Turkish Journal of Zoology

Volume 47 | Number 5 | Article 4

9-13-2023

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Recommended Citation

RASID, RASINA; TAJUDDIN, AMIRA LIYANA; ZARIM, MOHAMAD ABU UBAIDAH AMIR ABU; THANAKODI, SURESH; RAHAYU, SYARIFAH BAHIYAH; KAMARUDIN, NUR DIYANA; OMAR, AMY AINEDA; AZIZ, MOHD FAZRUL HISAM ABDUL; and HABIB, AHASAN (2023) "Tolerance limit of physicochemical water parameters in giant freshwater prawn (Macrobrachium rosenbergii) in a captive condition," *Turkish Journal of Zoology*: Vol. 47: No. 5, Article 4. https://doi.org/10.55730/1300-0179.3142
Available at: https://journals.tubitak.gov.tr/zoology/vol47/iss5/4

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Turkish Journal of Zoology

http://journals.tubitak.gov.tr/zoology/

Research Article

Turk J Zool
(2023) 47: 291-298
© TÜBİTAK
doi:10.55730/1300-0179.3142

Tolerance limit of physicochemical water parameters in giant freshwater prawn (*Macrobrachium rosenbergii*) in a captive condition

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Received: 31.05.2023 • **Accepted/Published Online:** 19.07.2023 • **Final Version:** 13.09.2023

Absrtact: Water quality is important in natural environments for an organism to sustain optimum physical conditions, as poor physicochemical characteristics can result in poor production and economic losses. The present study sets out to determine the subcritical of minimum and maximum physicochemical water parameters (pH, temperature, and dissolved oxygen) tolerance limit for the giant freshwater prawn, *Macrobrachium rosenbergii*. To explore this, samples of 48 juvenile prawns (2.70 ± 0.27 g) were randomly subject to 6 replicates. The trials on dissolved oxygen were conducted by reducing dissolved oxygen levels in water over 12 h duration, pH test was conducted by lowering the pH level by adding 5% hydrochloride acid over 2 h. A temperature test was conducted by increasing the temperature with the water heater over 4 h, and ice was added to reduce the water temperature over 3 h. The critical point of dissolved oxygen was observed at 2.9 to 1.9 mg O_2 L^{-1} before *M. rosenbergii* displayed signs of stress until fatality occurred at 0.5 mg O_2 L^{-1}. The subcritical point range of pH was recorded between 2.6 (min) and 4.6 (max). The critical tolerance cold shock temperature limit for *M. rosenbergii* was observed at a range of 16.8 °C to 23.2 °C. *M. rosenbergii* showed stress signs between 32.9 °C and 36.7 °C. The results provide critical information on the subcritical tolerance levels of each water parameter, including dissolved oxygen, temperature and pH for giant freshwater prawns, which could prevent massive mortality due to changes in physicochemical water parameters.

Key words: Freshwater prawn, critical water level, thermal tolerance, *Macrobrachium rosenbergii*

1. Introduction

Aquaculture is one of the emerging food-producing sectors globally and is flourishing daily (Habib et al., 2020; Syed et al., 2022). Among all finfish, crustacean production and rearing has become a developing industry in recent years, and about 1.8 kg is per capita global consumption of crustaceans (FAO, 2020; Harlioglu and Farhadi, 2022). Giant freshwater prawn, *M. rosenbergii* is a major and highly valued fisheries product that contributes to global aquaculture (Nadella et al., 2018; Tinikul et al., 2017; Tinikul et al., 2023) and throughout the Asia Pacific region (Liew et al., 2022). However, one of the major factors affecting the success of aquatic species cultivation in aquaculture is the optimal aquatic environment. In addition, temperature alters the rates of the organism's physiological process, which may involve harmful effects, and organisms may not recover (Reyes-Avalos et al., 2023). To achieve optimum production, the physicochemical conditions of the water, such as temperature, oxygen and pH, should be within its tolerance limits. The physicochemical properties of water are an indicator of water quality, and a small variation can affect the aquatic animal, leading to mortality (Pinheiro et al., 2021). Direct communication with farmers reported an inability to identify the critical point of physicochemical properties such as dissolved oxygen, temperature, and pH for freshwater prawns before mortality occurs. Previous studies' optimum range of water parameters includes a dissolved oxygen ratio of 3–8 mg L^{-1}, pH 7–8, and a water temperature of 25–30 °C suggested for *M. rosenbergii* (Habashy and Sharshar, 2020). However, mortalities occurred within the optimum rate suggested as farmers have trouble finding the water parameter's exact value when mortality transpires. Optimal management and monitoring of water parameters during culturing of any aquatic organism are essential to ensure good growth and survival.

