Fishery Technology 62 (2025) : 409 - 418

Evaluating the Impact of Acute Thermal Stress in GIFT Tilapia Using a Custom-Designed Portable Thermal Aquarium Setup

Paul Nathaniel T.[1], Tincy Varghese[2], Tejpal C. S.[2], Anandan R.[2], Ashutosh D. Deo[1], Vasanthakumaran K.[1], Naveen S. K.[1], Shivangi Bhatt[1], Akhila S.[1] and Subodh Gupta[1,*]
[1]*Fish Nutrition, Biochemistry and Physiology Division, ICAR-CIFE, Mumbai, India.*
[2]*Biochemistry and Nutrition Division, ICAR-CIFT, Kochi, India.*

Abstract

A portable thermal aquarium was custom-designed in this study to assess the physiological response of Genetically Improved Farmed Tilapia (GIFT) to acute thermal stress. The design included a modified aquarium heater and temperature controller unit, equipped with an external temperature sensor. A total of 144 fish (average weight: 27.13 ± 0.19 g) were assigned to three groups, viz., Control (28 °C), and two acute thermal stress exposed group (34 °C and 36 °C), with exposure durations of two and twelve hours (n=4 replicates/group) in temperature-controlled aquariums. The impact of acute thermal stress was assessed by measuring liver metabolism, stress hormones, and enzyme activities. Results revealed significant alterations ($p < 0.05$) in serum cortisol, T3, and T4 levels in fish exposed to thermal stress. Similarly, liver enzyme activities including SOD, CAT, GPx, GST, and GR, along with the brain AChE activity, were markedly elevated under thermal stress. The portable thermal aquarium designed for this study proved to be cost-effective, versatile, and adaptable to tanks of varying volumes. This study also highlights the importance of simulation studies to understand thermal stress-related perturbations in fish. The use of portable thermal aquariums offers a means to replicate warming scenarios in aquaculture systems.

Keywords: Thermal stress, aquaculture, climate change, stress physiology, Sustainability

Received 14 May 2025; Revised 25 July 2025; Accepted 28 July 2025

Handling Editor: Dr. P. S. Shyne Anand

Email: sgupta@cife.edu.in

Introduction

The advent of global warming and its increasing impacts on aquaculture and fisheries are more significant than ever before. Each passing year is recorded as the hottest year on record, surpassing the thresholds set by previous warming scenarios (WMO, 2025). Temperature is a critical master regulator of fish physiology, influencing metabolism, growth, and immune status (Jobling, 1997). The scientific community has actively pursued mitigation strategies at the organism level to ensure sustainable fish production by studying the adverse effects of thermal stress and its implications for fish biology and welfare (Akhila et al., 2025). Fish being ectotherms makes them highly prone to the adverse effects of thermal stress (Somero, Lockwood, & Tomanek, 2016).

India, being a tropical country, experiences its hottest months between March and June; for instance, the Ganga River basin of India faces intense heatwaves with water temperatures reaching up to 43.5 °C (Sarkar et al., 2022). Acute thermal stress during the summer primarily manifests as heatwaves. During such episodes, fish reared in ponds and reservoirs are more susceptible to acute thermal stress, which can result in immune suppression and summer fish kills. Genetically Improved Farmed Tilapia (GIFT) - *Oreochromis niloticus* is a recognized commercially important aquaculture species (Mannur et al., 2025), yet studies related to the effect of thermal stress and heatwave exposure on GIFT are lacking, especially in the context of climate change.

In fish, the stress response typically involves the immediate release of catecholamines and cortisol, which facilitates energy mobilisation from various

sources (Fabbri & Moon, 2016). Additionally, alterations in neurotransmission, characterised by changes in acetylcholinesterase (AChE) activity, have been reported (Kim, Kim, Lee, Han, & Lee, 2019). Elevated temperatures increase metabolic rates, leading to enhanced generation of reactive oxygen species (ROS) in mitochondria (Slimen et al., 2014), which has a detrimental effect on the redox potential and impair cellular function (Chen et al., 2024). Antioxidant enzymes such as Superoxide dismutase (SOD), Catalase (CAT), Glutathione peroxidase (GPx), Glutathione reductase (GR), and Glutathione S-transferase (GST) are known to regulate the antioxidant defence system against ROS (Martínez-Álvarez, Morales, & Sanz, 2005).

Simulation studies conducted in controlled laboratory settings using a thermal aquarium to mimic heatwave conditions in aquatic environments are indispensable for understanding the physiological adaptations of fish reared in ponds and reservoirs. These studies benefit from a controlled, reliable data compared to the inconsistent temperature data from field conditions. However, the high cost associated with fabricating thermal aquariums remains a limitation and poses a challenge for continuous pilot-scale experimentation. Therefore, in this study, an attempt was made to design a cost-effective portable thermal aquarium with precise temperature control that can be locally fabricated for thermal simulation studies while maintaining sufficient accuracy. Additionally, this study investigates the changes in stress hormones, antioxidant enzymes, and metabolic enzymes in GIFT exposed to acute thermal stress at 34 °C and 36 °C. These temperatures were selected based on their relevance to aquaculture during the peak summer months.

Materials and Methods

Commercially available glass aquarium heaters (JRB-250, Sun Sun, China 500 W, range: 28–34 °C) were modified by bypassing the built-in thermostat to allow unregulated heating, as explained in Fig. 1. The heaters were altered by disabling the bimetallic chip assembly, as shown in Fig.1 (right side). In brief, the glass casing of the heater was carefully removed to expose the heating elemen and thermostat assembly. The bimetallic chip was disabled by soldering a galvanised wire from the cable directly to the resistor. The modified heating element was then carefully reinserted into the heater casing, and an epoxy-based waterproof adhesive (Araldite®) was used to seal any openings that might cause water leakage. These modified heaters were then capable of increasing water temperature uncontrollably.

