Journal of Fisheries
Volume 14 Issue 2 August 2026
Peer Reviewed | Open Access

eISSN 2311-3111
pISSN 2311-729X

DOI: https://doi.org/10.17017/j.fish.1071

Original Article

Effects of temperature on somatic growth, gastric pepsin activity, and endocrine dynamics in orangefin labeo (*Labeo calbasu*)

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Manuscript history
Received 28 August 2025 | Accepted 4 April 2026 | Published online 19 April 2026

Citation
Mimi MS, Ghaffar MA, Hossain MA, Das SK, Islam MN, Rahman ML, Kader MA, Mazumder SK (2026) Effects of temperature on somatic growth, gastric pepsin activity, and endocrine dynamics in orangefin labeo (*Labeo calbasu*). Journal of Fisheries 14(2): 142207. DOI: 10.17017/j.fish.1071

Abstract
This study investigated the effects of varying water temperatures on somatic growth, digestive enzyme activity, and endocrine responses in orangefin labeo (*Labeo calbasu*), a commercially important freshwater fish species. The experiment was performed in triplicate using 100 L glass aquaria at four temperature levels: 22, 26, 30, and 34°C. Over a 60-day experimental period, growth performance, pepsin activity, and hormonal dynamics were analyzed to identify optimal thermal conditions for aquaculture. Results demonstrated that 30°C was the most favorable temperature, yielding the highest specific growth rate (SGR: 0.45±0.05% day^{-1}, n = 10), weight gain (3.07±0.70 g, n = 10), and length increase (1.29±0.57 cm, n = 10). In comparison, 22°C exhibited the lowest growth metrics (one-way ANOVA: $p < 0.05$). A thermal threshold for enzymatic efficiency was shown by significantly lower pepsin activity at both lower (22°C) and higher (34°C) temperatures, whereas digestive pepsin activity peaked at 30°C (8.34±0.71 U_{37} mg protein^{-1}). Endocrine analysis revealed that the hormonal dynamics also temperature dependent. Reproductive hormones-follicle-stimulating hormone (FSH: 1.45±0.10 mIU mL^{-1}), luteinizing hormone (LH: 1.61±0.04 mIU mL^{-1}), testosterone (1.50±0.01 ng dL^{-1}), and estradiol-17β (E2) reached peak at 30°C which were higher than in other treatments ($p < 0.05$). Overall *L. calbasu* exhibited optimal growth, digestive efficiency, and reproductive performance within a narrow temperature range of 26–30°C ($p < 0.05$). These findings underscore the species' sensitivity to temperature variation and highlight the importance of maintaining stable thermal conditions to ensure sustainable productivity and conservation in aquaculture.

Keywords: growth; hormones; *Labeo calbasu*; pepsin; temperature

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1 | INTRODUCTION

Global warming brought on by man-made activities and its well-known influence on climate change is among the biggest environmental issues of our day, both in the short and long term. The threat posed by global climate change to natural systems and their species is grave and is only becoming worse (Rosenzweig *et al*. 2008; Legg 2021). Changes in temperature patterns are severely affecting aquatic habitats by altering fundamental ecological processes, reducing biodiversity, and threatening species survival (Poff *et al*. 2002; Hassan *et al*. 2019). Fish metabolism, enzymatic processes, and endocrine dynamics are adversely affected by temperature changes in the water, which causes fish abundance to decline in extreme cases (Abram *et al*. 2017; Mazumder *et al*. 2018; Guo and Chen 2023; Mazumder *et al*. 2024). Because aquaculture fish cannot change their distribution, the effects on them are little understood, yet they are crucial to maintaining aquaculture's sustained contribution to food security worldwide (Katersky and Carter 2005; Little *et al*. 2013; Qiang *et al*. 2022). Minimizing the environmental impacts of thermal stress for enhancing growth performance and modulation of the physiological systems in tropical freshwater fish like orangefin labeo (*Labeo calbasu*) is a major challenge. Detailed insights into the physiological adaptations and their thermal stress tolerances for sustainable fisheries management are essential.

One of the most important variables in determining the economic feasibility of commercial fish culture is growth, which is known to be influenced by a variety of biotic and abiotic factors (Islam *et al*. 2020). As fish's body is temperature sensitive, their metabolic rates and growth rates also fluctuate with changing temperature causes decrease the aquaculture production (Fu *et al*. 2018). Conversely, it is said that higher temperatures speed up metabolism; yet, fish may experience physiological stress if the temperature rises too much (Maulvault *et al*. 2017). Fish somatic growth is impacted by temperature, with different species and habitats having different ideal ranges (Mazumder *et al*. 2019a; Islam *et al*. 2020). The significance of evolutionary adaptations in thermal physiology is shown by these variations.

