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Climate-driven physiological changes in Mahseer (*Tor tambroides*) juveniles

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Climate change, characterized by rising atmospheric carbon dioxide (CO_2) levels and increasing global temperatures, poses significant threats to aquatic ecosystems. This study examines the impact of elevated CO_2 concentrations and water temperature on the growth, survival, and hematological condition of mahseer juveniles. A controlled experiment was conducted to analyze growth parameters, including specific growth rate (SGR), relative growth rate (RGR), feed conversion ratio (FCR), and hematological indices across varying CO_2 and temperature conditions. The findings indicate that CO_2 levels significantly influence fish weight, with higher concentrations promoting growth up to a threshold. Elevated temperature negatively affects fish weight gain, particularly at extreme levels. Hematological responses suggest that prolonged exposure to high CO_2 and temperature alters blood parameters, indicating physiological stress. The interaction between CO_2 and temperature suggests that optimal growth occurs at high CO_2 and moderate temperatures, whereas excessive warming exacerbates metabolic stress and mortality. These results provide essential insights for sustainable aquaculture practices and conservation strategies in the face of climate change. The significance of these findings extends to aquaculture industries aiming to optimize fish production under changing environmental conditions.

Keywords Climate change, Mahseer, Growth performance, Hematology, Aquaculture

Mahseer is one of the most ecologically and economically valuable freshwater fish species found across South and Southeast Asia. As a keystone species, mahseer plays a crucial role in maintaining the ecological balance of riverine ecosystems by influencing trophic interactions and nutrient cycling[1]. Additionally, the species holds significant commercial value in the aquaculture and recreational fishing industries, contributing to local economies and food security. However, increasing environmental stressors, particularly climate change-driven factors such as rising atmospheric CO_2 levels and global warming, threaten the sustainability of mahseer populations[2]. Understanding how these factors affect mahseer growth, survival, and physiological responses is vital for developing effective conservation and aquaculture strategies.

Rising atmospheric CO_2 levels lead to increased CO_2 dissolution in freshwater bodies, causing shifts in water chemistry that can influence fish physiology and metabolism. Freshwater acidification, driven by elevated CO_2, disrupts ion exchange processes, acid-base regulation, and oxygen transport, leading to impaired growth and reduced survival rates in many fish species[3,4]. Similarly, global warming is altering aquatic habitats, with increasing water temperatures imposing additional physiological challenges. Elevated temperatures can accelerate metabolic processes, increasing energy demands while reducing dissolved oxygen availability, creating stressful conditions that may compromise growth performance and overall fitness in fish[5,6]. The extent to which mahseer can tolerate these environmental changes remains largely unexplored, necessitating rigorous experimental investigations.

Climate change affects fish populations through direct physiological stress as well as indirect ecological changes such as altered food web dynamics, habitat loss, and shifts in competitive interactions. For species like mahseer, which thrive in pristine riverine environments, habitat degradation due to warming waters and

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fluctuating pH levels could significantly impact recruitment and population viability[7]. Prolonged exposure to suboptimal environmental conditions could lead to metabolic inefficiencies, disrupted endocrine function, and compromised immune responses[8,9]. Despite these concerns, limited studies have investigated the interactive effects of CO_2 induced acidification and elevated temperatures on freshwater fish, particularly mahseer. This study aims to bridge this knowledge gap by assessing the impacts of increased CO_2 levels and rising temperatures on the growth, survival, and hematological responses of mahseer juveniles. By simulating projected climate change scenarios under controlled laboratory conditions, this study provides essential insights into the potential resilience of mahseer to environmental stressors and informs future conservation and aquaculture management efforts.

Methodology

Fish sampling and experimental design

A total of 144 mahseer juveniles (mean length: 5.0 ± 0.1 cm; mean weight: 2.4 ± 0.01 g) were obtained from local hatchery at Kuala Berang, Terengganu, Malaysia and acclimated for two weeks in controlled freshwater tanks at Universiti Kebangsaan Malaysia. The experimental trials were conducted using rectangular fiberglass tanks measuring 1.96 m in length, 1.02 m in width, and 0.61 m in height, with a total water capacity of approximately 1200 L. The tanks were filled with dechlorinated water and equipped with individual thermostatic heaters and aeration systems to maintain consistent temperature and dissolved oxygen levels. Juvenile *T. tambroide*s were randomly assigned to each tank at a stocking density of one fish per twenty litres of water to reduce crowding stress and allow natural swimming behaviour.

Each CO_2 treatment: 400 ppm (current atmospheric level/control), 600 ppm, 800 ppm and two temperature conditions (24 °C and 36 °C) were applied in triplicate (Fig. 1), based on future climate projections by the Intergovernmental Panel on Climate Change (IPCC). CO_2 levels were adjusted using controlled infusions of carbon dioxide gas and monitored daily using a calibrated handheld CO_2 meter. To achieve and maintain the desired CO_2 levels, CO_2 gas was automatically injected into the water using a calibrated system that regulated gas input based on the target concentration. The system was designed to maintain stable partial pressure of CO_2 rather than directly control pH, allowing water acidity to fluctuate naturally as CO_2 dissolved and formed carbonic acid. This approach accurately reflects freshwater acidification processes that occur under elevated atmospheric CO_2 conditions.

Water temperature in each tank was controlled using submersible aquarium heaters connected to thermostats. The heaters were set to maintain the experimental temperatures of 28 °C and 34 °C, depending on the treatment group. Temperature was continuously monitored using digital thermometers placed in each tank, with measurements taken twice daily to ensure stability. The heating system provided uniform temperature distribution within the tank water column, although it is acknowledged that this does not perfectly replicate natural temperature gradients. Temperature adjustments were made as necessary to maintain the set points within ± 0.5 °C throughout the experiment. The temperature was checked twice daily to prevent fluctuations that could stress aquatic organisms[10]. Temperature and water quality parameters were recorded twice daily to ensure consistency across all treatments.

