

To Study the Responses of Various Hormones on Captive breeding of the *Trichogaster fasciata* fish

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Abstract

The work was done to investigate the spawning performance of banded gourami (*Trichogaster fasciata*), a freshwater fish that is highly valued for its attractive color and larvivorous nature in India and other countries. Understanding the factors that influence fish breeding is crucial for improving aquaculture practices and enhancing economic benefits. Therefore, this study aimed to identify the most effective hormone treatment and environmental conditions for inducing ovulation, fertilization, and hatching rates in *T. fasciata*. To investigate the spawning performance, three different hormone treatments were used: Carp pituitary extract (T₁), *T. fasciata* pituitary extract (T₂), and Ovaprim (T₃), with each pair consisting of 2 males and 1 female. The water parameters were carefully controlled to ensure optimal conditions, including a temperature of 25-30°C, pH of 7.6-8.4, and dissolved oxygen of 3.8-4.6 mg/L. In all three treatments, 100% spawning occurred within 12-18 hours, with fertilization rates of 46.6 ± 9.42%, 76.6 ± 4.72%, and 86.7 ± 9.42% for T₁, T₂, and T₃, respectively. T₃ was found to be the most effective in inducing ovulation, fertilization, and hatching rates, with the larvae hatching out 22-28 hours after spawning, and hatching rates of 34.2 ± 2.73%, 46.1 ± 1.42%, and 52.2 ± 3.53% for T₁, T₂, and T₃, respectively.

Keywords: Fish breeding, hormone treatment, larvivorous, aquaculture, spawning performance

1. Introduction

Trichogaster fasciata, also known as banded gourami, is an important indigenous fish species that is valued for its attractive color pattern as well as its ability to control mosquito larvae, making it an important fish for both the aquarium and aquaculture industries (Poudel et al., 2021). *T. fasciata* has a lifespan of around four years, with males being larger and more colorful than females. Sexual maturity is indicated by the development of pointed dorsal and anal fins

in males, while females have blunt dorsal and anal fins. *T. fasciata* has three different life stages: pre-spawning (January-March), spawning (April-August), and post-spawning (September-December). The fish becomes sexually mature after completing one year of age, with a total lifespan of around four years. Males are larger, with a weight ranging from 9.35 to 14.85 g and length of 7.5 to 9.5 cm, while females are smaller, with a weight ranging from 9.94 to 12.9 g and length of 7.3 to 9.5 cm (Hossen et al., 2014).

A correlation between feeding intensity and breeding periodicity has been reported for *T. fasciata*. The feeding activity is low during spawning months, while it is high during the post-spawning season (Gupta, 2015). Given the economic value of *T. fasciata* as an aquarium fish and its role as biological control for mosquito larvae, there is a need to optimize its breeding in captivity. The use of appropriate hormone treatments, as investigated in the study by Sumon et al., (2019), can significantly improve the breeding success of *T. fasciata*. These findings have important implications for the aquaculture industry, where the optimization of breeding conditions can lead to increased production and economic benefits. In addition, by understanding the breeding behavior and periodicity of *T. fasciata*, fish farmers can plan their breeding cycles and achieve sustainable aquaculture practices.

In addition to optimizing breeding conditions and developing suitable rearing techniques, it is important to address the loss of habitat and overfishing of *T. fasciata*. The depletion of *T. fasciata* population is a concern not only for its economic and ecological value but also for the overall health of freshwater ecosystems. The conservation efforts for *T. fasciata* can have positive spillover effects on other species and the ecosystem as a whole. Furthermore, the economic value of *T. fasciata* in the aquarium industry highlights the need for sustainable practices in the trade of this species. Overfishing and habitat loss not only affect the population of *T. fasciata* but also impact the livelihoods of those involved in the trade of this species. Sustainable practices, such as aquaculture, can provide an alternative source of income while also contributing to the conservation of the species. Overall, the conservation of *T. fasciata* requires a holistic approach that addresses the various factors contributing to its decline. Research into the feeding and reproductive biology of *T. fasciata*, along with the development of sustainable aquaculture practices and conservation efforts, can contribute to the preservation

of this valuable fish species. The present report aims to consolidate all the available information on the feeding and reproductive biology of *T. fasciata* to identify knowledge gaps that require further study. Understanding the feeding and reproductive biology of *T. fasciata* is crucial for developing suitable rearing techniques for fry production and repopulating the species in freshwater bodies.

