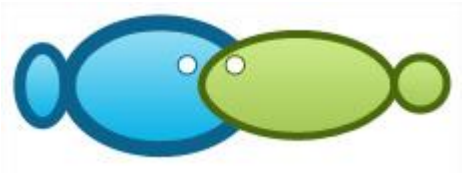

Exploring physiological and histological responses of juvenile red spotted grouper (*Epinephelus akaara*) under varied water temperatures

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Abstract. The current research aimed to examine how red spotted grouper, *Epinephelus akaara*, responds physiologically and histologically to different water temperatures, considering the impact of rising temperatures due to climate change. Juvenile *E. akaara* (total length, TL: 8.28±0.10 cm and body weight, BW: 8.53±0.27 g) were exposed to different water temperature regimes (25°C as control, 28°C, 31°C, and 34°C) for 42 days after a 2-week acclimation period at 25°C. Blood and tissue samples were collected at three intervals (2, 7, and 42 days) from a total of 180 fish. Hematolgical results showed that higher temperatures (31°C and 34°C) led to increased red blood cell count and hemoglobin levels. Additionally, biochemical analysis revealed elevated levels of glucose, cortisol, glutamic pyruvic transaminase (GPT), glutamic oxaloacetic transaminase (GOT), lactate dehydrogenase (LDH) and alkaline phosphatase (ALP) along with decreased total protein (TP), triglyceride (TG) and total cholesterol (TCHO) levels in the 34°C test group compared to other temperature groups (25°C, 28°C, and 31°C) ($p < 0.05$). Histological examination demonstrated abnormalities in gill and liver tissues in the 34°C group, including hyperplasia and curling in secondary gill lamellae, as well as hepatocyte coalescence, vacuolization, and sinusoid dilation in the liver. These findings indicate that exposure to 34°C induces significant physiological and histological changes in *E. akaara*, suggesting it as a sub-lethal temperature, while 31°C also causes disturbances after prolonged exposure.

Key Words: biochemical response, elevated water temperature, hematological response, histological response, red spotted grouper.

Introduction. Water temperature plays a pivotal role in aquaculture, with its fluctuations being influenced by both natural phenomena (like climate change) and human activities. This increase in temperature is a major concern for those involved in aquaculture and fisheries (Langford 2001; Somero 2010). Fluctuations in temperature can significantly disrupt the normal physiological functions, survival rates, and growth of teleost fish species (Person-Le Ruyet et al 2004; Fazio et al 2018). Moreover, temperature variations can impact metabolic processes (Lu et al 2016), weaken the innate immune system (Qiang et al 2013), and heighten susceptibility to diseases (Karvonen et al 2010). Essentially, temperature variations affect nearly all biochemical and physiological functions in animals. Thermal stress emerges as a significant environmental challenge/hurdle that fish face (Portner & Peck 2010).

Fish are highly sensitive to temperature changes, which can lead to various physiological and morphological adaptations (Mora & Maya 2006). The hematological and biochemical parameters are reliable markers for assessing fish responses to both internal and external stressors (Cataldi et al 1998; Lermen et al 2004; Fazio et al 2018; Panase et al 2019). Cortisol, the main stress hormone, is released by the hypothalamus-pituitary-interrenal (HPI) axis when fish face environmental disruptions. When fish experience prolonged or acute thermal stress, cortisol levels rise, causing an increase in plasma glucose to meet the heightened energy demands (Lima et al 2006). Enzyme activities such as glutamic pyruvic transaminase (GPT), glutamic oxaloacetic

transaminase (GOT), and lactate dehydrogenase (LDH) in the blood serve as biomarkers for monitoring fish's physiological reactions to thermal stress (Cheng et al 2018). Alterations in total protein concentrations in plasma indicate liver dysfunction (Fırat & Kargın 2010), while blood lipids are considered important indicators of chronic stress during thermal fluctuations (Ming et al 2012). Additionally, fish may undergo morphological alterations in the gills and liver tissues while responding against prolonged or abrupt thermal exposure (Liu et al 2015; Hernandez-Lopez et al 2018).

Groupers typically thrive within the temperature range of 24 to 30°C (Heemstra & Randall 1993). *Epinephelus akaara*, commonly known as the red spotted grouper, belongs to the Serranidae family and is predominantly found in the southern regions of Japan, Korea, Southern China, Hong Kong, and Taiwan (Heemstra & Randall 1993). Among various grouper species, *E. akaara* holds notable commercial significance in Southeast Asia due to its high market demand (Rimmer et al 2004). However, natural landings of this species are insufficient to meet the growing demand. Overexploitation has led to decreased populations of this species, rendering natural stocks vulnerable (Tupper & Sheriff 2008). Hence, aquaculture can secure the supply of this highly sought-after species. Juvenile *E. akaara* typically inhabit shallow coastal areas in their natural habitat, making them particularly susceptible to fluctuations in water temperature (Sadovy & Cornish 2000). Therefore, from an aquaculture perspective, it is crucial to understand how thermal fluctuations, particularly elevated water temperatures, affect the physiology and histology of *E. akaara* after prolonged exposure.

Numerous studies have investigated how water temperature affects blood biochemical and hematological parameters in different species (Shahjahan et al 2018; De et al 2019; Islam et al 2019; Mattioli et al 2019; Panase et al 2019). However, there's a knowledge gap regarding this aspect in *E. akaara*. Previous research on *E. akaara* focused on a narrow temperature range (24-28°C) to study physiological and growth responses (Lee & Baek 2018). Yet, a more comprehensive examination of *E. akaara*'s physiological and histological reactions across a wider thermal spectrum is essential, especially considering rising water temperatures due to climate change. Thus, this study aims to deepen our understanding of how *E. akaara* responds physiologically and histologically to different rearing temperatures. The findings can aid in monitoring the physiological condition of *E. akaara* and contribute to better management practices in grouper aquaculture.

Material and Method

Procurement and maintenance of experimental fish. Juvenile *E. akaara* were acquired from the Marine Science Institute at Jeju National University, Korea, and housed in the laboratory of the Marine Biology Department at Pukyong National University (PKNU), Busan, Korea. Upon their arrival at the laboratory, the fish underwent an immediate 30 ppm oxytetracycline dip (Chamshin Pharma Co. Ltd., Seoul, Korea). Prior to the experiment, the fish were acclimated for a period of two weeks. The study was conducted over two months, from June to July 2020. All procedures related to the maintenance, handling, and sampling of the fish followed the guidelines set forth by the Animal Ethics Committee of Pukyong National University (PKNU) (Regulation No. 554).

