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Research Article

Degree-Hours to Spawning Response, Fertilization Rate and Hatching of *Hypophthalmichthys nobilis* and *Cyprinus carpio* through Induced Breeding by using Ovaprim under a Captivity

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Abstract

A breeding experiment was conducted on *Hypophthalmichthys nobilis* and *Cyprinus carpio* April to August 2022 at Tawakkal Fish Hatchery at Muzaffargarh, Punjab, Pakistan. A single dose of intramuscular injection of Ovaprim with 0.7 ml/kg for female and 0.2 ml/kg for male brooders of both species were administrated. After hormonal adminteration brooders were kept in the circular tank for spawning. The 100% ovulation rate was observed in both species. Spawning response was observed after the latency period of (8.80±0.1-10.84±0.02 hours) and (8.1±0.26-9.8±0.01 hours) in *Hypophthalmichthys nobilis* and *Cyprinus carpio* respectively at water temperature range from 22-28 °C. Fertilization rate (61-73%) was observed in *Hypophthalmichthys nobilis* while, in *Cyprinus carpio* it was (60-76%) at water temperature range 22-28 °C respectively. Degree-hours to spawning were calculated in *Hypophthalmichthys nobilis* (238.2±1.46-246.4±1) while in *Cyprinus carpio it was* (215.6±1.3- 226.8±1.28) respectively. Hatching rate of *Hypophthalmichthys nobilis* was calculated as (52-70 %), while in *Cyprinus carpio* it was (56 - 71 %) and degree-hours to hatching was calculated as (748-784) and (682-702) respectively at water temperature range 22-28 °C respectively. Overall, the rates of fertilization and hatching increases with the increase of water temperature up to limit. These findings can be used to calculate spawning probabilities to enable future spawning predictions for the *Hypophthalmichthys nobilis* and *Cyprinus carpio.*

Keywords: Degree-hours; Induced breeding; *Hypophthalmichthys nobilis; Cyprinus carpio*.

Introduction

The demand of food rich in nutrients such as proteins, carbohydrates, fats, minerals and vitamins are increasing day by day due to over population(Merino *et al.*, 2012; Cai and Leung, 2022). The human population is expanding at very high rate and predicted to hit 10 billion people in 2050 (Faheem *et al.*, 2022). In the coming decades, the availability of food, particularly animal based protein, will be indispensable (MK *et al.*, 2022). To overcome food security and poverty some innovation in technologies (Feeds, breeding system and vaccines) and mechanization has been introduced in aquaculture and fisheries sectors(Yue and Shen, 2022). Aquaculture and fisheries sectors are flourishing worldwide and provide

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more than double the availability of aquatic food, and continuously expanding from 13.1 million mt in 1990 to 82.1 million mt in 2018(Garlock *et al.*, 2022). Aquaculture and fisheries industry are providing the 91% global production in Asia to fulfill the needs of overpopulated countries. Freshwater carps belong with Family Cyprinidae contributing about 53.1% of total production in the aquaculture industry (Tahseen, 2022). Aquaculture and fisheries industry is also increasing day by day and trend of almost all carp cultivable fish species is being practiced in Pakistan(Kalsoom *et al.*, 2021; Altaf *et al.*, 2020). Pakistan has vast aquaculture history of inland area about 79,200 km^2 and have 1120 km coastal belt but still only 31 species of warm water and cold water are cultivated. Main reason is poor management system in aquaculture and fisheries sectors(Noman *et al.*, 2022; Laghari, 2018). Bighead carp (*Hypophthalmichthys nobilis*) and Common carp (*Cyprinus carpio*) are exotic freshwater species commonly cultured in China and Thailand but introduced into Pakistan in 1959 and 1964 respectively, to fulfill and overcome the need of nutrients and food shortage (Khan *et al.*, 2016; Dastagir *et al.*, 2016). These carp are economically very important and played significant role in the aquaculture due their rapid growth rate, disease resistance, effective spawning response, high fecundity and hatching rate (Xiao *et al.*, 2017; Wang *et al.*, 2022). To overcome the food shortage some innovations are introduced in the breeding methods such as induced breeding.

