MARINE REPORTS
e-ISSN: 2822-5155
Journal homepage: https://scopesscience.com/index.php/marep/

Received: 12 December 2025; Received in revised form: 04 January 2026
Accepted: 04 January 2026; Available online: 04 January 2026
RESEARCH PAPER

Citation: Dola, S. I., Zilany, Md. A. K., Akram, W., Islam, Md. R., & Rahi, Md. L. (2026). Combined effect of temperature and salinity on the fertilization, hatching, larval development and expression of selected genes of major carp (*Labeo rohita*). *Marine Reports*, https://doi.org/10.5281/zenodo.18147292

COMBINED EFFECT OF TEMPERATURE AND SALINITY ON THE FERTILIZATION, HATCHING, LARVAL DEVELOPMENT AND EXPRESSION OF SELECTED GENES OF MAJOR CARP (*Labeo rohita*)

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Abstract

Temperature and salinity play a vital role in organismal physiological status including growth, immunity and survival. Any deviation from the optimum temperature and salinity level can adversely affect the aquaculture productivity because of imposed stress on organisms. The present study was conducted to assess the role of temperature and salinity on the fertilization, hatching and larval development of *Labeo rohita* under the combined effect of three different experimental temperatures (30°C, 33°C and 35°C) and two salinity levels (0‰, 2‰) for a period of 10 days. The experimental species reared at 2‰ and 35°C, showed significantly higher ($p<0.05$) relative expression pattern of two osmotic stress genes (NKA and HSP70) whereas the result also revealed lower expression levels of three growth and cell proliferation genes (MBL, SLB and IGF-1). The highest levels of physiological traits and gene expression changes were obtained at 2‰ and 35°C (T5) while the lowest levels of physiological-genetic changes were observed at 0‰ and 30°C (control treatment). Findings of this study demonstrate the adverse effect of temperatures and salinity stress on the fertilization, hatching, yolk sac absorption and larval development and gene expression pattern.

Keywords: Climate change, larval development, Major carp

Introduction

Bangladesh is known as the land of rivers and is naturally enriched with a large number of freshwater fish diversity with 265 well recognized species (Islam et al., 2011; Rahi & Shah, 2012; Zeehad et al., 2024). The inland aquaculture sector alone contributes 55% of the total fish production in Bangladesh (DoF, 2016; Siddika et al., 2025). Carps (both exotic and indigenous) dominate the inland freshwater aquaculture production of Bangladesh; constituting 80% of the total recorded aquaculture production (Shah et al., 2011; Shamsuzzaman, et. al., 2017; Lema et al., 2024). Three indigenous Indian major carps, *i.e.* rohu, catla and mrigal, are important candidates for the freshwater aquaculture in Bangladesh (Hasan & Ahmed, 2002; Mitu et al., 2024). Of these major carps, rohu (*Labeo rohita*) is the most demandable species because of its excellent taste and comparatively lower market price. Because of its high demand and well-established seed production technology, *L. rohita* is farmed throughout Bangladesh (Ali et al., 2008; Nath et al., 2008; Sabbir et al., 2017). Several factors can limit the growth and production of any aquaculture species including salinity, temperature, pollution, hypoxia, and feed quality (Sabbir et al., 2010; Ali et al., 2016; Chakrapati et. al., 2017). Any deviation of these factors from the optimum range can have negative impact on physiology as well as embryonic development, growth and overall yield or production (Portz et. al., 2006; Afroz et al., 2021; Muhammad et al., 2023). Temperature plays an important role in the molecular, biochemical, and physiological properties of aquatic animals (Strüssmann et al., 1996; Rahi et al., 2023). Fishes, the most abundant ectotherms, cannot regulate their body temperature by endogenous heat production, and are therefore susceptible to environmental temperature change; temperature is consequently the key determinant of biological systemic functioning in fishes (Islam et al., 2014; Scott & Johnston, 2012; Aziz et al., 2017; Rahi et al., 2021a). The survival, distribution, metabolism, and reproduction of fish are all contingent on water temperature (Moshtaghi et al., 2016; Aziz et al., 2018; Rahman et al., 2018) and the rates of biochemical reactions can approximately double in response to a temperature increase of 10 °C (Moshtaghi et al., 2018; Rahi et al., 2021b; Mridul et al., 2024). Rohu (*L. rohita*) is a primary freshwater species and is intolerant of any environmental salinity levels (Choudhury et al., 2023). Even 1‰ rise in aquatic salinity level can cause significant amount of mortality of this species (Moshtaghi et al., 2017; Rahi et al., 2021c). Due to climate change and sea level rise, a huge proportion of the inland freshwater bodies are at severe risk of salinity intrusion (Rahi et al., 2020). Large scale mortality of carps due to salinity intrusion (1-2‰ salinity levels) has become a common phenomenon in some upper coastal districts of Bangladesh (Zeehad et al., 2024). Sudden salinity shock can impose severe stress and consequently cause mortality. With gradual acclimation, species can tolerate a particular amount of salinity shock. Global warming has been accelerating the effects of temperature and salinity on ectotherms, clearly with impacts on the aquaculture industry, such as decreases in abundance and production (Cheng et al., 2003; Rahi et al., 2017; Islam et al., 2025). Furthermore, fishes from early life stages (embryo, hatchlings and larvae) are more sensitive to environmental stressors (particularly temperature and salinity changes) compared to juveniles and adults since these changes affect the timing of embryonic development, formation, and function of key tissues and structures (Rahi et al., 2018; Akram et al., 2023; Mou et al., 2024; Chowdhury et al., 2025). Minor temperature fluctuations can result in significant phenotypic variation during development with subsequent ontogenetic consequences (Rahi et al., 2019; Chowdhury et al., 2023; Rupa et al., 2025). For these reasons, it is necessary to clarify the impact of water temperature on the early life stages in fishes, particularly during embryonic development. Upper coastal areas of Bangladesh are at severe risk of saline water intrusion due to sea level rise and also occasionally by the coastal storm surges (Islam et. al., 2015; Rogl et al., 2018; Rahi et al., 2022). As a result, farmers face a greater challenge of production loss. The upper freshwater bodies near the coastline are being used for freshwater fish production, but this trend is reducing gradually due to salinity intrusion