Experimental setup and thermal exposure. In this study, 180 juvenile *E. akaara* (with a total length, TL, of 8.28±0.10 cm and body weight, BW, of 8.53±0.27 g) were exposed to four distinct water temperatures (25°C as control, 28°C, 31°C, and 34°C), with three replications for each temperature, over a period of 42 days. The fish were randomly allocated into 12 glass tanks, each with a volume of 120 L and capable of accommodating 15 fish. All aquaria were uniform in size (75 cm × 45 cm × 45 cm) and equipped with a recirculating filtration system. The experimental tank temperature was gradually increased by 1°C per hour using a thermostat (OKE-6422H; OKE, Busan, Korea) until reaching the desired experimental temperature. The experimental phase began upon achieving the target temperature.

Using a water quality meter (HI9829; Hanna Instrumentals, USA), water parameters such as temperature, dissolved oxygen (DO), salinity, and pH were monitored daily throughout the study period. Additionally, ammonium levels were quantified at two-day intervals with a NH_3/NH_4^+ test kit (Tetra GmbH, Germany). The water quality parameters recorded during the experimental period are detailed in Table 1. During both the acclimation and experimental phases, the fish in the experimental were provided with a commercial diet (Marubeni Nisshin Feed Co., Ltd., Japan; containing 50~52% protein and 7~10% lipids) twice a day, at 09:00 and 18:00 hours, until they were fully satiated. Each day, approximately 10% of the water in the tank was replaced, and any waste material and debris were removed from the tank using a siphon.

Table 1

Water quality parameters (mean±SEM) measured during the experimental period

Parameters	*Temperature groups*			
	25°C	*28°C*	*31°C*	*34°C*
Temperature	24.89±0.04	27.77±0.04	30.90±0.05	33.78±0.04
Salinity (psu)	33.80±0.05	33.89±0.06	33.72±0.05	33.77±0.07
pH	8.05±0.02	8.06±0.01	7.99±0.02	7.99±0.02
DO (mg L^{-1})	6.86±0.04	6.72±0.04	5.68±0.05	4.16±0.03
NH_4 (mg L^{-1})	< 0.25	< 0.25	< 0.25	< 0.25

Sampling procedure. Sampling was conducted at 2, 7, and 42 days' post thermal exposure, following a 24-hour fasting period for all fish. Fifteen fish were randomly chosen from each temperature group (5 fish from each aquarium; n = 15) on each sampling day. Prior to blood collection, the fish were gently anaesthetized using 300 mg L^{-1} 2-phenoxyethanol (Sigma Aldrich, St. Louis, MO, USA). Following morphometric measurements (TL and BW), blood samples were obtained from the caudal vein using capillary tubes treated with heparin. These samples were then properly labelled, and placed in 1.5 mL centrifuge tubes for subsequent hematological and biochemical analysis. Simultaneously, gill and liver tissues were surgically excised on ice and stored for histological examination.

Hematological analysis. Hematocrit (Ht) values were determined by centrifuging blood samples in glass capillary tubes for 5 minutes at 12,000 rpm using a microhematocrit centrifuge apparatus (VS-12000; Vision Co. Ltd., Korea). Hemoglobin (Hb) levels were quantified by applying a 10 µL blood sample onto Hb slides and analyzing them with an automatic analyzer (FUJI DRI-CHEM 400i, Japan). Red blood cell (RBC) count was performed by examining the samples under a microscope with the assistance of a hemocytometer, employing Hayem diluting fluid (Ricca Chemical Co., USA). Mean corpuscular volume (MCV), mean cell hemoglobin (MCH), and mean cell hemoglobin concentration (MCHC) were computed based on the average Hb % values (Dacie & Lewis 1984).

Biochemical analysis. Upon collection, plasma was promptly separated from blood samples through centrifugation at 4°C (15 min at 13,000 g), and the resulting supernatant was preserved at −70°C for subsequent analysis. Glucose, glutamic pyruvic transaminase (GPT), glutamic oxaloacetic transaminase (GOT), total protein (TP), triglyceride (TG), total cholesterol (TCHO), lactate dehydrogenase (LDH) and alkaline phosphatase (ALP) concentrations in the obtained plasma samples were quantified using an automated analyzer, which had previously been validated as an appropriate fish plasma analysis method (Krome 2014).

Radioimmunoassay. To quantify plasma cortisol levels, steroids were twice extracted with 2 mL of diethyl ether. The extracted steroids samples were then desiccated with nitrogen gas and reconstituted in a phosphate buffer with a pH of 7.5. Cortisol values were assessed using radioimmunoassay (RIA), adopting the methodology established by

Kobayashi & Mikuni (1987). The antiserum for cortisol was obtained from Cosmo-Bio Co. Ltd., Japan, while non-radioactive steroid standards were purchased from Steraloids Inc., USA. The radio-labeled steroids ([3H]-cortisol) were procured from Amersham Life Sciences, Piscataway, USA).

Gill and liver histology. For histological examinations, gills and liver tissues were promptly dissected and preserved in 10% neutral formalin. Subsequently, they underwent a dehydration process involving a series of graded ethanol concentrations, and were embedded in paraffin to create tissue blocks. Sections with a 5 µm thickness were stained using Mayer's Hematoxylin and Eosin (H & E) and subsequently examined under a light microscope (BX-50, Olympus, Japan).

Statistical procedure. Data analysis was conducted using SPSS Statistics software (ver. 21.0; IBM Corp., USA). Unless otherwise specified, all data were expressed as mean± standard error of mean (SEM). Prior to statistical analysis, we verified normality through the Shapiro–Wilk test, and ensured homogeneity of variance through Levene's test. To assess the impact of various temperature treatments on the hemato-biochemical parameters at various sampling intervals, a one-way analysis of variance (ANOVA) was employed. Using Duncan's multiple range test ($p < 0.05$), significant distinctions among the treatment groups were observed.

Results

Behavioral changes and mortality. Fish subjected to 34°C displayed rapid opercular movements and erratic body motions. Mortality was observed within the 34°C group 42 days after thermal exposure (Table 2). Conversely, fish within the 25°C, 28°C, and 31°C groups exhibited regular body movements without any apparent signs of external distress.

Table 2

Survival rate (%) of juvenile red spotted grouper, *Epinephelus akaara*, exposed to varying water temperatures (25°C, 28°C, 31°C, and 34°C) for a duration of 42 days

	Treatments	*Exposure time (day)*		
		2	*7*	*42*
Survival (%)	25°C	100.00±0.00[a]	100.00±0.00[a]	100.00±0.00[a]
	28°C	100.00±0.00[a]	100.00±0.00[a]	100.00±0.00[a]
	31°C	100.00±0.00[a]	100.00±0.00[a]	100.00±0.00[a]
	34°C	100.00±0.00[a]	100.00±0.00[a]	80.95±4.76[b]

Data are expressed as mean±SEM of three replicates (n = 15); different superscript letters within the same column denote significant differences among treatment groups at corresponding time intervals (ANOVA, Duncan's multiple range test; $p < 0.05$).