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Dissolved oxygen is one of the most important indicators of water quality. It is essential for the survival of fish and other aquatic organisms (Ali et al., 2022). Previous studies on the dissolved oxygen optimum range of *M. rosenbergii* have been reported from the lowest level of 1.21 mg L^{-1} to the highest 8.36 mg L^{-1} (Hai et al., 2015) 6.02–6.91 mg L^{-1} (Chand et al., 2015) and 3–7 mg L^{-1} (New, 2002). The *M. rosenbergii* observed within 24 hours after exposure to hypoxia and hyperventilation, start taking quick and loud gasps of air at below 2.75 mg L^{-1} to 1.75 mg L^{-1} (Cheng et al., 2003).

Optimum water temperature allows aquatic organisms to perform metabolism and improve growth. Temperature is an important water quality parameter as it affects the amount of dissolved oxygen in the water and increases the rate of chemical reaction (Suantika et al., 2017). The optimum temperature range was reported to be 25.8–29.4 °C for *M. rosenbergii larvae* (Crisp et al., 2017), whereas for *M. rosenbergii* adults ranging between 26.13 °C and 34.2 °C (Hai et al., 2015) 31.44–33 °C (Chand et al., 2015) and 28–31 °C (Chowdhury, 1993). A study observed temperature-induced metabolic response (TIMR) on *Macrobrachium tenellum* decreased when the temperature was high at 35 °C (Hernández-Sandoval et al., 2018). In addition, a trial by (García-Guerrero et al., 2022) found that oxygen delivery appears insufficient to sustain aerobic energy generation at a much lower temperature of 28 °C, which affects *Macrobrachium americanum* fundamental biological function. Sudden temperature changes can result in changes in the mobility of *M. rosenbergii* where stress behaviour characteristics such as pleopod twitch, early disorientation or total disorder were observed (Hernández-Sandoval et al., 2018). Locomotion is almost lost due to presynaptic failure and neuromuscular blockade (White, 1983).

The pH is the measurement of hydrogen ion concentration and one of the most critical indicators of water that can affect the survival of shrimp (Duan et al., 2019). Reported suitable pH optimum range for *M. rosenbergii* is steadily around 7.63–8.07 (Hai et al., 2015), 7.86–8.32 (Chand et al., 2015) and 7.0–8.5 (Boyd and Zimmermann, 2000; Chowdhury et al., 1993; New, 2005). The prawn exposed to pH 6 and 9 were very weak, and their feeding behaviour was very poor (Habashy and Sharshar, 2020). McLuckie et al., (2021) reported that *Metapenaeus macleayi* did not survive under acidic pH 5 circumstances and showed signs of stress. Shrimp exhibits behaviour characteristics such as being shocked, weakened, and stopping eating at low pH, thrashing, darting, gasping, swimming near the water's surface and trying to jump out of the tank (Jayalakshmi et al., 2016). According to Habashy and Sharshar (2020), lower 6 and higher 9 pH affected the moulting, growth, and survival of *M. rosenbergii.* Aquaculture species in captive conditions living in confined environment without migration opportunity will suffer from water pH fluctuation caused by terrestrial run-off or eutrophication issue. These conditions might negatively impact freshwater prawn health status or mass mortality (Liew et al., 2022). The lack of research on the subcritical point of minimum and maximum level rather than the optimum ready value of widely available water parameters would provide a greater understanding to prevent early mortality of the specific aquatic animal. Therefore, this study investigated subcritical minimum and maximum physicochemical properties levels, including dissolved oxygen, temperature, and pH for *M. rosenbergii*.

2. Materials and method

2.1. Prawns and experimental conditions

This experiment was conducted within the Faculty of Fisheries and Food Science (FPSM) Marine Hatchery, University of Malaysia Terengganu (UMT) compound. A total of 48 juvenile *M. rosenbergii* (mean weight of 2.70 ± 0.27 g) were obtained from a single batch of fertilised eggs from brood stock maintained within the marine hatchery. Two prawns were randomly allocated into the individual aquarium (L cm × W cm × H cm) with 20 L water capacity.

