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ORIGINAL RESEARCH

Effect of increasing water temperature on the physiology and gill histology of Barramundi, *Lates calcarifer* (Pisces, Perciformes) fingerlings

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Abstract Barramundi, *Lates calcarifer* is one of the commercial fish in Indonesia and other Southeast Asian countries. The species inhabits the coastal waters and is vulnerable to changes in the terrestrial environment due to coastal degradation, pollution, and global warming. The increase in temperature as a consequence of global warming affects the distribution and survival of aquatic organisms including fish. Therefore, this study aims to examine the effect of increasing water temperatures on the physiological conditions and gill histology of barramundi. The fish were exposed to eight levels of water temperature, ranging from 28°C to 35°C, for 15 days. The initial temperature was 28°C, which increased gradually by 1°C per 12-hour. Blood samples were taken at the end of the experiment for hemoglobin, glucose, cortisol, erythrocytes, leucocytes, and blood abnormality analyses. Gill samples were also taken for histological analysis. The results showed that water temperature significantly affected the level of glucose, cortisol, and erythrocyte cells ($P<0.05$), but did not influence hemoglobin levels and survival rate ($P>0.05$). A rapid increase in glucose and cortisol levels was also discovered at a temperature of 31°C above. The highest erythrocyte cell count was found at 35°C, gill cell abnormalities were occurred at temperature of 31 °C and above, and the blood abnormalities increased linearly with water temperature. Based on these results, it is concluded that the water temperature

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did not affect the survival rate but the increase in temperature causes intense physiological stress.

Keywords Global warming . Survival rate . Hematology . Gill cell abnormality . Barramundi

Introduction

Barramundi, *Lates calcarifer* is distributed widely in Southeast Asian countries, including Indonesia, Brunei Darussalam, Cambodia, Malaysia, Philippines, Singapore, Thailand, Viet Nam, and Timor-Leste (Vij et al. 2014; Suman et al. 2016). The species is one of the commercial fish in Indonesia, however, the productivity of barramundi fishery in the country is highly variable and seasonal. Based on statistical data, the production of barramundi in 2009 was 1,366.9 tons, which decreased to 872.4 tons in 2014 (BPS 2016), but increased again in 2020 to 1,950.8 tons (KKP, 2022).

The species is a catadromous and protandrous hermaphrodite, commonly found in coastal waters and estuaries. It has a wide salinity tolerance (euryhaline), spawns in the open sea, and grows in coastal waters (Blaber et al. 2008). Although the species can be cultured, Barramundi grows faster in the wild (Chan 1982; Rayes et al. 2013), with a lower survival rate of larvae (Cotton et al. 2003). Since Barramundi inhabits coastal waters, it is vulnerable to coastal environmental changes due to habitat degradation, pollution, and climate change. According to Solomon et al. (2007), global surface temperatures of the ocean have increased by approximately 0.8°C - 1.0°C from 1860 to 2000. However, since 1950, it has increased by 1.1°C - 1.3°C during the last 50 years. The significant effect of global warming on the marine climate and monsoonal patterns had also been reported (Caldeira and Wickett 2003; Xu et al. 2009; Bezuijen 2011; Oczkowski et al. 2015).

Water temperature affects the metabolic activity of fish (Muchlisin 2017) and increases the temperature of a species beyond the thermal range which affects survival rates and distributions (Freund and Petty 2007; Bilotta and Brazier 2008; Gupta et al. 2012; Kjelland et al. 2015; Bartnicki et al. 2021). Extreme water temperature changes also cause physiological stress, limits reproductive fitness (Arantes et al. 2011; Pankhurst and Munday 2011; Miller et al. 2015), and alter feeding habits (Tort et al. 2004; Walberg 2011; Volkoff and Rønnestad 2020). It also damages fish organs, especially gills and kidneys, causing stress which may lead to death (Aliza and Sipahutar 2013; Sumantri et al. 2017; Nur et al. 2020).