Hematological changes. The findings from the hematological analysis are presented in Table 3. There were no significant effects ($p > 0.05$) of temperature variations on Hb, Ht, and MCHC in juvenile *E. akaara* throughout the experimental duration. However, erythrocyte counts notably increased in the 34°C group at each sampling interval (2, 7, and 42 days) compared to other temperature groups (25°C, 28°C, and 31°C), coupled with significant alterations in MCV and MCH values ($p < 0.05$). Furthermore, changes in erythrocyte count, MCV, and MCH were observed in the 31°C group after 42 days of exposure, although these changes were not statistically significant for MCV and MCH ($p > 0.05$; Table 3).

Table 3

The hematological values (HB, Ht, RBC, MCV, MCH, and MCHC) of blood samples obtained from juvenile red spotted grouper, *Epinephelus akaara*, subjected to different water temperatures (25 °C, 28 °C, 31 °C, and 34 °C) over a 42-day period

Parameters	*Treatments*	*Exposure time (day)*		
		2	*7*	*42*
Ht (%)	25°C	36.92±0.68^{b}	32.55±0.62^{b}	34.78±0.22^{c}
	28°C	36.83±1.08^{b}	35.78±0.62^{ab}	36.67±0.84^{bc}
	31°C	38.17±1.02^{ab}	34.11±1.82^{ab}	38.89±0.59^{ab}
	34°C	40.92±1.16^{a}	36.34±0.67^{a}	40.13±0.99^{a}
Hb (mg dL^{-1})	25°C	7.84±0.11^{a}	7.80±0.18^{c}	7.62±0.09^{b}
	28°C	7.83±0.27^{a}	7.84±0.16^{bc}	8.69±0.09^{a}
	31°C	8.23±0.33^{a}	8.23±0.04^{ab}	8.90±0.15^{a}
	34°C	8.39±0.12^{a}	8.68±0.13^{a}	9.18±0.27^{a}
RBC (×10^{6} mm^{-3})	25°C	3.99±0.15^{b}	3.89±0.05^{bc}	3.68±0.04^{c}
	28°C	3.92±0.09^{b}	3.75±0.16^{c}	3.61±0.07^{c}
	31°C	4.06±0.19^{b}	4.16±0.09^{b}	4.53±0.19^{b}
	34°C	5.18±0.24^{a}	5.14±0.04^{a}	5.30±0.09^{a}
MCV (fl)	25°C	93.25±5.46^{a}	83.71±1.78^{b}	95.25±1.84^{ab}
	28°C	94.90±4.62^{a}	95.83±3.87^{a}	101.80±0.74^{a}
	31°C	94.62±2.27^{a}	78.60±1.95^{bc}	86.53±4.08^{b}
	34°C	79.60±2.08^{b}	71.97±1.19^{c}	76.01±2.05^{c}
MCH (pg)	25°C	19.72±0.88^{ab}	20.07±0.37^{a}	20.89±0.48^{b}
	28°C	20.16±1.03^{ab}	20.97±0.45^{a}	24.11±0.53^{a}
	31°C	20.54±1.69^{a}	20.03±0.33^{a}	19.85±1.13^{b}
	34°C	16.31±0.82^{b}	16.87±0.25^{b}	17.34±0.36^{c}
MCHC (g dL^{-1})	25°C	21.33±0.38^{a}	24.04±0.26^{ab}	21.96±0.19^{a}
	28°C	21.33±0.19^{a}	22.02±0.49^{c}	23.78±0.37^{a}
	31°C	21.75±1.56^{a}	25.54±0.39^{a}	22.97±0.61^{a}
	34°C	20.58±0.83^{a}	23.58±0.79^{bc}	22.97±1.11^{a}

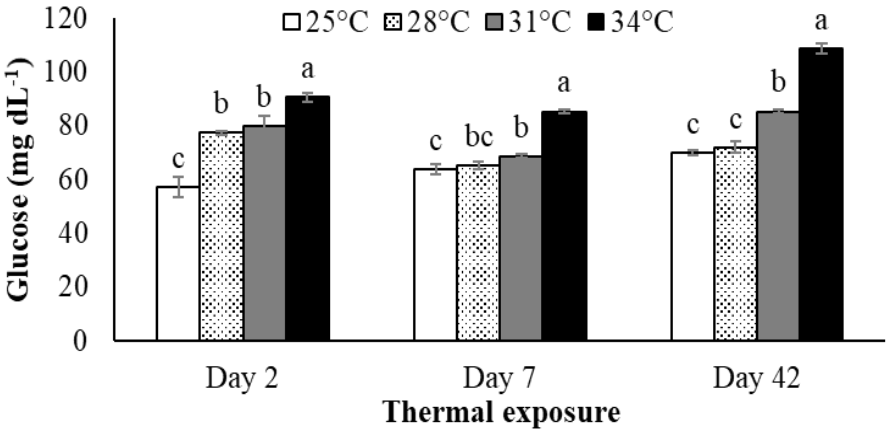
Data are expressed as mean±SEM of three replicates (n = 15); different superscript letters within the same column denote significant differences among treatment groups at corresponding time intervals (ANOVA, Duncan's multiple range test; $p < 0.05$).

Biochemical changes. Following exposure to thermal stress, there was a significant increase ($p < 0.05$) in plasma levels of glucose, cortisol, GPT, and GOT in the 34°C group compared to the other groups 25°C and 28°C) throughout the 42-day exposure period. Notably, in the 31°C group, a significant increase ($p < 0.05$) was observed after 2 and 42 days of sampling (Figures 1A-D).

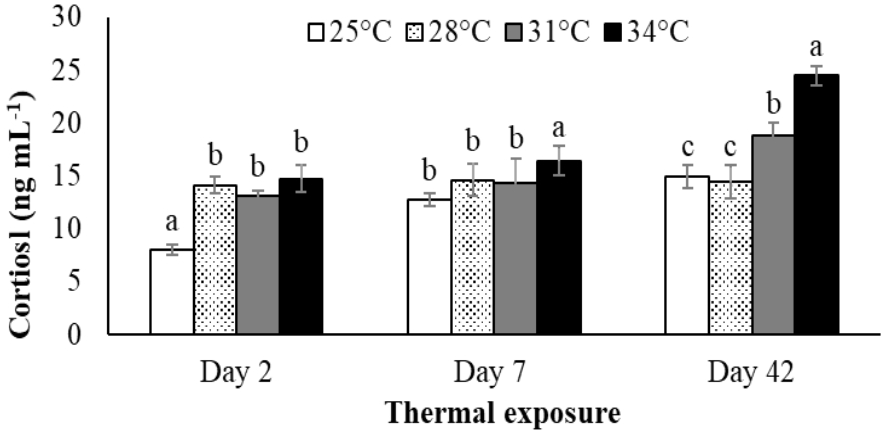
Plasma concentrations of TP, TG, and TCHO were significantly lower in the 34°C group ($p < 0.05$) at each sampling interval (2, 7, and 42 days) compared to the other temperature groups (25°C, 28°C, and 31°C) (Figures 1E-G). Additionally, decreased TG and TCHO values ($p < 0.05$) were noted in the 31°C group after 42 days of exposure (Figures 1F-G).

