bc}$	Complete spawning
T_4	4.9±0.31	6.6±0.23	0^a	0^a	0^a	0^a	No spawning
p-value	-	-	0.024	0.025	0.037	0.026	

Note: Values with different superscripts in the column differ significantly at $p<0.05$ (n=3)Table 3. Induced Reproductive Performance of Danio dangila.

	Survival (%)	**Final weight (g)**	**Average daily gain (g)**	**Specific growth rate (%)**
Rearing system				
FRP tanks	60.53^b	1.078^b	0.034^b	0.128^b
Nursery Raceways	62.86^a	1.229^a	0.039^a	0.132^a
SEM	0.123	0.031	0.001	0.001
p-value	0.02	0.003	0.003	0.016

Stocking density (numbers/L)				
10	81.00[a]	1.272[b]	0.040[a]	0.134[a]
30	80.55[a]	1.323[a]	0.042[a]	0.134[a]
50	58.05[b]	1.073[b]	0.034[b]	0.130[a]
70	28.07[c]	0.945[c]	0.029[b]	0.123[b]
SEM	0.783	0.044	0.001	0.001
p-value	0.000	0.000	0.000	0.000
Rearing system × Stocking density (numbers/L)				
p-value	0.041	0.043	0.038	0.022

TABLE 4. Growth performance of D. dangila

Breeding behavior and Latency Period

In almost all induced sets, brooders showed mating behavior after 2.0–2.5 h, except in the control and low-dose sets. Mating was preceded by elaborate courtship behavior where the male started chasing the female and sprayed milt over the eggs released by the female. In the case of T0 and T1, no breeding behavior was seen in all groups, whereas in the case of high dosage (T4), although the males were active, the females were stressed. In captive conditions, most fish lose their natural breeding behavior due to the non-availability of an optimum environment for breeding, and the breeding behavior can be stimulated through hormone administration(Motilan et al., 2014). Captive breeding using a suitable inducing agent is the best tool for the breeding of fish, and it ensures hatchery-bred seed throughout the year(Mariappan et al., 2021). Similarly, in two different study, Behera et al. (2007) and Karim et al. (2016) reported no inducement in the low dosage sets, whereas higher dosages of synthetic hormones caused stress to Labeo bata(Behera et al., 2007)and Mugil cephalus(Karim et al., 2016). It is expected that excess dosage may have caused negative stimulation(Bondarenko et al., 2015). Almost all brood fishes were spawned within 7-8 h of inducement. Differences in latency time might be due to the varied levels of dopamine activity and target organs of the hormone, as the luteinizing hormone-releasing factor of the anterior pituitary acts on the brain to produce well-matured sperm or eggs. A similar report on the variations in the latency time of tench (Tinca tinca) with different spawning agents was observed(Jamróz et al., 2008). The latency or response time is also related to the water temperature, which decreases with sudden changes. In the present study, the latency period of 6-8 h at ambient water temperature (20-25°C) was similar to the reports of in Labeo bata(Behera et al., 2007), HemichromisBimaculatus(Bhosale, et al., 2017), Dawkinsiarohani(Mariappan et al., 2021) at different doses of WOVA-FH.

Spawning Fecundity

The significantly highest ($p<0.05$) number of spawned eggs was observed in T3 (1002±52.6) followed by T2 (675.50±52.61). Nonetheless, fishes injected with excess and low doses showed poor performance. Similarly, the maximum number of spawned eggs in P. manipurensis (6218.75±32.75) and P. chola (893.34±41.34) using WOVA-FH at 0.4 ml/kg compared with other synthetic agents(Motilan et al., 2014). The highest spawning capacity (1142.3±40.20) in D. rohani when induced with WOVA-FH at 0.7 ml/kg for females and 0.3 ml/kg for males(Mariappan et al., 2021). In contrast, Udit et al. (2014) reported the maximum number of spawned eggs after inducing a higher dosage of Ovatide (1.0 ml/kg) for P. sarana(78500±124).

