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# Degree-Hours to Spawning Response, Fertilization Rate and Hatching of *Labeo rohita* and Cirrhinus Mrigala through Induced Breeding under Hatchery Environment

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# ARTICLE INFO

## A B S T R A C T

Major carp is a group of fish species commonly cultured in Indian Subcontinent. Fish

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Keywords Degree-hours Induced breeding Spawning Fertilization rate Hatching rate is the major source of protein, omega-3 fatty acids, vitamins and minerals. With the robust increase in human population, the demand of food is also increasing. To overcome the food, demand many food sectors are working in line to cope with the protein requirements management of brood stock and successful breeding is also very important. Estimation of degree-hours for the spawning response, hatching of brood stock and their fertilization rate, hatching rate of fish eggs is one of the major aspects of hatchery management. Degree-hours is a standard unit that is used to measure the heat requirement for spawning and hatching while data is scarce for many species. The current study was designed to investigate the degree-hours to spawning and hatching for major carps i.e., Rohu (Labeo rohita) and Mrigala (Cirrhinus mrigala) during May to August 2022 at Punjab Fish Seed Hatchery, Rawal Town Islamabad, Pakistan. Two triplets (one male and two females) of each species were selected for induced breeding. Ovaprim was administrated at the rate of 0.5ml/kg for female and 0.2ml/kg for male to stimulate ovulation and spermitation respectively. Both species showed ovulation rate (100%). Spawning was occurred after 9.35±0.4299 hours in Rohu and in Mrigala 10.18±0.4393 hours at average water temperature of 26.3±0.575. Degree-hours to spawning in Rohu and Mrigala were calculated as 248.6±9.35 and 264.6±5.625 respectively. Higher fertilization rate (87.23±2.029%) and hatching rate (84.88±1.7747%) were observed in Mrigala (Cirrhinus mrigala) as compared to Rohu (Labeo rohita) i.e. (85.75±1.856%) and (81.75±1.525%) respectively. Hatching activity proceeded rapidly in Rohu and completed in 741.025±14.532 degree-hours while, in Mrigala it occurred in 778.43±9.1972 degree-hours. This was a first documented report on effect of degreehours on induced breeding of indigenous carps of Pakistan i.e., Rohu and Mrigala under controlled hatchery environment.

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#### INTRODUCTION

Population of freshwater fish species is declining due to robust changes in environmental conditions like temperature, pH, migration pattern, and interaction with other species (Costa *et al.*, 2021). Variation may appear due to inbreeding, overexploitation, and food supply (Su *et al.*, 2021). The fisheries industry and aquaculture are flourishing worldwide with the development of new

modern mechanization in fish farming as well as to acknowledge the biology of culturable species (Fu and Niu, 2022; N'Souvi *et al.*, 2021). The aquaculture industry is also flourishing day by day and the trend of almost all carp-cultivable fish species is being practiced in Pakistan (Kalsoom *et al.*, 2021; Altaf *et al.*, 2020). Pakistan has satisfactory aquaculture functional operational history of the inland area of about 79,200 km<sup>2</sup> and has 1120 km coastal belt but still, only 31 species of warm water and cold water are cultivated. The reason behind is the lack of a management system in the aquaculture and fisheries sector (Noman *et al.*, 2022; Laghari, 2018).

Problem of aquaculture industry is the insufficiency of the desired qualities of seed production which acquire diseased and pathogens during stocking (Asche et al., 2022; Rehman et., 2019). Besides that, there are certain factors like environmental conditions like temperature, photoperiod, and rainfall which limit fish productivity due to natural divergence. These conditions vary from species to species (Hoffle et al., 2018). The reproductive cycle of fishes is primarily stimulated by water temperature (Igbal et al., 2021). Indian major carps required water temperature range 21-31°C for inducing ovulation and hatching (Day, 2014). To get control of this problem induced breeding method is applied in which the exogenous hormones ovaprim administrated to the body of mature brooder (Nargesi et al., 2022 Ameer et al., 2021). Synthetic hormone is administrated to stimulate ovulation and spermitation (Zadmajid et al., 2018; El-Hawarry et al., 2016).