2.2. Physicochemical parameters test: dissolved oxygen, temperature, and pH

M. rosenbergii. were subject to physicochemical parameters tests, including dissolved oxygen, temperature, and pH tolerance limits. YSI multiparameter (Horiba-U-52) was used to measure each water parameter. Each treatment was conducted in six replicates to obtain frequency and consistent results. The surface of the experimental tanks was tightly sealed with polystyrene to prevent air from entering the tank, and aeration was removed beforehand. The dissolved oxygen level was measured using the YSI every 1 h for 12 h. The pH test was conducted by adding a 5% pure HCL solution to 500 mL of distilled water. The HCL solution was titrated into the experimental tanks containing the animals every 10 min. pH readings and animal stress behaviour were observed for 2 h. The cold temperature trial was commenced by adding ice to the aquarium to lower the water temperature readings. Ice was added every 10 min, and the trial was conducted for 3 h. The hot temperature experiment was tested by setting the 25 watts heater (Aquaspeed 100W) to the required temperature setting. An increment of 1 °C temperature was set every 30 mins for 4 h. Observation of the prawn's behaviour including swimming pattern and stress signs from the beginning of the trial throughout the experiment and at each critical point was also recorded.

2.3. Statistical analysis
The data were tested for normality and homoscedasticity using the Shapiro–Wilks and Levene tests. The results are presented as means unless otherwise stated. All data were analysed by one-way analysis of variance (ANOVA), and Duncan's test at a significance level of $p = 0.05$. All analyses were performed using SPSS software (version 26) for Windows.

3. Results
3.1. Dissolved oxygen
In the present study, dissolved oxygen's maximum and minimum critical levels ranged between 2.7–3.2 mg O_2 L^{-1} and 1.4–2.4 mg O_2 L^{-1} (Table and Figure 1). The maximum critical point level observed in replicate 6 (at 24.8 °C) was significantly higher ($p < 0.05$) than in other replicates. On the other hand, minimum critical point levels were observed in replicates 3 and 5 (at 16.4 °C), which were significantly lower ($p < 0.05$) compared to other replicates (Figure 1).

3.2. pH
The pH values of the maximum and minimum critical levels resulted in intervals 4.23–4.9 ppm and 2.5–2.94 ppm (Table and Figure 2). The maximum critical point limits for replicates 5 and 6 at pH 4.9 were significantly higher ($p < 0.05$) than those of other replicates. On the other hand, the minimum critical point limit of replicate 4 pH of 2.16 was significantly lower ($p < 0.05$) compared to that of another replicate (Figure 2).

3.3. Cold temperature
Cold maximum and minimum temperature values were recorded in a narrow range of 20.4–24.8 °C and 16.4–17.4 °C (Table and Figure 3). The maximum critical point limit was observed in replicate 6 at 24.8 °C, significantly higher ($p < 0.05$) than the other replicates. On the other hand, minimum critical point limits were significantly lower ($p < 0.05$) in replicates 3 and 5 at 16.4 °C (Figure 3).

3.4. Hot temperature
Hot temperature maximum and minimum critical levels ranged between 35.7–38.2 °C and 32.0–33.7 °C (Table and Figure 4). The maximum critical point limits in replicate 3 at 38.3 °C were significantly higher ($p < 0.05$) than those of other replicates. The minimum critical point limits were observed in replicate 5 at 32.0 °C (Figure 4). Variations of different pH and temperature showed in Figures 5 and 6 during the experimental period.

4. Discussion
The present study explored the physicochemical parameter of dissolved oxygen, pH, cold and hot temperature critical point level maximum and minimum on *M.rosenbergii.*

4.1. Dissolved oxygen
In the present study, the critical point level between six replicates presented to *M. rosenbergii* in significant differences ($p < 0.05$). The mean DO critical point limit was 2.9 mg O_2 L^{-1} up to 1.9 mg O_2 L^{-1} as a minimum tolerance level before fatality. The prawns were also observed to be static and remained in the bottom of the tank during hypoxia. This trend follows the DO critical point level presented in Table, and the *M. rosenbergii* stressed when DO was below 2 mg O_2 L^{-1} and exhibited hypoxia. Prawn fatality occurred at 0.5 mg O_2 L^{-1}. Previous studies reported freshwater prawns observed within 24 h after exposure to hypoxia and hyperventilation started taking quick and

