Egyptian Journal of Aquatic Biology & Fisheries
Zoology Department, Faculty of Science,
Ain Shams University, Cairo, Egypt.
ISSN 1110 – 6131
Vol. 28(3): 1481 – 1499 (2024)
www.ejabf.journals.ekb.eg

Effects of Elevated Water Temperatures on Hemato-Biochemical and Histological Changes in the Juvenile Red Spotted Grouper (*Epinephelus akaara*)

Md Mofizur Rahman[1, 3], Young-Don Lee[2], Hea Ja Baek[1*]

[1]Department of Marine Biology, Pukyong National University, Busan-48513, Republic of Korea
[2]Marine Science Institute, Jeju National University, Jeju-63333, Republic of Korea
[3]Department of Fisheries and Marine Science, Noakhali Science and Technology University, Noakhali-3814, Bangladesh

[*]**Corresponding Author: hjbaek@pknu.ac.kr**

ARTICLE INFO

Article History:
Received: May 10, 2024
Accepted: June 10, 2024
Online: June30, 2024

Keywords:
Epinephelus akaara,
Thermal stress,
Hematological responses,
Biochemical responses,
Histological responses

ABSTRACT

The increase in water temperature attributed to global warming poses a significant concern for aquaculture and fisheries experts. Consequently, the current study explored the impacts of elevated temperatures on the hemato-biochemical and histological aspects in the juvenile red spotted groupers (*Epinephelus akaara*). Juveniles with a length (TL) of 9.4± 0.12cm and body weight (BW) of 12.89± 0.61g were subjected to thermal exposure at 24 (Control), 28, 32 and 36°C for 4 weeks, following a two-week acclimation period at 24°C. A total of 180 juvenile fish were sacrificed at three different time points (1, 14, and 28 days) for blood and tissue samples. Following 1 day of exposure, the thermal stress resulted in a significant increase in hematocrit [Ht], red blood cell [RBC] count, cortisol, glucose, glutamic oxaloacetic transaminase [GOT], and glutamic pyruvic transaminase [GPT] levels at 36°C, in comparison with the other temperature groups (24, 28, and 32°C) ($P< 0.05$). After 14 days, no noticeable changes were observed. Interestingly, after an exposure of 28 days, a significant rise in Ht and cortisol levels was reported in the group exposed at 32°C. Severe histological damage such as epithelial necrosis [EN] and shortening of secondary gill lamellae [SSL] in the gills, and cytoplasmic vacuolization [CV], shrinkage, and coalesce of hepatocytes [CHP] in the liver, was observed in the 36°C group after 1 day. Furthermore, secondary lamellar hyperplasia [HSL] in the gills and swollen hepatocytes [SHP] in the liver became evident at 32°C following the exposure of 28 days. In summary, our findings indicate that 36°C is a lethal temperature for the red spotted grouper, and prolonged exposure to 32°C can induce some degree of physiological stress in juvenile individuals.

INTRODUCTION

In aquaculture farming, the temperature in water is one of the most important regulatory factors. The temperature increase in water can occur due to anthropogenic interventions and natural causes like change in the climate. This rising regulatory factor in the aquatic environment is a great concern for aquaculture farms and fishery biologists **(Langford, 2001; Somero, 2010)**. Fish typically maintain physiological homeostasis in accordance with the temperature of their natural environment. However, alterations in

water temperature can profoundly affect the normal physiological process and overall survival of teleost fish **(Fazio *et al*., 2018)**. Moreover, change in the water temperature can affect the activities related to the non-specific immunity **(Qiang *et al*., 2013)** and metabolism **(Lu *et al*., 2016)**. Additionally, it can also increase the susceptibility to different diseases **(Karvonen *et al*., 2010)**. Temperature affects an animal's physiological and biochemical activities. Among all kinds of environmental challenges, stress due to the rising tempreature is the most significant factor **(Portner & Peck, 2010)**.

Fish being susceptible to fluctuations in temperature show a variety of changes in morphological and physiological characters **(Mora & Maya, 2006)**. Hemato-biochemical parameters are considered as useful indicators in fish for physiological stress responses **(Lermen *et al*., 2004; Fazio *et al*., 2018; Panase *et al*., 2018)**. Cortisol which is a stress hormone is secreted via hypothalamus-pituitary-interrenal (HPI) axis whenever fish encounters any fluctuations in the physico-chemical factors. Sudden or prolonged exposure to higher temperature triggers the release of cortisol, subsequently leading to a rise in the glucose levels in plasma to facilitate the enhanced utilization of energy **(Lima *et al*., 2006)**. The activity of enzymes like glutamic pyruvic transaminase (GPT) and glutamic oxaloacetic transaminase (GOT) can serve as stress indicators for monitoring the hemato-biochemical responses in fish to temperature-induced stresses. The increase in blood GPT and GOT levels is an indication of liver tissue injury in animals **(Cheng *et al*., 2018)**. Alterations in plasma total protein levels are employed as a marker for assessing liver dysfunction **(Fırat & Kargın, 2010)**. Furthermore, reports suggested that fish exhibit morphological alterations in gill and liver tissues while responding against prolonged or abrupt thermal exposure **(Liu *et al*., 2015; Hernandez-Lopez *et al*., 2018)**.

Warm-water fish like groupers prefer to grow between 24 and 30°C **(Rimmer *et al*., 2004)**. The red spotted grouper is a sub-tropical species of the Serranidae family, which is typically found in the southern regions of Japan, Korea, Hong Kong, Taiwan. and Southern China **(Heemstra & Randall, 1993)**. Among groupers, the red spotted grouper due to its high market value holds significant commercial importance in Southeast Asia **(Rimmer *et al*., 2004)**. Juvenile *E. akaara* usually inhabits shallow coastal areas where they are susceptible to thermal fluctuations **(Sadovy & Cornish, 2000)**. Understanding how thermal fluctuations, particularly elevated water temperatures, affect the physiology and histology of the red spotted grouper after a prolonged exposure is crucial from an aquaculture perspective. Consequently, a thorough understanding of the physiology of the juvenile red spotted grouper is essential for an effective aquaculture and efficient management practices.

Numerous studies have been undertaken to enhance our understanding of the hemato-biochemical responses of aquatic inhabitants to temperature-induced stresses **(Bevelhimer & Bennet, 2000; Panase *et al*., 2018; Shahjahan *et al*., 2018; Islam *et al*., 2019)**. However, while several studies on the red spotted grouper biology have explored various aspects, including feeding frequency **(Kayano *et al*., 1993)**, salinity stress effects

(Lee *et al*., 2016), and reproductive behavior **(Park *et al*., 2016)**, there is still a lack of information concerning hemato-biochemical and histological responses to high temperatures. Nevertheless, very little is known about the red spotted groupers' adaptation and physiological changes following a sudden or an extended exposure to thermal fluctuations. Thus, our aim was to observe the hemato-biochemical and histological responses of *E. akaara* under elevated water temperatures over extended periods.

