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RESEARCH ARTICLE

Oxygen delivery does not limit thermal tolerance in a tropical eurythermal crustacean

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ABSTRACT

In aquatic environments, rising water temperatures reduce water oxygen content while increasing oxygen demand, leading several authors to propose cardiorespiratory oxygen transport capacity as the main determinant of aquatic animal fitness. It has also been argued that tropical species, compared with temperate species, live very close to their upper thermal limit and hence are vulnerable to even small elevations in temperature. Little, however, is known about physiological responses to high temperatures in tropical species. Here we report that the tropical giant freshwater shrimp (*Macrobrachium rosenbergii*) maintains normal growth when challenged by a temperature rise of 6°C above the present day average (from 27°C to 33°C). Further, by measuring heart rate, gill ventilation rate, resting and maximum oxygen uptake, and hemolymph lactate, we show that oxygen transport capacity is maintained up to the critical maximum temperature around 41°C. In *M. rosenbergii* heart rate and gill ventilation rate increases exponentially until immediately below critical temperatures and at 38°C animals still retained more than 76% of aerobic scope measured at 30°C, and there was no indication of anaerobic metabolism at the high temperatures. Our study shows that the oxygen transport capacity is maintained at high temperatures, and that other mechanisms, such as protein dysfunction, are responsible for the loss of ecological performance at elevated temperatures.

KEY WORDS: Critical temperature, Crustacean, Growth rate, *Macrobrachium rosenbergii*, Oxygen supply capacity, Temperature

INTRODUCTION

Metabolism of resting, fasting animals (standard metabolic rate, SMR) increases exponentially as temperature rises, whereas maximal oxygen uptake typically plateaus or even decreases as the structural and physiological ceilings of the cardiorespiratory systems to transport oxygen are reached at high temperatures (Fry and Hart, 1948). The capacity to increase oxygen uptake ($\dot{M}_{O_2}$) and delivery by the cardiorespiratory systems is often quantified as the aerobic scope, which is the difference between $\dot{M}_{O_2}$ of the resting fasting animal (SMR) and its maximal $\dot{M}_{O_2}$ ($\dot{M}_{O_2,max}$), typically measured during or immediately after intense exercise (Clark et al., 2013). Since SMR represents the basic cost of living, the capacity to increase $\dot{M}_{O_2}$ reflects the amount of aerobically derived, and hence sustainable, energy production that can be allocated to functions beyond body maintenance, i.e. physical activity, digestion, growth and reproduction (Fry and Hart, 1948; Nilsson et al., 2009; Eliason et al., 2011). These temperature relationships have led to the development of the oxygen and capacity limited thermal tolerance (OCLTT) model, proposing that aquatic animal fitness in rising temperatures is closely correlated to the capacity of the cardiorespiratory system to maintain oxygen supply, which thus provides the key mechanism defining the upper temperature limit of aquatic animals (Pörtner, 2010). In crustaceans, several studies over the past decade have reported such limitations in the oxygen supply capacity at high temperatures, revealed as a decline in cardiac and ventilatory performance as well as reductions in oxygen levels in the hemolymph with increasing temperature (Frederich and Pörtner, 2000; Frederich et al., 2009; Marshall et al., 2011; Jost et al., 2012). As further support for the OCLTT model, several marine invertebrates, including crustaceans, resort to anaerobic metabolism when approaching their upper thermal limit (Taylor et al., 1973; Taylor et al., 1977; Sommer et al., 1997; Frederich and Pörtner, 2000; Peck et al., 2002; Sokolova and Pörtner, 2003).

