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Thermal tolerance of crustacean larvae (zoea I) in two different populations of the kelp crab *Taliepus dentatus* (Milne-Edwards)

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SUMMARY

Studies of thermal tolerance in marine ectotherms are key in understanding climate effects on ecosystems; however, tolerance of their larval stages has rarely been analyzed. Larval stages are expected to be particularly sensitive. Thermal stress may affect their potential for dispersal and zoogeographical distribution. A mismatch between oxygen demand and the limited capacity of oxygen supply to tissues has been hypothesized to be the first mechanism restricting survival at thermal extremes. Therefore, thermal tolerance of stage zoea I larvae was examined in two populations of the Chilean kelp crab *Taliepus dentatus,* which are separated by latitude and the thermal regime. We measured temperature-dependent activity, oxygen consumption, cardiac performance, body mass and the carbon (C) and nitrogen (N) composition in order to: (1) examine thermal effects from organismal to cellular levels, and (2) compare the thermal tolerance of larvae from two environmental temperature regimes. We found that larval performance is affected at thermal extremes indicated by decreases in activity, mainly in maxilliped beat rates, followed by decreases in oxygen consumption rates. Cardiac stroke volume was almost temperature-independent. Through changes in heart rate, cardiac output supported oxygen demand within the thermal window whereas at low and high temperature extremes heart rate declined. The comparison between southern and central populations suggests the adaptation of southern larvae to a colder temperature regime, with higher cardiac outputs due to increased cardiac stroke volumes, larger body sizes but similar body composition as indicated by similar C:N ratios. This limited but clear differentiation of thermal windows between populations allows the species to widen its biogeographical range.

Key words: oxygen consumption, heart rate, swimming, mass, C:N ratio, zoea I, *Taliepus dentatus*, larvae, thermal tolerance, temperature.

INTRODUCTION

Temperature is often invoked as the main determinant of distribution ranges and boundaries for marine and terrestrial species (Brown and Lomolino, 1998; Gaston, 2003; Sanford et al., 2006). Although evidence is mostly based on studies of thermal tolerance of adults, water temperature can strongly influence planktonic larvae by affecting survival, developmental time and growth, limiting recruitment and determining species distribution (Gilman, 2006; Sanford et al., 2006). However, our understanding of thermal tolerance and the physiological limits of larval stages is quite limited, despite the fact that larvae might experience stronger short- and long-term temperature fluctuations and are more vulnerable to thermal and osmotic stresses than adults (Anger et al., 2003). Physiological studies can help to explain distribution patterns and understand the mechanisms behind thermal sensitivity of organisms, which becomes particularly important under a global warming scenario (Pörtner and Knust, 2007).

A mismatch between oxygen demand and the limited capacity of oxygen supply to tissues is hypothesized to be the first mechanism restricting survival at the limits of the thermal tolerance window of marine organisms (Frederich and Pörtner, 2000; Mark et al., 2002; Pörtner, 2001; Pörtner, 2002; Pörtner and Knust, 2007). Thermal limitation becomes effective firstly at high hierarchical levels of organization, the whole organism and its oxygen delivery system and then at lower cellular and molecular levels (Mark et al., 2002; Pörtner et al., 2005). Pejus temperatures are thought to represent the long-term physiological temperature limits for a given species and are characterized by falling oxygen levels in the body fluids (hypoxemia) and the resulting decrease in the animal's aerobic scope. The progressively limited capacities of circulatory and ventilatory mechanisms upon warming and cooling indicate a mismatch between oxygen supply and demand (Frederich and Pörtner, 2000). Further cooling or warming leads to low or high critical threshold temperatures where aerobic scope vanishes and transition to an anaerobic mode and progressive insufficiency of cellular energy supply occurs (Pörtner, 2001; Pörtner et al., 2005).

