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## Hormonal (sGRnH) Doses Optimization with the Ages of Brood Fish in Induced Breeding of *Mystus tengara* (Hamilton, 1822)

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### ABSTRACT

The age of brood fish is an inevitable factor in induced breeding technology worldwide. This study aimed to determine the optimal age for *M. tengara* for induced breeding and identify the most suitable age based on hormonal doses. Three age groups (1, 1.5, and 2-year-old) of brood fishes were used as three different treatments (T1, T2, and T3), with 0.8 ml kg<sup>-1</sup>, 1 ml.kg<sup>-1</sup>, and 1.2 ml kg<sup>-1</sup> body doses of Ovasis (sGRnH) as the replications (R1, R2, and R3) of each treatment. Results showed that the highest mean fertilization rate (77.5 ± 1.73 %) was achieved in T1R2 for 1-year-old fish, while 1 ml kg<sup>-1</sup> dose was ideal for them. For 1.5-year-old *M. tengara*, the highest mean fertilization rate was observed in T2R1 (79.25 ± 2.99 %) with 0.8 ml kg<sup>-1</sup> dose being the most suitable. Two-year-old fish had the highest mean fertilization rate in T3R1 and T3R2 (76.25 ± 5.5 %) in June. Based on the fertilization rate and hatching rate, 1 ml kg<sup>-1</sup> dose was identified as ideal for 1-year-old, and 0.8 ml kg<sup>-1</sup> was suitable for 1.5-year-old *M. tengara*. Among all the treatments, T2R1 (1.5 year age, 0.8 ml kg<sup>-1</sup>) showed the highest hatching rate (78.25 ± 2.36 %) and most successful breeding performance over the study period, and June was identified as the best time for induced breeding. This scientific evidence provides valuable insight into the optimal age and hormonal doses for *M. tengara* in induced breeding technology.

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### Introduction

The Bagrid catfish is an endemic tiny fish species in Bangladesh that is critically important both environmentally and commercially. It is a member of the Bagridae family, which is grouped within the Asian striped dwarf catfish [1]. The Bagrid catfish were once teeming with the ascendant presence in the rivers, creeks, canals, reservoirs, lakes, ponds, and other bodies of water throughout Bangladesh [2]. Nowadays, the populations of the Bagrid catfish have come down significantly and putting them at a heightened risk of near-threatened status due to overexploitation and numerous ecological changes in their natural habitats [3]. A few species from the Bagrid catfish family has brought under commercial aquaculture through induced breeding of their broods in hatcheries. Different Bagrid catfishes brood age impacts on induced breeding has been studied and documented [4-7]. Brood age is regarded to be a crucial factor to their offspring's development and survival in most wild fish species [4]. Mature female brood produces relatively larger eggs, and as a result, the hatching rate and other

growth factors increase. Similar results documented in different studies indicating enhanced fecundity and higher egg quality with increasing female brood age, such as in Nile tilapia *Oreochromis niloticus* Linnaeus, 1758, common carp *Cyprinus carpio* Linnaeus, 1758 [8,9]. and Indian major carp *Labeo rohita* Hamilton, 1822 [10]. Along with the age of broods, hormonal doses have a critical influence in the induced breeding of most fishes. A successful induced breeding is greatly dependent on the proper optimization of hormonal doses according to the age of the broods. During the induced breeding, the ripeness of eggs and gonadal maturation can be estimated through precise histological analysis of the gonad and have studied the *Mystus tengara* ovary and revealed the reproductive biology [11-13]. There hasn't been much thorough research looking at the connection between *M. tengara*'s age and hormone dosages. A clear grasp of how to choose the ideal aged brood and the optimization of hormone doses according to that age were the main goals of this study. Future-induced breeding of *M. tengara* may benefit greatly from the findings of this work. *M. tengara* broods were taken from the wild Chalan bill in Natore, Bangladesh, and kept in captivity until gonadal maturation in order to prevent inbreeding. Many elements must come together

for the Bagrid catfish to successfully undergo induced breeding; one of these elements is the delivery of the right hormone dosage. Environmental conditions and good brood control are other crucial variables [14]. In this study, broods of *M. tengara* were injected with three separate doses of salmon Gonadotropin-Releasing Hormone (sGRnH) for each age group, resulting in successful spawning. This study aims to discover the ideal hormone doses in relation to the age of the broods and to recommend the best age for excellent performance during the induced breeding of Bagrid catfish.

**Methods and Materials**

**Study Site**

This study was carried out at Arifa Fish Hatchery, Bogura, Bangladesh (Fig. 1), in between April to July 2022. This commercial hatchery offered essential facilities for the induced breeding of Bagrid catfish.



**Figure 1:** Map Shows the Study Site

**Brood Collection and Set up**

To conduct this experiment, different age groups of fish were stocked in a small brood-rearing pond. We considered three different brood group having six-month age gap. Three age-group Bagrid catfish (1, 1.5, and 2-year-old) were obtained in February 2022. The fish were kept apart by netting inside a 20 decimal brood rearing pond. A total of 500 female and 500 male broods of *M. tengara* were also obtained for this experiment. The *M. tengara* brood stocks were managed in the same brood rearing pond, which was separated by a net. To prepare the brood pond, predatory and unwanted fish were removed by dewatering and drying the pond, and rotenone (5 grams.decimal-1.foot-1) was used to eradicate any undesirable fish species and insects. Manual removal of aquatic vegetation was done. After cleaning the pond, lime was applied at a rate of 1 kg decimal-1, and 4-5 days later, Urea and TSP were also added at rates of 150g and 100g per decimal, respectively. The brood pond was prepared for stocking with brood fish seven days following fertilization.