Several studies have evaluated the impacts of increased water temperature on fish behavior and physiology. Nur et al. (2020) examined the impact of water temperature changes on the physiological responses of *Betta rubra*, the freshwater endemic species in Sumatra Island Indonesia. The results showed that *B. rubra* suffered physiological stress and gill damage at temperatures above 28°C. Takata et al. (2018) also reported in a Neotropical freshwater fish *Lophiosilurus alexandri* that the higher temperature, with a value of 29-32°C causes damage to the gills morphology. Rojas et al. (2013) exposed the *Colossoma macropomum* juvenile to three temperature levels (18, 29, 35 °C), and discovered that the normal histoarchitecture of the gills, liver, and kidneys occurred at 29°C. At lower a temperature of 18°C, the gills showed branchiallipid droplets and disorganization of the branchial tissue at 35°C. Furthermore, Slesinger et al. (2019) studied the effect of water temperature on the aerobic and hypoxia tolerance of black sea bass, *Centropristis striata*, which decreased respiration capacity at temperatures above 24°C. According to Park et al. (2016), the cortisol hormone and blood glucose in the red grouper *Epinephelus akaara* increased with changes in water temperature. It was also stated that as chronic temperature increases, it alters reproductive pathways and decreases fish fitness (Dadras et al. 2017; Alfonzo et al. 2021).

Hobday et al. (2006) reported that an increase in water temperature changed fish migration patterns, damaging mangroves, and coral reef ecosystems. The declines in fish production also occur due to changes in fish migration pathways from one water body to another or an increase in the natural mortality of fish due to environmental changes (Gough 2012; Brink et al. 2018; Chen et al. 2018). For example, the impact of climate change on decreasing productivity of mackerel *Trachurus trachurus* fisheries have been discovered in European waters and was related to the decline of plankton biomass (Reid et al. 2001; Stenseth et al. 2002). The skipjack, *Katsuwonus pelamis* fisheries in the Pacific Ocean have also experienced declines (Lehodey et al. 1997). Anchovy *Stelephorus* sp. fisheries off the coast of Peru decreased due to the El-Nino that brought in warm water masses and reduced upwelling (Reid et al. 2001; Stenseth et al. 2002). Stenseth et al. (2002) also reported that changes in the North Atlantic Oscillation (NAO) off the West Coast of Canada

and Alaska impacted sea surface temperature, leading to decreased nutrient levels, reduced salinity, changes in the food web, and alterations in habitat, which impacted pelagic fisheries in these areas.

Therefore, this study aims to examine the impact of temperature changes on the physiological conditions and gill histology of the barramundi fingerling, as a model for migratory fishes. This information is important to inform mitigation planning for coastal fisheries under climate change.

Materials and methods

Experimental design

A completely randomized experimental design with eight treatment levels was used in this study. The treatment was exposure of water temperature, namely T1= 28°C, T2= 29°C, T3= 30°C, T4= 31°C, T5= 32°C, T6= 33°C, T7= 34°C, and T8= 35°C. Each treatment was applied with three replicates.

Experimental fish

A total of 240 experimental fish were purchased from a local fish farmer in Lhokseumawe City, Indonesia. The average size and length of the fish were 1.03±0.5 g and 4.2±0.4 cm, respectively. Fish were acclimatized in a circular fiber tank (Vol 240L) for 21 days and fed on a commercial diet, containing 42% crude protein, 10% lipid, 3% fiber, and 14% ash, three times a day (9 AM, 12 AM, and 5 PM) to satiation, and feces were siphoned one hour after feeding. The procedures used in this study followed the animal ethics in the research of Universitas Syiah Kuala (Ethic Code No: 958 /2015).

Temperature exposure

A total of 24 plastic jars (vol. 20 L) were used as an experimental medium. The jar was filled with 10 L seawater at a salinity of 22 ppt and equipped with an aerator pump (Amara AA-350, China), and a heater (Kandila KD-50, China). Seawater was taken from the waters of Alue Naga, Banda Aceh City, pooled in a fiber tank, and deposited for one week before being used. Each jar was stocked with 10 fish equivalent to 1 fish L^{-1} of water. The initial water temperature was 28°C and the heater was turned on to increase the temperature gradually by 1°C per 12 hours (Islam et al. 2019) until the treatment temperature was reached. Subsequently, fish were reared at the treatment temperature for 15 days in three replicate jars. The water temperature, salinity, pH, and dissolved oxygen were monitored at 6-hour intervals to ensure levels were optimum. The water quality was kept within the following ranges, dissolved oxygen 6.0 - 8.5 mg L^{-1}, pH 8.11 - 8.39, and salinity 21-23 ppt, with an average of 6.9±0.05 mg L^{-1}, 8.29 ±0.01, and 22 ±0.17 ppt, respectively. Moreover, the survival rate was assessed for each treatment.

