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Effect of Temperature on the Embryonic Development of Nilgiri Melon Barb *Haludaria fasciata*

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ABSTRACT

Background: External factors affecting the embryonic development of fishes have importance in aquaculture and conservational studies. The temperature directly affects the development rate and hatching success of fish eggs. An optimum level of temperature is required for each species for the highest hatching rate and proper development. The Nilgiri melon barb *Haludaria fasciata* is an indigenous ornamental fish of India and this study aims to find the effect of temperature on its embryonic development and find the optimum temperature for hatcheries.

Methods: The *H. fasciata* were bred in a controlled condition and fertilized eggs were selected for the study. The embryonic development stages were observed under a digital microscope. The eggs were incubated at different temperatures ranging from 10 to 35°C to find their effect on embryonic development on achieving each stage and hatching success.

Result: Among the temperature, the eggs incubated at 35°C have developed and hatched earlier (within 23 hrs 50 min±3 min) than in other treatments. The slowest development and late hatching (within 29 hrs 20 min±14 min) were observed at 20°C. At 26°C, the incubated eggs had a higher hatching rate and higher survival rate (97.68%). Based on the observations, it is suggested fish hatcheries incubate the eggs of *H. fasciata* at 26°C for attaining better production.

Key words: Embryonic development, Haludaria, Temperature, Teratogenic.

INTRODUCTION

The range of temperature required for the ontogenic development of fish species varies and an optimum level is needed for the proper development and hatching of eggs (Nissling *et al.*, 2006; Sapkale *et al.*, 2011 Nwosu and Holzlohner, 2000). Identifying an optimum level of temperature for egg incubation has application in fish hatchery operations. Global warming and increasing water temperature do affect the ecosystem and species dwelling in the system. It is also essential to know the tolerable temperature range of fish eggs in its ecosystem for their conservational studies.

The influence of temperature on the embryonic development of cyprinids was studied on *Tinca tinca* and *Rutilus rutilus* (Kokurewicz, 1970), *Leuciscus idus* (Florez, 1972), *Leuciscus leuciscus* (Mills, 1980), *Abramis brama*, *Chalcalburnus chalcoides mento* and *Vimba Vimba* (Herzig and Winkler, 1986), *Cyprinus carpio* and *Ctenopharyngodon Idella* (Korwin-Kossakowski, 2008), *Leuciscus leuciscus*, *L. idus* and *L. cephalus* (Kupren *et al.*, 2011), *Hypophthalmichthys nobilis* and *H. molitrix* (George and Chapman, 2013), *Henicorhynchus siamensis* (Avakul and Jutagate, 2015), *D. rerio* (Zhang *et al.*, 2018). These literatures observed that egg incubation temperature influenced the rate of embryonic growth, hatching, deformity and survival and optimum temperature varies with the species.

Nilgiri melon barb *Haludaria fasciata* is a small-sized (4-7 cm) cyprinid fish, endemic to India and found in streams and rivers of Southern India (Jayaram, 2010; Pethiyagoda, 2013). It is widely known in the aquarium industry as Ember barb and Panda barb (Daniels, 2002). The commercial trade of this species as an ornamental fish depends on their wild collection (Sekharan, 2006). Developing hatchery protocols for the species needs to be standardized to reduce the dependence on the wild collection and develop conservational practices. We studied the effect of temperature on the progress of ontogenic development and the hatching success of eggs. The findings of this work may be useful for setting hatchery protocol and conservation plans.

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MATERIALS AND METHODS

Adult fishes having 4-5 cm were collected from a freshwater stream in Eruvessi village in Kerala, India (12°05′19.5N 75°34′24.3E). For spawning, the brood fishes were sorted and stocked in a spawning tank two days before the experiment. The stocking sex ratio in each spawning tank was 3 ♀:1♂ and ten tanks were facilitated for the collection of sample eggs. The mating and spawning occurred on the second day in the morning hours from 10 am to 1 pm after stocking. The tank was siphoned at 11 am to collect all the

eggs that fall on the bottom and those eggs were discarded. Another siphoning was done at noon to collect fresh eggs from the bottom of the tank and eggs at stage initial stage of the zygote were selected for the study. Fifteen eggs for each treatment were immediately transferred into plastic tubs (15 l) filled with water having a pre-determined temperature range before stocking the eggs for treatment.