MATERIALS AND METHODS

1. Experimental fish

The juveniles of *E. akaara* were relocated from the Marine Science Institute at Jeju National University in Korea and reared in the laboratory of the Marine Biology Department at Pukyong National University (PKNU), Republic of Korea. Fish care, handling, and sampling procedures were conducted following the ethical guidelines outlined by Pukyong National University's Animal Ethics Committee (PKNU) (Regulation No. 554). Upon arrival at the laboratory, the fish were promptly treated with a 30ppm oxytetracycline solution obtained from Chamshin Pharma Co. Ltd. in Seoul, Korea. The fish underwent a two-week acclimation period in their new environment, maintaining a water temperature of 24°C, a salinity of 34g/ L, dissolved oxygen levels of ≥ 6.6mg/ L, a pH of 7.8, and a 12-hour light : 12-hour dark photoperiod. The fish in the experiment were provided with a commercial diet (Marubeni Nisshin Feed Co., Ltd., Japan) twice a day, at 09:00 and 18:00 hours, until they were fully satiated. The feeding regimen continued until 24 hours before the start of the trial. Any uneaten food was promptly removed within 30 minutes after feeding to maintain water quality.

2. Experimental setup and thermal exposure

A total of 180 juveniles of the red spotted grouper, initially weighing 12.89± 0.61g in body weight (BW) and measuring 9.4± 0.12cm in total length (TL), were randomly allocated among 12 rectangular glass tanks (measuring 75 × 45 × 45cm in width, length, and height), with each tank accommodating 15 fish. Each tank was fitted with a recirculating filtration system holding 120 liters of seawater. The experiment consisted of four temperature treatments: 24 (as control), 28, 32, and 36°C, where the juveniles of the red spotted grouper were maintained for 4 weeks. Each temperature treatment group was maintained in a set of three independent replicate tanks. To achieve the specified temperature for each treatment, the tank temperatures were gradually increased at a rate of 1°C per hour using a thermostat (OKE-6422H, Korea). Temperature of each aquarium was constantly maintained at ± 0.5°C with additional aeration and skimming. The survival rate of the fish was continuously observed throughout the rearing period. Using a water quality meter (HI9829; Hanna Instrumentals, USA), water parameters such as temperature, dissolved oxygen, salinity, and pH were monitored daily.

Additionally, the ammonium levels were quantified at two-day intervals with a NH_3/NH_4^+ test kit (Tetra GmbH, Germany). During the experimental trial, the recorded values for pH, salinity, NH_4, and DO values were within the ranges of 7.89– 7.97, 33.67–33.81g/ L, < 0.25mg/ L, and 4.16– 6.96mg/ L, respectively. 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.

3. Sampling protocol

Fish were sampled at intervals of 1, 14, and 28 days from the initiation of thermal exposure. The second sampling (at 14 days) and the third sampling (at 28 days) were conducted specially within the 24, 28, and 32°C test groups, as all fish in the 36°C group succumbed to thermal stress within a day of exposure. All fish underwent a 24-hour fasting period before sampling. At each sampling point, 5 fish were randomly selected from each experimental tank, with triplicate tanks for each temperature treatment. The fish were gently anaesthetized using 300mg/ L 2-phenoxyethanol (Sigma Aldrich, St. Louis, MO, USA). Following morphometric measurements (TL, Body length: BL, 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 1.5mL centrifuge tubes for subsequent hematological analysis (hemoglobin [Hb], hematocrit [Ht], and red blood cell [RBC] count) and biochemical indices (plasma glucose, GPT, GOT, total protein). Simultaneously, gill and liver tissues were surgically excised on ice and stored for histological examination.

4. Hematological analysis

Hematocrit (Ht) measurements were conducted through the collection of blood samples in glass capillary tubes, which were subsequently centrifuged for 5 minutes at 12,000rpm using a microhematocrit centrifuge apparatus (VS-12000; Vision Co. Ltd., Korea). Hemoglobin (Hb) levels were quantified by applying a 10µL blood sample onto Hb slides, which were subsequently analyzed using an automated analyzer (FUJI DRI-CHEM 400i, Japan). The 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).

5. Biochemical analysis

Upon collection, plasma was promptly separated from blood samples through centrifugation at 4°C (15min at 13,000g), and the resulting supernatant was preserved at –70°C for a subsequent analysis. Glucose, glutamic pyruvic transaminase (GPT), glutamic oxaloacetic transaminase (GOT), and total protein 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)**.

6. Radioimmunoassay

To quantify plasma cortisol levels, steroids were twice extracted with 2mL of diethyl ether. The extracted steroid 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 and 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 ([^{3}H]-cortisol) were procured from Amersham Life Sciences, Piscataway, USA.

7. Histological analysis

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 5µm thickness were stained using Mayer's Hematoxylin and Eosin (H & E) and subsequently examined under a compound microscope (BX-50, Olympus, Japan).

8. Statistical procedures

Data analysis was conducted using SPSS 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, the 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

1. Behavioral changes and mortality

In the 36°C treatment group, the fish displayed signs of distress, including gasping, swift movements of the operculum, erratic movements, efforts to escape the experimental tank, spinning along their longitudinal axis, loss of balance, and ultimately succumbed to acute heat stress, resulting in mortality. In contrast, fish in the remaining groups (24, 28, and 32°C) exhibited vigorous swimming and normal opercular movements, indicating no apparent signs of external distress. The 36°C group experienced a complete mortality as a result of the thermal shock on day 1, while no mortality was found in the other test groups (24, 28, and 32°C).

2. Hematological parameters

After 1 day of thermal exposure, there was a notable rise in Ht levels at 28°C, reaching its peak at 36°C. After 28 days of exposure, significantly increased Ht values were observed in the 32°C temperature group. RBC counts were significantly elevated on the first day of the experiment in the 36°C group, while no alterations were noted in the remaining thermal groups (24, 28, and 32°C) following 28 days of exposure. After 1 day of exposure, fish subjected to 36°C exhibited significantly lower Hb levels, whereas no effects on Hb levels were evident in the other test groups, even after 28 days of exposure (Table 1).

Table 1. Changes in hematocrit, red blood cell (RBC) count, and hemoglobin levels of *E. akaara* subjected to various water temperatures (24, 28, 32, and 36°C) for 28 days