A portable temperature control unit was fabricated using a stainless-steel housing equipped with an external thermostat and a Proportional Integral Derivative (PID) controller switch, with provisions for connecting aquarium water heaters (Fig. 2). The control box included a digital interface for setting the desired temperature, a solid-state relay (SSR), a PID controller, a temperature sensor, and power supply units to connect the aquarium heaters placed inside the tanks. The temperature sensor measures the water temperature using a resistance temperature detector (RTD). The controller will deliver a certain DC voltage to the relay if the water temperature is below the predetermined value. The relay will turn on, causing the AC to flow to the connected aquarium heaters. As a result, the heater starts to warm the water. The PID controller will constantly activate and deactivate the relay when the actual temperature of the water approaches the desired value, maintaining a nearly precise water temperature with a maximum variance of ± 0.1 °C.

Genetically Improved Farmed Tilapia were used for the experiment. Fish were acclimatised under well-aerated conditions and fed with commercial feed (GROWFIN®, Growel) containing 30% crude protein and 8% crude lipid, twice daily to satiation. Rectangular glass aquariums (50 x 40 x 35 cm) with a 75 L capacity were equipped with the portable temperature control unit for thermal stress exposure studies (Fig. 2C). Physicochemical parameters of the rearing water were monitored using an Aquaprobe® AP2000 multi-parameter water quality meter (Aquaread® Ltd, Kent, England).

A total of 144 fish with an average weight of 27.3 ± 0.46 g were randomly distributed in twelve rectangular tanks (4 tanks per treatment), viz., Control (C) (28 °C); T_1 (34 °C); and T_2 (36 °C). Fish were maintained in these tanks for 15 days for acclimatisation prior to thermal stress exposure. On each experimental day, only one temperature treatment was conducted. Necessary care was taken to minimise the variability with the time of the day the samples were collected across treatments. T_1 was conducted on the first day, the temperature of the aquaria was raised from 28 °C to 34 °C within 2 h, with the 2 h exposure sampling done at 10 a.m., and

12 h exposure sampling at 8 p.m. This sampling time frame was followed for the T_2 (36 °C) group as well, to avoid the changes induced by the circadian rhythm.

Following thermal stress exposure, fish were anaesthetised with clove oil (50 μL L^{-1}), and samples were collected from two fish per replicate at each time interval. Tissue and serum were collected following previously established procedures in the same laboratory (Akhila et al., 2025). Brain, gill, and liver tissues were dissected, and a 5% homogenate was prepared in 0.1 M Phosphate buffer (pH 7.5) using a tissue homogeniser, centrifuged at 10,000 rpm for 15 min at 4 °C, and stored at -20 °C until analysis. Blood was collected via caudal venipuncture using a 1 mL syringe into Eppendorf tubes, left undisturbed for 30 min at room temperature to clot, and centrifuged at 5,000 rpm for 10 min to collect the supernatant serum.

Protein concentrations of different tissues were estimated using the Bradford method (Bradford, 1976) using BSA (Bovine Serum albumin) as the standard. Brain acetylcholine esterase (AChE; EC 3.1.1.7) activity was measured spectrophotometrically at 412 nm, standardised for a 96-well plate assay, and expressed as moles of acetylcholine hydrolysed/min/mg protein (Ellman, Courtney, Andres, & Featherstone, 1961). SOD (EC 1.15.1.1) activity was assessed based on the inhibition of epinephrine auto-oxidation (Misra & Fridovich, 1972), expressed as 50% inhibition of epinephrine autooxidation/mg protein/min. CAT (EC 1.11.1.6) activity was estimated following Takahara et al. (1960), and results were expressed as nanomoles of H_2O_2 decomposed/min/mg protein. Glutathione reductase (EC 1.6.4.2) activity was measured by monitoring NADPH oxidation, expressed as units of GR/mg protein (Morales, Pérez-Jiménez, Hidalgo, Abellán, & Cardenete, 2004). Glutathione Peroxidase (EC 1.11.1.9) activity of the liver was assayed using the method of Flohé and Günzler (1984), with modifications, and expressed as units of GPx/mg protein. GST (EC 2.5.1.18) activity was estimated using 1-chloro-2,4-dinitrobenzene (CDNB) in a 96-well plate method (Sigma, CS0410), and expressed as micromoles of GST formed/mL/min.

Serum glucose was estimated using a clinical glucose assay kit (ERBA, Manheim, Germany), based on the GOD-POD (glucose oxidase-peroxidase) method, and results were expressed as mg/dL. Commercial ELISA kits (Calbiotech, Inc., India) were used to estimate serum cortisol, T3 (Triiodothyronine) and T4 (Thyroxine) (Catalogue no. CO368S, T3379T, and T4224T, respectively). Standard curves were created using concentrations of the stock solution provided with the kits to compute the results, and hormone values were reported in ng/mL.

Liver metabolic enzymes, aspartate aminotransferase (AST; EC2.6.1.1) and alanine (ALT; EC2.6.1.2) aminotransferases, were estimated using the original method by Wooten (1964), later modified for microplate assay by Akhila et al. (2025). AST activity was expressed as micromoles of oxaloacetate formed/mg protein at pH 7.4, 37 °C and ALT activity as micromoles of pyruvate formed/mg protein under the same conditions.

Statistical analysis performed using one-way and two-way analysis of variance (ANOVA) using SPSS 25.0 (IBM Corp., Armonk, NY, USA) to study the effects of time and temperature on thermal stress. Post hoc analysis was done using the Duncan multiple range test (DMRT). The confidence of ANOVA was set at 5% significance level ($p < 0.05$), and the results were expressed as mean ± standard error (SE).