Table. Physicochemical parameter pH maximum and the minimum point of dissolved oxygen, temperature, and within 2–12 hours observations in *M. rosenbergii.*

	Parameter							
Replicate	Maximum				Minimum			
	DO[1] (mg L^{-1})	pH	Temperature (°C)		DO[1] (mg L^{-1})	pH	Temperature (°C)	
			Cold	Hot			Cold	Hot
1	2.8	4.29	20.4	36.3	2.0	2.82	16.7	32.6
2	3.0	4.82	22.5	37.3	1.8	2.47	17.0	33.3
3	2.9	4.50	23.8	38.2	1.4	2.50	16.4	33.7
4	2.7	4.23	23.1	36.0	1.8	2.16	16.9	33.5
5	2.8	4.90	24	36.6	1.8	2.90	16.4	32.0
6	3.2	4.90	24.8	35.7	2.4	2.94	17.4	32.5

[1]Dissolved oxygen.
Data are means of six replicates.
Different superscripts indicate significant differences ($p < 0.05$).

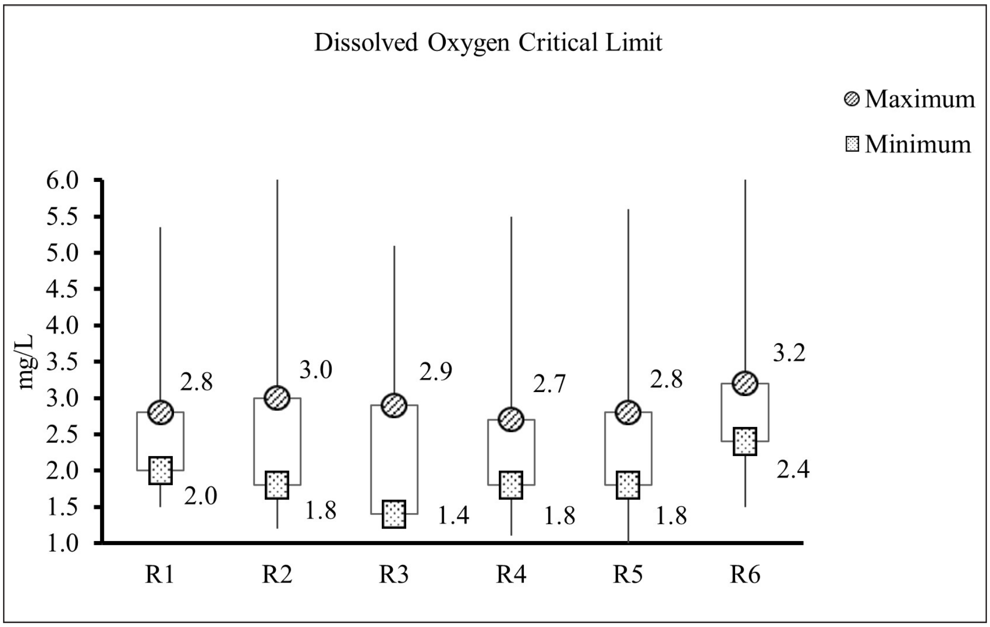

Figure 1. Dissolved oxygen critical tolerance limit (maximum and minimum) for *M. rosenbergii*.