This recently developed conceptual framework of oxygen and capacity-limited thermal tolerance was elaborated in adult individuals from various phyla but has not yet been systematically explored in early life stages. In crustaceans, thermal tolerance of larval stages is particularly interesting considering their potential for dispersal and the strong short-term variations in temperature that they may experience in the water column. We therefore investigated whether a mismatch between oxygen demand and limited capacity of oxygen supply to tissues occurs in larvae of a marine crustacean. We determined the thermal tolerance of *Taliepus dentatus* (Milne-Edwards) larvae (zoea I stage), integrating variables from organismal (whole animal activity and

oxygen consumption and body mass) to physiological [cardiac performance: heart rate (f_H), stroke volume (V_S) and cardiac output ($\dot{Q}$)] and elemental levels [carbon (C) and nitrogen (N) content]. We compared tolerance of acute temperature changes in larvae from two different climatic zones in order to identify differences in thermal adaptation. We used the kelp crab *T. dentatus* as a model of a eurythermal species exhibiting a wide range of latitudinal distribution along the coast of Chile. Two populations were chosen from southern and central habitats more than 10 deg. apart in latitude along the Pacific coast of Chile. To our knowledge our mechanistic analysis is the first to address how temperature might affect the physiology of crab larvae and how the thermal tolerance of the zoea might reflect the differentiation of the species into thermally specialized sub-populations, thereby leading to a widening of the distribution range of this species.

MATERIALS AND METHODS

Larval collection and experimental conditions

T. dentatus is distributed from 12 deg.S to 51 deg.S along the southeastern Pacific (Fagetti and Campodonico, 1971). Ovigerous *T. dentatus* were collected in southern Chile (SC) (Melinka, 43 deg.54′S, 73 deg.44′W)] and central Chile (CC) (Las Cruces, 33 deg.29′S, 71 deg.38′W)] by local professional divers during autumn/winter 2006 and brought to the Estación Costera de Investigaciones Marinas of Las Cruces, Chile. Mean annual seawater temperature ranges from 10.5°C in SC to 15.6°C in CC (Astorga et al., 2003). Extreme seawater temperatures varied between 8°C and 18°C at the southern Chilean site and between 10°C and 21°C at the central Chilean site. Five female crabs from each site were held individually in 50 l aquaria set up with running seawater at 11°C, 34 psu, constant aeration and a 12 h:12 h light:dark photoperiod until spawning. Newly hatched larvae were transferred to 0.5 l culture vessels with a constant density of 20 individuals. Culture water was changed once every 24 h by transferring all individuals using a glass pipette to a clean vessel containing filtered, well-aerated seawater and freshly hatched *Artemia* nauplii (Sanders Brine Shrimp Company, Ogden, UT, USA). The larvae were starved for one day prior to experimentation in order to provide uniform conditions. Experiments were conducted between autumn and spring 2006.

The experimental protocol was standardized with respect to temperature changes and acclimation time, and measurements of all parameters were taken synchronously. All experiments were conducted within two days using 9–10-day-old zoea I because thermal tolerance might change during the zoea l stage, depending on larval age. The 10th day is the middle of the developmental period of zoea I, which lasts around 20 days at a rearing temperature of 11°C (D.S., K.C. and M.F., in preparation). The rearing temperature of 11°C will be referred to as the control temperature. Immediately after the measurements at 11°C were finished, the system was cooled/warmed to the next experimental temperature. One set of larvae was progressively cooled from 11°C to 3°C and the second set was warmed from 11°C to 27°C, at a rate of 4°C within two hours. Experimental temperatures were 11°, 7° and 3°C for cooling experiments and 11°, 15°, 19°, 23° and 27°C for warming experiments. Therefore, the number of replicates was doubled at 11°C. Larvae were allowed to acclimate at each temperature for two hours before continuing to decrease or increase temperature to the next experimental period. At each experimental temperature, we measured: (1) larval activity, (2) oxygen consumption rate, (3) cardiac performance, and (4) C:N ratio. Larval activity (maxilliped and abdomen beat rates), oxygen consumption and cardiac performance (f_H, V_S and $\dot{Q}$) were determined in individual zoea. Five larvae from the different experimental mothers of each population were used for each experimental condition. Five replicates from each population were also used for fresh mass (FM), dry mass (DM) and C:N ratios, although in this case, a pool of zoea larvae from each female was used.