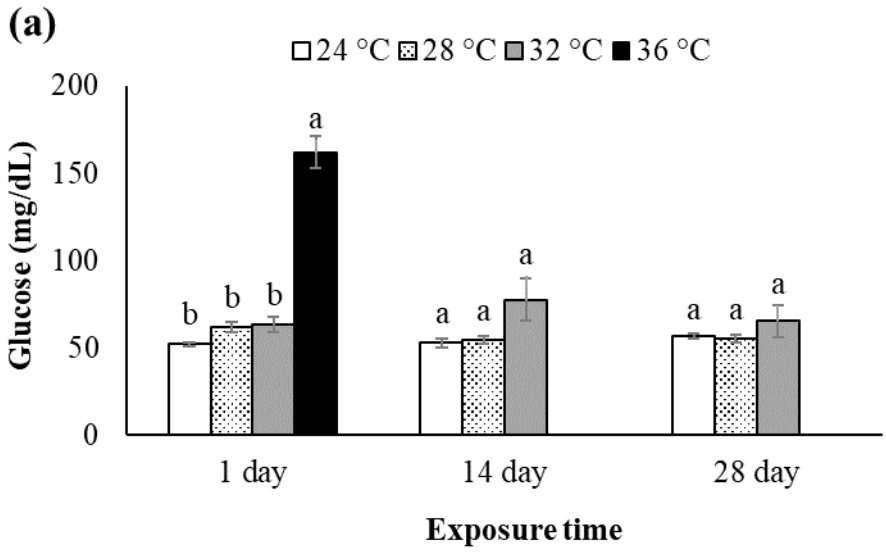
Parameter	Treatments	Thermal exposure (days)		
		1	14	28
Ht (%)	24 °C	31.44 ± 1.11^{c}	37.83 ± 0.25^{a}	33.66 ± 0.28^{b}
	28 °C	34.92 ± 1.12^{b}	38.95 ± 0.69^{a}	33.93 ± 0.34^{b}
	32 °C	36.16 ± 0.25^{b}	39.89 ± 0.95^{a}	38.13 ± 0.07^{a}
	36 °C	41.24 ± 0.12^{a}	-	-
RBC ($\times 10^6$/mm^3)	24 °C	5.31 ± 0.05^{bc}	5.48 ± 0.07^{a}	5.31 ± 0.28^{a}
	28 °C	5.12 ± 0.07^{c}	4.87 ± 0.05^{b}	5.18 ± 0.14^{a}
	32 °C	5.53 ± 0.17^{b}	5.15 ± 0.13^{ab}	5.53 ± 0.18^{a}
	36 °C	6.01 ± 0.05^{a}	-	-
Hb (g/dL)	24 °C	8.10 ± 0.27^{a}	8.75 ± 0.23^{a}	8.54 ± 0.54^{a}
	28 °C	8.13 ± 0.14^{a}	8.45 ± 0.15^{a}	8.53 ± 0.07^{a}
	32 °C	8.18 ± 0.39^{a}	7.67 ± 0.19^{b}	8.02 ± 0.18^{a}
	36 °C	7.42 ± 0.18^{b}	-	-

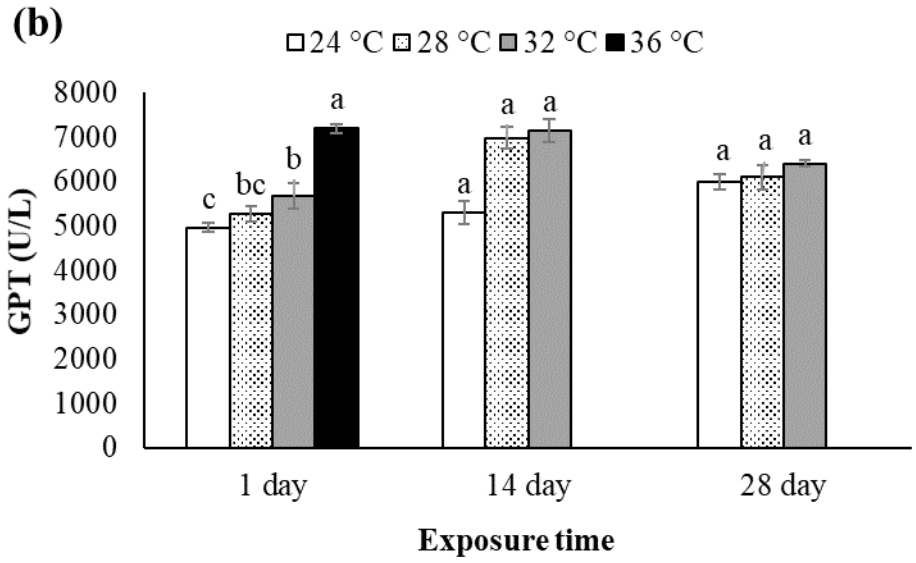
N.B.: Sampling occurred at three distinct time intervals (1, 14 and 28 days). The values represent the mean ± SEM of three replicates (n=15; comprising 5 fish per tank). Lowercase letters indicate statistically significant differences among groups at corresponding time points (ANOVA, Duncan's multiple range test; $P < 0.05$).

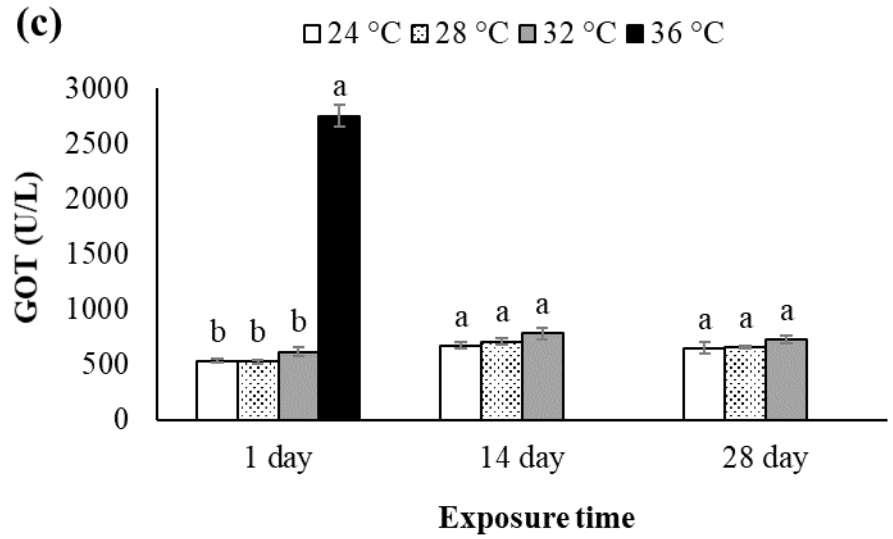
3. Biochemical parameters

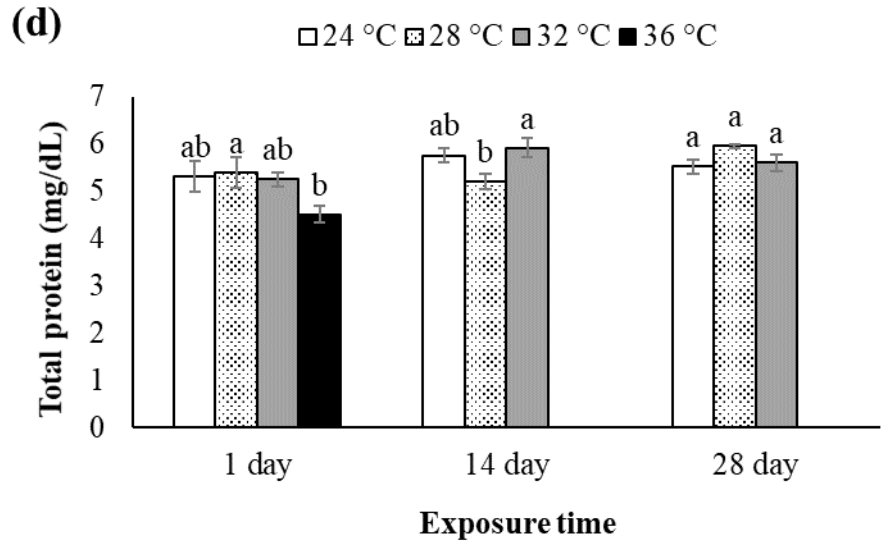
On day 1, the plasma glucose levels exhibited a sharp increase in the 36°C group, whereas they remained stable in the other experimental groups (24, 28, and 32°C). After 14 and 28 days, slightly elevated glucose levels were observed in the 32°C group;

however, these changes were not statistically significant (Fig. 1a). Plasma GPT levels exhibited a significant increase after 1 day of exposure in the 36°C group, but the other groups did not show any significant changes at the same sampling points. GPT levels remained unaffected after 14 and 28 days of heat exposure (Fig. 1b). On the first day of experiment, thermal exposure caused a significant increase in the plasma GOT levels in the 36°C group, reaching five times higher levels compared to the other test groups. However, no significant changes were noted upon comparing to the other experimental groups after 14 days and 28 days of exposure, as shown in Fig. (1c). Following the 1st day of exposure, the total protein of plasma levels showed a reduction in the 36°C test group in comparison with the other test groups. However, no change was recorded in any experimental group after 14 and 28 days of exposure (Fig. 1d). Moreover, following the 1st day of exposure, the 32 and 36°C groups exhibited significantly higher plasma cortisol levels compared to the 24 and 28°C groups. There was a notable rise of cortisol in the 32°C group following 28 days of exposure (Fig. 1e).




