

Variations in the reproductive performance of rohu *Labeo rohita* (Hamilton, 1822) under different agroclimatic zones of India

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Abstract

This study aimed to evaluate the induced breeding performance of the Indian major carp, rohu (*Labeo rohita*), in various agroclimatic zones of India. Breeding trials were conducted in 14 commercial hatcheries across four different Indian states: Andhra Pradesh, Tamil Nadu, Tripura and West Bengal. Except for Tamil Nadu, no significant variations in the breeding performance of rohu were observed. Breeding trials from Thanjavur District in Tamil Nadu showed a comparatively extended latency period, and lower fertilisation as well as hatching rates. The overall latency period was found to be more constant and lower (339 ± 13 min) in temperatures between 24 and 31°C. Below 24 and above 31°C, a delay in spawning was observed. Fertilisation and hatching rates were optimal in the 24-30°C range, however at 31°C, these rates became inconsistent and began to decline. At 32°C, both fertilisation (~60%) and hatching (~50%) rates dropped significantly. Early season (pre-monsoon) breeding exhibited a significantly extended latency period (484.89 ± 32.92 min), lower fertilisation rates (47-81%) and reduced broodstock response rates ($\leq 75\%$). Despite these constraints, the hatching rates during the pre-monsoon period were significantly higher ($93.13 \pm 3.09\%$) compared to monsoon breeding ($86.77 \pm 6.02\%$).

Introduction

Rohu (*Labeo rohita*) is the most important Indian major carp species for commercial aquaculture across India owing to its high growth potential, nutritional value, palatable flesh, and strong market value (Khan, 1972; Jhingran and Pullin, 1985; Jana, 2023). It is extensively farmed in countries like India and Bangladesh, global farmed production reaching 2.08 million t in 2020 (FAO, 2022). Considering its importance in the aquaculture systems, considerable emphasis has been placed on its genetic improvement through selective breeding in India. On the other hand, it is increasingly concerning that the physiology of fish species like *L. rohita* is being profoundly influenced by shifting precipitation patterns and rising global temperatures.

Temperature is one of the most decisive environmental variables that have profound effects on the life of poikilothermic animals such as fish. Since the body temperature of most fish species is essentially the temperature of the surrounding water, environmental temperature has a significant direct impact on their development, growth, behaviour and overall performance representing their physical wellbeing (Angilletta *et al.*, 2002; Islam *et al.*, 2019). Typically, most tropical fish are adapted to and live with optimum physiological activities in the temperature range of 25-35°C (Ficke *et al.*, 2007; Barange *et al.*, 2018). The majority of fish have developed their own specific behavioural and physiological adaptive mechanisms, which enable them to survive within the temperature range, through acclimation and adaptation to the changing temperature conditions in their environments (Kua *et al.*, 2020).



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A confined increase in water temperature within their natural tolerance range generally increases the metabolic rates and growth rates of the fish, and a decrease in temperature results in reduced performance (Auer *et al.*, 2018; Baerum *et al.*, 2021). However, a temperature beyond the optimum limits of a particular species can adversely affect the overall health of the species due to metabolic stress, increased oxygen demand and vulnerability to diseases (Wedemeyer *et al.*, 1999; Shahjahan *et al.*, 2018).

In recent years, climate change has led to a perceptible warming of the atmosphere. The Inter-governmental Panel on Climate Change (IPCC, 2014) reported that, the mean global temperature has increased by 0.85°C over the period from 1880 to 2012 and projected an additional temperature rise of 0.5-5.5°C by the year 2100. This temperature rise has and will have noticeable impact on fish populations in a broad way, either positively or negatively, as most fish species lack physiological ability to regulate their body temperature and also have limited dispersal ability within hydrographic networks in which they live (Angilletta *et al.*, 2002; Buisson *et al.*, 2008; Realis-Doyelle *et al.*, 2016).

Fish reproductive processes, starting from gamete development or maturation to spawning and fertilisation are also closely associated with ambient temperature (Pankhurst and Munday, 2011; Realis-Doyelle *et al.*, 2016). It also has an important role in the modulation of post-fertilisation processes, through its rate-determining effects on embryogenesis and hatching (Pauly and Pullin, 1988; Thepot and Jerry, 2015) as well as subsequent larval development, growth and survival (Landsman *et al.*, 2011). Changes in water temperature are transduced into effects on the reproductive processes of fish through the hypothalamo-pituitary-gonadal (HPG) axis (Bock *et al.*, 2021). These effects vary depending on species and even within species, depending upon geographic location (Pankhurst and Munday, 2011). Investigations have shown that the fishes exhibit strong upper thermal thresholds for reproductive activity and reduced number and quality of offsprings near this upper threshold (Donelson *et al.*, 2010). Moreover, eggs are considered one of the most thermally sensitive life stages, as for many fish species the tolerance limits appear to be within $\pm 0.6^\circ\text{C}$ of the spawning temperature (Rombough, 1997). Hence, a

small increase in temperature beyond the thermal tolerance range can significantly increase the mortality of eggs, particularly in tropical fish species (Barange *et al.*, 2018).