**Brood Rearing and Management**

For the course of the study, *M. tengara* broods were raised in a 20-decimal brood-rearing pond with a water depth of 1-1.5 meters and a stocking density of 6-7 kg decimal-1. The right amount of lime, fertilizer, and other treatments were applied at one-month intervals. Furthermore, TSP and urea were applied at rates of 100 and 150 g.decimal-1 each month, respectively. Moreover, brood fish were given additional feed at a rate of 1 to 1.5% of their body weight. At midnight, supplemental food was given out once daily.

The following table includes a list of the components added to the supplementary feed formulation.

**Brood Collection**

During brood selection, healthy and ready broods (male and female) of *M. tengara* were taken from brood-rearing ponds. The brood had been reared previously in the brood-rearing pond using a nursery net (small mesh size, 1 cm). Male broods were separated from females based on their secondary sexual characteristics, such as the external view of their abdomen and pectoral fins. Males were identified by seeing the slightly pointed genital papilla, and females were identified by seeing the swollen abdomen. On the first week of April 2022, a total of 180 females and 180 males of *M. tengara* were collected; among these, 30 males and 30 females were from the 2-year-old age group, and the same were from the 1.5-year-old and 1-year-old broods. They were kept for 5–6 hours in a resting tank to make them stress-free and get the best performance.

**Brood Injection**

Three age groups were examined as three different treatments (T1, T2, and T3), and every treatment had three replications. 0.8 ml kg<sup>-1</sup>, 1 ml kg<sup>-1</sup>, and 1.2 ml kg<sup>-1</sup> doses were applied in three different replications (R1, R2, and R3). At 8.00 pm, females of *M. tengara* of three different ages were injected with different doses (Tables 2, 3, and 4). *Ovasis* (sGnRH) hormone was injected into the muscular basal part of the dorsal fin. Broods were collected from the tank just before injection. A specific dose was administered to individuals of both male and female genders as a single dosage (ml kg<sup>-1</sup> body weight of fish) of *Ovasis* (sGnRH) hormone. Broods were caught by a net and handled carefully during the hormonal injection. The broods were injected with inducing hormone at 8.00 pm so that the fish could ovulate in the morning. The injection administrations were done very carefully with different doses of three treatments. The needle was inserted at 45° angles to the body surface according to the body weight of the Tengra (*M. tengara*). The first injection of *M. tengara* was done in the first week of April 2022. Following table 2, 3, and 4 are showing different treatments with different replication.

**Table 1: Ingredients of Fish Feed**

Ingredients	Amount (%)
Fish-meal or highly digestive protein	28
Brown Rice-bran	21
Molasses	5
Mustered-oil- cake	33
Wheat/ flour	13
Total=	100

**Table 2: Treatment 1 with its Replication (T1R1, T1R2, and T1R3)**

Age of Fish (year) (T1)	Replication	Hormonal Doses		No of female	No of male	Species
		Female (ml kg <sup>-1</sup> )	Male (ml kg <sup>-1</sup> )			
1	R1	0.8	0.5	10	10	<i>M. tengara</i>
	R2	1.0	0.5	10	10	
	R3	1.2	0.5	10	10	
Total				30	30	

**Table 3: Treatment 2 with its Replication (T2R1, T2R2, and T2R3)**

Age of Fish (year) (T2)	Replication	Hormonal Doses		No of female	No of male	Species
		Female (ml kg <sup>-1</sup> )	Male (ml kg <sup>-1</sup> )			
1.5	R1	0.8	0.5	10	10	<i>M. tengara</i>
	R2	1.0	0.5	10	10	
	R3	1.2	0.5	10	10	
Total				30	30	

**Table 4: Treatment 3 with its Replication (T3R1, T3R2, and T3R3)**

Age of Fish (year) (T3)	Replication	Hormonal Doses		No of female	No of male	Species
		Female (ml kg <sup>-1</sup> )	Male (ml kg <sup>-1</sup> )			
2	R1	0.8	0.5	10	10	<i>M. tengara</i>
	R2	1.0	0.5	10	10	
	R3	1.2	0.5	10	10	
Total				30	30	

Injected fishes were kept in nine different small tanks (1 m × 1.5 m). After seven hours of injection, female broods started to release eggs, and it takes more than 3-4 hours to complete ovulation. At 8 am of the following day, the broods were collected from tanks to release in brood-rearing ponds. Before being released in the brood-rearing ponds, fishes were treated with an antibiotic so that they don't get infected by bacteria.

**Estimation of Fertilization and Hatching Rate (In percent %)**

Fertilization and hatching rate are important aspects of this research to identify the best age and suitable hormonal dosages. Initially, 10 grams of eggs and, after 12–16 hours, 10 grams of spawn were taken to count the number of eggs and the number of spawns per gram and recorded on the sheet for further analysis. The same procedure was done for each injection. Fish were injected for 16 weeks and were injected once a week. The fertilization rate was determined according to formula (i) and the hatching rate was calculated according to formula (ii) The fertilization and the hatching rate were recorded in the below table 5.