(IAB, 2000; Rahi et al., 2013; Islam et al., 2014; Rahi et al., 2023). Many freshwater species were found to tolerate a certain level of salinity with gradual acclimation (Nath et al., 2008; Sabbir et al., 2010; Rahi, 2017). Although *L. rohita* is a prime aquaculture species in Bangladesh (and across the entire Indian Sub-continent), no studies have been directed to test the combined stress by salinity and temperature change on early stages of this species.

Therefore, it will be insightful to investigate the salinity and temperature stress on the early developmental stages (fertilized embryo, larvae and hatchlings) of Rohu. This will act as a foundation to predict the potential role of climate change for the vulnerable early developmental stages of major carp rohu (*Labeo rohita*).

Materials and Methods

Experimental fish

Healthy broods of Rohu were collected from a commercial carp hatchery (Sardare Hatchery, Jashore, Bangladesh). The mature and brood fishes (3-4 kg) were used for artificial breeding in the hatchery complex for subsequent experimental condition specific investigations including fertilization rates, hatching rates, survival performance of the hatchlings and expression profiling of some selected candidate genes.

Induced breeding and fertilization of eggs

Two male and two female fish were selected for breeding purposes. Matured males were identified by a slightly pointed genital papilla, and females by a swollen abdomen and a reddish swollen vent (Shah et al., 2011; Afroz et al., 2021). The maturity of the female was confirmed by slightly pressing the ventral side of the fish for oozing of eggs (Rahi & Shah, 2012). Male and female were placed in separate tanks for about 6 h with a gentle shower of water to induce spawning. Both male and female fishes were artificially induced by intra-muscular injection with carp pituitary gland (PG) hormone with a dose of 2 mg/kg and 6 mg/kg body weight, respectively (Shah et al., 2011; Sabbir et al., 2017). After injection, the male and female broodfish were placed in spawning tanks. After 8h of PG hormone administration, when ovulation appeared to have been completed, the broodstock fish was removed from the spawning tank and placed in a blanket for stripping. Male and female fishes were stripped by gentle pressure on the abdomen to collect the milt and eggs in a plastic bowl. Milt and eggs were mixed thoroughly by using clean and soft poultry feather to accomplish fertilization in well-aerated freshwater at ambient temperature (30 °C).

Experimental Design

Fertilized eggs were exposed to five different experimental conditions and each of the experimental units was maintained under three replications (Table 1).

Table 1. Experimental Design

Treatment name	Temperature (°C)	Salinity (‰)
Control	30	0
T_1	33	0
T_2	35	0
T_3	30	2
T_4	33	2
T_5	35	2

Methods for counting Fertilization, Hatching, and Larval development

Immediately after mixing the sperm with eggs, they were kept in the 50L fertilization tanks connected to water circulating system for proper fertilization. After 30 min, they were examined to determine the fertilization rate using magnifying glass. The fertilized eggs were differentiated and separated from the unfertilized eggs by the presence of transparent shell with grey spot within the egg shell compared with opaque appearance of the unfertilized eggs. Fertilization rate was determined as follows:

$$\text{Fertilization rate (\%)} = \frac{Number\ of\ fertilized\ eggs}{Number\ of\ total\ eggs} * 100$$

Generally, the eggs were hatched after 18-24 hours of fertilization. The hatching rate was determined by counting the number of hatchlings as follows:

$$\text{Hatching rate (\%)} = \frac{Number\ of\ hatchlings}{Number\ of\ total\ fertilized\ eggs} * 100$$

Gene Expression Study

As the experimental individuals were too small in size, whole body of the individuals were required to assess the relative expression pattern of five selected genes. Sampling was done in triplicate across seven different time series (Day 1, Day 2, Day 3, Day 4, Day 5, Day 7 and Day 10) to observe the expression level of the genes. From the experimental individuals, total RNA was extracted using the TRIzol/chloroform extraction method and a commercial RNA extraction kit (Serva, Germany). For each sample, total RNA integrity (quality and quantity) was evaluated using a 2% agarose gel electrophoresis and NanoDrop 2000 Spectrophotometer (Nabi, South Korea). The high-quality RNA samples were preserved at -80°C for further analysis. By applying the SensiFAST cDNA synthesis kit (Bioline, UK) according to the manufacturer's protocol, the complementary DNA (cDNA) was extracted from the total RNA samples (using 1 µg RNA for each sample). Then the samples of cDNA were preserved at -20ºC until the experimental gene expression study.