Results and Discussion

During hyperthermal stress, fish adapt to changes in their environment through various physio-metabolic responses involving endocrine and metabolic alterations. This study aimed to evaluate the effects of acute thermal stress (34 °C and 36 °C) on GIFT tilapia by examining secondary stress responses such as cortisol, glucose, thyroid hormones, and metabolic and antioxidant enzymes using a portable thermal aquarium setup. These temperatures were selected based on the available field data and diurnal temperature variations observed in previous experiments conducted at a wet laboratory in Mumbai, India (19°.8′23.92″ N, 72°48′58.2″ E). Temperatures in the region varied from 26 °C in the morning, 33 °C at 14:00 h, 34 °C at 16:00 h, 29.5 °C at 18:00 h, and 28 °C at 20:00 h. This serves as a reference data for selecting the test temperatures used in the thermal stress experiment.

No mortality was observed in any treatment group throughout the experiment. Water quality parameters remained within optimum limits for tilapia culture. Dissolved oxygen (DO) content was moni-

tored every 30 minutes using a portable oxygen probe. The DO values exhibited minimum variation during the experimental period, fluctuating between 5.3 – 6.9 mg/mL. This ruled out the possibility of hypoxia contributing to thermal stress response.

Prior to use, the modified heaters were tested for water leakage and reliability. No leakage was observed inside the glass casing after resealing the thermostat assembly, and the heaters were found to be heating uncontrollably, without the thermostat. The built-in resistance temperature detector in the portable temperature controller was calibrated using an external handheld calibrated thermometer, and any offset was updated in the Proportional Integral Derivative controller. This allowed precise temperature regulation within ± 0.1 °C. The portable temperature controller was then used for thermal stress experiments.

The cost of a single thermal aquarium was INR 1,200 (Indian Rupees), the glass heater was INR 1,300, and the fabricated temperature controller was INR 18,000 including the stainless-steel casing. The total cost of the thermal aquarium setup amounted to INR 20,500. The portability of the temperature controller enables its application to aquariums of varying volumes, compared to a single built-in tank that comes with a fixed thermal aquarium. Based on the wattage of the aquarium water heaters (500 W, 1000 W, 2000 W, 5000 W), different tank sizes (500 L, 1000 L, 2000 L and 5000 L) can be used for thermal stress experiments by modifying the appropriate heaters.

According to two-way analysis (Table 1), tempera-

Table 1. Changes in the activities of enzymatic stress markers in GIFT (*Oreochromis niloticus*) exposed to acute thermal stress.

One-way ANOVA											
[1]Treatments	[2]AChE	[3]SOD (L)	[3]SOD (G)	[4]CAT (L)	[4]CAT (G)	[5]GPx (L)	[5]GPx (G)	[6]GST (L)	[6]GST (G)	[7]GR (L)	[7]GR (G)
28 °C_12h	0.51^{a}±0.09	0.34^{a}±0.02	15.57±1.13	2.40^{a}±0.11	0.96±0.01	5.48^{ab}±0.11	6.15±0.13	13.73^{a}±0.02	4.12±0.02	0.87^{a}±0.12	0.93±0.07
28 °C 12h	0.50^{a}±0.05	0.34^{a}±0.01	15.57±0.65	2.47^{a}±0.10	0.94±0.02	5.48^{ab}±0.06	6.15±0.08	14.09^{a}±0.36	4.12±0.01	0.87^{a}±0.07	0.93±0.04
34 °C_2h	0.59^{ab}±0.03	0.62^{b}±0.03	13.65±0.42	8.05^{b}±0.14	0.77±0.1	7.39^{c}±0.61	6.17±0.09	16.40^{c}±0.11	4.2±0.14	1.29^{b}±0.10	1.13±0.13
34 °C 12h	0.72^{b}±0.01	0.38^{a}±0.01	15.13±1.11	7.77^{b}±0.42	1.06±0.16	5.04^{a}±0.58	7.05±0.56	14.71^{b}±0.11	5.04±0.28	1.17^{b}±0.06	1.11±0.01
36 °C_2h	0.72^{b}±0.03	0.55^{b}±0.08	14.06±0.21	7.35^{b}±0.70	0.89±0.13	4.60^{a}±0.69	7.45±1.48	16.56^{c}±0.15	4.28±0.17	1.26^{b}±0.03	0.96±0.2
36 °C 12h	0.67^{b}±0.03	0.37^{a}±0.04	14.53±1.36	7.73^{b}±0.73	1±0.13	6.74^{bc}±0.30	8.75±0.64	18.32^{d}±0.05	4.57±0.54	1.10^{b}±0.02	1.02±0.02
p-value	0.006	0.001	0.578	< 0.001	0.603	0.014	0.168	< 0.001	0.225	0.004	0.632
Two-way ANOVA											
Effect of Temperature											
28 °C	0.495^{a}	0.349^{a}	15.570	2.473^{a}	0.944	5.479	6.154^{a}	14.088^{a}	4.123	0.866^{a}	0.927
34 °C	0.651^{b}	0.499^{b}	14.391	7.912^{b}	0.915	6.216	6.614^{a}	15.556^{b}	4.622	1.231^{b}	1.119
36 °C	0.692^{b}	0.460^{b}	14.295	7.539^{b}	0.946	5.668	8.100^{b}	17.440^{c}	4.424	1.179^{b}	0.989
SEM	0.026	0.027	0.588	0.321	0.076	0.327	0.493	0.161	0.186	0.046	0.071
p-value	< 0.001	0.003	0.272	< 0.001	0.949	0.291	0.040	<0.001	0.203	< 0.001	0.189
Effect of Time											
2 h	0.625	0.363^{a}	16.124	5.992	1.137	5.754	8.196	15.70	4.910	1.046	1.146
12 h	0.600	0.503^{b}	15.473	5.957	1.005	5.821	7.471	15.68	4.531	1.138	1.130
SEM	0.022	0.022	0.480	0.262	0.062	0.267	0.403	0.131	0.152	0.038	0.058
p-value	0.423	0.001	0.357	0.926	0.162	0.861	0.227	0.883	0.103	0.114	0.852
Interaction of Temperature × Time											
p-value	0.086	0.019	0.670	0.768	0.449	0.001	0.648	<0.001	0.303	0.477	0.897

