



Acclimation temperature influences the critical thermal maxima (CT_{max}) of red-spotted grouper

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Abstract

The present study investigated the critical thermal maxima (CT_{max}) of red-spotted grouper, *Epinephelus akaara* under different acclimation temperatures (T_{acc}). Fish were acclimated at 24 °C, 28 °C, and 32 °C water temperature for 2 weeks. Water temperature was increased at a rate of 1 °C/h and CT_{max} level was measured following the critical thermal methodology (Paladino et al., 1980). The results showed that CT_{max} values of *E. akaara* were 35.61 °C, 36.83 °C, and 37.65 °C for fish acclimated at 24 °C, 28 °C, and 32 °C, respectively. The acclimation response ratio (ARR) was 0.26. The CT_{max} values were significantly correlated with body size. Collectively, it is said that the CT_{max} value of red-spotted grouper can be affected by different adaptation temperature (24 °C, 28 °C, and 32 °C) and the fish acclimated to a higher temperature has a higher CT_{max} level. Besides, the CT_{max} value of 35.61 °C–37.65 °C indicating the upper thermal tolerance limit for *E. akaara* under different T_{acc} (24 °C, 28 °C, and 32 °C). Understanding the thermal tolerance of *E. akaara* is of ecological importance in the conservation of this species.

Keywords: Red-spotted grouper, *Epinephelus akaara*, Acclimation temperature, Critical thermal maxima, Acclimation response ratio

Introduction

Temperature is considered one of the most important criteria in species selection for aquaculture. Temperature tolerance of fish varies with species, acclimation time, and magnitude of thermal exposure (Das et al., 2004). Understanding thermal acclimation and critical temperature are two important aspects in evaluating the thermal tolerance of fish (Díaz-Herrera et al., 1998). The critical thermal maxima (CT_{max}) is recognized as

an effective indicator of thermal tolerance of an organism that allows identifying the first stress occurring temperature point (Beitinger et al., 2000). CT_{max} is the temperature for a given species, above which most individuals respond with unorganized locomotion, subjecting to likely death. The CT_{max} procedure is more preferable for working with the endangered or threatened species as it does not require sacrificing the fish as an endpoint (Lutterschmidt & Hutchison, 1997).

The critical thermal methodology is the most common

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method used to determine the CT_{max} of an aquatic organism. Cowles & Bogert (1944) first introduced this methodology and then used it by various researchers on fish or other aquatic animals (Beitinger et al., 2000; Lutterschmidt & Hutchison, 1997). Critical thermal methodology data provides a relative comparison of the thermal tolerance between fish species in extreme temperature. The reaction of the aquatic animals to temperature change is mathematically expressed by acclimation response ratio (ARR), which is also used in thermal tolerance studies (Díaz-Herrera et al., 1998). ARR is defined as the change of the CT_{max} value with per degree change in the acclimation temperature (T_{acc}).

The red-spotted grouper (*Epinephelus akaara*) is a subtropical species, which has a promising aquaculture value due to its high market demand in Korea, southern Japan, southern China, Hong Kong and Taiwan (Sadovy de Mitcheson et al., 2013). Currently, this species is being developed as an export item in Korea. The optimal growth temperature of the red-spotted grouper is reported to be 24°C–28°C (Lee & Baek, 2018). However, the seawater temperature is likely to be increased above 30°C in Korea during the summer season. The seawater temperature is exceeding beyond the normal tolerance range of fish due to the climate change effect (Noyes et al., 2009). The rising water temperature due to the climate change effect is anticipated to affect the productivity of red-spotted grouper in wild as well as in aquaculture conditions. Therefore, it is essential to determine the upper thermal tolerance limit of *E. akaara* in terms of CT_{max}.

CT_{max} provides helpful information on the ecology and distribution of aquatic animals (Bennett & Beitinger, 1997). Therefore, determining the CT_{max} level would be helpful to improve the management and conservation strategies of endangered species like red-spotted grouper (Deslauriers et al., 2016). The effects of T_{acc} on CT_{max} have been explored in many fish species (Beitinger et al., 2000; Currie et al., 1998; Yanar et al., 2019; Zhang & Kieffer, 2014). However, the thermal acclimation effect on *E. akaara* has so far not been studied. Therefore, the purpose

of the present study was to assess the CT_{max} of red-spotted grouper under different T_{acc}.