There are multiple genes which are particularly known for their different functional impacts including ionic regulation or osmoregulation (NKA: Na^+/K^+-ATPase), stress response (HSP70: heat shock protein 70), brain development (MBL: masterblind), morphogenesis (SBL: Silberblick) and growth and cell proliferation (IGF-I: insulin like growth factor I). Elongation factor 1 alpha (EF 1α) was used as the reference gene for the gene expression due to the suitability of this gene as an important reference gene in other studies (Sahu et al., 2015). The five candidate genes were chosen because of their indicative functional roles in some earlier studies (Sahu et al., 2015; Islam et al., 2020).

Gene specific primer sequences of the candidate genes were collected from some previous studies (Sahu et al., 2015). The gene study was conducted by running reactions in RT-PCR including: 3 µL of ultra-pure water, 20 µL mixtures including 10 µL of 2x SensiFAST SYBR No-ROX Mix (Bioline, UK), 5 µL of template cDNA, and 1 µL each of forward and reverse primer. Using three technical replicates for each of the experimental sample, the final observing reactions were then executed in a RT-qPCR (real time PCR system) (Bio-Rad, California, USA). To finalize the amplification of a single qPCR product, a standard melt-curve analysis was accomplished at the closure of the reaction (Rotllant et al., 2017; Chowdhury et al., 2025;

Siddika et al., 2025). Relative Gene Expression was estimated according to the following equation:

$$\text{Relative Gene Expression (R)} = 2^{-[\Delta Ct \text{ Target Gene} - \Delta Ct \text{ Reference Gene}]}$$

Then the relative gene expression levels of the candidate genes were analyzed through the ΔΔCt method (Nitzan et al., 2019).

Treatment specific investigations

The experiment was conducted for a period of 10 days. Combined effects of temperature and salinity was investigated on:

i) Fertilization rates,
ii) Hatching rates,
iii) Survival performance and
iv) Developmental durations and relative gene expressions.

Statistical analysis

Embryonic hatching success and mortality of larvae will be determined by calculation of the means of three replicates ± standard deviation (SD). Data will be analyzed by one-way analysis of variance (ANOVA) followed by Tukey's post hoc test for statistical significance of differences among treatment groups. Statistical analyses will be performed using SPSS Version 20.0 for Windows (SPSS Inc., Chicago, IL).

Results

In the current study, the combined effect of temperature and salinity on the fertilization, hatching and larval development of *Labeo rohita*. were assessed under two different experimental salinity levels (0‰ and 2‰) and three temperature variations (30°C, 33°C and 35°C) for a period of 10 days. Each of the experimental units was maintained under five replications along with one controlled unit.

Combined effect of temperature and salinity on the success rate (%) for different traits (fertilization, hatching, survival to yolk sac absorption and survival rate)

There was a strong impact of salinity, temperature and sampling time on the rates of fertilization, hatching, survival to yolk sac absorption and survival of *Labeo rohita*. Significant differences were observed between the two salinity levels and three temperature variations for the rates of fertilization, hatching, survival to yolk sac absorption and survival throughout the entire experimental time frame (Figure 1).

Experimental *Labeo rohita* individuals showed significant changes with the changes of salinity and temperature on the fertilization rate in the five experimental units throughout the experiment. The fertilization rate started to decline gradually at a stable trend in the replications up to the end. The highest level of fertilization rate was observed in the treatment one (T_1= 0‰ +33°C).

The lowest level of fertilization rates was observed in treatment five (T_5= 2‰ +35°C) from beginning to the end. Similar trends were observed in other traits such as hatching rate, survival to yolk sac and survival rate. Each of the traits showed higher success rates in treatment one and lowest at the treatment five.