The majority of studies supporting the OCLTT model have been on stenothermal species (Pörtner, 2010) and it remains to be investigated whether this model also applies to eurythermal species that may have evolved a more thermoresistant cardiorespiratory system. Furthermore, numerous authors have argued that tropical species in general are close to their upper thermal limit and vulnerable to even small temperature increases (Deutsch et al., 2008; Tewksbury et al., 2008; Nilsson et al., 2009). Here we measure the effect of a 6°C increase in temperature from 27 to 33°C on growth capacity over a 3 month period in the giant freshwater shrimp (*Macrobrachium rosenbergii* De Man 1879), a crustacean of global importance to tropical aquaculture with annual production exceeding 400,000,000 kg per year (FAO, 2005–2013). Furthermore, we directly investigate whether increased temperature causes collapse of the oxygen supply capacity by the cardiorespiratory systems, by measuring $\dot{M}_{O_2}$ of resting and exercising shrimp at various temperatures, as well as heart rate, ventilation rate and hemolymph lactate during acute rises in temperature.

RESULTS

Growth rate

The growth rate of *M. rosenbergii* was not reduced when temperature was increased from 27 to 33°C (Fig. 1). At 27°C, mean body mass increased from 7.4±0.5 to 17.8±1.1 g, while body mass at 33°C rose from 7.6±0.7 to 18.6±1.4 g over the same period. As a result, the specific growth rates (SGR) of 0.0102 and 0.0106 g g^{-1} day^{-1} at 27 and 33°C, respectively, were unaffected by the 6°C rise in temperature. Mortality during this period was 43.6 and 52.7% at 27 and 33°C, respectively, both of which are in the normal range for this species in aquaculture ponds primarily due to cannibalism during molting (FAO, 2005–2013).

Oxygen uptake

SMR and $\dot{M}_{O_2,max}$ both increased exponentially when temperature was increased from 30 to 38°C (Fig. 2). However, Q_{10} was higher

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List of symbols and abbreviations

$\dot{M}_{O_2}$	oxygen uptake
$\dot{M}_{O_2,max}$	maximum oxygen uptake
SGR	specific growth rate
SMR	standard metabolic rate
T_{crit}	critical temperature
T_d	denaturation temperature
T_p	pejus temperature

for SMR than for $\dot{M}_{O_2,max}$ (2.3 and 1.3, respectively), such that aerobic scope decreased slightly with rising temperatures. Nevertheless, at 38°C, 2–3°C below the upper critical temperature (T_{crit}), the animals still retained 76% of the aerobic scope at 30°C.

Hemolymph lactate concentrations

That adequate capacity for oxygen supply is maintained at high temperatures is also supported by the lack of transition to anaerobic metabolism even when temperatures approached T_{crit} (Fig. 3). This was not due to an inability of *M. rosenbergii* to perform anaerobic metabolism, as exercise at 30°C was accompanied by an immediate and almost fourfold (*t*-test, $P<0.001$) rise in hemolymph lactate concentration (Fig. 3).

Heart rate and gill ventilation rate

Each heartbeat consisted of a double-spiked top and each gill ventilation cycle consisted of a short spike followed by a longer spike, corresponding to the elevation and depression of the scaphognathite (Fig. 4A). At low temperatures, shrimps exhibited prolonged heartbeats associated with spontaneous cardiac arrest, and intermittent breathing patterns where ventilatory periods of around 10 s were interspersed amongst apneas of ~20 s. These patterns were replaced by short heartbeats and continuous ventilation when temperatures exceeded ~34°C. From 30 to 40°C, both heart rate (Fig. 4B) and gill ventilation rate (Fig. 4C) increased exponentially (Q_{10} values of 2.3 and 4.8 between 30 and 40°C, respectively). Only at temperatures immediately below T_{crit} (~41°C) did the shrimp exhibit a sudden and very pronounced drop in these rates of convective oxygen transport. The collapse of cardiovascular and respiratory functions coincided with the loss of buoyancy and the shrimp became clearly moribund.