Materials and Methods

Experimental fish

The experimental fish were collected from Jeju National University, Korea and were gradually acclimated to 120L aquariums for 2 weeks at 24°C, 28°C, and 32°C water temperature. Temperatures of the tank were maintained by using a thermostat (OKE-6422H, Sewon Oke, Pusan, Korea). Each aquarium had equal size and height (75 cm × 45 cm × 45 cm) and was equipped with a recirculating filtration system. Temperature, salinity, pH, and dissolved oxygen (DO) were monitored daily by using a multi-parameter (HI9829, Hanna Instrumentals, Woonsocket, RI, USA). During the acclimation period, salinity, pH, DO, and photoperiod was maintained at 33.67–33.81 psu, 7.89–7.97, and 5.62–6.96 mg/L, 12L : 12D, respectively. Fish were fed 2 times daily (09:00 and 18:00 h; 2% of body weight [BW]) and uneaten food was cleaned after 30 minutes of feeding. 10% of the water in each aquarium was changed daily with filtered clean seawater during the acclimation time. The total length (TL) and BW of fish were recorded individually from each acclimated groups before conducting the CT_{max} test (Table 1).

Determination of CT_{max}

The fish were starved for 1 day before the CT_{max} test. 7 fish from each temperature group were randomly selected and determined the CT_{max} value individually. Thus, 21 individuals from three T_{acc} were used in this process. The CT_{max} levels were assessed following the critical thermal methodology described by Paladino et al. (1980). The temperature increase rate of 1°C/h was used as recommended by Wedemeyer & McLeay (1981). The individual fish from the designated adaptation temperatures (24°C, 28°C, and 32°C) were transferred into a 40L aquarium and subjected to thermal stress by using a thermostatically controlled aquarium heater. Water temperature was constantly

Table 1. Total length and body weight of *E. akaara* at different acclimation temperature (24°C, 28°C, and 32°C). Values are expressed as mean ± SEM (n = 10)

Parameters	Temperature groups		
	24°C	28°C	32°C
Total length (TL, cm)	10.23 ± 0.13	19.33 ± 0.54	18.81 ± 0.31
Body weight (BW, g)	14.80 ± 0.65	144.19 ± 8.25	128.16 ± 0.68

increased ($1^{\circ}\text{C}/\text{h}$) until loss of equilibrium (LOE) was reached (Fig. 1). LOE was designated as the endpoint used for the CT_{max} test, at which fish were first time unable to keep the position in dorsoventrally for 10 sec (Ziegeweid et al., 2008). The final temperature was recorded after reaching the LOE. The temperature was carefully monitored before and during the thermal test. During each test, the DO level was maintained above 6.5 mg/L in the test tank through continuous aeration. Fish were transferred to their respective T_{acc} immediately after the CT_{max} test and monitored for survival for the next 24 h. During the CT_{max} trial, changes in fish behavior (body movement, opercular movement, slime secretion, gasping, revolution along the axis, and LOE) were noted. The thermal tolerance period (TT_p) of fish was also recorded during each test. The ARR was calculated according to Claussen (1977).

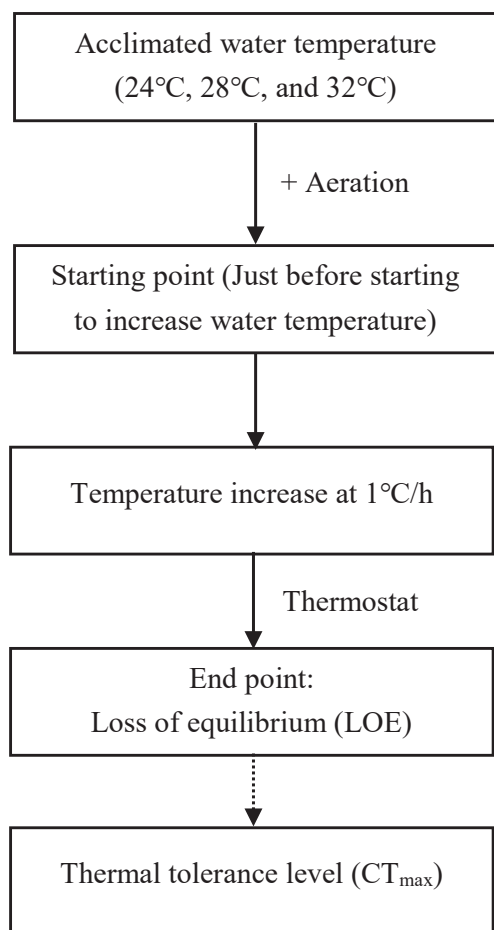


Fig. 1. Flow diagram of the experimental design for the determination of CT_{max} of red-spotted grouper, *Epinephelus akaara*. CT_{max} critical thermal maxima.