Indian major carps i.e., Rohu (*Labeo rohita*) and Mrigala (*Cirrhinus mrigala*) belong to family Cyprinidae, which is dominant about (53.7%) of the total families (Mohanty *et al.*, 2019). Family Cyprinidae is most diverse and has 84 species and 35genera (Mogalekar *et al.*, 2017). Major carps are the inhabitants of Ganga River channels in North India, Pakistan, Nepal and Burma (Sarma *et al.*, 2017). Major carp i.e., Mrigala and Rohu breed in the river naturally. They can also breed artificially in bundhs. They need stimulation for spawning (Bais, 2018). Water temperature played an important role for inducing ovulation and hatching in major carps (Ashaf-Ud-Doulah *et al.*, 2021).

Degree-hours are standard units to measure and elaborate the heat requirement for major carps (Heer *et al.*, 2019). It is an emerging and acceptable method for elaborating variation in fish growth and development. It is used for the investigation of the spawning response and

hatching time of fish brooders in hatcheries (Phelps *et al.*, 2007). Spawning is a natural process in fishes in which gametes i.e., eggs or milt released from females and males respectively (Urooj *et al.*, 2018). Spawning is due to the complex correlation between the different hormones and different tissues or organs in the fish body (Berlinsky *et al.*, 2020). Lower water temperature increases the latency period for carp (Sule and Adikwu, 2021).

Hatching is a physiological change in which the secretion of different hatching enzymes resulted in the breakdown of eggs shell and emerging of larvae (Yanagitsuru et al., 2021). Induced spawning, fertilization rate and hatching duration are directly affected by water temperature (Li et al., 2018). The latency period and hatching activity in major carps during development accelerate with an increase of temperature up to the upper threshold temperature (EL-GAMAL, 2006). There is little literature cited about degree hours for spawning response, fertilization rate and hatching activity in Rohu (Labeo rohita) and Mrigala (Cirrhinus mrigala). Therefore, a point study was conducted to determine the relationship between time and temperature during development in Rohu (Labeo rohita) and Mrigala (Cirrhinus mrigala). The current study was performed to evaluate the degreehours for a successful spawning response, hatching activity and to determine the influence of water temperature on fertilization rate and hatching rate in circular tanks.

# **MATERIALS AND METHODS**

#### Study area

This study was conducted in Punjab Fish Seed Hatchery at Rawal Town, Islamabad, Pakistan. Islamabad has subtropical humid weather with summers are sultry, humid, soaked and having latitude 33° 41' 5" N and longitude 73° 7' 4" E. Winters are short, unconcerned and partly cloudy. This area has minimum temperature of 1°C to maximum 42°C and having the average cloud cover 12% (Streimikiene etal., 2019). Experiment was performed during the months of May until August 2022 during the breeding seasons of major carps Rohu and Mrigala in this region.

#### **Experimental design**

There were two comprehensive parts of activities in this experimental study. The first part of the activity related to the selection of brood stock, acclimatization in holding tanks, induced breeding via. ovaprim hormone injection and keeping brood in circular tanks till spawning. In the second part, the estimation of degree-hours to spawning, hatching activity, and determine the impact of temperature variation on fertilization rate and hatching rate were done (Nazir *et al.*, 2022).

## Water quality parameters

Good quality water is a basic positive outcome to fish hatchery. There are three water quality parameters that

Table 1. Physico-chemical parameters of water.

assist to measure the quality of water, which include biological parameters, physical parameters, and chemical parameters. The physical parameters include taste, color, odor, turbidity, temperature, and electrical conductivity. Physico-chemical parameters of hatchery water were measured (Table 1).

Tuble 1.1 Hysico chemical parameters of water.							
Sr. NO.	Parameters	Measurements					
1	Temperature (°C)	25.9±0.43					
2	рН	8.97±0.085					
3	Dissolved Oxygen (mgL <sup>-1</sup> )	7±0.71					
4	Total alkalinity (mgL <sup>-1</sup> )	34.2±0.66					
5	Calcium hardness (mgL <sup>-1</sup> )	35±1					
6	Chlorides (mgL <sup>-1</sup> )	30±0.23					
7	Electrical conductivity (uScm <sup>-1</sup> )	193±0.69					
8	Total dissolved solid (mgL <sup>-1</sup> )	118±0.8					
9	Salinity (ppt)	0.15±0.02					

# Preparation of Holding tank and Circular tank

The holding and circular tanks were managed for spawning and hatching. Two holding tanks having the size of  $8 \times 5 \times 7$  feet (length × width × depth) and two circular tanks having a diameter of 1 m and depth of 4 feet were selected for spawning and hatching respectively. Circular and holding tank were washed with potassium per magnate KMnO<sub>4</sub> prior to stocking.