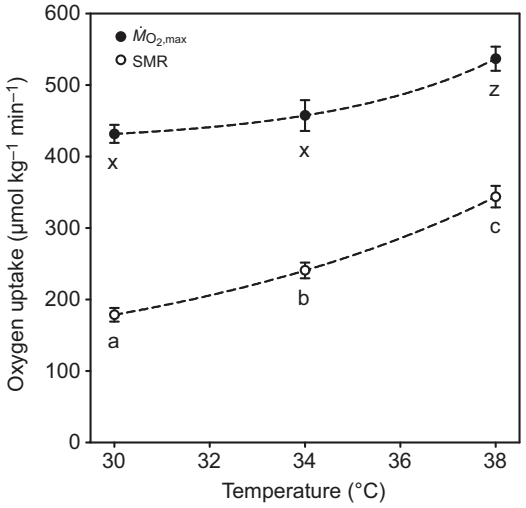

Fig. 2. Oxgen uptake. Standard metabolic rate (SMR, white circles) (*N*=10, body mass=24.2±1.5 g) and maximum oxygen uptake ($\dot{M}_{O_2,max}$, black circles) (*N*=10, body mass=24.3±1.3 g) measured at 30, 34 and 38°C in the giant freshwater shrimp (*Macrobrachium rosenbergii*). Data at each temperature were fitted with exponential function. Different superscript letters indicate a significant difference (one-way ANOVA, $P<0.05$). Values are means ± s.e.m.

DISCUSSION

Our observations that aerobic scope, as well as heart rate and ventilation were maintained at temperatures immediately below T_{crit}, and that anaerobic metabolism is not utilized in resting animals, document that the upper thermal tolerance of resting *M. rosenbergii* is not determined by inadequate oxygen delivery. These results are in striking contrast to data on the European spider crab (*Maja squinado*) during acute temperature elevation

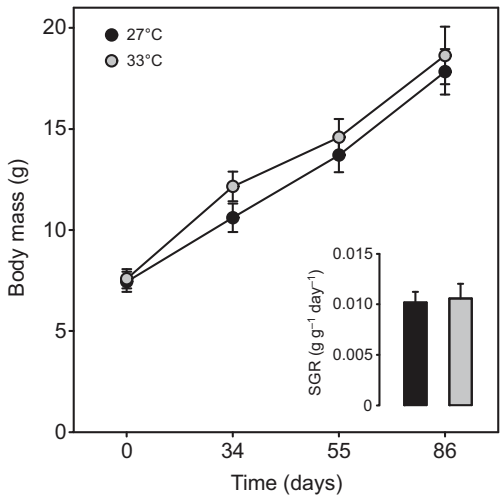

Fig. 1. Growth rate. The increases in body mass (g) and specific growth rate (SGR, g g^{-1} day^{-1}) of the giant freshwater shrimp (*Macrobrachium rosenbergii*) during the 86 day growth experiment at 27°C (black) and 33°C (gray) are shown. There was no significant difference between SGR at 27°C (*N*=55) and 33°C (*N*=57), respectively (one-way ANOVA, $P=0.834$). Values are means ± s.e.m.

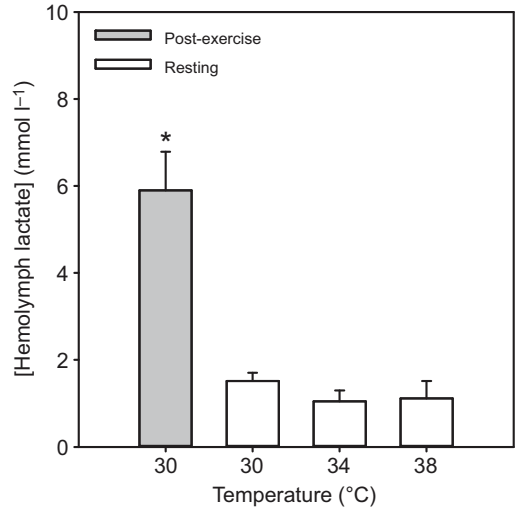

Fig. 3. Anaerobic metabolism. Hemolymph lactate concentrations (mmol l^{-1}) in the giant freshwater shrimp (*Macrobrachium rosenbergii*) measured in resting shrimp at 30, 34 and 38°C and in exercised shrimp at 30°C. In resting shrimp hemolymph lactate concentration was unaffected by temperature (one-way ANOVA, $P=0.494$). At 30°C exercised shrimp showed a significant (*t*-test, $P<0.001$) almost fourfold rise in hemolymph lactate concentration compared with resting shrimp. Values are means ± s.e.m. (*N*=6, body mass=87.1±7.3 g).