The effect of temperature on embryonic development was studied in two-phased experimental sets-ups: the preliminary and main. In the preliminary experiment, the eggs were incubated at a coarse set of values: 10°C (T1), 20°C (T2), 30°C (T3) and 35°C (T4). In the main experiment, the eggs were incubated at more fine values based on the optimal value recorded from the preliminary set. In the main study, 23°C (T5), 26°C (T6) and 32°C (T7) were used. The combination of these two sets helped in finding a viable range to improve the hatching and survival rate of eggs and hatchlings.

Incubation was carried out using three different temperatures stable closed systems such as a refrigerator for 10°C, an air-conditioned room for 19-24°C and water heater with a thermostat with automatic cut-off for maintaining the temperature between 25-35°C. The water temperature was measured using a thermometer and temperature was retained at variations below 1°C. The water used for egg incubation was dosed with 1.5 mg/l methylene blue to control fungal infection (Chacko and Sekharan, 2022). The water quality parameters maintained during the rearing and breeding were at pH 6.8, dissolved oxygen at 5 ppm and general hardness at 5 dGh.

The eggs were observed during the incubation period using a compound microscope (ESAW, India). The time taken for each treatment to achieve the stages: yolk bulge, 2-cell, 8-cell, 64-cell, 128-cell, 1028-cell, germ ring, shield, the blastopore, 3-somite, 5-somite, bud, twitching and hatching were noted from the ten selected eggs in each treatment at every five minutes. The number of eggs hatched, embryo deformed and hatchling survived 24 hours post hatched were noted. The percentage of egg hatching, deformity and hatchling survival was calculated using the following equations:

$$\text{Hatching (\%)} = \frac{\text{No. of eggs hatched}}{\text{No. of eggs incubated}} \times 100$$

$$\text{Deformity (\%)} = \frac{\text{No. of deformed hatchlings}}{\text{No. of hatched eggs}} \times 100$$

$$\text{Survival rate (\%)} = \frac{\text{No. of survived hatchlings}}{\text{No. of hatched eggs}} \times 100$$

The influence of temperature was characterized by a comparison of development stages with timescale and embryo viability. Significant differences (0.05 level) in the effect of treatments on parameters were analysed by ANOVA. All the statistical analysis was carried out using the software Jamovi (Navarro and Foxcroft, 2018). The work was carried out in the School of Industrial Fisheries, Cochin University of Science and Technology in December 2019.

Results and Discussion

The required temperature for the ontogeny of each species varies and an optimum level should be maintained for proper development and hatchings (Nissling *et al.*, 2006). In freshwater fish hatcheries, maintaining optimal temperature during incubation of the eggs was advised for successful hatching (Woynarovich and Horvath, 1980). We have tried to note the variation in the embryonic developmental rate at different temperatures and find a better incubation temperature for *H. fasciata* eggs.

In the preliminary experiment, the eggs incubated at treatment T3 had a higher hatching rate and comparatively fewer deformities than other groups (Table 1). In T1, the eggs had developed up to the two-celled stage and later deactivated or became dormant. These eggs were alive for up to 35 hrs (hours) and later egg mortality was marked with opaque white color. In T2 and T4, the hatching rates were about 4.44% and 2.22% respectively. However, all of the hatchlings were deformed and mortality occurred within 24 hours post-hatch (hph). The eggs incubated at T4 hatched earlier (23 hr 50 min±3 min). Whilst the eggs incubated at T2 hatched slower (29 hr 20 min±14 min). The hatching rate was significantly high in T3 ($p<0.05$). There was no significant difference in hatchling deformity between the treatment. In the main experiment, the T7 and T5 treatments resulted in lower hatching rates than T6. The survival rates were lower at T7 than at T5. The eggs incubated at T6 had the highest hatching rate about 95.55% and survival rate of about 97.32% and a lower percentage of about 2.68% of deformity (Fig 1). The eggs hatched slower (28 hr 20 min±26 min) at T5 than (24 hr 10 min±10) at T7. In treatment (T6), the eggs hatched at 27 hrs 40 min±30 min. The hatching rate was significantly higher in T6 than in other treatments ($p<0.05$). The deformity was significantly higher in T7 ($p<0.05$).