# **Fish Brooders Selection**

Brooders of Rohu and Mrigala (Triplets i.e., two females and one male of each species) were selected on the basis of health, activity, weight, length, colour, belly size and sexual dimorphism (Kumar *et al.*, 2021). The belly of the female brooder was rounded in shape while that of male brooder was slick or streamlined. The brooders were held for 10-12 hours without feeding so their bellies become empty and free of feces to avoid any kind of contamination during spawning. Four replicates for each species of major carps i.e., Rohu (*Labeo rohita*) and Mrigala (Cirrhinus mrigala) were studied.

# **Induced Breeding**

The synthetic hormone ovaprim was injected according to gender and weight of the brood stock (Abbas *et al.*, 2019). The sex ratio of the brooders was kept at 2:1 for females and male respectively. Recommended dose of ovaprim for a female is 0.5ml/kg and for male 0.2ml/kg were administrated according to the weight of brooders (Table 2)2. Ovaprim hormone stimulated ovulation and spermitation (Urooj *et al.*, 2018). This kind of injection was administrated between the area of the dorsal line and lateral line of scale at an angle of 40<sup>o</sup> to 50<sup>o</sup>. After hormone injection, the brooders were held in a circular tank until spawning. Continuously flowing water was maintained in the circular tank (khan *et al.*, 2022).

# **Ovulation Rate**

After hormonal administration, female ovulation occurred. The rate of ovulation was determined by using the formula of (Islam *et al.*, 2016).

Ovulation rate % = No. of female ovulated/Total No. of female injected ×100

# **Calculation of Degree-Hours for Spawning**

Spawning occurs due to the hormonal change in the reproductive parts of fishes induced by ovaprim (Marshall *et al.*, 2022). Sum of water temperatures in a circular tank after every hour is expressed as degree-hours (Urooj *et al.*, 2018; Nash and Koningsberger, 1981). A digital thermometer was used to measure the

temperature of continuously flowing water in circular tank. Water temperature of upper, middle and bottom area of circular tank was measured on hourly basis until spawning complete. In major carps Rohu (*Labeo rohita*) and Mrigala (*Cirrhinus mrrigala*) spawning occurred within 8.30-10.40 hours and 9.23-11.20 hours respectively. Degree hours were calculated for successful

Species	Months	Gender	Weight (kg)	Ovaprim (ml)
Labeo rohita	Мау	Male	$1.78 \pm 0.004$	0.36
		Female	2.4±0.04	1.5
		Female	$1.7 \pm 0.01$	0.83
Labeo rohita	June	Male	2.1±0.7	0.42
		Female	2.3±0.9	1.15
		Female	$1.8 \pm 0.01$	0.9
Labeo rohita	July	Male	$1.6 \pm 0.01$	0.32
		Female	2.0±0.1	1.0
		Female	$1.6 \pm 0.01$	0.80
Labeo rohita	Aug.	Male	$1.9 \pm 0.03$	0.36
		Female	1.3±0.1	0.64
		Female	$1.4 \pm 0.04$	0.70
Cirrhinus mrigala	May	Male	$1.4 \pm 0.04$	0.28
		Female	$1.6 \pm 0.01$	0.8
		Female	2.0±0.1	1.0
Cirrhinus mrigala	June	Male	1.6±0.1	0.32
		Female	$1.8 \pm 0.01$	0.90
		Female	$1.7 \pm 0.1$	0.85
Cirrhinus mrigala	July	Male	$1.6 \pm 0.01$	0.33
		Female	2.1±0.7	1.05
		Female	$1.7 \pm 0.10$	0.87
Cirrhinus mrigala	Aug.	Male	1.1±0.02	0.21
-	-	Female	$1.8 \pm 0.01$	0.88
		Female	$1.4 \pm 0.04$	0.71

spawning in each species of major carps at different temperatures. Table 2. Ovaprim for *Labeo robita* and *Cirrhinus mriagla* during breeding months

#### **Stripping and Fertilization**

When fish was ready to spawn, acquisition of eggs from female and milt from male was done by hand stripping method. At first acquisition of eggs from female were collected in a bowl. At the same time respective male was stripped and collected milt was assorted well with previously accumulated eggs. To get better outcome of fertilization physiological saline was applied and assorted well with a clean and sterile feather. The inseminated eggs were then transferred into a circular tank providing with continuous water flow. Fertilized eggs hatched in this condition (Haque *et al.*, 2021).