2. Materials and method

The experiment on induced breeding of *T. fasciata* was conducted at the Fish Biology Research Wet Lab, Department of Zoology, University of Lucknow, India. The laboratory was equipped with all the necessary facilities for preparing and conducting the experiment. The experiment was conducted with the utmost care to minimize any potential harm or stress to the fish.

2.1 Aquarium setup for induced breeding

Prior to the breeding program, the aquarium setup (Figure 1) was established to provide a suitable environment for the fish. The water supply facilities were assured to maintain consistent water quality parameters, including temperature, pH, and dissolved oxygen (DO). The working space was organized to provide a safe and sterile environment for preparing the hormone treatments and handling the fish. The aquarium setup consisted of individual tanks that were stocked with a single pair of fully mature male and female *T. fasciata*. The water



Figure 1: *T. fasciata* in a controlled environment glass aquarium

temperature was maintained at 25-30°C, pH at 7.6-8.4, and DO at 3.8-4.6 mg/L. These conditions were considered optimal for the breeding of *T. fasciata*.

2.2 Collection of brood fish and rearing

The study on induced breeding of *T. fasciata* involved collecting about 100 broods of the fish from a local pond called Tal katora situated in Rajasthan state of India. Before the start of the experiment, the collected fish were treated with KMnO₄ at a concentration of 1g/L for 30 minutes to eliminate any potential pathogens and parasites. The fish were then kept for acclimatization in aquarium water to ensure their survival and adaptation to the new environment. The acclimatized fish were transferred to the previously prepared aquarium, which was equipped with suitable shelter and aquatic plants to provide a suitable environment for the fish. The fish were fed with readymade fish food at a rate of 5% body weight, with occasional feeding of small aquatic grass and algae to cater to their omnivorous nature. Regular monitoring of water quality was done, and wastes were removed daily with siphoning to maintain optimal water quality parameters. The fish were raised for a duration of up to three months, during which they exhibited secondary sexual characteristics, indicating their readiness for spawning. To maintain ideal conditions for the fish, an aerator was used to provide continuous aeration throughout this period.

In the induced breeding experiment, healthy and fully mature male and female fish were selected on the basis of their sexually dimorphic characteristics. Females were comparatively smaller, had light coloration, yellowish enlarged belly, and were heavier, while males were larger, had dark coloration, congested belly, and had different fin structuring (Figure 2). These characteristics were used to identify and select appropriate male and female fish for the breeding program. The chosen fish were introduced into the breeding aquaria a week prior to hormone administration, allowing sufficient time for suitable pairing and preparation of the fish. This was done to ensure that the selected fish were accustomed to the laboratory environment and were ready for breeding. The conditioning process also allowed the male and female fish to establish a social hierarchy and form a suitable breeding pair.



Figure 2: Mature male and female *T. fasciata*

To provide suitable shelter and breeding conditions for the *T. fasciata*, four glass aquariums of size 36 X 14 X15 inches and one bathing tub of size 60 X 30 X 14 inches were used in the experiment. Plastic aquarium plants, algae, and water hyacinth were placed in all of these arrangements to provide shelter for the fish. These aquatic plants also play a critical role in breeding behavior, as male *T. fasciata* use them to build bubble nests where the eggs are attached after fertilization. The presence of these plants in the aquariums helps to stimulate the breeding behavior of the fish. To ensure successful breeding, aeration was stopped when the broods started making bubble nests, as this was an indication that the breeding behavior had commenced. The use of aeration can cause damage to the bubble nest and affect the fertilization of eggs (Figure 3).