Fertilization and Hatching Rates

The fertilized eggs were spherical andtranslucent with a pale brownish color measuring approximately 0.8–1.0 mm. The twitching movement of the embryos was observed within 60:00 h of spawning, and the young embryos hatched out within 68–72 h at 25.5±1.5oC. In the experiment, the administered hormone and its dosage apparently affected the rate of fertilization and hatching. Significantly highest ($p<0.05$) fertilization (84.0±2.72%) and hatching rate (86.5±2.9%) were observed in T3 (Table 3). Similarly, increased percentage of egg production, fertilization and hatching rate in carps, L. bata(Behera et al., 2007), P.manipurensis(Motilan et al., 2014), O. belangri(Das et al., 2016),H.bimaculatus(Bhosale, et al., 2017) and D. rohani(Mariappan et al., 2021)when induced with WOVA-FH at 0.4-0.7 ml/kg for females and 0.2–0.4 ml/kg for males.

However, a lower fertilization rate was observed with excess inducement, which might be due to its mode of action of forcing the gonad to open up even when it is underdeveloped for fertilization(Zohar & Mylonas, 2001). Over-dosing of the inducing agents caused early milting and under-dosing caused late inducement in one of the mating partners, whichtriggered fertilization and ultimately the hatching rate(Karim et al., 2016). The dose of hormone obviously affected the hatching rate, and the optimum range of water quality parameters in the breeding tanks may have also led to higher fertilization and hatching rates. The ideal incubation temperature (25.5±1.5oC) for tropical barb and carp varieties resulted in higher fertilization and hatching rates(Bondarenko et al., 2015; Svinger et al., 2013). Similar findings were reported in L. bata(Behera et al., 2007)and O. belangeri(Das et al., 2016). The chronological development of the embryo has been described in Fig. 1.