To understand the effects of temperature variations on the breeding performance and larval development, we selected the widely cultured Indian major carp (IMC), rohu *Labeo rohita* (Hamilton 1822) for the present study. Fish were sampled from different agroclimatic regions of India. The species is eurythermal, and a temperature range of 22-31°C is considered optimal for its spawning (Talwar and Jhingran, 1991). Previous studies by Ashaf-Ud-Doula *et al.* (2021) and Das *et al.* (2006) demonstrated the thermal sensitivity of rohu embryos under laboratory conditions, reporting developmental deformities and low survival at elevated temperatures. However, most existing literature on the impact of temperature on spawning and fertilisation has been limited to hatchery or laboratory settings, with very few studies reflecting field conditions under the current climatic scenarios. Therefore, in this study, we assessed the induced breeding performance of rohu in commercial hatcheries across wide geographical and agro-climatic conditions of India, with a particular focus on evaluating the potential impacts of elevated temperature on spawning, fertilisation and hatching rate.

Materials and methods

Study sites and timeline

The study was conducted at 14 commercial rohu hatcheries located in four districts across four different states in India, viz., Tanjavur District, Tamil Nadu ($10^\circ 39' 32''\text{N}$; $79^\circ 14' 42''\text{E}$), Krishna District, Andhra Pradesh ($16^\circ 41' 00''\text{N}$; $80^\circ 43' 30''\text{E}$), South Tripura District, Tripura ($23^\circ 13' 54''\text{N}$; $91^\circ 33' 34''\text{E}$) and Murshidabad District, West Bengal ($24^\circ 13' 44''\text{N}$; $88^\circ 14' 46''\text{E}$), between March 2018 and August 2019 (Fig. 1). Breeding trials were conducted at the hatcheries during the monsoon season, and the data were recorded from a total of 132 monsoon breeding cycles (Table 1).

To evaluate the breeding performance of rohu during pre-monsoon (March-April) and monsoon (June-August) seasons, additional

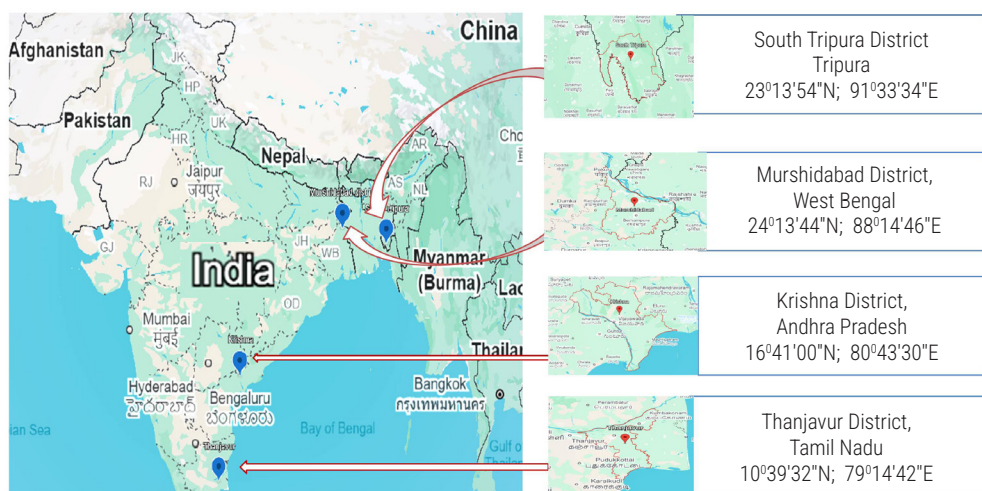


Fig. 1. Map showing the study sites

Table 1. Summary of sampling and mean rainfall (mm) and annual mean daily temperature of the study locations

Study site (District and State)	No. of hatcheries	No. of breeding cycle	Mean rainfall (mm) §†		Annual mean daily temperature (°C)†	
			Monsoon	Annual	Maximum	Minimum
South Tripura, Tripura	03	26	1422.6 ± 19.1 ^a	2380.4 ± 18.6 ^a	30.5	20.1
Murshidabad, West Bengal	05	43	1418.7 ± 13.9 ^a	1851.4 ± 13.5 ^b	32.1	21.2
Krishna, Andhra Pradesh	03	34	512.6 ± 21.2 ^b	903.6 ± 18.0 ^c	32.3	20.6
Thanjavur, Tamil Nadu	03	27	311.7 ± 26.3 ^c	898.1 ± 25.5 ^c	32.9	24.2

§: Mean rainfall (± Coefficient of variation) in the state during monsoon months (June-September total) and annually during the period 1989-2018.

Values of mean rainfall in a column with different superscripts differ significantly ($p < 0.05$).

†Source: IMD 2008; IMD 2019 and <https://imd pune.gov.in/hydrology/rainfall%20variability%20page/rainfall%20trend.htm>

breeding data were collected from 23 pre-monsoon breeding cycles conducted at four hatcheries in West Bengal. These data were then compared with the results of 32 monsoon breeding cycles conducted in the same four hatcheries. In this study, the term 'breeding cycle' refers to 'a complete sequence of events starting from broodstock selection and hormone administration to spawning, fertilisation and the completion of hatching of fertilised eggs'. The breeding performance was evaluated based on spawning response, fertilisation and hatching rates, as well as viability and production of hatchlings.