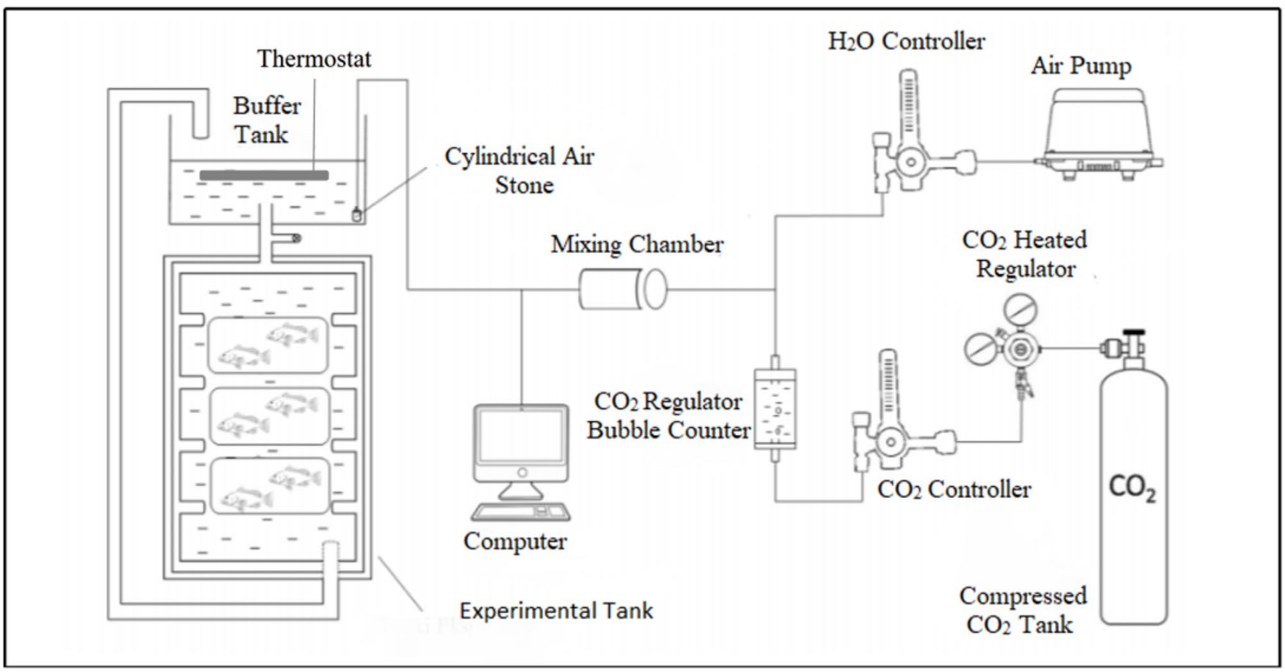

Fig. 1. Experimental tank setup with flow-through system.

Each tank was maintained with continuous aeration, and water quality parameters, including dissolved oxygen, ammonia, and pH levels, were regularly monitored to ensure stable experimental conditions. Water exchange was performed at a rate of 10% per day to minimize fluctuations in water chemistry[11]. The photoperiod was set at 12 h light and 12 h dark to mimic natural conditions and minimize external environmental influences on fish behavior. All laboratory protocols in this study were approved by the Animal Ethics Committee of Universiti Kebangsaan Malaysia (UKM) (approval code no: FST/2016/SIMON/27-JULY/763-JULY-2016-MAY-2017). We have followed the guidelines set forth by the UKM Animal Ethics Committee, ensuring that all methods were carried out in accordance with relevant regulations. Furthermore, all methods are reported in accordance with the ARRIVE guidelines (https://arriveguidelines.org).

Although natural climate change unfolds gradually over extended periods, this experiment adopted an endpoint simulation approach to represent projected environmental conditions rather than mimic the rate of progression. Sudden exposures to elevated temperature and carbon dioxide were applied to juvenile *T. tambroides* under controlled conditions to assess physiological thresholds and short-term adaptability. This approach is widely accepted in laboratory-based aquatic stressors studies as it allows isolation of individual variables without interference from fluctuating environmental factors. While it is acknowledged that this method does not reflect the full complexity of natural climate dynamics, it provides essential baseline data on how freshwater species respond to specific environmental extremes predicted under climate change scenarios. The controlled environment also ensures consistency, repeatability, and minimisation of external confounding variables. These experimental endpoints were selected based on established climate projection models and reflect potential future conditions that may be encountered by sensitive freshwater species such as *T. tambroides*. Nonetheless, it is important to emphasise that future studies should adopt more ecologically realistic designs, such as gradual thermal and carbon dioxide ramping or mesocosm systems, to investigate acclimation capacity, adaptive responses, and multi-stressor interactions over extended periods. These steps will further enhance the ecological relevance of laboratory findings and improve predictive accuracy for aquaculture and conservation under climate stress. While this study focused on temperature and carbon dioxide as primary drivers, other factors associated with climate change, such as altered photoperiod, hypoxia, ultraviolet radiation, and hydrological shifts, were beyond the present scope and merit further investigation under integrated multi-stressor frameworks.

Water quality

Water quality was maintained through a structured protocol to ensure stable environmental conditions for the fish. Carbon dioxide gas was automatically injected into each experimental tank using a regulated gas delivery system to maintain the target CO_2 concentrations of 400, 600, and 800 parts per million (ppm), corresponding to the treatment groups. The CO_2 injection was controlled by solenoid valves linked to CO_2 sensors that continuously monitored dissolved CO_2 levels in the water. This setup ensured consistent and stable partial pressures of CO_2 throughout the experimental period. As expected, the increase in dissolved CO_2 resulted in a natural decrease in water pH due to the formation of carbonic acid, causing acidification proportional to the CO_2 concentration. The system did not independently regulate or fix the pH level but allowed it to vary naturally in response to CO_2 dosing. Water pH was regularly monitored twice daily using a calibrated pH meter to track changes and confirm the expected acidification under elevated CO_2 treatments.

A 25% water exchange was conducted weekly to reduce waste accumulation and maintain overall water chemistry balance, a crucial factor in intensive aquaculture systems[12]. To ensure proper buffering capacity and prevent pH instability, alkalinity was adjusted biweekly[13]. Additionally, continuous biological filtration was implemented to facilitate the breakdown of organic matter and promote a stable nitrogen cycle, while protein skimming was utilized to remove dissolved organic waste, helping to maintain optimal water clarity and quality[14]. The water quality data are collected and recorded.

Growth performance

Fish were provided with a commercially formulated diet twice daily, ensuring a consistent and adequate nutrient intake to support their growth and overall health. The amount of feed offered was measured using a precision electronic balance with an accuracy of ± 0.01 g and carefully adjusted based on fish biomass and consumption patterns to minimize feed wastage and maintain optimal water quality. Uneaten feed was collected approximately 30 min after feeding, dried, and weighed to estimate actual feed intake, allowing for accurate adjustment of subsequent feeding amounts. Feed consumption was meticulously recorded throughout the experimental period to enable precise analysis of feeding efficiency and growth performance. To assess growth dynamics, key parameters including Specific Growth Rate (SGR), Relative Growth Rate (RGR), and Feed Conversion Ratio (FCR) were calculated using well-established standard formulas, following the methodology described by Odeyemi and Awopetu (2022)[15].

