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# Thermal tolerance and oxygen consumption of *Macrobrachium rosenbergii* acclimated to three temperatures

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## Abstract

1. Critical thermal maxima ($CT_{max}$), critical thermal minima ($CT_{min}$) and oxygen consumption rate of four-month-old adult *Macrobrachium rosenbergii* (38 ± 3.1 g) were measured after acclimating to 3 preset temperatures (25°C, 30°C and 35°C) for 30 days.
2. $CT_{max}$ and $CT_{min}$ were 40.73±0.16°C, 41.06±0.17°C, 41.96±0.17°C and 14.9±0.13°C, 15.4±0.14°C, 16.98+0.21°C, respectively, and were significantly different ($p<0.05$).
3. Rate of oxygen consumption with increasing acclimation temperatures were 2.11±0.11, 2.95±0.13, 3.36±0.11 mg $O_2\,kg^{-1}\,h^{-1}$ at three different temperatures and were significantly different ($p<0.05$).
4. The thermal tolerance polygon for the specified temperatures was calculated as 255°$C^2$.

*Keywords:* Critical thermal maxima; Critical thermal minima; Thermal tolerance polygon; Oxygen consumption rate; Giant freshwater prawn; *Macrobrachium rosenbergii*; crustacea

## 1. Introduction

Biologists have been studying thermal tolerance of poikilotherms for more than hundred years considering its multifaceted significance (Heath, 1884; Hezel and Prosser, 1974; Monica et al., 1996; Beitinger et al., 2000; Fernando et al., 2000, 2002; Monica and Fernando, 2002). Rising temperature up to certain limit favours aquaculture by reducing the time required to produce marketable sized animal and producing more generations per year. On the contrary, temperature adversely affects the health of aquatic animal by increasing metabolic rates and subsequent oxygen demand, and assisting proliferation, invasiveness and virulence of bacteria and other pathogens that causes a variety of pathophysiological disturbances in the host (Wedemeyer et al., 1999).

Temperature affects virtually all biochemical and physiological activities of the organism. It should be viewed as an environmental resource (Magnuson et al., 1979), which evokes multiple effects on organisms (Fry, 1947). Metabolic activities of *Macrobrachium* sp. are controlled by temperature and oxygen consumption increases with increasing temperature. Prawns require high amounts of oxygen for converting feed into flesh. Optimum temperature for prawns is in the range of 28–32°C and dissolved oxygen concentration of 5–8 mg $l^{-1}$ (Sebastian, 1996). Sudden change in temperature induces stress in prawns.

The giant freshwater prawn, *Macrobrachium rosenbergii* is a prime inland cultured species, which has recently emerged as an important shellfish species for culture in India after significant losses of penaid shrimp

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culture in mid-1990s due to viral diseases. Although the former act as carriers, adult stages have shown resistance against viral diseases (Flegel, 1996; Rajendran et al., 1999). Temperature tolerance studies on this species are essential to assess its culture potential in different agro-climatic zones across the world. In this context, we evaluated thermal tolerance and oxygen consumption in the giant freshwater prawn, *M. rosenbergii*.

## 2. Materials and methods

Adult prawns (*M. rosenbergii*) with an average size [mean ± S.E = 38 ± 3.1 g] were brought in aerated open containers from Bhivandi Farm, Mumbai to Wet Laboratory, Central Institute of Fisheries Education, Mumbai and were held for 15 days at ambient water temperature (30°C). During this period, prawns were fed with supplementary feed before thermal tolerance studies.

Acclimation of prawns (6/aquarium) to test temperatures were carried out in a thermostatic aquarium (52 l water capacity, sensitivity ±0.2°C). Acclimation was carried out at one degree/day over ambient water temperature (30°C) to reach test temperatures (25°C, 30°C and 35°C) and maintained for a period of 30 days prior to the critical thermal methodology (CTM) experiments. Pretrial acclimation periods and experimental acclimation temperatures suggested for conducting experiments in shellfishes still remains as a debatable topic among physiologists across the globe. As the thermal acclimation period of adult stages of *M. rosenbergii* was unknown, other life stages were considered. *M. rosenbergii* post larvae were completely acclimated after four weeks of acclimation (Herrera et al., 1998). Therefore we assumed our experimental animals were completely acclimated prior to CTM tests.