Plasma LDH enzyme levels were significantly higher ($p < 0.05$) in the 31°C and 34°C groups compared to the 25°C and 28°C groups after 2 days of exposure. However, LDH levels normalized ($p > 0.05$) in the 31°C group after 7 and 42 days, while continuing to increase in the 34°C group (Figure 1H). Furthermore, ALP levels exhibited significantly higher values ($p < 0.05$) in the 31°C and 34°C groups compared to the 25°C and 28°C groups at each sampling point (Figure 1I).

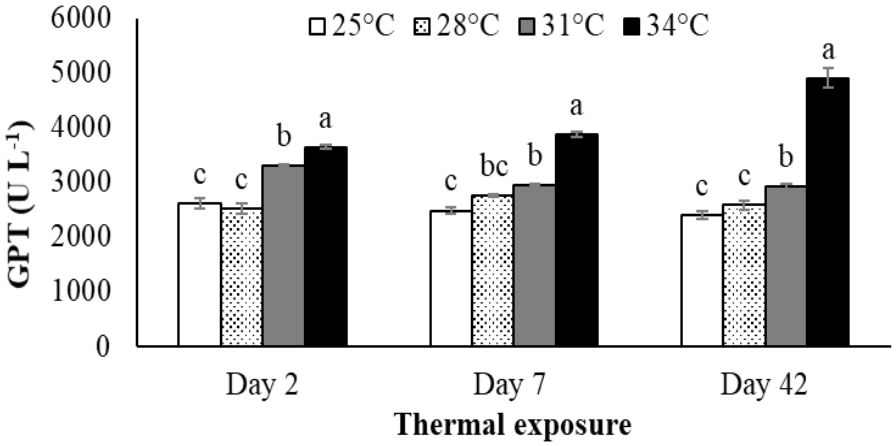
A


25°C 28°C 31°C 34°C
Glucose (mg dL-1)
120
100
80
60
40
20
0
c b b a
c bc b a
c c b a
Day 2
Day 7
Day 42
Thermal exposure

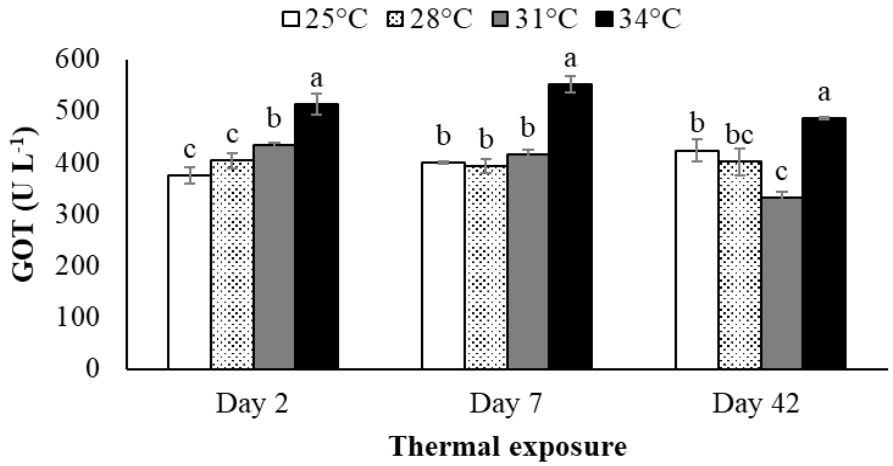
B


25°C 28°C 31°C 34°C
Cortiosl (ng mL-1)
30
25
20
15
10
5
0
a b b b
b b b a
c c b a
Day 2
Day 7
Day 42
Thermal exposure

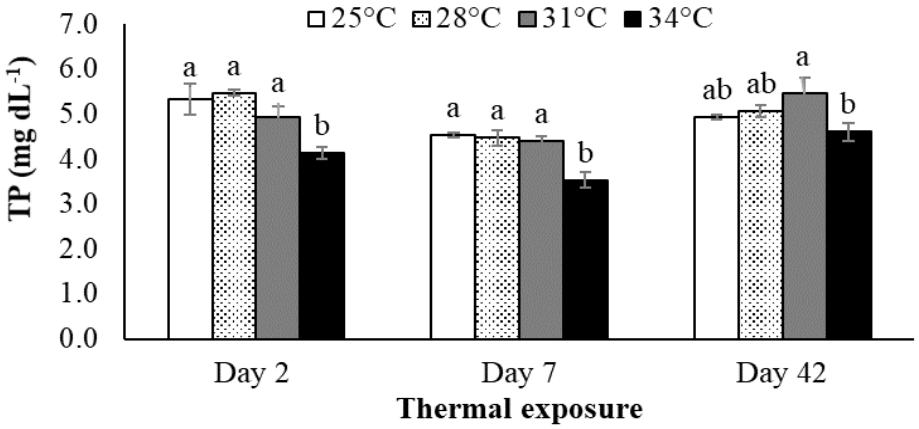
C


25°C 28°C 31°C 34°C
GPT (U L-1)
6000
5000
4000
3000
2000
1000
0
c c b a
c bc b a
c c b a
Day 2
Day 7
Day 42
Thermal exposure

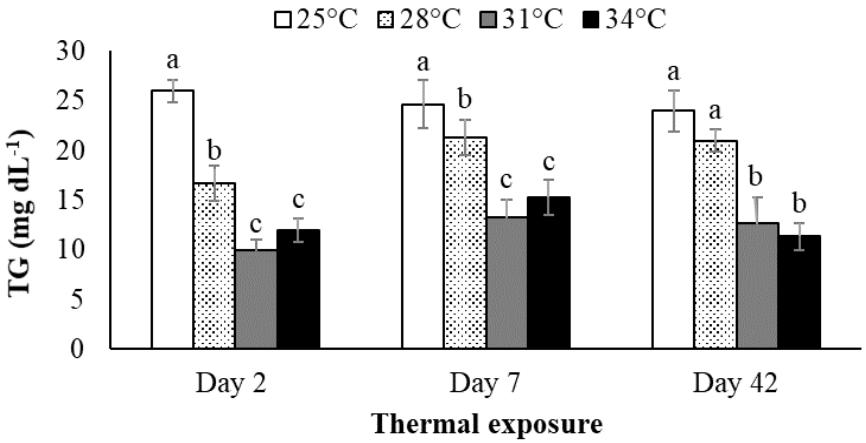
D


25°C 28°C 31°C 34°C
GOT (U L-1)
600
500
400
300
200
100
0
c c b a
b b b a
b bc c a
Day 2
Day 7
Day 42
Thermal exposure

E


25°C 28°C 31°C 34°C
TP (mg dL-1)
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
a a a b
a a a b
ab ab a b
Day 2
Day 7
Day 42
Thermal exposure