Survival rate assessment

Mortality was closely monitored on a daily basis throughout the study, with any deceased fish promptly removed from the tanks to prevent potential impacts on water quality. Each instance of mortality was carefully recorded to ensure accurate documentation of survival trends. The survival rate was calculated using the initial and final fish counts per tank, allowing for a precise assessment of overall mortality patterns. Additionally, any observable signs of distress, such as reduced activity levels, erratic swimming behavior, loss of equilibrium, or other abnormal symptoms, were systematically noted. These observations were analyzed to identify potential correlations between mortality events and environmental conditions, such as temperature fluctuations, dissolved oxygen levels, or changes in water chemistry[16].

Behaviour assesment

Behavioral assessments were conducted throughout the experimental period to evaluate the effects of varying temperature and CO_2 levels on mahseer. Fish behavior was monitored daily, focusing on activity levels, feeding responses, and any signs of distress such as erratic swimming, gasping at the water surface, or lethargy[17]. Observations were conducted during both feeding and non-feeding periods to determine changes in appetite and overall vitality[18]. Each tank was observed for 10 min at three different time intervals daily, ensuring consistent behavioral data collection. Additionally, any abnormal behavior, including loss of equilibrium or excessive mucus secretion, was recorded to assess the physiological impact of experimental conditions[19].

Mucus secretion was assessed qualitatively through routine visual inspections conducted daily during fish handling and health monitoring procedures. During each assessment, fish were gently netted to minimise stress, and their external body surfaces, including the skin and gill areas, were carefully examined for visible signs of excessive mucus production. Indicators such as increased mucous thickness, cloudiness, surface clumping, or unusual film formation were noted as markers of elevated mucus secretion. Observations were also made for accompanying behavioural cues, including signs of respiratory distress (e.g., rapid opercular movement) or reduced activity, which are commonly associated with excessive mucus production. Although no biochemical quantification of mucus components was undertaken, these standardised and consistent visual assessments provided reliable comparative insights into mucus secretion levels across all experimental treatments. This clarification has been incorporated into the revised methodology to enhance transparency regarding the evaluation of this physiological response. These observations were crucial in correlating stress responses with growth performance and survival rates under different temperature and CO_2 conditions.

Hematological & biochemical assessment

A comprehensive hematological and biochemical analysis was conducted by selecting five *T. tambroides* individuals from each replicate under all experimental treatments. Fish were anesthetized using MS-222 solution (20 mg/L; Sigma Aldrich) for ten minutes to minimize handling stress. Blood samples were collected via caudal vein puncture using 5 mL syringes pre-rinsed with 10% EDTA to prevent coagulation. The collected blood was divided into two portions: one mixed with anticoagulant for hematological assessments, including leukocyte counts, hemoglobin concentration (measured using the cyanmethemoglobin method), and erythrocyte counts; and the other portion centrifuged without anticoagulant at 3000 rpm for 10 min to obtain serum for biochemical analyses. Serum biochemical parameters measured included total protein, glucose, and cortisol levels. Cortisol quantification was performed using a Vitros DTEII analyzer (Johnson & Johnson, New Brunswick, NJ, USA), following the manufacturer's quality control protocols. Although cholesterol measurement was initially planned, it was omitted due to limitations in available serum volume. Statistical analyses were performed using one-way analysis of variance (ANOVA) followed by Tukey's HSD test to identify significant differences among treatments ($p < 0.05$) via OriginPro 6.0 software. These methods provided critical insights into the physiological and metabolic stress responses of *T. tambroides* juveniles exposed to elevated CO_2 and temperature.

Statistical analysis

All data were analyzed using two-way analysis of variance (ANOVA) to examine the interaction effects of carbon dioxide concentrations (400, 600, and 800 ppm) and water temperatures (26 °C and 34 °C). Where significant interactions were found ($p < 0.05$), Tukey's HSD post hoc test was applied to determine differences between treatment groups. Statistical analyses were performed using OriginPro 6.0, and all data are presented as mean ± standard deviation (SD). The assumptions of normality and homogeneity of variance were tested before conducting ANOVA.

Results

Water quality by treatments

The water quality data presented in Table 1 demonstrate that both CO_2 concentration and temperature had significant main effects ($p < 0.05$) on pH, dissolved oxygen (DO), ammonia (NH_3), and alkalinity, as confirmed by two-way ANOVA. In addition, significant interaction effects ($p < 0.05$) were observed for all parameters, indicating that the effect of one factor was dependent on the level of the other. As CO_2 increased from 400 to 800 ppm and temperature rose from 26 °C to 34 °C, pH values declined from 8.0 ± 0.15 to 7.4 ± 0.25, reflecting acidification due to increased carbonic acid formation. DO concentrations followed a similar declining pattern, dropping from 7.0 ± 0.4 mg/L to 5.5 ± 0.65 mg/L under the highest CO_2 and temperature condition, suggesting thermal oxygen depletion compounded by elevated CO_2. Meanwhile, ammonia levels increased progressively with both stressors, doubling from 0.015 ± 0.008 mg/L to 0.030 ± 0.015 mg/L, indicating impaired ammonia excretion possibly due to gill dysfunction. Alkalinity also decreased significantly, falling from 175 ± 12 mg/L

Parameter/Temperature (°C) + CO2 (ppm)	26 °C + 400	34 °C + 400	26 °C + 600	34 °C + 600	26 °C + 800)	34 °C + 800
pH	8.0 ± 0.15	7.9 ± 0.2	7.7 ± 0.2	7.6 ± 0.25	7.5 ± 0.2	7.4 ± 0.25
Dissolved oxygen (mg/L)	7.0 ± 0.4	6.0 ± 0.5	6.8 ± 0.4	5.8 ± 0.6	6.6 ± 0.45	5.5 ± 0.65
Ammonia (NH_3) (mg/L)	0.015 ± 0.008	0.025 ± 0.012	0.018 ± 0.009	0.028 ± 0.014	0.020 ± 0.010	0.030 ± 0.015
Alkalinity (mg/L $CaCO_3$)	175 ± 12	170 ± 15	155 ± 15	150 ± 16	145 ± 15	140 ± 17

Table 1. Water quality parameters under different CO_2 concentrations and temperature conditions where ($p < 0.05$).

to 140 ± 17 mg/L $CaCO_3$, reflecting reduced buffering capacity. These changes are tightly interrelated: as acidification and thermal stress intensify, oxygen solubility and waste excretion efficiency decline, leading to physiochemical instability that may directly compromise fish health.