(b)
24 °C
28 °C
32 °C
36 °C
GPT (U/L)
8000
7000
6000
5000
4000
3000
2000
1000
0
c
bc
b
a
a
a
a
a
a
a
a
1 day
14 day
28 day
Exposure time


(c)
24 °C
28 °C
32 °C
36 °C
GOT (U/L)
3000
2500
2000
1500
1000
500
0
b
b
b
a
a
a
a
a
a
a
a
1 day
14 day
28 day
Exposure time



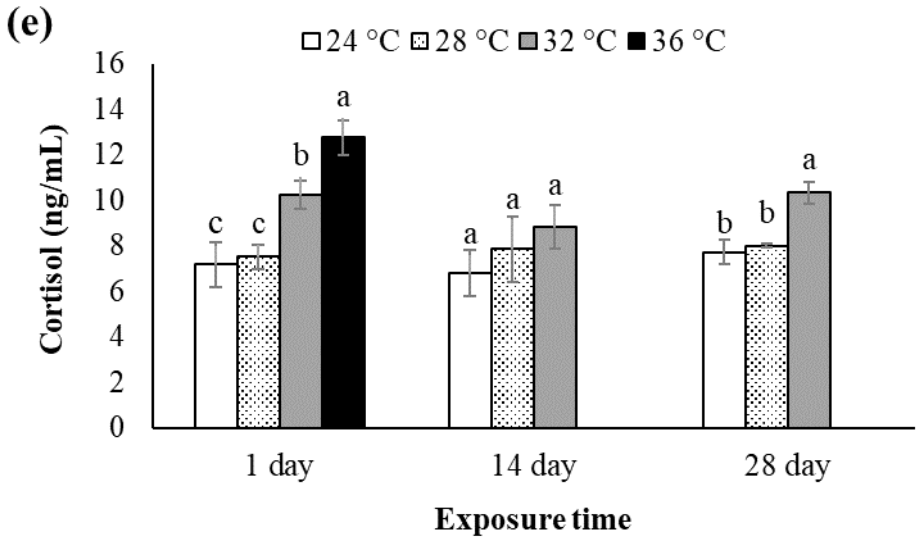


Fig. 1 (a- e) Alterations in plasma glucose, glutamic pyruvic transaminase (GPT), glutamic oxaloacetic transaminase (GOT), total protein and cortisol levels of *E. akaara* following exposure to various water temperature (24, 28, 32, and 36°C) for 28 days. Sampling was conducted at three distinct time intervals (1, 14 and 28 days). The values represent the mean ± SEM of three replicates (n=15; comprising 5 fish per tank). Lowercase letters indicate statistically significant differences among groups at corresponding time points (ANOVA, Duncan's multiple range test; $P< 0.05$)

4. Histological observations in gills

After the 1st day of exposure, the gills of fish in the 24, 28, and 32°C groups showed no significant effects (Fig. 2a- c), whereas those in the 36°C group were notably affected by heat exposure. Significant changes including epithelial necrosis (EN), reduction in the length of secondary gill lamellae (SSL), and disorganization of lamellae were observed in the 36°C test group (Fig. 2d). However, after 14 and 28 days of exposure, there were no substantial alterations detected in the thermal exposure groups (Fig. 3a– e), with the exception of secondary lamellar hyperplasia [HSL] in the gills observed in the 32°C experimental group on the 28th day (Fig. 3f).

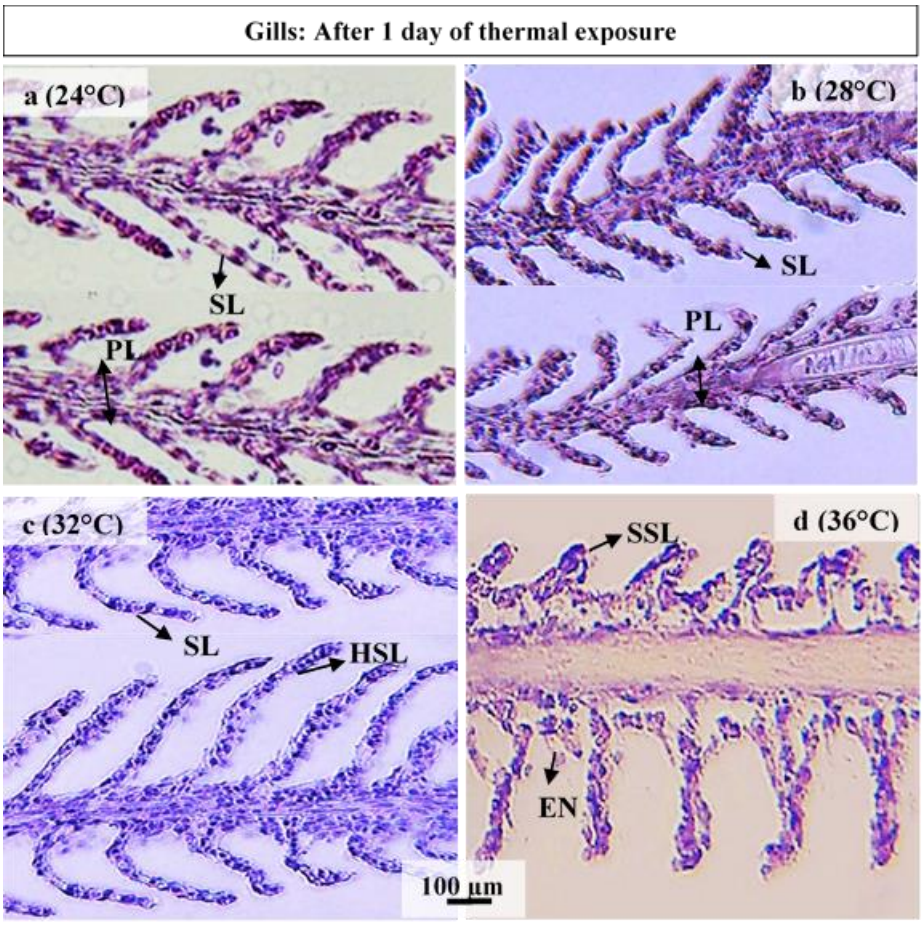


Fig. 2 (a- d) Histological changes in the gills of *E. akaara* following the 1st day of thermal exposure at varying temperatures (24°, 28°, 32°, and 36°C). **a**(24°C): Normal primary (PL) and secondary gill lamellae (SL); **b**(28°C): Normal PL and SL; **c**(32°C): Hyperplasia of secondary gill lamellae (HSL); **d**(36°C): Epithelial necrosis (EN); Shortening of secondary gill lamellae (SSL). Stain: Hematoxylin and Eosin (H & E); Scale bars = 100µm