All values in the table are expressed as Mean ± SE (n =3). Means in the same column with different superscript are significantly different ($p < 0.05$) among treatments. [1] Treatments include the exposure of GIFT juveniles to acute thermal stress of 34 and 36 °C for 2 and 12 h exposure, whereas fish maintained at 28 °C served as control. (L) – Liver, (G) – Gill. [2]AcHE (Brain), Acetylcholine Esterase activity expressed as moles of acetylcholine hydrolyzed/min/mg protein); [3]SOD, Superoxide Dismutase activity expressed as 50% inhibition of epinephrine autoxidation/mg protein/min; [4]CAT, Catalase activity expressed as nano moles H_2O_2 decomposed/ min/mg protein; [5]GPX, Glutathione Peroxidase activity expressed as Units of GPX/ mg protein; [6]GST, Glutathione-S- transferase activity expressed as μmol/ml/min; [7]GR, Glutathione reductase activity expressed as Units of GR/mg protein.

Table 2. Serum cortisol, glucose and thyroid hormones of GIFT (*Oreochromis niloticus*) exposed to acute thermal stress

One-way ANOVA	Parameters			
[1]Treatments	Glucose (mg/dL)	Cortisol (ng/mL)	[2]T3 (ng/mL)	[3]T4 (ng/mL)
28 °C_2h	54.75 ± 13.04	88.42^{a} ± 6.65	1.28^{a} ± 0.07	1.36^{ab} ± 0.08
28 °C 12h	54.75 ± 7.53	82.00^{a} ± 1.73	1.28^{a} ± 0.04	1.44^{ab} ± 0.09
34 °C_2h	44.91 ± 1.73	349.93^{d} ± 9.34	1.14^{a} ± 0.02	2.63^{c} ± 0.02
34 °C 12h	57.50 ± 2.97	313.14^{c} ± 8.25	1.30^{a} ± 0.06	1.17^{a} ± 0.05
36 °C_2h	56.29 ± 2.76	340.23^{cd} ± 14.06	1.18^{a} ± 0.06	1.46^{b} ± 0.10
36 °C 12h	59.06 ± 0.39	215.60^{b} ± 10.01	1.64^{b} ± 0.04	1.29^{ab} ± 0.10
p-value	0.444	< 0.001	< 0.001	<0.001
Two-way ANOVA				
Effect of Temperature				
28 °C	54.75	85.21^{a}	1.27^{a}	1.43^{a}
34 °C	51.20	331.53^{c}	1.21^{a}	1.89^{b}
36 °C	57.67	277.91^{b}	1.41^{b}	1.37^{a}
SEM	3.32	6.45	0.03	0.05
p-value	0.415	< 0.001	0.003	<0.001
Effect of Time				
2 h	57.10^{b}	259.52^{b}	1.40^{b}	1.30^{a}
12 h	51.98^{a}	203.57^{a}	1.19^{a}	1.84^{b}
SEM	2.71	5.27	0.02	0.04
p-value	0.208	< 0.001	< 0.001	<0.001
Interaction of Temperature × Time				
p-value	0.401	< 0.001	0.001	<0.001

All values in the table are expressed as Mean ± SE (n =3). Means in the same column with different superscript are significantly different ($p < 0.05$) among treatments. Different superscripts in the same column differ significantly ($p < 0.05$). [1] Treatments include the exposure of GIFT juveniles to acute thermal stress of 34 and 36 °C for 2 and 12 h exposure, whereas fish maintained at 28 °C served as control. [2]T3 (Triiodothyronine); [3]T4 (Thyroxine).

ture significantly ($p < 0.05$) influenced the antioxidant activity of brain AChE, gill GPx, and liver enzymes SOD, CAT, GST, and GR. The exposure time, on the other hand, did not significantly ($p < 0.05$) alter the enzyme activity except SOD activity of the liver. No significant ($p > 0.05$) difference was observed in the enzyme activity of the gills, except for GPx. AChE activity increased significantly ($p < 0.05$) with temperature, peaking at 36 °C. Exposure time had no influence on the activity, nor was there a significant ($p > 0.05$) interaction effect of time and temperature. The elevated AChE activity at higher temperatures at both 34 °C and 36 °C indicates termination or inhibition of cholinergic signalling, potentially leading to the activation of proinflammatory responses in fish (Zivkovic et al., 2023).

GPx activity in the gills was significantly ($p<0.05$) influenced by temperature alone. The highest GPx activity was found at 36 °C. SOD, CAT, GST, and GR activities in the liver were also significantly ($p < 0.05$) influenced by temperature, and the values increased with an increase in temperature. Liver SOD activity was significantly ($p < 0.05$) higher in both thermal stressed groups at 34 °C and 36 °C compared to the control, and the highest activity was found after 12 h exposure. A significant ($p < 0.05$) interaction effect between temperature and time was found for liver SOD activity, with the highest activity in the 34°C_2h group. Liver CAT activity was significantly ($p < 0.05$) influenced only by temperature, with the highest levels at 34 °C. No significant difference ($p > 0.05$) was observed between 34 °C and 36 °C exposed groups, and no

significant ($p > 0.05$) interaction effect with time was noted. Liver GPx activity was not influenced by temperature and time; only a significant ($p < 0.05$) interaction effect of both time and temperature was observed, with the highest activity in the 34°C_2h group. The liver GST activity increased significantly ($p < 0.05$) with temperature, reaching the highest levels in the 36 °C exposed groups. However, exposure time had no significant effect on the GST activity. There was a significant ($p < 0.05$) interaction effect of temperature and time, and the highest value was found in the 36°C_12h group, followed by the 36°C_2h group. Similar to the CAT activity, liver GR activity was significantly ($p < 0.05$) influenced only by temperature and not by the time interval, with the highest values at 34 °C. No significant difference ($p > 0.05$) was observed between the 34 °C exposed and 36 °C-exposed groups.

