

Spatial-environmental context of carbon dioxide induced stress in Malaysian Mahseer (*Tor tambroides*) cultured in inland aquaculture systems

Nur Syuhada Iskandar¹, Noorashikin Md Noor^{1,2}, Zaidi Che Cob^{2,3}, Simon Kumar Das^{4,5},
Mohamad Amir Aiman Abdullah³

¹Earth Observation Centre, Institute of Climate Change, Universiti Kebangsaan Malaysia

²Marine Ecosystem Research Centre (EKOMAR), Natural & Physical Laboratories Management Centre (ALAF-UKM), Universiti Kebangsaan Malaysia

³Department of Earth Sciences and Environment, Faculty of Science and Technology, Universiti Kebangsaan Malaysia

⁴Centre for Sustainable Tropical Fisheries and Aquaculture, James Cook University

⁵Tropical Aquafeed Innovations Lab, James Cook University

Correspondence: Noorashikin Md Noor (email: noor@ukm.edu.my)

Received: 3 May 2025; Accepted: 13 January 2026; Published: 25 February 2026

Abstract

Elevated carbon dioxide (CO₂) concentrations in freshwater aquaculture systems represent an emerging environmental stressor, particularly in inland and highland settings where watershed confinement and limited gas exchange can influence water chemistry. This study examined the physiological stress responses of Malaysian mahseer (*Tor tambroides*), an indigenous and economically important freshwater species, under controlled CO₂ exposure. Juvenile fish were exposed to three CO₂ concentrations: control (400 ppm), moderate (600 ppm), and high (800 ppm) for 30 days in a flow-through experimental system conducted at a single inland aquaculture facility in Hulu Terengganu, Malaysia. Plasma cortisol and glucose levels were quantified as biomarkers of physiological stress. Fish exposed to moderate and high CO₂ concentrations exhibited significantly elevated cortisol and glucose levels compared with the control group ($p < 0.05$), indicating heightened physiological stress under chronic CO₂ enrichment. The findings are interpreted within the spatial–environmental context of inland highland aquaculture systems characterized by hydrological confinement and site-specific water exchange conditions. These results provide empirical evidence that local environmental settings can mediate physiological responses to CO₂ exposure, with implications for water quality management in inland freshwater aquaculture.

Keywords: Aquaculture, carbon dioxide, inland freshwater, spatial environmental context, stress response

Introduction

Aquaculture has become one of the fastest-growing food production sectors globally, playing an increasingly important role in enhancing food security, reducing fishing pressure on wild stocks,

and supporting rural livelihoods, particularly in tropical and developing regions such as Southeast Asia (Noor & Harun, 2022). The rapid intensification of aquaculture practices, including the adoption of closed and semi-closed systems such as recirculating aquaculture systems (RAS), has improved production efficiency, biosecurity and water-use control (Martins et al., 2010; Van Rijn, 2013). However, intensification also increases sensitivity to water quality deterioration, especially in systems where chemical parameters are strongly influenced by site-specific environmental conditions.

One of the most critical yet frequently underestimated challenges in intensive freshwater aquaculture systems is the accumulation of dissolved carbon dioxide (CO₂). Carbon dioxide is continuously produced through fish respiration and microbial degradation of organic matter and may accumulate to biologically harmful levels when degassing capacity is limited (Colt, 2006). Elevated CO₂ conditions, commonly referred to as hypercapnia, are particularly problematic in systems with high stocking density, restricted water exchange or insufficient aeration. In inland aquaculture facilities, especially those located in highland or river-fed catchments, hydrological confinement and system design may further constrain gas exchange, increasing the likelihood of CO₂ retention.

Chronic exposure to elevated CO₂ has been shown to disrupt multiple physiological processes in freshwater fish. Documented effects include acid base imbalance, impaired oxygen transport, altered ion regulation, and reduced metabolic stability (Skov et al., 2023; Iskandar et al., 2023). These physiological disturbances can translate into reduced growth performance, compromised health status and diminished production efficiency, ultimately affecting the economic sustainability of aquaculture operations (Mansour et al., 2017). Understanding the physiological consequences of CO₂ exposure is therefore essential for improving water quality management strategies, particularly in intensive freshwater systems. The primary endocrine pathway mediating physiological stress responses in teleost fish is the hypothalamic-pituitary-interrenal (HPI) axis. Environmental stressors such as hypercapnia activate this axis, leading to the release of cortisol, the principal glucocorticoid hormone in fish (Barton, 2002; Guerreiro et al., 2022). Cortisol regulates a wide range of physiological functions, including energy mobilization, ion balance, and immune modulation. A secondary stress response commonly observed under prolonged stress exposure is an elevation in plasma glucose concentration, resulting from cortisol-stimulated gluconeogenesis and glycogenolysis (Mommsen et al., 1999; Wang et al., 2023). Plasma cortisol and glucose are therefore widely used as reliable biomarkers for assessing chronic physiological stress in aquaculture species (Samaras et al., 2021).

