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Physiological tolerance of the early life history stages of fresh water prawn (*Macrobrachium rosenbergii* De Man, 1879) to environmental stress

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The present study examines the effect of temperature and salinity on the larval development and survival of *Macrobrachium rosenbergii.* The larvae showed 100 % mortality at higher temperature (33.5 ± 0.5 °C) in all the salinity conditions (12, 15, and 20 PPT). The survival rate varied between 76-96 % on exposure to lesser temperature conditions. Likewise, the post-embryonic yolk lasted for 4 days at ambient temperature (29 °C); whereas, at 33.5 ± 0.5 °C, it lasted only for 2-3 days. There was an increase in total length of larvae, when exposed to higher temperature and salinity. For the cardiac performance, larval heart beat (f_H) significantly increased for higher temperature and salinity conditions (20 PPT; 33.5 °C) and lowered at ambient condition 12 PPT; 29 °C. Larval stroke volume V_s, Cardiac output (Qt) were higher in ambient conditions and lowest in higher temperature and salinity conditions. The larval activity decreased significantly at higher temperature and salinity conditions, compared to ambient conditions.

[Keywords: Climate change, Cardiac performance, Early life history stages, Larval activity, Prawns yolk consumption]

Introduction

Earth's climate is changing at a rapid pace, mainly because of the increased carbon dioxide emission caused due to anthropogenic activities[1]. Though climate change is a global phenomenon, its effects on living organisms manifest at very local levels[2], and the magnitudes of these changes/effects could considerably fluctuate from location to location. Estuaries are one such ecosystem that is influenced by a variety of anthropogenic stressors[3]. Estuaries act as a natural shelter for all myriad forms of aquatic life on earth where the spawning and feeding of early life forms of fish and shellfish happen[4]. The diversity, distribution and biological functions of the organisms living in estuaries are influenced by climate change stressors[5-7]. Climate change stressors including, rise in temperature, precipitation, salinity changes, sea level rise and ocean acidification pose deleterious impact on marine organisms and ecosystems[8].

The giant fresh water prawn, *Macrobrachium rosenbergii*, is an indigenous species to south and south-east Asia[9]. Lately, this prawn has been introduced to several other countries as commercially important aquaculture species[10]. In their natural environment, *M. rosenbergii* is inhabited in various environments including fresh water streams, estuarine waters and canals connected to the sea[11-13]. The life history of *M. rosenbergii* is amphidromous in nature. The adults spend their life in the fresh water; after spawning, brooders migrate to estuarine waters for hatching. The larval development takes place in estuarine water, and after settling down, it returns to the fresh water[10,14]. However, fresh water prawn culture in India increased steadily since 1999 reaching a peak output of 42,780 tons in 2005, but then declined to 6,568 tons in 2009–2010 due to poor seed and brood stock quality[15].

In general, elevated temperature and salinity variations affect the metabolic rate, calorific intake and energy budget of decapods[16]. Temperature and salinity are the important abiotic factors that control the growth and development of decapods crustaceans[16-18]. Salinity around 12-15 PPT and temperature range from 28 to 30 °C appeared to be optimal for the adults and larvae[19]. Salinity plays a critical role on egg, embryo and larval development during the life cycle of *M. rosenbergii*. Yen and Bart[20] studied the negative effects of elevated salinity on the reproduction and growth of female *M. rosenbergii*. Salinity influences all aspects of larval biology including survival, development, morphology, the moulting cycle, growth, feeding, metabolism, energy partitioning, and behavior[16]. Likewise, Guest and Durocher[21] reported the

necessity of brackish water for the completion of larval development in *M. amazonicum*.