#### **Determination of Fertilization Rate**

After some times (1-2hrs) the eggs were scrutinized to observe fertilization rate. For this motive 500ml of water sample from the bottom of the circular tank was taken in trough from the circular hatching tank and counted. Then the eggs were discerned under a magnifying glass and fertilized eggs were counted with the assist of a soft thin brush. The fertilized eggs were easily disparate from the unfertilized eggs by the appearance of transparent shell with gray/ black spot within the eggshell, while the unfertilized eggs were non-transparent or opaque (Muhammad *et al.*, 2011).

The fertilization rate was calculated by following the formula:

Fertilization rate % = Numbers of fertilized eggs in sample/Total numbers of eggs ×100

#### **Estimation of Hatching Rate**

Hatching rate was determined by using formula of (Islam *et al.*, 2016).

Hatching rate %= Number of hatching eggs in sample/Total numbers of fertilized eggs×100

### **Calculation of Degree-Hours for Hatching**

Degree-hours for hatching of two species of major carp Rohu (*Labeo rohita*) and Mori (*Cirrhinus mrigala*) was noted until successful completion of hatching.

# RESULTS

The current study was carried out to observe the comparison of calculated degree-hours for successful spawning, hatching and determination of their ovulation rate, fertilization rate and hatching rate though induced breeding by using synthetic ovaprim hormones in both species of major carps i.e., Rohu (*Labeo rohita*) and Mrigala (*Cirrhinus mrigala*). This experiment was conducted in four trails (each trail of one month). In every trail each species was divided into four groups. Outcomes were calculated after treatments of ovaprim hormone (Nowosad *et al.*, 2016; Naeem *et al.*, 2005). These results show that degree-hours directly affect the spawning response and

hatching in major carp i.e., Rohu (Labeo rohita) and Mrigala (Cirrhinus mrigala). This study shows that successful spawning occurred after the latency period of Rohu (Labeo rohita) within 9.35±0.4299 hours (561 minutes) at 248.6±9.35 degree-hours and in Mrigala (Cirrhinus mrigala) within 10.18±0.4393 hours (610.8 minutes) at 264.6±5.625 degree-hours respectively (Table 5). Both species showed (100%) ovulation rate. Average fertilization rate of Rohu (Labeo rohita) was found (85.75±1.856 %) while, in Mrigala (Cirrhinus mrigala) it was (87.23±2.029%) respectively (Fig. 1 and Fig. 2). Hatching completed in Rohu (Labeo rohita) within 29.75±0.85 hours at 741.025 ±14.532 degree-hours and in Mrigala (Cirrhinus mrigala) occurred within 31.2±0.648 hours at 778.43±9.1972 degree-hours respectively while, average hatching rate of Rohu (Labeo rohita) and Mrigala (Cirrhinus mrigala) were observed as (81.75±1.525%) and (84.88±1.7747 %) respectively (Table 5).

Table 5. Comparison of estimated degree-hours for spawning response time, hatching time, fertilization rate and hatching rate of Rohu (*Labeo rohita*) and mrigala (*Cirrhinus mrigala*).

Sr No	Devemptor	Ovaprim treatment			
51.100.	Falalletei	Rohu	Mrigala		
1	Total No. of females treated	8	8		
2	Total weight of females (kg)	14.5	14.12		
3	Aver. temperature for spawning (°C)	26.3±0.575	26.3±0.575		
4	Spawning response time (hrs.)	9.35±0.4299	10.18±0.4393		
5	Ovulation rate%	100	100		
6	Degree-hours for spawning	248.6±9.35	264.6±5.625		
7	Overall fertilization rate%	85.75±1.856	87.23±2.029		
8	Avar.temperature for hatching ( <sup>o</sup> C)	24.93±0.25	24.93±0.25		
9	Hatching time (hrs.)	29.75±0.85	31.2±0.648		
10	Degree-hours for hatching	741.025 ±14.532	778.43±9.1972		
11	Hatching rate%	81.75±1.525	84.88±1.7747		



Figure 1. Fertilization rate of Rohu (Labeo rohita) induced by ovaprim.