F


25°C 28°C 31°C 34°C
TG (mg dL-1)
30
25
20
15
10
5
0
a b c c
a b c c
a a b b
Day 2
Day 7
Day 42
Thermal exposure

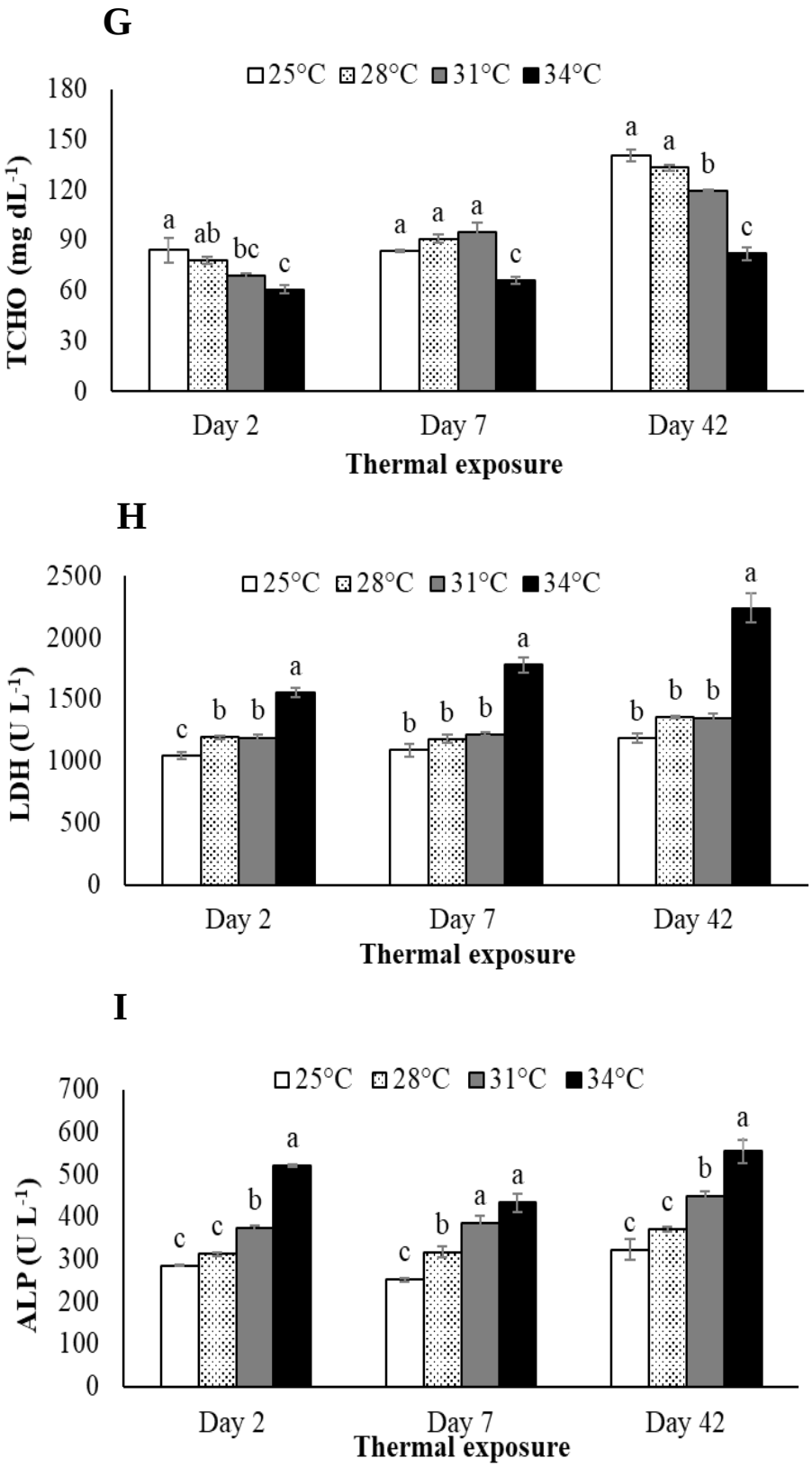
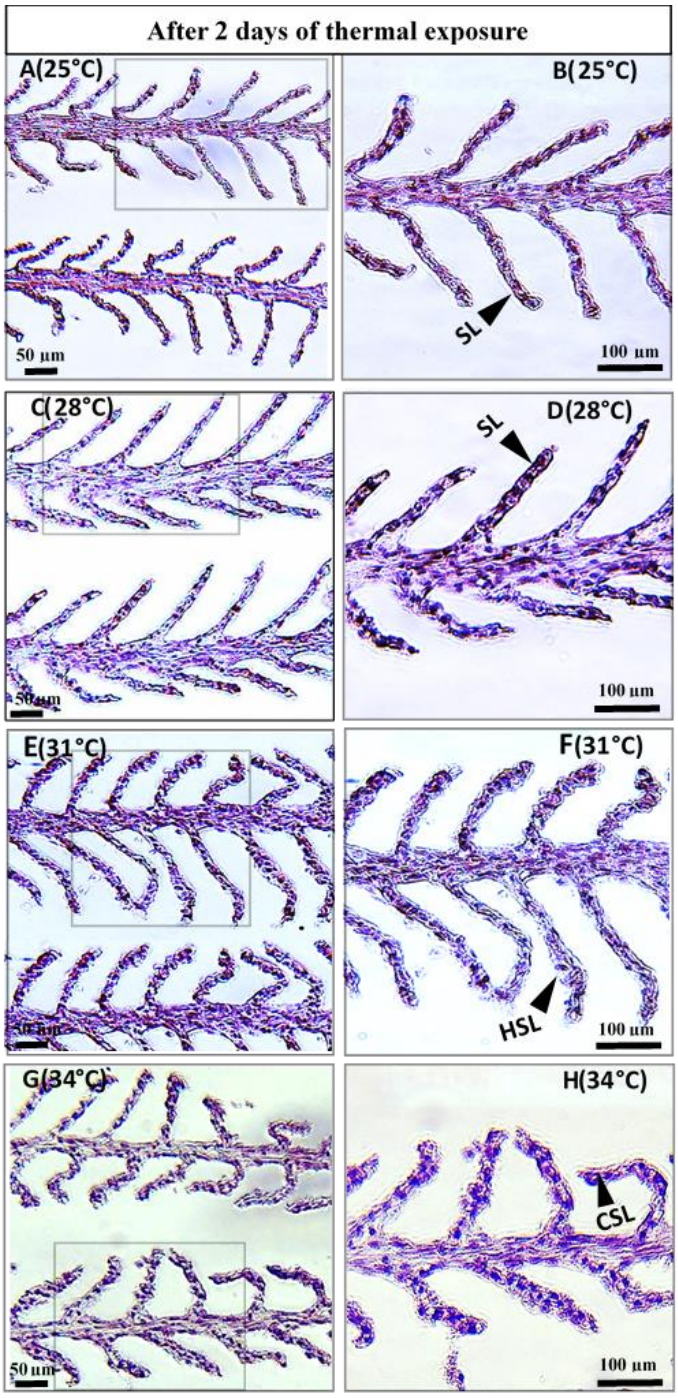


Figure 1. A-I. The biochemical values of A. glucose, B. cortisol, C. GPT, D. GOT, E. TP, F. TG, G. TCHO, H. LDH, and I. ALP in blood samples obtained from juvenile red spotted grouper, *Epinephelus akaara*, subjected to various water temperatures (25°C, 28°C, 31°C, and 34°C) over a period of 42 days. The values are expressed as mean±SEM of three replicates (n = 15). Distinct lowercase letters indicate significant differences among the temperature groups at corresponding time points (ANOVA, Duncan's multiple range test; $p < 0.05$).