Feed consumed by fish undergoes a series of mechanical and chemical processes. After being chewed and fragmented into smaller particles, it is subjected to the action of various enzymes (Caruso *et al*. 2009). Investigations into digestive secretions in fish can clarify aspects of their nutritional physiology and assist in addressing dietary challenges, such as aligning feed composition with the fish's digestive capabilities (German and Bittong 2009; German *et al*. 2010). Although bony fish possess a similar range of digestive enzymes as other vertebrates (Stevens and Hume 2004; Mazumder *et al*. 2024), their specific enzymatic functions remain insufficiently understood. Among these enzymes, pepsin plays a crucial role in protein digestion in fish diets (Mazumder *et al*. 2018). Its activity is regulated by both external and internal factors, including age, diet type, and seasonal or acclimation temperatures (Chen *et al*. 2022; Magouz *et al*. 2022). A major challenge in interpreting enzyme-mediated processes, particularly the cumulative catalytic conversion, lies in their strong dependence on temperature (Tijskens *et al*. 2001), as this sensitivity complicates accurate evaluation of enzymatic efficiency.

In fish, hormones fundamentally regulate physiological development, reproduction, and adaptation to environmental changes. Produced by endocrine glands, these chemical messengers play a vital role in controlling growth, maturation, and stress responses (Islam *et al*. 2025). The fish pituitary gland secretes two types of gonadotropins (GTH), namely follicle-stimulating hormone (FSH) and luteinizing hormone (LH) (Nagahama *et al*. 1994). Reproductive success of fish in captivity is strongly regulated by FSH and LH. FSH initiates early testicular development by stimulating Leydig cells to produce 11-ketotestosterone, which in turn activates Sertoli cells to release activin B, thereby promoting spermatogonial mitosis and the onset of spermatogenesis (Hatef and Unniappan 2019). During the spawning phase, LH supports sperm maturation by inducing the production of maturation-inducing steroids in most fish species (Zohar *et al*. 2010). The temporal secretion patterns observed in several fish species indicate that FSH plays a dominant role in regulating vitellogenic follicle growth, partly by stimulating the synthesis of 17β-estradiol (E2) in ovarian follicles. In turn, E2 regulates ovarian development through its control of vitellogenin synthesis. LH subsequently triggers a cascade leading to the production of maturation-inducing hormone (MIH), followed by the activation of maturation- or metaphase-promoting factor (MPF) (Sato *et al*. 1990; Nagahama 1994). Water temperature plays a crucial role in the reproductive processes of many fish species by influencing gamete maturation, ovulation, and spawning (Anguis and Canavate 2005). However, the interaction of these physiological reactions in *L. calbasu* at different temperatures remains unknown. Therefore, this study investigates the temperature-mediated changes in growth, pepsin activity, and hormonal dynamics in *L. calbasu*. The results will help guide aquaculture methods and conservation plans for this species in a warming climate.

2 | METHODOLOGY

2.1 Sample collection and experimental site

The experiment was carried out at the laboratory and hatchery complex of the Department of Genetics and Fish Breeding, Gazipur Agricultural University (GAU), Gazipur. The specimens of *L. calbasu* with an average body weight of 10.10±0.36 g and a total length of 10.06±0.23 cm were collected from the Bangladesh Fisheries Research Institute (BFRI) in Mymensingh Bangladesh and transported to the Genetics and Fish Breeding laboratory of GAU, Bangladesh (24°02′10″N 90°23′45″E).

The fish were maintained in a 4000 L cemented stocking tank at 26±0.5°C for 21 days prior to the start of the experiment to allow recovery from handling and transportation stress, as well as to facilitate acclimation to the new environment and diet. During this period, the fish were fed a commercial starter diet (Mega fish feed containing 31.2% crude protein, 6.72% lipid, 13.2% ash, 11.58% crude fiber, and 12.9% moisture) twice daily to satiation. All possible measures were taken to ensure animal welfare and to minimize any potential stress or discomfort.