Figure 2. Fertilization rate of Mrigala (Cirrhinus mrigala) induced by ovaprim.

### DISCUSSION

# Effects of Temperature Variation on Spawning Response Time

Ovulation rate, spawning response, fertilization and hatching rate were directly influenced by temperature (Iqbal et al., 2021). It was observed that increased in temperature decreased the latency period in fishes (Phelps et al., 2007). Ovulation rate in Rohu and Mrigala were found (100%) with single dose of ovaprim. The similar outcomes were reported by (Alam et al., 2022; Naeem et al., 2013). It was noted that spawning response time in Rohu (Labeo rohita) occurred within 8.30 hours, 9.27 hours, 9.43 hours and 10.40 hours and in Mrigala (Cirrhinus mrigala) it was 9.23 hours, 9.58 hours, 10.38 hours and 11.20 hours at water temperature range 25.9±0.028°C 27.4±0.03°C, 26.9±0.024°C, and 24.8±0.03°C respectively (Table 3 and Table 4). This study shows that Rohu (Labeo rohita) spawns earlier and required less heat as compared to Mrigala (Cirrhinus mrigala) at same temperature. In India researcher performed an experiment on major carps i.e., Rohu (Labeo rohita) and Mrigala (Cirrhinus mrigala) for seed production through induced spawning observed that spawning occurred after latency period 5.7-6.7 hours (342-400minutes) at temperature range from 27-34°C (Mohapatra et al., 2018). In India researcher reported that increase in temperature up to optimum decreased the spawning response time which supports the current findings (Mohapatra et al., 2016). The similar idea was reported that spawning response time directly influenced with water temperature (Urooj et al., 2018).

Spawning response, fertilization rate and hatching activity in major carp i.e., Rohu (*Labeo rohita*) and Mrigala (*Cirrhinus mrigala*) during development accelerated with increase of temperature up to upper thresh hold temperature (EL-GAMAL, 2006). When water temperature increases above upper thresh hold temperature or drop from lower threshold temperature, then spawning and hatching activity is non-linear or effectively zero (Chezik *et al.*, 2014).

# Impact of Temperature Variation on Fertilization Rate

In the last few decenniums, the artificial stimulation of fish fertilization rate has acquired a great awareness of scientists. Temperature has great impact on fish fertilization rate. Increase in temperature up to optimum boosting the fertilization rate (Iqbal et al., 2021). The present study also reveals the comparison of Rohu (Labeo rohita) and Mrigala (Cirrhinus mrigala) fertilization rate. This study shows that Mrigala (Cirrhinus mrigala) has maximum fertilization rate (81.6%), (86.2%), (91.2%) and (87.5%) as compared to Rohu (Labeo rohita) (80.4%), (86.2%), (88.7%) and (87.57%) at water temperature range 24.4±0.007°C, 24.6±0.027°C, 25.4±0.021°C and 25.3±0.018°C respectively (Fig.1 and Fig.2). Both species showed maximum fertilization rate at high temperature 25.4±0.021°C, but at low temperature 24.4±0.007°C both species showed minimum fertilization rate. In India researcher recorded that highest fertilization rate (97.5%) in Mrigala (Cirrhinus mrigala) followed by Rohu (92%) due to influence of temperature range 27-34°C (Mohapatra et al., 2018).

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Months	Females	Aver. Temp.	Spawning	Degree-	Fertilization	Aver. Temp. for	Hatching	Hatching	Degree-hours
		for spawning	time	hours for	rate (%)	hatching	time	rate (%)	for
		(ºC)	(hours)	spawning		( <u>°</u> C)	(hours)		hatching
May	2	24.8±0.03	10.40	272.8	80.4	24.4±0.007	32	77.4	780.8
June	2	25.9±0.028	9.43	244.9	86.2	24.6±0.027	30	82.1	738.6
July	2	27.4±0.03	8.30	227.4	88.7	25.4±0.021	28	84.4	711.2
Aug	2	26.9±0.024	9.27	249.2	87.7	25.3±0.018	29	83.1	733.5

Table 3. Estimated degree-hours for spawning response and hatching in Labeo rohita.