**Table 5: Fertilization Rate (%) and Hatching rate (%) of Treatment and Replication (FR= Fertilization Rate (%), HR= Hatching Rate (%))**

Month	Weeks	T1R1		T1R2		T1R3		T2R1		T2R2		T2R3		T3R1		T3R2		T3R3	
		FR	HR	FR	HR	FR	HR	FR	HR	FR	HR	FR	HR	FR	HR	FR	HR	FR	HR
April	W1	50	46	55	48	48	52	55	50	53	48	50	56	60	61	60	58	46	58
	W2	50	49	55	50	48	59	55	60	56	60	55	55	65	55	60	58	51	58
	W3	50	48	60	50	56	47	60	60	59	61	55	60	55	60	60	60	56	60
	W4	55	59	62	55	54	56	60	65	62	62	65	60	65	60	60	62	55	62
May	W5	60	56	65	60	60	57	65	65	55	65	53	60	53	60	55	60	60	60
	W6	60	58	65	61	55	65	70	70	68	68	70	65	70	65	70	65	55	65
	W7	70	65	70	68	65	70	70	72	70	72	72	70	72	70	70	70	56	70
	W8	75	76	80	70	70	75	80	75	78	75	75	75	75	75	75	70	73	75
June	W9	77	82	79	80	77	76	82	80	79	80	75	78	75	78	75	78	72	78
	W10	77	78	78	76	78	68	80	80	81	78	81	79	81	79	80	80	75	80
	W11	78	76	78	78	77	81	80	78	80	75	80	70	80	70	80	72	69	72
	W12	70	67	75	75	72	67	75	75	69	75	69	72	69	72	70	68	71	68
July	W13	70	65	75	70	75	61	70	70	70	70	70	71	70	71	68	70	64	70
	W14	65	65	65	68	65	65	60	70	60	65	60	70	60	70	65	70	55	70
	W15	60	62	65	65	57	68	60	65	62	66	62	68	62	68	61	65	51	65
	W16	60	59	65	62	60	62	60	65	62	63	62	68	62	68	62	60	48	60

(i). Fertilization rate (%) = (no. of fertilized eggs/total no. of eggs) × 100

(ii). Hatching rate (%) = (no. of eggs hatched/total no. of fertilized eggs) × 100

In every treatment, there were three replications. These were indicated as T1R1, T1R2, T1R3, T2R1, T2R2, T2R3, T3R1, T3R2, and

T3R3. Data was recorded individually for each treatment and replication. In this experiment, the amount and number of eggs and spawn, hatching, and fertilization rate were considered.

### Data Analysis

All the data for this study were recorded and analyzed using Microsoft Excel and SPSS version 26.

### Result

#### Relation of One-Year-Old Brood with Hormonal Doses

This study investigated one-year-aged *M. tengara* as treatment 1. Three different doses of hormones including 0.8, 1, and 1.2 ml kg<sup>-1</sup> body weight were used as replicates to find out the best dose for 1-year-old broods of *M. tengara*. Each replicates of hormone doses were injected to 10 females where the dose for the males were maintained constant of 0.5 ml kg<sup>-1</sup> body weight and 10 males were used against 10 females maintaining 1:1 sex ratio. Broods were injected once a week, four injections per month, and a total of 16 times during the whole experiment period. Every week, fish from every treatment were injected with three different doses. We recorded the total egg production in grams, number of eggs, fertilization rate, and hatching rate every week, and also calculated the mean and standard deviation of the fertilization rate. The highest mean fertilization rate (%) was recorded in June in replication 2 (T1R2), and the lowest (51.5 %) in April and replication 3 (T1R3) (Table 06 and 08 and Figure 2). The highest mean hatching rate (77.5 %) was recorded in the same month and replication and the lowest was in April (50.5 %) in replication 1 (Table 07 and 09 and Figure 08).

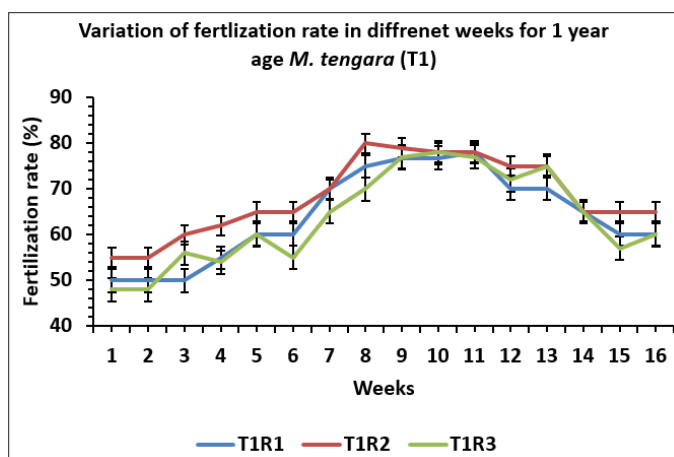
**Table 6: Shows the Fertilization Rate (%) in Different Treatments. Data are Presented as Mean ± Standard Deviation**

Months	T1			T2			T3		
	T1R1	T1R2	T1R3	T2R1	T2R2	T2R3	T3R1	T3R2	T3R3
April	54.00 ± 2.5	58.00 ± 3.56	51.50 ± 4.12	57.50 ± 2.89	57.50 ± 3.87	56.25 ± 6.29	61.25 ± 4.78	60.00 ± 2.01	52.00 ± 4.54
May	66.25 ± 7.5	70.00 ± 7.07	62.50 ± 6.45	71.25 ± 6.29	67.75 ± 9.53	67.50 ± 9.88	67.50 ± 9.88	66.25 ± 7.50	61.50 ± 9.26
June	75.50 ± 3.7	77.50 ± 1.73	76.00 ± 2.71	79.25 ± 2.99	77.25 ± 5.56	76.25 ± 5.5	76.25 ± 5.5	76.25 ± 4.78	71.75 ± 2.5
July	63.75 ± 4.78	67.50 ± 5.0	64.25 ± 7.89	62.50 ± 5.0	63.50 ± 4.43	63.50 ± 4.43	63.50 ± 4.43	64.00 ± 3.16	54.50 ± 6.95