In the present study, ammonia (NH_3) concentrations increased progressively across treatments, from 0.015 ± 0.008 mg/L at 400 ppm CO_2 and 26 °C to 0.030 ± 0.015 mg/L at 800 ppm CO_2 and 34 °C (Table 1). This trend suggests impaired nitrogenous waste excretion efficiency in T. tambroides under combined hypercapnia and thermal stress. Elevated temperatures are known to accelerate fish metabolism, resulting in increased ammonia production, while higher CO_2 levels can disrupt ammonia excretion through gill ion transport mechanisms, leading to internal accumulation and greater environmental toxicity. The concurrent decrease in pH and alkalinity further reduces the buffering capacity of the system, potentially increasing the proportion of un-ionized ammonia (NH_3), which is highly toxic to fish. This rise in ammonia correlates with the behavioral signs of stress and reduced survival rates observed under these treatment combinations. These findings align with those of Das et al.[8] and Iskandar et al.[20], who reported similar physiological impairments in freshwater fish under acidified and thermally stressed conditions. Therefore, ammonia accumulation under elevated CO_2 and temperature represents a critical water quality challenge in aquaculture settings that could compromise fish health, survival, and overall productivity.

Two-way ANOVA revealed that both CO_2 concentration and temperature had significant main effects on pH, dissolved oxygen, ammonia, and alkalinity ($p < 0.05$). Additionally, significant interaction effects ($p < 0.05$) were found for all parameters, indicating that the impact of CO_2 depended on the temperature level and vice versa. These interactions were particularly strong for dissolved oxygen and ammonia, which decreased more sharply under high CO_2 combined with elevated temperature.

Growth performance assessment

The growth performance of mahseer (Table 2) was significantly influenced by varying temperature conditions and carbon dioxide (CO_2) concentrations, as reflected in body weight gain, specific growth rate (SGR), daily growth rate (DGR), and feed conversion ratio (FCR). At 26 °C with 400 ppm CO_2, the fish exhibited moderate growth with a body weight gain of 1.17 ± 0.031 g, an SGR of 2.54 ± 0.031%/day, and a DGR of 0.039 ± 0.010 g/day, with an FCR of 1.55 ± 0.022. However, when the temperature increased to 34 °C under the same CO_2 concentration, growth performance declined, with body weight gain decreasing to 0.86 ± 0.030 g, SGR dropping to 2.09 ± 0.029%/day, and FCR increasing to 1.72 ± 0.025, indicating a lower feed efficiency. In contrast, higher CO_2 concentrations at 600 ppm and 800 ppm showed improved growth at both temperature levels, with body weight gain peaking at 2.85 ± 0.023 g at 34 °C with 800 ppm CO_2. The highest SGR (4.47 ± 0.021%/day) and DGR (0.095 ± 0.008 g/day) were recorded under these conditions, along with the lowest FCR (1.13 ± 0.007), suggesting enhanced feed utilization and metabolic efficiency. These findings indicate that while elevated CO_2 may enhance growth at optimal levels, excessive temperature can negatively impact growth performance, highlighting the importance of balancing environmental factors to optimize aquaculture productivity[21–23].

Statistical analysis showed that both temperature and CO_2 had significant main effects on all growth parameters ($p < 0.05$). Significant interaction effects ($p < 0.05$) were also observed for body weight gain, SGR, DGR, and FCR, confirming that the growth response to CO_2 was dependent on the temperature condition. The best growth performance occurred at 34 °C with 800 ppm CO_2, while the poorest results were recorded at 34 °C with 400 ppm, confirming the modifying effect of CO_2 under thermal stress.

Survival rate assesment

The survival rates in this study ranged from 80.4 to 92.5%, with the highest at 92.5% (± 0.042) and the lowest at 80.4% (± 0.048). Intermediate values included 85.3% (± 0.046), 90.2% (± 0.045), 83.7% (± 0.046), and 87.6% (± 0.043), reflecting variability across conditions. The differences in survival rates recorded in Fig. 2 may be influenced by various factors, including environmental conditions, handling methods, and biological characteristics of the fish. Higher survival rates observed in some groups suggest that optimal conditions were maintained, while lower survival rates may indicate stressors affecting fish health. The variations in survival highlight the importance of identifying key parameters that contribute to improved survival outcomes. Understanding these differences can help in formulating better management strategies to enhance fish survival and productivity in aquaculture settings.

The main effects of both CO_2 and temperature were statistically significant ($p < 0.05$) in influencing survival rate. Moreover, a significant interaction was found between the two factors ($p < 0.05$), where survival

Temperature (°C) + CO_2(ppm)	Body weight gain (g)	SGR (%/day)	DGR (g/day)	FCR	Survival rate (%)
26 °C + 400	1.17 ± 0.031^c	2.54 ± 0.031^c	0.039 ± 0.010^c	1.55 ± 0.022^c	92.5 ± 0.042^a
34 °C + 400	0.86 ± 0.030^d	2.09 ± 0.029^d	0.029 ± 0.008^d	1.72 ± 0.025^d	85.3 ± 0.046^{bc}
26 °C + 600	2.05 ± 0.021^b	3.73 ± 0.018^b	0.068 ± 0.007^b	1.27 ± 0.007^b	90.2 ± 0.045^{ab}
34 °C + 600	2.07 ± 0.034^b	3.76 ± 0.031^b	0.069 ± 0.011^b	1.26 ± 0.012^b	83.7 ± 0.046^c
26 °C + 800	2.75 ± 0.021^a	4.38 ± 0.019^a	0.092 ± 0.007^a	1.15 ± 0.006^a	87.6 ± 0.043^{bc}
34 °C + 800	2.85 ± 0.023^a	4.47 ± 0.021^a	0.095 ± 0.008^a	1.13 ± 0.007^a	80.4 ± 0.048^d

Table 2. Growth performance and survival rate of *Tor tambroides* reared at different treatment (Temperature condition and carbon dioxide concentration) whereas ($p < 0.05$). Different superscript letters indicate statistically significant differences ($p < 0.05$) among treatment.

