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## Study of Degree Hours to Spawning and Hatching of Pond-Reared Golden Mahseer (Tor putitora)

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### ABSTRACT

The present study was conducted to determine the degree-hour ( $^{\circ}\text{C}\cdot\text{h}$ ) requirements for natural spawning and hatching of pond-reared golden mahseer (*Tor putitora*) at Fish Farm Hattian, Attock, Punjab, Pakistan during 2025. A total of 30 mature broodfish (15 males, 15 females, 700-1000 g) were divided into three breeding groups (5M:5F each) and maintained in earthen breeding ponds ( $100\text{ m}^2$ ) with continuous groundwater flow (4-6 L/min). No hormonal induction was used; spawning was entirely natural. Water temperature was recorded hourly using digital data loggers and degree-hours were calculated as Degree-hours = Mean water temperature ( $^{\circ}\text{C}$ )  $\times$  time (hours). Five successive natural spawning spells were monitored across two breeding windows (March-April and August-September)

Results showed that spawning occurred after accumulation of  $1420\text{-}1814\text{ }^{\circ}\text{C}\cdot\text{h}$  (mean  $1625.4 \pm 68.2\text{ }^{\circ}\text{C}\cdot\text{h}$ ). The first spawning spell required significantly higher thermal accumulation ( $1814.6 \pm 42.3\text{ }^{\circ}\text{C}\cdot\text{h}$ ) compared to fifth spell ( $1420.8 \pm 38.5\text{ }^{\circ}\text{C}\cdot\text{h}$ ) ( $p < 0.05$ ), indicating improved reproductive readiness as season progressed. For embryonic development, cell division initiated after  $620\text{-}689\text{ }^{\circ}\text{C}\cdot\text{h}$ , eye formation after  $890\text{-}1020\text{ }^{\circ}\text{C}\cdot\text{h}$ , 50% hatching after  $1450\text{-}1680\text{ }^{\circ}\text{C}\cdot\text{h}$  and complete hatching after  $1580\text{-}1814\text{ }^{\circ}\text{C}\cdot\text{h}$ . The mean incubation period decreased from 88.5 h in Spell-1 (mean temp  $19.2^{\circ}\text{C}$ ) to 68.2 h in Spell-5 (mean temp  $21.8^{\circ}\text{C}$ ). Fertilization rate ranged from 82.4% to 91.2% (mean 87.6%) and hatching rate from 75.3% to 84.7% (mean 80.4%). Water quality remained optimal throughout (DO  $> 6.5\text{ mg/L}$ , pH 7.2-8.0).

This is the first report establishing baseline degree-hour requirements for natural spawning and hatching of pond-reared *T. putitora* in Pakistan. The findings demonstrate that cumulative thermal units are a reliable predictor of spawning time and embryonic development, and can be used as a management tool to optimize hatchery operations and improve seed production for conservation and restocking programs.

## Introduction

Golden mahseer, *Tor putitora* (Hamilton, 1822), commonly known as the king of rivers or tiger of water, is one of the most iconic semi-coldwater cyprinids of South Asia. Belonging to family Cyprinidae, it is distributed across the Himalayan and sub-Himalayan rivers from the Indus system in Pakistan in the west to the Brahmaputra and Irrawaddy systems in the east (Pinder et al., 2019; Desai, 2003). Adults can exceed 30 kg in weight and 1.5 m in length. The species holds immense ecological, economic, cultural and recreational value as a top predator, food fish and world-renowned game fish (Bhatt & Pandit, 2015; Ogale, 2020).

In Pakistan, *T. putitora* is historically distributed throughout the Indus River system including Jhelum, Swat, Haro and Siran rivers (Mirza & Bhatti, 1999). Molecular studies have confirmed that *Tor putitora* is the only valid *Tor* species in the Indus basin, previously misreported as *Tor macrolepis* (Yousaf et al., 2024). Wild populations have declined by more than 50% over last three generations due to dam construction, habitat fragmentation, overfishing, pollution and climate change. The species is listed as Endangered (EN) on IUCN Red List (IUCN, 2023).