In induced breeding synthetic hormones were administrated into their bodies for successful ovulation, spawning response, high fertilization and hatching rate (Biswas *et al.*, 2021; Kumar *et al.*, 2021). Ovaprim hormone is a synthetic product used as a spawning aid and contains Salmon GnRH analogues and dopamine antagonist (Brzuska, 2021; Nargesi *et al.*, 2022). Ovaprim hormone is widely used in Family Cyprinidae for successful spawning (Muchlisin *et al.*, 2014). Spawning is a natural process in which eggs or milt are expelled from the body of brooders in to surrounding of the water(Amezawa *et al.*, 2018; Superio *et al.*, 2021; Kucharczyk *et al.*, 2022). Hatching is physiological change in which secretion of different hatching enzyme resulted in the breakdown of eggs shell and emerging of larvae (Yanagitsuru *et al.*, 2021). Spawning response time, fertilization and hatching rate has direct relation with different factors such as environment, seasons, photoperiod, rainfall, temperature, mechanism of reproduction (Jia *et al.*, 2020; Wang *et al.*, 2022). Water temperature has direct influence on fish breeding and played a very effective role in spawning response, fertilization and hatching rate (Gonzal *et al.*, 1987).

Degree-hours are standard unit that is used to determine the heat requirement of carps i.e., *Hypophthalmichthys nobilis* and *Cyprinus carpio* (Heer *et al.*, 2019). It is the most suitable for explaining variations in fish growth and development (Phelps *et al.*, 2007; Urooj *et al.*, 2018). There is little literature cited about degree-hours for spawning response, fertilization rate and hatching activity in *Hypophthalmichthys nobilis* and *Cyprinus carpio*. Therefore, a point study was evaluated to determine the relationship between time and temperature during development in *Hypophthalmichthys nobilis* and *Cyprinus carpio*. This experimental study was performed to calculate the degree-hours for successful spawning response, hatching activity and to determine the impact of water temperature on fertilization rate and hatching rate in circular tanks.

Methodology

Study area

This experimental study was conducted in Tawakkal Fish Hatchery at Muzaffargarh, Punjab, Pakistan during breading seasons of *Hypophthalmichthys nobilis* and *Cyprinus carpio* April to August 2022.

Experimental Design

This study was portioned into two comprehensive sections, first part brooders selection, acclimatization in rectangular tank, induced breeding through hormonal administration,

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and holding brooders in circular tank till spawning. In second part, degree-hours to spawning response, fertilization rate, incubation, and hatching rate were calculated, effect of temperature fluctuation on latency period, fertilization and hatching was evaluated (Urooj *et al.*, 2018).

Physicochemical parameters of hatchery water

The physicochemical parameters such as colour, odor, taste, electrical conductivity, turbidity of hatchery water was measured (Table 1).

Table 1. Physico-chemical parameters of water.

Sr. NO.	Parameters	Measurements
1	Temperature range (°C)	22.4-28.7
2	pH	8.77±0.082
3	Dissolved Oxygen (mgL^{-1})	7±0.61
4	Total alkalinity (mgL^{-1})	33.2±0.56
5	Calcium hardness (mgL^{-1})	34±1
6	Chlorides (mgL^{-1})	31±0.69
7	Electrical conductivity ($uScm^{-1}$)	194±0.71
8	Total dissolved solid (mgL^{-1})	119±0.22
9	Salinity (ppt)	0.16±0.09

Brooders selection and acclimatization

Fish Brooders were selected on the basis of weight, sexual dimophism, health and their bellies. Female brooders have soft bulging abdomen and smooth pectoral fin while, for male the roughness of the pectoral fin and flat abdomen were examined. Brooders were acclimatized for 10-12 hours in holding tank.

Induced breeding

A single dose of Ovaprim hormone (Syndel Laboratory, Canada) was administrated at intramuscular junction to the lateral line of fish body. Ovaprim hormone was administrated 0.7ml/kg and 0.2 ml/kg for female and male respectively. After ovaprim administration brooders were kept in the circular tank for spawning purposes. Male and female brooder were placed together in the pair form to facilitate spawning (Ameer *et al.*, 2021).

Ovulation rate

After hormonal injection, ovulation occurred. The rate of ovulation was calculated by applying the formula of (Islam et al., 2016). Ovulation rate %= No. of female ovulated/Total No. of female injected ×100

Degree-hours to spawning

Hourly water temperature of circular tank was noted using the digital thermometer until spawning occurred. Water temperature from three surface, upper layer, middle layer and bottom layer of circular tank was noted. At the end, the sum of all water temperature readings was used to calculate the degree-hours for the spawning of *Hypophthalmichthys nobilis* and *Cyprinus carpio*.

Stripping and Fertilization

Brood fish was examined after 6-8 hours of injection whether they were ready for stripping. Acquisition of eggs and milt was carried out using hand stripping followed by dry methods. Female/male was held with vent oriented over a dry bowl. Gametes were released into the bowl by gently pressing abdomen toward vent from anterior to posterior. Gametes were mixed for artificial fertilization in water immediately with bird feathers. After water hardening, fertilized eggs were transferred into the circular tank for hatching.