Temperature, the other major factor, influences the species distribution, range of thermal tolerance and acclimatization of ectotherm organisms[22]. In tropical environment, ectotherms have narrow range of thermal tolerance due to lack of seasonality in this region and most of them are living at the verge of their maximum thermal limit, making them vulnerable under global warming scenarios[23]. In crayfish, significant difference on the gonad development and spawning were observed at different temperatures[24]. Similarly, the effect of high temperature showed irregular patterns of egg development in *M. americanum*[25]. The growth pattern of the *M. rosenbergii* adults also changed, as temperature increased from low to normal/optimum, with the growth declining at the higher temperature[18]. Furthermore, synergistic effects in combination with one or more environmental variables (e.g., temperature and salinity) also play a key role in the ecological and geographical distribution of a species. Nelson *et al*.[26] reported interactive effects of salinity and temperature on the metabolic rate of juveniles of *M. rosenbergii*.

The persistence or the failure of a population is determined by the successful completion of all larval stages[27]. Even though the impact of varying salinities and temperature have been extensively studied in adult and juveniles of *M. rosenbergii*, the understanding on the physiological consequences of individual as well as interactive impacts of salinity and temperature on the early life stages of this species is still scarce. Under the predicted climate change scenario, understanding the physiological constrains and energy cost for the completion of larva stages are vital to know the adaptive capability of successive population. Hence, in the present study, we used yolk utilization, cardiac performance, larval activity, growth as proxy to know the physiological fitness of the organism under future climate change condition. These data may give insight on the impact of climate change stress on early life history stages of this important aquaculture species.

Materials and Methods

Animal collection and maintenance

The adult male and female of *M. rosenbergii* were procured from a fisherman based at Cuddalore, Tamil Nadu. The shrimps were transported to the demonstration hatchery at the Centre for Climate Change Studies, Sathyabama Institute of Science and Technology, Chennai in an oxygen filled poly-ethylene bags. After transfer, the shrimps were acclimatized for 2-4 hours to the laboratory condition and shifted to 4×500 l fiber tank filled with the fresh water and fitted with biological filters. The water temperature was maintained at 29 °C and salinity at 0 PPT. The animals were fed three times a day with grated potatoes and commercial prawn feeds. Ten to 30 % of the water was exchanged once in 3 days to maintain the quality.

Experimental set up

Twenty spawned brooders were kept in 4×300 l fiber tank at 29 °C and salinity 12 PPT and observed for the embryonic stages until hatching took place. Organogenesis and developmental changes were recorded under a light microscope equipped with computer aided software (Nikon Eclipse E600). On 19th day, most of the brooders released embryos which were pooled together in 20 l fiber tank. Equal numbers of larvae were distributed among tanks with different experimental conditions. The following combinations of temperature and salinity were used: 29 °C/12 PPT, 31°C/12 PPT, 33.5 °C/12 PPT, 29 °C/15 PPT, 31 °C/15 PPT, 33.5 °C/15 PPT, 29 °C/20 PPT, 31 °C/20 PPT and 33.5 °C/20 PPT. Unfortunately, due to technical faults, all the larvae in tank with 20 PPT and 31°C were lost on day 1st, hence excluded from the analysis. The desired temperature in the tank was maintained using aquarium thermostat (Aqua Zonic, Singapore). The desired salinity was achieved by mixing fresh water with seawater of 35 PPT. The experimental tank had stock of 3540 ± 82.76 larvae in it. During the experiment, larvae were fed with live Artemia. The experiment lasted for 5 days from the day of hatching till most of the larvae were at 4th stage. The yolk utilization was measured everyday till fully consumed. Following 5th day, larval survival rate, growth rate, activity, and cardiac performance were measured.

Yolk utilization

Depletion of yolk in larvae was determined by staining the live larvae with Nile Red. To quantify the yolk, 10 larvae from each treatment condition were stained with Nile Red (10 mg/ml) for 15 minutes, followed by washing using distilled water. The images were captured using the Epifluorescence microscope (Nikon Eclipse E 600, excitation filter BP 490; barrier filter O515) equipped with digital camera and computer aided software (NIS-Elements).

The images were analysed by color threshold function in the image processing software Image J[28]. The total area and total intensity were calculated and represented in mm and pixels, respectively.