Larval activity

Maxilliped and abdomen beat rates were monitored using a video flex camera (Ken-A-Vision Mfg. Co. Inc., Kansas City, MO, USA). The camera was mounted onto a binocular and connected to a time-lapse video recorder (Sony Deutschland GmbH, Berlin, Germany). During the experiment, larvae were placed beneath the binocular in a temperature-controlled microchamber filled with seawater, which allowed the temperature to be changed according to the experimental protocol without disturbing the larvae. The zoea was positioned in the centre of the microchamber by gluing the dorsal spine of the cephalothorax to a thin glass spine using rapid glue. The glass spine, in turn, was attached to a glass table. The zoea was allowed to freely move its maxillipeds and abdomen in the experimental chamber filled with filtered seawater. The glass chamber was enclosed by aluminium foil to avoid visual disturbance. After a 2 h recovery period from handling stress, the experiment started at the control temperature. Afterwards, temperature was changed according to the protocol (see above). At each experimental temperature, the zoea was videotaped for 2 min. Maxilliped beating was calculated as the mean number of beats per minute (beats min^{-1}) from three 10 s intervals. The beating of the abdomen was counted over a 90 s time interval and was calculated as beats min^{-1}. The timeframe for measurements was adjusted based on the frequency of each movement. A two-way analysis of variance (ANOVA) was conducted to test for the effect of site of origin and temperature on maxilliped and abdomen beating. Maxilliped beat data were square-root-transformed to meet the homocedasticity assumptions of the ANOVA. Tukey tests were conducted for a posteriori analysis.

Oxygen consumption

Oxygen consumption rates were measured in individual zoea using a closed respirometry system. Hamilton microliter precision syringes (volume: 500 µl; Hamilton Bonaduz AG, Bonaduz, Switzerland) were used as chambers. Oxygen partial pressures were recorded by oxygen micro-optodes (needle-type, fiber-optic microsensor, flat broken tip, diameter: 140 µm) connected to a Microx TX2 (PreSens GmbH, Regensburg, Germany). Syringes were placed upside down in a temperature-controlled seawater bath, containing air-saturated, filtered (0.45 µm filter) seawater (salinity: 34 psu). The needle of the microsensor was inserted from the side of the cannula. Prior to insertion, optodes were calibrated in the same temperature-controlled seawater bath where measurements took place. Larvae were carefully introduced into the barrel by removing the plunger. After larvae were placed in the syringe, the plunger was inserted and carefully brought to the desired volume of 40 µl. The procedure took place entirely underwater to avoid introducing air bubbles. Subsequently, the optode was inserted and the sensitive tip was positioned in the middle of the respiration chamber. During the experimental trials, maximum oxygen depletion did not exceed 20%. Maintaining oxygen levels above 80% of saturation minimizes stress effects and the effects of hypoxia on thermal tolerance. In order to correct for bacterial oxygen consumption, blanks were run before and after measurements took place. At the end of the experiments, larvae were taken out of the chambers, carefully dried with a paper towel and weighed on a Sartorius bp 211 D balance (Göttingen, Germany). The overall picture of temperature-dependent oxygen

consumption of the zoea did not change when expressed as μg O_2 per fresh mass (μg O_2 FM^{-1}), μg O_2 per dry mass (μg O_2 DM^{-1}) or μg O_2 per individual (μg O_2 $individual^{-1}$). Therefore, mean rates of oxygen consumption are given as μg O_2 per mg fresh mass per hour (μg O_2 mg FM^{-1} h^{-1}) to highlight the variation in mass-specific physiological rates, which is crucial to understanding the underlying physiological mechanisms when comparing the two populations. A two-way ANOVA was conducted to test for the effect of site of origin and temperature on larval oxygen consumption. Data were log-transformed to meet the assumptions of the ANOVA. A posteriori analysis (Tukey test) was used to assess the differences among treatment levels.

Cardiac performance

f_H and V_S were determined using the same video sequences as for maxilliped and abdominal activity. The zoea stage of *T. dentatus* is transparent and the beating heart is clearly visible. f_H was obtained by using frame-by-frame analysis of the videotape on an editing tape player counting the number of contractions per unit time. The f_H was calculated as the mean number of beats min^{-1} from three 10 s intervals for each zoea.

V_S was determined by advancing the tape frame-by-frame until the heart reached its maximal dimension (end-diastolic volume) and its minimal dimension (end-systolic volume). The images of a 10 s video sequence were captured using a video frame grabber and digitizing program. The dimensions of the heart were measured and used in a geometric equation to calculate cardiac volumes. The heart was modelled as a prolate spheroid [$V=(4/3)\pi ab^2$ according to Harper and Reiber (Harper and Reiber, 2004)], where V is the cardiac volume, a is the length and b is the height of the heart). V_S was calculated as the difference between end-diastolic and end-systolic ventricular volume and expressed as nl per beat (nl $beat^{-1}$). $\dot{Q}$ was calculated as the product of f_H and V_S.