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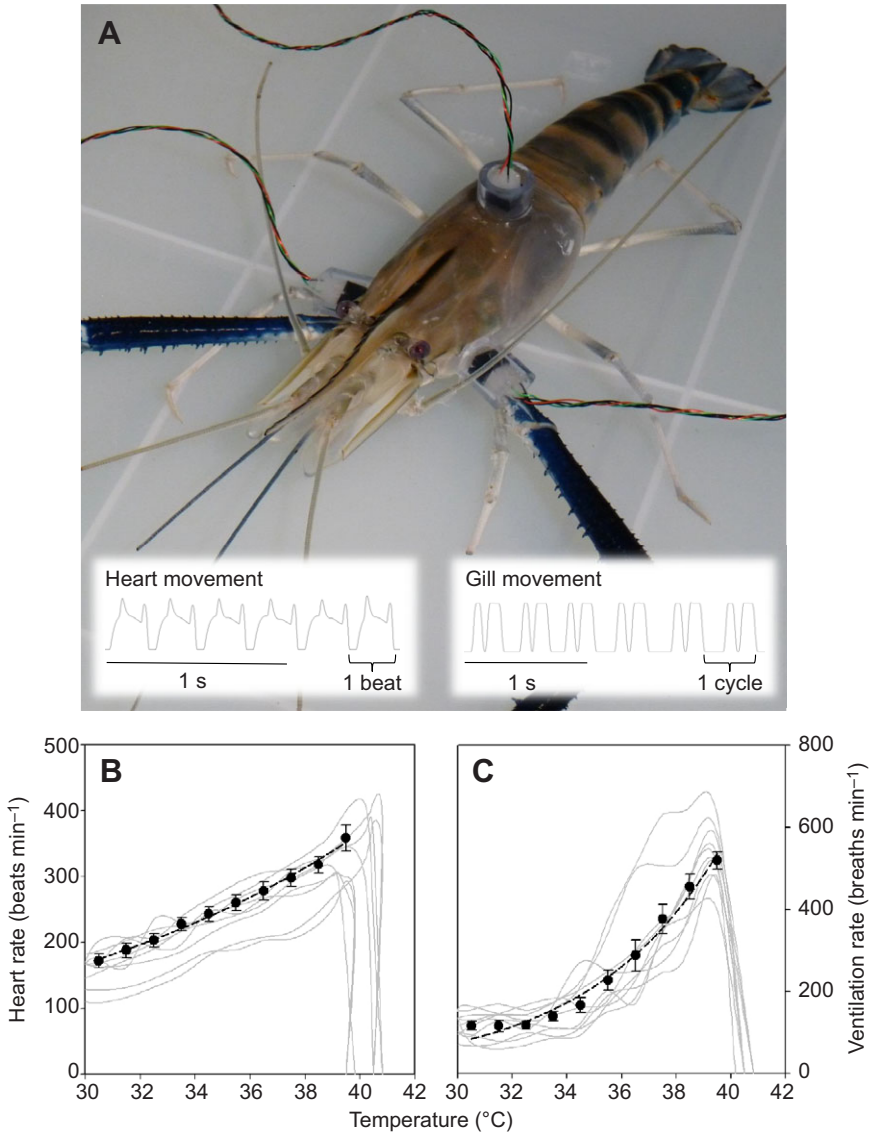

Fig. 4. Heart and gill ventilation. (A) The giant freshwater shrimp (*Macrobrachium rosenbergii*) with reflective infrared sensors glued onto the carapace above the heart and the scaphognathites for measurements of cardiac and ventilatory activities (see typical trace in inset). (B,C) Effect of acute temperature increase (2°C h^{-1}) on heart rate (*N*=9, body mass=90.4±4.2 g) and ventilation rate (*N*=10, body mass=91.6±4.2 g) in resting shrimp. Data for each individual animal are shown as gray lines and the means ± s.e.m. of all animals are shown as black circles. Mean data were fitted with an exponential function.