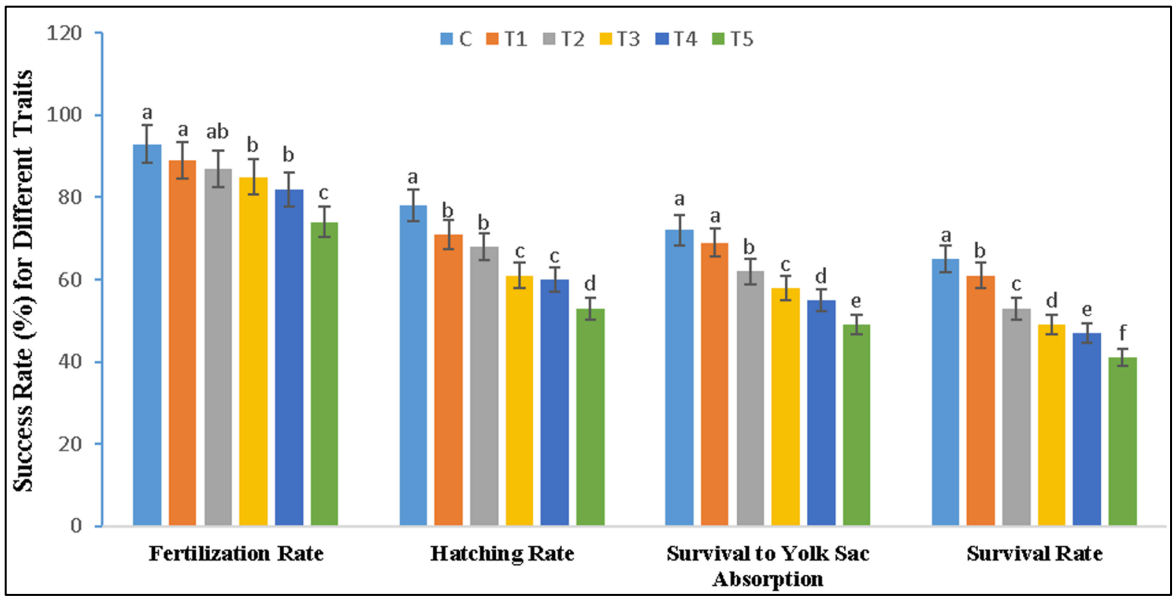

Figure 1. Effect of temperature and salinity on the success rate (%) for different traits. Error bars indicates ± SD.

Temperature and salinity induced changes on the relative expression levels of NKA gene

A significant interaction between salinity, temperature, sampling time and expression of NKA was obtained from the result of two-way analysis of variance (ANOVA). Significantly higher expression levels of NKA was observed for treatment five (T_5) and treatment four (T_4) over the controlled treatment throughout the experimental period (Figure 2). Significant differences among the five treatments were observed from day 2 sharply but the results of relative expression of NKA were seen in T_3, T_4, and T_5 basically. The generalized expression pattern of NKA gene at T_5 showed rapid increase in expression pattern from day 2 to day 3 and then the expression levels were then gradually started to decrease up to day 7, following which a stable expression trend was observed throughout the remainder.

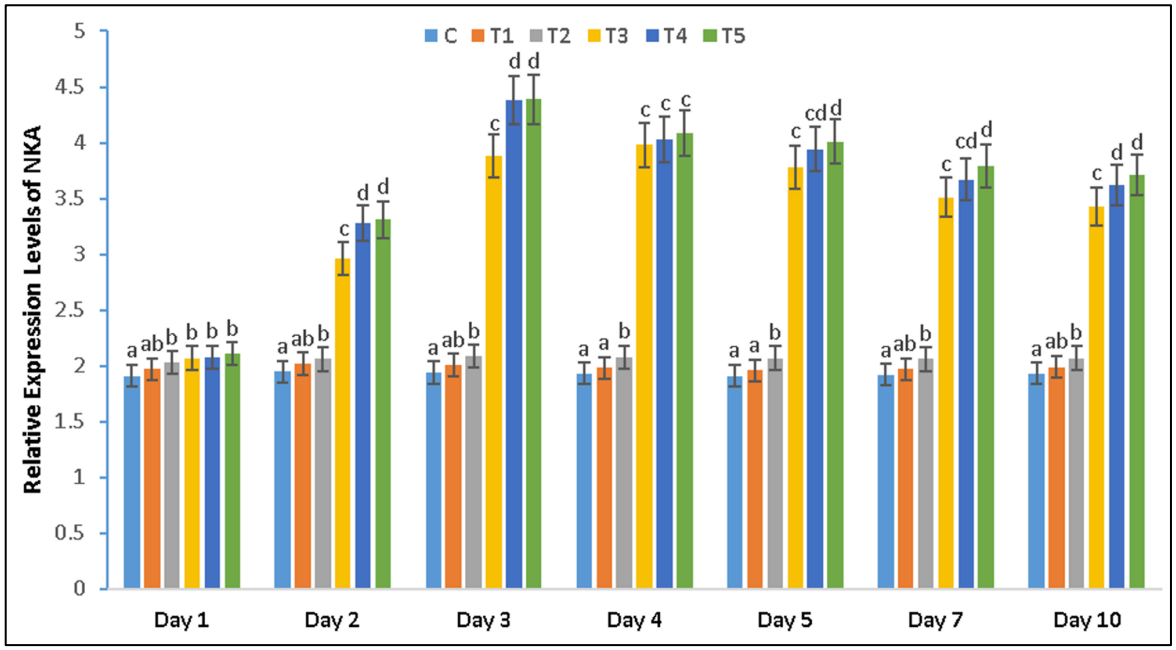

Figure 2: Relative expression level of NKA gene at different temperature and salinity levels. Error bars indicates ± SD.

Relative expression levels of HSP70 gene with the changes of Temperature and salinity levels

A significant interaction between salinity, temperature, sampling time and expression of HSP70 was obtained from the result of two-way analysis of variance (ANOVA). Significantly higher expression levels of HSP70 were observed for five experimental treatments over the controlled treatment throughout the experimental period (Figure 3). Significant differences among the five treatments were observed from day 2 which shows a higher expression of HSP70 sharply. The generalized expression pattern of HSP70 gene at T_5 showed rapid increase in expression pattern from day 2 and then the expression levels were then gradually started to decline from day 3 to day 7, following which a stable expression trend was observed throughout the remainder.