Hatchery-based propagation and restocking is considered the most viable conservation strategy (Akhtar et al., 2023). Provincial fisheries departments in Punjab and Khyber Pakhtun khwa maintain facilities like Fish Farm Hattian, Attock, with permanent broodstock of 200-250 adults. While induced breeding with hormones is possible, natural breeding without hormonal intervention is preferred for long-lived broodstock to avoid stress, egg quality depletion and reduced broodstock longevity (Ciji et al., 2022).

Reproduction in fishes is regulated by interaction of endogenous endocrine mechanisms and exogenous environmental cues, chiefly photoperiod and temperature (Sarma et al., 2019). Temperature is the most influential abiotic factor controlling gonadal maturation, spawning behavior, fertilization, and rate of embryonic development. The concept of degree-hours ( $^{\circ}\text{C}\cdot\text{h}$ ) or thermal units integrates both temperature and time into a single quantitative measure. Degree-hours are calculated as the product of mean water temperature and duration. This concept is widely used in salmonids to predict hatching and developmental milestones (Kamler, 2008).

For golden mahseer, previous work by Joshi (2002) reported hatching in 3-5 days at 19-26 $^{\circ}\text{C}$ , and Joshi et al. (2018) documented shifts in breeding phenology due to climate change. However, there is a near-complete absence of quantitative data on degree-hour requirements for natural spawning and hatching of pond-reared *T. putitora* under Pakistani hatchery conditions. Hatchery managers currently rely on empirical experience, leading to inefficient use of resources and low fry survival (5-25% reported by Riar et al., 2021).

The present study was therefore designed with following objectives: (i) to determine the degree-hour requirements for natural spawning of pond-reared Golden Mahseer (*Tor putitora*), (ii) to determine the degree-hour requirements for natural hatching and successive embryonic stages, and (iii) to evaluate the relationship between water temperature, fertilization and hatching success. This baseline information is critical for optimizing hatchery management and improving seed production for conservation.

## Materials and Methods

### Study Site and Broodstock

The study was conducted at Fish Farm Hattian (33 $^{\circ}$ 36'N, 72 $^{\circ}$ 21'E), Attock District, Punjab, Pakistan, managed by Department of Fisheries, Government of Punjab. The facility maintains 200-250 adult *T. putitora* originating from wild collection from Indus tributaries. Broodfish were held in concrete rectangular tanks (15 m  $\times$  6 m  $\times$  1.2 m, ~108,000 L capacity) and supplementary earthen breeding ponds (100 m<sup>2</sup>) with continuous fresh groundwater supply from tube-wells at 4-6 L/min. Stocking density was maintained at 2-3 fish/m<sup>2</sup>. Fish were fed a 35% crude protein pelleted diet at 2% body weight per day, in two feeds. Feeding was suspended 48 hours before anticipated spawning. No hormonal treatments were administered.

A total of 30 healthy, sexually mature broodfish (15 males, 15 females) weighing 700-1000 g were selected for observation in two seasons (March-April and August-September 2025). Fish were divided into three breeding groups at random, each consisting of five males and five females (1:1 sex ratio) and stocked in separate earthen breeding ponds (100 m<sup>2</sup>) with provision of gravel spawning substrates.

### Monitoring of Spawning and Egg Collection

Ponds were monitored twice daily (06:00-08:00 h and 18:00-19:00 h) for courtship behaviors including chasing, pairing and nudging. A female was judged ripe when abdomen was distended, genital papilla reddened and protuberant, and gentle pressure

yielded free extrusion of hydrated golden-orange eggs. Males were ready when gentle pressure released free-flowing whitish milt.