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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 bottom of circular tank was taken in trough from circular hatching tank and counted. About 100 eggs were collected with the beaker. The dark black spot was examined with the help of a magnifying glass in the fertilized egg (Table 3 and Table 4).

Fertilization rate = No. of fertilized eggs / total no of eggs × 100

Calculation of hatching rate

Hatching rate was calculated by applying formula of (Islam et al., 2016).

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

Calculation of degree-hours to hatching

Degree-hours to hatching of both species i.e *Hypophthalmichthys nobilis* and *Cyprinus carpio* was noted until successful completion of hatching.

Result and Discussion

The present study was conducted to calculate degree hours for successful spawning response, fertilization and hatching in Chinese carp i.e *Hypophthalmichthys nobilis* and *Cyprinus carpio*. through induced breeding by using single intramuscular injection of synthetic product Ovaprim. This study was designed into Five trails (each trail of one month). Outcomes were calculated after Ovaprim administration (Nowosad et al., 2016; Ghosh et al., 2012; Naeem et al., 2011). The current findings indicate that degree-hours directly affect the spawning response time, fertilization rate and hatching rate of *Hypophthalmichthys nobilis* and *Cyprinus carpio*. Both species showed 100% ovulation rate. Spawning response time was calculated in *Hypophthalmichthys nobilis* and *Cyprinus carpio* with in (8.80±0.14 10.84±0.02 h) and (8.1±0.26·9.8±0.01 h) after the latency period at water temperature range 22-28 °C respectively. Fertilization and hatching rate of *Hypophthalmichthys nobilis* was calculated as 61, 65, 72, 73 and 71% and 52, 57, 68, 70 and 69 %, while in *Cyprinus carpio* it was 60, 69, 74,76 and 75 % and 56, 69, 70, 71 and 70 % at water temperature range 22, 24 26, 27 and 28 °C respectively. Degree-hours to spawning response time, Fertilization rate and hatching in both species were calculated (Table 2 and Table 3).

Degree-hours to spawning response time

In this experimental study both *Hypophthalmichthys nobilis* and *Cyprinus carpio* were induced to spawn administration with single dose of Ovaprim hormone during breading seasons. Outcomes of the hormonal stimulation in the current work are similar to effectiveness by using Ovaprim in different experimental studies (Urooj *et al.*, 2018; Naeem *et al.*, 2013). The Present study determined the time and temperature relationship for spawning and hatching of *Hypophthalmichthys nobilis* and *Cyprinus carpio.* Finding shows that *Hypophthalmichthys nobilis* spawned after the latency period of 8.80-10.84 hours of hormonal injection at 22-28 °C and have close resemblance with (Naeem *et al.*, 2011). *Cyprinus carpio* showed spawning after latency period of 8.1-9.8 hours of hormonal injection at water temperature 22-28 °C. Spawning response time of *Hypophthalmichthys molitrix* after hormonal injection was reported in literature in 8-10 hours at 26-30°C (Ali *et al.*, 2015) and in 9-10 hours at 24-28°C (Urooj *et al.*, 2018) and in *Cyprinus carpio* showed spawning response time 8-12 hours at 24-27°C (Naeem *et al.*, 2011).

In the current study, spawning response time increased as water temperature decreased vice versa. Latency period reported in literature increased with decreased in water temperature (Phelps *et al.*, 2007; Urooj *et al.*, 2018). In this experimental study, degree-hours to *Hypophthalmichthys nobilis* and *Cyprinus carpio* was calculated 238, 244, 345.1, 245.7, 246.4 and 215.6, 232.2, 223.6, 221.4 and 226.8 water temperature

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22, 24, 26, 27, 28°C respectively.

Outcomes of current study was that degree-hours to spawning response time decreased with increased in water temperature vice versa. Finding of present study have resemblance with the finding of (Phelps *et al.*, 2007).

Degree-hours to fertilization rate

In this experimental study, fertilization rate of both Chinese carp i.e *Hypophthalmichthys nobilis* and *Cyprinus carpio* were calculated as 61, 65, 72, 73, 71 % and 60, 69,74,76 and 75% at water temperature range 22, 24, 26, 27, 28 °C respectively.