Histological changes in gill tissue. The impact of elevated temperature was notably observed in the gills of the 34°C group compared to those in the 25°C, 28°C, and 31°C groups. Following 2 days of exposure, well-organized secondary gill lamellae (SL) were observed in the 25°C and 28°C groups (Figures 2A-D). However, hyperplasia of secondary gill lamellae (HSL) was evident in the 31°C group (Figures 2E-F), while curling of secondary gill lamellae (CSL) was observed in the 34°C group after 2 days of thermal exposure (Figures 2G-H). No noticeable changes were observed among the temperature-treated groups after 7 days of exposure (Figure not shown). However, after 42 days, significant histological alterations were noted in the gills of the 34°C group due to prolonged thermal stress. These alterations included hyperplasia in secondary gill lamellae (HSL), telangiectasia (TL), swelling of primary gill lamellae (SPL), and curling of secondary gill lamellae (CSL) (Figures 2I-N). Conversely, no significant changes were observed in the 25°C, 28°C, and 31°C temperature groups (Figure not shown).



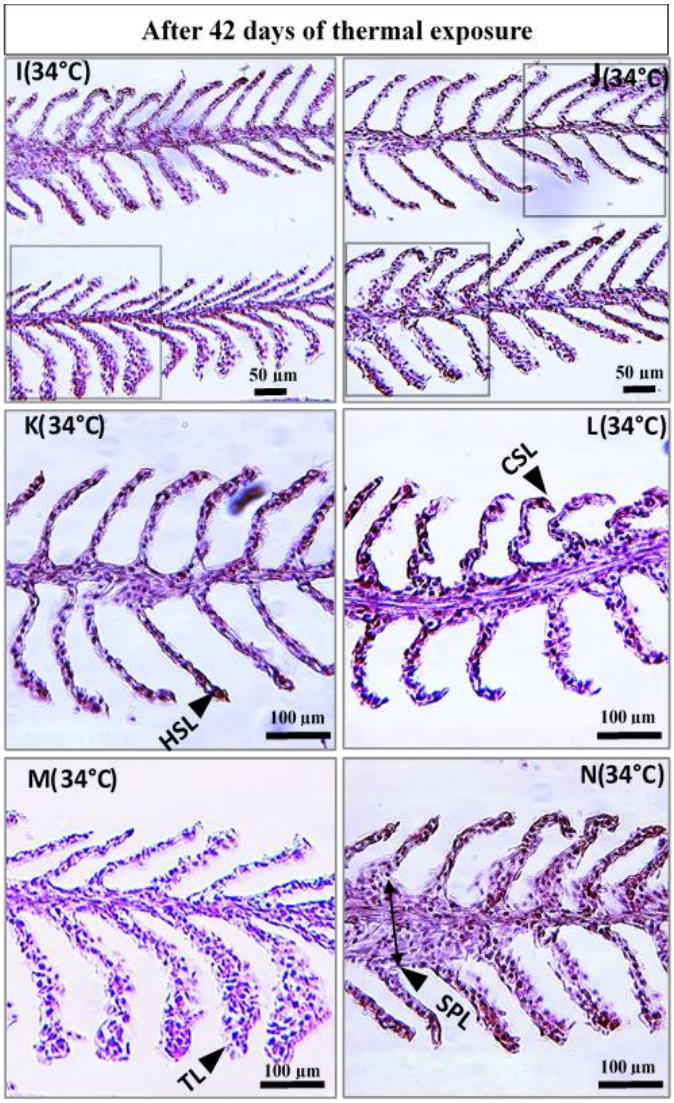
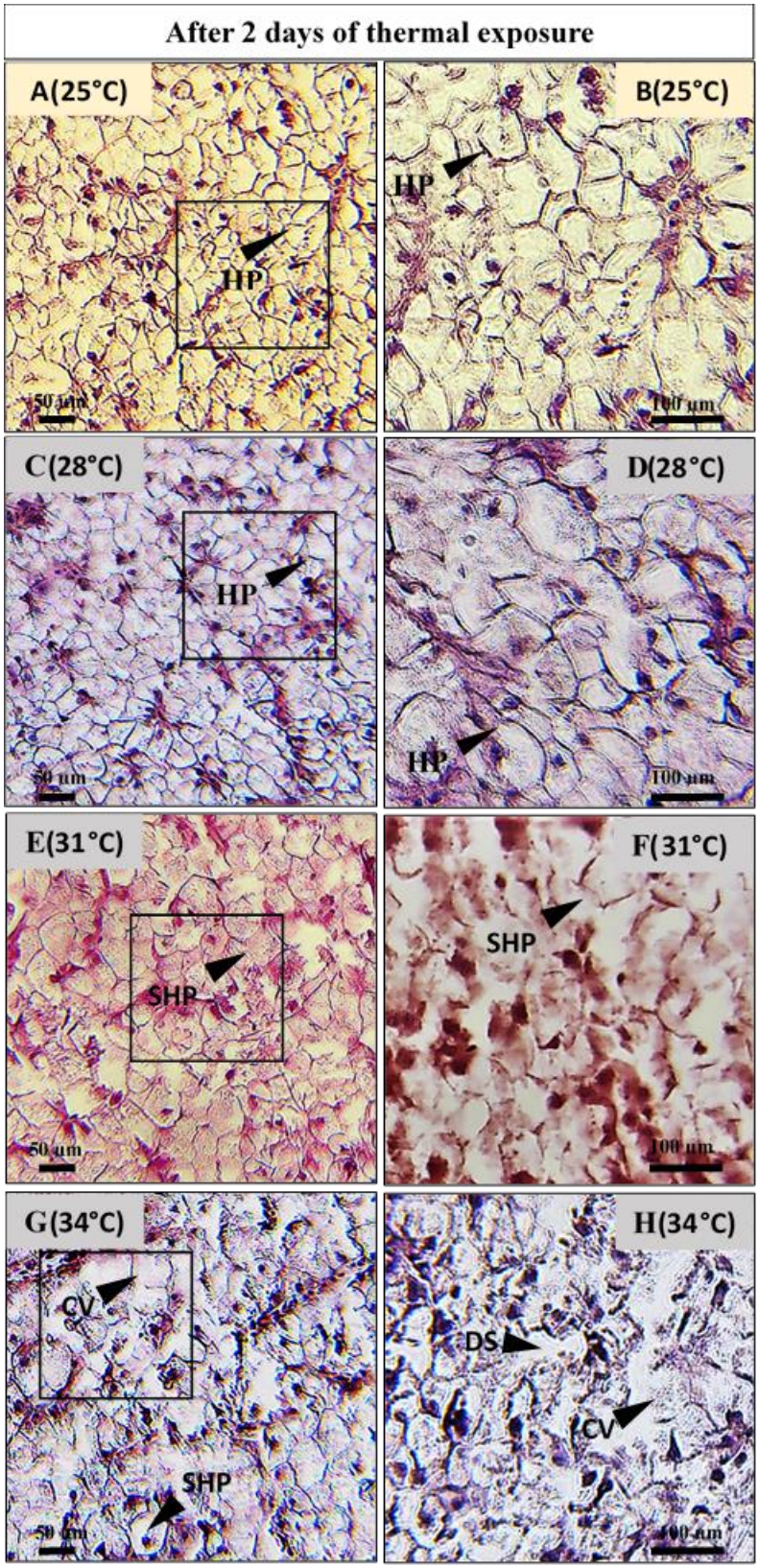