A two-way ANOVA was conducted to test for the effects of site of origin and temperature on f_H, V_S and $\dot{Q}$. For a posteriori analysis, Tukey tests were used.

Larval mass and C:N ratio

Larval FM was measured in samples containing between 45 and 50 individuals, which were carefully dried with a paper towel and weighed using a Sartorius bp 211D balance. In order to measure DM and C and N contents, we followed a standard method; samples of five zoea were briefly rinsed in distilled water, blotted dry on paper and subsequently frozen for storage at –20°C in pre-weighed tin cartridges. Later, samples were dried to constant mass at 60°C, weighed on a microbalance (Sartorius) and analyzed for C:N ratio on a Euro EA CHNSO Analyser (HEKAtech GmbH, Wegberg, Germany), using acetanilide as the standard. DM is reported as μg per individual (μg $individual^{-1}$) and as a % of FM. The commonly used C:N ratio was calculated to assess the differences in the lipid:protein ratio between temperature and populations.

A two-way ANOVA was conducted to test for the effects of site of origin and temperature on larval FM and DM, C and N contents and C:N ratio. Data were not transformed as the assumptions of the ANOVA were met. Tukey tests were used for a posteriori analysis.

RESULTS

Larval activity

A significant interaction in the ANOVA between temperature and site of origin precluded us from testing the main effects on maxilliped beat rates (F=13.16, d.f.=6,66; P<0.0001). The interaction was significant because larvae from SC showed higher maxilliped beat rates at temperatures between 3°C and 11°C and lower rates at temperatures between 15°C and 27°C in comparison with larvae from CC (Fig. 1A). For zoea from CC, it is interesting that maxilliped beat rate increased significantly as experimental temperature was reduced from 11°C to 7°C (P<0.05) whereas no changes were observed at increasing temperatures between 11°C and 15°C (P>0.05) (Fig. 1A). This contrasts with constant maxilliped beat rate in zoea from SC between 7°C and 11°C (P>0.05) whereas a decrease was detected at increasing temperatures between 11°C and 15°C (P<0.05) (Fig. 1A). Maxilliped beat rate decreased between 7°C and 3°C in larvae from both populations (P>0.05). The lowest maxilliped beat rates were recorded at the two highest temperatures in larvae from SC (23°C and 27°C; P<0.05) and the two extreme temperatures in larvae from CC (3°C and 27°C; P<0.05) (Fig. 1A). It is remarkable that larvae from SC exposed to 3°C showed beat rates similar to those observed in larvae from CC at 11°C (P>0.05) whereas larvae from CC exposed at 3°C were virtually moribund (P<0.05). A significant difference in maxilliped beat rates was found between sites at the control temperature of 11°C (lower in CC than in SC; P<0.05) (Fig. 1A).