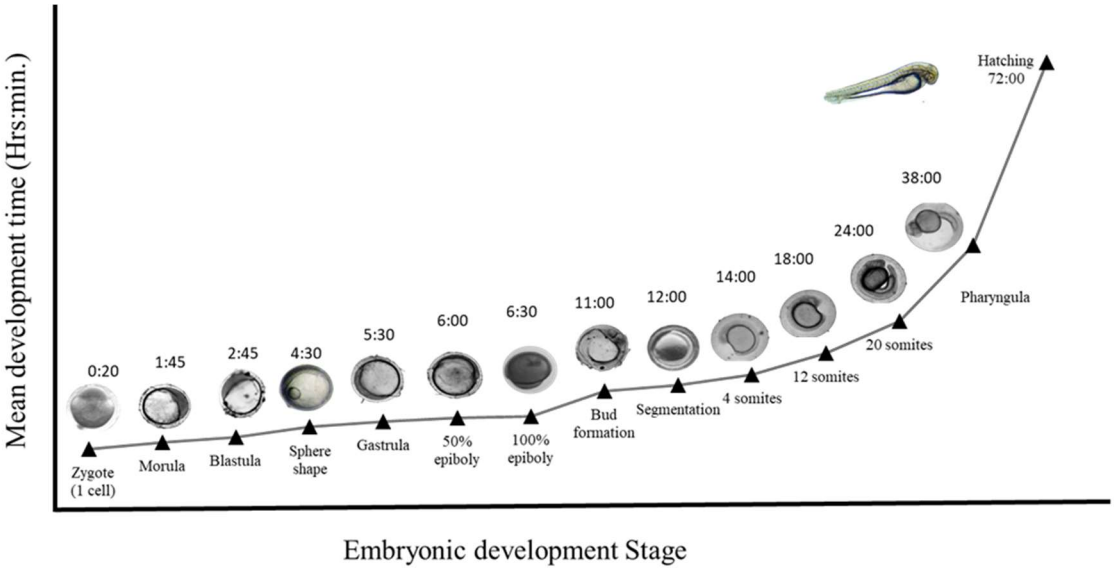

Fig. 1: Embryonic development of D. Dangila with mean development time

Larval Rearing

The newly hatched larvae were transparent and measured approximately 1.8–2.1 mm length. Yolk sac was fully absorbed within 68-72 h. Intensive aquaculture systems are used to efficiently produce large quantities of fish or shrimp under controlled conditions(Watanabe et al., 2002). One such system is RAS-based nursery raceways, which constantly circulate water by infusing sufficient oxygen by providing riverine conditions, leading to increased production rates more than conventional culture systems(Felix et al., 2021; Tidwell, 2012). In the present experiment, the raceways and FRP tank-based nursery rearing systems at four cumulative stocking densities showed a significant interaction effect ($p = 0.041$) (Table 4). The spawns (DPH 4) reared in nursery raceways significantly ($p<0.05$) exhibited higher survival rate (62.86%), daily weight gain (0.039), final weight gain (1.229 g), and specific growth rate (0.132%) compared with FRP tanks. Similarly, the highest survival (98%) and 10-fold increase in weight gain of genetically improved farmed tilapia (GIFT) fry reared in nursery raceways(Felix et al., 2021). Furthermore, they shows that the stocking density could be increased again up to 33% in an aerobic microbial flock-driven raceway system. As with any aquaculture species, the initial developmental stages are the bottleneck of finfish and shellfish hatchery systems and are relatively susceptible to pathogens, especially ciliate parasites and bacterial infections(Plumb & Hanson, 2010). However, in the present experiment, no such incidents were observed throughout the study, even at higher stocking densities.

Mortality is an important indicator of fish adaptation to the rearing system. In several studies, high stocking densities resulted in huge mortality(Ashley, 2007; Ellis et al., 2002). In our study, the highest and almost equal survival rates were observed at 10 numbers/L (81.00%) and 30 numbers/L (80.55%) stocking densities. However, mortality was observed in higher stocking densities, which might be due to overcrowding, aggressive behavior of fast-growing fishes, and mechanical injuries against the system components, mainly air lift pumps and water outlet systems.

Similar results were observed inClarias gariepinus(Hengsawat et al., 1997), Oncorhynchus mykiss(North et al., 2006), Dicentrarchuslabrax(Sammouth et al., 2009), Pagruspagrus(Laiz-Carrión et al., 2012), Oreochromis niloticus(Elliot Haruna Alhassan, 2012), and Leuciscus idus(Kucharczyk et al., 2020).

The stoking density is connected to the production parameters in commercial farming systems even though the fishes were fed to satiation(Ashley, 2007; Jha & Barat, 2005). In traditional open culture systems, fishes are grown at a very low stocking ratio (0.2–0.3 fish/L)(Jahedi et al., 2012). The well-studied physiological parameter in relation to the rearing environment is growth(Sloman & Armstrong, 2002). It can be measured easily and used as an indicator of stress and other related issues. In our experiment, the average daily weight gain (0.042±0.002 g) and mean weight gain (1.323±0.051 g) were found to be maximum at 10 and 30 numbers/L. Furthermore, the specific growth rate remained constant (0.134%) at 10 and 30 numbers/L with a significant interaction effect ($p = 0.016$). However, an increased stocking density of more than 30 numbers/L significantly affected survival and growth parameters. Stocking more than the optimum rate increases stress on the fish and leads to heavy mortality(Jha & Barat, 2005; Tan et al., 2018). Furthermore, the findings shows that stocking densities lower than the optimum range might not alter the growth efficiency of cultured fish but rather the production potential. These results are consistent with the trials conducted on guppies(Olivier & Kaiser, 1997), Scortumbarcoo(Luo et al., 2013), and Cyprinus carpiovr. Koi(Jha & Barat, 2005).

CONCLUSION

D.dangila can be successfully bred and reared under captive conditions by obtaining sustainable techniques and standard management practices. The WOVA-FHTM, a gonadotropic signaling molecular analog, was found effective to induce at 0.5 ml/kg for females and 0.3 ml/kg for males with the highest egg production, fertilization, and hatching rate Furthermore, the raceway-based larval rearing system gave surprising results and recommended rearing of fish at a 30 number/L stocking rate. The subject matter in this paper is useful for fish breeders and aquarium keepers for expanding aquaculture, species restoration, and conservation.

CONFLICT OF INTEREST

The authors declare that they do not have any conflict of interest.

ETHICAL APPROVAL - Not Required.

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