Larval survival rate

Larval survival rate was estimated after 5 days of incubation at different conditions using the formulae:

Larval survival rate (%) = {initial number - (initial number - final number)/initial number} *100.

Morphometrics of larvae

Morphometric analyses were conducted on the images taken by stereomicroscope equipped (Motic (Xiamen) Electric Group Co., Ltd, China) with digital camera and computer aided software (Motic image plus 3.0). Total length was estimated after 5 days of incubation by subtracting initial length from final length and represented in millimeter (mm).

Larval activity

Larval activity, defined as the rate of maxilliped movement, was determined in the video taken using stereomicroscope[29]. Larva was trapped in a drop of water on cavity slide and covered with a cover slip. The water drop was taken from the respective experimental tank, and video was recorded at 5X magnification using stereomicroscope (Motic (Xiamen) Electric Group Co., Ltd, China) for 2 minutes. Videos were parsed into 10s segments (free video cutter v 10.4) and slowed to 25 % from original speed (VLC media player V. 2.4.4) for counting maxilliped movements (first three feeding legs) for at least 3 individuals. The results were represented in beats per minute (bpm).

Cardiac performance

Heart rate (f_H) and stroke volumes (V_S) were determined from the same video recorded for the larval activity. The videos were slowed down to 25 % from original speed (VLC media player V. 2.4.4) and zoomed to count accurate f_H. Screen marker (Epic pen V. 3.0) was used to mark end-diastolic and end-systolic perimeter. V_S were determined by calculating the difference between the end-diastolic volume (EDV) and end-systolic volume (ESV), assessed using Image J.

$$V_S = EDV\text{-}ESV$$

EDV and ESV were assumed as prolate spheroids[23,30-31] so following equation was used to calculate volume:

$$Volume = 4/3\pi\ ab^2$$

where, a is the radius of major diameter and b is the radius of minor diameter (from image analysis).

Individual cardiac output (Q) was determined as a product of V_S and f_H.

Statistical analysis

Data were tested for normality and homogeneity using Shapiro–Wilk and variance test. After successful completion of these parameters, two-way ANOVA and post hoc tukey's tests on the mean values for finding the independent and dependant effect of temperature and salinity were analyzed. All the statistical analyses were performed using SPSS v. 22[32].

Results

Larval survival rate

Immediately after hatching, the larvae were collected and concentrated in 12 PPT seawater with a density of 71 ± 3.7 individuals /ml^{-1}. Soon after, equal volume of water assuming equal numbers of larvae (approx. 3500 ± 82.76 individuals) were distributed among all the experimental conditions. On day 5th, 100 % mortality was observed at the higher temperature conditions (33.5 ± 0.5 °C) in all the salinity conditions (12 PPT, 15 PPT, and 20 PPT). However, among other experimental conditions, the survival rate varied between 76-96 % (Table 1). Unfortunately, due to technical faults, all the larvae in tank with 20 PPT and 31°C were lost on day 1st hence it was not included in further analysis. At the ambient temperature of 29 °C and different salinity, larvae survival rates were 96 %, while at 31 °C; 12 PPT and 31 °C; 15 PPT showed 86 % and 76 % survival rate, respectively.

Yolk consumption

Post-embryonic yolk was depleted at different rates under varying experimental conditions. In the ambient

Table 1 — Survival rate of larvae exposed under different conditions

Condition	Rate of survival (in %)
29 °C/12 PPT	95.8
31 °C/12 PPT	87.6
33.5 °C/12 PPT	0
29 °C/15 PPT	96.8
31 °C/15 PPT	76
33.5 °C/15 PPT	0
29 °C/20 PPT	95.3
33.5 °C/20 PPT	0

condition (12 PPT; 29 °C), yolk lasted till day 4th. Moderate increase in temperature (31 °C) and salinity alone did not show any effect on the rate of yolk consumption, whereas higher temperature (33.5 °C), alone and in combination with low and high salinity (12 PPT & 20 PPT, respectively) caused faster depletion of yolk. In these cases, the yolk was almost depleted either on day 2nd or day 3rd (Fig. 1). The utilization of the yolk under different conditions in terms of total area and intensity has been shown in Figure 1.