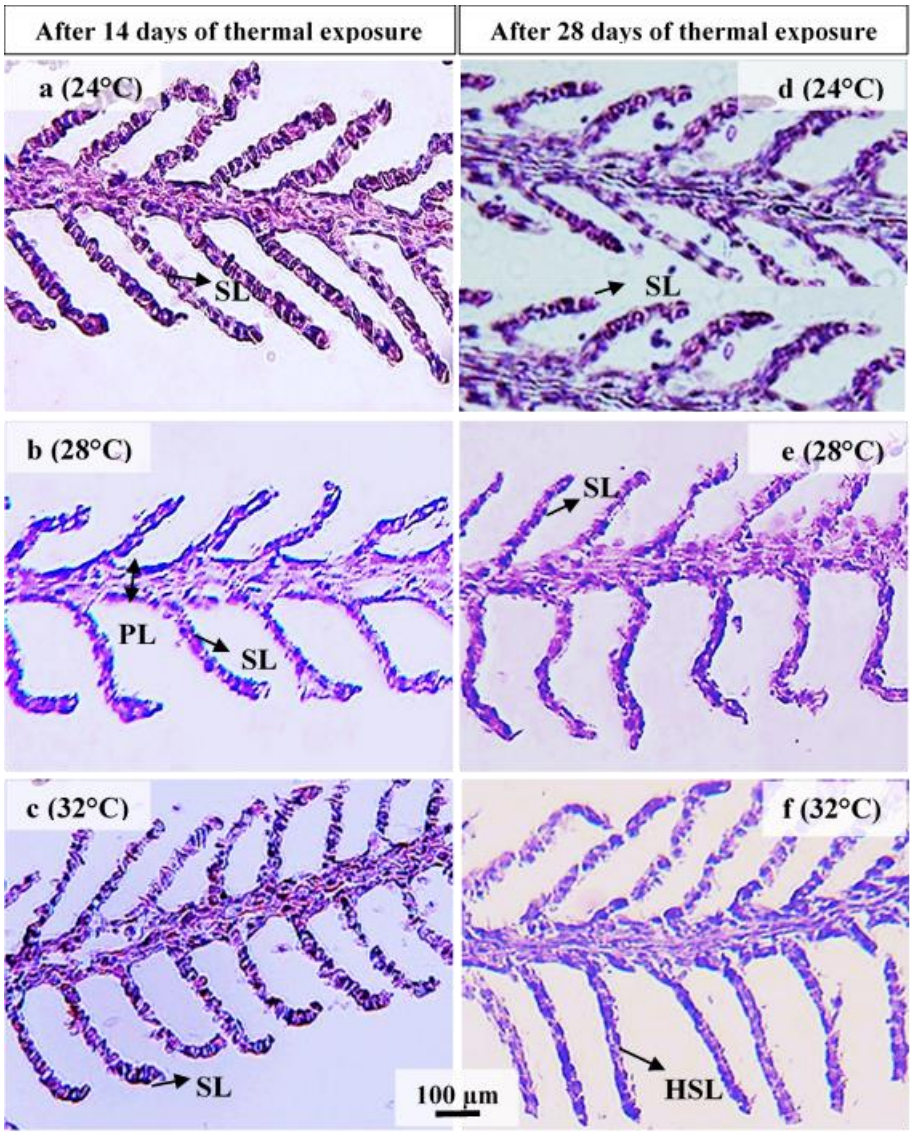


Fig. 3 (a- f) Histological changes in the gills of the *E. akaara* after 14 **(a- c)** and 28 days **(d- f)** of thermal exposure at various temperatures (24, 28, and 32°C). **a, d** (24°C); **b, e** (28°C); **c**(32°C): Well organized primary (PL) and secondary gill lamellae (SL); **f**(32°C): Hyperplasia of secondary gill lamellae (HSL) after 28 days; Stain: Hematoxylin and Eosin (H & E); Scale bars = 100µm

5. Histological observations in the liver

Following one day of exposure, the liver tissues in the 32 and 36°C exhibited a severe damage compared to the tissues in the 24 and 28°C test groups (Fig. 4a, b). In the 36°C group, indications of this damage encompassed coalescence of hepatocytes (CHP), blood clot (BC) formation within the sinusoid, and the contraction of hepatocytes (SH) (Fig. 4d). Meanwhile, the 32°C group exhibited swollen hepatocytes (SHP) and cytoplasmic vacuolization (CV) (Fig. 4c). After 14 and 28 days, there were no observable

alterations in the thermal exposure groups (Fig. 5a– e), except for the presence of cytoplasmic vacuolization (CV) in the 32°C group on the 28th day, as depicted in Fig. (5f).

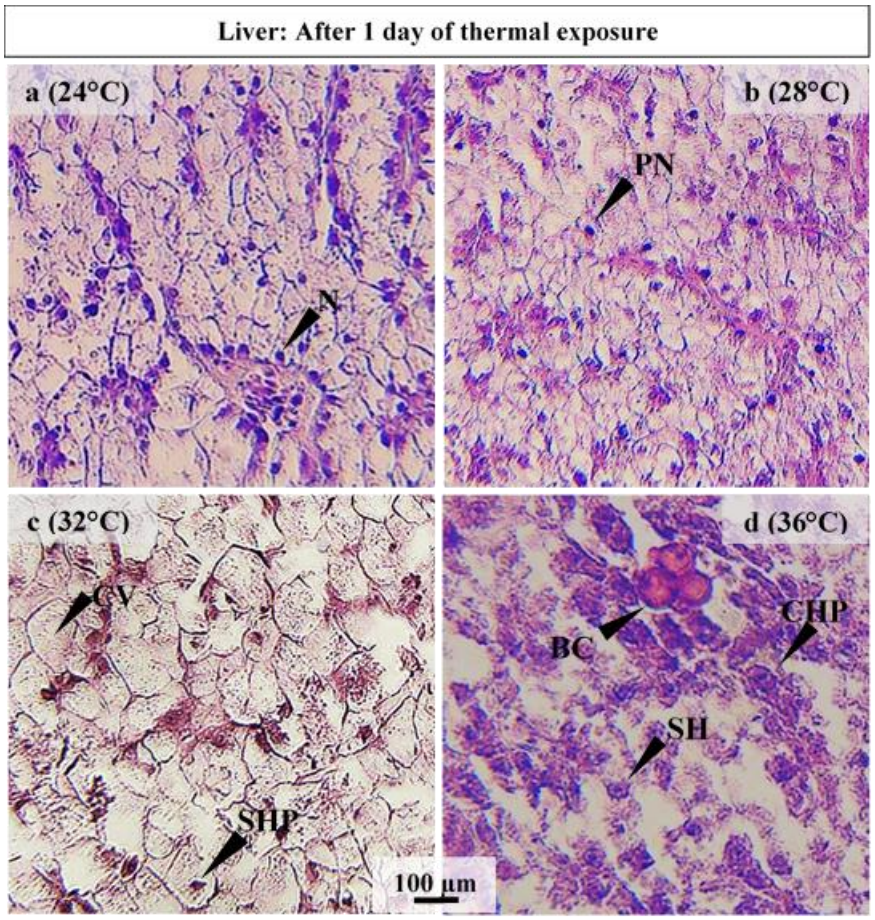


Fig. 4 (a- d) Histological alterations in the liver of the *E. akaara* after the 1st day of thermal exposure at various temperatures (24, 28, 32, and 36°C). **a**(24°C): Hepatocytes with centrally located nuclei (N); **b**(28°C): Hepatocytes with prominent nuclei (PN); **c**(32°C): Cytoplasmic vacuolization (CV), Swollen hepatocytes (SHP); **d**(36°C): Shrinkage of hepatocytes (SH), Blood clotting (BC) in sinusoid, Coalesce of hepatocytes (CHP); Stain: Hematoxylin and Eosin (H & E); Scale bars = 100µm