Experimental animals

Brood fishes for the study were selected from the stocks of the respective hatcheries. As broodstock management was carried out independently at each hatchery, some variation in management practices was expected. However, a standard protocol was followed, *i.e.*, broodfish were maintained at a stocking density of 1,500 kg ha⁻¹ and fed floating fish feed containing 24% crude protein, at 3% body weight, starting 60 to 75 days prior to the breeding operations. Only fish in advanced gonadal maturity stages were selected for the experiments. Females were selected by external morphological characteristics, such as pinkish-red urogenital papilla and a bulging coelomic cavity, while males were selected based on the release of milt with light pressure on the coelomic region. All fish handling procedures complied with national and international guidelines for the ethical treatment of animals used in scientific research. Healthy rohu brooders were collected from the broodstocks of the respective hatcheries. To minimise variability due to brooder size, which can significantly influence breeding performance (Kamler, 2005), efforts were made to maintain uniform body weights across hatcheries and breeding cycles. The average weight (mean ± standard deviation) of male and female broods selected for the study was 1.34 ± 0.21 and 1.56 ± 0.32 kg, respectively.

Induced spawning

For each breeding cycle, eight to twelve broodfishes were selected at a 1:1 male-to-female ratio. The selected fish were

conditioned in well-oxygenated water for 6 to 8 h prior to hormone administration. A synthetic spawning-stimulating hormone, Ovotide [Salmon Gonadotropin-Releasing Hormone analogue (SGnRHa) + Domperidone] was used. Male brooders received a single intraperitoneal injection (IP injection) at dose of 0.20 ml kg⁻¹ body weight, while female brooders received a single dose of 0.40 to 0.50 ml kg⁻¹ body weight (Chaudhury, 1960; Chakrabarti *et al.*, 2017). An exception was noted in one hatchery in Tamil Nadu, where the doses were modified to 0.30 ml kg⁻¹ for males and 0.8 ml kg⁻¹ body weight for females. Following hormone administration, the brooders were released into the breeding pool of the Chinese hatchery system for spawning. After spawning, the fertilised eggs were transferred to the hatching pool and incubated under a continuous flow of water until hatching was complete.

Water quality

All experiments were performed under natural temperature, photoperiod and climatic conditions. Throughout the breeding trials, the water temperature in each hatchery was monitored twice daily using a probe with a precision of 0.10°C. Additional water quality parameters, *viz.*, pH, dissolved oxygen, alkalinity and hardness were measured, on each sampling day throughout the experimental period, using a portable meter (Model HI9829, HANNA Instruments Co.) and a chemical test kit (Model HI3817, HANNA Instruments Co.) (Table 2).

Breeding performance

Latency periods, fertilisation rates and hatching rates were recorded for each breeding cycle. The latency period or response time is the time between the hormone administration and the onset of spawning. To estimate the fertilisation rates, three subsamples of eggs (at least 100 eggs per sample) were collected from the spawning pools 4-6 h after the onset of ovulation. Only fertilised eggs with clearly visible and intact nuclei were counted for calculating the percentage of fertilisation. Fertilisation (%) was calculated using the formula:

Table 2. Water quality of parameters at the study locations over the experimental period

Parameters	Tripura	West Bengal	Andhra Pradesh	Tamil Nadu
Temperature (°C)	26.53 ± 2.77 ^a	26.96 ± 2.85 ^{ab}	25.5 ± 2.62 ^{bc}	28.18 ± 2.29 ^b
pH	7.46 ± 0.31 ^a	7.85 ± 0.22 ^b	7.78 ± 0.16 ^b	7.69 ± 0.19 ^c
Dissolved oxygen (mg l ⁻¹)	5.71 ± 0.47 ^a	5.86 ± 0.35 ^a	5.56 ± 0.49 ^a	5.16 ± 0.81 ^b
Alkalinity (mg l ⁻¹)	71.15 ± 4.37 ^a	121.42 ± 4.57 ^b	94.51 ± 9.39 ^c	114.21 ± 8.78 ^d
Hardness (mg l ⁻¹)	56.46 ± 6.92 ^a	146.82 ± 6.28 ^b	129.38 ± 7.44 ^c	135.36 ± 4.58 ^d

Values of each water quality parameter in a row with different superscripts differ significantly ($p < 0.05$). All values expressed as mean ± SD.

Fertilisation rate = $(A/B) \times 100$, where A = No. of fertilised eggs and B = No. of eggs counted.

Post-hatching, early larvae were counted approximately 24 h after the outset of hatching and the hatching rate was calculated using the following formula:

Hatching rate = $(C/D) \times 100$, where C = No. of hatched larvae and D = No. of fertilised eggs.

Statistical analysis

All the results were expressed as mean $\pm$ standard deviation (SD), unless otherwise stated. One-way analysis of variance (ANOVA) followed by Tukey's *post hoc* test was used to assess statistically significant differences among the states and across different temperature conditions. Regression analyses were performed to determine the overall correlation of latency time, fertilisation rate and hatching rate with temperature. All results were considered significant at the level of $p < 0.05$. Statistical analyses were carried out using SPSS 14.0 for Windows (SPSS Inc., Chicago, IL).