While fish stress physiology has been extensively studied, most existing research has focused on marine species or temperate freshwater taxa. In contrast, comparatively little attention has been given to indigenous tropical freshwater species cultured under inland aquaculture conditions. Environmental settings such as inland river basins, highland catchments and enclosed freshwater systems exhibit distinct hydrological and physicochemical characteristics that may influence CO₂ dynamics and stress vulnerability. These spatial-environmental characteristics are particularly relevant in Malaysia, where freshwater aquaculture is increasingly practiced away from coastal zones.

The Malaysian mahseer (*Tor tambroides*), locally known as *ikan kelah*, represents an ecologically and economically significant freshwater species native to upland rivers and fast-flowing streams of Southeast Asia (Ingram et al., 2005; Khairul-Adha et al., 2016). The species is highly valued for food, conservation, and restocking programs, yet is known to be sensitive to changes in water quality, including fluctuations in pH, dissolved oxygen and temperature. As *T.*

tambroides is increasingly cultured in controlled aquaculture environments, particularly in inland and highland regions such as Hulu Terengganu, its physiological tolerance to chronic environmental stressors such as elevated CO₂ warrants closer investigation. Despite its growing importance, information on the physiological responses of *T. tambroides* to elevated CO₂ remains limited. Existing studies on CO₂-induced stress in fish are largely biased toward marine or model species, leaving a gap in understanding how tropical indigenous freshwater species respond under aquaculture conditions. Addressing this gap is critical for improving species-specific management strategies and ensuring the sustainability of inland freshwater aquaculture systems operating under diverse environmental settings.

Accordingly, the present study evaluated the physiological stress responses of juvenile *T. tambroides* exposed to chronic CO₂ enrichment under controlled experimental conditions. Fish were subjected to three CO₂ concentrations: control (400 ppm), moderate (600 ppm), and high (800 ppm) over a 30-day period and plasma cortisol and glucose levels were measured as indicators of endocrine and metabolic stress. The experiment was designed to reflect operational conditions typical of inland freshwater aquaculture systems in Malaysia. By focusing on physiological responses within a clearly defined environmental setting, this study provides site-specific evidence on CO₂ tolerance in *T. tambroides* and contributes to a better understanding of how local environmental context can influence fish stress responses in intensive freshwater aquaculture.

Materials and methods

Study area and spatial environmental context

This study was conducted as a site-specific inland aquaculture case study using juvenile Malaysian mahseer (*Tor tambroides*) sourced from a government certified freshwater hatchery located in Hulu Terengganu, within the Kenyir Lake basin of Peninsular Malaysia (Figure 1). The hatchery is situated in a highland interior catchment, characterized by elevated terrain, forest-dominated land cover and perennial freshwater inflows originating from upstream river systems.

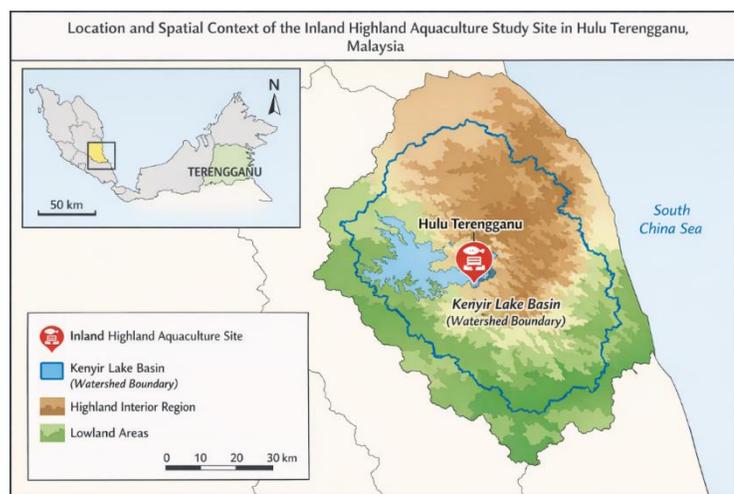


Figure 1. Location map showing the inland highland aquaculture study site in Hulu Terengganu within the Kenyir Lake basin, Peninsular Malaysia. The map illustrates the relative elevation setting and watershed context of the hatchery, highlighting its position within a confined inland freshwater system relevant to CO₂ accumulation risk

The spatial setting represents a typical inland highland aquaculture environment increasingly favored in Malaysia due to relatively clean water sources, limited exposure to coastal salinity intrusion, and proximity to conservation landscapes. However, such inland systems differ from coastal or lowland aquaculture in their hydrological confinement, reduced natural degassing capacity and limited atmospheric exchange, which may increase the risk of dissolved CO₂ accumulation in flow-through and semi-closed systems. The surrounding catchment is dominated by tropical rainforest with minimal urban land use, providing buffering against abrupt salinity or pollutant inputs. At the same time, elevated topography and controlled water exchange typical of inland hatcheries may constrain gas release, particularly under increasing biomass density or reduced flow rates. While the study was conducted at a single location, the site was selected as a representative inland highland freshwater aquaculture system and all spatial interpretations are framed within a case-study context, rather than as a regional or geospatial assessment.