Blood analysis

Haemoglobin, erythrocyte cell, leukocyte cell, glucose, and cortisol levels were measured at the end of each experiment. Subsequently, a total of 3 fish were taken randomly from each jar and anesthetized using MS222 solution, prepared by dissolving 0.8 g of MS 222 in one liter of tap water (Muchlisin et al. 2004), and sacrificed. The blood sample was collected from the gills using a syringe by cutting with the sterile scissor, and mixed with the anticoagulant EDTA- K2 in a test tube (Nur et al. 2017). Hemoglobin and blood glucose levels were measured using a glucometer (Easy Touch - GCU, China), while erythrocyte and leucocyte cells were quantified using a haematometer. Blood cortisol was analyzed by applying the ELISA method, while the blood cell abnormalities were observed using a stereomicroscope (Primo Star Zeiss, Switzerland).

The ELISA analysis was performed based on the standard procedure as follows, a total of 20 µl of standard, sample, and quality control (QC) solutions were put into a microplate. Subsequently, 200 µl of HRP Cortisol enzyme conjugate was added, shaken gently for 10 sec, and incubated for 60 min. After incubation, the sample was washed four times using a microplate strip washer, followed by the addition of approximately 100 µl of TBM substrate solution, and incubated for 20 min. A total of 100 µl of 0.5 M H_2SO_4 solution was added to the wells of the plate and the absorbance was read with an ELISA reader (Absorbance

Microplate Reader, Biorad) at a wavelength of 450 nm.

Gill histology preparation

Gill samples were taken randomly from three fish in each jar at the end of the experiment for histological analysis by dissecting the mouth to the operculum and extracting gill tissue from the gill cavity. The gill samples were fixed with 10% formalin and stored for one week before further processing. After one week, the gill tissue was processed for histological preparations using the paraffin method and the Hematoxylin--Eosin (HE) staining based on Muchlisin et al. (2010) and Shah and Parveen (2022).

Red blood cell abnormality analysis

Red blood cell abnormalities were analyzed using the blood smear method (Adewoyin and Nwogoh 2014) as follows, approximately 20 µl of blood was taken from the gills then dropped onto a glass slide. It was swabbed using another glass slide and dried at room temperature for 10 min. The sample was fixed in 98% methanol for 10 min, followed by staining with 5% Giemsa solution for 20 min. Subsequently, the sample was washed under running water and dried at room temperature for 24 h. The cells were observed for any abnormality under a stereomicroscope (Primo Star Zeiss, Switzerland) at 100X magnification by sampling 1,000 red blood cells randomly. Shahjahan et al. (2018) and Islam et al. (2019) identified five types of blood cell abnormalities, namely echinocytes, thorn-shaped red blood cells, elliptocytes that are oval or elongated, fusion, consisting of several uninuclear combine to become multinuclear cells, teardrop-shaped, and twin, where two red blood cells bind to each other.

Data analysis

The data on survival rate, glucose, hemoglobin, cortisol, erythrocyte, and erythrocyte abnormalities were subjected to one-way Analysis of Variance (ANOVA) followed by Duncan's multiple range test using SPSS software ver.19.0 (IBM22). Subsequently, the data on gill tissue abnormalities were analyzed descriptively.

Results

Survival and blood physiology

The percentage of survival rate of barramundi fingerlings ranged between 80% and 90%, where the lower value was recorded at a treatment temperature of 35°C. Fig. 1 showed that the group mean survival rates