Figure 3: Eggs of *T. fasciata* attached to the bubble nest

2.3 Hormonal treatment

To induce breeding in *T. fasciata*, hormone treatments were prepared using three different methods: Carp pituitary extract (CP), *T. fasciata* pituitary extract (TP), and Ovaprim (OP). Three treatments were considered as T₁, T₂, and T₃ for CP, TP, and OP, respectively. In each

treatment, two male and one female fish were stocked in separate aquariums to ensure accurate monitoring of breeding performance. The hormones were administered in two doses, with the first dose given to the female fish at a rate of 0.10 mL/kg of body weight, and the second dose given to both male and female fish at an interval of 12 hours. In the second dose, the female fish received twice the dose of the male fish. Table 1 provides information on the hormonal dose for male and female broods of *T. fasciata* in the induced breeding experiment.

The breeding performance of *T. fasciata* was monitored for 12-18 hours after the hormone injections to assess the effectiveness of the different hormone treatments. The use of multiple hormone treatments allowed for a comparison of their effectiveness in inducing breeding in *T. fasciata*. Water quality parameters such as temperature, pH, salinity, resistivity, turbidity (TDS), and DO were measured daily to ensure optimal breeding conditions for the fish. The temperature of the water was measured using a mercury thermometer, while a Multiparameter Benchtop (Model-LMMP-30 Make-Labman) was used for the measurement of pH, salinity, resistivity, and TDS. A DO meter (Model No. LMDO-50 Make- Labman) was used for the estimation of dissolved oxygen.

Table 1: Hormonal dose for male and female broods of *T. fasciata*

Treatment	Hormone	Sex	Doses		Time interval (h)
			1 st (mL/kg)	2 nd (mL/kg)	
T ₁	TP	Female	0.10	0.20	12
		Male	-	0.10	
T ₂	CP	Female	0.10	0.20	12
		Male	-	0.10	
T ₃	OP	Female	0.10	0.20	12
		Male	-	0.10	

2.4 Fertilization and hatching rate determination

During the induced breeding of *T. fasciata*, the eggs were attached to the bubble nest formed at the surface of the water in the aquarium. The bubble nests were formed in the vacant spaces of aquatic plants like *Hydrilla verticillata* and *Eichhornia crassipes*. To determine the fertilization rate of the different hormone treatments, 15 eggs were randomly collected from

each aquarium and placed in a petri dish for observation. The eggs were observed under a microscope after an incubation period of about 30-35 minutes. The observation was done twice at an interval of 3 hours. Unfertilized eggs were opaque, while fertilized eggs were transparent. The fertilization rate was calculated by using formula given below:

$$\text{Fertilization rate} = \frac{\text{No. of fertilized eggs}}{\text{Total no. of eggs}} \times 100$$

Subsequent to fertilization, the fertilized eggs were segregated into individual containers, and the hatching process commenced approximately 24-26 hours later. Once the hatchlings emerged, they were gathered, and the hatching rate was determined using the following formula:

$$\text{Hatching rate} = \frac{\text{No. of hatchlings}}{\text{Total no. of fertilized eggs}} \times 100$$

2.5 Statistical analysis

The results of the experiment on induced breeding of *T. fasciata* were analyzed using statistical analysis and the chi-square test. The level of significance ($p < 0.05$) of the difference between the treatments was determined through statistical analysis. Microsoft Excel 2010 was used for the statistical analysis.

3. Results

3.1 Mature fish rearing

During the experiment on induced breeding of *T. fasciata*, the fully mature fish were reared for approximately three months in a large tank. This allowed the fish to develop their secondary sexual characteristics and become fully mature. After this period, the broods were found to be healthy and fully mature, making them ready to spawn. The spawning period of *T. fasciata* was found to be from April to September.