Table 4. Estimated degree-hours for spawning response and hatching in *Cirrhinus mrigala*.

				-	-				
Months	Females	Aver. Temp	Spawning	Degree-	Fertilization rate	Aver. Temp.	Hatching	Hatching	Degree-hours
		for spawning	time	hours for	(%)	for hatching	time	rate (%)	for
		(ºC)	(hours)	spawning		(ºC)	(hours)		hatching
May	2	24.8±0.03	11.20	277.8	83.3	24.4±0.007	32.6	80.0	799.5
June	2	25.9±0.028	10.38	269.5	87.5	24.6±0.027	32	85.7	788.16
July	2	27.4±0.03	9.23	252.8	91.2	25.4±0.021	30	88.5	762.0
Aug	2	26.9±0.024	9.58	258.1	88.6	25.3±0.018	30.2	85.33	764.06

The similar outcomes regarding impact of temperature variation on fertilization rate was found by (Ashaf-Ud-Doulah *et al.*, 2021; Mohapatra *et al.*, 2016). The similar results are reported on effect of temperature variation on fertilization rate (85.98%), (91.58%), (79.39%) and (75.49%) in Mrigala (*Cirrhinus mrigala*) at water temperature range 26°C, 29°C, 32°C, and 34°C respectively (Iqbal *et al.*, 2021). **Effects of Temperature Variation on Hatching Response** 

It was observed that after fertilization hatching occurred in Rohu (*Labeo rohita*) earlier within 28 hours, 29 hours, 30 hours and 32 hours while, in Mrigala (*Cirrhinus mrigala*) it occurred within 30 hours, 30.2 hours and 32 hours and 32.6 at 25.4±0.021°C, 25.3±0.018°C, 24.6±0.027°C and

24.4±0.007°C respectively. These results show that hatching activity proceeds rapidly with the increase of temperature up to optimum (Table 3 and Table 4). In India a study was conducted for seed production of Rohu (Labeo rohita) showed that hatching in Rohu preceded rapidly within 15.4-16.16 hours (920-970 minutes) at optimum temperature range 28.3-32.6ºC (Chakrabart et al., 2016). The similar outcome was reported regarding impact of temperature variation on hatching activity of Rohu (Labeo rohita) was completed within 18-30 hours after fertilization at temperature range 20-24.5°C (Naeem et al., 2013). In India researcher conducted a study which supports the current findings that hatching activity of Rohu (Labeo rohita) and Mrigala (Cirrhinus mrigala) species are directly affected with

the temperature (Mohapatra et al., 2016). The current study shows that Rohu (Labeo rohita) hatched earlier at 741.025 ±14.532 degree-hours as compared to Mrigala (Cirrhinus mrigala) at 778.43±9.1972 degree-hours. Maximum hatching rate in Mrigala was observed (80%), (85.7%), (88.5%) and (85.3%) as compared to Rohu (77.4%), (82.1%), (84.4%), and (83.1%) at temperature range 24.4±0.007°C, 24.6±0.027°C, 25.4±0.021°C and 25.3±0.018°C (Table 3 and Table 4). Both species showed maximum hatching rate at high temperature 25.42ºC. Previous studies have illustrated that increase in temperature up to optimum has direct effect on hatching rate which supports the current findings (Alam et al., 2022; Naeem et al., 2013).

# CONCLUSIONS

Degree-hours have a direct effect on fish breeding. Increase in water temperature up to upper threshold decrease the latency period for spawning and decrease the duration for hatching in major carps i.e., Rohu (Labeo rohita) and Mrigala (Cirrhinus mrigala). The findings of the current study indicate that both species of major carps showed maximum spawning response at high temperature 27.4±0.03°C, maximum fertilization rate and hatching rate were also calculated at high temperature 25.4±0.021°C. With the current level of practical support and training made available by Government hatchery, the mechanization has performed well and would be able to improve further in future. The situations like accessibility of brood-stock, suitable environmental conditions such as temperature would enable the farmers to authorize the potential level of performance of the technology. This work could provide useful perception into role of degree-hours for spawning response, ovulation rate and hatching rate of fishes.

# **DECLERATION OF COMPETING INTEREST**

The author declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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