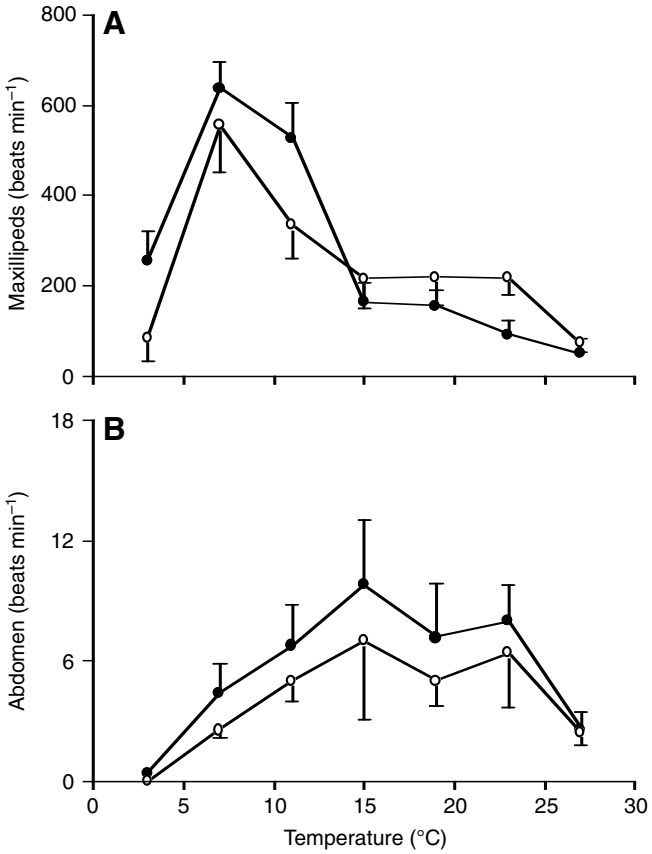

Fig. 1. The effect of temperature on (A) maxilliped beat rate and (B) abdomen beat rate in zoea I of *Taliepus dentatus* from southern Chile (closed circles) and central Chile (open circles). Data points are means ± s.e.m.

The influence of temperature was less pronounced on abdominal beat rate of the zoea than on maxilliped beat rate but was still significant (F=3.85; d.f.=6,66; P=0.002) (Fig. 1B). Lower abdomen beat rates were found at the extreme temperatures of 3°, 7° and 27°C (Tukey test, P<0.05). The highest abdomen beat rates were observed at the intermediate temperatures of 11°, 15°, 19° and 23°C (Fig. 1B) (P<0.05). No differences were detected between sites of

origin (F=2.04; d.f.=1,66; P=0.16) and the interaction term was not significant (F=0.10; d.f.=6,66; P=0.99).

Oxygen consumption

Oxygen consumption rates did not follow a typical exponential function, which is expected if standard metabolic rate is measured under rising temperature. As the zoea is actively swimming in the water column, larval oxygen consumption comprises standard metabolism and oxygen demand for swimming, thereby including a term for temperature-dependent performance. Oxygen consumption rates were significantly lower in zoea from CC than from SC (F=16.42; d.f.=1,66; P<0.001) (Fig. 2). Temperature affected oxygen consumption rates of the larvae (F=20.34; d.f.=1,66; P<0.001), following a similar pattern at both sites (the interaction term was not significant; F=0.45; d.f.=6,66; P<0.85) (Fig. 2). The lowest temperature of 3°C showed the lowest oxygen consumption rates (P<0.05) (Fig. 2). Oxygen consumption increased significantly between 3°C and 7°C (P<0.05), levelled off between 7°C and 11°C (P>0.05) and increased again at 15°C (P<0.05) (Fig. 2). The highest oxygen consumption rates were found between 19°C and 23°C (P<0.05). Then oxygen consumption rates dropped at 27°C (P<0.05) (Fig. 2) in zoea from SC whereas no changes were observed for zoea from CC between 15°C and 27°C (P>0.05).

Cardiac parameters

The pattern of f_H of the zoea from both populations was remarkably similar over the range of experimental temperatures analyzed in the present study (F=1.05; d.f.=1,66; P=0.31) (Fig. 3A). Temperature significantly affected f_H (F=130.14; d.f.=6, 66; P<0.0001) (Fig. 3A), which increased significantly between 3°C and 7°C (P<0.05), remained constant between 7°C and 11°C (P>0.05) and increased again between 11°C and 19°C (P<0.05) (Fig. 3A). f_H did not vary between 19°C and 27°C (P>0.05) (Fig. 3A). At control temperature of 11°C, f_H in zoea from SC and CC were 211±33 beats min^{-1} and 219±36 beats min^{-1}, respectively, and did not differ (P>0.05) (Fig. 3A). However, at 3°C, f_H were lower in zoea from CC than from SC (P<0.05).

V_S was independent of temperature (F=1.62; d.f.=6,66; P=1.15) but was significantly different between sites (F=18.44, d.f.=1,66; P<0.0001) (Fig. 2B). V_S was two times higher in zoea from SC at temperatures lower than 11°C (P always<0.05) (Fig. 3B). No differences between populations were detected at higher temperatures (P>0.05) (Fig. 3B).