Experimental designs

A total of ninety juvenile *Tor tambroides* were used in the experiment. The fish had a mean length of 5.0 ± 0.1 cm and average weight of 2.4 ± 0.01 g. Upon arrival, the fish were acclimated for 30 days in fiberglass tanks containing aerated, dechlorinated freshwater with a continuous flow system, maintained at a temperature of 26 to 28°C under natural photoperiod cycles (12 h light: 12 h dark). Acclimation included daily monitoring of water parameters, feeding with commercial floating pellets (32% protein), and routine tank cleaning to maintain water quality. No mortality or abnormal behavior was observed during this period.

After acclimation, the fish were randomly divided into three experimental treatment groups based on target CO₂ concentrations: control (400 ppm), moderate (600 ppm), and high (800 ppm). These concentrations were selected based on the following rationale: (i) 400 ppm represents ambient dissolved CO₂ conditions commonly reported in well-aerated freshwater aquaculture systems; (ii) 600 ppm reflects moderate CO₂ enrichment reported in intensively stocked or partially degassed freshwater systems and (iii) 800 ppm represents a high but sub-lethal CO₂ concentration observed in poorly buffered or inadequately aerated inland systems. Together, these treatments simulate a realistic gradient of CO₂ exposure relevant to inland freshwater aquaculture under varying system efficiencies and management conditions. Each group consisted of three replicate tanks, with ten fish per tank. The tanks used were rectangular glass aquaria, each measuring 1.23 m in length, 0.63 m in width and 0.46 m in height, with a water-holding capacity of 356 liters. Water inflow was adjusted to maintain a complete turnover every 6–8 hours, and filtration was supported by mechanical and biological systems. CO₂ was administered into each tank through a fine-bubble ceramic diffuser connected to a CO₂ gas cylinder and concentrations were adjusted using a precision gas flow regulator.

Dissolved CO₂ concentrations were continuously regulated and monitored. Although treatment levels are expressed as ppm for consistency with aquaculture operational literature, all measurements represent dissolved CO₂ concentration in water. CO₂ was measured twice daily using a calibrated portable CO₂ meter (Model AZ-7752, AZ Instrument Corp., Taiwan). To validate meter readings, dissolved CO₂ concentrations were cross-checked using calculated values derived from measured pH and total alkalinity following APHA (2017) standard methods. For clarity, ppm values are equivalent to mg L⁻¹ under standard freshwater conditions (1 ppm ≈ 1 mg L⁻¹), assuming equilibrium between dissolved CO₂ and the aqueous carbonate system at experimental temperature and ionic strength. All reported values reflect operational dissolved CO₂

concentrations within the tanks, not atmospheric CO₂. Water temperature, dissolved oxygen (DO), pH, and conductivity were recorded using a YSI ProDSS multiparameter probe. Dissolved oxygen was maintained above 6.0 mg L⁻¹ across all treatments through continuous aeration to avoid hypoxia-related confounding effects. pH was allowed to fluctuate naturally in response to CO₂ enrichment (Figure 2).

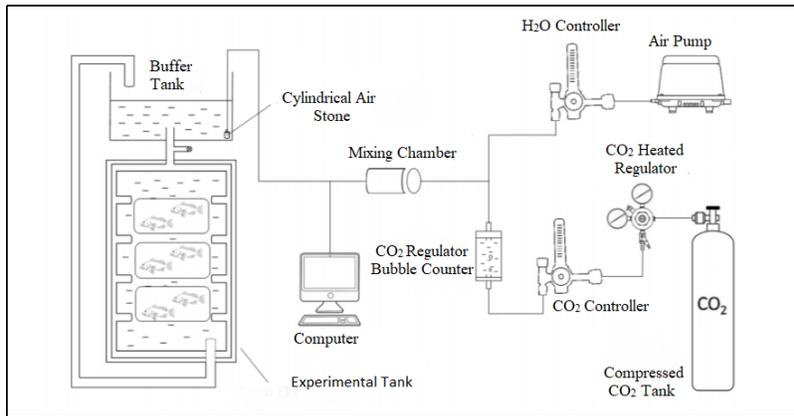


Figure 2. Experimental Tank setup with flow-through system (1.23 × 63 × 46 cm, 356L in size and capacity)

Sampling procedure

At the end of the 30-day exposure period, all fish were sampled during the early morning (0800–1000 h) to minimize diel variation in hormone levels. Prior to blood collection, fish were gently netted and anesthetized using a buffered solution of Transmore® at a concentration of 0.22 mL/L, following the manufacturer’s guidelines. Anesthesia was confirmed by the loss of righting reflex and cessation of opercular movement. Blood samples were collected within 3 minutes of handling to minimize stress-induced artefacts. Approximately 0.3–0.5 mL of blood was withdrawn via caudal venipuncture using a sterile 1 mL heparinized syringe. The blood was transferred immediately into 1.5 mL microcentrifuge tubes and kept on ice. Samples were centrifuged at 3,000 rpm for 10 minutes at 4°C using a refrigerated centrifuge to separate plasma. The plasma was then transferred into labeled microtubes and stored at –20°C until biochemical analysis.