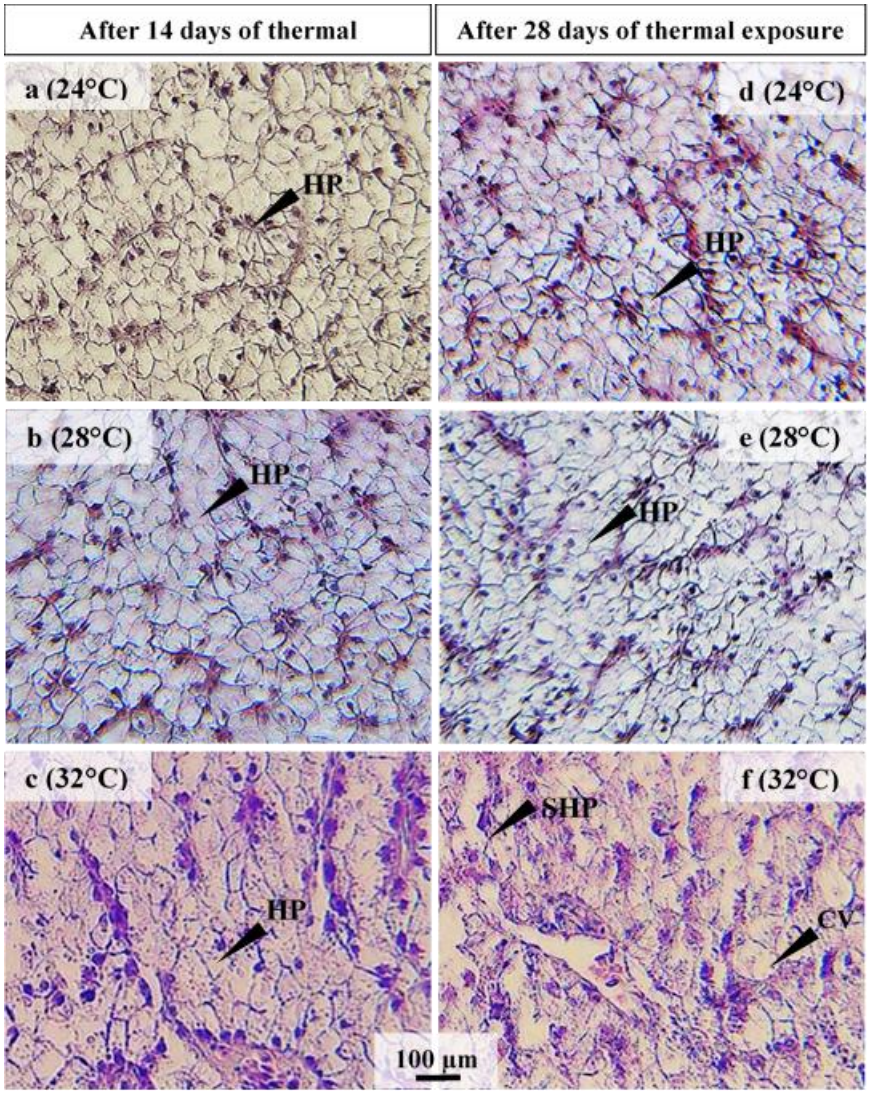


Fig. 5 (a- f) Histological alterations in the liver of *E. akaara* after 14 (**a- c**) and 28 days (**d- f**) of thermal exposure at various temperatures (24, 28, and 32°C). **a, d**(24°C); **b, e** (28°C); **c**(32°C): Normally arranged hepatocytes (HP); **f**(32°C): Swollen hepatocytes (SHP) and cytoplasmic vacuolization (CV) after 28 days; Stain: Hematoxylin and Eosin (H & E); Scale bars = 100µm

DISCUSSION

Thermal fluctuations exceeding the tolerance level frequently result in fish mortality **(Cheng *et al*., 2013)**. In this study, the juveniles of the red spotted grouper were found to experience severe thermal stresses, and their mortality was observed at 36°C within the first day of experiment, clearly representing the lethal nature of this temperature for *E. akaara*. A similar finding was observed by **Cheng *et al*. (2013)**, who stated in their study that 50% of juvenile brown-marbled grouper (*E. fuscoguttatus*) died

when temperature raised from 35.9 to 38.3°C. The orange spotted grouper (*E. coioides*) exhibited temperature sensitivity and experienced mortality within 12 hours of transfer from 27 to 35°C, as reported by **Cheng *et al*. (2009)**.

Hematological parameters were employed to evaluate the efficient state of oxygen-carrying capacity within the blood circulatory system **(Shah & Altindag, 2004)**. In this study, we observed an increase in Ht values and RBC counts, along with a decrease in Hb levels, at 36°C following one day of exposure. Remarkably, the increased levels of Ht and RBC enhance the blood's ability to transport oxygen, thereby meeting the oxygen needs of vital organs in reaction to elevated metabolic demands, which is an indicative of stress response **(Ruane *et al*., 1999)**. It was observed that the Hb levels decreased as Ht levels increased in the Nile tilapia (*Oreochromis niloticus*) when subjected to a temperature of 37°C for 4 hours **(Panase *et al*., 2018)**. However, it was found that hematological responses to higher water temperatures usually vary depending on the specific species and thermal exposure time **(Radoslav *et al*., 2013)**.

In this present study, a substantial rise in plasma glucose levels was evident in the *E. akaara* juveniles under higher temperatures (36°C) in contrast to other temperature groups (24, 28, and 32°C). This increase signifies an elevated energy demand required to cope with the challenging physiological conditions at higher temperatures. After the onset of thermal stress, elevation in plasma blood glucose levels occurs as a result of glycogenolysis **(Naour *et al*., 2017)**, which is essential to fulfill additional energy requirements **(Hsieh *et al*., 2003)**. Our findings align with those of previous studies on the Nile tilapia that were exposed to heat stress at 37°C **(Panase *et al*., 2018)**. Alterations in blood GPT and GOT levels have served as markers for hepatic dysfunction and injury, leading to increased transaminase activity **(Gholami-Seyedkolaei *et al*., 2013)**. In the current study, the elevation in plasma GPT and GOT concentrations at high temperatures (36°C) indicated a state of liver stress, revealing heightened hepatic activity. Our results coincide with the observations made by **Cheng *et al*. (2018)**, who noted significant rises in GPT and GOT values in the puffer fish (*Takifugu obscurus*) when subjected to 37°C. Total protein serves as a common diagnostic tool for assessing fish immune status as well as nutritional and metabolic health **(Ortuno *et al*., 2001)**. In this study, the reduction in the total protein levels observed at elevated temperatures (36°C) could be linked to compromised protein synthesis resulting from liver damage. Similar findings were also documented in the common carp, *Cyprinus carpio communis* **(Ahmad *et al*., 2011)**, and in the puffer fish, *T. obscurus* **(Cheng *et al*., 2018)**.