(1°C h^{-1}), showing an initial increase in heart rate and ventilation rate from 0 to 17°C, followed by a plateau phase from 17 to 30°C and finally a decrease from 30 to 40°C until death at 40°C (Frederich and Pörtner, 2000). In *M. squinado*, the plateau phase was accompanied by a decrease in arterial hemolymph P_{O_2} consistent with the decline in heart and ventilation rates from 30 to 40°C, and the subsequent death. Although resting heart and ventilation rates in *M. rosenbergii* increased exponentially with temperature (Fig. 3B,C), a drop in stroke volume of either system could reduce cardiac or gill output and with these the overall oxygen transport capacity of the cardiorespiratory system. However, both SMR and $\dot{M}_{O_2,max}$ also increased exponentially from 30 to 38°C (Fig. 2), indicating that the overall capacity of the cardiorespiratory system was fully sufficient to meet the rise in oxygen demand by the tissue. Aerobic scope was measured in South Vietnam during the dry season where the normal water temperature in the tanks was around 30°C. This temperature was therefore chosen as the starting temperature. Furthermore, growth rates at 27 and 33°C did not differ, indicating that performance was not compromised at 30°C. In a previous study of the effects of salinity on metabolism in *M. rosenbergii*, SMR in freshwater at 27°C was estimated to be 79.8±3.1 µmol kg^{-1} min^{-1} (Ern et al., 2013). Extrapolating the exponential equation fitted to SMR values between 30 and 38°C in the present study back to 27°C and rescaling for the differences in animal size (White et al., 2006), we estimate a 27°C SMR of around 125 µmol kg^{-1} min^{-1}. Animals in the present study seemed quiescent for the entire 9 h of measuring. The SMR value in the present study may nevertheless be slightly higher than found by Ern et al. (Ern et al., 2013), which may be a result of our protocol, kept short to avoid the very rapid increase in background $\dot{M}_{O_2}$ due to the bacterial growth that inevitably becomes very evident above 33°C. The Q_{10} for SMR in the present study is 2.3 and is within the range 2.0–2.4 reported on *M. rosenbergii* in the literature (Chen and Kou, 1996; Manush et al., 2004). We conclude therefore that while the SMR values reported here may be slightly overestimated, this overestimation would have resulted in a similar underestimation of aerobic scope and that the conclusion that this species retains a large percentage of its aerobic scope up to a few degrees below T_{crit} is valid. The lack of reduced oxygen transport capacity at high temperatures may in part be due to the cardiovascular anatomy of crustaceans. The single-chambered crustacean heart is situated within the pericardial sinus and supplied with oxygen-rich hemolymph entering the heart through ostia from the gills (McGaw, 2005). This is in contrast to

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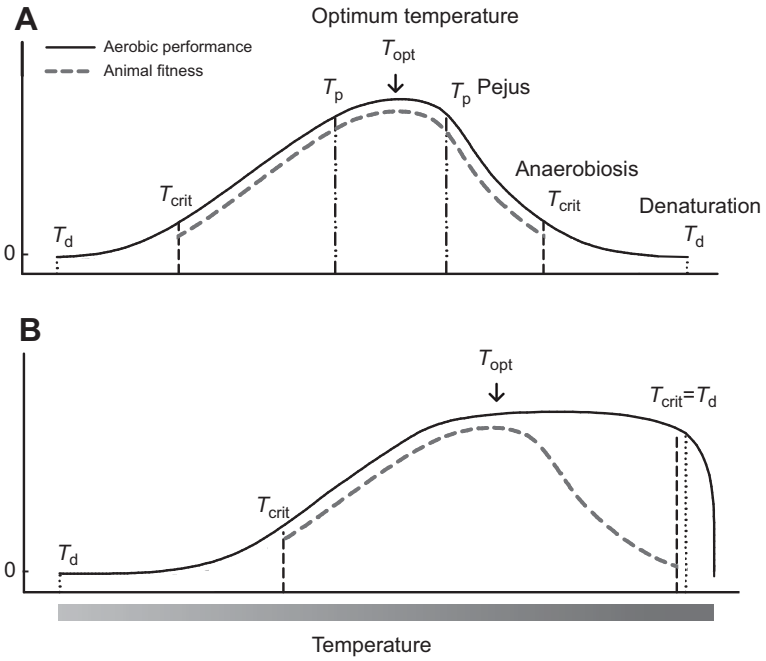