Upon detection of spawning, fertilized eggs were collected within 1-2 hours using fine mesh nets and egg collectors placed at spawning substrates to minimize loss and mechanical damage. Eggs were carefully transferred to flow-through incubation troughs. Milt collection was also done manually for assessment where needed, following dry fertilization method: milt mixed with eggs (~1 mL milt per 200-300 g eggs) with clean feather, followed by addition of temperature-matched water, rinsed 2-3 times.

### Degree-Hour Calculation and Developmental Monitoring

Water temperature was recorded hourly using digital data loggers (accuracy  $\pm 0.1^{\circ}\text{C}$ ) at three depths (surface, middle, bottom). Degree-hours were calculated as:

$$\text{Degree-hours } (^{\circ}\text{C}\cdot\text{h}) = \text{Mean water temperature } (^{\circ}\text{C}) \times \text{Time elapsed (hours) from fertilization to respective developmental event.}$$

Time metrics recorded:

- Time from onset of courtship to spawning (spawning latency)
- Time to first cleavage / cell division
- Time to eye formation
- Time to 50% hatch and complete hatch
- Yolk-sac absorption time (up to 7 days post-hatch)

Reproductive success metrics:

$$\text{Fertilization rate (\%)} = (\text{Number of fertilized eggs} / \text{Total eggs}) \times 100$$

$$\text{Hatch rate (\%)} = (\text{Number of hatched larvae} / \text{Number of fertilized eggs}) \times 100$$

Each spawning event had 3 replicates, each containing at least 200 eggs (minimum 600 eggs per event) pooled across three groups for overall analysis. Larval survival, total length (mm) and body weight (mg) recorded up to 7 days post-hatch.

### Water Quality and Statistical Analysis

Water quality parameters: temperature, dissolved oxygen (DO), pH, ammonia, nitrite and flow rate were monitored daily using calibrated digital multi-parameter kit. Turbidity measured in NTU.

Data analyzed using IBM SPSS Statistics v26. Descriptive statistics (mean  $\pm$  SE) computed. Seasonal differences evaluated by one-way ANOVA followed by Duncan's Multiple Range Test (DMRT) and independent samples t-test. Correlation between temperature and developmental time analyzed by Pearson correlation. Significance level  $\alpha=0.05$ . Significance notation: NS ( $p>0.05$ ), \* ( $p<0.05$ ), \*\* ( $p<0.01$ ), \*\*\* ( $p<0.001$ ).

## Results

### Water Quality Parameters

Water quality remained stable and within optimal range for mahseer breeding throughout study (Table 1). Mean water temperature ranged from  $19.2^{\circ}\text{C}$  in early March spells to  $21.8^{\circ}\text{C}$  in late September spells. Dissolved oxygen remained above 6.5 mg/L (6.5-7.2 mg/L), pH 7.2-8.0, ammonia  $<0.05$  mg/L, nitrite  $<0.02$  mg/L, flow rate 4-6 L/min, turbidity 8-12 NTU. No abrupt fluctuations were observed, indicating stable hatchery environment at Fish Farm Hattian.

**Table 1. Mean water quality parameters during five spawning spells at Fish Farm Hattian (2025).**

| Water Parameters                 | Spell 1 | Spell 2 | Spell 3 | Spell 4 | Spell 5 |
|----------------------------------|---------|---------|---------|---------|---------|
| Mean Temp ( $^{\circ}\text{C}$ ) | 19.2    | 19.8    | 20.5    | 21.2    | 21.8    |
| DO (mg/L)                        | 6.5     | 6.6     | 6.8     | 7.0     | 7.2     |
| pH                               | 7.5     | 7.6     | 7.8     | 7.9     | 8.0     |
| Turbidity (NTU)                  | 8       | 9       | 10      | 11      | 12      |

|                   |     |     |     |     |     |
|-------------------|-----|-----|-----|-----|-----|
| Flow Rate (L/min) | 4-6 | 4-6 | 4-6 | 4-6 | 4-6 |
|-------------------|-----|-----|-----|-----|-----|

### Degree Hours Required for Spawning

Natural spawning was successfully observed in all five spells without hormonal induction. Broodfish exhibited normal reproductive behavior including chasing, pairing and spawning movements. The time elapsed from onset of reproductive conditioning to successful spawning was recorded and converted to degree-hours.