2.2 Experimental design

Twelve aquaria (80 × 45 × 40 cm), each containing 150 L of freshwater, were set up in the laboratory of the Department of Genetics and Fish Breeding, GAU, Bangladesh. After acclimation, fifteen individuals of *L. calbasu* were stocked in each aquarium, with adequate aeration provided. The fish were exposed to four temperature treatments (22, 26, 30, and 34°C) for 60 days (De *et al*. 2019; Das *et al*. 2021), with 26°C considered the control. Each temperature treatment was maintained in triplicate tanks (3 tanks × 4 temperatures). Temperature adjustments were carried out at a rate of 2°C day^{-1} using a water heater (E-JET Heater 200W, Penang, Malaysia) and a cooler (HS-28A 250–1200 L/H, Guangdong Hailea Group Co., Ltd., Chaozhou, China) until the target temperatures of 22°C (lowest) and 34°C (highest) were reached. A natural daylight photoperiod was maintained throughout the experiment. During the trial, 20% of the water was replaced daily. Fish were sampled and data were recorded at 15-day intervals. A commercial pellet diet (proximate composition: crude protein 39.93%, ash 11.63%, moisture 2.59%, and fat 2.94%) was provided twice daily at 09:00 and 16:00 h until satiation. Additionally, feces and uneaten feed were removed daily.

2.3 Growth rate study

The fish from each experimental tank were weighed and measured separately at the start of the experiment and then every two weeks after that α-methyl quinoline (TransmoreR; Nika Trading, Puchong, Malaysia) (0.22 mL L^{-1} of seawater) was used to gently anesthetize the fish for 10 to 15 minutes before sampling. Using an electronic balance (Model: KD-300KC, Mazumder *et al*. 2018) and a measuring board, the final individual total weight and length were measured to the nearest 0.01 g and 1.0 cm at the end of the experiment. The body weight gain (BWG, g) specific growth rates (SGR) were determined using the following formula:

Body weight gain (BWG, g) = $(W_2 – W_1) \times n$

Specific growth rate (SGR, % day^{-1}) = $100 \times [\ln(W_2) - \ln(W_1)] / t$

Here, W_1 and W_2 represent the starting (0 d) and final (60 d) average individual weights per treatment, n represents the final number of fish, t represents the length of the experimental period.

2.4 Pepsin enzymatic activity study

To assess pepsin activity, gut tissues were collected. Each fish was dissected from the oesophagus to the anus, and the entire gut was excised. After a longitudinal incision, the gut was gently rinsed with 0.1 mol L^{-1} ice cold phosphate-buffered saline (PBS, pH 7.4), blotted dry with filter paper, and weighed. Individual samples were homogenized using a glass and electric Teflon homogenizer (Model: D160, China) in 20 volumes (v/w) of ice-cold 50 mM Tris-HCl buffer (pH 7.4). The homogenates were centrifuged at 10,000 × g for 15 minutes at 4°C (Centrikon H-401 Kontron), and the resulting supernatants were collected and stored at −80°C for enzyme analysis (Bowyer *et al*. 2012; De *et al*. 2021; Mazumder *et al*. 2024).

Total protein content in the supernatants was determined using the Bradford method (Bradford 1976), following De la Fuente-Betancourt *et al*. (2009), with bovine serum albumin (1 mg mL^{-1}) as the standard. A standard curve of absorbance versus protein concentration was used to calculate protein levels. Pepsin activity was measured using 2% bovine hemoglobin in 0.06 N HCl as a substrate following the method of Natali *et al*. (2004), based on Anson's (1938) stop-point assay. For each reaction, 500 µL of substrate was mixed with 100 µL of crude enzyme extract and incubated at 37°C for 10 minutes. The reaction was terminated by adding 1 mL of 5% trichloroacetic acid (TCA). After cooling and centrifugation at 12,000 × g for 5 minutes, absorbance was recorded at 280 nm. Pepsin activity (U37 mg protein^{-1}) was calculated using: {Absorbance value at 280 (supernatant) - Absorbance value at 280 (blank)} × 1000/ (10 min × mg protein)

2.5 Hormonal concentration analysis

For hormonal analysis, blood samples were collected from three fish per replicate in each treatment group. The fish were fasted for 24 hours following the experiment and then anesthetized using α-methyl quinoline (Transmore®; Nika Trading, Puchong, Malaysia). Blood samples were collected from the caudal vein of each fish in the experimental groups using a sterile 2.5 mL plastic syringe. After collection, blood was allowed to clot at room temperature and centrifuged at 12,000 × g for 10 minutes to separate the serum. The clear supernatant (serum) was carefully aspirated and stored at −80°C until hormonal assays were performed. The concentrations of key reproductive hormones - follicle-stimulating hormone (FSH), luteinizing hormone (LH), testosterone, and estradiol-17β (E2) were quantified using commercial ELISA kits obtained from Uscnlife Science Inc. (Wuhan, China) following the manufacturer's instructions as previously described by Yu *et al*. (2018). These assays are based on competitive inhibition ELISA with high specificity and sensitivity (FSH < 0.77 ng mL^{-1}; LH < 127.4 pg mL^{-1}; testosterone < 162.3 pg mL^{-1}; E2 < 4.93 pg mL^{-1}). The kits showed excellent specificity with negligible cross-reactivity. Validation parameters included intra- and inter-assay coefficients of variation below 10% and 12%, respectively, recovery rates of approximately 80–105%, and good linearity across serial dilutions. Hormone concentrations were calculated by interpolating the absorbance values against the standard curves generated for each hormone. All samples were measured in duplicate to ensure the reliability and accuracy of the results.