Baseline (0-day) physiological values presented in figures were obtained from a subsample of fish drawn from the acclimation population prior to treatment allocation. These fish were not reused in the experimental treatments. Baseline sampling followed the same anesthesia, blood collection, and analytical procedures described below, ensuring methodological consistency while avoiding repeated sampling of the same individuals.

Biochemical analysis of stress biomarkers

Plasma cortisol concentrations were determined using a commercial enzyme-linked immunosorbent assay (ELISA) kit (Cayman Chemical, USA) validated for use with teleost fish. The assay procedure followed the manufacturer’s protocol, including sample dilution (1:20), incubation at 37°C, and measurement of absorbance at 450 nm using a microplate reader (Bio-Rad iMark). Standards and controls were run in parallel, and each plasma sample was analyzed in

duplicate. The detection limit for the assay was 2 ng/mL. Plasma glucose levels were measured using the glucose oxidase-peroxidase (GOD-POD) method with a commercial colorimetric kit (Randox Laboratories, UK). Samples (10 µL plasma) were mixed with 1 mL of working reagent, incubated at 37°C for 10 minutes, and absorbance was read at 505 nm. Glucose concentrations were determined from a standard curve and expressed in mmol/L.

Statistical and spatial analysis

Physiological data were tested for normality (Shapiro–Wilk) and homogeneity of variance (Levene’s test). One-way ANOVA was used to evaluate differences among CO₂ treatments, followed by Tukey’s HSD post hoc tests. All analyses were performed using IBM SPSS Statistics v25.0, with significance set at $p < 0.05$. While no geospatial modelling or multi-site spatial comparison was conducted, spatial interpretation was applied at the contextual level. Elevation setting, inland watershed confinement, and aquaculture system design were explicitly considered when interpreting CO₂ accumulation risk and stress responses. This framework links local physiological outcomes to broader spatial characteristics typical of highland and river-fed aquaculture systems, providing an evidence-based foundation for future spatially explicit assessments of CO₂ vulnerability in inland aquaculture planning.

Results

Water quality parameters

Water quality parameters were monitored daily throughout the 30-day experimental period and are summarized in Table 1. Across all treatments, water temperature remained stable between 26.3°C and 26.5°C, aligning with the thermal range considered optimal for *Tor tambroides* culture (Khairul-Adha et al., 2016). Dissolved oxygen levels were maintained above 6.0 mg/L in all tanks due to continuous aeration. However, pH levels declined consistently with increasing CO₂ concentrations, consistent with the known acidifying effects of dissolved CO₂ (Colt, 2006). The control group recorded a mean pH of 7.11, while the moderate and high CO₂ groups recorded 6.78 and 6.41, respectively. Total alkalinity remained relatively stable (86–88 mg/L CaCO₃), indicating a consistent buffering capacity and supporting the validity of the CO₂ treatments. These data confirm that the experimental conditions were effectively controlled and representative of CO₂-induced acidification commonly observed in recirculating aquaculture systems (Martins et al., 2010; Van Rijn, 2013).

Table 1. Water quality parameters (mean ± SD) in *T. tambroides* aquaculture tanks during the 30-day CO₂ exposure period

Parameter	Control (400 ppm)	Moderate CO ₂ (600 ppm)	High CO ₂ (800 ppm)
Temperature (°C)	26.3 ± 0.2	26.4 ± 0.2	26.5 ± 0.3
pH	7.11 ± 0.04	6.78 ± 0.05	6.41 ± 0.06
Dissolved Oxygen (mg L ⁻¹)	6.5 ± 0.3	6.3 ± 0.4	6.1 ± 0.4
Total Alkalinity (mg L ⁻¹ CaCO ₃)	88 ± 2	87 ± 2	86 ± 3

Plasma cortisol levels

Plasma cortisol concentrations differed significantly among CO₂ treatments following 30 days of exposure (Figure 3). At day 30, fish exposed to moderate (600 ppm) and high (800 ppm) CO₂ exhibited significantly higher cortisol levels than the control group ($p < 0.05$). The highest cortisol concentrations were observed in the high CO₂ treatment, indicating a dose-dependent activation of the hypothalamic-pituitary-interrenal (HPI) axis under chronic hypercapnia. Baseline (0-day) cortisol values shown in represent measurements obtained from a subsample of fish collected prior to treatment allocation, as described in the Materials and Methods. These baseline values did not differ appreciably among groups, confirming comparable initial physiological status before CO₂ exposure. The elevation in cortisol observed at day 30 reflects a sustained primary stress response rather than acute handling effects.