Statistical analysis

All data are presented as mean \pm SEM. The significant differences between the means were analyzed with ANOVA (one-way analysis of variance) followed by Duncan's multiple range test using SPSS statistics software (ver. 21.0; IBM, Armonk, NY, USA). Regression analysis was used to estimate the relationship between T_{acc} and other studied parameters. The significance level was set at $p < 0.05$.

Results

Effects of T_{acc} on thermal tolerance (CT_{max}) level

CT_{max} was significantly influenced by T_{acc} (ANOVA, $p < 0.05$; Fig. 2). A significant positive relationship was observed between CT_{max} and T_{acc} ($R^2 = 0.965$, $p < 0.01$, $\text{CT}_{\text{max}} = 29.55 + 0.26 T_{\text{acc}}$). Fish acclimated to 24°C had the lowest CT_{max} (35.61°C) and fish acclimated to 32°C had the highest CT_{max} (37.65°C ; Fig. 2). The ARR ($\text{ARR} = \Delta\text{CT}_{\text{max}} / \Delta T_{\text{acc}}$; Claussen, 1977) was 0.26 between 24°C and 32°C .

Effects of T_{acc} on thermal tolerance period (TT_p)

The thermal tolerance period was significantly affected by T_{acc} (ANOVA, $p < 0.05$; Fig. 3). Fish acclimated at 32°C had higher TT_p (388.57 min) compared with those acclimated at 24°C (318.15 min) and 28°C (325.14 min).

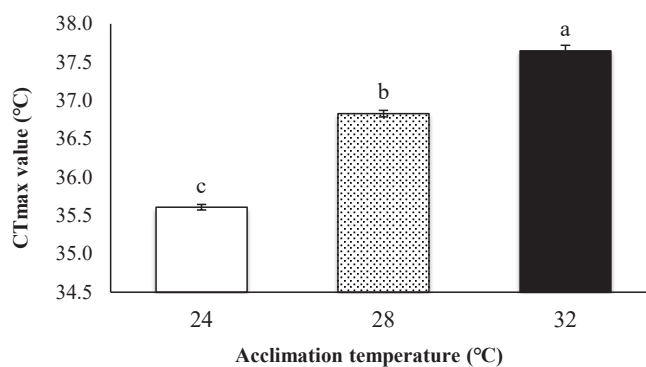


Fig. 2. Critical thermal maximum (CT_{max}) level of red-spotted grouper, *Epinephelus akaara*, under different acclimation water temperatures (24°C , 28°C , and 32°C). Values are presented as mean \pm SEM ($n = 7$). Different lowercase letters indicate the significant difference across temperatures (ANOVA, Duncan's multiple range test; $p < 0.05$). CT_{max} critical thermal maxima.

Effects of T_{acc} on behavioral changes and operculum movement number (OPMN)

Fish of the differently acclimatized groups (24 °C, 28 °C, and

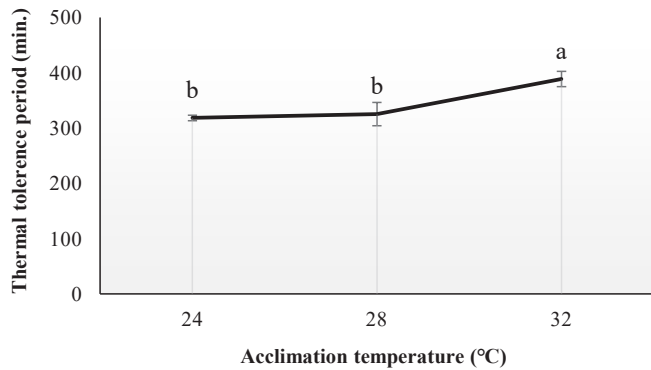


Fig. 3. Thermal tolerance period (TT_p) of red-spotted grouper, *Epinephelus akaara*, under different acclimation water temperatures (24 °C, 28 °C, and 32 °C). Values are presented as mean ± SEM (n = 7). Different lowercase letters indicate the significant difference across temperatures (ANOVA, Duncan’s multiple range test; p < 0.05).

32 °C) showed extreme behavioral changes including rapid body movement, increased opercular movement, slime secretion, gasping, revolving along their axis, and finally LOE during the CT_{max} trial (Table 2).