2.6 Data analysis

All information is displayed as mean values ± SE. One-way analysis of variance (ANOVA) was used to analyze all the data. A pairwise post-hoc Tukey test was performed to identify the precise groups that differed when the ANOVA revealed significant differences (Zar 1999). Unless otherwise indicated, a significance level (α) of 0.05 was applied. Minitab™ software version 17 and Origin™ software version 9.0 were used for all statistical analyses.

3 | RESULTS

3.1 Effect of temperature on growth

For all four temperature regimes, the mean starting weight and length values were similar (Figure 1). The average individual weight was approximately 10.10±0.36 g, and the mean overall length was about 10.06±0.23 cm. Fish survival was 100% across all treatments. Figure 1a illustrates the average length gain of *L. calbasu* over 60 days under four temperature treatments (22°C, 26°C, 30°C, and 34°C). Growth followed a power model, with the highest gain observed at 30°C, indicating enhanced somatic growth at this temperature regime. The model fits were strong (r^2 > 0.98), confirming the reliability of the trend lines. Growth differences became more pronounced over time, especially after day 30, with 30°C consistently outperforming other treatments. Similar to the length gain, all groups displayed an upward growth trend, with the highest gain recorded at 30°C by day 60, indicating enhanced weight accumulation under this temperature. Although fish at 22°C initially had higher weight, those at 30°C surpassed others in the later stages. These results suggest that 30°C may be near-optimal for promoting length and weight gain in *L. calbasu* under experimental conditions.

The graph illustrates a cubic polynomial model describing the relationship between specific growth rate (SGR) and temperature, ranging from 22°C to 34°C. The fitted equation, $y = 4.98 - 0.657x + 0.030x^2 - 4.029x^3$, reflects a nonlinear trend with possible inflection points. The model shows a strong fit, as indicated by the high coefficient of determination ($r^2 = 0.998$). The graph depicts that temperature significantly influenced SGR, with a marked increase observed as water temperature rose from 22°C to 30°C ($p < 0.05$). However, further temperature elevation to 34°C led to a decline in SGR. The highest SGR was recorded at 30°C (0.45±0.05% day^{-1}), significantly greater than both the lower and higher temperatures (22 and 34°C, $p < 0.05$), while the lowest value occurred at 22°C (0.25±0.04% day^{-1}), highlighting the temperature sensitivity of growth in *L. calbasu* (Figure 2).