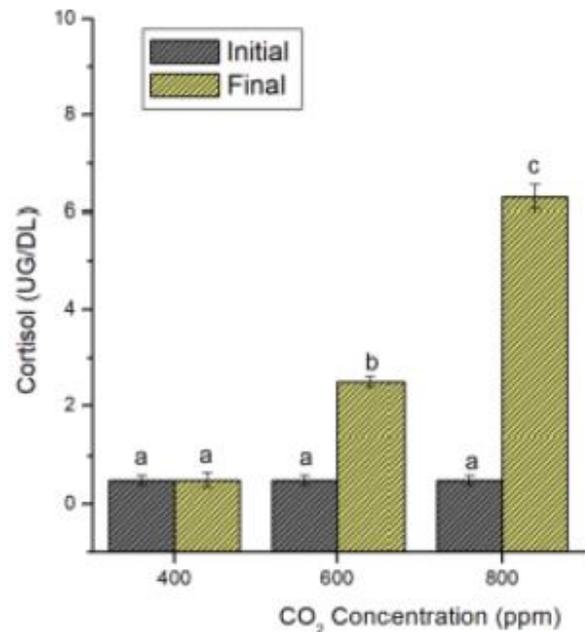


Figure 3. Plasma cortisol concentration (mean \pm SD) in juvenile *T. tambroides* measured at baseline (0 d; subsample prior to treatment allocation) and after 30 days of CO₂ exposure. Different superscript letters indicate significant differences among treatments at day 30 ($p < 0.05$)

Plasma glucose levels

Plasma glucose concentrations showed a pattern consistent with cortisol responses (Figure 4). After 30 days of exposure, fish in the moderate and high CO₂ treatments exhibited significantly higher glucose levels than those in the control group ($p < 0.05$). The highest glucose concentrations were recorded in the high CO₂ group, indicating a pronounced secondary metabolic stress response under elevated CO₂ conditions. Baseline (0-day) glucose values were obtained from the same subsampling procedure described for cortisol and served as reference values for physiological status prior to experimental exposure. The concurrent elevation of glucose and cortisol at day 30

indicates that prolonged CO₂ exposure imposed sustained metabolic demands on *T. tambroides* juveniles.

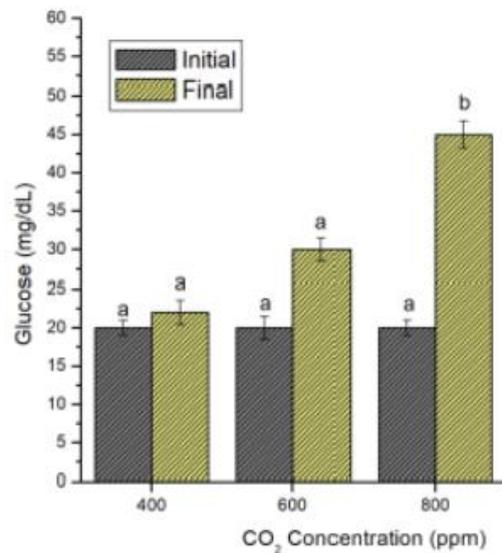
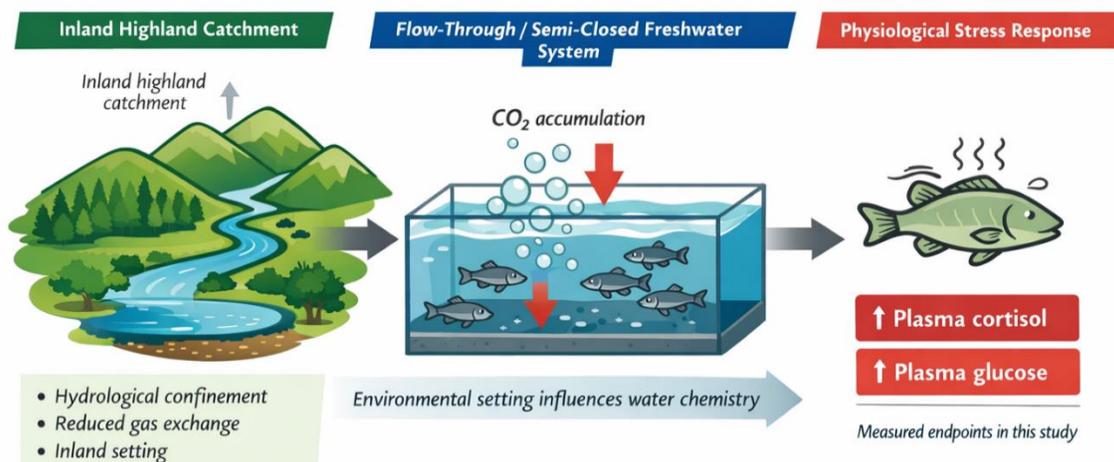


Figure 4. Plasma glucose concentration (mean \pm SD) in juvenile *T. tambroides* measured at baseline (0 d; subsample prior to treatment allocation) and after 30 days of CO₂ exposure. Different superscript letters indicate significant differences among treatments at day 30 ($p < 0.05$)