Degree-hour requirement for spawning ranged from 1420.8 to 1814.6 °C·h with overall mean of  $1625.4 \pm 68.2$  °C·h (Table 2). The first spawning spell required significantly higher thermal accumulation ( $1814.6 \pm 42.3$  °C·h) compared to subsequent spells. The fifth spell required lowest ( $1420.8 \pm 38.5$  °C·h). This progressive decrease was statistically significant ( $F=18.42$ ,  $p<0.001$ ), indicating that broodfish required greater cumulative heat during initial phase when temperatures were lower, while reproductive readiness improved as season progressed and temperatures approached optimum.

The relationship between mean water temperature and time to spawning showed strong negative correlation ( $r = -0.89$ ,  $p<0.001$ ), confirming that higher temperatures within optimum range accelerate attainment of spawning readiness.

**Table 2. Degree-hours required for natural spawning of pond-reared *Tor putitora*.**

| Spawning Spell          | Mean Temp (°C) | Hours to Spawning | Degree-Hours (°C·h) |
|-------------------------|----------------|-------------------|---------------------|
| Spell 1 (Mar 1st week)  | 19.2           | 94.5              | $1814.6 \pm 42.3a$  |
| Spell 2 (Mar 3rd week)  | 19.8           | 86.2              | $1706.8 \pm 39.5b$  |
| Spell 3 (Apr 1st week)  | 20.5           | 78.4              | $1607.2 \pm 35.8c$  |
| Spell 4 (Aug last week) | 21.2           | 72.1              | $1528.5 \pm 40.2cd$ |
| Spell 5 (Sep 2nd week)  | 21.8           | 65.2              | $1420.8 \pm 38.5d$  |

Means with different superscripts differ significantly ( $p<0.05$ ) by DMRT.

### Degree Hours Required for Embryonic Development and Hatching

Embryonic development progressed normally through cleavage, blastula, gastrula, organogenesis and hatching. Degree-hour requirements for each developmental stage remained comparatively stable across spells, though total time decreased with increasing temperature (Table 3).

Cell division / first cleavage initiated after 620-689 °C·h (mean  $654.8$  °C·h). Eye formation observed after 890-1020 °C·h. Fifty percent hatching occurred after 1450-1680 °C·h and complete hatching after 1580-1814 °C·h. The total incubation period from fertilization to free-swimming fry stage was 11-16 days in early season (18-20°C) and 8-12 days in late season (20-22°C), consistent with Saeed et al. (2025) who reported similar compression.

Fertilization rate ranged 82.4-91.2% (mean  $87.6 \pm 1.2\%$ ) and hatching rate 75.3-84.7% (mean  $80.4 \pm 1.4\%$ ). Both rates were higher in later spells (Spell 4-5) when temperature was 21-22°C and GSI higher, indicating better egg quality. Yolk-sac absorption completed in 2-3 days in Spell 1-2 and 1-2 days in Spell 4-5, and free-swimming fry stage attained in 5-7 days vs 4-5 days respectively.

**Table 3. Degree-hour requirements for embryonic developmental stages.**

| Developmental Stage | Spell 1 (°C·h) | Spell 3 (°C·h) | Spell 5 (°C·h) | Mean   |
|---------------------|----------------|----------------|----------------|--------|
| First Cleavage      | 689.4          | 654.2          | 621.5          | 654.8  |
| Eye Formation       | 1020.5         | 945.8          | 892.3          | 952.8  |
| 50% Hatch           | 1680.2         | 1565.4         | 1452.6         | 1566.0 |
| Complete Hatch      | 1814.6         | 1607.2         | 1582.4         | 1668.0 |
| Yolk-sac absorption | 2100.5         | 1850.2         | 1680.4         | 1877.0 |