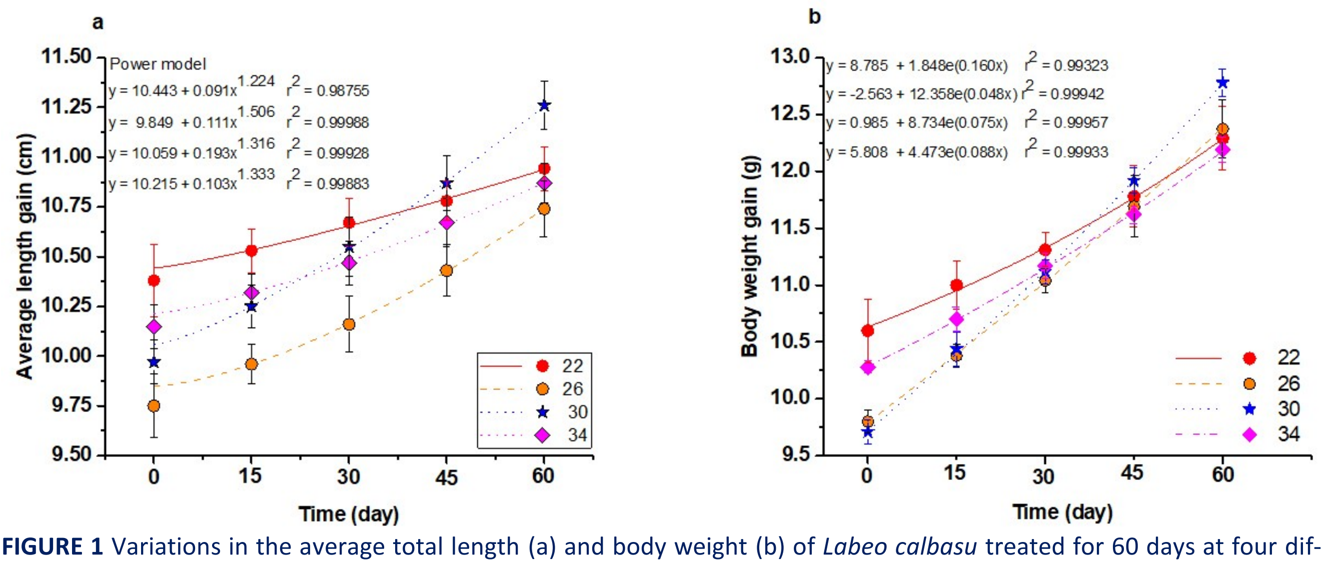

FIGURE 1 Variations in the average total length (a) and body weight (b) of *Labeo calbasu* treated for 60 days at four different temperatures.

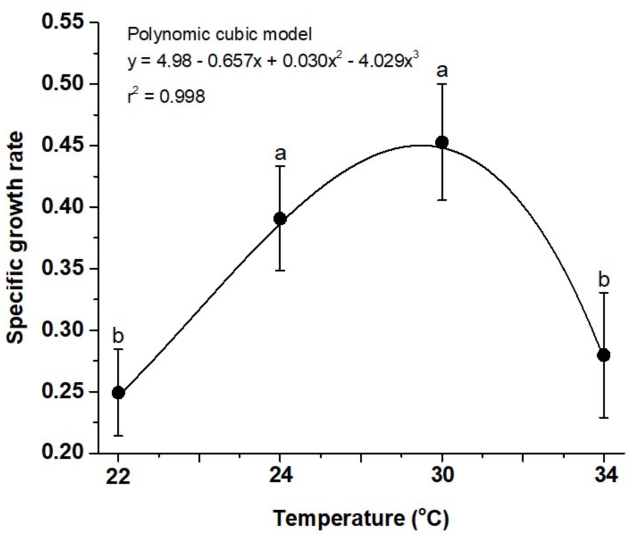

FIGURE 2 Specific growth rate (SGR) of *Labeo calbasu* under four temperatures. The solid circle represents SGR at a specific temperature while the solid line represents non-linear fit.

3.2 Effect of temperature on digestive pepsin enzyme activity

Pepsin activity is plotted across four temperature treatments: 22°C, 26°C, 30°C, and 34°C. The activity increases steadily from approximately 3.37±0.22 U37 mg protein^{-1} at 22°C to around 7.6 at 26°C, reaching its maximum around 8.34±0.71 U_{37} mg protein^{-1} at 30°C (Figure 3). Beyond this optimal point, activity declines to about 5.14±1.09 U_{37} mg protein^{-1} at 34°C indicating a thermal threshold beyond which enzyme function is impaired. The fitted model shows an asymmetric Gaussian distribution, with separate standard deviations (σ_1 and σ_2) for the ascending and descending phases, indicating a sharper decline post-optimum. The results suggest that pepsin activity is temperature-dependent, with an optimum near 30°C, and both lower and higher temperatures reduce enzymatic efficiency, likely due to reduced enzyme-substrate affinity at low temperatures and denaturation or instability at high temperatures. This pattern reflects the thermal sensitivity of digestive enzymes in fish, influencing nutrient assimilation efficiency.

3.3 Effect of temperature on hormonal activity

3.3.1 Effects of water temperature variations on follicle-stimulating hormone (FSH) and luteinizing hormone (LH)

The effects of water temperature on FSH and LH levels are presented in Figure 4. Both hormones were lowest at 22°C, measuring 1.07±0.03 mIU mL^{-1} for FSH and 1.16±0.05 mIU mL^{-1} for LH. As temperatures rose, both hormone levels increased sharply, peaking at 30°C (FSH: 1.45±0.10 mIU mL^{-1}; LH: 1.61±0.04 mIU mL^{-1}). Beyond 30°C, concentrations began to decline as temperature continued to increase. FSH levels at 30°C and 34°C were significantly higher than at 22°C and 26°C ($p < 0.05$), though there was no notable difference between 30°C and 34°C. In contrast, LH concentration reached its maximum at 30°C and was significantly greater than at other temperatures. No difference in LH was observed between 22°C and 26°C (Figure 4), indicating minimal variation in that lower range. These data show that both FSH and LH exhibit temperature-dependent patterns, with optimal levels around 30°C. The pronounced hormonal response at this temperature suggests it is an ideal condition for endocrine activity, while lower or higher temperatures correspond with reduced hormone concentrations.