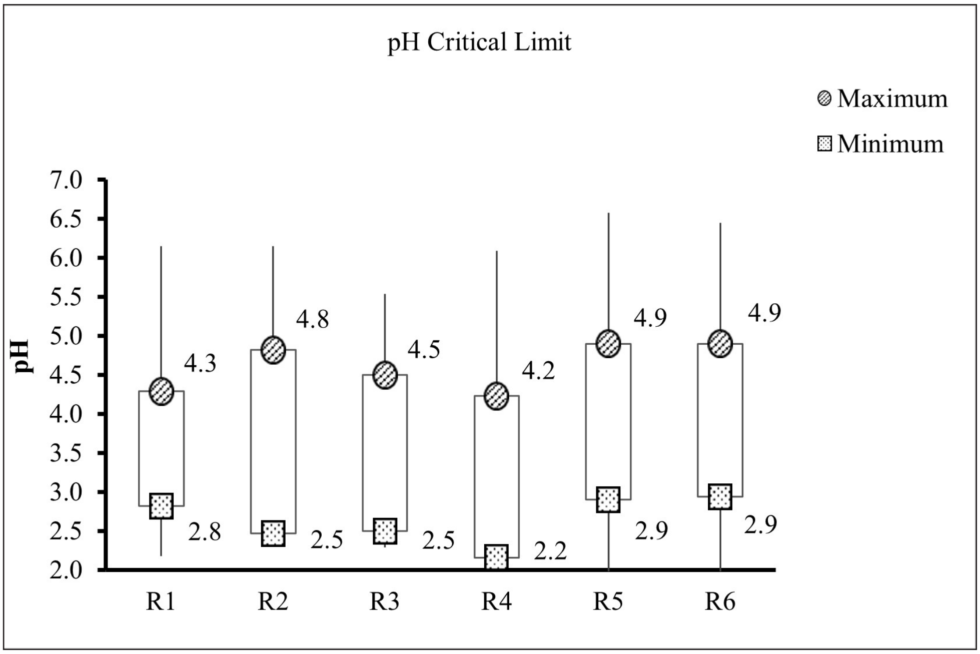

Figure 2. *M. rosenbergii* mean pH critical tolerance limit (maximum and minimum).

loud gasps of air when oxygen levels were low (2 mg O_2 L^{-1}) (Cheng et al., 2003). DO has often been considered an important environmental factor determining the success and intensification of prawn culture, and DO values higher than 5 mg O_2 L^{-1} have often been recommended for intensive culture practice (Cheng et al., 2003). The low DO adversely affected crustaceans' behaviour and normal physiology, such as the survival and respiration of penaeid shrimps (Aquacop and Soyez, 1988; Clark, 1986; Seidman and Lawrence, 1985). Clark (1986) reported that mortality occurred and moulting was inhibited when *Penaeus semisulcatus* were kept at an oxygen level of 2 mg O_2 L^{-1} for 17 days.

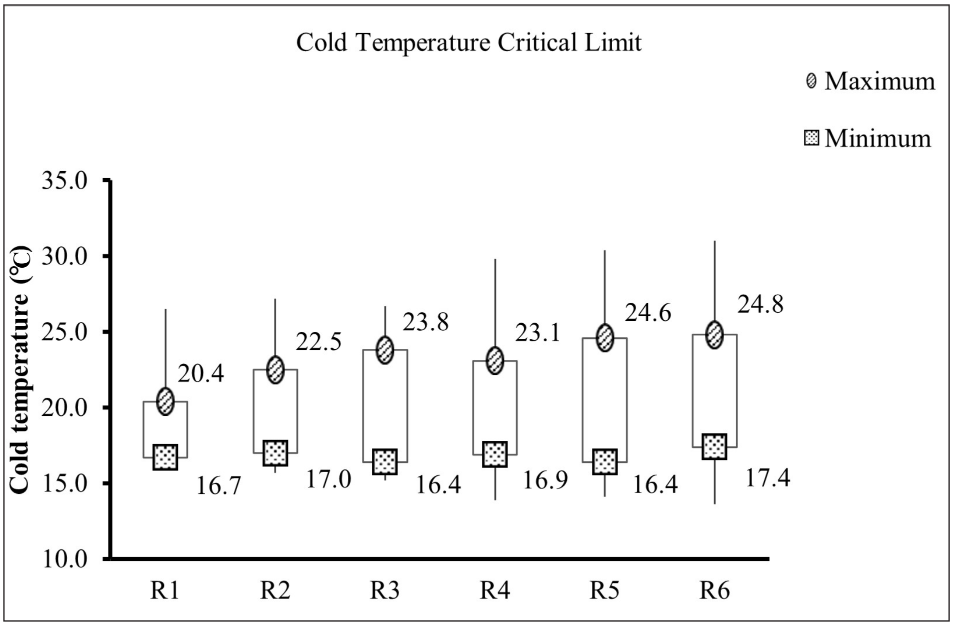

Figure 3. Six replicates of the mean of cold temperature critical limit (maximum and minimum) subject to *M. rosenbergii.*