Results

The goal of the current study was to assess the breeding performance of rohu in different agro-climatic zones of India. The states and districts included in this study are located in three distinct climatic zones, differing in terms of rainfall pattern and temperature. South Tripura, situated in the Eastern Himalayan Region, has a sub-humid climate with annual rainfall ranging from 200 to 400 cm. Temperatures in July typically range from 25 to 33°C, while those in January range from 10 to 24°C. Murshidabad District of West Bengal, located in the Lower Gangetic Plains Region, experiences a hot and humid climate with 100-200 cm of annual rainfall and the temperature ranges from 26 to 41°C in July and from 9 to 24°C in January. The Krishna District of Andhra Pradesh and the Thanjavur District of Tamil Nadu, in the East Coast Plains and Hills Region, experience warm and sub-humid climates. These regions receive 75 to 150 cm of rainfall annually and their July and January temperatures range from 26 to 32°C and 20 to 29°C, respectively. The influence of rainfall was considered negligible in this study because all the breeding operations were conducted in Chinese hatcheries. Temperature was therefore the most significant environmental factor influencing breeding and spawning performance of rohu in these hatcheries, which we have investigated in detail during the present study.

Overall breeding performance of rohu in relation to temperature during monsoon months

The mean latency period (343 $\pm$ 13 min) was found to be significantly lower ($p < 0.05$) in the temperature range of 24-31°C (Fig. 2a), based on the data collected from the four states. With the exception of a significant difference between 24 and 28°C ($p = 0.014$), no significant differences in latency period were observed between temperatures in the 24-31°C range ($p = 0.06$ to 0.89). However, the latency period was found significantly higher at the temperature range of 22-23°C and even at higher temperature of 32°C ($p < 0.05$). Moreover, the variations in latency periods within the same temperature were also found to be greater at lower (22°C) and higher (30°C and above)

temperatures. Overall, a significant, yet small negative relationship ($R = -0.2925$, $p < 0.05$) was observed between the temperatures and latency periods of rohu (Fig. 2a).

No significant differences in the fertilisation rates of rohu ($\geq 88\%$) were observed within the temperature range of 22-30°C ($p > 0.07$), except very low rates of fertilisation (68 – 79%) at 24–25°C and 30°C at Thanjavur, Tamil Nadu (Fig. 2b). However, in all the states, overall mean fertilisation rates were found significantly low at temperatures of 31°C (81.47 $\pm$ 7.21%, $p < 0.05$) and 32°C (73.66 $\pm$ 8.73%, $p < 0.01$) (Fig. 2b and 3). Although a significant inverse relationship ($R = -0.6759$, $p < 0.01$) existed between temperature and fertilisation rates of rohu, highest percentage of fertilisation ($> 90\%$) with less variations (SD < 3.5) were observed in the temperature range of 26-29°C (Fig. 2b).

Although no significant differences were observed in the hatching rate of rohu among different temperatures from 22-30°C ($p > 0.05$), the mean percentage of hatching (87.20 $\pm$ 6.72%) was observed better at the temperature range of 24-30°C (Fig. 2c; Fig. 3). The hatching rate (82.14 $\pm$ 7.23%) at 31°C was found to be somewhat lower than that of the 24-30°C temperature range. However, the hatching rate of rohu was drastically (68.80 $\pm$ 12.14%) and significantly ($p < 0.01$) reduced at a higher temperature of 32°C. Overall, a significant negative correlation ($R = -0.4891$, $p < 0.01$) was observed between temperature and hatching rate of rohu (Fig. 2c).

Variations in breeding performance of rohu in relation to temperature

Variations in the breeding performance of rohu in relation to temperature within the hatchery and between the hatcheries of different states were observed. Comparing the breeding trials across all temperatures (with the exception of 24°C) of the states, a more extended latency period was observed in Tamil Nadu. However, no significant difference in latency period against corresponding temperature was observed among Tripura, West Bengal and Andhra Pradesh ($p > 0.05$), except significantly longer ($p = 0.036$) latency period at 25°C in West Bengal compared to Tripura (Fig. 4a). No definite trend was observed between water temperature and latency period of rohu in Tripura ($R = -0.1022$; $R^2 = 0.0104$; $p = 0.62$). However, the latency period was significantly reduced with increasing temperatures in West Bengal ($R = -0.5713$; $R^2 = 0.3264$; $p < 0.01$) and Andhra Pradesh ($R = -0.8706$; $R^2 = 0.7579$; $p < 0.01$). Whereas, in Tamil Nadu a significant increase in latency period was observed with an increase in temperature ($R = 0.7238$; $R^2 = 0.5239$; $p < 0.01$).