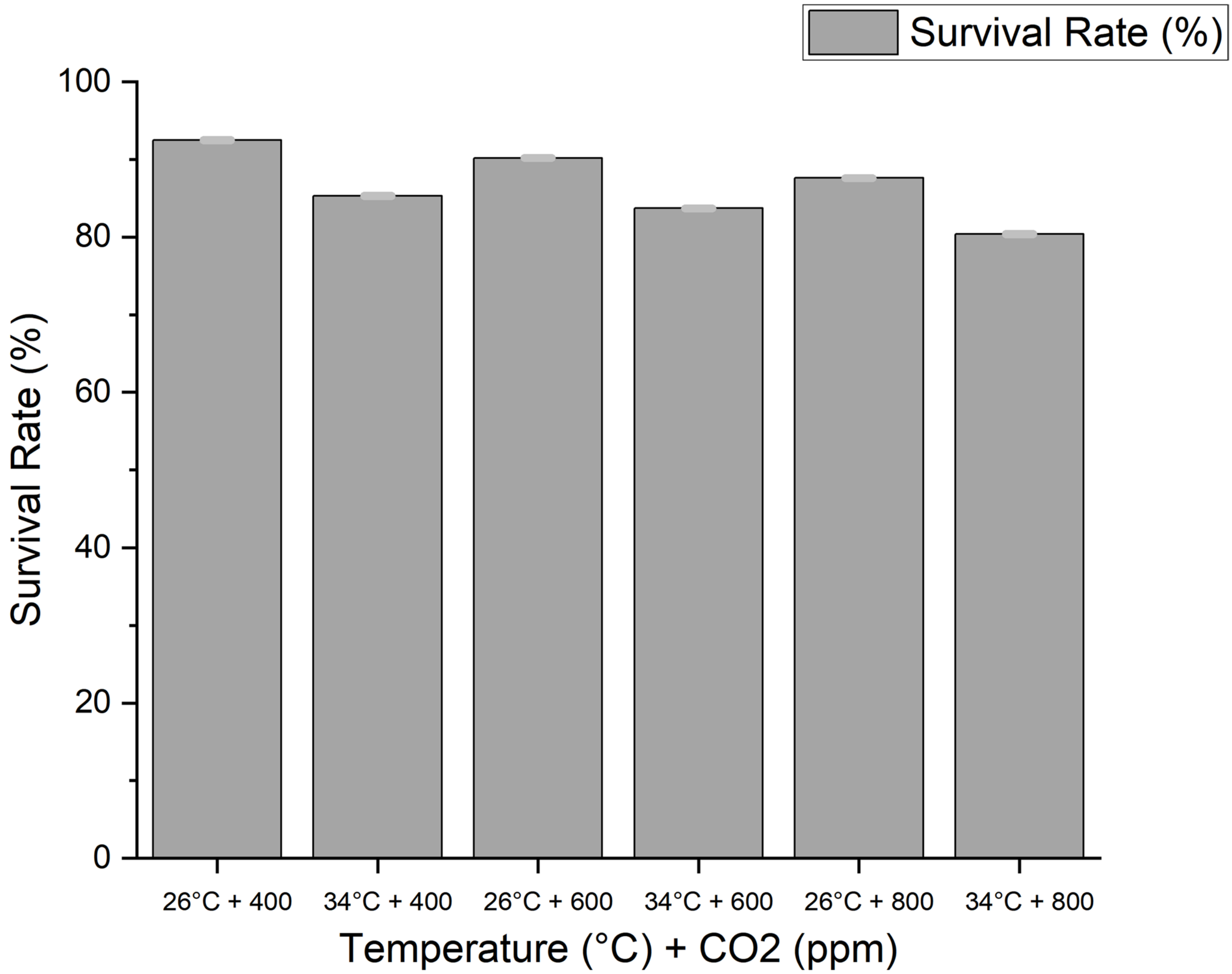

Fig. 2. Survival rates of *Tor tambroides* reared at different treatment (Temperature condition and carbon dioxide concentration).

declined most notably under the highest CO_2 and temperature treatment. The highest survival was recorded at 26 °C + 400 ppm, while the lowest was observed at 34 °C + 800 ppm, indicating that elevated CO_2 may exacerbate temperature-induced mortality in juvenile *T. tambroides.*

Behavior assessment

The behavioral responses of mahseer to different temperature and CO_2 conditions indicate a clear trend in physiological adaptability and stress tolerance (Table 3). At optimal temperature (26 °C) with low to moderate CO_2 levels, mahseer exhibit stable behavior, normal feeding, and improved growth rates. However, increased temperature (34 °C) leads to mild stress, evident in heightened opercular movement and occasional erratic swimming. Higher CO_2 concentrations (600–800 ppm) at moderate temperatures enhance growth efficiency, while excessive CO_2 combined with high temperatures induces severe metabolic stress, respiratory difficulties, and potential immune suppression. The most extreme condition (34 °C + 800 ppm) poses the greatest risk, with signs of labored breathing, reduced feed intake, and elevated mortality rates, aligning with previous studies on climate-induced fish stress[24,25].

Although behavioral observations were qualitative, trends in stress-related symptoms corresponded closely with the statistically significant growth and survival data. The most severe behavioral disruptions, such as labored breathing and erratic swimming, were consistently observed under treatment combinations with significant physiological stress (34 °C + 800 ppm).

Hematological assessment

The study examined how increased temperature and elevated CO_2 levels affect key blood and physiological parameters.From Table 4, Leukocyte counts were significantly higher at 34 °C than at 26 °C across all CO_2 levels, with the highest count ($28.5 \times 10^3/\mu L$) recorded under 34 °C + 800 ppm, suggesting an immune response or stress reaction. Hemoglobin and erythrocyte levels, crucial for oxygen transport, decreased at higher temperatures, with the lowest values (7.1 g/dL and $1.86 \times 10^6/\mu L$, respectively) at 34 °C + 800 ppm, indicating potential heat-

Temperature (°C) + CO_2 (ppm)	Behavior and symptoms
26 °C + 400	Normal swimming patterns, active feeding behavior, optimal growth, stable oxygen consumption, and no significant stress signs
34 °C + 400	Mild stress with increased opercular movement, moderate feed intake reduction, slower growth and occasional
26 °C + 600	Slight increase in feed efficiency and growth rates, stable ammonia excretion, minor acid-base regulatory adjustments, no major behavioral abnormalities
34 °C + 600	Increased gill ventilation, surfacing behavior due to lower dissolved oxygen, moderate metabolic stress, reduced immune function, potential vulnerability to diseases
26 °C + 800	Enhanced growth performance, efficient feed conversion, minor metabolic adjustments, stable swimming behavior, slight increase in ammonia excretion
34 °C + 800	High metabolic stress, labored breathing, erratic swimming, reduced feed intake, hypoxia-induced distress, suppressed immune function, potential for high mortality

Table 3. Behaviour of *Tor tambroides* reared at different treatment (Temperature condition and carbon dioxide concentration).