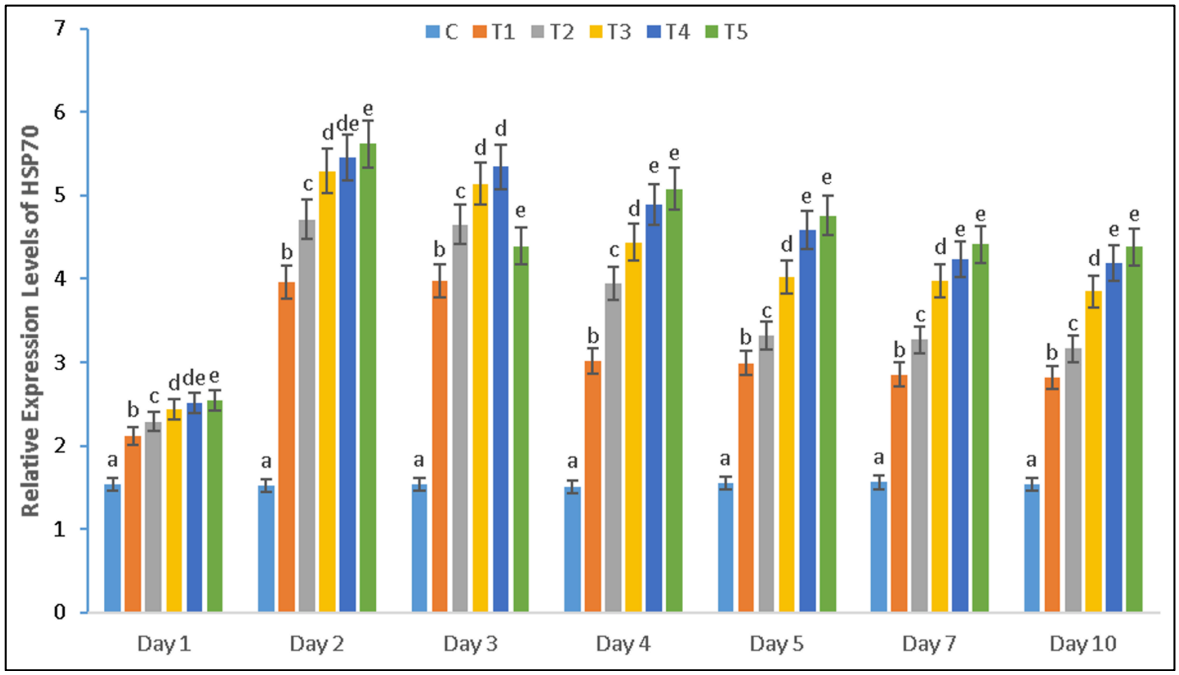

Figure 3. Relative expression level of HSP70 gene at different temperature and salinity levels. Error bars indicates ± SD.

Temperature and salinity induced changes on the relative expression levels of Masterblind (MBL), Silberblick (SBL) and IGF-I gene

The two-way ANOVA revealed a strong impact of temperature, salinity and sampling time on the masterblind (MBL), silberblick (SBL) and IGF-I gene expression of *Labeo rohita*. Significantly lower expression levels of masterblind (MBL), silberblick (SBL) and IGF-I were observed for five experimental treatments over the controlled treatment throughout the experimental period (Figure 4, 5 and 6). Significant differences among the five treatments were observed from day 2 which shows a lower expression of masterblind (MBL), silberblick (SBL) and IGF-I consequently. The highest level of relative expression of masterblind (MBL), silberblick (SBL) and IGF-I was found at treatment one(T_1) and the lowest one was at treatment five(T_5). The expression pattern of masterblind (MBL), silberblick (SBL) and IGF-I gene in T_1, T_2, T_4 and T_5 showed a gradual decrease in expression pattern from day 2 to day 5 and from day 7 a stable expression trend was observed throughout the remainder.