The fertilisation rate was consistently lower across the temperature range in Tamil Nadu with a greater degree of variation compared to other three States. Mean fertilisation rates were greater than 90% across all individual temperatures in the range of 24-29°C in Tripura, West Bengal and Andhra Pradesh and the differences were statistically insignificant ($p > 0.05$). The mean fertilisation rates varied between 77-89% in Tamil Nadu. At a temperature of 30°C, the mean fertilisation rates were 93.8% in Tripura, 82.76% in West Bengal and 79.3% in Tamil Nadu, although the differences were not significant ($p = 0.07$ -0.15). Overall, at the temperature range of 30-31°C, the average fertilisation rates were in the range of 75-90%, which further reduced to 60-85% at 32°C (Fig. 4b). Furthermore, 58% of the fertilisation rate was observed in one of the hatcheries

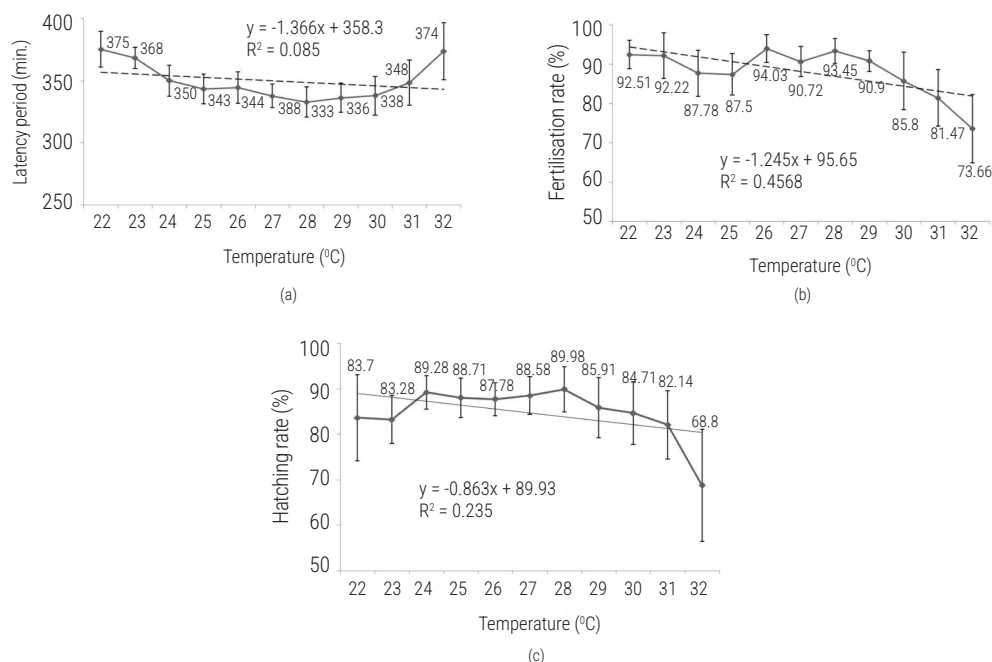


Fig. 2. (a) Latency period; (b) Fertilisation and (c) Hatching rate (as percentage of eggs) of *L. rohita* at different water temperatures. Each data point represents the mean $\pm$ SD of observations ($n>3$; range 4-18), pooled across all four states corresponding to each temperature

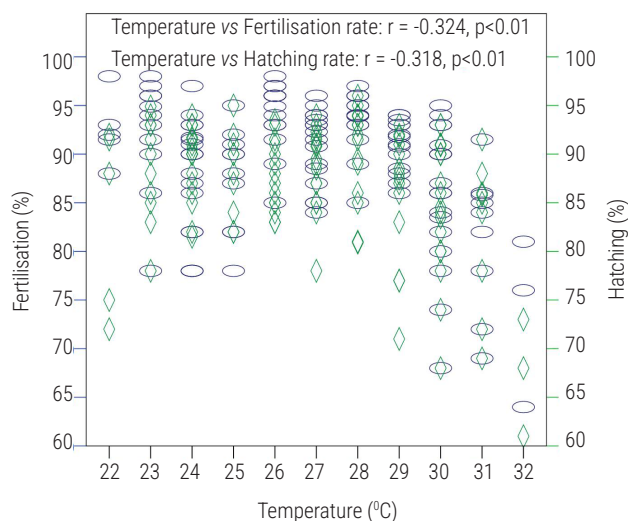


Fig. 3. Average fertilisation and hatching rates across 132 monsoon breeding cycles in relation to the corresponding water temperature ($^{\circ}\text{C}$)

in Tamil Nadu at 34°C through hormonal dose manipulation ($0.3\text{--}0.8\text{ ml kg}^{-1}$ body weight). Intra-state variation analysis showed significant negative relationships between water temperatures and fertilisation rates for Tripura ($R = -0.5103$; $R^2 = 0.2604$; $p < 0.01$), West Bengal ($R = -0.5412$; $R^2 = 0.2929$; $p < 0.01$), Andhra Pradesh ($R = -0.3425$; $R^2 = 0.1173$; $p < 0.05$) and Tamil Nadu ($R = -0.413$; $R^2 = 0.1706$; $p < 0.05$).