Treatment	Leukocytes (10^3/μL)	Hemoglobin (g/dL)	Erythrocytes (10^6/μL)	Glucose (mg/dL)	Cortisol (ng/mL)
26 °C + 400 ppm	24.3 ± 0.021^b	$7.9 \pm 0.0.041^b$	2.15 ± 0.018^b	52.3 ± 0.015^c	28.6 ± 0.024^c
34 °C + 400 ppm	26.8 ± 0.032^a	7.5 ± 0.035^c	1.98 ± 0.022^c	64.7 ± 0.032^b	48.9 ± 0.026^b
26 °C + 600 ppm	24.7 ± 0.023^b	8.1 ± 0.033^{ab}	2.23 ± 0.015^{ab}	54.8 ± 0.029^c	30.4 ± 0.038^c
34 °C + 600 ppm	27.2 ± 0.035^a	7.3 ± 0.036^c	1.92 ± 0.025^c	68.3 ± 0.019^b	53.2 ± 0.025^b
26 °C + 800 ppm	25.1 ± 0.024^b	8.3 ± 0.033^a	2.29 ± 0.014^a	59.3 ± 0.022^c	33.7 ± 0.045^c
34 °C + 800 ppm	28.5 ± 0.038^a	7.1 ± 0.037^c	1.86 ± 0.028^c	75.1 ± 0.024^a	$62.8 \pm 0.03.3^a$

Table 4. Blood profile of *Tor tambroides* reared at different treatment (Temperature condition and carbon dioxide Concentration). Mean values ± SD at the same raw with different superscript are significantly different ($P < 0.05$).

induced anemia or impaired red blood cell production. Glucose levels increased with temperature and CO_2, peaking at 75.1 mg/dL under 34 °C + 800 ppm, reflecting an elevated metabolic rate likely linked to stress-induced energy demands. Similarly, cortisol, a well-known stress hormone, reached its highest concentration (62.8 ng/mL) under the same conditions, indicating heightened physiological stress. These findings suggest that rising temperature and CO_2 levels may significantly impact the organism's health by altering immune function, oxygen transport, and stress responses.

Two-way ANOVA indicated significant main effects of both CO_2 and temperature on all blood parameters ($p < 0.05$). Additionally, interaction effects between CO_2 and temperature were statistically significant ($p < 0.05$) for leukocyte counts, glucose, and cortisol levels. These findings suggest that the physiological stress response in *T. tambroides* is driven by both individual and combined environmental stressors. The most extreme values, including elevated cortisol (62.8 ng/mL) and reduced hemoglobin (7.1 g/dL), were observed at 34 °C + 800 ppm, indicating a compounded physiological stress response.

Statistical analysis

To better illustrate the influence of environmental stressors on the physiological and performance metrics of *T. tambroides*, a summary of the two-way ANOVA results is presented in Table 5. The analysis revealed that both CO_2 concentration and water temperature exerted significant ($p < 0.05$) main effects on all measured water quality variables, including pH, dissolved oxygen, ammonia, and alkalinity. Similarly, growth-related parameters such as body weight gain, specific growth rate (SGR), daily growth rate (DGR), and feed conversion ratio (FCR) were significantly affected by both factors. Importantly, the interaction between CO_2 and temperature was also statistically significant ($p < 0.05$) for most parameters, indicating that the response of T. tambroides to one stressor was influenced by the presence of the other. Hematological parameters, including leukocyte count, glucose, and cortisol, also showed significant interactive effects, while hemoglobin and erythrocyte counts exhibited only main effects without significant interaction. These statistical findings reinforce the complex and synergistic nature of environmental stress responses in freshwater fish, particularly under climate change scenarios. The identification of such interactions provides critical insights for aquaculture management and future research on thermal and CO_2 tolerance thresholds in tropical species.

Discussion

Water quality

The significant alterations in water quality parameters observed in this study reflect the synergistic impact of elevated CO_2 and temperature on freshwater aquaculture systems. As shown in Table 1, the progressive decline in pH with rising CO_2 levels and temperature is attributed to carbonic acid formation, a common outcome of hypercapnia in closed aquatic systems. Two-way ANOVA confirmed that both factors had significant main effects on all parameters, with notable interaction effects ($p < 0.05$) amplifying the severity of the changes. Dissolved oxygen levels decreased significantly in response to increased temperature and CO_2, demonstrating the dual impact of lower oxygen solubility at higher temperatures and greater oxygen demand from stressed fish.

Variable	CO_2 effect (*p*-value)	Temperature effect (*p*-value)	CO_2 × Temperature interaction (*p*-value)	Significant effect(s)
pH	< 0.05	< 0.05	< 0.05	All
Dissolved oxygen	< 0.05	< 0.05	< 0.05	All
Ammonia (NH_3)	< 0.05	< 0.05	< 0.05	All
Alkalinity	< 0.05	< 0.05	< 0.05	All
Body weight gain	< 0.05	< 0.05	< 0.05	All
Specific growth rate (SGR)	< 0.05	< 0.05	< 0.05	All
Daily growth rate (DGR)	< 0.05	< 0.05	< 0.05	All
Feed conversion ratio (FCR)	< 0.05	< 0.05	< 0.05	All
Survival rate	< 0.05	< 0.05	< 0.05	All
Leukocyte count	< 0.05	< 0.05	< 0.05	All
Hemoglobin	< 0.05	< 0.05	NS	CO_2, Temp only
Erythrocyte count	< 0.05	< 0.05	NS	CO_2, Temp only
Glucose	< 0.05	< 0.05	< 0.05	All
Cortisol	< 0.05	< 0.05	< 0.05	All

Table 5. Summary of two-way ANOVA results showing the main and interaction effects of carbon dioxide concentration and temperature on water quality, growth, survival, and hematological parameters in *T. tambroides*. Significant effects are indicated where $p < 0.05$; ns = not significant.

The decline in alkalinity further exacerbates the problem by weakening the system's buffering capacity, making the aquatic environment more susceptible to pH fluctuations. Simultaneously, the accumulation of ammonia under combined CO_2 and thermal stress is likely the result of impaired gill function and acid–base disruption, as documented in previous research[26,27]. The increase in ammonia concentration corresponds with observed reductions in growth and survival rates, suggesting a cascade of physiological consequences initiated by deteriorating water conditions. These findings illustrate how CO_2 and temperature act not only as independent stressors but also interactively degrade water quality in ways that directly impact the viability of *T. tambroides* in culture environments under climate stress scenarios.

Furthermore, the combination of elevated CO_2 and temperature can increase ammonia (NH_3) concentrations in aquaculture systems. Higher temperatures enhance the metabolic rates of fish, leading to increased ammonia excretion. Simultaneously, elevated CO_2 levels can impair ammonia excretion efficiency, resulting in ammonia accumulation, which is toxic to fish and can lead to reduced growth and increased mortality rates[28]. Studies indicate that elevated CO_2 levels affect the growth, development, and survival of fish during early life stages, underscoring the potential risks associated with ammonia toxicity[22]. Moreover, prolonged exposure to elevated CO_2 and ammonia levels can lead to chronic stress, which suppresses immune responses and increases mortality risks[23]. These findings underscore the importance of monitoring and managing CO_2 levels and temperature in aquaculture systems to maintain optimal water quality and ensure the health and productivity of aquatic organisms.