Increased activity of hepatic antioxidant enzymes SOD, CAT, GPx, GST, and GR indicates elevated production of reactive oxygen species due to thermal stress (Musa et al., 2021; Akhila et al., 2025). The lipid alterations occurring in the liver likely contributed to enhanced free radical production. The observation that most of these enzyme activities peaked at 34 °C, followed by a slight decline at 36 °C, suggests a strong initial response at 34 °C as the fish attempts to maintain homeostasis. However, at 36 °C, the rising temperature may disrupt metabolic balance. The decrease in enzyme activity at this temperature could result from temperature-induced conformational changes leading to enzyme denaturation (Daniel, Dines, & Petach, 1996) or oxidative damage to the enzymes themselves.

According to the Two-way ANOVA (Table 2), serum cortisol, T3, and T4 levels were significantly ($p < 0.05$) influenced by both temperature and time (Table 2), with a significant interaction ($p < 0.05$) also observed. However, thermal stress did not significantly affect glucose levels ($p > 0.05$). Cortisol and T4 levels were significantly ($p < 0.05$) higher at 34 °C compared to 36 °C, while T3 levels were highest at 36 °C. Serum glucose, cortisol, and T3 levels were significantly ($p < 0.05$) higher in the 2 h exposure groups compared to the 12 h groups, whereas T4

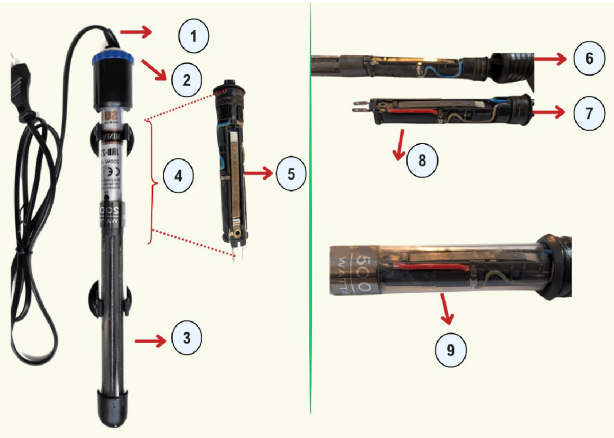

Fig. 1. Modification of commercially available aquarium glass heaters

The aquarium glass heater has the following components: (Left) 1. Power cord; 2. Screw (blue) to adjust the temperature; 3. Heating rods; 4. Thermostat assembly; 5. Bi-metallic chip inside the thermostat assembly. Thermostat assembly modification (Right): 6. Thermostat assembly without modification; 7. Thermostat assembly with modification; 8. Soldering of a galvanic wire (red) directly to the resistor to disable the bi-metallic chip; 9. Modified thermostat assembly inside the glass casing of the heater.

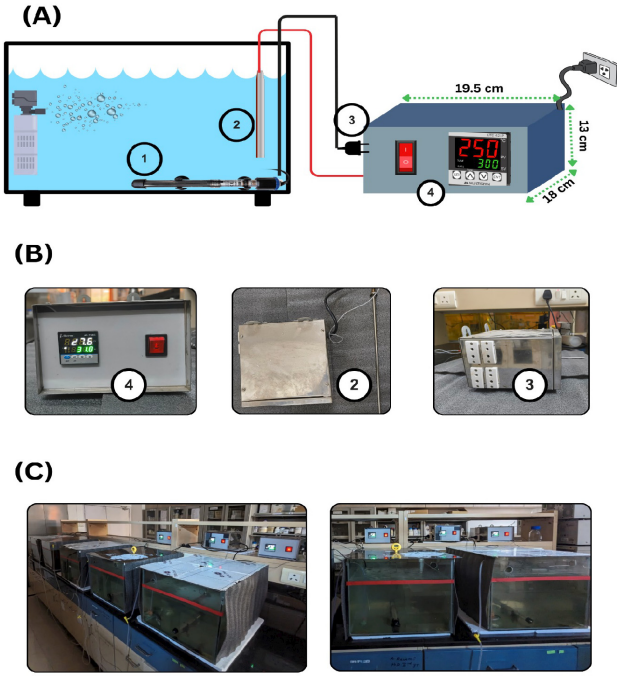

Fig. 2. Portable temperature control unit and thermal stress set-up

Graphical representation of the portable temperature control and thermal stress set up along with its components (A); Front, top and rear view of the portable temperature control unit (B); Functional set-up for conducting thermal stress experiments (C). 1. Modified aquarium heaters from (Fig.1); 2. Stainless temperature sensor connecting to the control panel; 3. Sockets for connecting heaters to the control unit; 4. PID control unit for maintaining the temperature.

levels were highest in 12 h exposure groups. Time had a significant effect ($p < 0.05$), with glucose, cortisol, and T3 levels decreasing with an increase in exposure time, except T4 levels, which increased after 12 h exposure. According to one-way analysis, significantly ($p < 0.05$), the highest cortisol levels were found in fish exposed to the 34°C_2h group, while the lowest were in the 28 °C exposed groups. Cortisol levels increased with rising temperature. T3 levels were significantly ($p < 0.05$) higher in the 36°C_12h group compared to all other groups. T4 levels increased at 34 °C and declined at 36 °C compared to the control; the lowest T4 levels were observed in the 34°C_12h group and the highest in the 34°C_2h group.