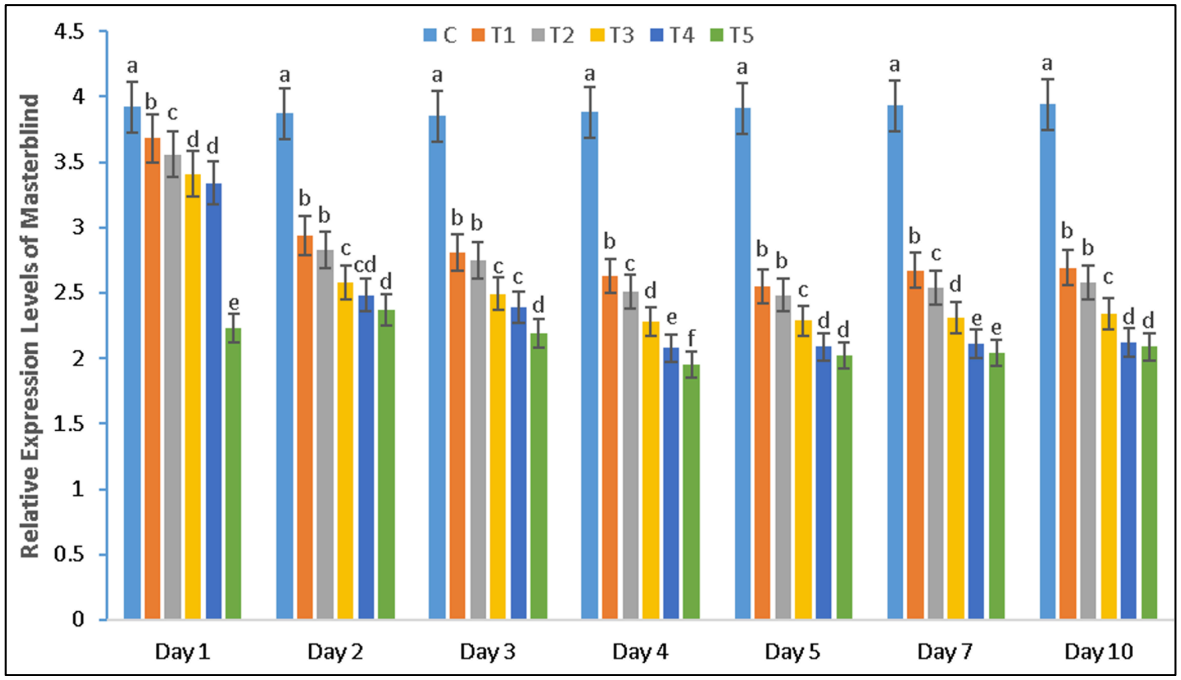

Figure 4: Relative expression level of masterblind at different temperature and salinity levels. Error bars indicates ± SD.

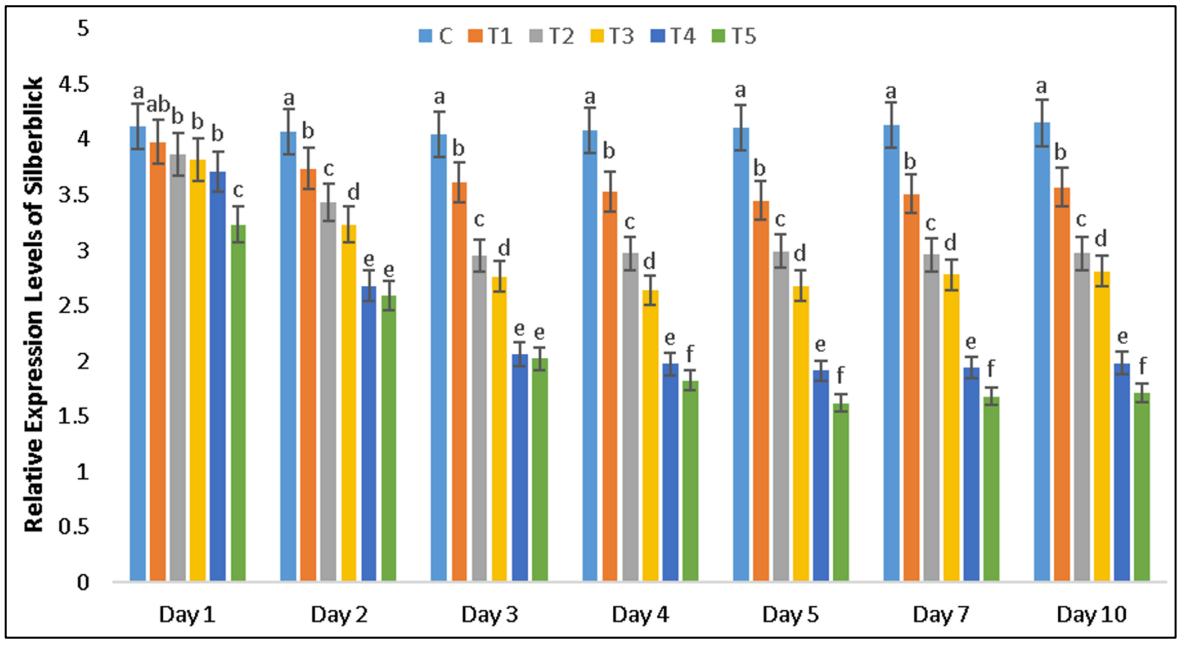

Figure 5: Relative expression level of Silberblick gene at different temperature and salinity levels. Error bars indicates ± SD.