Cortisol levels exhibited a significant increase, reaching a 2-fold elevation compared to the control group after one day of exposure to 36°C. Subsequently, they returned to their basal levels, but a second rise in cortisol level was observed in the 32°C group following 28 days of exposure, which may be attributed to the stress induced by the prolonged confinement. These observations align with prior research conducted on the black Sea trout **(Balta *et al*., 2017)** and the Nile tilapia **(Panase *et al*., 2018)**.

Corticosteroid levels usually experience an increase in response to stress and subsequently revert to their baseline levels upon recovery **(Iwama *et al*., 2006)**. Alterations in plasma cortisol levels have been documented to result in increased glucose levels **(Hur *et al*., 2008)**, a finding which concurs with the observation of the current study.

Morphological changes in both the gill and liver were examined to evaluate the impact of stress, and these effects also show a strong correlation with hemato-biochemical findings. Our findings revealed significant histological alterations in the gill, including EN, SSL, and disorganization of lamellae after the 1st day of exposure to 36°C. Additionally, secondary lamellar hyperplasia was observed after 28 days of exposure to 32°C. The findings of this study are in alignment with those of previous research on gill morphology in the thermally stressed Japanese flounder, *Paralichthys olivaceus* **(Liu *et al*., 2015)** and the bighead carp, *Aristichthys nobilis* **(Aboka *et al*., 2017)**, where both the species were submitted to temperatures of 32 and 38°C for a duration of 1 day, respectively. The histological analysis of this study of the liver revealed hepatocyte coalescence, blood clot within the sinusoid, and contraction of hepatocyte following a single day of exposure to 36°C. Additionally, hepatocyte hypertrophy was evident after 28 days of exposure to 32°C. The observed morphological disturbances in liver tissues signifies an adaptive and immediate response to thermal stress, aiming to counteract the temperature-induced effects. Comparable effects were documented in different fishes stressed thermally such as the Japanese flounder, *P. olivaceus* **(Liu *et al*., 2015)**, and the Pacific sardine, *Sardinops sagax caeruleus* **(Hernandez-Lopez *et al*., 2018)**.

CONCLUSION

In summary, this study highlights the profound physiological changes induced by elevated temperatures in the red spotted grouper. Our findings suggest that the temperature of 36°C can be considered lethal for *E. akaara*, as the fish could not endure exposure to this temperature. Moreover, *E. akaara* begins to exhibit physiological disturbances when reared for prolonged periods at 32°C, indicating that this temperature also creates a stressful environment for this species. The outcomes of this study could be valuable for monitoring the physiological status of *E. akaara*, thereby facilitating an improved management approach in aquaculture, particularly in response to the challenge of rising water temperatures.

ACKNOWLEDGEMENT

This research was supported by the Korean Ministry of Agriculture, Food and Rural Affairs (MAFRA), the Korean Ministry of Oceans and Fisheries (MOF), the Korean Rural Development Administration (RDA), and the Korea Forest Service (KFS) under grant number 213008-05-3-WT511.

REFERENCES

Aboka, E.R.; Jian, Z.; Shengming, S.; Wuxiao, Z.; Han, Y.; Li, S.Y.; Jiahui, Y. and **Jiabin, Z.** (2017). Histopathological changes in gills, liver, and kidney tissues of Bighead Carp (*Aristichthys nobilis*) due to the effects of acute high-temperature stress. Isr. J. Aquac., *69*: 1372.

Ahmad, S.M; Shah, F.A.; Bhat, F.A.; Bhat, J.I.A. and **Balkhi, M.H.** (2011). Thermal adaptability and disease association in common carp (*Cyprinus carpio communis*) acclimated to different (four) temperatures. J. Therm. Biol., *36*(8): 492-497.

Baillie, J.E.; Hilton-Taylor, C. and **Stuart, S.N.** (2004). IUCN red list of threatened species: a global species assessment: IUCN, 191pp.

Balta, Z.D.; Akhan, S. and **Balta, F.** (2017). The physiological stress response to acute thermal exposure in Black Sea trout (*Salmo trutta labrax* Pallas, 1814). Turk. J. Vet. Anim. Sci., *41*(3): 400-406.

Bevelhimer, M. and **Bennett, W.A.** (2000). Assessing cumulative thermal stress in fish during chronic intermittent exposure to high temperatures. Environ. Sci. Pol., *3*(1): 211-216.

Cheng, A.C.; Cheng, S.A.; Chen, Y.Y. and **Chen, J.C.** (2009). Effects of temperature change on the innate cellular and humoral immune responses of orange-spotted grouper (*Epinephelus coioides*) and its susceptibility to *Vibrio alginolyticus*. Fish. Shellfish. Immun., *26*(5): 768-772.

Cheng, S.Y.; Chen, C.S. and **Chen, J.C.** (2013). Salinity and temperature tolerance of brown-marbled grouper (*Epinephelus fuscoguttatus*). Fish. Physiol. Biochem., *39*(2): 277-286.

Cheng, C.H.; Guo, Z.X.; Luo, S.W. and **Wang, A.L.** (2018). Effects of high temperature on biochemical parameters, oxidative stress, DNA damage and apoptosis of pufferfish (*Takifugu obscurus*). Ecotox. Environ. Safe., *150*: 190-198.

Cho, H.C.; Kim, J.E.; Kim, H.B. and **Baek, H.J.** (2015). Effects of water temperature change on the hematological responses and plasma cortisol levels in growing of red spotted grouper (*Epinephelus akaara*). Dev. Reprod., *19*(1): 19.

Constantz, J.; Thomas, C.L. and **Zellweger, G.** (1994). Influence of diurnal variations in stream temperature on streamflow loss and groundwater recharge. Water. Resour. Res., *30*: 3253-3264.

Fazio, F.; Ferrantelli, V.; Piccione, G.; Saoca, C.; Levanti, M. and **Mucciardi, M.** (2018). Biochemical and hematological parameters in European sea bass (*Dicentrarchus labrax* Linnaeus, 1758) and Gilthead sea bream (*Sparus aurata* Linnaeus, 1758) in relation to temperature. Vet. Arh., *88*(3): 397-411.

Fırat, Ö. and **Kargın, F.** (2010). Individual and combined effects of heavy metals on serum biochemistry of Nile tilapia (*Oreochromis niloticus*). Arch. Environ. Con. Tox., *58*(1): 151-157.

Gallant, M.J.; LeBlanc, S.; MacCormack, T.J. and **Currie, S.** (2017). Physiological responses to a short-term, environmentally realistic, acute heat stress in Atlantic salmon (*Salmo salar*). Facets., *2*(1): 330-341.

Gholami-Seyedkolaei, S.J.; Mirvaghefi, A.; Farahmand, H.; Kosari, A.A.; Gholami-Seyedkolaei, S.J. and **Gholami-Seyedkolaei, S.J.** (2013). Optimization of recovery patterns in common carp exposed to roundup using response surface methodology: evaluation of neurotoxicity and genotoxicity effects and biochemical parameters. Ecotox. Environ. Safe., *98*: 152-161.

Heemstra, P.C. and **Randall, J.E.** (1993). Groupers of the world. FAO Fisheries synopsis., *16*(125), I.