Environmental and spatial-environmental contextual interpretation

The observed responses are interpreted within the spatial-environmental setting of the study site, which represents an inland highland freshwater aquaculture environment (Figure 5). The hatchery location within the Kenyir Lake basin is characterized by upland catchment hydrology, limited lateral water exchange and reliance on river-fed freshwater inputs. Such spatial-environmental characteristics can influence the persistence of dissolved gases, particularly CO₂, within aquaculture systems even when flow-through management is applied. Under these conditions, CO₂ generated through fish respiration and microbial activity may accumulate more readily compared with open or coastal systems where atmospheric exchange and tidal flushing enhance degassing. Within this spatial context, the significant elevation of plasma cortisol and glucose observed in the moderate and high CO₂ treatments indicates that local environmental setting and system confinement may amplify physiological sensitivity to CO₂ enrichment. The consistency of water quality parameters across treatments, aside from CO₂-driven pH changes, further supports the interpretation that physiological stress responses were linked to CO₂ exposure rather than to broader physicochemical instability. The results provide site-specific physiological evidence relevant to inland and highland aquaculture systems where topography, watershed confinement, and system design interact to shape water quality dynamics. These findings highlight that environmental setting should be considered a background determinant when interpreting stress responses in freshwater aquaculture, particularly in inland catchments with limited natural degassing capacity.



Conceptual framework illustrating spatial–environmental context.

This figure is not based on spatial or geospatial analysis.

Figure 5. Conceptual spatial–environmental framework illustrating how inland highland aquaculture settings may influence dissolved CO₂ accumulation and associated physiological stress responses in freshwater fish. The framework highlights key environmental features of inland systems, including watershed confinement, limited natural degassing and controlled water exchange, which may interact with aquaculture system design to affect CO₂ persistence and endocrine stress responses

Discussion

This study provides physiological evidence that chronic exposure to elevated dissolved carbon dioxide (CO₂) disrupts homeostasis in juvenile *Tor tambroides*, as reflected by significant increases in plasma cortisol and glucose under moderate (600 ppm) and high (800 ppm) CO₂ treatments. Elevated cortisol is a well-established indicator of sustained stress in teleost fishes and reflects activation of the hypothalamic–pituitary–interrenal (HPI) axis under unfavourable environmental conditions (Barton, 2002; Guerreiro et al., 2022; Morón-Elorza et al., 2024). The dose-dependent cortisol response observed here is consistent with previous studies on freshwater species such as *Salmo salar* and *Oncorhynchus mykiss*, where chronic hypercapnia and associated acid–base disturbance elicited prolonged endocrine stress responses (Fivelstad et al., 2003; Baker et al., 2009). Beyond confirming activation of the stress axis, the present results highlight the functional consequences of CO₂-driven acidification for fish physiology. Accumulation of dissolved CO₂ reduces ambient pH and challenges internal acid–base balance, placing additional demands on osmoregulatory and respiratory processes (Perry & Gilmour, 2002; Iskandar et al., 2023). Compensatory mechanisms required to maintain internal stability, including ion regulation and acid–base adjustment, are energetically costly and may divert resources away from growth and other vital processes (Heuer & Grosell, 2014; Jensen et al., 2022). The persistence of elevated cortisol throughout the exposure period therefore suggests a chronic physiological load rather than a transient stress response.

The concurrent elevation of plasma glucose further supports this interpretation. Rather than reiterating the mechanistic pathway linking cortisol to glucose mobilisation, the key implication is

that sustained CO₂ exposure imposes a prolonged metabolic cost. Increased circulating glucose reflects heightened energy demand under stress, which, when maintained over extended periods, has been linked to reduced growth performance, immunosuppression and increased disease susceptibility in cultured fishes (Mommsen et al., 1999; Wendelaar Bonga, 1997; Iwama et al., 2011; Wang et al., 2023). These outcomes are of particular concern in intensive aquaculture, where even sublethal physiological stress can translate into economic losses through reduced feed efficiency and compromised stock condition. The physiological responses documented in this study should be interpreted within the operational context of intensive freshwater aquaculture systems, including recirculating and semi-closed designs. While such systems offer advantages in water use efficiency and biosecurity, they are also prone to CO₂ accumulation when gas exchange is limited or biomass loading is high (Martins et al., 2012; Van Rijn, 2013). Previous studies have emphasized that CO₂ can become a chronic and often underestimated stressor in these systems, particularly when management focuses primarily on oxygen, ammonia or temperature control (Das et al., 2021; Gupta et al., 2024).

Importantly, the spatial relevance of this study lies in its implications rather than spatial conclusions. The experiment was conducted at a single inland highland hatchery site, and no spatial or geospatial analysis was performed. Nevertheless, the environmental setting characterized by inland watershed confinement, river-fed water sources, and limited natural degassing provides a relevant background for understanding why CO₂ persistence may be more pronounced in similar inland or highland aquaculture facilities. Within this context, the physiological sensitivity of *T. tambroides* observed here suggests that system design, water exchange capacity and degassing efficiency may play critical roles in moderating CO₂ exposure in such environments, rather than implying broad spatial patterns across regions.