**Table 7: Shows the Hatching rate (%) in Different Treatments. Data are Presented as Mean ± Standard Deviation**

Months	T1			T2			T3		
	T1R1	T1R2	T1R3	T2R1	T2R2	T2R3	T3R1	T3R2	T3R3
April	50.50 ± 5.80	50.75 ± 2.99	53.50 ± 5.20	58.75 ± 6.29	57.75 ± 6.55	57.75 ± 2.63	59.0 ± 2.71	59.50 ± 1.91	59.50 ± 1.91
May	63.75 ± 9.03	64.75 ± 4.99	66.75 ± 7.67	70.50 ± 4.2	70.0 ± 4.4	67.50 ± 6.45	67.50 ± 6.45	67.0 ± 5.71	67.0 ± 5.71
June	75.75 ± 6.34	77.25 ± 2.21	73.0 ± 6.68	78.25 ± 2.36	77.0 ± 2.45	74.75 ± 4.42	76.25 ± 5.5	76.25 ± 4.79	74.50 ± 5.51
July	62.75 ± 2.87	66.25 ± 3.5	64.0 ± 3.16	67.5 ± 2.89	66.0 ± 2.94	69.25 ± 1.50	69.25 ± 1.50	66.25 ± 4.79	66.25 ± 4.79

The results of treatment 1 indicates that the lowest fertilization rate was recorded in the 1<sup>st</sup> and 2<sup>nd</sup> weeks of replication 3, with a dosage of 1.2 ml kg<sup>-1</sup> body weight (Table 05). This suggests that 1.2 ml kg<sup>-1</sup> body weight is not suitable for *M. tengara* induction at the beginning. The highest fertilization rate was observed in the 8<sup>th</sup> week with a dosage of 1 ml kg<sup>-1</sup> body weight, with the highest mean value recorded in replication 2 (Table 06 and 08, and Figure 2). That means for 1 year-aged brood, 1 ml kg<sup>-1</sup> body weight was the best dose for induced breeding of *M. tengara*. On the other hand, the highest hatching rate was also recorded in the 9<sup>th</sup> week (Table 05) with 1 ml kg<sup>-1</sup> body weight of induced hormone, and the highest mean value (Table 07 and 09 and Figure 03) was also recorded in replication with 1 ml kg<sup>-1</sup> body weight. Results indicated that 1 ml kg<sup>-1</sup> body weight is the suitable dose for 1-year-old broods for the peak breeding season to get the highest success in both fertilization rate and hatching rate. Moreover, the fertilization rate and hatching rate showed an increasing trend over time, particularly from the 8<sup>th</sup> to 12<sup>th</sup> week (May - June 2022), as seen in Figure 2 and Figure 3.



**Figure 2: Fertilization rate in Different Weeks in Treatment 1**

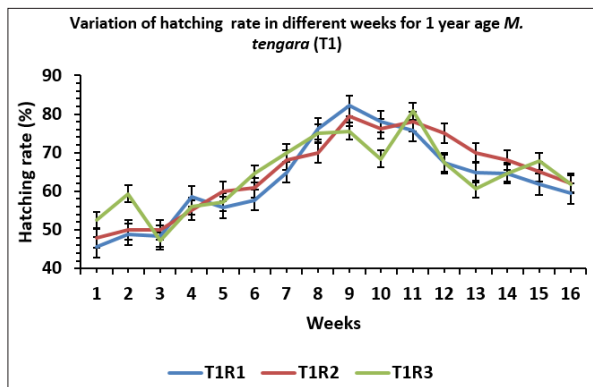


Figure 3: Hatching Rate in Different Weeks in Treatment 1

### Relation of 1.5-year-old *m. Tengara* and Hormonal Doses

In treatment 2, 1.5-year-old *M. tengara* was used. The similar replications and number of broods were used for treatment 2 to find out the best dose for 1.5-year-old broods of *M. tengara*. The experiment started in April and continued up to July 2022. Breeding data for 16 weeks were recorded during the experiment period. Every week, fish from every treatment were injected by three different doses. Total egg production in grams, the number of eggs, fertilization rate, and hatching rate were recorded every week. The highest fertilization rate was recorded in the 9<sup>th</sup> week of June (82%) in replication 1 (T2R1) and the lowest (50%) in the 1<sup>st</sup> week of April and replication 3 (Table 05). But the highest mean fertilization rate was recorded at 79.25% in treatment 2 with 0.8 ml.kg<sup>-1</sup> body weight hormonal doses, and the lowest was 56.25% with 1.2 ml.kg<sup>-1</sup> body weight fish (Table 06 and 08 and Figure 4). On the other hand, the highest mean hatching rate (78.25%) was recorded in June with 0.8 mL.kg<sup>-1</sup> doses and the lowest in April (57.75%) and replications 2 and 3 (Table 07 and 09 and Figure 05). In treatment 2, replication 1, which means T2R1 (1.5-year-old brood and 0.8 ml kg<sup>-1</sup> body weight hormone doses) showed the best result for 1.5-year-old broods of *M. tengara*. In treatment T2R1, the lowest fertilization rate was 55 % and the highest was 82 %, which was the best in treatment 2. If we consider the month, then June was the best month for *M. tengara* breeding in terms hatching rate and fertilization rate (Figure 09 and Figure 10). Furthermore, the lowest mean hatching rate was found 48% in T2R2 and the highest mean fertilization rate was 80% in both T2R1 and T2R2. From table 06, it can be considered that T2R1 (0.8 ml kg<sup>-1</sup>) and T2R2 (1 ml kg<sup>-1</sup>) were the best doses to get the highest hatching rate of eggs from 1.5 year aged broods of *M. tengara*. June is also the best month to get the highest hatching rate. From the figure (Figure 03 and Figure 04), 1.5-year-aged broods showed the best result for doses. Mean hatching rate was also found highest for all doses in the 1.5-year-aged broods.