The operculum movement was significantly influenced by T_{acc} (ANOVA, p < 0.05). Fish acclimated to 24 °C and 28 °C had higher OPMN in comparison to the fish acclimated at 32 °C (Fig. 4).

Correlation between body size and CT_{max} level

A significant positive relationship exists between CT_{max} and body size of red-spotted grouper. CT_{max} was found positively correlated with the TL (T_L, R² = 0.741; p < 0.01; Fig. 5A) and BW (B_w, R² = 0.695; p < 0.01; Fig. 5B) of *E. akaara*. The relationships are described by the equations, CT_{max} = 33.94 + 0.17T_L and CT_{max} = 35.56 + 0.01B_w.

Discussions

The CT_{max} test is the most relevant among the various studied approaches for determining the thermal tolerance of animals

Table 2. Behavioral changes of differently acclimated fish (24 °C, 28 °C, and 32 °C) in response to increased water temperature

Behavioral changes	Acclimated temperature ¹⁾	Exposed temperature (°C)														
		24	25	26	27	28	29	30	31	32	33	34	35	36	37	38
Body movement	24 °C	+	+	+	+	+	+	+	+	++	++	+++	+++	+++	-	-
	28 °C					+	+	+	+	+	++	++	+++	+++	+++	-
	32 °C									+	+	++	++	+++	+++	+++
Opercular activity	24 °C	+	+	+	+	+	+	+	+	++	++	+++	+++	+++	-	-
	28 °C					+	+	+	+	+	++	++	++	+++	+++	-
	32 °C									+	+	+	+	++	+++	+++
Slime secretion	24 °C	+	+	+	+	+	+	+	+	+	++	++	+++	-	-	
	28 °C					+	+	+	+	+	+	++	++	+++	-	
	32 °C									+	+	+	+	++	+++	+++
Gasping	24 °C	-	-	-	-	-	-	-	-	-	++	+++	+++	-	-	-
	28 °C					-	-	-	-	-	-	++	+++	+++	-	-
	32 °C									-	-	-	++	+++	+++	-
Revolving along the axis	24 °C	-	-	-	-	-	-	-	-	-	-	-	++	+++	-	-
	28 °C					-	-	-	-	-	-	-	-	++	+++	-
	32 °C									-	-	-	-	-	++	+++
Loss of equilibrium (LOE) ²⁾	24 °C	-	-	-	-	-	-	-	-	-	-	-	++	+++	-	-
	28 °C					-	-	-	-	-	-	-	-	++	+++	-
	32 °C									-	-	-	-	-	++	+++

¹⁾ Temperature of each experimental group was increased from their respective acclimated temperature.

²⁾ LOE was the end point for CT_{max} test.

-, none; +, normal; ++, high; +++, severe; CT_{max} critical thermal maxima.

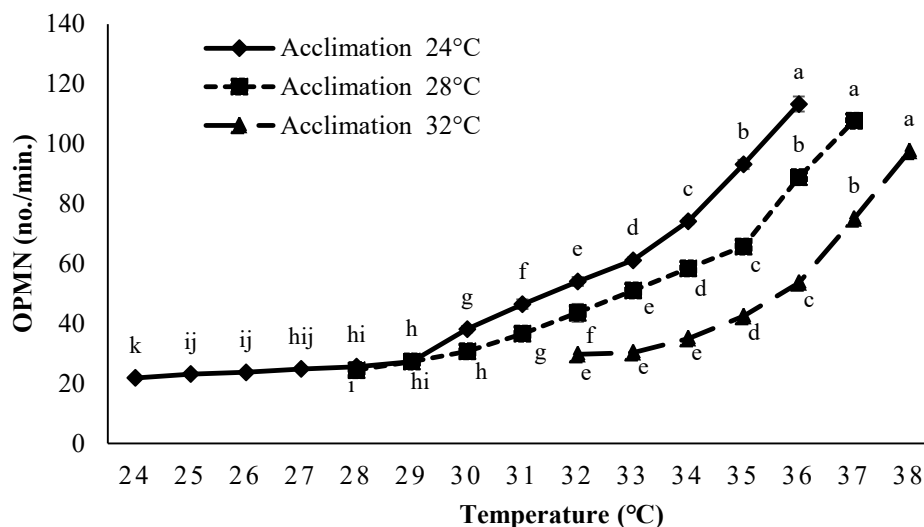


Fig. 4. Operculum movement number (OPMN) of red-spotted grouper, *Epinephelus akaara*, under different acclimation water temperatures (24°C, 28°C, and 32°C). Values are presented as mean \pm SEM ($n = 7$). Different lowercase letters indicate the significant difference across temperatures (ANOVA, Duncan's multiple range test; $p < 0.05$).