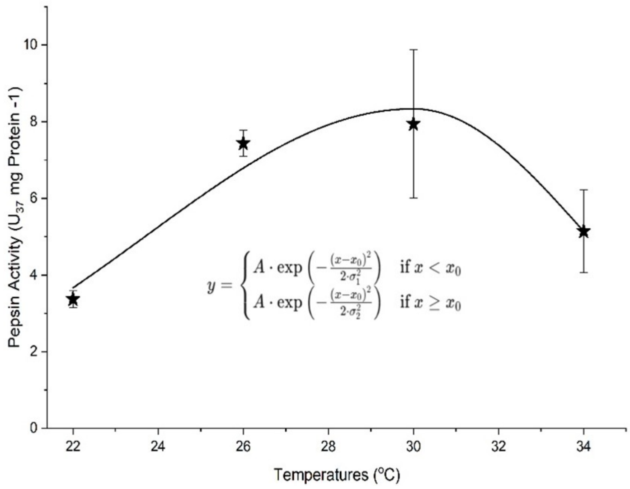

FIGURE 3 Specific pepsin enzyme activity of *Labeo calbasu* under four temperatures. The solid star represents the activity at a specific temperature, while the solid line represents the non-linear fit using an asymmetric Gaussian model.

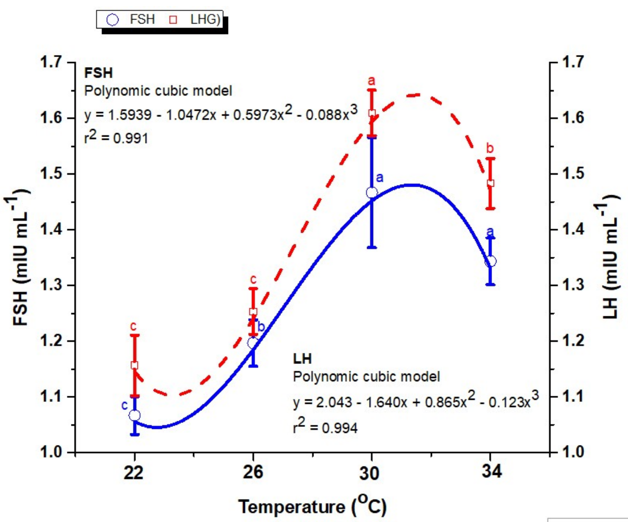

FIGURE 4 FSH, LH, and temperature acclimation to four different temperatures (22, 26, 30, and 34°C) are related. Values are presented as mean ± SE (n = 15), and trends corresponding to different letters differ substantially (p < 0.05).

3.3.2 Effects of water temperature changes on testosterone and estradiol 17-*β* (E2) concentrations

Water temperature significantly influenced serum testosterone and estradiol 17-β (E2) concentrations in *L. calbasu* (Figure 5). The highest testosterone level (1.50±0.01 ng dL^{-1}) was recorded at 30°C, which was greater than other groups (ANOVA: $p < 0.05$). Fish kept at the lowest temperature (22°C) exhibited the lowest testosterone concentration, indicating a suppressive effect of cold stress. Although fish reared at 26°C showed a moderate increase, testosterone levels at 26°C and 34°C were not significantly different, suggesting that both moderately low and high temperatures may impair testosterone synthesis.

Similarly, E2 levels varied significantly among the temperature treatments ($p < 0.05$). The highest plasma E2 concentration was recorded at 30°C, followed by 26°C, 34°C, and the lowest at 22°C. The difference in E2 levels between 30°C and all other temperatures was significant ($p < 0.05$), whereas no significant variation was observed between 26°C and 34°C ($p > 0.05$). Overall, there was a clear increasing trend in E2 concentration as temperature rose from 22°C to 30°C, followed by a slight decline at 34°C. These findings suggest that both testosterone and estradiol synthesis in *L. calbasu* are temperature-dependent, with 30°C being optimal for steroid hormone production, while deviations from this temperature either lower or higher can suppress endocrine activity.