Morphometrics of larvae

Larval morphometrics were analyzed by measuring total length after 4th day (Fig. 2). In general, an increase in the total length of larvae were observed upon exposure to higher temperature and salinity independently or in combination up to 4th day ($p < 0.001$(temperature); $p < 0.01$(salinity); $p < 0.05$ (temperature*salinity)), however the larvae in the higher temperature (33.5 °C) in all three salinity (12, 15, and 20 PPT) conditions died on 5th day.

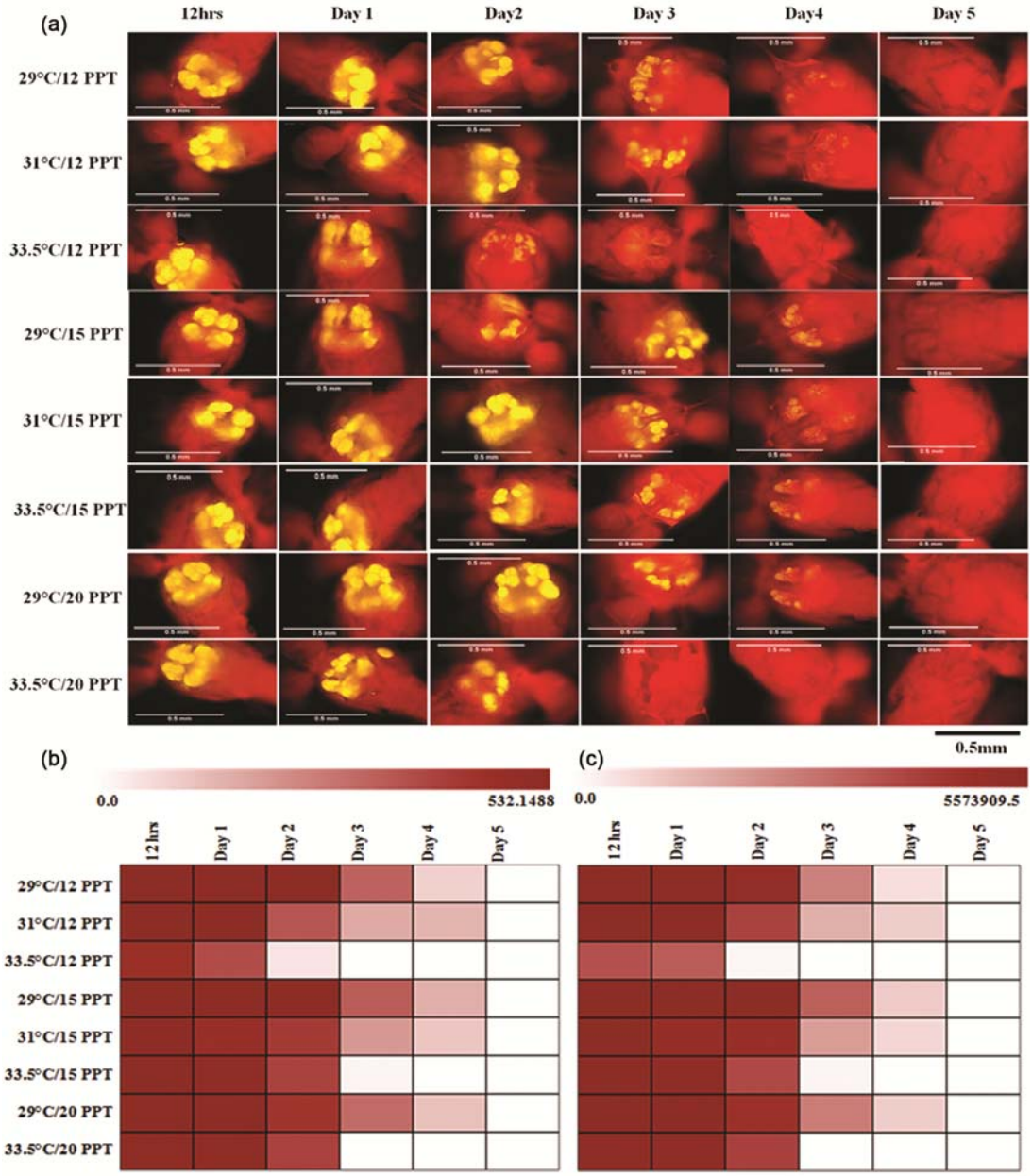
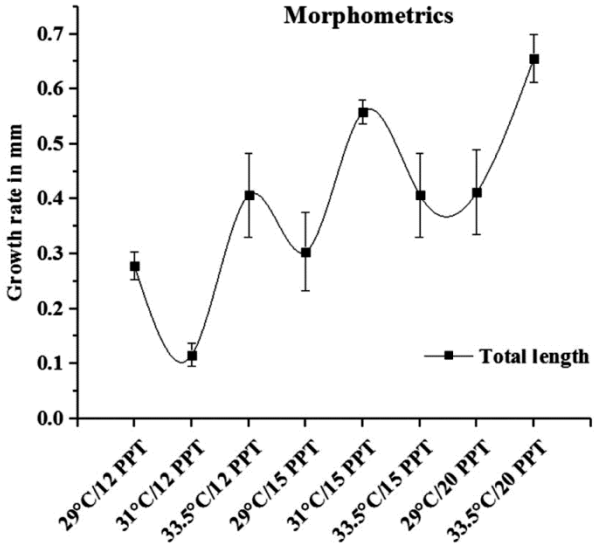