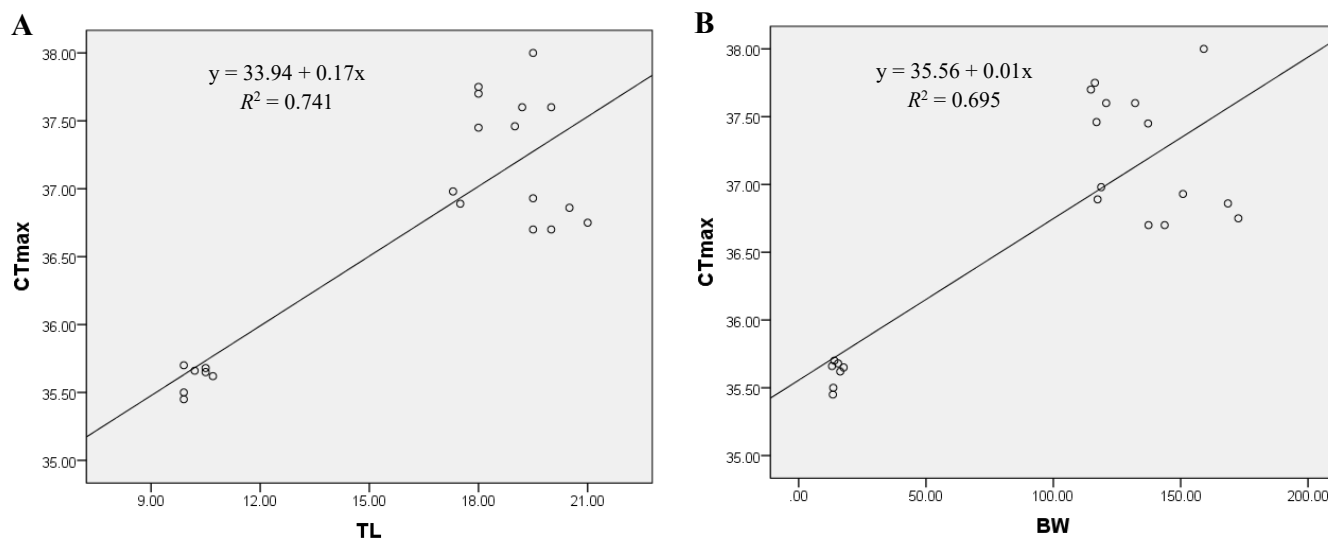


Fig. 5. Correlation between (A) total length (T_L) and CT_{max} , (B) body weight (B_W) and CT_{max} of red-spotted grouper, *Epinephelus akaara*, under different acclimation water temperatures (24°C, 28°C, and 32°C). Values are presented as mean \pm SEM ($n = 7$). Different lowercase letters indicate the significant difference across temperatures (ANOVA, Duncan's multiple range test; $p < 0.05$). CT_{max} , critical thermal maxima.

(Beitinger et al., 2000). CT_{max} provides an ecologically and physiologically valuable reference point that can signal an early sign of thermal stress (Stewart & Allen, 2014). The CT_{max} of *E. akaara* has not been determined. This is the first study that has examined the thermal tolerance, measured as CT_{max} in red-spotted grouper. The results of the present study showed that chang-

es in T_{acc} can significantly affect the CT_{max} level of *E. akaara*.

The T_{acc} has been suggested to be the most important factor that affects the thermal tolerance of fish (Beitinger & Lutterschmidt, 2011). In addition to the T_{acc} , the thermal tolerance limits of fish are also influenced by a variety of factors like species (Das et al., 2004), size (Zhang & Kieffer, 2014), and

condition factor (Baker & Heidinger, 1996). The relationship between CT_{max} and T_{acc} have been reported in many aquatic species (Akhtar et al., 2012; He et al., 2014; Yanar et al., 2019; Zhang & Kieffer 2014) as obtained in the present study for *E. akaara*. In this study, the highest CT_{max} value observed at 32 °C T_{acc} compared with the 24 °C and 28 °C indicating that CT_{max} level increased significantly with increasing T_{acc}. The result of the present study confirms that fish's prior thermal exposure history or T_{acc} largely affects the thermal tolerance limit of fish. Our result is in line with the findings of Cheng et al. (2013), who reported that the CT_{max} value of brown-marbled grouper (*Epinephelus fuscoguttatus*) was ranged from 35.90 °C to 38.30 °C under different T_{acc}. Besides, the CT_{max} values for the tropical bonefish (*Albula vulpes*) were reported to be 36.4 °C and 37.9 °C for fish acclimated at 27.3 °C and 30.2 °C, respectively (Murchie et al., 2011). The CT_{max} values in different T_{acc} were also observed in many other fish species including shortnose sturgeons (*Acipenser brevirostrum*) (Zhang & Kieffer, 2014), climbing perch (*Anabas testudineus*) (Sarma et al., 2010), and carp (*Cyprinus carpio*) (Chatterjee et al., 2004).