A total of 36 prawns were distributed in three groups and critical thermal maxima ($CT_{max}$) and critical thermal minima ($CT_{min}$) were determined (Beitinger et al., 2000). Animals acclimated to three temperatures were subjected to a constant rate of increase or decrease ($0.3°C\,min^{-1}$) until loss of equilibrium (LOE) was reached. LOE was designated as $CT_{max}$ and $CT_{min}$, respectively (Paladino et al., 1980; Beitinger et al., 2000). This technique has been critically evaluated by numerous workers (Hutchinson, 1976; Reynolds and Casterlin, 1979) and is well established as a powerful tool for studying the physiology of stress and adaptation in fishes (Paladino et al., 1980; Beitinger and McCauley, 1990). Thermal tolerance range was determined by the difference between $CT_{max}$ and $CT_{min}$. Dissolved oxygen concentration was maintained at $5.5 \pm 0.5\,mg\,l^{-1}$ throughout the temperature tolerance studies by continuous aeration using a 2 HP centralized air blower.

Oxygen consumption rate was determined using another set of 18 prawns in three groups after acclimating to 25°C, 30°C and 35°C for 30 days. Acclimation was carried out at one degree/day over ambient water temperature (30°C) to reach test temperatures (25°C, 30°C and 35°C). After reaching their acclimation temperatures prawns were held for 30 days prior to oxygen consumption measurements.

They were kept individually in a sealed glass chamber (5 l) with 6.4 mm thick glass lid cut to cover the top portion completely. An opening in the lid fitted with a gasket to ensure an air tight seal permitted the insertion of a dissolved oxygen probe. A magnetic stir bar was used to maintain constant water circulation. A plastic-mesh shield was placed over the stir bar to prevent incidental contact with the animal. The chamber was placed inside the thermostatic aquarium at their respective temperatures for an hour. All four sides of the aquarium were covered with opaque screen to minimize visual disturbances of the experimental animal. The initial and final oxygen content were measured using a digital oxy-meter 330 (sensitivity $0.01\,mg\,O_2\,mg\,l^{-1}$) (Merck, Germany). Another lot of 18 prawns were acclimated to 25°C at one degree/day over ambient water temperature (30°C) and maintained for 30 days. The test temperature was immediately increased to 30°C and 35°C so as to delineate the effect of sharp increase of temperature *vs* acclimation procedure on oxygen consumption rate. Temperature quotients ($Q_{10}$) were calculated from the rate of oxygen consumption as

$$Q_{10} = (\text{Rate1}/\text{Rate2})^{(10/\text{Temp2}-\text{Temp1})}.$$

Statistical analyses of $CT_{max}$, $CT_{min}$ and the rate of oxygen consumption were carried out using one-way analysis of variance (ANOVA via SPSS 11.0 for Windows). Duncan's multiple range test was carried out for post hoc mean comparisons ($p \leqslant 0.05$).

## 3. Results and discussion

$CT_{max}$ of prawn (mean ± SE) (40.73 ± 0.16°C, 41.06 ± 0.17°C, 41.96 ± 0.170°C) increased with increasing acclimation temperatures (25°C, 30°C and 35°C) and the mean $CT_{max}$ were significantly ($p < 0.05$) different (Fig. 1). Similarly, mean $CT_{min}$ (14.9 ± 0.13°C, 15.4 ± 0.14°C, 16.98 ± 0.21°C) increased with increasing acclimation temperatures (25°C, 30°C and 35°C) and were significantly ($p < 0.05$) different (Fig. 2). A thermal tolerance polygon over a range of 25–35°C was generated from mean $CT_{max}$ and mean $CT_{min}$ based on 36 points (Fig. 3) and was calculated as $255°C^2$.