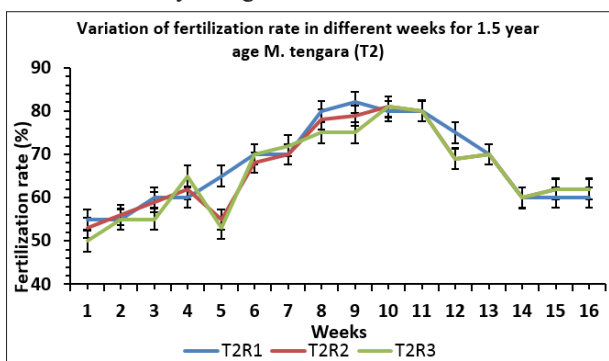


Figure 4: Fertilization Rate in Different Weeks in Treatment 2

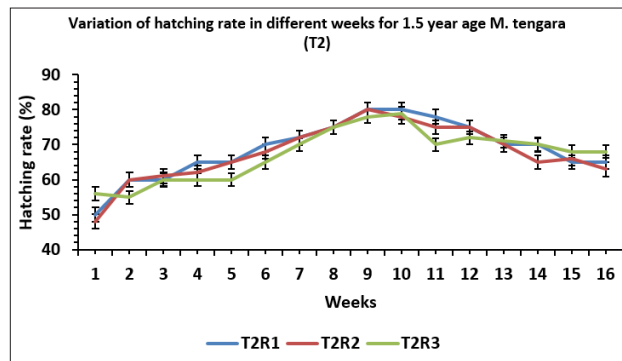


Figure 5: Hatching Rate in Different Weeks in Treatment 2

### Relation of 2-year-old *M. Tengara* and Hormonal Doses

We investigated 2-year-old *M. tengara* as treatment 3. Like the other treatments, different doses, such as 0.8, 1, and 1.2 ml kg<sup>-1</sup> body weight, were used as three replications to find the best dose for 2-year-old broods of *M. tengara*. The experiment was started in April and continued up to July 2022. Total egg production in grams, the number of eggs, fertilization rate, and hatching rate were recorded every week. The highest fertilization rate was recorded in the 10<sup>th</sup> week of June (81%) in replication 1 (T3R1) and the lowest (46%) in the 1<sup>st</sup> week of replication 3 (T3R3). On the other hand, the highest hatching rate was also recorded in the 10<sup>th</sup> week in replication 2 (T3R2). The highest mean fertilization rate was 76.25 % in replications 1 and 2, and the lowest was 52 % in replication 3 (Table 06 and 08 and Figure 07). On the other hand, the highest mean hatching rate (76.25%) was recorded in June in replications 1 and 2. and the lowest in April (59.00%) and in replication 1 (Table 07 and 09 and Figure 08).