Hernandez-Lopez, J.R.; Hernandez-Rodriguez, M.; Rivas-Manzano, P. and **Buckle-Ramirez, L.F.** (2018). Thermal Effect of Acute and Chronic Stress on Hepatic and Renal Tissue of the Pacific Sardine (*Sardinops sagax caeruleus* Jenyns, 1842). Int. J. Morphol., *36*(1): 212-221.

Hsieh, S.L.; Chen, Y.N. and **Kuo, C.M.** (2003). Physiological responses, desaturase activity, and fatty acid composition in milkfish (*Chanos chanos*) under cold acclimation. Aquaculture., *220*(1-4): 903-918.

Hur, J.W.; Lim, H.K. and **Chang, Y.J.** (2008). Effects of repetitive temperature changes on the stress response and growth of olive flounder (*Paralichthys olivaceus*). J. Appl. Anim. Res., *33*(1): 49-54.

Iwama, G.K.; Afonso, L.O.B. and **Vijayan, M.M.** (2006). Stress in fishes In: Evans DH & Claiborne JB, editors. The Physiology of Fishes: Boca Raton, FL: CRC Press.

Islam, M.A.; Uddin, M.H.; Uddin, M.J. and **Shahjahan. M.** (2019). Temperature changes influenced the growth performance and physiological functions of Thai pangas (*Pangasianodon hypophthalmus*). Aquac. Rep., *13*: 100179.

Karvonen, A.; Rintamäki, P.; Jokela, J. and **Valtonen, E.T.** (2010). Increasing water temperature and disease risks in aquatic systems: climate change increases the risk of some, but not all, diseases. Int. J. Parasitol., *40*(13): 1483-1488.

Kobayashi, Y. and **Mikuni, T.** (1987). A fundamental and clinical study of ferritin'Eiken'radioimmunoassay kit. Radioisotopes., *36*(1): 28-31.

Krome, C.A. (2014). Studies on the suitability of Jatropha curcas kernel meal as an alternative protein source in diets for carp (*Cyprinus carpio*) and tilapia (*Oreochromis niloticus*). University of Stirling, 243pp.

Langford, T.E. (2001). Thermal discharges and pollution. Encyclopedia of Ocean Sciences. Academic Press, pp. 2933-2940.

Lee, J.W.; Kim, H.B. and Baek, H.J. (2016). Plasma Stress Responses in Juvenile Red Spotted Grouper (*Epinephelus akaara*) exposed to Abrupt Salinity Decrease. Dev. Reprod., *20*(3):187.

Lermen, C.L.; Lappe, R.; Crestani, M.; Vieira, V.P.; Gioda, C.R.; Schetinger, M.R.C.; Baldisserotto, B.; Moraes, G. and **Morsch, V.M.** (2004). Effect of

different temperature regimes on metabolic and blood parameters of silver catfish (*Rhamdia quelen*). Aquaculture., *239*(1-4): 497-507.

Lima, L.C.; Ribeiro, L.P.; Malison, J.A.; Barry, T.P. and Held, J.A. (2006). Effects of temperature on performance characteristics and the cortisol stress response of Surubim (*Pseudoplatystoma* sp). J. World. Aquac. Soc., *37*(1): 89-95.

Liu, Y.; Ma, D.; Xiao, Z.; Xu, S.; Wang, Y.; Wang, Y.; Xiao, Y.; Song, Z.; Teng, Z.; Liu, Q. and **Li, J.** (2015) Histological change and heat shock protein 70 expression in different tissues of Japanese flounder (*Paralichthys olivaceus*) in response to elevated temperature. Chin. J. Oceanolo. Limn., *33*(1): 11-19.

Lu, Y.; Wu, Z.; Song, Z.; Xiao, P.; Liu, Y.; Zhang, P. and **You, F.** (2016) Insight into the heat resistance of fish via blood: Effects of heat stress on metabolism, oxidative stress and antioxidant response of olive flounder (*Paralichthys olivaceus*) and turbot (*Scophthalmus maximus*). Fish. Shellfish. Immun., *58*: 125-135.

Mora, C. and **Maya, M.F.** (2006). Effect of the rate of temperature increase of the dynamic method on the heat tolerance of fishes. J. Therm. Biol., *31*(4): 337-341.

Naour, S.; Espinoza, B.M.; Aedo, J.E.; Zuloaga, R.; Maldonado, J.; Bastias-Molina, M.; Silva, H.; Meneses, C.; Gallardo-Escarate, C.; Molina, A. and **Valdés, J.A.** (2017). Transcriptomic analysis of the hepatic response to stress in the red cusk-eel (*Genypterus chilensis*): Insights into lipid metabolism, oxidative stress and liver steatosis. PloS. One., *12*(4): e0176447.

Ortuno, J.; Esteban, M.A. and **Meseguer, J.** (2001). Effects of short-term crowding stress on the gilthead seabream (*Sparus aurata* L.) innate immune response. Fish. Shellfish. Immun., *11*(2): 187-197.

Panase, P.; Saenphet, S.; Saenphet, K.; Pathike, P. and **Thainum, R.** (2018). Biochemical and physiological responses of Nile tilapia (*Oreochromis niloticus* Linn.) subjected to rapid increases of water temperature. Comp. Clin. Pathol., *28*(2): 493-499.

Portner, H.O. and **Peck, M.A.** (2010). Climate change effects on fishes and fisheries: towards a cause-and effect understanding. J. Fish. Biol., *77*:1745-1779.

Qiang, J.; Yang, H.; Wang, H.; Kpundeh, M.D. and **Xu, P.** (2013). Interacting effects of water temperature and dietary protein level on hematological parameters in Nile tilapia juveniles, (*Oreochromis niloticus* L.) and mortality under *Streptococcus iniae* infection. Fish. Shellfish. Immunol., *34*: 8-16.

Radoslav, D.; Aleksandar, I.; Rajko, G.; Goran, T.; Danijela, Ć. and **Svjetlana, L.** (2013). Effect of thermal stress of short duration on the red blood cell parameters of *Barbus balcanicus* Kotlik, Tsigenopulos, Rab, Berrebi, 2002. Afr. J. Biotechnol., *12*(18): 2484-2491.

Rimmer, M.A.; McBride, S. and **Williams, K.C.** (2004). Advances in grouper aquaculture. Australian Centre for International Agricultural Research (ACIAR), 67pp.

Ruane, N.M.; Bonga, S.W. and **Balm, P.H.M.** (1999). Differences between rainbow trout and brown trout in the regulation of the pituitary-interrenal axis and physiological performance during confinement. Gen. Comp. Endocr., *115*(2): 210-219.

Sadovy, Y. and **Cornish, A.S.** (2000). Reef fishes of Hong Kong. Hong Kong University Press, 344pp.

Shah, S.L. and **Altindag, A.** (2004). Hematological parameters of tench (*Tinca tinca* L.) after acute and chronic exposure to lethal and sublethal mercury treatments. B. Environ. Contam. Tox., *73*(5): 911-918.

Shahjahan, M.; Uddin, M.H.; Bain, V. and **Haque, M.M.** (2018). Increased water temperature altered hemato biochemical parameters and structure of peripheral erythrocytes in striped catfish (*Pangasianodon hypophthalmus*). Fish. Physiol. Biochem., *44*: 1309-1318.

Somero, G. (2010). The physiology of climate change: how potentials for acclimatization and genetic adaptation will determine 'winners' and 'losers'. J. Exp. Biol., *213*(6): 912-920.