The rates of oxygen consumption at different acclimation temperatures significantly ($p < 0.05$) increased with

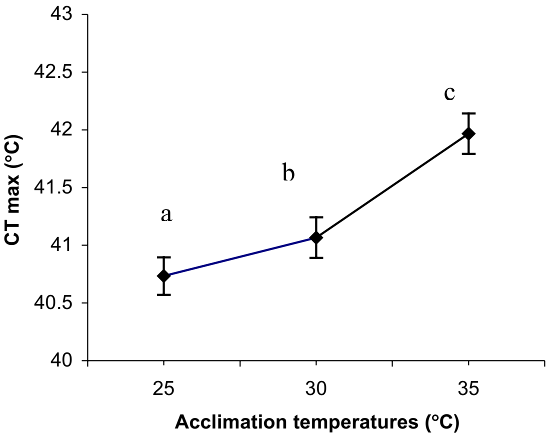

Fig. 1. $CT_{max}$ of *M. rosenbergii* acclimated at 25°C, 30°C and 35°C. Different superscripts indicate significant difference ($p<0.05$) among the treatments. Values are expressed as mean ± SEM ($n=6$).

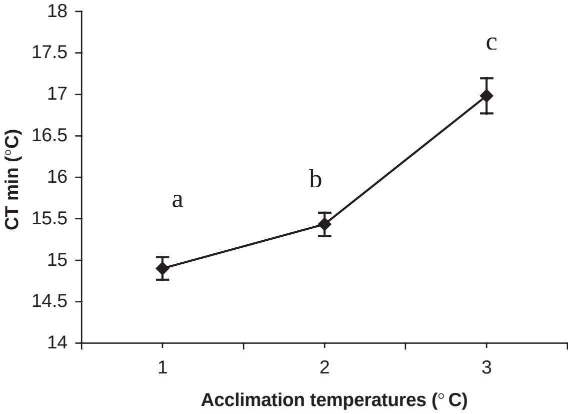

Fig. 2. $CT_{min}$ of *M. rosenbergii* acclimated at 25°C, 30°C and 35°C. Different superscripts indicate significant difference ($p<0.05$) among the treatments. Values are expressed as mean ± SEM ($n=6$).

increasing temperatures (Table 1). $Q_{10}$ values were calculated as 1.25 in acclimated and 1.95 in non-acclimated condition.

Differences in behavior of prawns subjected to CTM (critical thermal methodology) tests were recorded. At 30°C, they were resting on the bottom of the aquarium. As temperatures increased, prawns had excessive movement and appeared to be trying to escape. At temperatures beyond 39°C, their abdominal muscle segments were contracted, accompanied by an erratic movement, finally loosing their balance and becoming inverted, which was used as our CTM end point. At temperatures below 18°C, animals remained motionless and at 14°C, prawns lost their balance. The animals at $CT_{max}$ and $CT_{min}$ were then immediately transferred to the pretrial acclimation temperatures for rescue and recovery (Beitinger et al., 2000). All the animals survived the CTM test.

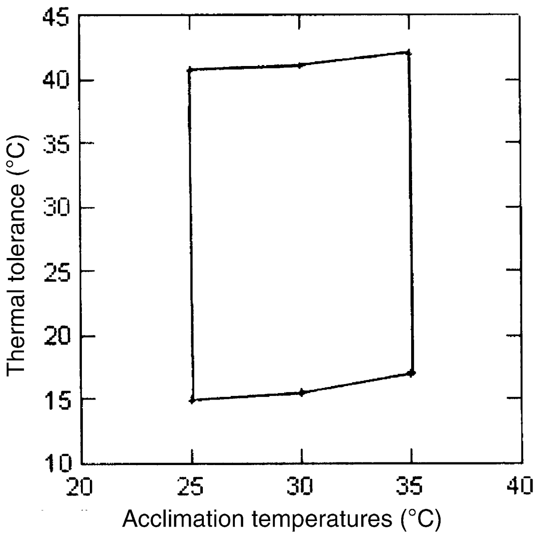

Fig. 3. Thermal tolerance polygon of freshwater prawn, *Macrobrachium* sp. over three acclimation temperatures (25°C, 30°C and 35°C) using CTM values. The areas of the thermal tolerance polygon was calculated as 255°$C^2$.