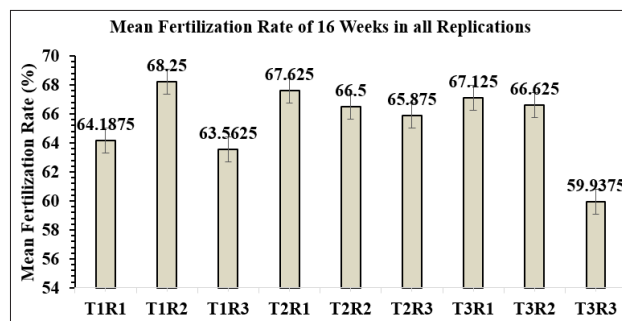


Figure 6: Mean Fertilization Rate in Different Treatments and Replication

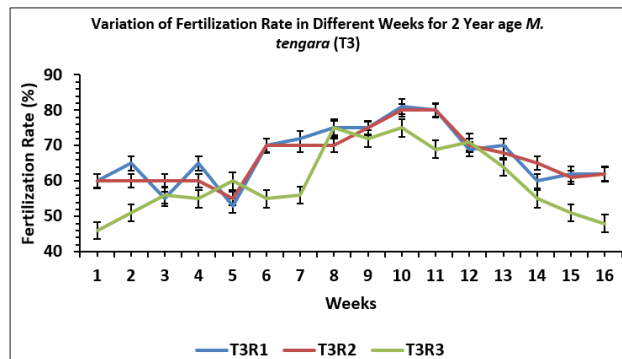


Figure 7: Fertilization Rate in Different Weeks in Treatment 3

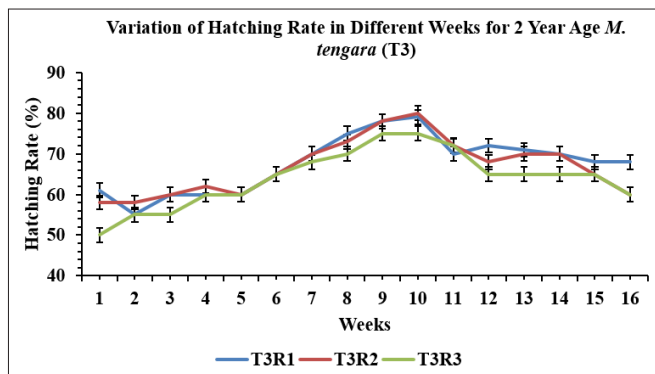


Figure 8: Hatching Rate in Different Weeks in Treatment 3

The results of treatment 3 showed that the best mean fertilization rate was observed in replication 1 (T3R1), indicating that a dose of 0.8 ml kg<sup>-1</sup> body weight is the optimal dosage for induced breeding of 2-year-old *M. tengara* broods. Moreover, the highest hatching rate was recorded in both replications 1 and 2 (Table 07 and 08 and Figure 05). In contrast, the lowest fertilization and hatching rates were observed in replication 3 (T3R3), suggesting that a hormonal dose of 1.2 mL.kg<sup>-1</sup> body weight is unsuitable for 2-year-old *M. tengara* broods. Therefore, doses of 0.8 and 1 mL.kg<sup>-1</sup> body weight are recommended for breeding 2-year-old broods of *M. tengara*. The fluctuations in fertilization rate and hatching rate in different weeks are presented in Figure 04 and Figure 05, respectively, providing valuable insights into the optimal timing for the induced breeding of 2-year-old *M. tengara* broods.

**Performances of Brood with Times based on Fertilization Rate**

Figure 10 illustrates the fertilization rates observed in different treatments during April, May, June, and July. In April, the highest fertilization rate was recorded in T3R1, whereas the lowest was recorded in T1R1. This indicates that, from 2-year-old and onwards, the broods responded well to a hormonal dose of 0.8 mL.kg<sup>-1</sup> body weight, because of previous gonadal maturation and ripe eggs. In contrast, the 1-year-old fish showed a weaker response to the same dose, presumably due to less maturation. In May, the highest fertilization rate was recorded in T1R2, whereas the lowest was recorded in T3R3. This indicates that the 1-year-old fish responded better to a hormonal dose of 1 mL.kg<sup>-1</sup> body weight, while the 2-year-old fish showed a weaker response to 1.2 mL.kg<sup>-1</sup> hormonal doses. In June, the 1.5-year-old fish responded better to a hormonal dose of 0.8 mL.kg<sup>-1</sup>, whereas the 2-year-old broods exposed to 1.2 mL.kg<sup>-1</sup> hormonal doses showed fewer responses. The broods showed similar results in July. Overall, the results suggest that the 1.5-year-old broods exhibited the best response to a hormonal dose of 0.8 mL.kg<sup>-1</sup> body weight. However, for optimal results, the hormonal dose should be adjusted based on the age of the fish. Notably, June appeared to be the best month for the induced breeding of *M. tengara*.

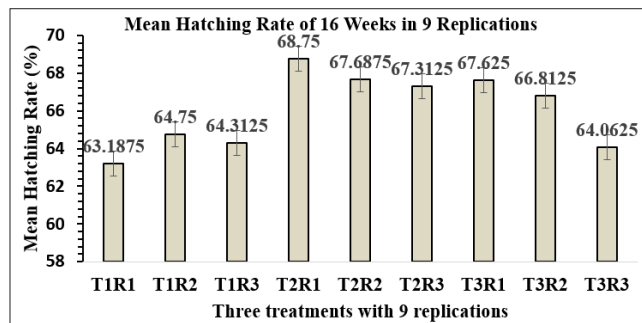


Figure 9: Mean Hatching Rate in Different Treatments and Replication

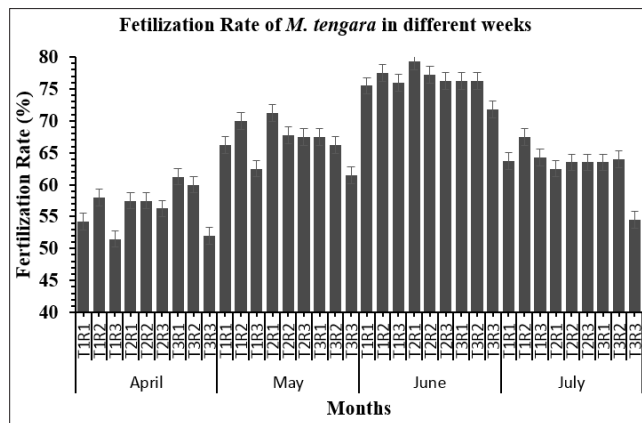


Figure 10: Fertilization Rate in Different Replication in Experimental Weeks

**Performances of Brood with Times Based on Hatching Rate**

According to Figure 11, the highest hatching rate was observed in June, which appeared to be the best month for fertilization rate. The broods exhibited similar results for both parameters. In the beginning, the 2-year-old brood showed better hatching rates than the 1-year-old brood, presumably due to early maturation. However, the 1.5-year-old broods exhibited the best overall hatching rates, achieving the highest rates with a hormonal dose of 0.8 mL.kg<sup>-1</sup> body weight. The other two age groups can also be used based on hormonal dose optimization.