Growth performance

The growth performance of mahseer under different temperature and CO_2 conditions reveals critical insights into the effects of climate change on freshwater aquaculture. The results indicate that elevated CO_2 levels, particularly at 600 ppm and 800 ppm, enhance growth performance, with the highest body weight gain, specific growth rate (SGR), and daily growth rate (DGR) recorded at 34 °C with 800 ppm CO_2. This suggests that increased CO_2 may have a stimulatory effect on fish growth, possibly due to its influence on water chemistry, improving bicarbonate availability for acid-base regulation and metabolic function[8]. However, when temperature increases beyond optimal levels, it negatively impacts feed conversion efficiency, as seen in the higher feed conversion ratio (FCR) at 34 °C with 400 ppm CO_2. Elevated temperatures can increase metabolic rates, leading to higher energy demands for maintenance rather than growth, which may explain the lower weight gain and reduced SGR under these conditions[29]. Additionally, prolonged exposure to high temperatures can cause physiological stress, impairing digestion and nutrient absorption, ultimately affecting growth performance and overall health[30].

The interplay between CO_2 and temperature also influences ammonia excretion and dissolved oxygen availability, which are crucial for fish health and growth. At higher CO_2 levels, fish may experience respiratory acidosis, leading to compensatory changes in ion exchange processes that enhance ammonia accumulation in tissues and plasma[31]. This could explain the improved growth at higher CO_2 concentrations, as moderate acidification has been reported to enhance energy efficiency in some fish species[32]. However, excessive CO_2 levels, coupled with high temperatures, can lead to hypoxic conditions, as observed in the reduction of dissolved oxygen at 34 °C with 800 ppm CO_2. Low dissolved oxygen levels can cause oxidative stress, reducing the ability of fish to utilize nutrients effectively and increasing susceptibility to disease[7]. Moreover, increased ammonia levels under elevated temperatures and CO_2 conditions may contribute to toxic buildup, further compromising fish survival and reducing aquaculture productivity[3]. These findings highlight the importance of maintaining optimal water quality parameters to balance the benefits of CO_2 enhanced growth while mitigating the risks associated with temperature-induced stress and oxygen depletion.

The implications of these findings for aquaculture management are significant, particularly in the context of climate change, where rising global temperatures and CO_2 levels are expected to impact freshwater systems[6]. Adaptive strategies such as regulating water temperature through shading, aeration, or controlled water exchange can help mitigate temperature stress while maintaining optimal CO_2 concentrations to enhance growth performance. Additionally, dietary modifications, including the use of high-energy or protein-rich feeds, may help counteract the increased metabolic demands associated with elevated temperatures, improving feed efficiency and overall production outcomes[1]. Future research should focus on long-term exposure studies to determine the cumulative effects of warming and acidification on physiological and biochemical responses in mahseer, as well as on developing strategies for selective breeding to enhance resilience to changing environmental conditions (20,8). These insights contribute to a growing body of literature on climate change adaptation in aquaculture, emphasizing the need for proactive management approaches to sustain fish production under shifting environmental conditions.

Behavior assesment

The impact of temperature and CO_2 concentration on fish behavior and physiology is a critical area of study in aquatic ecology and fisheries management. The data presented indicate that fish exhibit optimal growth and stable physiological functions at 26 °C with 400 ppm CO_2, which aligns with findings from previous studies that suggest this temperature range falls within the preferred thermal range for many freshwater species[33]. At this baseline condition, fish maintain normal swimming behaviors and feeding patterns without evident physiological stress. However, when temperature increases to 34 °C while CO_2 remains at 400 ppm, mild stress symptoms emerge, characterized by increased opercular movement and occasional erratic behavior. This response is consistent with the concept of thermal stress, where elevated temperatures accelerate metabolic rates, leading to increased oxygen demand and a potential mismatch between oxygen supply and demand in fish tissues[34]. Moreover, the reduction in feed intake and slower growth rates suggest that prolonged exposure to this temperature could compromise energy allocation strategies, affecting long-term survival and fitness.

When CO_2 concentrations rise to 600 ppm at 26 °C, the data indicate slight improvements in feed efficiency and growth rates, stable ammonia excretion, and only minor acid-base regulatory adjustments. These findings align with previous research that suggests moderate increases in CO_2 within tolerable limits can enhance physiological efficiency by slightly acidifying blood pH, which can, in turn, improve oxygen transport capacity in some species[35]. However, when temperature increases to 34 °C at the same CO_2 level, fish exhibit increased gill ventilation, surfacing behavior, and signs of moderate metabolic stress. This phenomenon can be attributed to a reduction in dissolved oxygen availability at higher temperatures, exacerbated by elevated CO_2 levels, which may impair respiratory function and overall metabolic homeostasis[36]. The observed reduction in immune function and heightened disease susceptibility further underscores the compounded physiological burden of concurrent temperature and CO_2 stress, which could have significant implications for fish populations under future climate scenarios[37].

At 800 ppm CO_2, a divergent pattern emerges based on temperature differences. At 26 °C, fish exhibit enhanced growth performance and efficient feed conversion, suggesting that increased CO_2 levels may provide a compensatory advantage under stable temperature conditions. This observation supports the hypothesis that some freshwater species possess adaptive mechanisms to moderate CO_2 fluctuations within specific thresholds[38]. However, at 34 °C with 800 ppm CO_2, fish experience severe metabolic stress, characterized by labored breathing, erratic swimming, and hypoxia-induced distress. The suppressed immune function and high mortality potential highlight the critical interplay between hypercapnia and thermal stress, which may push fish beyond their physiological tolerance limits[39]. These findings emphasize the urgency of understanding species-specific thermal and CO_2 thresholds to inform conservation and aquaculture strategies in response to climate change.

Survival rate assessment

The observed survival rates suggest notable variability, which may be attributed to differences in environmental conditions, handling practices, or intrinsic biological factors among the studied groups. The highest survival rate (92.5%) aligns with findings from previous studies, which emphasize the role of optimal water quality parameters in promoting fish health and survival[30]. On the other hand, the lowest survival rate (80.4%) might be indicative of suboptimal conditions or stress-related factors, consistent with observations by[7] that identified handling stress and inadequate nutrition as key contributors to mortality. A comparison of survival rates across different conditions suggests that even minor variations in husbandry practices can significantly influence outcomes. Research by Aliabad et al. 2022[40] has shown that water temperature, dissolved oxygen levels, and stocking density are critical determinants of fish survival, with deviations from optimal ranges resulting in increased mortality. The differences observed in this study may, therefore, reflect the influence of these parameters, warranting further investigation into specific environmental variables that might have played a role.

Moreover, the findings underscore the importance of adopting best management practices to maximize survival rates. Studies have demonstrated that improvements in feed quality, controlled feeding regimens, and stress mitigation strategies can enhance fish survival[24]. The results presented here are in line with such conclusions, highlighting the necessity of refining aquaculture techniques to achieve consistently high survival outcomes. Future research should explore the mechanistic basis of the observed survival variations, particularly by incorporating physiological and molecular assessments. Additionally, a longer-term study monitoring survival trends across different developmental stages could provide deeper insights into factors influencing fish viability under aquaculture settings. By addressing these aspects, aquaculture practitioners can develop more effective strategies for enhancing survival rates and overall productivity[41].