Hatching rates were found to be significantly lower ($p < 0.05$) and also inconsistent across temperature ranges in Tamil Nadu compared

to all other three States. The highest hatching rate ($>90\%$) was observed at a temperature range of $27\text{--}29^{\circ}\text{C}$ in Tripura, at 25 and $27\text{--}30^{\circ}\text{C}$ in West Bengal and at temperature range of $25\text{--}29^{\circ}\text{C}$ in Andhra Pradesh. Except for Tamil Nadu, the overall hatching rates in all other three states (with an exemption of a single case at 26°C in West Bengal) were found better ($>85\%$) in the temperature range of $24\text{--}30^{\circ}\text{C}$ in the order of Andhra Pradesh $\geq$ Tripura $>$ West Bengal (Fig. 4c). The intra-state variations analysis showed no detectable relationship between hatching rates and water temperatures ($R = 0.0154$; $R^2 = 0.0002$; $p = 0.92$) in West Bengal; although a negative relationship existed ($R = -0.4876$; $R^2 = 0.2378$; $p < 0.05$) for Tripura and Tamil Nadu ($R = -0.9099$; $R^2 = 0.8279$; $p < 0.01$). However, a significant positive linear relationship ($R = 0.8168$; $R^2 = 0.6672$; $p < 0.01$) was observed between hatching rate and water temperature in Andhra Pradesh.

Comparison between monsoon and pre-monsoon breeding performance of rohu

A summary of monsoon and pre-monsoon breeding performance of rohu is presented in Table 3. Overall, the latency period during pre-monsoon breeding was found to be significantly longer ($p < 0.01$) than breeding during monsoon months (Fig. 5a). With the increase in temperature, shorter latency periods were observed in both pre-monsoon ($R = -0.8591$; $R^2 = 0.7381$; $p < 0.01$) and monsoon ($R = -0.6166$; $R^2 = 0.3802$; $p < 0.01$).

Fertilisation rates across temperature ranges were found to be significantly higher ($p < 0.05$) in monsoon breeding than pre-monsoon breeding. In monsoon breeding, fertilisation rates were $79\text{--}98\%$ at a temperature range of $23\text{--}31^{\circ}\text{C}$, whereas they were $47\text{--}81\%$ during pre-monsoon breeding at a temperature range

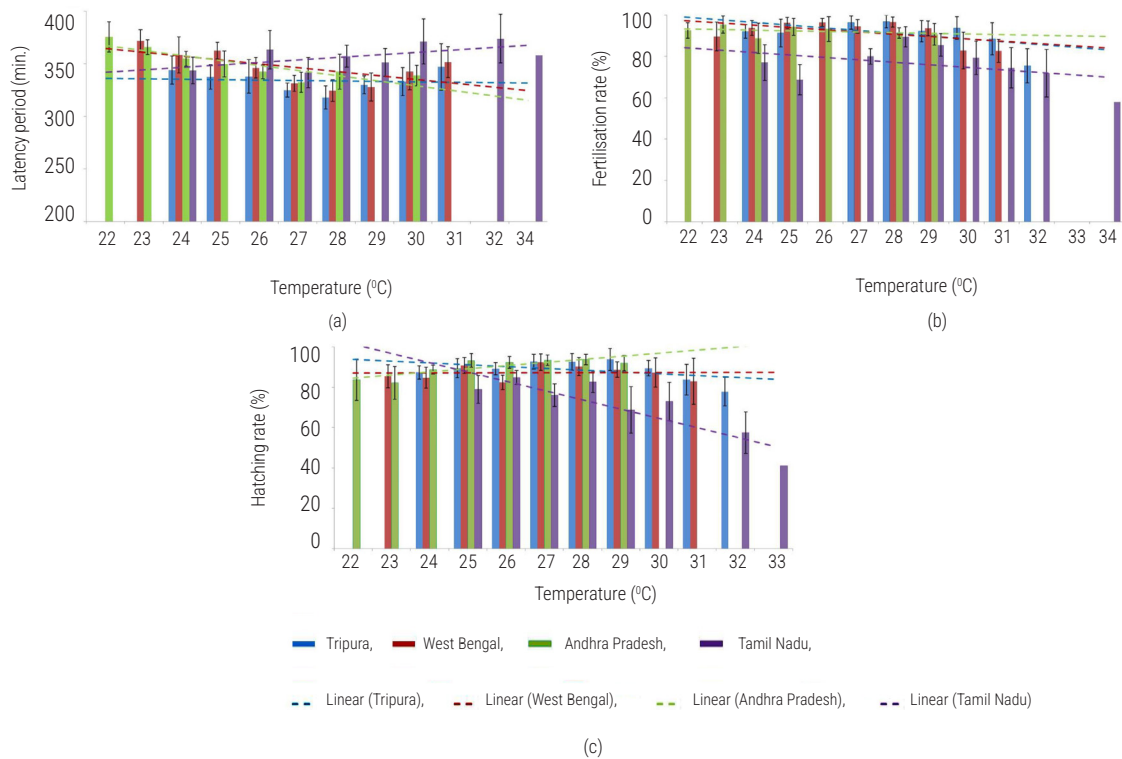


Fig. 4. Intra- and inter-state variations in (a) Latency period; (b) Fertilisation and (c) Hatching (expressed as percentage of eggs) of *L. rohita* at different water temperatures. Values are presented as mean±SD from two to six observations (mostly n>3), except at the terminal temperature values. Dotted lines represent the linear relationships between reproductive parameter and temperature