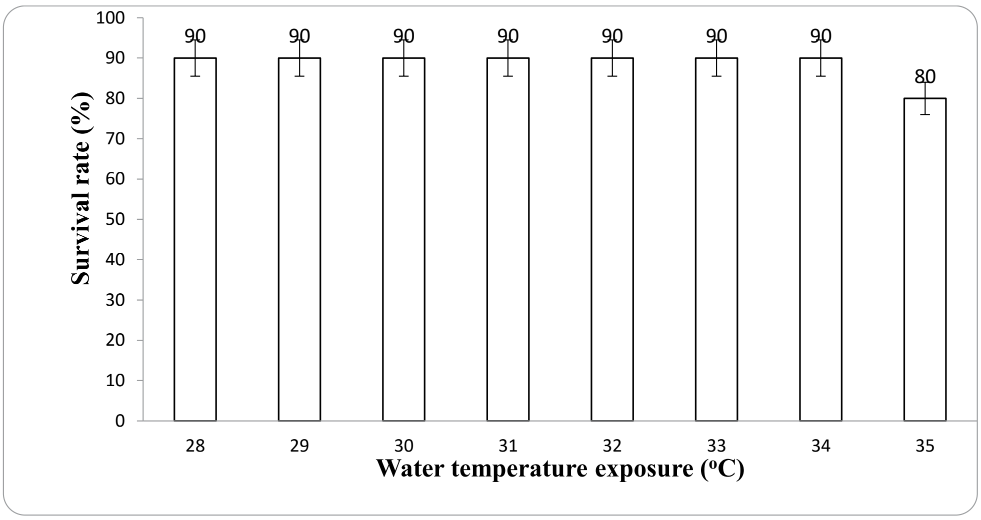

Fig. 1 Survival rate of barramundi fingerling exposure at different temperature (28-35 °C)

were not significantly different among the temperature treatments (P>0.05). A significant effect of water temperature treatment was also discovered on glucose as well as cortisol levels and the number of erythrocyte cells (P<0.05). Blood glucose ranged from 3.57 mmol L^{-1} to 8.30 mmol L^{-1}, where the highest level was recorded at 34°C (8.3±1.01 mmol L^{-1}). This value was significantly greater than glucose levels in the 28°C and 29°C treatments (P<0.05) as presented in Table 1. Furthermore, cortisol levels ranged between 21.9 ng ml^{-1} and 72.7 ng ml^{-1}, with the highest cortisol hormone concentration at 35°C (72.7±5.94 ng ml^{-1}), which is significantly greater compared to others (P<0.05). The lowest cortisol concentration was recorded at 29°C (21.9±0.78 ng ml^{-1}). Total erythrocyte cells ranged from 4.5 x10^5 to 41.4 x10^5 cells, with the highest number obtained at 35°C. This value was significantly different from all other treatments (P<0.05).

There was a significant effect of water temperature on red blood cell abnormalities (P<0.05), with the highest value obtained at 35°C. The six types of abnormalities observed were echinocyte, elliptocytes, tear-drop shape, fusion, and twins as shown in Fig. 2.

Gills histology

The five types of cell abnormalities in the gill tissue of barramundi recorded were lamella fusion, hypertro-

Table 1 Physiological condition of the blood of barramundi exposure with different temperatures. Mean±SD in the same line with different superscript are significantly different (P<0.05). The number of fish examined was 3 in each treatment (N= 3).

Parameter	Exposure temperature (°C)							
	28	29	30	31	32	33	34	35
Heamoglobin (mmol L^{-1})	$6.90±0.55^a$	$7.90±0.66^a$	$7.53±0.34^a$	$8.53±0.99^a$	$7.57±0.19^a$	$9.13±0.48^a$	$8.33±0.55^a$	$8.20±0.74^a$
Glucose (mmol L^{-1})	$3.57±0.20^a$	$3.90±0.87^{ab}$	$5.2±1.47^{abc}$	$7.70±2.55^{bc}$	$7.43±1.94^{bc}$	$6.23±0.55^{abc}$	$8.30±1.01^c$	$8.03±0.30^c$
Cortisol hormone (ng ml^{-1})	$25.0±0.03^a$	$21.9±0.78^a$	$24.8±1.55^a$	$39.9±3.14^b$	$42.6±2.84^{bc}$	$39.7±1.48^b$	$50.0±4.10^c$	$72.7±5.94^d$
Erythrocyte cell (x10^5)	$7.6±1.9^a$	$7.7±2.8^a$	$8.4±2.4^a$	$4.5±8.5^a$	$10.5±4.6^a$	$19.8±3.4^a$	$12.00±7.6^a$	$41.4±3.1^b$