Figure 2 (A-N). The histological changes observed in the gills of red spotted grouper, *Epinephelus akaara*, subjected to various water temperatures (25°C, 28°C, 31°C, and 34°C) over a period of 42 days. A-B: 25°C group (Control); C-D: 28°C group; E-F: 31°C group; G-H: 34°C group after 2 days of thermal exposure; I-N: 34°C group after 42 days of thermal exposure; SL, secondary gill lamellae; HSL, hyperplasia of secondary gill lamellae; CSL, curling of secondary gill lamellae; TL, telangiectasia; SPL, swelling of primary gill lamellae. Scale bars = 50 and 100 µm.

Histological changes in liver tissue. Histological examination of the liver revealed substantial impacts of elevated water temperatures, particularly evident in the 34°C group compared to the other temperature groups (25°C, 28°C, and 31°C) following 42 days of thermal exposure. At the 2-day mark, nucleated hepatocytes (HP) were observed in the 25°C and 28°C groups (Figures 3 A-D). In 31°C group, swollen hepatocytes (SHP) was noted, while the 34°C group displayed dilation of the sinusoid (DS), cytoplasmic vacuolization (CV), and SHP (Figures3 E-H). No noticeable changes were recorded after 7 days of exposure among the temperature-treated groups (Figure not shown).

Following 42 days of thermal exposure, the 25°C and 28°C groups showed no noticeable changes (Figure not shown). However, the 31°C group exhibited lipid globules (LG) around hepatocytes (Figures 3 I-J). Severe liver damage, including coalescence of hepatocytes (CHP), blood clotting in the sinusoid (BC), cell death (CD), and DS, was observed in the 34°C group (Figures 3 K-N).


After 2 days of thermal exposure
A(25°C)
HP
50 µm
B(25°C)
HP
100 µm
C(28°C)
HP
50 µm
D(28°C)
HP
100 µm
E(31°C)
SHP
50 µm
F(31°C)
SHP
100 µm
G(34°C)
CV
SHP
50 µm
H(34°C)
DS
CV
100 µm

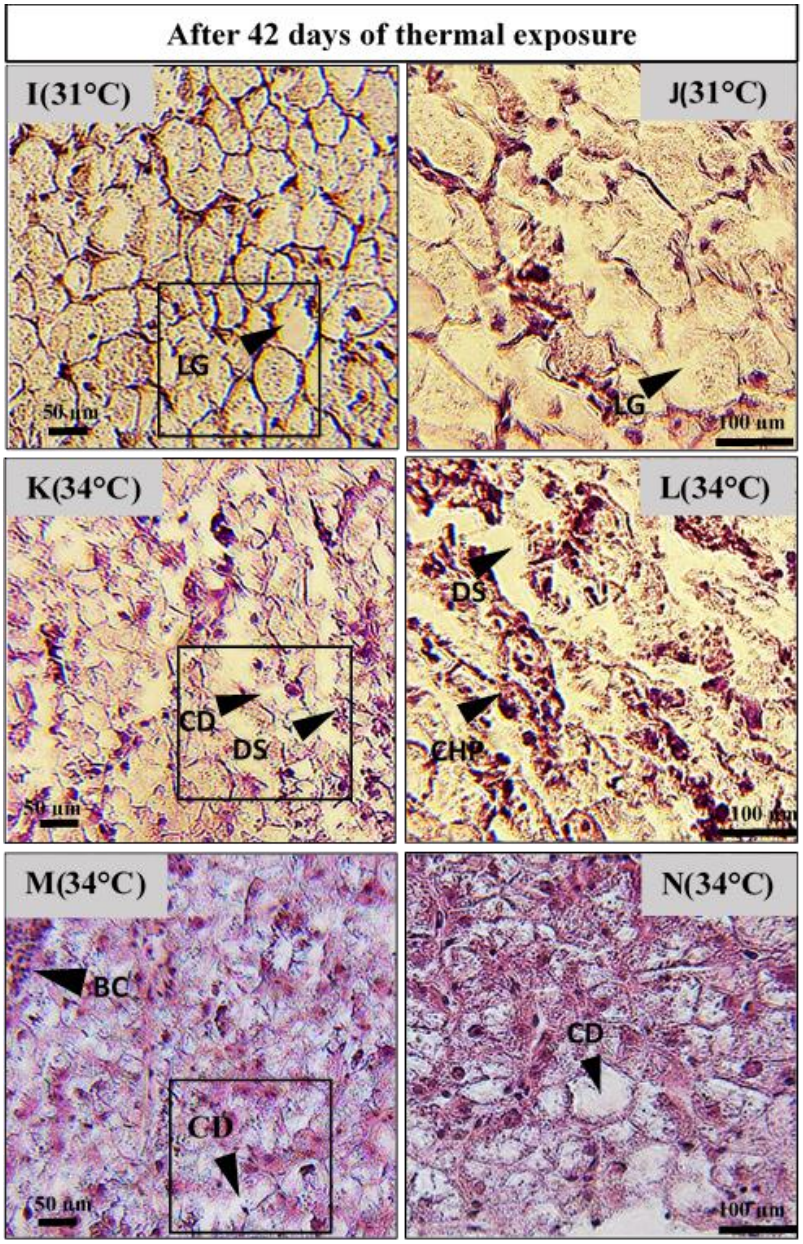


Figure 3 (A-N). The histological changes observed in the liver of red spotted grouper, *Epinephelus akaara*, subjected to various water temperatures (25°C, 28°C, 31°C, and 34°C) over a period of 42 days. A-B: 25°C group (Control); C-D: 28°C group; E-F: 31°C group; G-H: 34°C group after 2 days of thermal exposure; I-J: 31°C group; K-N: 34°C group after 42 days of thermal exposure; HP, hepatocytes; SHP, swollen hepatocytes; CV, cytoplasmic vacuolization; LG, lipid globule, BC, blood clotting in sinusoid; DS, dilation of sinusoid; CHP, coalesce of hepatocytes; CD, cell death. Scale bars = 50 and 100 µm.