Table 1
Rate of oxygen consumption in acclimated and non-acclimated condition of adult *M. rosenbergii* at three temperatures (25°C, 30°C and 35°C). Unit is expressed as mg $O_2\,kg^{-1}\,h^{-1}$. Values are expressed as mean ± SEM ($n=6$). Superscripts with different alphabets in a row differ significantly ($p<0.05$)

| Temperatures (°C) | 25 | 30 | 35 |
|---|---|---|---|
| Acclimated | $2.11 \pm 0.11^a$ | $2.95 \pm 0.13^b$ | $3.36 \pm 0.11^c$ |
| Non-acclimated | $2.18 \pm 0.09^a$ | $3.17 \pm 0.14^b$ | $4.13 \pm 0.16^c$ |

In the present study, both $CT_{max}$ and $CT_{min}$ increased significantly with increasing acclimation temperatures. It indicates that acclimation temperature affect prawns (Figs. 1 and 2), which is consistent with the findings of previous studies on the responses of rising thermal stress in *Macrobrachium tenellum* acclimated to four temperatures (Monica et al., 1996). But Herrera et al. (1998) reported no significant differences in $CT_{max}$ and $CT_{min}$ when *M. rosenbergii* post larvae and juveniles were acclimated for four weeks at five different temperatures. However, data extrapolated from Herrera et al. (1998) and Fernando et al. (2002) in *Macrobrachium* sp. to three acclimation temperatures (25°C, 30°C and 35°C) were consistent with our CTM findings (Table 2). But, in one of our CTM investigations on adult *Macrobrachium* with shorter acclimation periods, the values were lesser

Table 2
Summary of laboratory thermal tolerance data for *Macrobrachium* sp. (extracted from previous investigations)

| $T_{\text{acclimation}}$ (°C) | Species and stage | Method | Tolerance data (°C) | Reference |
|---|---|---|---|---|
| 25 | *M. rosenbergii* PL | $CT_{max}$ | 39.0 | Herrera et al. (1998) |
| 25 | *M. rosenbergii* PL | $CT_{min}$ | 12.5 | Herrera et al. (1998) |
| 25 | *M. rosenbergii* juveniles | $CT_{max}$ | 39.0 | Herrera et al. (1998) |
| 25 | *M. rosenbergii* juveniles | $CT_{min}$ | 12.7 | Herrera et al. (1998) |
| 25 | *M. acanthurus* | $CT_{max}$ | 34.7 | Fernando et al. (2002) |
| 25 | *M. acanthurus* | $CT_{min}$ | 11.7 | Fernando et al. (2002) |
| 30 | *M. rosenbergii* PL | $CT_{max}$ | 40.9 | Herrera et al. (1998) |
| 30 | *M. rosenbergii* PL | $CT_{min}$ | 15.4 | Herrera et al. (1998) |
| 30 | *M. rosenbergii* juveniles | $CT_{max}$ | 41.4 | Herrera et al. (1998) |
| 30 | *M. rosenbergii* juveniles | $CT_{min}$ | 15.2 | Herrera et al. (1998) |
| 30 | *M. acanthurus* | $CT_{max}$ | 37.7 | Fernando et al. (2002) |
| 30 | *M. acanthurus* | $CT_{min}$ | 13.7 | Fernando et al. (2002) |
| 35 | *M. rosenbergii* PL | $CT_{max}$ | 42.8 | Herrera et al. (1998) |
| 35 | *M. rosenbergii* PL | $CT_{min}$ | 18.3 | Herrera et al. (1998) |
| 35 | *M. rosenbergii* juveniles | $CT_{max}$ | 43.7 | Herrera et al. (1998) |
| 35 | *M. rosenbergii* juveniles | $CT_{min}$ | 17.7 | Herrera et al. (1998) |
| 35 | *M. acanthurus* | $CT_{max}$ | 40.7 | Fernando et al. (2002) |
| 35 | *M. acanthurus* | $CT_{min}$ | 15.8 | Fernando et al. (2002) |