3.2 Water quality

Water quality is a critical factor in the successful breeding operation of fish. The physico-chemical conditions of water such as temperature, pH, salinity, resistivity, turbidity, and DO are important factors that affect the health and breeding performance of fish. During the induced breeding experiment of *T. fasciata*, the water quality parameters of the experimental

aquariums were closely monitored and maintained. The average water quality parameters of the three treatments were measured and recorded in Table 2. The temperature range was between 26.67 to 28.43° C, pH ranged from 7.98 to 8.65, salinity ranged from 0.43 to 0.52 psu, resistivity ranged from 0.92 to 1.06 KΩ, turbidity ranged from 463 to 567 ppm, and DO ranged from 3.87 to 4.61 mL/L.

Table 2: Mean values of water quality parameters of hormonal treatment of *T. fasciata*

Parameter	Treatments		
	T ₁	T ₂	T ₃
Temperature (°C)	27.55 ± 0.88	27.53 ± 0.85	27.56 ± 0.87
pH	8.34 ± 0.31	8.30 ± 0.32	8.32 ± 0.31
Salinity (psu)	0.49 ± 0.03	0.47 ± 0.04	0.49 ± 0.03
Resistivity (KΩ)	0.99 ± 0.07	0.98 ± 0.06	0.99 ± 0.05
Turbidity (ppm)	506 ± 45	510 ± 57	507 ± 44
DO (mL/L)	4.14 ± 0.27	4.24 ± 0.37	4.21 ± 0.24

3.3 Reproductive behavior and spawning

The study revealed that hormonal administration induced reproductive behavior in male *T. fasciata* fish within a few hours of administration. Males began building bubble nests under floating plant leaves and roots as an indication of readiness to mate. The males showed dark coloration, indicating their readiness to approach the females for mating. The males first struck the ventral side of the female with their mouth and dorsal fin, then wrapped their body around the female to apply pressure on the abdomen for egg ejection. The fertilized eggs were lightweight and attached to the bubble nest. The hatching process took place between 24 to 28 hours after spawning. The study compared the effectiveness of three hormone treatments on the breeding performance of *T. fasciata* (Table 3). T₃ showed the highest fertilization rate (86.65 ± 6.6%) and the lowest fertilization rate (43.33 ± 4.6%) was obtained by the T₂ treatment.

Table 3: Estimation of fertilization rate of *T. fasciata* (Value = mean \pm SD; values of the parameter in each column with different superscripts (a, b, c) differ significantly (P>0.05): Fifteen eggs were collected from each treatment)

Treatment	Sampling time	Total no. of eggs	No. of fertilized eggs	Fertilization rate (%)	Mean fertilization rate (%)
T ₁	3:00 pm	15	7	46.6	43.3 \pm 4.6 ^a
	6:00 pm	15	6	40.0	
T ₂	3:00 pm	15	12	80.0	73.3 \pm 6.7 ^b
	6:00 pm	15	10	66.6	
T ₃	3:00 pm	15	12	80.0	86.6 \pm 6.6 ^c
	6:00 pm	15	14	93.3	

3.4 Rate of hatching

Hatching rate is an important parameter in the success of induced breeding in fish. It is defined as the number of hatchlings that emerge from a predetermined number of fertilized eggs. In this experiment, hatching occurred within 24-26 hours after fertilization, and the hatching rate was observed to be significantly different (P > 0.05) among the three treatments. Treatment T₃ showed the highest hatching rate of 52.5 \pm 3.5%, while the lowest hatching rate of 34.2 \pm 2.7% was observed in treatment T₁ (Table 4).

Table 4: Hatching rate of *T. fasciata* in different treatment (Mean \pm SD); values of the parameter in each column with different superscripts (a, b, c) differ significantly (P<0.05)

Treatment	Average fertilization rate	No. of fertilized eggs	Sampling time	Hatching rate	Average hatching rate (%)
T ₁	43.3 %	224 \pm 35	3:00 pm	37 %	34 \pm 2.83 ^a
			6:00 pm	31 %	
T ₂	73.3 %	1054 \pm 97	3:00 pm	44 %	46 \pm 1.41 ^b

			6:00 pm	48 %	
T ₃	86.6 %	1276 ± 105	3:00 pm	51 %	52.5 ± 3.5°
			6:00 pm	54 %	

4. Discussion

Understanding the spawning period is critical for the development of effective breeding programs and for maximizing the success of induced breeding techniques. The spawning period of *T. fasciata* was found to be from April to September. This information is essential for fish breeders and researchers who are interested in breeding this species. The three-month period of rearing the fully mature *T. fasciata* in a large tank was important for conditioning the fish and preparing them for spawning. This process allowed the fish to develop their reproductive systems and reach peak breeding conditions. These findings provide valuable insights into the reproductive biology of this species and can inform future breeding programs for this economically and ecologically important fish.