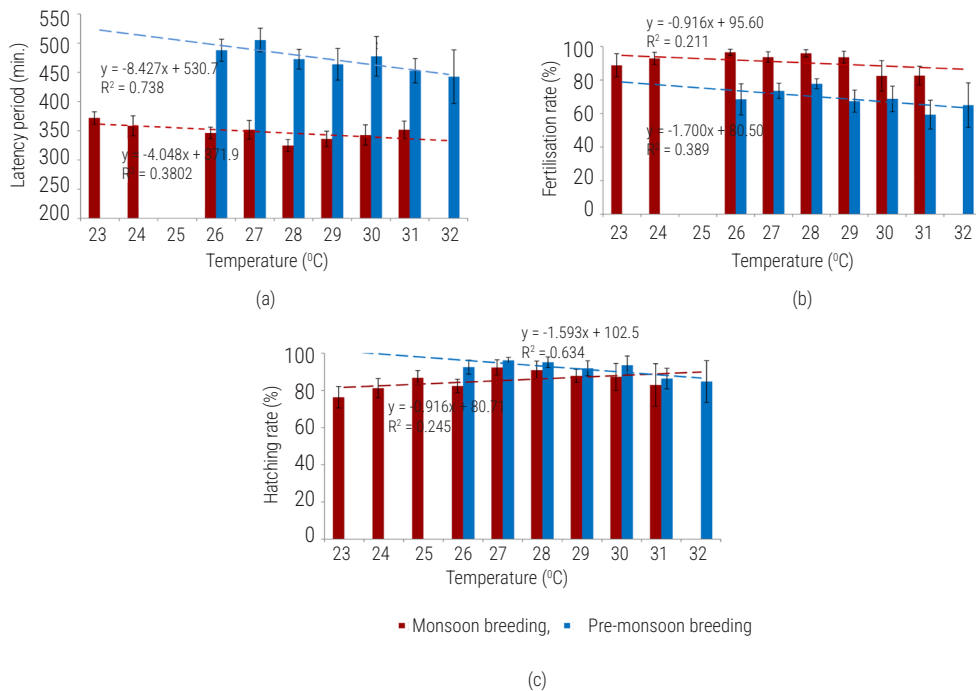


Fig. 5. Variations in (a) Latency period, (b) Fertilisation rate and (c) Hatching rate (expressed as percentage of eggs) of *L. rohita* during pre-monsoon and monsoon breeding seasons. All values expressed as mean±SD of two to five observations (mostly n>3)

Table 3. Summary of monsoon and pre-monsoon breeding performance of rohu

Parameter	Pre-monsoon breeding (March-April)	Monsoon breeding (June -August)	p- value
Temperature	28.92±1.64°C ^a (26-32°C)	26.45±2.45°C ^b (23-31°C)	0.0023
Latency period (min)	484.89±32.92 ^a (390-530)	338.19±17.89 ^b (310-380)	<0.0001
Fertilisation rate (%)	62.75±9.81 ^b (47-81)	91.05±5.23 ^a (79-98)	<0.0001
Hatching rate (%)	93.13±3.09 ^a (87-97)	86.77±6.02 ^b (74-97)	0.0006

Values in a row with different superscripts are significantly different ($p < 0.05$). All values expressed as mean±SD. Values in parenthesis represent the lowest and highest values

of 26-32°C. The highest rate of fertilisation was 96.2±1.92% at 26°C during monsoon breeding followed by 77.75±2.75% at 28°C during pre-monsoon breeding (Fig. 5b). Overall a strong negative correlation between fertilisation rate and water temperature were observed in both monsoon ($R = -0.46$; $R^2 = 0.2116$; $p < 0.01$) and pre-monsoon ($R = -0.6242$; $R^2 = 0.3896$; $p < 0.01$) seasons.

At a particular temperature, the hatching rates during pre-monsoon breeding was found consistently higher than the hatching rates during monsoon months. The differences, however, were not statistically significant ($p > 0.05$). During pre-monsoon breeding, the mean hatching rate against temperatures in the 26-30°C range was 91-96%, whereas during monsoon, it was 82-92%. Furthermore, the mean hatching rates were above 80% (82.90-86.33%) regardless of the season, even at higher temperatures of 31-32°C. The highest rate of hatching observed during pre-monsoon and monsoon breeding were 95.80±1.64 and 92.40±4.21%, respectively and both were at 27°C. Correlation analysis showed a significant negative relationships between water temperatures and hatching rates during the pre-monsoon season ($R = -0.7966$; $R^2 = 0.6346$; $p < 0.01$) and a significant positive relationships between the two during the monsoon season ($R = 0.4959$; $R^2 = 0.2459$; $p < 0.01$).

Discussion

Environmental parameters such as temperature, pH and dissolved oxygen can influence oocyte maturation, ovulation and spawning (Dou *et al.*, 2008; Landsman *et al.*, 2011). There is a strong correlation between spawning latency periods and water temperature, which generally decreases as temperatures rise (Pankhurst and Munday, 2011). A mean latency period of 339±13 min was recorded for rohu in the temperature range of 24-31°C during the present study, which is in line with the findings of Chakrabarti *et al.* (2017). However, the spawning was observed to be significantly delayed (*i.e.*, the latency period increased) at temperatures above 31°C. Moreover, 22.2% of the female fish did not spawn at all at the higher temperature of 32°C. Similar detrimental effects of temperature have been documented for a variety of fish species, and are thought to occur when fishes are exposed to temperatures outside their thermal tolerance range (Das *et al.*, 2006; Ashaf-Ud-Doula *et al.*, 2021). The decrease in spawning success caused by the stress resulting from exposure to high temperatures, which leads to the release of catecholamines and cortisol, resulting in the suppression of fish reproductive hormones (Shahjahan *et al.*, 2018; Cockrem *et al.*, 2019). Delayed spawning at lower temperatures ($\leq 24^\circ\text{C}$) observed in the present study is in agreement with reports of similar interference for other carp species, such as *Labeo catla* (Ghosh *et al.*, 2019). According to Dou *et al.* (2008), fish typically ovulate and spawn within a limited range of water temperature. Here the results indicated that a water temperature range of 24-31°C is more suitable for optimal ovulation and spawning of rohu.