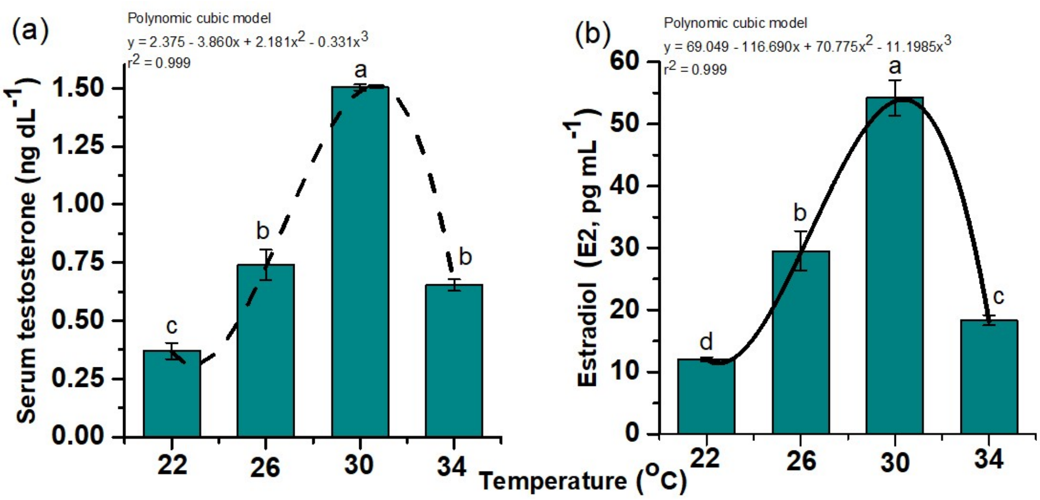

FIGURE 5 Relationship between testosterone (a) and Estradiol 17-*β* (E2) (b) with temperature. Mean ± SE (n = 15) is used to express values. Significant differences exist between trends with different letters ($p < 0.05$).

4 | DISCUSSION

The current study provides thorough insights into how the temperature of *L. calbasu* can affect somatic growth, digestive enzyme activity, and endocrine dynamics. In the present study, the optimum temperature for somatic growth, activity of pepsin enzyme and hormonal profiles is around 30°C. However, these observations are based on measured indicators and should be interpreted cautiously rather than as direct casual relationships. Other temperature ranges, such as lower (22°C) or higher (34°C), were associated with less than ideal physiological and biochemical reactions. In line with the thermal optima documented for other tropical and subtropical fish species, *L. calbasu* showed the maximum specific growth rate (SGR: 0.45±0.05% day^{-1}) and weight gain at 30°C. Katersky and Carter (2005) observed the maximum SGR between 33–36°C for *Lates calcarifer* and a decrease in the growth efficiency beyond this range. Mazumder *et al*. (2019b) observed similarly on *Lutjanus malabaricus*, although species-specific differences should be considered when making such comparisons. Hybrid sturgeon (*Acipenser baerii* × *A. schrenckii*) reached its growth apex at intermediate temperature and declines steadily at extreme temperature (Chen *et al*. 2022). As fish rely on the optimum temperature for regulating their physiological states, the reduced growth at lower temperature may be associated with reduced metabolic rates (Fu *et al*. 2018) which is supported by Islam *et al*. (2020) in European seabass (*Dicentrarchus labrax*). On the other hand, the decrease in SGR at 34°C may indicate thermal stress, supporting findings by Maulvault *et al*. (2017). However, these explanations are inferred and should be considered as plausible rather than confirmed mechanisms. The thermal growth coefficient (TGC) pattern of *L. calbasu* is similar to that of rohu (*Labeo rohita*), which Mridul *et al*. (2024) found to have an ideal TGC of 30°C. However, due to latitudinal adaptations, species such as Atlantic cod (*Gadus morhua*) might have lower thermal optima (~12°C; Little *et al*. 2013).

Temperature has a profound impact on enzyme activity, particularly digestive enzymes, which play a crucial role in the breakdown and assimilation of food. Pepsin activity in *L. calbasu* peaked at 30°C (8.34±0.71 U37 mg $protein^{-1}$), mirroring trends observed in other fish species. Lazo *et al*. (2007) reported maximal digestive enzyme activity in red drum (*Sciaenops ocellatus*) larvae at temperatures close to their natural habitat, while Xiong *et al*. (2011) noted similar thermal optima for pepsin in *Glyptosternum maculatum*. In this study, higher pepsin activity at 30°C may suggest enhanced digestive capacity; however, as digestion efficiency was not directly measured, this should be interpreted as an indirect indication. The reduction of pepsin activity at lower temperatures might be the cold-induced suppression of activity. A similar scenario of reduced protein digestion and nutrient absorption was also documented by Mazumder *et al*. (2024) in *Barbonymus gonionymus*. In keeping with research by De la Fuente-Betancourt *et al*. (2009), which found decreased enzyme stability in jumbo squid at high temperatures, the drop-in pepsin activity at 34°C may indicate enzyme instability or metabolic disruption under elevated temperatures, although this remains a possible explanation rather than a confirmed outcome. On the other hand, the Rainbow trout (*Oncorhynchus mykiss*) as a cold-water fish species, pepsin activity was retained at cold temperatures (Handeland *et al*. 2003) while the activity of pepsin in *L. calbasu* follow the similar pattern as *L. malabaricus* (Mazumder *et al*. 2018), suggesting the necessity of species-specific temperature management for sustainable aquaculture.