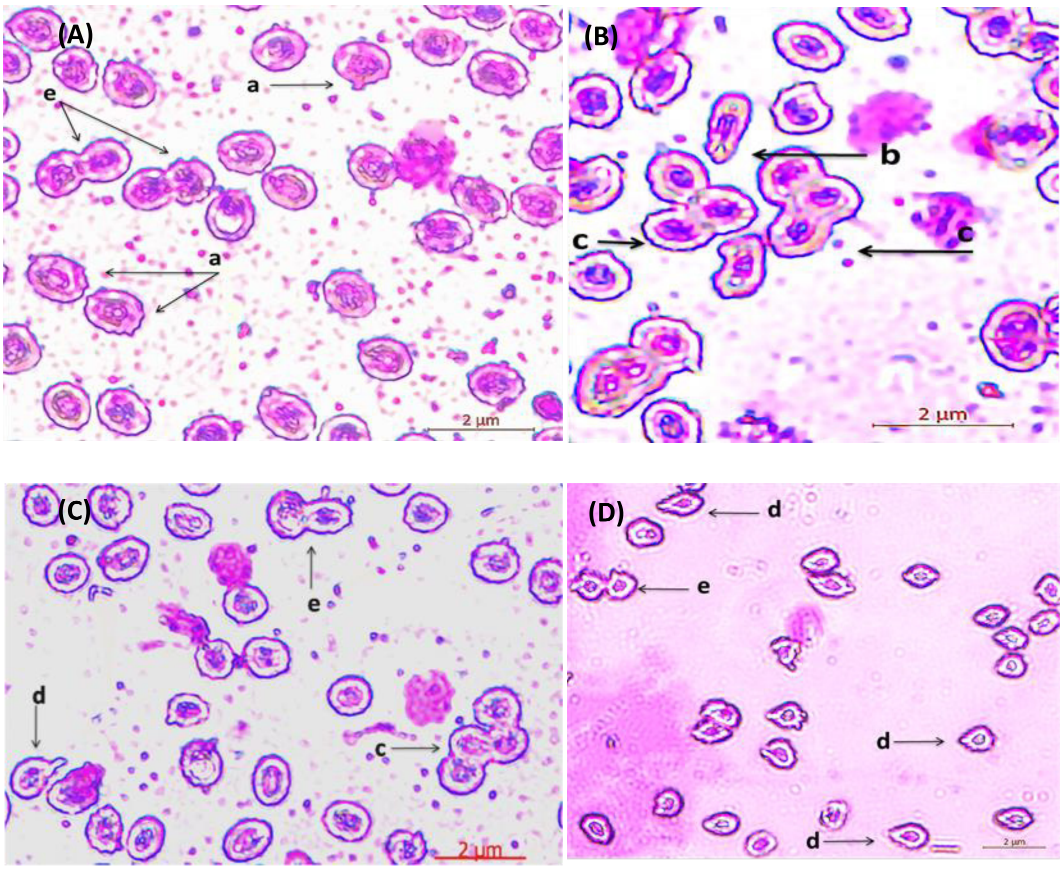

Fig. 2 Erythrocyte cell abnormalities found in barramundi exposure with different temperature. (A) The erythrocyte cell showed echinocytes abnormality, (B) The erythrocyte cell displayed elliptocytes abnormality, (D) The erythrocyte cell showed tear drop shapes and twins abnormalities. (a)= echinocytes, (b)= elliptocytes, (c)= fussion, (d)= tear drop shaped, (e)= twins.

phy, hyperplasia, telangiectasis, and shortening of secondary gill lamellae. The abnormalities occurred at exposure temperatures of 31°C and above. For example, hypertrophy was found at 31°C and 32°C. Furthermore, temperatures of 33°C and 34°C caused hyperplasia, cell fusion, and telangiectasis, while shortening of the secondary lamellae was observed at 35°C as shown in Fig. 3. The intensity of the abnormality of the gills was presented in Table 3.