Cortisol is the major corticosteroid in teleost fish and is the primary stress hormone acting via the hypothalamic–pituitary–interrenal (HPI) axis. Its serum levels are known to increase under thermal stress (Vazzana, Cammarata, Cooper, & Parrinello, 2014). In the present study, serum cortisol levels significantly increased with exposure to thermal stress at both 34 °C and 36 °C, and were higher after 2 h rather than 12 h exposure, indicating an acute thermal stress response. These results were consistent with previous studies in *O mossambicus* (Basu, Nakano, Grau, & Iwama, 2001; Panase, Saenphet, Saenphet, Pathike, & Thainum, 2019). There was a significant elevation in cortisol levels at 34 °C than 36 °C, suggesting that an increase in temperature lowers cortisol levels, as previously reported by Seo, Lee, Jeong, Mun, and Lin (2023). In parallel with another stress axis, the hypothalamic-pituitary-thyroid (HPT) axis via hypothalamic thyrotropin-releasing hormone (TRH) and pituitary thyroid-stimulating hormone regulates the T4 and T3 levels (Gorissen & Flik, 2016). In fish, thyroid hormones are critical for growth, development, metabolism, reproduction, and stress response (Arjona et al., 2008). Elevated T4 and T3 levels at high temperatures indicate increased metabolic response to thermal stress. The decline in T4 levels at 36 °C compared to 34 °C suggests a partial failure of metabolic adaptation at thermal extremes, possibly due to mitochondrial dysfunction and oxygen limitation at the tissue level (Ern, Andreassen, & Jutfelt, 2023). However, there are contrasting evidence, with some studies reporting increased (Giroux, Gan, & Schlenk, 2019) and others decreased (Akhtar et al., 2013; Roychowdhury, Aftabuddin, & Pati, 2020) T4 levels at high temperatures. The significant increase in serum cortisol, T3, and T4 levels confirms the activation of the hypothalamic-pituitary-interrenal axis, a primary stress response pathway in fish. However, the hyperglycemic response triggered by the cortisol was not reflected in serum glucose levels, possibly due to the inherently low responsiveness of serum glucose to regulatory hormones in teleost (Ma et al., 2023). These enhanced endocrine response observed suggest that tilapia can respond rapidly to high temperatures (Basu et al., 2001; Panase, Saenphet, & Saenphet, 2018), similar to other species such as carp (Akhtar et al., 2013).

According to two-way ANOVA (Fig. 3), temperature significantly ($p < 0.05$) influenced hepatic AST and ALT activities, while exposure time significantly affected only ALT activity. No significant ($p > 0.05$)

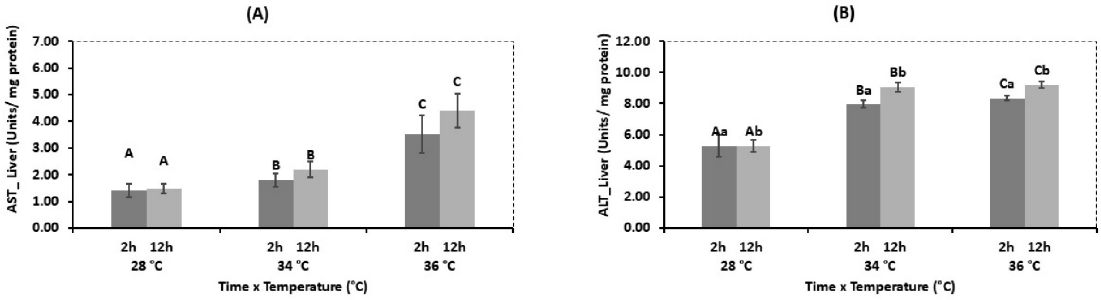

Fig. 3. Metabolic enzyme activities of GIFT juveniles (*Oreochromis niloticus*) exposed to acute thermal stress Fig. 3(A). AST (Liver), Aspartate aminotransferase activity expressed as micromoles of oxaloacetate formed/mg protein/min at pH 7.4, 37 °C; Fig. 3(B). ALT (Liver), Alanine aminotransferase activity expressed as micromoles of pyruvate formed/mg protein/min at pH 7.4, 37 °C. Values are expressed as mean ± SE (n=4). Different superscripts in lowercase above each bar of the same group signify statistical differences in time exposure ($p < 0.05$). Different superscripts in uppercases above each bar of the same group signify statistical differences in the temperature ($p < 0.05$).

interaction effect between time and temperature was observed for either enzyme. Both AST and ALT activities (Fig. 3.A) increased with an increase in temperature, with the highest activities observed in the 36 °C temperature-exposed groups. The exposure time significantly ($p < 0.05$) increased the ALT activity; more the exposure time, higher the activity, which was evident in both 34 °C and 36 °C exposed groups. Liver enzymes, AST, and ALT were selected as representative metabolic enzymes, as their availability from the extrahepatic tissues, such as blood and mucus, increases during stress and is part of the liver function test (Roychowdhury et al., 2020; Abdel-Ghany, El-Sisy, & Salem, 2023). Elevated enzyme activities indicate high amino acid catabolism during thermal stress, likely to meet the increased metabolic energy demand (Ma et al., 2021). The significantly higher activity at 36 °C suggests that this temperature induces more pronounced metabolic stress than 34 °C. The absence of a significant difference in ALT levels between 34 °C and 36 °C might indicate that the increase in amino acid catabolism at extreme hyperthermal exposure is mainly mediated by AST rather than ALT (Panase et al., 2018; Panase et al., 2019; Liu et al., 2023).