Research has indicated that temperature is also crucial for controlling fish reproductive hormones, for example, FSH and LH, which regulate reproduction influenced by different environmental factors (Housh *et al*. 2024; Qiang *et al*. 2022). Current investigations showed that the FSH and LH levels also declined at both the lower and higher extreme temperatures. However, reproductive performance was not directly assessed in this study, and therefore hormonal changes should be interpreted as physiological indicators rather than direct evidence of reproductive optimization. When Atlantic salmon (Andersson *et al*. 2013), *Leporinus elongatus* (Sato *et al*. 2000), *Anguilla japonica* (Dou *et al*. 2008), and carp (Hanna *et al*. 2006) were exposed to different temperatures, similar findings were observed, although differences among species and experimental conditions should be considered. These species showed impaired hypothalamic pituitary gonadal (HPG) axis function, which is reflected in the suppression of these hormones at 22 and 34°C (Bock *et al*. 2021).

FSH, LH, testosterone, and estradiol-17β (E2) peak values at 30°C correspond to the temperature optima for multiple fish species' reproductive hormonal activity. Arantes *et al*. (2011), for instance, found that *Prochilodus argenteus* exhibited comparable patterns, with gonadotropin levels and steroidogenesis peaking at intermediate temperatures (24–30°C). In the present study, similar hormonal trends were observed; however, these do not necessarily confirm optimal reproductive outcomes. Differential heat sensitivity in gonadotropin regulation is suggested by the fast drop in LH and the lack of a discernible variation in FSH between 30°C and 34°C. This is consistent with research by Qiang *et al*. (2022) that found that FSH was more resistant to heat stress than LH in Nile tilapia (*Oreochromis niloticus*). As shown by Lubzens *et al*. (2010) in fish, the ideal E2 and testosterone levels at 30°C further bolster the significance of this temperature in vitellogenesis and oocyte maturation. The drop in E2 at 34°C is consistent with findings made by Housh *et al*. (2024) in *Cyprinodon nevadensis*, where high temperatures hampered the production of estrogen and the viability of eggs. In contrast to temperate species such as the European eel (*Anguilla anguilla*), where optimal steroidogenesis occurs at colder temperatures (Dou *et al*. 2008). The evolutionary adaptations to local climates are highlighted by this divergence.

The identification of 26–30°C as the optimal range for *L. calbasu* has direct applications in aquaculture, particularly in tropical regions like Bangladesh, where rising temperatures due to climate change threaten fish health. Resilience may be improved by mitigation techniques such food changes (Gheytasi *et al*. 2021) or selective breeding for heat tolerance (Zhou *et al*. 2019).

5 | CONCLUSIONS

This study explains *L. calbasu*'s temperature-dependent physiological responses and reveals that 30°C is the optimal setting for growth, pepsin activity, and the synthesis of stress and reproductive hormones. The observed decrease in SGR and digestive efficiency at 22°C and 34°C further supports ectotherms' susceptibility to severe heat. The stress caused by high temperatures is further shown by the peak in cortisol and ACTH at 34°C. A preserved temperature optimum for gonadotropin activity in tropical species is suggested by the reproductive hormone profiles (FSH, LH, testosterone, and E2) at 30°C. However, subtle regulation mechanisms are highlighted by the differing sensitivity of FSH and LH to high temperatures. These findings highlight the necessity of temperature-controlled aquaculture systems to sustain the productivity of *L. calbasu*, especially in areas where water temperatures are rising due to climate change. Future research should explore molecular pathways (e.g. heat shock proteins, thyroid hormones) underlying these responses and investigate adaptive strategies, such as selective breeding or dietary supplements to enhance thermal resilience. By bridging gaps between tropical and temperate fish physiology, this study contributes to global efforts in sustainable aquaculture and climate adaptation.

ACKNOWLEDGEMENTS

This research was financially supported by the University Grants Commission of Bangladesh through research grant #37.01.0000.073.01.150.22.1776, which is gratefully acknowledged.

ETHICAL APPROVAL

All applicable international, national and/or institutional guidelines for the care and use of animals were followed in this study.

CONFLICT OF INTEREST

The author declares no conflict of interest.

AUTHORS' CONTRIBUTION

S.K.M., M.A.G. and S.K.D. conceived and designed the experiments. M.S.M., M.N.I. and M.L.R. performed the statistical analysis and prepared the manuscript, the table and the figures. M.S.M., M.N.I. and M.A.K. conducted the experiment. M.N.I., M.S.M., M.A.K. and M.L.R. collected the samples.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on a reasonable request from the corresponding author.

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