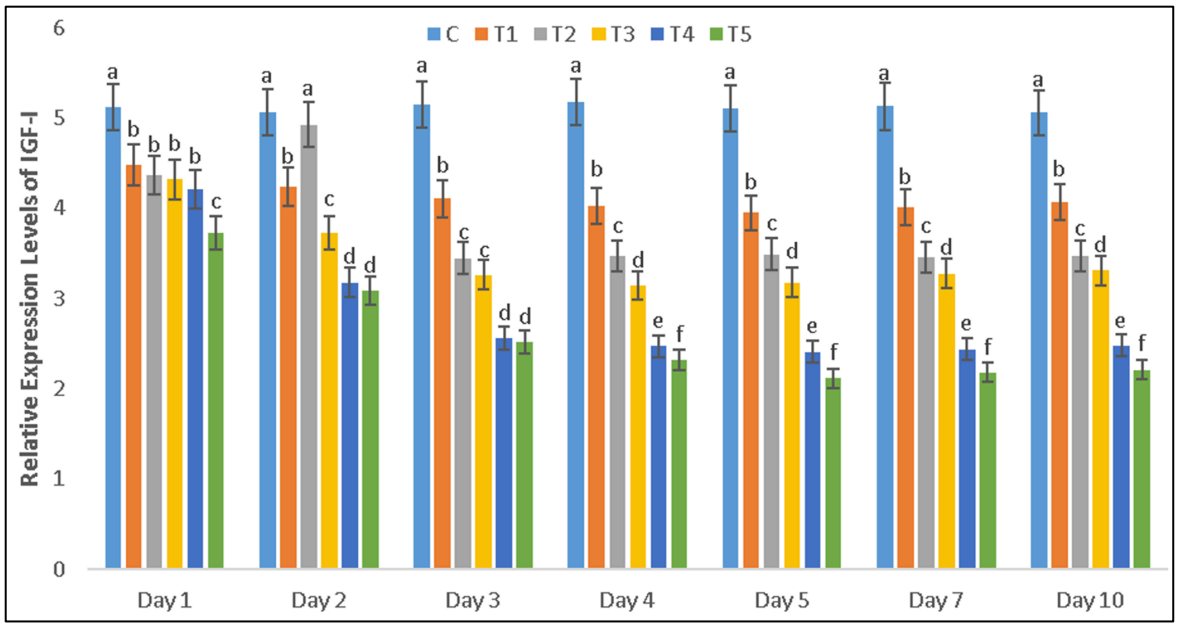

Figure 6. Relative expression level of IGF-1 at different temperature and salinity levels. Error bars indicates ± SD.

Discussions

Combined effect of temperature and salinity on the success rate (%) for different traits (fertilization, hatching, survival to yolk sac absorption and survival rate)

Successful embryonic and larval development are considered as the key factor for the propagation of hatchery as well as intensive aquaculture systems for *Labeo rohita*. There are some several factors that controls the embryonic and larval development and such development can be deterred by some factors like genetic, nutritional, environmental, and toxic factors (Backiel et al., 1984). Among environmental factors, temperature and salinity induced embryonic and larval deformities are well established (Steinarsson & Bjornsson 1999; Wang & Tsai 2000).

In the present study, maximum fertilization rate, hatching rate, yolk sac absorption and survival rate were recorded at 0‰ and 30°C in the controlled treatment. But the alteration of temperature and salinity level shows a significant impact of temperature and salinity over the controlled treatment (Figure 1). The fertilization rate, hatching rate, yolk sac absorption and survival rate constantly decreased with the increase of temperature and salinity level. Das et al. (2006) reported the optimal temperature for *L. rohita* is 31°C while Kaur and Ram (2017) found optimal embryonic development at temperatures 30–32°C which is similar to the findings of the present study. Impacts of incubation temperature and salinity on the hatching success in fish also demonstrated in several other fisheries organisms (Pérez-Sánchez et al., 2002; Rechulicz et al., 2002; Das et al., 2006; Korwin-Kossakowski, 2008; Kupren et al., 2008; Thépot & Jerry 2015).

Any change in environmental salinity levels can impose ionic (osmoregulatory) stress on aquatic organisms (Pequeux, 1995). Osmotic stress can potentially hamper Biological traits at different magnitudes (Cheng et al., 2003). At optimum temperature, organisms show better growth performance, higher survivability, higher fertilization rate and equal proportions of sex ratios (Serezli et al., 2017; Acquafredda et al., 2019). In tropical environments, aquatic species inhabit at temperature ranges very close to their maximum thermal tolerance limit (Val et al.,

2006). If any temperature treatment crosses the limit, it can impose severe stress that results in slower growth with massive mortality (Mridul et al., 2024). On the other hand, it also is widely believed that salinity fluctuation can put organisms under severe threat of extinction coupled with massive mortality because ionic balance with the surrounding medium requires much more energy to compensate with the water conditions (Anger, 1991; Moshtaghi et al., 2018; Rahi et al., 2019). Ability to rapidly respond to stress mitigation determines organismal success to acclimate with the changed environmental conditions. At Treatment five (T_5= 2‰ +35°C), we observed the lowest fertilization rate, hatching rate, yolk sac absorption and survival rate. Therefore, 35°C can be considered as the severe stressful condition while 32°C could be the maximum upper thermal tolerance limit for *Labeo rohita*. It is also likely that the maximum thermal tolerance limit for *P. monodon* could be 33°C; further study is required for precise estimation of this temperature and salinity limit.

Temperature and salinity induced changes on the relative gene expression pattern (NKA, HSP70, MBL, SLB, IGF-1)

Significant differences between different temperature (30°C, 33°C and 35°C) and salinity levels (0‰ and 2‰) for the expression of NKA (Na+-K+-ATPase), HSP70 (Heat shock protein), MBL (Masterblind), SLB (Silberblick) and IGF-1 (Growth and cell proliferation) genes clearly indicate imposed stress on the experimental *Labeo rohita* (Figures 2-6). In aquatic organisms, NKA is considered as a vital gene for osmoregulation which is primarily involved with establishing a strong electromechanical gradient to facilitate active transport and exchange (absorption or release depending on the salinity levels of surrounding environment) of Na+ and other critical cations (Boudour-Boucheker et al., 2014; Freire et al., 2008; Henry et al., 2012). Therefore, NKA shows higher expression levels with salinity changes (high salinity to low salinity or vice versa) for efficient ionic balance and surrounding aquatic environments (Furriel et al., 2010; Moshtaghi et al., 2018; Rahi et al., 2019). Constant expression pattern of NKA between sampling times at 0‰ (control salinity) revealed no imposed stress on experimental individuals (Figure 2). At 2‰, NKA showed significantly higher expression levels at day 3 over the other sampling times. Though temperature does not instigate the expression pattern of NKA but salinity plays a major role. In this present study we found no change in the expression level of T_1 and T_2 but observed a higher expression level of NKA in T_3, T_4 and T_5 that revealed the salinity induced changes of the expression level of NKA. Then the relative expression of NKA gradually started to decrease up to day 7, following which a stable expression trend was observed throughout the remainder which indicates the adaptability with the changes of the temperature and salinity level.