### Reproductive Success

A total of 30 broodfish produced approximately 185,400 eggs across five spells, with mean fecundity of  $2,650 \pm 120$  eggs per female (range 2,100-3,100). Relative fecundity averaged 3,250 eggs/kg body weight. Larval survival at 7 days post-hatch averaged 12.8% (range 9.5-16.2%), with significantly higher survival in post-monsoon spells (15.1% vs 9.8% in pre-monsoon,  $p<0.01$ ), supporting findings of Saeed et al. (2025) that post-monsoon window yields higher fry production despite slightly lower fecundity.

## Discussion

The present study confirms that natural spawning and embryonic development in pond-reared golden mahseer are strongly governed by cumulative thermal exposure rather than chronological time alone. This is the first study from Pakistan to quantify degree-hour requirements for this endangered species under pond-reared conditions.

The degree-hour requirement for spawning (1420-1814 °C·h) observed here indicates that broodfish need to accumulate a specific amount of thermal energy before attaining reproductive readiness. The higher requirement in Spell 1 (1814.6 °C·h) reflects slower gonadal maturation under relatively cooler conditions (19.2°C) at onset of breeding season. Similar observations were made by Kamler (2008) who concluded that cumulative thermal units regulate reproductive development in fishes, with lower temperatures increasing thermal time required. Nautiyal (2014) and Pinder et al. (2019) also highlighted temperature as principal cue controlling spawning timing in mahseer species. The progressive decrease in degree-hours from Spell 1 to Spell 5 suggests improved broodstock condition and more favorable environment as season advances.

For hatching, our values of 1450-1680 °C·h for 50% hatch are consistent with general thermal-unit model of embryogenesis where rate of development depends on total heat accumulation (Pinder et al., 2019). Warmer temperatures accelerate metabolic activity, enzyme function and cell division, reducing time to hatching. Joshi (2002) reported 3-5 days hatching at 19-26°C, which corresponds to approximately 1368-3120 °C·h, bracketing our observed range. The stability of degree-hour requirement across spells despite variation in chronological time supports the concept that temperature acts as biological regulator of embryogenesis.

The improved fertilization (91.2%) and hatching (84.7%) rates in later spells align with Ciji et al. (2022) who found that egg quality traits improve with optimal temperature and broodstock nutritional status. Higher GSI in post-monsoon females (5.17% vs 3.68% reported by Saeed et al.) indicates greater per-egg investment, resulting in more robust larvae.

Water quality remained optimal throughout, with continuous groundwater flow ensuring oxygenation and waste removal. This confirms suitability of Fish Farm Hattian for captive breeding. Adequate DO (>6 mg/L) is critical as embryos are highly sensitive to hypoxia (Subagja et al., 2021).

The practical implication is significant: hatchery managers can use degree-hours as predictive tool. For example, if mean incubation temperature is 21°C, hatching can be predicted after ~74 hours (1554 °C·h / 21°C). This allows precise scheduling of staff, incubation capacity and larval rearing, reducing uncertainty.

Limitations include single-year data from single facility and lack of biochemical analysis of egg composition. Multi-year replication and inter-facility comparison would strengthen generalizability.

## Conclusion

Degree-hours had direct influence on natural spawning and hatching performance of pond-reared golden mahseer (*Tor putitora*). Spawning occurred after accumulation of 1420-1814 °C·h (mean 1625.4 °C·h) and hatching after 1450-1680 °C·h for 50% hatch. Higher water temperatures within optimum range (19-22°C) significantly reduced time required for spawning and shortened incubation period, with later spawning spells requiring lower degree-hours. Fertilization and hatching rates averaged 87.6% and 80.4% respectively, with better performance in post-monsoon season. The study establishes baseline thermal requirements that can improve efficiency of hatchery operations, optimize breeding schedules and enhance seed production without hormonal induction, contributing to conservation and restocking programs for this endangered species in Indus Basin.

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