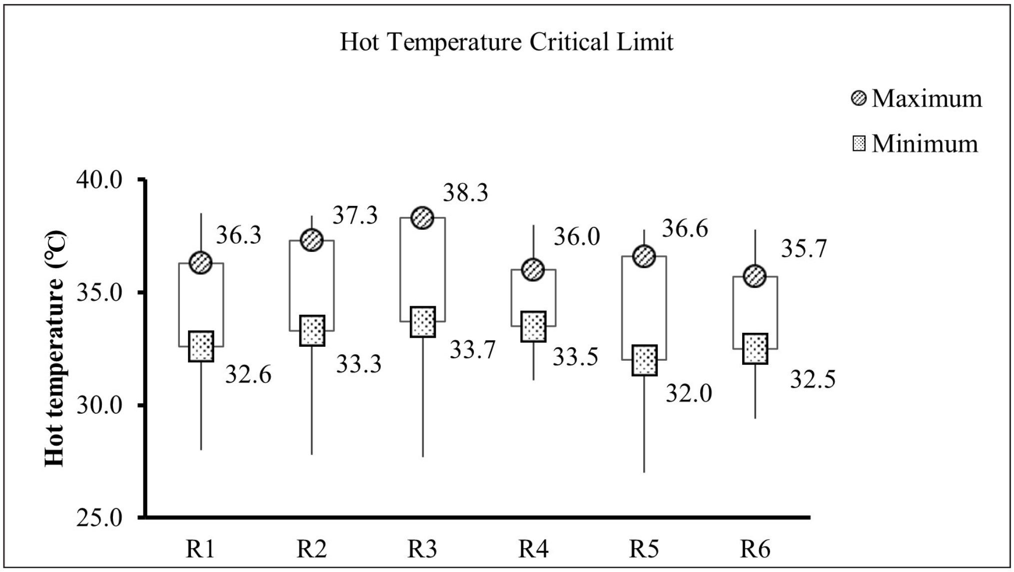

Figure 4. Mean of hot temperature critical level (maximum and minimum) conducted on *M. rosenbergii* in six replicates.

4.2. pH

pH is an important factor in freshwater bodies and a significant factor for understanding the environmental conditions in an ecosystem affecting biochemical and chemical reactions and controlling activities relating to the distribution of aquatic flora and fauna. The present trial found that the mean pH critical point maximum limit was pH 4.6 and the minimum of 2.6 for *M. rosenbergii*. The prawns were observed to be static, weak, and unstable at the bottom of the tanks. Das and Sahu (2005) observed that a pH of 5 and hazardous relative toxicity lead to *Penaeus monodon* and *Penaeus indicus* mortality within 1.61 times per interhour. Prawns have been reported to exhibit crucial behavioural traits at pH 2.5, causing irreparable harm to the linings of the skin and organs (Kawamura et al., 2018).

4.3. Cold and hot temperature

Water temperature is considered one of the crustaceans' most important environmental factors since it influences

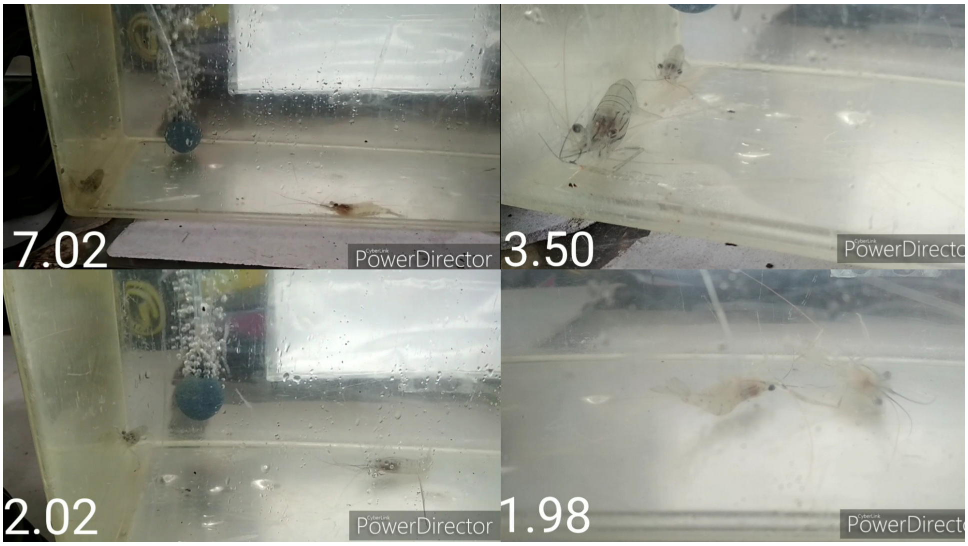

Figure 5. Variation of different pH levels (7.02, 3.50, 2.02, and 1.98) in the experimental tank.