HSP70 (Heat shock protein) showed quick response with temperature change (at all temperature ranging from low to high). HSP70 is well known as a thermal stress response gene that shows immediate response with higher levels (Figure 3). Changes in expression pattern of HSP70 were noticed initially in all of experimental treatments, followed by a declining trend and finally a stable trend at day 7 when organisms acclimate well with the experimental conditions (McLennan & Miller, 1990; Rahi et al., 2017; Junprung et al., 2019). We found a similar trend in this study confirming an important role of HSP70 in *Labeo rohita* to deal with any thermal stress tolerance. To counterbalance the adverse effects, any deviation from optimum condition (e.g. control temperature) can impose stress on individuals at different scales depending on the intensity of temperature change causing increased expression of stress response genes (HSP70) (De et al., 2011; Mridul et al., 2024; Rahi et al., 2018).

Masterblind (MBL) and Silberblick (SLB) are well known for the brain development and morphogenesis of aquatic organisms. Silberblick (SLB) acts directly on the biological process

that causes a tissue or organ to develop its shape by controlling the spatial distribution of cells during embryonic development (Gabillard et al., 2003). The present study results of masterblind and silberblick gene revealed a significant impact of temperature and salinity levels alteration over the controlled treatment group. The highest level of relative expression of masterblind (MBL) and silberblick (SBL were found at treatment one(T_1) and the lowest one was at treatment five(T_5) (Figure 4 and 5). The expression pattern of masterblind (MBL) and silberblick (SBL) gene in T_1, T_2, T_4 and T_5 showed a gradual decrease in expression pattern from day 2 to day 5 and from day 7 a stable expression trend was observed throughout the remainder. The decline of the relative expression of masterblind and silberblick gene can be concluded as temperature and salinity induced changes hamper the brain development of the experimental species which can cause larval deformities.

A number of past studies have also been revealed comparable results in terms of plasma IGF-I gene expression and growth performance in contrast to an increased temperature in Atlantic salmon, rainbow trout, gilthead seabream, coho salmon and Nile Tilapia (Gabillard et al., 2003). A positive relationship was also observed in plasma IGF-I, growth, and water temperature in rainbow trout (Siddika et al., 2025). The expression levels of IGF-1 were significantly different in the treatment groups in contrast to the controlled treatment at different temperature and salinity levels in *L. rohita*, which indicates that normal growth mechanisms can be hampered at these different ranges of temperature and salinity levels (Figure 6). However, a significant decrease in the expression pattern of IGF-1 with subsequently declined growth performance was observed in fish at 35◦C indicates the inhibitory effect of higher temperature on the IGF system in *L. rohita.* Considering the previous findings, the present results indicate that higher acclimation temperature and salinity negatively affects the growth through the suppression of IGF system in *L. rohita.*

Conclusion

Temperature and salinity alteration can impose severe osmotic stress on aquatic organisms that can bring major changes in fertilization rate, hatching rate, yolk sac absorption and survival rate that are directly controlled by genetic means (i.e. changes in the expression of candidate genes regulating organismal physiology). The current study on combined effect of temperature and salinity on the fertilization, hatching and larval development of *Labeo rohita* revealed remarkable differences on some physiological traits. The Phenotypic and gene expression study showed significantly higher values at 0‰ and 30°C compared to 2‰ and 35°C. Findings demonstrate the role of temperature and salinity on the fertilization, hatching and larval development of *Labeo rohita*. Further study is required using more genes and other biological aspects including biochemical changes and physiological changes to clearly understand the mechanisms involved with acclimation to high temperature and salinity environments by *Labeo rohita*.

Ethical approval

The animal study protocol was approved by the Institutional Review Board of ANIMAL ETHICS COMMITTEE of KHULNA UNIVERSITY (protocol code: KUAEC-2021/09/21 and date of approval: 20 September, 2021).

Informed consent

Not available

Data availability statement

The authors declare that data can be provided by corresponding author upon reasonable request.

Conflicts of interest
There is no conflict of interests for publishing this study.

Funding organizations
No funding available for this study.

Contribution of authors
Sadia Islam DOLA: Conceptualization, Data curation, Formal analysis, Writing original draft
Md. Abdul Kadir ZILANY: Resources, Supervision, Validation, Review, Editing.
Wasim AKRAM: Investigation, Methodology, Writing original draft, Software
Md. Rashedul ISLAM: Resources, Supervision, Validation, Visualization, Review, Editing.
Md. Lifat Rahi: Resources, Supervision, Validation, Visualization, Review, Editing.
"All authors have read and agreed to the published version of the manuscript."

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