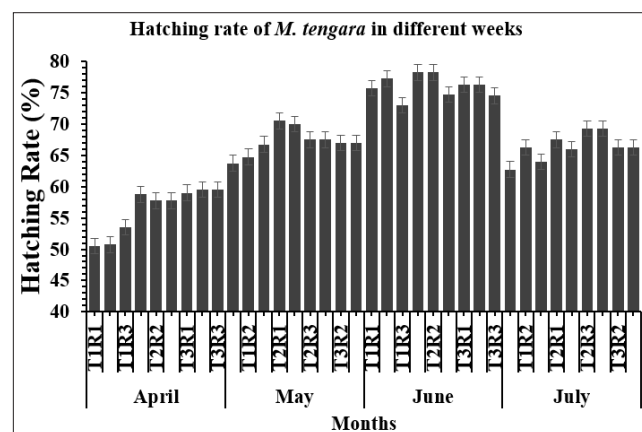


Figure 11: Hatching Rate in Different Replication in the Experimental Time Period

## Discussion

Hormonal doses are the most important aspect of induced breeding which varies based on species, breeding time and maturity of broods. In this study, we investigated the effect of hormonal doses on induced breeding success in different age groups of *M. tengara* broods. Three different doses of Ovasis (sGnRH), namely 0.8, 1, and 1.2 ml kg<sup>-1</sup> body weight, were used, and observed successful breeding across all doses. The individual hormonal dose was kept the same for the whole experimental period so that the perfect dose at the perfect time for the specific age group of fish can be identified. However, we found that fertilization and hatching rates varied with the age of the broods. Our experimental design involved keeping the individual hormonal dose constant throughout the study period to identify the optimal dose at the best time for the specific age group of fish. In comparison, Beilunf et al. (2017)[15] used different doses of Ovaprim (0.4, 0.6, 0.8, and 1.0 ml kg<sup>-1</sup>) with a 1:1, 2:1, and 3:1 ratio of male to female fish. Their results showed successful breeding under all conditions.

In this study, Ovasis (sGnRH) doses of 0.8 ml kg<sup>-1</sup>, 1 ml kg<sup>-1</sup>, and 1.2 ml kg<sup>-1</sup> body weight of fish resulted in successful induced breeding, but fertilization and hatching rate varied with the age of broods. We found that males were more readily involved in mating after 5-9 hours of hormone injection, which is similar with the findings of Alam et al. (2006) and Islam et al. (2011). However, we did not include this finding in the results section of this paper [16,17]. In this study, the optimum rate of fertilization and hatching for induced breeding of *M. tengara* was found to be at doses of 1.00 mL per kg body weight, with a 1:1 male to female sex ratio assimilate with result of a previous study by [17]. These findings suggest that carefully controlling the dosage and sex ratio can improve the effectiveness of induced breeding programs for *M. tengara*.

[10] investigated the effect of different doses of ovatide on the induction of female and male brood in three treatment groups (T1, T2, and T3). The brood fish in all treatments were induced with a specific single dose of ovatide. The ovulation rates were found to be 0%, 100%, and 63%, and the fertilization rates were 0%, 97%, and 90% for treatments T1, T2, and T3, respectively. The hatching rates for these treatments were 0%, 95%, and 76%. Additionally, the study found that a dose of 0.8 mL kg<sup>-1</sup> body weight resulted in an 81% fertilization rate, and a dose of 1 mL kg<sup>-1</sup> body weight resulted in an 82% hatching rate for 1-year-old and 1.5-year-old brood. These findings suggest that the effectiveness of ovatide induction may vary depending on the specific dose and the age of the brood fish. Similar variation was observed in this study where the fertilization rate and hatching rate significantly varied with different treatment and within their replications.

In a study the effectiveness of PG extract for inducing breeding was evaluated, and the optimal dosage was found to be 8 mg/kg for female brood and 4 mg/kg for male brood, resulting in a sex ratio of 2:1 [17]. Flash hormone (CPG) was used in a similar study the range of 0.4 to 1.2 mg/kg body weight for induce breeding of *Mystus vittatus*, and high fecundity was observed in mid-July [18]. However, in this study, sGnRH was used in the range of 0.8 to 1.2 ml/kg body weight, and the best results were obtained in the period from mid-May to mid-June, for all three age groups of brood fish. The present study used a dosage of 0.8 mL/kg body weight, which was higher than the minimum dosage used in Paul, 2022 study [18]. The strength of this study lies in the fact that it used brood fish from three different age groups, which helped to identify the optimal dosage for each group and the appropriate

timing for induction. The results of this study are expected to be beneficial for hatchery owners in the near future.

The breeding season of *Mystus tengara* typically starts in April and stretched up to August in the hatchery. But August do not considered as the perfect month for induce breeding of this species, because most of the hatchery do not get good result in this month. A study on the reproductive biology of *Mystus tengara* found the breeding season of this species as the typical practice of hatchery [12]. But this study identified the season last from April to July to get the highest fertilization rate and hatching and June is the best month for induce breeding *Mystus Tengara*.

Typically study show fertilization as greater than hatching rate if one apply the formula Fertilization (%) = (No. of fertilized eggs\*100)/Total number of eggs and hatching rate by applying Hatching (%) = (No. of hatching appeared\*100)/ No. of eggs in subsample [16,17] But this study applied another formula to calculate the fertilization rate and hatching which resembled to recent study on embryonic and larval development of stinging catfish[19]. Consequently hatching rate was found to be higher than fertilization Rate in some weeks which also assimilate with the study of Mahalder et al [19].

## Conclusion

Bagrid catfish species are at risk of being threatened due to overexploitation and the lack of seed availability. Induced breeding has become an essential aspect of protecting these species from disappearing from their natural habitat. Although induced breeding is practiced for many species in Bangladesh, limited data is available for *M. tengara*. Recent success in the induced breeding in *Mystus vittatus* by administering injections of carp pituitary hormone at an appropriate sex ratio, resulted in successful fertilization and spawning. The use of sGnRH in this experiment also showed promising results. These hormonal doses were used on different age groups of brood fish, and spawn production of *M. tengara* can be initiated through proper hatchery operation. Incorporating *M. tengara* into the carp polyculture of the country will not only help protect this threatened species but also contribute to the overall sustainability of the aquaculture industry. Further research on the effectiveness of different hormones and dosages for inducing breeding in other fish species can help in the conservation of endangered fish populations.