Fig. 5. Aerobic performance and animal fitness. Conceptual model showing optimum temperature (T_{opt}) and the effect of temperature on aerobic performance and animal fitness in animals with a cardiorespiratory system adapted to lower (i.e. temperate and arctic species) (A) and higher (i.e. tropical species) (B) temperatures (modified from Pörtner and Knust, 2007; Wang and Overgaard, 2007).

the cardiovascular layout in fish where most of the cardiac oxygen supply derives from the oxygen-depleted venous return (Pörtner and Farrell, 2008). Given the different position of the heart relative to the respiring tissue, the crustacean heart may be less vulnerable to the lowering of venous P_{O_2} arising from the elevated metabolic demand argued to be responsible for cardiac failure in fish at high temperatures (Heath and Hughes, 1973; Wang and Overgaard, 2007; Farrell, 2009). Hence as long as the diffusive conductance of gills can meet oxygen demands, the heart will be perfused with oxygen-rich hemolymph and low venous oxygen levels would therefore not limit the ability to maintain pressure and cardiac output. However, while this argument can explain why *M. rosenbergii* appears to maintain cardiac work up to temperatures immediately below T_{crit}, it does not provide a satisfactory explanation of why other invertebrates resort to anaerobic metabolism when approaching their thermal thresholds (Frederich and Pörtner, 2000; Peck et al., 2002; Sokolova and Pörtner, 2003).

The crustaceans in which high temperatures cause collapse of the cardiorespiratory systems and increases in anaerobic metabolism are primarily temperate or arctic species with relatively low T_{crit} values (reviewed in Pörtner, 2010). In these species, the cardiorespiratory system is adapted to relatively low temperatures, and at least the intertidal crabs of the genus *Petrolisthes* from cooler habitats have increased temperature sensitivity of heart function compared with warmer habitats (Stillman and Somero, 1996). Thus a parsimonious explanation may be that the OCLTT model (Pörtner, 2010) adequately applies to species where T_{crit} is well below the debilitating effects of temperatures around 40°C where proteins denature and where cell membrane lipid function deteriorates (Somero, 2012). In these species animals are unable to sustain oxygen transport capacity (i.e. aerobic performance) below and above the 'pejus' temperatures (T_p), leading to critical temperatures, beyond which oxygen delivery is insufficient to sustain aerobic energy production causing mobilization of anaerobic metabolism. As a result animal fitness is determined by limitations in oxygen transport capacity of the cardiorespiratory system and the two therefore correlate over the entire temperature range of the animal (Fig. 5A). However, in tropical species such as *M. rosenbergii* and the cane toad (*Rhinella marina*) (Overgaard et al., 2012), the cardiorespiratory systems are adapted to function at high temperatures and these animals, therefore, do not experience a gradual decrease in aerobic performance at high temperatures.

We therefore propose a modification to the OCLTT model for the relationship between oxygen supply capacity, animal fitness and thermal tolerance in tropical species (Fig. 5B). Here aerobic performance is maintained until denaturation temperatures (T_d) and the primary determinants of T_{crit} are therefore not inadequate oxygen delivery from organ malfunction, but rather a more generalized heat-induced loss of protein structure or membrane integrity leading to a very sharp decline in metabolic and physiological functions. Fitness-related parameters decline in a number of crustaceans when approaching T_{crit} (Brown et al., 1992; King, 1994; O'Brien, 1994). Growth rates did not differ at 27 and 33°C in *M. rosenbergii* (Fig. 1), but animals die within a few days after being exposed to 38°C after a gradual rise in temperature (0.33°C day^{-1}) (R. Ern, unpublished observation). Thus given that acute aerobic performance was maintained up to T_{crit}, future studies should address whether this performance is amendable to acclimation to higher temperatures. It is also imperative to establish how aerobic scope correlates with the decline in growth in *M. rosenbergii* between 33 and 38°C, as well as other physiological and biochemical functions immediately below T_{crit}.