than the present CTM findings (unpublished data). Thus *Macrobrachium* sp. shows variations in response to the acclimation temperatures and acclimation period. Another report in black crappie, *Pomoxis nigromaculatus* revealed that CTM was influenced by differential rate of change of temperature during thermal tolerance studies, size and condition factor (K) of the animals (Baker and Heidenger, 1996). Thus various factors may influence CTM data apart from acclimation temperatures and acclimation period.

In the present study, thermal tolerance polygon for adult *M. rosenbergii* with three preset temperatures was found to be 255°C$^2$. We found some of the previous investigations, which measured the zone of thermal tolerance of freshwater prawns. *Macrobrachium acanthurus*, a species commonly cultured in Southwestern region of Mexico when acclimated at four different temperatures, had a thermal tolerance zone of 644°C$^2$ (Fernando et al., 2002) *M. rosenbergii* post larvae (821.2°C) and juveniles (816.9°C) (Herrera et al., 1998). But both have reported an extrapolated value in the range 15–38°C and 10–42°C based on five acclimation temperatures. However, the thermal tolerance polygon data extracted from these investigations for post larvae (257°C$^2$) juveniles (261°C$^2$) of *M. rosenbergii* and *M. acanthurus* (239°C$^2$) to three acclimation temperatures (25°C, 30°C and 35°C) were consistent with our findings (255°C$^2$). It indicates that the area of thermal tolerance polygon is dependent on acclimation temperatures during the experiment. In our investigation, thermal tolerance polygon of *M. rosenbergii* was estimated over a range of 25–35°C. Further research is required to elucidate the zone of thermal tolerance over the entire tolerance range of this species.

Over the last few years, there has been an emerging interest in the effects of temperature on metabolic activity in crustaceans (Whiteley et al., 1996; Whiteley and El Haj, 1997; El Haj and Whiteley, 1997). Rate of oxygen consumption is an indication of energy utilization by the animals (Brett, 1964; Kutty, 1968).

The present study indicates that thermal tolerance and oxygen consumption of *M. rosenbergii* is dependent on experimental acclimation period and acclimation temperatures. This preliminary study on thermal tolerance poses new avenues of research including its physiological changes occurring at extreme temperatures, induction of stress proteins at different life stages and the importance in cross protection against several forms of biotic and abiotic stress (Sarah et al., 1998; Wedmeyer et al., 1999). But there are no reports available about any such mechanism in *M. rosenbergii*. More research on its adaptability to high temperatures and the possibility of culturing this commercially important decapod crustacean in high temperatures is essential in this era of global warming.

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## References

Baker, S.C., Heidinger, R.C., 1996. Upper lethal temperature of fingerling black crappie. J. Fish Biol. 48, 1123–1129.

Beitinger, T.L., McCauley, R.W., 1990. Whole animal physiological processes of the assessment of stress in fishes. J. Great Lakes Res. 16, 542–575.

Beitinger, T.L., Bennett, W.A., McCauley, R.W., 2000. Temperature tolerances of North American freshwater fishes exposed to dynamic changes in temperature. Environ. Biol. Fish. 58, 237–275.

Brett, J.R., 1964. The respiratory metabolism and swimming performance of young sockeye salmon. J. Fish. Res. Board Can. 21, 1183–1226.

El Haj, A.J., Whiteley, N.M., 1997. Molecular regulation of muscle growth in crustacea. J. Mar. Biol. Assoc. 77, 95–106.