From a management perspective, these findings underscore the importance of recognizing CO₂ as a standalone water-quality parameter of concern, particularly for environmentally sensitive species such as *T. tambroides* (Ingram et al., 2005; Khairul-Adha et al., 2016). Defining species-specific tolerance thresholds and integrating CO₂ monitoring into routine farm management may help mitigate chronic stress and improve production outcomes, especially under tropical conditions where thermal regimes and organic loading may exacerbate gas accumulation. While broader spatial assessments would be required to generalize risk across landscapes or aquaculture zones, the present study provides essential site-level physiological evidence to support such future work.

Conclusion

This study provides empirical evidence that chronic exposure to elevated dissolved carbon dioxide (CO₂) induces measurable physiological stress in juvenile *Tor tambroides* under controlled freshwater aquaculture conditions. Significant increases in plasma cortisol and glucose under moderate (600 ppm) and high (800 ppm) CO₂ treatments demonstrate sustained endocrine and metabolic stress responses, indicating that hypercapnia can act as a chronic water-quality stressor even when other physicochemical parameters remain within acceptable ranges. Importantly, the findings of this study are based on a single-site, controlled physiological experiment, and conclusions are therefore restricted to the biological responses measured. The environmental setting of the study of an inland highland hatchery within a confined freshwater catchment provides a relevant contextual backdrop for interpreting CO₂ persistence in similar aquaculture systems. The results suggest that system design and water exchange characteristics, rather than

broad geographic patterns, are likely to play a key role in moderating CO₂ exposure risk for cultured fish.

From a practical perspective, the physiological thresholds identified here support the inclusion of CO₂ as a routine water-quality parameter in freshwater aquaculture management, particularly for environmentally sensitive species such as *T. tambroides*. Improved monitoring and management of dissolved CO₂ may help reduce sublethal stress and safeguard fish condition in intensive and semi-closed culture systems. However, any recommendations regarding site selection, spatial planning, or zoning should be considered prospective implications that require validation through broader, multi-site investigations.

Future research should build upon these findings by examining longer-term effects of chronic CO₂ exposure on growth performance, immune competence and reproductive capacity. In addition, comparative studies across multiple inland aquaculture sites with differing elevations, hydrological settings, and system designs would be necessary to evaluate the spatial variability of CO₂ risk more explicitly. Such work would provide the evidence base required for developing spatially informed aquaculture planning tools, grounded in both physiological response data and environmental context.

Acknowledgement

The author would like to acknowledge financial support by UKM through grant GUP-2024-033.

References

- Anderson, W. G., McKinley, R. S., & Colavecchia, M. (1997). The use of clove oil as an anesthetic for fish: An assessment of its suitability for routine anaesthesia of salmonids. *North American Journal of Fisheries Management*, 17(3), 119-129.
- Baker, D. W., Wood, C. M., & McDonald, D. G. (2009). The effects of chronic acidification on the growth and health of juvenile Atlantic salmon, *Salmo salar* L. *Aquaculture*, 295(3-4), 216-224.
- Barton, B. A. (2002). Stress in Fish: A Diversity of Responses with Particular Reference to Salmonids. In *Fish Stress and Health in Aquaculture* (pp. 1-24). Springer.
- Bernier, N. J., & Craig, P. M. (2005). Effects of elevated CO₂ on the health and welfare of freshwater fish in aquaculture systems. *Journal of Aquatic Animal Health*, 17(2), 85-101.
- Colt, J. (2006). Overview of CO₂ control strategies in recirculating aquaculture systems. *Aquacultural Engineering*, 34(3), 89-105.
- Das, S. K., Tou, W. X., Noor, N. M., De, M., & Samat, A. (2021). Length-weight relationship, condition factor, and age estimation of commercially important trawl species from Mersing coastal waters, Johor, Malaysia. *Sains Malaysiana*, 50(1), 1-7.
- Fivelstad, S., Glover, K., & Nilsen, T. (2003). Effects of elevated CO₂ on Atlantic salmon (*Salmo salar*) growth, oxygen consumption, and blood chemistry in a recirculating aquaculture system. *Aquaculture*, 224(1-4), 405-413.
- Guerreiro, P. M., Silva, S., Louro, B., Alves, A., Couto, E., & Canário, A. V. M. (2022). The stress response in Antarctic fish: HPI modulation, cortisol profiles, interrenal sensitivity, and