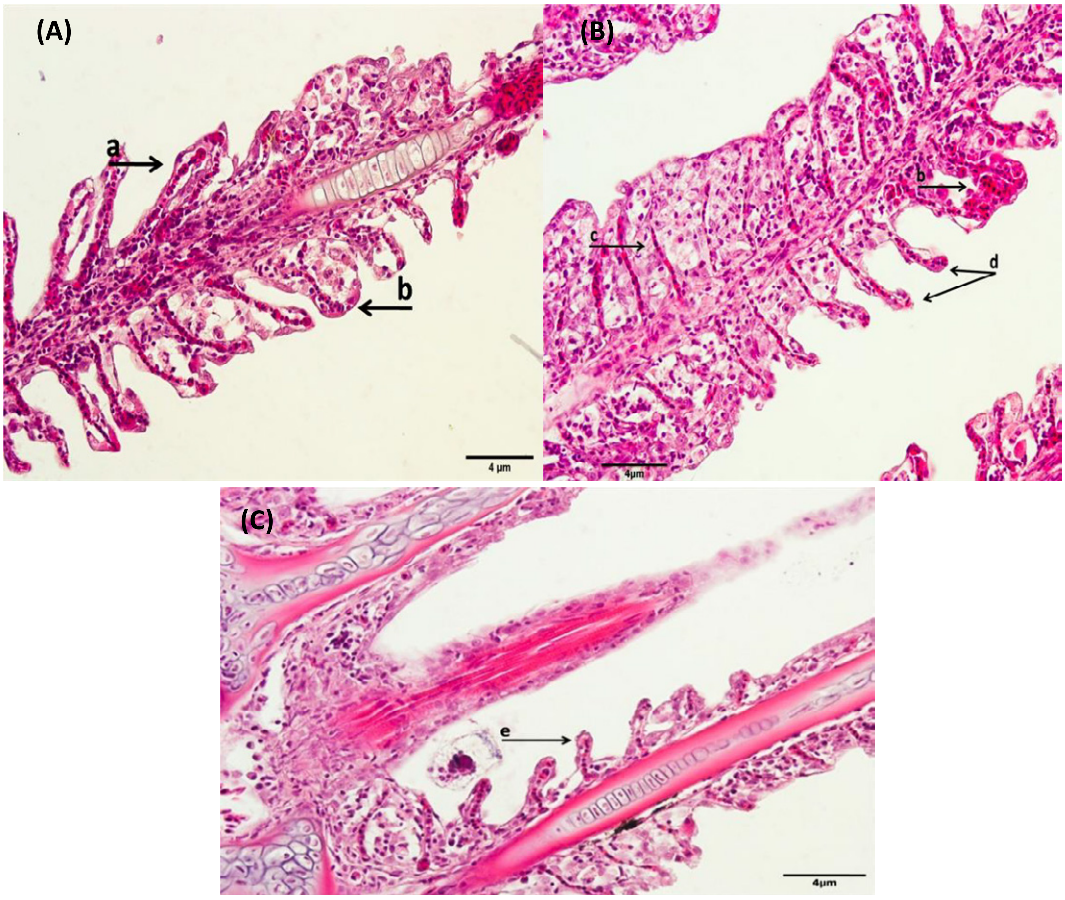

Fig. 3 The gills abnormalities due to temperature exposure. (A) The gills displayed lamella fussion and hypertropia, (B) The gills displayed hyperplasia and secondary lamella shortening, (C) The gills displayed telangiektasis abnormality. (a)= Lamella fusion, (b)= Hypertropi, (c)= Hyperplasia, (d)= Telangiektasis, (e)= Secondary lamella shortening.

Table 2 The Eryhrocite cell abnormalities of barramundi exposure with different temperatures. Mean±SD in the same line with different superscript are significantly different (P<0.05). The number of fish examined was 3 in each treatment (N= 3).

Abnormality	Exposure temperature (°C)							
	28	29	30	31	32	33	34	35
Ecinocytes (%)	0.0 ± 0.0^{a}	0.23 ± 0.1^{a}	0.13 ± 0.1^{a}	0.53 ± 0.1^{a}	0.83 ± 0.1^{ab}	1.6 ± 0.2^{bc}	2.4 ± 1.0^{c}	5.3 ± 1.2^{d}
Elliptocytes (%)	0.0 ± 0.0^{a}	0.07 ± 0.1^{a}	0.13 ± 0.1^{a}	0.30 ± 0.1^{ab}	0.70 ± 0.2^{b}	0.63 ± 0.1^{b}	0.73 ± 0.1^{b}	1.7 ± 0.6^{c}
Fussion (%)	2.90 ± 1.7^{a}	3.10 ± 1.2^{a}	7.30 ± 4.6^{ab}	8.40 ± 2.5^{ab}	13.7 ± 4.9^{abc}	13.6 ± 4.4^{bc}	12.1 ± 1.6^{bc}	18.6 ± 6.0^{c}
Tear drop shape (%)	0.0 ± 0.0^{a}	0.30 ± 0.0^{a}	0.17 ± 0.1^{a}	0.13 ± 0.1^{ab}	0.33 ± 0.2^{ab}	0.57 ± 0.1^{c}	0.77 ± 0.1^{c}	1.17 ± 0.4^{d}
Twins (%)	3.80 ± 0.5^{a}	6.40 ± 0.5^{ab}	9.27 ± 1.7^{bc}	10.8 ± 1.4^{cd}	12.7 ± 1.5^{cd}	11.8 ± 3.9^{cd}	13.4 ± 1.8^{de}	16.5 ± 1.5^{e}

Table 3 The histopathological changes in Barramundi gills exposed with different temperature levels. The number of fish examined was 3 in each treatment (N= 3).