Fernando, D., Miguel, A., Elizabeth, S., Marisela, A., Denisse, R., 2000. Preferred temperature and critical thermal maxima of red abalone *Haliotis rufescens*. J. Therm. Biol. 25, 257–261.

Fernando, D., Elizabeth, S., Ana Denisse, R., Leticia, R., 2002. Behavioural thermoregulation and critical thermal limits of *Macrobrachium acanthurus* (Weigman). J. Therm. Biol. 27, 423–428.

Flegel, T.W., 1996. A turning point for substainable aquaculture: the white spot crisis in Asian shrimp culture. Aquaculture Asia 1, 29–34.

Fry, F.E.T., 1947. Effects of the environment on animal activity. University of Toronto Studies in Biology Series 55. Publication of the Ontario Fisheries Research Laboratory, 68, pp. 1–62.

Heath, N., 1884. Effect of cold on fishes. Bull. US Fish. Comm. 4, 369–371.

Herrera, F.D., Uribe, E.S., Ramirez, L.F.B., Mora, A.G., 1998. Critical thermal maxima and minima of *Macrobrachium rosenbergii* (Decapoda: Palaemonidae). J. Therm. Biol. 23, 381–385.

Hezel, J.R., Prosser, C.L., 1974. Molecular mechanisms of temperature compensation in poikilotherms. Physiol. Rev. 54, 19–42.

Hutchinson, V.H., 1976. Factors influencing thermal tolerances of individual organisms. In: Esch, G.W., McFarlene, R.W. (Eds.), Thermal Ecology, ERDA Symposium Series, pp.10–26.

Kutty, M.N., 1968. Respiratory quotient in gold fish and rainbow trout. J. Fish. Res. Board Can. 25, 2689–2728.

Magnuson, J.J., Crowder, L.B., Medwick, P.A., 1979. Temperature as an ecological resource. Am. Zool. 19, 331–343.

Monica, H.R., Fernando, B.R., 2002. Temperature tolerance polygon of *poecilia sphenops* (Pisces: Poeciliidae). J. Therm. Biol. 27, 1–5.

Monica, H.R., Fernando, B.R., Fernando, D.H., 1996. Critical thermal maximum of *Macrobrachium tenellum*. J. Therm. Biol. 21, 139–143.

Paladino, F.V., Spotila, J.R., Schubauer, J.P., Kowalski, K.T., 1980. The Critical thermal maximum: a technique used to elucidate physiological stress and adaptation in fish. Rev. Can. Biol. 39, 115–122.

Rajendran, K.V., Vijayan, K.K., Santigo, T.C., Krol, R.M., 1999. Experimental host range and histopathology of white spot syndrome virus (WSSV) infection in shrimp, prawns, crabs, lobsters from India. J. Fish Dis. 22, 183–191.

Reynolds, W.W., Casterlin, M.E., 1979. Behavioural thermoregulation and the final preferandum paradigm. Am. Zool. 19, 211–224.

Sarah, F.D., Feng, P., George, C.T., Terence, M.B., 1998. Thermal shock of salmon invivo induces the heat shock protein (hsp 70) and confers protection against osmotic shock. Aquaculture 168, 311–323.

Sebastian, C.D. (Ed.), 1996. A manual on seed production and farming of giant freshwater prawn, *Macrobrachium rosenbergii*. The Marine Products Export Development Authority, Ministry of Commerce, Govt. of India, Cochin, 58pp.

Wedemeyer, G.R., Meyer, F.P., Smith, L., (Eds.), 1999. Environmental Stress and Fish Diseases. Narendra Publishing House, Delhi, India, 107p.

Whiteley, N.M., El Haj, A.J., 1997. Regulation of muscle gene expression over the moult cycle. Comp. Biochem. Physiol. 117B, 30–32.

Whiteley, N.M., Taylor, E.W., El Haj, A.J., 1996. A comparison of metabolic cost of protein synthesis in stenothermal and eurythermal isopod crustaceans. Am. J. Physiol. 40, R1295–R1303.