- gene expression of *Notothenia rossii* acclimated to temperature challenges. *Biology and Life Sciences Forum*, 13(1), 58.
- Gupta, A., Zhang, Y., Kim, D., & Hernandez, M. (2024). Recent developments in recirculating aquaculture systems: A review. *Aquaculture Research*, 2024(1), 6096671.
- Heuer, R. M., & Grosell, M. (2014). The effects of elevated CO₂ on fish health and aquaculture performance. *Journal of Fish Biology*, 84(5), 1336-1357.
- Hrubec, T. C., & Smith, S. A. (2000). Blood and plasma chemistry values for the common carp, *Cyprinus carpio*, and the goldfish, *Carassius auratus*. *Journal of Aquatic Animal Health*, 12(4), 317-323.
- Ingram, B. A., Moos, M. T., & Haug, C. (2005). Aquaculture potential of mahseer (*Tor* spp.) in Malaysia. *Asian Fisheries Science*, 18(2), 309-320
- Iskandar, N. S., Noor, N. M., Cob, Z. C., Das, S. K., & Kasihmuddin, S. (2024). Mahseer conservation in Asia: trends and insights from scientometric analysis. *Marine and Freshwater Research*, 75(14), MF24073.
- Iskandar, N. S., Md Noor, N., Che Cob, Z., & Das, S. K. (2023). Elevated carbon dioxide and its impact on growth, blood properties, and vertebral column of freshwater fish mahseer, *Tor tambroides* juveniles. *Fishes*, 8(6), 307.
- Ismail, A., Rahman, N. A., & Abdullah, M. 2022. Efficacy of Transmore® as an anesthetic agent in red tilapia (*Oreochromis* sp.) under laboratory conditions. *Journal of Aquatic Animal Health*, 34(3), 210–217.
- Iwama, G. K., Vijayan, M. M., & Pickering, A. D. (2011). Stress in fish: The Role of Cortisol and Other Hormones. In *Fish Physiology* (Vol. 31, pp. 39-64). Academic Press.
- Jensen, F. B., Berenbrink, M., & Perry, S. F. (2022). Acute stress response on Atlantic salmon: a time-course study of the physiological changes. *Journal of Experimental Biology*, 225(18), jeb220251.
- Khairul-Adha, M., Mohd-Setapar, S. H., & Zulkifli, S. (2016). The potential of freshwater fish species *Tor tambroides* (Malaysian mahseer) in aquaculture: A review. *Aquaculture Research*, 47(3), 724-733.
- Mansour, O., Idris, M., Noor, N. M., Ruslan, M. S., & Das, S. K. (2017). Effects of organic and commercial feed meals on water quality and growth of *Barbonymus schwanenfeldii* juvenile. *Aquaculture, Aquarium, Conservation & Legislation*, 10(5), 1037-1048.
- Martins, C. I. M., Penha-Lopes, G., & Mota, A. P. (2010). Recirculating aquaculture systems (RAS): A review of environmental, management, and welfare considerations. *Aquaculture*, 300(1-4), 1-7.
- Martins, C. I. M., Schneider, H., & Sampaio, L. (2012). Effects of environmental stressors on fish health in aquaculture systems. *Reviews in Aquaculture*, 4(3), 200-214.
- Mommsen, T. P., Vijayan, M. M., & Moon, T. W. (1999). The effects of stress on the physiology of fish: The role of cortisol in energy metabolism and osmoregulation. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*, 132(2), 183-202.
- Morón-Elorza, P., David, H., Batista, H., Quina, V., Baylina, N., & Pereira, N. (2024). Blood collection under anesthesia, peripheral blood cells, plasma biochemistry, and plasma protein electrophoresis in a living fossil: the Spotted Ratfish (*Hydrolagus coliei*). *Frontiers in Veterinary Science*, 10, 1305968.
- Noor, N. M., Sani, M. A. H. M., Hazri, M. I. N. M., Maulud, K. N. A., & Abas, A. (2025). Sistem Maklumat Geografi dalam perikanan: Analisis sistematik ke arah kelestarian sumber. *Geografia-Malaysian Journal of Society and Space*, 21(1), 100-114.

- Noor, N. M., & Harun, S. N. (2022). Towards sustainable aquaculture: A brief look into management issues. *Applied Sciences*, *12*(15), 7448.
- Perry, S. F., & Gilmour, K. M. (2002). Acid-base balance and CO₂ excretion in fish: Responses to environmental perturbations. In *Fish Physiology* (Vol. 21, pp. 101-155). Academic Press.
- Skov, P. V., Munubi, R. N., & Hamad, M. I. (2023). Effects of hypercapnia on the metabolism and production performance in Nile tilapia (*Oreochromis niloticus*). World Aquaculture Society Meetings.
- Samaras, A., Boeck, G., & Verberk, W. C. E. P. (2021). Stress responses of freshwater fish under environmental challenges: Effects of hypercapnia and temperature fluctuations. *Aquatic Toxicology*, *240*, 105986.
- Ullah, I., Zuberi, A., Rehman, H., Ali, R., Thornqvist, P.-O., & Winberg, S. (2020). Effects of early rearing enrichments on modulation of brain monoamines and hypothalamic–pituitary–interrenal axis (HPI axis) of fish mahseer (*Tor putitora*). *Fish Physiol Biochem*, *46*(1), 75–88.
- Van Rijn, J. (2013). Waste treatment in recirculating aquaculture systems. *Aquaculture*, *432*, 35-44.
- Wang, C.-Y., Tseng, Y.-C., & Tang, D.-Y. 2023. Glucocorticoid receptor mediates cortisol regulation of glycogen metabolism in the gills of Nile tilapia (*Oreochromis niloticus*). *Fishes*, *8*(5), 267.
- Wendelaar Bonga, S. E. (1997). The stress response in fish. *Physiological Reviews*, *77*(3), 591-625.