Fig. 1 — Yolk utilization in total area and intensity. A- Depletion of yolk in different conditions, B- Total yolk area in mm and C- Total yolk intensity in pixel.



ANOVA results

Total length	d.f.	F	P
Temperature	2	5.713	.005
Salinity	2	10.658	.000
Temp*Salinity	3	5.024	.003

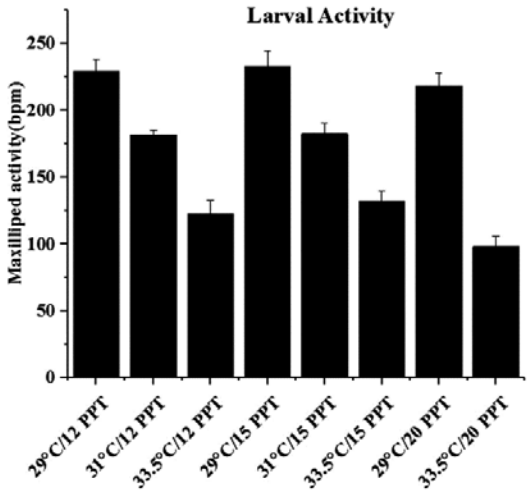
Fig. 2 — Growth rate of larvae. Growth rate of larvae on 4th day was calculated by subtracting the growth in mm on 1st day from 3rd day.

Larval activity

Larval activity was measured in terms of maxilliped movement which ranged from 90 to 250 bpm across different conditions (Fig. 3). Mean maxilliped frequency decreased significantly at higher temperature and salinity conditions than the ambient conditions ($p < 0.05$*(temperature)*; $p < 0.001$ *(salinity)*), however, the interaction of temperature and salinity did not show any effect on them (p = 0.760).