The T_{acc} exerts a major effect on thermal tolerance showing a strong linear correlation with CT_{max} (Beitinger et al., 2000). In this study, the linear regression slope indicates that, for each 1 °C T_{acc}, the CT_{max} of *E. akaara* increased by 0.26 °C. This indicates a gain in thermal tolerance (CT_{max}) with an increase in T_{acc}. However, this is fairly smaller than the previously studied shortnose sturgeons (*A. brevirostrum*), where CT_{max} increment rate was 0.52 °C for each 1 °C T_{acc} (Zhang & Kieffer, 2014). This may be due to the regional difference as *E. akaara* is a subtropical species and *A. brevirostrum* is a cold-water species.

The ARR indicates the physiological response of aquatic organisms to the temperature change (Díaz et al., 2002). In the present study, the ARR values of *E. akaara* was 0.26. Similar ARR values have been reported in many warm water fishes such as 0.36 °C for *Pelteobagrus vachelli* (Wang, 2009), 0.40 °C for channel catfish (*Ictalurus punctatus*) and 0.32 °C for large mouthbass (*Micropterus salmoides*) (Currie et al., 1998). However, the present study had smaller ARR value in comparison with the shortnose sturgeons (*A. brevirostrum*) (Zhang & Kieffer, 2014). The ARR values are dependent on the geographic zone where the organisms dwell (Díaz et al., 2002).

In this experiment, the TT_p of *E. akaara* was significantly affected by T_{acc}. The fish acclimated at high temperature may have a higher TT_p. Zhang & Kieffer (2014) reported a similar variation in the TT_p of shortnose sturgeons (*A. brevirostrum*). In our study,

the differently acclimated fish (24 °C, 28 °C, and 32 °C) exhibited behavioral changes including extreme mobility, increased operculum activity, mucus secretion, gulping for air, revolution along the axis, and finally loss of balance after reaching their respective CT_{max} level. Our results are consistent with those of Cheng et al. (2013), who observed similar behavioral changes in the differently acclimated brown-marbled grouper, *E. fuscoguttatus* (20 °C, 26 °C, and 32 °C) during the CT_{max} test. Counting the operculum movement rate is a way to calculate the respiration rates. In this study, the increased operculum movement (OPMN) observed in fish acclimated to 24 °C and 28 °C indicates a higher respiratory frequency compared to fish acclimated at 32 °C. Seol et al. (2008) reported a similar variation in opercular activity in Far Eastern catfish, *Silurus asotus* after exposed to different acclimatized temperatures (20 °C, 25 °C, and 30 °C). In our study, the body size of *E. akaara* was found to be correlated with the CT_{max} value. A similar result was observed in shortnose sturgeons (*A. brevirostrum*) by Zhang & Kieffer (2014). CT_{max} level increases with fish size because larger fish have a lower surface area to volume ratios, which causes a longer period to penetrate the heat into the fish's body (Ziegeweid et al., 2008).

Conclusion

Collectively, it is said that the CT_{max} value of red-spotted grouper can be affected by different adaptation temperature (24 °C, 28 °C, and 32 °C) and the fish acclimated to a higher temperature has a higher CT_{max} level. Besides, the obtained CT_{max} value of 35.61 °C–37.65 °C indicating the upper thermal tolerance limit for *E. akaara* under different T_{acc} (24 °C, 28 °C, and 32 °C). Understanding the critical thermal tolerance limit of *E. akaara* is of ecological significance in the conservation of this species.

Competing interests

The authors declare no potential conflict of interest.

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Acknowledgements

Not applicable.

Availability of data and materials

Upon reasonable request, the datasets of this study can be available from the corresponding author.

Ethics approval and consent to participate

All experiments were conducted following the fish maintenance, handling, and sampling guidelines of the Animal Ethics Committee of Pukyong National University (PKNU; Regulation No. 554).

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