Hematological assessment

The study's findings align with existing research indicating that elevated temperatures significantly impact fish hematological parameters. Increased temperatures have been associated with elevated leukocyte counts, suggesting an immune response or physiological stress in fish[42]. This response may indicate that fish experience heightened immune activation as a compensatory mechanism to counteract the stress caused by rising temperatures[35]. Conversely, hemoglobin and erythrocyte levels showed a decreasing trend under elevated temperatures, with the lowest values observed at 34 °C + 800 ppm. These reductions in oxygen-carrying capacity may result from disruptions in hematopoiesis or heat-induced anemia, leading to reduced oxygen transport efficiency and potentially affecting metabolic functions[43]. The decline in erythrocyte levels at higher temperatures could also be due to increased oxidative stress and hemolysis, which have been reported in previous studies examining the physiological effects of climate change on aquatic organisms[35]. Therefore, these findings suggest that elevated temperature alone, even without additional CO_2 stress, can compromise fish health and survival, particularly in species already vulnerable to environmental changes.

Elevated CO_2 levels further exacerbate these physiological stressors by influencing metabolic and hormonal responses. Higher CO_2 concentrations have been linked to increased glucose levels in fish, likely due to a stress-induced hyperglycemic response[35]. This finding aligns with studies showing that fish experiencing elevated CO_2 levels exhibit heightened metabolic rates as part of an adaptive response to environmental challenges[42]. The highest glucose concentration (75.1 mg/dL) was recorded at 34 °C + 800 ppm, reinforcing the idea that combined stressors amplify metabolic disruption. Additionally, cortisol, a key biomarker of physiological stress, reached its peak concentration under the same conditions, suggesting that fish exposed to both high temperatures and increased CO_2 undergo significant endocrine stress[43]. Chronic exposure to elevated cortisol levels may lead to immunosuppression, reduced growth, and impaired reproductive success, further emphasizing the detrimental effects of environmental stressors[42]. These findings highlight the complex interactions between temperature and CO_2 in modulating fish physiology, necessitating further investigation into species-specific resilience mechanisms.

The combined effects of elevated temperature and CO_2 levels pose significant challenges to fish health, particularly in the context of ongoing climate change. The observed alterations in hematological and metabolic parameters suggest that rising environmental stressors could lead to long-term population declines in susceptible fish species[43,44]. Disruptions in immune function, oxygen transport, and energy metabolism could compromise overall fitness, increasing susceptibility to disease and reducing survival rates. Additionally, species that inhabit thermally sensitive regions may face habitat loss or migration pressures, altering ecosystem dynamics[35]. Given the rapid pace of global temperature rise and ocean acidification, understanding these physiological impacts is essential for predicting future shifts in fish populations and developing mitigation strategies for conservation and fisheries management.

Experimental design and environmental relevance

It is important to acknowledge certain limitations inherent to the experimental design of this study. The abrupt increases in water temperature and carbon dioxide concentrations employed, while consistent with approaches commonly used to simulate near-future climate scenarios, do not fully replicate the gradual, seasonal changes that occur in natural freshwater ecosystems. Additionally, the use of static fiberglass tanks with uniform heating lacks the ecological complexity of natural habitats, such as vertical thermal stratification, flowing water, heterogeneous substrates, and diel temperature fluctuations. These factors limit the opportunity for fish to express natural behaviours, including thermoregulatory movements and habitat selection, which could influence their physiological responses[45]. Nonetheless, this controlled environment allowed for the isolation of temperature and CO_2 effects, providing valuable baseline data on the acute responses of juvenile *T. tambroides* to projected climate change conditions. We recommend that future studies incorporate longer-term exposure with gradual environmental changes and utilize mesocosm or semi-natural systems that better mimic natural complexity to assess behavioural plasticity and acclimatory capacity in this species.

Conclusion

The findings of this study highlight the significant influence of elevated CO_2 levels and increased temperatures on water quality, fish growth performance, survival, and behavior, underscoring the challenges posed by climate change to freshwater aquaculture. Elevated CO_2 concentrations lead to acidification, which affects fish respiration, osmoregulation, and metabolic processes, while rising temperatures exacerbate these effects by reducing dissolved oxygen availability and altering nitrogen cycling. The interplay of these factors results in increased physiological stress, oxidative damage, and higher energy demands for homeostasis, potentially compromising fish growth and health. However, the study also demonstrates that moderate levels of CO_2 can enhance growth performance in mahseer, likely due to improved acid-base regulation and metabolic efficiency. The highest growth rates observed at 34 °C with 800 ppm CO_2 suggest that under controlled conditions, elevated CO_2 may have a stimulatory effect on fish metabolism, though excessive temperature increases can negate these benefits by impairing feed conversion efficiency and increasing ammonia toxicity. The assessment of survival rates further reinforces the importance of optimal water quality management, as variations in temperature, dissolved oxygen, and CO_2 concentrations significantly influence fish viability, with suboptimal conditions leading to higher mortality. The observed differences in survival rates emphasize the need for refined aquaculture management practices, including enhanced feeding strategies, improved water quality monitoring, and stress mitigation techniques. Given the potential long-term consequences of climate change on freshwater systems, future research should focus on developing adaptive aquaculture strategies, such as selective breeding for resilience to environmental fluctuations and optimizing culture conditions to balance the benefits of CO_2-enhanced growth while mitigating risks associated with temperature-induced stress. Additionally, further investigation into physiological and hematological responses to prolonged exposure to elevated CO_2 and temperature will provide deeper insights into fish adaptability and inform sustainable aquaculture practices. These findings contribute to the growing body of knowledge on climate change adaptation in aquaculture, emphasizing the need for proactive management approaches to sustain fish production in an increasingly variable environment. Although the present study provides important insights into the physiological responses of juvenile *T. tambroides* under climate change scenarios, we recognise the limitations posed by the use of static, shallow tanks and abrupt environmental shifts. Future research should aim to integrate more gradual exposure protocols, larger aquatic systems with depth variation, and ecologically relevant settings to better reflect natural environmental changes and fish behavioural responses.

Data availability
The data produced during this study has been reported in this paper.

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Author contributions
Nur Syuhada Iskandar contributed to the conceptualization, methodology, data analysis, and writing of the original draft. Noorashikin Md Noor provided supervision and contributed to writing – review and editing. Zaidi Che Cob and Simon Kumar Das contributed to the writing – review and editing. Mohamad Amir Aiman Abdullah contributed to the writing – editing. All authors reviewed the manuscript.

Declarations

Competing interests
The authors declare no competing interests.

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