Discussion. Temperature stands as a crucial environmental factor, influencing biochemical reactions and thereby significantly impacting the health of aquatic animals (Person-Le Ruyet et al 2004). The outcomes of our study revealed that elevated water temperatures result in changes in the hemato-biochemical parameters and tissue structure of *E. akaara*. Specially, our results identified 34°C as the most stressful condition for *E. akaara*. Exposure to thermal fluctuations beyond the tolerance threshold can lead to diminished appetite, erratic swimming behavior, and prompting fish to surface for air, often resulting in mortality (Cheng et al 2013). In our investigation, *E. akaara* displayed reduced appetite, rapid opercular movements, and irregular body motions. Similar observations were reported by Islam et al (2019) in Thai pangas (*Pangasianodon hypophthalmus*) following exposure to 36°C for a duration of 28 days.

Hematological parameters are valuable indicators for evaluating the blood's ability to carry oxygen efficiently (Shah & Altindag 2004). In our study, we noted alterations in Ht, Hb levels, and RBC counts with rising water temperatures. Increased Ht and RBC levels enhance the blood's ability to carry oxygen, meeting the heightened metabolic demands of essential organs under stress conditions (Ruane et al 1999). Previous research has shown that Hb levels decrease as Ht rises when Nile tilapia (*Oreochromis niloticus*) are exposed to 37°C for 4 hours (Panase et al 2019). Hematological responses to elevated water temperatures vary across aquatic species and depend on the duration of thermal exposure (Radoslav et al 2013). Moreover, fluctuations in water temperature also impact other hematological parameters such as MCV and MCH. The decrease in MCV and MCH values at higher temperatures (34°C) correlates with the rise in RBC and Hb levels. Similar findings were reported by Ahmad et al (2011) in common carp (*Cyprinus carpio communis*) exposed to a water temperature of 32°C for 30 days.

In this research, plasma glucose levels notably increased in juvenile *E. akaara* at the higher temperature of 34°C compared to other treatment groups (25°C as control, 28°C, and 31°C), indicating a heightened energy demand to cope with this unstable physiological state. The rise in blood glucose levels following thermal stress is attributed to glycogenolysis, aimed at meeting additional energy demands (Hsieh et al 2003; Naour et al 2017). Our findings are consistent with studies on Nile tilapia exposed to thermal stress at 37°C (Panase et al 2019). Alterations in blood GPT and GOT levels indicate hepatic dysfunction and damage, leading to increased transaminase activity (Gholami-Seyedkolaei et al 2013). In our study, the elevated levels of blood GPT and GOT at high temperatures (34°C) suggest liver stress, reflecting heightened activity in hepatic metabolism. These results are in line with observations by Cheng et al (2018), who reported significant increases in GPT and GOT levels in pufferfish (*Takifugu obscurus*) at 37°C. Total protein levels are commonly used to evaluate fish immunity, nutritional status, and metabolic health (Ortuno et al 2001). The decrease in total protein observed at high temperatures (34°C) in our study may be attributed to impaired protein synthesis due to liver damage. These findings are in line with those reported for puffer fish, *T. obscurus* (Cheng et al 2018).

Furthermore, cortisol levels exhibited a significant increase ($p < 0.05$) at high water temperatures (34°C) in our study. The elevation in cortisol levels under high water temperatures could be attributed to stress induced by prolonged thermal exposure and extended confinement. Similar observations have been reported in Black Sea trout (*Salmo trutta labrax*) (Balta et al 2017) and Nile tilapia (Panase et al 2019). Typically, corticosteroid levels surge in response to stress and return to baseline levels upon recovery (Iwama et al 2006). Changes in plasma cortisol levels have been associated with increased glucose levels (Hur et al 2008), consistent with our study's observations.

In this research, the serum levels of TCHO and TG exhibited a significant decrease with increasing water temperature. This declining pattern suggests that elevated temperature might disrupt lipid mobilization and circulation between the liver and tissues. TCHO and TG act as energy sources during fish stress, fulfilling the additional energy demand under thermal stress conditions (Ming et al 2012). LDH is a crucial glycolytic enzyme responsible for converting lactate to pyruvic acid and is considered a potential indicator for assessing environmental toxicity. Our findings indicate a notable increase in serum LDH activity at 34°C. Similarly, previous studies have reported a sharp elevation in LDH activity in pufferfish at 34°C and 37°C (Cheng et al 2018). Alkaline phosphatase (ALP) is an essential enzyme involved in phosphate group transfer and the metabolism of calcium and phosphate. In our research, ALP levels rose with increasing water temperatures. Similar fluctuations in ALP activity were observed in hybrid grouper (*Epinephelus fuscoguttatus* ♀ × *E. lanceolatus* ♂) under various thermal conditions (22°C, 26°C, 30°C, and 34°C) (De et al 2019). The alterations in ALP levels at higher temperatures may stem from disruptions in both physiological and functional mechanisms under thermal stress conditions.

To evaluate the stress impact, we analyzed morphological changes in the gill and liver, which corresponded well with hemato-biochemical observations. Our results unveiled significant histological changes in the gills, encompassing hyperplasia, curling,

and shortening of secondary gill lamellae, along with swelling of primary gill lamellae, epithelial necrosis, and telangiectasia. These observations align with prior research on thermally stressed Japanese flounder (*Paralichthys olivaceus*) exposed to 32°C (Liu et al 2015). Histological examination of the liver revealed hepatocyte coalescence, blood clotting in sinusoid, hepatocyte shrinkage, swelling, vacuolization, lipid globule deposition, sinusoid dilation, and cell death at elevated water temperatures. These liver morphological alterations suggest an adaptive and prolonged response to thermal stress to mitigate its effects. Similar findings have been documented in thermally stressed fish from other species, such as Japanese flounder (Liu et al 2015), and Pacific sardine, *Sardinops sagax caeruleus* (Hernandez-Lopez et al 2018).

Conclusions. In summary, our study unveiled substantial physiological and morphological alterations in *E*. *akaara* due to elevated water temperatures. Through hemato-biochemical and histological analyses, we conclude that 34°C represents a sub-lethal threshold for *E*. *akaara*. Moreover, extended exposure to 31°C and 34°C also induced physiological disruptions in *E*. *akaara*, suggesting stress at these temperature ranges. The foundational information regarding plasma profiles and tissue morphology under heightened water temperatures can serve as valuable tools for monitoring the health status of *E. akaara*, thereby contributing to enhanced welfare and productivity outcomes.

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Conflicts of interest. The authors declare that there is no conflict of interest.

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