Global warming and its impacts on aquatic ecosystems and aquaculture are a major concern for food security and farmers' livelihoods. Understanding the effects of thermal stress through simulation studies in commercially important fish species is indispensable to formulate mitigation strategies relevant to the field conditions. However, limited facilities and high costs often hinder the execution of such studies in fish. This study demonstrated a cost-effective, portable thermal aquarium setup that can be easily adapted to any laboratory conditions. Furthermore, it demonstrated that acute thermal stress significantly affected stress-related signalling pathways in GIFT, indicating its adverse effects on fish physiology and metabolism, especially in tropical regions. This study could serve as a proof-of-concept analysis to understand the stress response associated with acute thermal stress in GIFT during heat waves in the context of climate change. Further studies are essential for understanding immune response, disease resistance, and the effects of chronic thermal stress in fish. Use of omics-based big data analysis, such as transcriptomics, proteomics, and metabolomics, can help provide a comprehensive understanding of thermal stress-induced alterations. Future research aimed at studying the stress response and mitigation strategies of different commercially important fish species is crucial for sustaining aquaculture production.

Acknowledgements

The authors express their sincere gratitude to the Director and Vice-Chancellor of ICAR-Central Institute of Fisheries Education, Versova, Mumbai, India, for facilitating the research work. The first author is grateful to the Indian Council of Agricultural Research, New Delhi, for providing the institutional fellowship at ICAR-CIFE, Mumbai.

References

Abdel-Ghany, H. M., El-Sisy, D. M., & Salem, M. E. S. (2023). A comparative study of effects of curcumin and its nanoparticles on the growth, immunity and heat stress resistance of Nile tilapia (*Oreochromis niloticus*). *Scientific Reports*, *13*(1), Article 2523. https://doi.org/10.1038/s41598-023-29343-z.

Akhila, S., Varghese, T., Sahu, N. P., Gupta, S., Dasgupta, S., Deo, A. D., Mannur, V. S., Nathaniel, T. P., & Chandan, N. K. (2025). Hyperthermal stress potentiates enhanced lipid utilisation in genetically improved farmed Tilapia, *Oreochromis niloticus* juveniles. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology*, *275*, Article 111033.https://doi.org/10.1016/j.cbpb.2024.111033.

Akhtar, M. S., Pal, A. K., Sahu, N. P., Ciji, A., Meena, D. K., & Das, P. (2013). Physiological responses of dietary tryptophan fed *Labeo rohita* to temperature and salinity stress. *Journal of Animal Physiology and Animal Nutrition*, *97*(6), 1075-1083. https://doi.org/10.1111/jpn.12017.

Arjona, F. J., Vargas-Chacoff, L., del Rio, M. P. M., Flik, G., Mancera, J. M., & Klaren, P. H. M. (2008). The involvement of thyroid hormones and cortisol in the osmotic acclimation of *Solea senegalensis*. *General and Comparative Endocrinology*, *155*(3), 796-803. https://doi.org/10.1016/j.ygcen.2007.09.007.

Basu, N., Nakano, T., Grau, E. G., & Iwama, G. K. (2001). The effects of cortisol on heat shock protein 70 levels in two fish species. *General and Comparative Endocrinology*, *124*(1), 97-105. https://doi.org/10.1006/gcen.2001.7688.

Chen, P., Yao, L., Yuan, M., Wang, Z., Zhang, Q., Jiang, Y., & Li, L. (2024). Mitochondrial dysfunction: A promising therapeutic target for liver diseases. *Genes & Diseases*, *11*(3), Article 101115. https://doi.org/10.1016/j.gendis.2023.101115.

Daniel, R. M., Dines, M., & Petach, H. H. (1996). The denaturation and degradation of stable enzymes at high temperatures. *Biochemical Journal*, *317*(1), 1-11. https://doi.org/10.1042/bj3170001.

Ellman, G. L., Courtney, K. D., Andres, V., & Featherstone, R. M. (1961). A new and rapid colorimetric determi-

nation of acetylcholinesterase activity. *Biochemical Pharmacology*, *7*(2), 88-95. https://doi.org/10.1016/0006-2952(61)90145-9.

Ern, R., Andreassen, A. H., & Jutfelt, F. (2023). Physiological mechanisms of acute upper thermal tolerance in fish. *Physiology*, *38*(3), 141-158. https://doi.org/10.1152/physiol.00027.2022.

Fabbri, E., & Moon, T. W. (2016). Adrenergic signaling in teleost fish liver, a challenging path. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology*, *199*, 74-86. https://doi.org/10.1016/j.cbpb.2015.10.002.

Flohé, L., & Günzler, W. A. (1984). Assays of glutathione peroxidase. In L. Packer (Ed.), *Methods in enzymology* (Vol. 105, pp. 114-120). Academic Press.

Giroux, M., Gan, J., & Schlenk, D. (2019). The effects of bifenthrin and temperature on the endocrinology of juvenile Chinook salmon. *Environmental Toxicology and Chemistry*, *38*(4), 852-861. https://doi.org/10.1002/etc.4372.

Gorissen, M., & Flik, G. (2016). The endocrinology of the stress response in fish: an adaptation-physiological view. In C. B. Schreck, L. Tort, A. P. Farrell, & C. J. Brauner (Eds.), *Fish physiology* (Vol. 35, pp. 75-111). Academic Press.

Jobling, M. (1997). Temperature and growth: modulation of growth rate via temperature. In C. M. Wood, & D. G. McDonald (Eds.), *Global warming: Implication for freshwater and marine fish* (pp. 225-253).

Kim, S., Kim, K., Lee, J. H., Han, S. H., & Lee, S. H. (2019). Differential expression of acetylcholinesterase 1 in response to various stress factors in honey bee workers. *Scientific Reports*, *9*(1), Article 10342. https://doi.org/10.1038/s41598-019-46842-0.

Liu, Y., Liu, J., Ye, S., Bureau, D. P., Liu, H., Yin, J., Mou, Z., Lin, H., & Hao, F. (2019). Global metabolic responses of the lenok (*Brachymystax lenok*) to thermal stress. *Comparative Biochemistry and Physiology Part D: Genomics and Proteomics*, *29*, 308-319. https://doi.org/10.1016/j.cbd.2019.01.006.