Significantly lower fertilisation rates recorded at temperatures of 31°C and above during the present study may be linked to sperm motility (Dzyuba *et al.*, 2019). Cosson and Alavi (2004) confirmed that the spermatozoa of *Cyprinus carpio* are motile for a longer duration at a lower temperature of 20°C than at 26 or 30°C. In the present study, optimal hatching rates of rohu were recorded in a temperature range of 24-31°C. Das *et al.* (2006) reported a higher hatching rate of rohu at a temperature range of 26-31°C. Whereas, Ashaf-Ud-Doula *et al.* (2021) in a controlled thermal tolerance study, recorded the highest hatching success and optimal embryonic development of rohu at temperatures between 30 and 32°C. However, in the present study significantly lower hatching rates were observed (51-84%) at 32°C, but embryonic development and the percentage of larval survival were found comparable with those at temperatures of 24-31°C. Das *et al.* (2006) also recorded significantly lower hatching percentages of rohu eggs at temperatures of 33°C (58%) and 36°C (45%).

Breeding performances of rohu, against corresponding temperatures, were good in all the states except Tamil Nadu. Although inter-state variations were observed in water quality parameters, the overall levels were found to be within the permissible range (Hussan *et al.*, 2017; Ghosh *et al.*, 2019; Hussan *et al.*, 2020) in all states. The lower fertilisation and hatching rates noted in Thanjavur, Tamil Nadu may be due to parental effects, local environment conditions, or both. Parental effects can originate from either parent and are generally related to sperm motility, sperm velocity, sperm enzyme levels and egg characteristics (Cosson and Alavi, 2004; Migaud *et al.*, 2013). While the abiotic and biotic conditions of the water body, broodstock nutrition and spawning induction protocols can influence the egg characteristics (Migaud *et al.*, 2013), which vary significantly depending on the age and size of female fish (Kamler, 2005). Moreover, higher temperatures coupled with lower rainfall during the monsoon months in the state (India Meteorological Department, IMD 2019) could also have some antagonistic effects on sexual maturity of the species (Dou *et al.*, 2008; Pankhurst and Munday, 2011). However, a lower fertilisation rate of only 58% (at 34°C) and hatching of only 41% of fertilised eggs (at 33°C), might be an indication of reduced gamete quality and/or stressful external conditions due to increased temperature (Wedemeyer *et al.*, 1999; Cosson and Alavi, 2004; Ashaf-Ud-Doula *et al.*, 2021).

In the present study, a prolonged latency period and lower fertilisation rate were observed during early season (pre-monsoon) breeding of rohu, despite higher average water temperatures. This may be linked with the physiological maturity of the brooders. A delayed surge of plasma concentration of carp gonadotropin (cGtH) and 17 α 20 β -dihydroxy-4-pregnen-3-one to induce final oocyte maturation and ovulation, might be responsible for longer latency period (Dasgupta *et al.*, 2009) despite the higher water temperature (28.92±1.64°C) that prevailed during the pre-monsoon period. Generally maturational competence in ovarian follicles increases

with an increase of concentration of cGtH during monsoon months (Dasgupta *et al.*, 2009), as maturity and spawning season of the species (*L. rohita*) coincides mainly with the south-west monsoon, which typically lasts from June to September (Talwar and Jhingran, 1991). As a result, a lower response rate was recorded for the male and female fish during the pre-monsoon period ($\leq 75\%$) than monsoon season (response increased to $\geq 90\%$). Similar result was recorded by Ghosh *et al.* (2019) in another Indian major carp, *L. catla* ($\sim$ *Catla catla*), where they found about 50% response for induced spawning of male and female fish in pre-monsoon. However, the higher hatching rate of the fertilised eggs during pre-monsoon period observed in the present study is likely to be associated with the relatively stable climatic condition (March-April). During the pre-monsoon season (March to May), weather in the Gangetic plains of West Bengal where the study area falls, is typically dry, with clear to moderately cloudy skies. The region experiences low average monthly rainfall (37.71 ± 25.49 mm) and a limited number of rainy days (days with ≥ 2.5 mm rainfall) averaging 2.17 ± 1.24 days per month. This period is also the hottest with a mean maximum air temperatures of 35.6°C (IMD, 2008).

The results of the present study clearly indicate the influence of temperature on rohu breeding performance, affecting the latency period, fertilisation rate and hatching rate. Optimal breeding performance of rohu was observed within the temperatures range of $24\text{--}30^\circ\text{C}$.

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