Cardiac performance of larvae

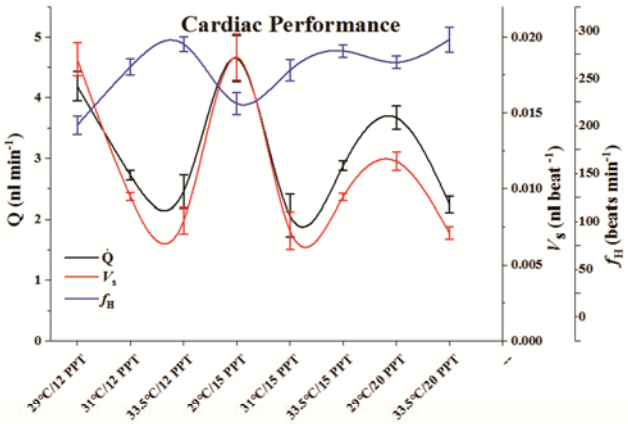
Mean cardiac performance in larval stage was significantly affected under the treated conditions. We found larval heart beat (f_H) in the range of 200-300 beats min^{-1}, with maximum beating in higher temperature and salinity conditions (20 ppt; 33.5 °C) and minimum in ambient condition 12 ppt; 29 °C. Temperature, salinity, and their interactions showed significant effect on f_H ($p < 0.001$*(temperature)*; $p < 0.01$*(salinity)*; $p < 0.05$*(temperature*salinity)*). Larval stroke volumes (V_s) across different conditions were in the range of 0.005-0.020 nl beats^{-1}, higher in ambient conditions and lowest in higher temperature and salinity conditions (Fig. 4). Temperature, salinity,



ANOVA results

Larval activity	d.f.	F	P
	2	108.842	.000
Temperature	2	3.899	.031
Salinity	3	.392	.760
Temp*Salinity			

Fig. 3 — Larval activity. Maxilliped activity of larvae on 4th day under different conditions.



ANOVA results

Heart beat	d.f.	F	P
Temperature	2	25.844	.000
Salinity	2	7.365	.002
Temp*Salinity	3	3.572	.025
Stroke volume	d.f.	F	P
Temperature	2	84.282	.000
Salinity	2	14.404	.000
Temp*Salinity	3	5.202	.011
Cardiac output	d.f.	F	P
Temperature	2	49.507	.000
Salinity	2	3.965	.040
Temp*Salinity	3	2.475	.099

Fig. 4 — Cardiac performance**.** Cardiac performance of larvae on 4th day.

and their interactions showed significant effect on V_s [(*p < 0.001(temperature); p < 0.001(salinity); p < 0.05(temperature*salinity*)]. Cardiac output (Qt) which is the product of $V_s * f_H$ was in the range of 2-5 nl min^{-1}, with higher in the ambient condition and lowest in the higher temperature and salinity conditions. Temperature and salinity independently showed significant effect on (Qt) (*p < 0.05 (temperature); p < 0.001(salinity)),* while their interaction seemed not to have any impact on (Qt) (*p = 0.099).* V_s and (Qt) were following similar patterns in wave manner; both of them were inversely related to f_H across different treatment conditions (Fig. 4).

Discussion

In this study, physiological performance of *M. rosenbergii* early life stages following exposure to varying temperature and salinity to understand their response to climate change stress conditions were assessed. Physiological performances are discussed in two broad categories: 1) survival and growth; 2) larval activity and metabolic performance. Overall, we find a small rise in temperature and salinity may result in sub lethal physiological rate reductions in *M. rosenbergii* early life stages, but the substantial increase in temperature and salinity may be detrimental.

Survival and growth

Salinity and temperature are the important environmental factors affecting survival, growth and distribution of many aquatic organisms[18,33]. In the adult *M. rosenbergii* the survival rate varied between 91 % (at 0 PPT) and 78 % (at 20 PPT), and the prawn exhibited lowest final average weight at 20 ppt seawater and higher at 10 PPT salinity[17]. Similarly, Habashy & Hassan[18] reared the juvenile prawns for eight months in different salinity and temperature conditions revealing that growth of the prawn increased as temperature increased from 24 to 29 ºC, but declined at the higher temperature (34 ºC). Also, with increase in salinity from 0 to 16 PPT, growth of female prawn decreased at all temperatures tested[18]. Recently, Mohanty *et al*.[34] conducted survival experiments on zoeae and post larvae of *M. rosenbergii* for combined effects of salinity and temperature, in which, post larvae showed maximum survival at 31 ºC which declined both at lower and higher temperature of 25 º C and 35 º C, respectively. In the line of these previous results on adults and post larvae, in the present study larval survival rates were higher at optimum temperature of 29 °C in 12 PPT, 15 PPT, and 20 PPT. However, survival rate decreased with increase in temperature at all tested salinity conditions. For the higher temperature conditions (33.5 ± 0.5 °C) with all salinity conditions (12 PPT, 15 PPT and 20 PPT) no survival was recorded. Similar to our results, Mohanty *et al*.[34] reported lower survival rate for *M. rosenbergii* zoeae (Z1-Z5) at 35 ºC and 15-18 PPT salinity.