The temperature requirements of different species have been studied by various researchers. For example, the minimum thermal requirement for egg incubation for silver carp and bighead carp is 12.1°C and 12.9°C, respectively (George and Chapman, 2015). In *Henicorhynchus siamensis*, the hatching success was highest at 30°C. However, at a high temperature of 32°C, hatching success decreased. The temperature between 24 and 34°C was the ideal thermal limit for generating viable larvae of *H. siamensis* (Avakul and Jutagate, 2015). Embryonic development of zebrafish was successful from 22 to 32°C and exceeding this range had resulted in an increment in mortality (Schnurr *et al.*, 2014). 31°C was found ideal temperature for egg incubation of *L. rohita* for better hatching rate and faster development (Das *et al.*, 2006). Our study suggests incubating the egg of *Haludaria fasciata* at 26°C for better hatching and larval survival and attained larval above 60% at 23°C, 26°C and 30°C (Fig 1).

The embryonic development rate varied with species and water temperature. It is generally observed that higher temperature accelerates the rate of embryonic development,

reduces incubation time and influences the hatching rate (Iglesias *et al.*, 1995; Polat *et al.*, 2018). The sequence of development of embryo of *H. fasciata* under each treatment is shown in the (Table 1). The yolk bulge was seen within 10 min in all treatments except T1, which took 15 min. The 2-cell stage was observed at 30 min in T3, T4, T6 and T7, while in T2 and T5 it was seen within 40 min. In T1, the two-cell stage was attained within 80 min; after that, no further development occurred in these treatments. The cleavage stage was completed by T3, T4 and T7 within 90 min, which was comparatively earlier than other treatments. The 128-cell stage was first achieved in T4 treatment within 125 min and completed blastulation within 170 min. The T2 was the slowest to achieve the 1028 cell stage which happened within 300 min. Germ ring in gastrulation stage was attained within 280 min in T4, while T3 and T6 attained the stage within 290 min. The treatment T3 and T6, which had the highest hatching rate, attained this stage within 290 and 370 min, respectively and completed the formation of blastopore within 420 and 480 min, respectively. Similar to the previous observation, the fastest attainment of the segmentation stage with the 3-somite stage was found in T4 within 460 min. The T2 continued to be slower among the treatments and achieved this stage within 710 min. The T6 attained the 3-somite stage within 550 min and attained the twitching stage within 730 min. The T2 and T5 treatments were slower in development and attained this stage within 980 and 880 min. The earliest was observed in T4 which occurred within 690 min. The ascending order of treatments for hatching time (within) was T4 (1410 min), T7 (1440 min), T3 (1450 min), T6 (1660 min), T5 (1690 min) and T2 (1760 min).

The results show that the eggs of *H. fasciata* hatched at 35°C earlier (23 hrs. 30 min) than in other treatments. The eggs kept at 20°C hatched later (29 hrs. 20 min) during the treatment. But both hyperthermia and hypothermia had a teratogenic effect and resulted in 100% deformity of hatchlings. The deformity increased at and above 32°C. The larval survival was highest observed at 26°C (97.68%), at

Table 1: Achievement of developmental stages at different time scales under each treatment.