Conclusion

In the giant freshwater shrimp (*Macrobrachium rosenbergii*) the current worst-case scenario predicting a 3°C increase in tropical water temperature by the end of this century (Nilsson et al., 2009) does not cause a negative effect on its growth performance. Furthermore, oxygen transport capacity of the cardiorespiratory system does not seem to be universally coupled to animal fitness in aquatic organisms as previously proposed (Pörtner, 2010). Too heavy a reliance on the relatively easily measured aerobic performance characters such as heart rate, gill ventilation rate, aerobic scope and anaerobic metabolites will therefore yield inaccurate predictions on the impact of climate change in these species.

MATERIALS AND METHODS

Animals and maintenance

Physiological measurements were performed on giant freshwater shrimp (*Macrobrachium rosenbergii*) obtained from a local hatchery outside Can Tho University (Vietnam). The shrimp were held in 1 m^3 freshwater tanks (29±1°C) at Can Tho University and fed to satiety on dry feed and freshly thawed shrimp every second day. Water was changed regularly to ensure that NH_4^+-N, NO_2^- and NO_3^- never exceeded 0.25, 0.3 and 20 mg l^{-1}, respectively. All measurements were performed in the intermolt period in animals fasted for 4 days.

Growth rate

Growth was measured at 27 and 33°C in shrimp shipped to Aarhus University (Denmark) from Can Tho University. Shrimp (N=112) were evenly distributed in six freshwater tanks (1 m^3) at 27±0.1°C, constantly supplied by particle-filtered, UV sterilized and protein skimmed water. The shrimp were fed *ad libitum* on dry feed and freshly thawed shrimp once a day. Temperature was maintained at 27±0.1°C in three tanks, while temperature was raised to 33±0.1°C over the course of 18 days (0.33°C day^{-1}) in the other three tanks. The body mass of each shrimp was measured at days 0, 34, 55 and 86, and specific growth rate (SGR, g g^{-1} day^{-1}) was calculated between days 0 to 86 using Eqn 1:

$$\mathrm{SGR} = \frac{\ln(m_{t_2} / m_{t_1})}{t_2 - t_1}, t_2 > t_1, \quad (1)$$

where m_{t1} and m_{t2} are body mass (g) at the different time points t_1 and t_2, respectively.

Oxygen uptake

Oxygen uptake ($\dot{M}_{O_2}$) was measured using computerized intermittent-flow respirometry (Steffensen et al., 1984; Lefevre et al., 2011). SMR was measured at 30, 34 and 38°C in the same animals. The shrimp were placed in the respirometer (volume, 1 l) at 30±0.1°C and left undisturbed for 4 h, after which $\dot{M}_{O_2}$ was measured over 1 h. Temperature was then elevated to 34±0.1°C (4°C h^{-1}) and the shrimp left for 1 h before $\dot{M}_{O_2}$ was measured again for 1 h. The 1 h delay before measurement was because the Hamilton oxygen probes fluctuate when temperature is changed and needs 1 h to stabilize after each temperature elevation for reliable measurements. The same procedure was then repeated at 38°C. Each $\dot{M}_{O_2}$ measurement lasted 15 min with a 10 min closed period followed by 5 min of flush. During measurements, water P_{O_2} in the respiration chamber did not fall below 100 mmHg. At each temperature, the lowest of the four measured $\dot{M}_{O_2}$ values was chosen as an estimate of SMR. $\dot{M}_{O_2}$ was calculated from the decline in P_{O_2} during the closed period, with a 2 min delay from the start of the closed period using Eqn 2:

$$\dot{M}_{O_2} = \frac{-\delta P_{O_2} \times \alpha O_2 H_2 O \times (V_{\mathrm{chamber}} - M_b)}{M_b}, \quad (2)$$

where $\dot{M}_{O_2}$ is oxygen uptake (μmol kg^{-1} min^{-1}), δP_{O_2} is slope of the decline in oxygen tension of the water (mmHg min^{-1}) during a closed respirometer cycle, $\alpha O_2 H_2 O$ is the solubility of oxygen in the water at the relevant temperature (μmol l^{-1} $mmHg^{-1}$) (Colt, 1984), V_{chamber} is the volume of the respirometer (l), and M_b is shrimp body mass (kg). It was assumed that the shrimp displaced a water volume similar to its body mass (i.e. a density of 1 g ml^{-1}).

Maximum oxygen uptake ($\dot{M}_{O_2,max}$) at 30, 34 and 38°C was measured in 30 animals, 10 at each temperature. The shrimp was placed in a water tank at 30±0.1°C and the temperature either maintained at 30±0.1°C or elevated to 34±0.1 or 38±0.1°C (2°C h^{-1}). At the target temperature, a vigorous escape response was induced by nudging the carapace with a soft brush. After 3–4 min, the shrimp was deemed exhausted, indicated by the inability to induce escape responses, and immediately moved to a respirometer and $\dot{M}_{O_2}$ measured over 20 min. The decline in P_{O_2} was divided into 3 min bins and the bin with the highest δP_{O_2} used to estimate $\dot{M}_{O_2,max}$. Randomly distributed blind tests to measure bacterial respiration after the shrimp had been removed from the respirometers showed that background $\dot{M}_{O_2}$ due to bacterial respiration never exceeded 5% of SMR.

Hemolymph lactate concentrations

Hemolymph lactate concentrations were measured in resting and exhausted shrimp at 30°C and in resting shrimp at 34 and 38°C, using a handheld lactate analyzer (Accutrend Plus, Cobas-Roche, Mannheim, Germany). At all three temperatures, shrimp were initially placed overnight in a water tank (radius: 60 cm) at 30±0.1°C. The following morning temperature was either maintained at 30±0.1°C or elevated to 34±0.1 or 38±0.1°C (2°C h^{-1}). At the target temperature a hemolymph sample was taken by inserting a needle into the pericardial space in gently handled shrimp where escape responses were avoided. At 30°C the shrimp was exhausted (as described above) after the first sampling and another hemolymph sample was taken for lactate measurements after exercise.

Heart rate and gill ventilation rate

Heart and ventilation rates in *M. rosenbergii* were measured using reflective infrared sensors (AMP03, Newshift Ltd, Leiria, Portugal) fitted into plastic tubes and glued onto the carapace above either the heart or the scaphognathites (Fig. 4A). The animal was submerged in an aerated water tank at 30±0.1°C and left overnight to obtain resting values. The following morning water temperature was elevated (2°C h^{-1}), while heart or ventilation rate were recorded using a Biopac (Varna, Bulgaria) MP100 data acquisition system at 200 Hz.

Statistical analysis

One-way analysis of variance (P<0.05) was used to test the effect of acclimation temperature on growth rate, the effect of temperature on hemolymph lactate concentrations and the effect of temperature on SMR and $\dot{M}_{O_2,max}$, respectively. Student's t-test (P<0.05) was used to test the effect of exercise on hemolymph lactate concentrations in resting shrimp at 30°C. All statistical analyses were performed using JMP statistical Discovery from SAS (www.jmp.com).

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Competing interests

The authors declare no competing financial interests.

Author contributions

This study was conceived by R.E., D.T.T.H., N.T.P., T.W. and M.B. The experiments were designed by R.E., D.T.T.H., N.T.P., T.W. and M.B., and executed by R.E. and D.T.T.H. The results were interpreted by R.E., T.W. and M.B. The manuscript was drafted and revised by R.E., T.W. and M.B.

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