For larval growth, there was an increase in total length of larvae up to 4th day for salinity ($p < 0.01$), temperature and combined temperature and salinity ($p < 0.05$). Although temperature was major determining factor for growth[33], the quality of larvae was compromised and died in 5th day for the higher temperature (33.5°C) in all salinity conditions.

Larval activity and metabolic performance

Lipid in yolk acts as energy sources for the early stages of larvae, ensuring the first successful molt and supporting the survival of early larvae before it started feeding[35]. In the present study, larval yolk in both ambient temperature and 31 °C lasted for 4 days, but for the higher temperature, the yolk depletion was completed either on day 2nd or day 3rd (Fig. 1). Under the increased temperature, the rate of metabolic processes hiked, demanding more energy, and causes the early depletion of energy reserve. The influence of temperature on the utilization of yolk content was reported in several aquatic species[36-38]

During the stressful condition, organisms try to maintain its homeostasis. The capacity of controlling cardiovascular function is one such function to maintain the organism's oxygen consumption rate and activity of the organism[39]. Temperature alters cardiac performance in crustaceans, as has been reported in several other organisms[40,41]. Salinity also affects the sensitivity of organism; thereby affecting their oxygen consumption[42,43]. In the present study, both salinity and temperature are shown to influence stroke volume and cardiac output, ultimately affecting the oxygen transport capacity of the animal. Larval heart beat (f_H) in *M. rosenbergii* larvae increased significantly with elevated temperature and salinity, but at the same time, stroke volume (V_s) decreased, that reduced cardiac output (Qt) as well as oxygen transport capacity. Ern *et al*.[39] showed that heart rates and ventilation rates increased, and stroke volume decreased with increasing temperature in adult *M. rosenbergii.* They also showed that the animals retained their 76 % of aerobic scope at 30 °C[39]. The lower stroke volume and cardiac output observed in the present study, which may have affected the oxygen consumption rate of organism. Hence, the

temperature and salinity beyond tolerance level could initiate anaerobic metabolism with detrimental effects.

Further, the decrease in aerobic scope could affect the function and behavior of larval activity in order to maintain pejus temperatures for long term survival[6,44]. For example, the rise of temperature in the kelp crab *Taliepus dentatus* constrained the aerobic scope and affected the level of maxilliped activity[31]. This was observed in the present study as well, in which the mean maxilliped frequency was significantly lowered in elevated temperature and salinity conditions. However, under the combined conditions of salinity and temperature, larval activity did not show significant effect as similar to our cardiac output results.

Conclusion

Global climate change is transforming life on earth, causing widespread effects on all ecosystems. Among marine ecosystems, estuaries are considered as nursery grounds for marine and fresh water species. This study shows that substantial increase of temperature and salinity may result in negative impact on the survival, growth, cardiac performance and activity of the early life stages of *M. rosenbergii*. The effect of climate change stressors thus could restrain the tolerance capability and physical fitness of the early life stages of this freshwater prawn, thereby affecting the successful persistence of the population.

Thus, the physiological responses to temperature and salinity by the early life stages of *M. rosenbergii* could restrain the tolerance capability of the organism, thereby interfering in the successful completion of the larval development under the altered climatic conditions

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