Major stage	Minor stage	The time scale in treatments (in minutes)						
		T1	T2	T3	T4	T5	T6	T7
		10±1°C	20±1°C	30±1°C	35±1°C	23±1°C	26±1°C	32±1°C
Cleavage	Yolk bulge	20	10	10	10	10	10	10
	2 cell	80	40	30	30	40	30	30
	8 cell	-	90	60	50	80	70	60
	64 cell	-	160	90	90	130	100	90
Blastulation	128 cell	-	215	130	125	180	145	130
	1028 cell	-	300	180	170	210	190	180
Gastrulation	Germ ring	-	490	290	280	380	370	290
	Shield	-	560	350	340	440	420	340
	Blastopore	-	650	420	410	510	480	420
Segmentation	3 somite	-	710	470	460	570	550	470
	5 somite	-	740	530	520	620	590	530
	Bud	-	830	640	630	710	670	640
	Twitching	-	980	710	690	880	730	700
Hatching	Hatching	-	1760 (29 hrs. 20 min)	1450 (24 hrs. 10 mins)	1410 (23 hrs. 30 mins)	1690 (28 hrs. 10 mins)	1660 (27 hrs. 40 mins)	1440 (24 hrs.)

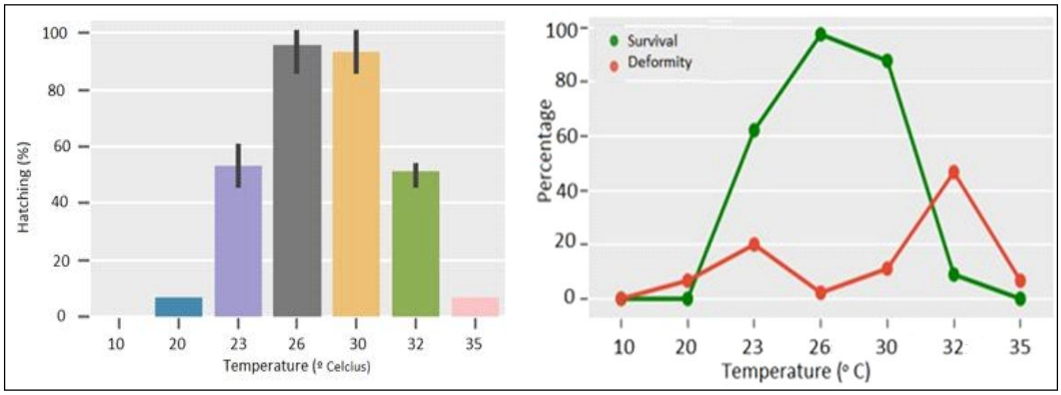

Fig 1: Graphical representation of egg viability variables in each treatment.

which eggs hatched at 27 hrs. 40 min. pf (post-fertilization) and at 30°C (88.09%) at which eggs hatched at 24 hrs. 10 min. pf. The hatching success was highest observed at 26°C with higher survival and less deformed hatchlings. The temperature at which broodstock was reared and spawning occurred had influenced the optimal thermal requirement of egg incubation (Hubbs and Bryan, 1974; Thépot and Jerry, 2015). In this study, the brood fishes were kept and spawning occurred at a water temperature range of 25-31°C and notably, the optimal range of hatching success was observed at this range. Therefore, more studies are required to understand whether hatching success relates to the temperature condition at which brood fish of *H. fasciata* was maintained in hatcheries and wild conditions.

CONCLUSION

The Nilgiri melon barbs *H. faciata* are endemic fishes of India which has commercial value in ornamental fisheries. The studies on embryonic development and egg incubation temperature have importance in setting hatchery protocols. The study on the influence of temperature on embryonic development and hatching success has revealed the hypothermia and hyperthermia during incubation can damage the embryo and reduce hatching and hatchling survival rates. In this study, eggs incubated at 26±1°C had a better hatching rate, low deformed hatchling and more survival rate when incubated between 10-35°C. The obtained data finds application in the optimization of the egg incubation process in hatcheries.

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