Type of abnormality	Temperature							
	28	29	30	31	32	33	34	35
Lamella fusion	0	0	0	0	0	++	+++	++
Hypertrophy	0	0	0	+	+	++	++	++
Hyperplasia	0	0	0	+	+	++	+++	++
Telangiectasis	0	0	0	0	0	+	+++	++
Shortening of secondary gill lamellae	0	0	0	0	0	0	++	+++

Note: 0 absent, + present at low frequency, ++ present at moderate frequency, +++ present at high frequency

Discussion

This study showed that the survival rate of barramundi fingerlings remained above 80% at all treatment temperatures and can tolerate a range of 28-35ºC. Fish is a poikilothermic animal that can regulate its body temperature from the ambient temperature (Giannetto et al. 2014; Maisano et al. 2016). However, a temperature change of more than 2ºC will trigger stress in fish marked by inactivity, disruption of respiration, and increased metabolism as well as excretion (Nur et al. 2020), leading to mortality when exposed for longer periods (Ashaf-Ud-Doulah et al. 2020). In the wild, barramundi generally lives in waters with temperatures above 20ºC with an optimum range of 26-30ºC and stops feeding at a value less than 20ºC (NSW, 2022). In Australian waters, the species has been recorded in the range of 18-36ºC with the ability to tolerate temperatures up to 40ºC (Glencross and Bermudes 2010). In Indonesia, the optimal temperature range is 28-32ºC, and survival decreases at a value above 35ºC. The fish experiences stress at this temperature, as recorded by the increase in cortisol hormone and blood glucose (Mahardika et al. 2020).

The cortisol hormone and blood glucose are the important parameters determining the physiological stress of fish (Martinez-Porchas et al. 2009). Bartoňková et al. (2017) stated that the level of cortisol hormone is correlated with blood glucose concentration. Similarly, this study reported that an increase in blood sugar concentration was followed by the higher hormone cortisol. According to Tahir et al. (2018), normal levels of cortisol hormone in Perciformes range from 5.65-26.3 ng ml^{-1}, while cortisol levels in barramundi fish were up to 39.9 ng ml^{-1}, indicating that the fish had experienced intense stress. The stressed condition stimulates the hypothalamus to release Corticotrophin Releasing Factor (CRF), which stimulates the anterior pituitary gland to release the Adrenocorticotropin Hormone (ACTH). The ACTH triggers the interrenal cells (adrenal medulla) to produce cortisol and catecholamine hormones such as epinephrine commonly known as adrenaline (Ohmura and Yoshioka 2018). These hormones play an important role in gluconeogenesis, converting glycogen reserves in the liver and muscles into glucose which is released into the blood (Hastuti 2004; Chaudhary 2019).

Blood glucose increase shows that the fish is experiencing stress (Martíneš-Porchas et al. 2009). Normal blood glucose levels in fish are 2.22-4.99 mmol L^{-1} (Nasichah et al. 2016), but in this study, the levels reached 5.2 mmol L^{-1} at a temperature of 30ºC and proportionately increased to 8 mmol L^{-1} at 34ºC and 35ºC. According to Masjudi et al. (2016), when fish are stressed, blood glucose increases due to the secretion of the adrenaline hormone. When the receptors receive a stress stimulus, namely a temperature change, it conveys the stimulus to the hypothalamus and the chromatin cells will secrete catecholamine hormones at the behest of the hypothalamus. This will cause the activation of glycogen catabolism enzymes and increase glucose levels (Masjudi et al. 2016). Under prolonged stress, it is unable to restore homeostasis, leading to maladaptive metabolism, hydromineral imbalance, respiratory malfunction, increased susceptibility to disease, and death (Barton 2002; Polakof et al. 2011). At high blood glucose levels, its appetite will also decrease due to a feeling of fullness, and thereby experience stunted growth (Polakof et al. 2012; Falcinelli et al. 2016). Furthermore, at high glucose levels, gastroparesis may also occur, causing damage to the vagus (digestive) nerve, which is connected to the stomach. When this occurs, digestion in the stomach is obstructed, leading to loss or reduced appetite (Masaoka and Tack 2009; Aljarallah 2011).