Ma, S., Lv, Y., Hou, L., Jia, Z., Lin, S., Wang, S., He, X., & Hou, J. (2023). Effect of acute temperature stress on energy metabolism, immune performance and gut microbiome of largemouth bass (*Micropterus salmoides*). *Aquaculture and Fisheries*, *10*(2), 260-270. https://doi.org/10.1016/j.aaf.2023.10.001.

Ma, B., Zhang, L., Li, J., Xing, T., Jiang, Y., & Gao, F. (2021). Heat stress alters muscle protein and amino acid metabolism and accelerates liver gluconeogenesis for energy supply in broilers. *Poultry Science*, *100*(1), 215-223. https://doi.org/10.1016/j.psj.2020.09.090.

Mannur, V. S., Kumar, S., Varghese, T., Satyakumar, A., Sardar, P., Deo, A. D., Kumar, H. S., Patel, A., & Mohanta, K. N. (2025). Reducing nitrogenous waste in RAS culture of gift fingerlings through dietary manipulation of protein and lipid levels. *Fishery Technology*, *62*(2), 297-306. https://doi.org/10.56093/ft.v62i3.164189.

Martínez-Álvarez, R. M., Morales, A. E., & Sanz, A. (2005). Antioxidant defenses in fish: biotic and abiotic factors. *Reviews in Fish Biology and Fisheries*, *15*, 75-88. https://doi.org/10.1007/s11160-005-7846-4.

Misra, H. P., & Fridovich, I. (1972). The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. *Journal of Biological Chemistry*, *247*(10), 3170-3175. https://doi.org/10.1016/S0021-9258(19)45228-9.

Morales, A. E., Pérez-Jiménez, A., Hidalgo, M. C., Abellán, E., & Cardenete, G. (2004). Oxidative stress and antioxidant defenses after prolonged starvation in *Dentex dentex* liver. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology*, *139*(1-3), 153-161. https://doi.org/10.1016/j.cca.2004.10.008.

Musa, N., Low, C. F., Ramli, H. R., Manaf, M. T. A., Lee, K. L., Tan, S. H. A., Aznan, A. S., & Musa, N. (2021). Prominent vulnerability of red hybrid tilapia (*Oreochromis* spp.) liver to heat stress-induced oxidative damage. *International Aquatic Research*, *13*(2), 109-118. https://doi.org/10.22034/iar.2021.1924489.1140.

Panase, P., Saenphet, S., & Saenphet, K. (2018). Biochemical and physiological responses of Nile tilapia *Oreochromis niloticus* Lin subjected to cold shock of water temperature. *Aquaculture Reports*, *11*, 17–23. https://doi.org/10.1016/j.aqrep.2018.05.005.

Panase, P., Saenphet, S., Saenphet, K., Pathike, P., & Thainum, R. (2019). Biochemical and physiological responses of Nile tilapia (*Oreochromis niloticus* Linn.) subjected to rapid increases of water temperature. *Comparative Clinical Pathology*, *28*, 493-499. https://doi.org/10.1007/s00580-018-2872-5.

Roychowdhury, P., Aftabuddin, M., & Pati, M. K. (2020). Thermal stress altered growth performance and metabolism and induced anaemia and liver disorder in *Labeo rohita*. *Aquaculture Research*, *51*(4), 1406-1414. https://doi.org/10.1111/are.14486.

Sarkar, U. K., Paul, T. T., Puthiyottil, M., Karnatak, G., Das, V. G., Lianthuamluaia, Bandyopadhyay, A., Bakshi, S., & Das, B. K. (2022). Inland heat waves (IHWs) and associated impacts on hydro-biology of aquatic ecosystems in lower Ganga basin, India. *International Journal of Biometeorology*, *66*, 235-245. https://doi.org/10.1007/s00484-021-02210-0.

Seo, Y. S., Lee, H. B., Jeong, J. H., Mun, S. J., & Lim, H. K. (2023). Change in plasma cortisol and glucose levels of *Oncorhynchus keta* according to water temperature. *Fisheries and Aquatic Sciences*, *26*(2), 117-132. https://doi.org/10.47853/FAS.2023.e10.

Slimen, I. B., Najar, T., Ghram, A., Dabbebi, H., Mrad, M. B., & Abdrabbah, M. (2014). Reactive oxygen species, heat stress and oxidative-induced mitochondrial damage: A review. International Journal of Hyperthermia, 30(7), 513-523. https://doi.org/10.3109/02656736.2014.971446.

Somero, G. N., Lockwood, B. L., & Tomanek, L. (2016). *Biochemical adaptation: Response to environmental challenges, from life's origins to the Anthropocene.* Oxford University Press.

Takahara, S., Hamilton, H. B., Neel, J. V., Kobara, T. Y., Ogura, Y., & Nishimura, E. T. (1960). Hypocatalasemia: a new genetic carrier state. *The Journal of Clinical Investigation*, *39*(4), 610-619. https://doi.org/10.1172/JCI104075.

Vazzana, M., Cammarata, M., Cooper, E. L., & Parrinello, N. (2002). Confinement stress in sea bass (*Dicentrarchus labrax*) depresses peritoneal leukocyte cytotoxicity. *Aquaculture*, *210*(1-4), 231-243. https://doi.org/10.1016/S0044-8486(01)00818-3.

WMO. (2025). *State of the Global Climate 2024*. World Meteorological Organization, Geneva. Retrieved from https://library.wmo.int/idurl/4/69455.

Wooten, I. D. P. (1964). *Microanalysis in medical biochemistry*. J & A Churchill Ltd.

Zivkovic, A. R., Paul, G. M., Hofer, S., Schmidt, K., Brenner, T., Weigand, M. A., & Decker, S. O. (2023). Increased enzymatic activity of acetylcholinesterase indicates the severity of the sterile inflammation and predicts patient outcome following traumatic injury. *Biomolecules*, *13*(2), Article 267. https://doi.org/10.3390/biom13020267.