Table 2 Calculated degree-hours to spawning response and hatching in *Hypophthalmichthys nobilis.*

Months	Females	Ovaprim for female (ml/kg)	Male	Ovaprim for male (ml/kg	Aver. Temp. for Spawning (°C)	Spawning time (hours)	Degree-hours for spawning	Fertilization rate (%)	Aver. Temp. for hatching (ºC)	Hatching time (hours)	Hatching rate (%)	Degree-hours for hatching
April	6	0.7	3	0.2	22±0.08a	10.84±0.02a	238.2±1.46a	61±0.2a	22±0.01a	34±0.7a	52±0.1a	748±2.81a
May	6	0.7	3	0.2	24±0.06b	10.18±0.02b	244.4±1.56b	65±0.5b	24±0.03b	32±0.3b	57±0.6b	768±2.27b
June	6	0.7	3	0.2	26±0.04c	9.43±0.02c	245.1±0.71b	72±0.1c	26±0.02c	30±0.7c	68±0.5c	780±1.96c
July	6	0.7	3	0.2	27±0.02d	9.10±0.12d	245.7±1.97b	73±0.4c	27±0.02d	29±0.8c	70±0.3c	783±4.49c
Aug	6	0.7	3	0.2	28±0.02e	8.80±0.14d	246.4±1.42b	71±0.2c	28±0.02e	28±0.6c	69±0.7c	784±2.97c

Mean values labelled with same alphabet are non-significantly different at α=0.05.

Table 3 Calculated degree-hours to spawning response and hatching in *Cyprinus carpio.*

Months	Females	Ovaprim for female (ml/kg)	Male	Ovaprim for male (ml/kg	Aver. Temp. for Spawning (°C)	Spawning time (hours)	Degree-hours for spawning	Fertilization rate (%)	Aver. Temp. for hatching (ºC)	Hatching time (hours)	Hatching rate (%)	Degree-hours for hatching
April	6	0.7	3	0.2	22±0.08a	9.8±0.01a	215.6±1.34a	60±0.1a	22±0.01a	31±0.23a	56±0.4a	682±2.21a
May	6	0.7	3	0.2	24±0.06b	9.3±0.03b	223.2±1.62b	69±0.4b	24±0.07b	29±0.56b	69±0.2b	696±2.24b
June	6	0.7	3	0.2	26±0.04c	8.6±0.02c	223.6±1.82b	74±0.3c	26±0.03c	27±0.66c	70±0.7c	702±2.32c
July	6	0.7	3	0.2	27±0.024d	8.2±0.12d	221.4±1.74b	76±0.1c	27±0.02d	26±0.54c	71±0.1c	702±4.24c
Aug	6	0.7	3	0.2	28±0.02e	8.1±0.26d	226.8±1.28b	75±0.3c	28±0.04e	25±0.34c	70±0.6c	700±3.64c

Mean values labelled with same alphabet are non-significantly different at α=0.05

Fertilization rate of was reported as 70- 80% for *Cyprinus carpio* and 72-78% for *Hypophthalmichthys molitrix* which supports the current findings respectively (Urooj *et al.*, 2018). In this study, fertilization rate increased as water temperature increased. In the present study, hatching response time was recorded within 28, 29, 30, 32 and 34 hours for bighead carp and 25, 26, 27, 29 and 31 hours for Grass carp at water temperature range 22,24,26,27 and 28 °C respectively. Hatching time duration increased as water temperature decreased vice versa. Findings of current study are thus almost in conformity with the finding of _in which hatching completed in 33 hours at 22°C and 24 hours at 28 °C water temperature (Woynarovich and Horváth, 1980).

Degree- hours to hatching response and hatching rate

In this experimental study, degree-hours to hatching of *Hypophthalmichthys nobilis* and was calculated as 748,768, 780, 783 and 784 and for *Cyprinus carpio it was* 682, 696,702, 702 and 700 at water temperature range 22, 24, 26, 27 and 28 °C respectively. Outcomes of this current study shows that degree-hours to hatching was decreased as water temperature increased

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(Urooj *et al.*, 2018; EL-GAMAL, 2006). Hatching rate for both species were recorded, *Hypophthalmichthys nobilis* showed hatching rate 52, 57, 68, 70 and 69%, while for *Cyprinus carpio* it was 56, 69, 70, 71 and 70% at water temperature range 22, 24, 26, 27 and 28 °C respectively. In this work, hatching rate of both species of Chinese carp increased with increased in water temperature up to limit (Phelps *et al.*, 2007; Urooj *et al.*, 2018).

Conclusion

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 *Hypophthalmichthys nobilis* and *Cyprinus carpio*. 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.

Declaration 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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Authors Contributions

All the authors have contributed equally to the research and compiling the data as well as editing the manuscript.

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