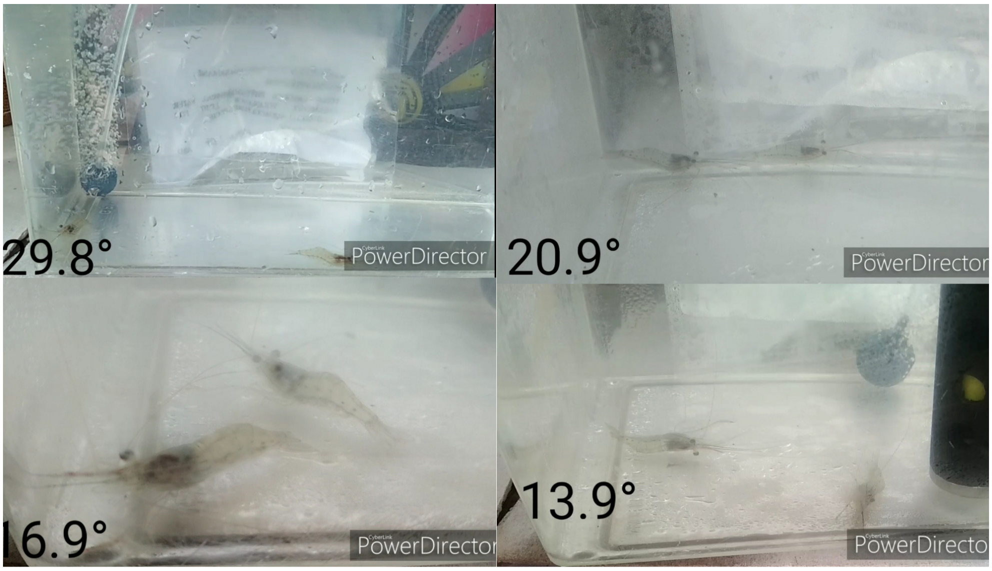

Figure 6. Variation of different temperatures (29.8 °C, 20.9 °C, 16.9 °C, and 13.9 °C) in the experimental tank.

metabolism, oxygen consumption, feeding, growth, moulting, survival, and tolerance to toxic metabolites (Ren et al., 2021). The rapid decrease in a river or sea water temperature results in cold shock affecting aquatic animal's health condition. The optimal temperature range of *Macrobrachium rosenbergii* is reported to be between 25 °C and 31 °C (Chang et al., 2015). In juvenile *P. vannamei*, the optimum temperature was higher than 30 °C for adult shrimp, which was around 27 °C (Wyban et al., 1995).

In the present study trial, the cold temperature max critical point limit was 23.2 °C and the minimum of 16.8 °C for *M. rosenbergii* before a fatality occurred. The min critical hot temperature tolerance limit was 32.9 °C and the max of 36.7 °C before the fatality of *M. rosenbergii* occurred (Table 1). The prawns were observed to be hyperactive and actively moving around the tank. On the other hand, they were inactive and did not survive for long periods below the tolerance min and max limit. The behaviour of the mobility and signs of stress, such as pleopod beating, initial disorientation, and total disorientation, were observed when exposed to temperatures beyond the min and max tolerance limit, similar to a previous study reported by Hernández-Sandoval et al. (2018). Inappropriate or extreme temperatures caused physiological stress or even death (Payette and McGaw, 2003).

5. Conclusion

The result of this study indicated the critical physiological water parameter tolerance limit for *M. rosenbergii.* The critical point limit of dissolved oxygen was 2.9–1.9 mg O_2 L^{-1} and fatality occurred at 0.5 mg O_2 L^{-1}. pH critical tolerance limit for *M. rosenbergii* is pH 4.6–2.6. The cold temperature critical tolerance limit is 23.2–16.8 °C, and the hot temperature tolerance limit is between 32.9 and 36.7 °C. Measures should be taken to prevent stress on the prawns when changes in the physicochemical water parameters occur in farms to prevent massive mortality.

Conflict of interest

The authors declare that there are no conflicts of interest.

Acknowledgements

The author would like to thank the Marine Hatchery, Faculty of Fisheries and Food Science (FPSM), University of Malaysia Terengganu (UMT) staff for their kind assistance with the technical aspects of this study.

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