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**Conflict of interest:** The authors declare that they have no conflict of interest.

## References

1. Mitu NR, Alam MM (2016) Feeding Ecology of a bagrid catfish *Mystus tengara* (Hamilton, 1822) in the Tanore wetland of Rajshahi, northwestern Bangladesh. *Journal of Applied Ichthyology* 32: 448-455.
2. IUCN, Bangladesh (2000) Red book of threatened fishes of Bangladesh: IUCN – The World Conservation Union, Dhaka, Bangladesh, Xii+116 <https://en.bdfish.org/2010/09/book-profile-red-book-threatened-fishes-bangladesh/>
3. Hossain MY, Jasmine S, Ibrahim AHM, Ahmed ZF, Rahman MM et al. (2009) Length-weight and length-length relationships of 10 small fish species from the Ganges, Bangladesh. *Journal of Applied Ichthyology* 25: 117-119.
4. Berkeley SA, Chapman C, Sogard SM (2004) Maternal age as a determinant of larval growth and survival in a marine fish, *Sebastes melanops*. *Ecology* 85: 1258-1264.
5. Birkeland C, Dayton P (2005) The importance in fishery management of leaving the big ones. *Trends in Ecology & Evolution* 20: 356-358
6. O'Farrell MR, Botsford LW (2006) Estimating the status of near shore Rockfish (*Sebastes* Spp.) populations with length frequency data. *Ecological Applications* 16: 977-986.
7. Shelton AO, Hutchings JA, Waples RS, Keith DM, Akçakaya HR et al. (2015) Maternal age effects on Atlantic cod recruitment and implications for future population trajectory. *ICES Journal of Marine Science* 72: 1769-1778.
8. Tsadik Getinet G (2008) Effects of maternal age on fecundity, spawning interval, and egg quality of Nile tilapia, *Oreochromis niloticus* (L.). *Journal of the World Aquaculture Society* 39: 671-677.
9. Weber MJ, Brown ML (2012) Maternal effects of common carp on egg quantity and quality. *Journal of Freshwater Ecology* 27: 409-417
10. Hasan MZ, Islam MF, Haque SA, Islam MS, Rahman MM et al. (2021) Dose Optimization of Ovatide Hormone for Induced Breeding of Freshwater Gang Magur, *Hemibagrus Menoda* (Hamilton, 1822). *Research in Agriculture Livestock and Fisheries* 8: 171-179.
11. Kokokiris L, Stamoulis A, Monokrousos N, Doulgeraki S (2014) Oocytes development, maturity classification, maturity size and spawning season of the red mullet (*Mullus barbatus barbatus* Linnaeus, 1758). *Journal of Applied Ichthyology* 30: 20-27.
12. Gupta S, Banerjee S, (2013) Studies on reproductive biology of *Mystus tengara* (Ham.-Buch., 1822), a freshwater catfish of West Bengal, India. *International Journal of Aquatic Biology* 1: 175-184.
13. Guraya SS, Kaur R, Saxena PK (1975) Morphology of ovarian changes during the reproductive cycle of the fish, *Mystus tengara* (Ham.). *Acta Anatomica* 91: 222-260.
14. Pillay TVR (1990) *Aquaculture: Principles and practices*: Fishing News Books Ltd. London. ;UK.175 pp. [chrome-extension://efaidnbmnnnibpcajpcglclefindmkaj/https://www.unipu.hr/fileadmin/datoteke/Znanost\\_o\\_moru/Obavijesti/MARIKULTURA\\_Aquaculture\\_Principles\\_and\\_Practices\\_2nd\\_edition.pdf](https://www.unipu.hr/fileadmin/datoteke/Znanost_o_moru/Obavijesti/MARIKULTURA_Aquaculture_Principles_and_Practices_2nd_edition.pdf)
15. Bailung B, Biswas SP (2017) Spawning biology and induced breeding of freshwater catfish *Mystus dibrugarensis* : an approach to conservation (preprint) *Zoology*.
16. Alam MJ, Begum M, Islam MA, Pal HK (2006) Spawning behavior and induced breeding of an estuarine catfish, *Mystus gulio* (Ham.). *Bangladesh Journal of Fishery Research* 10: 101-109.
17. Islam S, Shah S, Rahi L (2012) Study of fecundity and induced breeding of *Mystus vittatus*. *Bangladesh Journal of Zoology* 39: 205-212
18. Paul SK (2022) Breeding biology and dose optimization for captive breeding of striped dwarf catfish *Mystus vittatus* using different hormones: *Iranian Journal of Fisheries Sciences* 21.
19. Mahalder B, Haque MM, Siddique MAB, Hasan NA, Alam MM, et al. (2023) Embryonic and Larval Development of Stinging Catfish, *Heteropneustes fossilis*, in Relation to Climatic and Water Quality Parameters: *Life* 13: 583.
20. Fakhruddin, Md, Hossain Y, Jesmine S, Akther KR, Chandra Roy N (2022) Observation of water quality parameters on induced breeding of Bagrid catfish in Bangladesh. *American Journal of Agricultural Science, Engineering, and Technology* 6: 67-73.

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