Hemoglobin and erythrocyte levels fluctuated during the study, but the number of erythrocytes increased with higher temperatures. The hemoglobin levels in treated fish are higher than in the control, which showed that the fish is in poor condition (Mones 2008; Yanto and Hasan 2015). At 28ºC, 6.9 mmolL^{-1} of the treated fish was similar to the control, which was 6.3 mmol L^{-1} but increased at higher levels. This is due to the increase in the number of erythrocytes to allow efficient homeostasis, needed for the cellular respiration process (Marengo-Rowe 2006; Setiawati et al. 2007; Bozorgnia et al. 2011). Jagtap (2012) also explained that hemoglobin level is directly proportional to that of erythrocytes, which causes fish to experience anemia.

There was an increase in blood cell abnormalities at exposure temperatures of 33-35ºC. The five types of abnormalities observed were echinocyte, elliptocyte, teardrop shape, fusion, and twin. This is caused by an increase in lipid peroxidation of erythrocytes due to exposure to high temperatures (Ghaffar et al. 2015). Increased production of lipid peroxidation in erythrocytes causes defects in the cell membrane. In normal conditions, after passing the capillaries, the blood cells return to their normal shape (biconcave). However, it did not occur in this case, and the blood cells remain oval, called an elliptocyte abnormality (Warang

and Kedar 2018). The formation of more than three centrioles in blood cells also led to the cell pulling the chromosomes in various directions, which caused two or more cells to merge into one form called fusion or twin abnormalities (Baker 1972, Aliyu et al. 2018). Teardrop shape abnormalities occur due to changes in the erythrocyte membrane and cytoskeletal system because of fibrosis in the spinal cord (Khan et al. 2018). This occurs due to exposure to increased temperatures, while echinocyte is caused by disruption of erythrocyte membrane lipid solubility. Therefore, the cell becomes unstable and forms thorn-like protrusions, leading to apoptosis (Walia et al. 2013).

Gills are the most often organ of fish in contact with water and are sensitive to changes in the aquatic environment, including temperature (Flores-Lopes and Thomaz 2011). According to Ayoola et al. (2008) and Clark et al. (2003), exposure to high temperatures also causes damage to the gill organs, disturbing the respiration process. Based on histological examination, several types of gill tissue abnormalities in barramundi were shown in this study. For example, hypertrophy was found predominantly in fish gills exposed to temperatures of 31°C and 32°C. Meanwhile, hyperplasia, cell fusion, and telangiectasis, which predominantly occurred at 33°C and 34°C, as well as the shortening of the secondary lamellae dominantly occurred at 35°C exposure.

This study highlights that increased temperature beyond the optimal level has profound negative effects on physiology, metabolism, blood cells, and gill histopathological structure. It also influences the severity of alterations to the cell and the extent of physiological damage. These physiological, and morphological changes to the blood cell and gills provide helpful information about the environmental conditions. Furthermore, a higher temperature can act as an important biomarker to evaluate the general health and stress status of fish, specifically barramundi. The present data predict the future impacts of climate change on this species as well as other aquatic organisms and the urgent need for mitigation steps to be undertaken.

Conclusion

Water temperature did not significantly affect the survival rate of the barramundi fingerlings but influenced the physiological conditions and gills histology. The concentrations of blood glucose and cortisol, the level of the erythrocytes, and blood cell abnormalities were increased with high exposure to temperature. This indicates that the fish experienced stress under high temperatures. Although barramundi can survive up to a temperature of 35°C, the fish show symptoms of stress at a value above 30°C. Therefore, for aquaculture purposes, the recommended temperature is 28-30°C.

Conflict of interest The authors have no competing interests to declare.

Author contribution ZAM is the primary investigator, responsible for developing the research proposal, and approving the final draft of the manuscript; FA, ASB, and FMN are responsible for conducting laboratory work, data collection, and analysis; NF, UMT, and BH carried out the data analysis and report drafting; MNS, KM , FKK and MW are responsible for final draft checking and proofreading.

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