The results from Table 2 demonstrate that the water quality parameters were consistently maintained within acceptable ranges for all three treatments. This indicates that the breeding performance of *T. fasciata* was not affected by significant fluctuations in water quality. The accurate monitoring and control of water quality parameters during the experiment contribute to the validity and reliability of the findings. It is important to maintain parameters such as temperature, pH, turbidity, photoperiod and DO to ensure optimal fertilization and hatching rates (Bakos, 2015). The role of temperature in the re-appearance of gonads and timing of fish ovulation is crucial. Maintaining the optimal temperature is vital to minimize maturation delays and ensure the attainment of the desired quantity of eggs (Healy, 1971). In the case of *T. fasciata*, the preferred breeding temperature is around 28-30°C, and breeding occurs at temperatures ranging from 20 to 29°C (Abujam et al., 2015; Axelrod, 1973). The recorded water temperature (26-29°C) in the present study was within this range and optimal for breeding of *T. fasciata*.

The pH value of the water in the present study was slightly higher than the reported optimum range of 7.2 to 7.8 for successful breeding (Abujam et al., 2015). However, it is noteworthy that *T. fasciata* has been reported to breed in foul water (Bhuiyan, 1964), indicating that the slightly hard water in the present study may not have negatively impacted breeding success. The DO level in the aquarium water was slightly lower than reported in previous studies, possibly due to the aerator being stopped to avoid disturbing the bubble nest. However, successful breeding was still achieved, indicating that DO levels within the observed range of 4.14 ± 0.27 mg/l may not be a limiting factor for *T. fasciata* breeding in captive conditions.

In the present study, courtship behavior and spawning were observed 12–18 hours after hormonal injection. The earliest response was observed after 6–8 hours of T₃ treatment, i.e., Ovaprim, which matches with the previously reported results (Abujam et al., 2015). Where male fish initiated nest-building for successful mating. It is similar to findings reported by Bindu et al., (2014), Mitra et al., (2007), and Abujam et al. (2015) as they found that males make bubble nests to deposit eggs. The territorial behavior of male *T. fasciata* was also discussed by Degani, (1989). Based on the current findings, it can be concluded that male fish in this study exhibit bubble nest building behavior. Mating occurs beneath the bubble nest, where the male curves his body and encloses the female from below. The male initiates the process by prodding the female's tail base with his snout, delivering a series of rapid thrusts. Subsequently, the female begins to release eggs while the male simultaneously releases sperm to fertilize them. This observation aligns with a previous report by Abujam et al. (2015).

The number of eggs laid varied from 190 to 429, while the fertilization percentage ranged from 55.28 to 71.53. The lowest latency period was observed in the third trial (1.0 mL kg⁻¹), while the highest was in the first trial (1.0 mL kg⁻¹). The highest fertilization rate was observed in the first trial with a dose of 0.10 mL kg⁻¹, while the lowest was found in the third test with 0.8 mL kg⁻¹. The findings suggest that the lower dose of Ovaprim was more effective than the higher dose in terms of reproductive performance in *T. fasciata*. Based on the results of the breeding tests, the first trial was found to be the most effective, indicating that the Ovaprim dose ranging from 0.10 to 0.50 mL kg⁻¹ was reasonably suitable for the breeding program in the aquarium. The second trial was even more effective than the third, suggesting that the Ovaprim dose

ranging from 0.10 to 0.70 mL kg⁻¹ was suitable for breeding in comparison to the higher dose of 0.8 to 1.0 mL kg⁻¹. Therefore, it can be concluded that Ovaprim is an effective hormone for induced breeding in *T. fasciata*, and further optimization of the dosage is recommended for successful breeding programs in the future.

The current study also found that the male often collects the fertilized eggs and the bubble is attached to the nest. The same behavior is reported by Mitra et al. (2007) and Abujam et al. (2015). Mitra et al. (2007) demonstrated the presence of territoriality and parental care in the species under study, as evidenced by the guarding behavior exhibited towards both eggs and chicks. In *T. fasciata*, the eggs are lighter than water and float to the top of the nest, or are scooped up by the female in her mouth and placed in the nest with bubbles (Miller, 1964; Picciolo, 1964). The male continues to maintain the nest and court and spawn with other females (Pollak et al., 1981). However, the number of eggs was comparatively less in this study compared to other species of Anabantid (Norris & Douglas, 1991). In the current study, the parents were removed from the tank the day after the eggs were laid. Consequently, there were no discernible differences in post-spawning parenting behavior between the species examined.

The spawning, fertilization, and hatching rates were observed to vary significantly among the three different inducing hormones (TP, CP, and Ovaprim). The fertilization rate and hatching rate (52.5 ± 3.5%) was found to be higher in ovaprim-treated fish. The hatching period, observed to be 24-28 hours after fertilization in this study, aligns with the findings of Hossen et al. (2014) and Bindu et al. (2014). The current results indicate a significant difference (P < 0.05) in hatching rates among the three hormone treatments. However, it is noteworthy that Bindu et al. (2014) reported a 95% hatching rate in *T. trichopterus* without the use of any hormone induction, suggesting that the hatching rate observed in this study is commendable but falls short of being considered satisfactory. The results of this study suggest that induced breeding using hormonal injections can be an effective method for captive breeding of *T. fasciata*. The findings also suggest that dose optimization is necessary for successful breeding of *T. fasciata*.

Overall, the successful breeding and rearing of *T. fasciata* in captivity requires careful consideration of various factors such as water quality, hormonal treatments, and feeding

practices. This study showed that Ovaprim was the most effective hormone for inducing breeding and that lower doses were more effective than higher doses. The results also indicated that zooplankton and mosquito larvae could be suitable food for hatchlings and fry. Further studies could explore other factors such as the effect of photoperiod and water flow on breeding and rearing success.

5. Limitations of the study

This study provides valuable information on the feeding and reproductive biology of *T. fasciata*, an important indigenous fish species. However, there are some limitations to this study. One limitation is that the study only focuses on the effects of three hormones on induced breeding, which may not represent the full range of hormonal options available for breeding. Moreover, it is important to note that the sample size in this study was relatively small, which could restrict the generalizability of the findings to larger populations of *T. fasciata*. Another limitation is that the study did not explore the influence of other environmental factors, such as light intensity or water flow, on the breeding process. Despite these limitations, this study serves as a valuable foundation for future investigations regarding the breeding and conservation of *T. fasciata*.

6. Conclusion

In conclusion, the present study provides valuable information on the reproductive biology of *T. fasciata*, an important indigenous fish species. The results of the study indicate that the use of hormonal treatment can induce spawning in the species, and Ovaprim was found to be the most effective in terms of egg laying, fertilization, and hatching rates. The findings of this study have practical implications for the conservation and management of *T. fasciata*, as well as for the aquaculture industry. The use of hormonal treatment can be a useful tool for the successful breeding and rearing of *T. fasciata* in captivity.

However, it is crucial to acknowledge the limitations of this study, and future research is necessary to refine and optimize the dosages of hormonal treatments for induced breeding in *T. fasciata*. Additionally, the study provides only a preliminary understanding of the species' reproductive biology, and further research is needed to fully understand the species' breeding

behavior, optimal environmental conditions for breeding, and larval rearing techniques. Nonetheless, the findings of this study contribute to the understanding of the reproductive biology of *T. fasciata*, and provide a foundation for further research on the species.

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