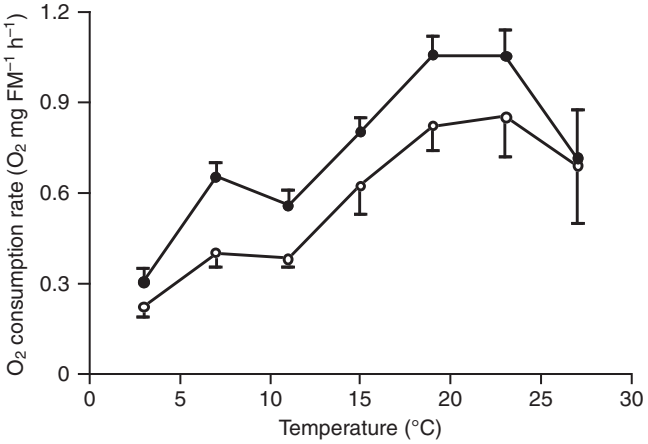

Fig. 2. The effect of temperature on oxygen consumption rate in zoea I of *Taliepus dentatus* from southern Chile (closed circles) and central Chile (open circles). Data points are means ± s.e.m.

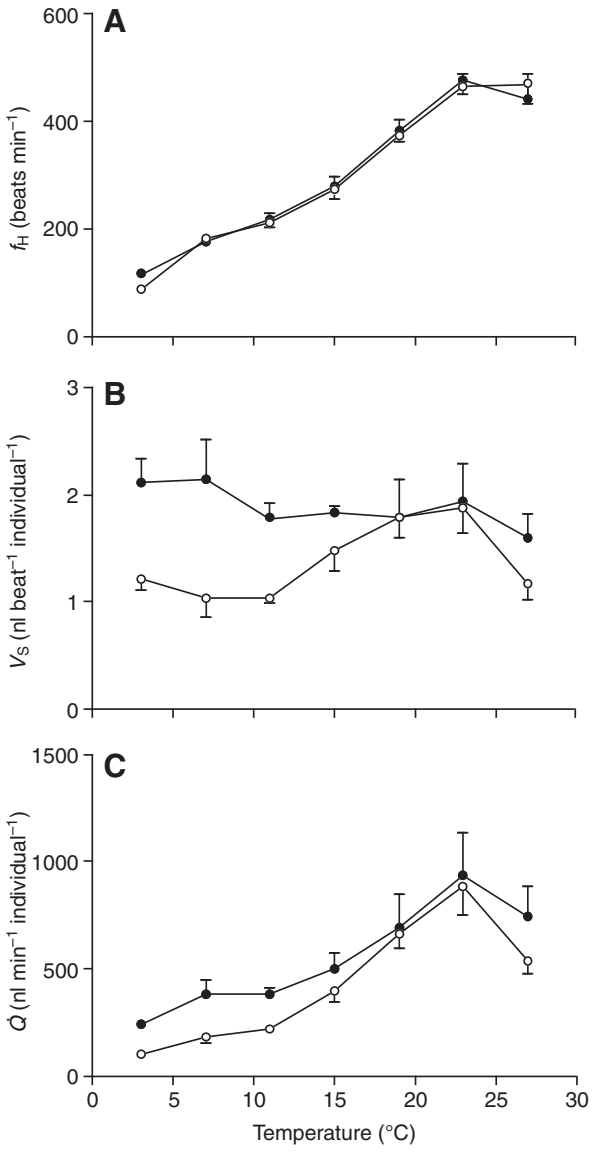

Fig. 3. The effect of temperature on (A) heart rate (f_H), (B) stroke volume (V_S) and cardiac output ($\dot{Q}$) in zoea I of *Taliepus dentatus* from southern Chile (closed circles) and central Chile (open circles). Data points are means ± s.e.m.

$\dot{Q}$ followed the trend of f_H and oxygen consumption rates. A significant interaction between site of origin and temperature in the ANOVA precluded us from testing the main effects (F=2.36; d.f.=6,66; P=0.04) (Fig. 3C). The interaction was significant because the differences between sites varied among temperatures. $\dot{Q}$ was two times higher in larvae from SC at 3°, 7° and 11°C (P<0.05) whereas no significant differences among sites were found at the higher temperatures (P>0.05) (Fig. 3C). The lowest $\dot{Q}$ was observed in larvae from CC exposed to 3°C (P<0.05).

Mass and elemental composition

Temperature had no effect on FM, DM, C and N contents and on C:N ratio of zoea I in the acute thermal tolerance experiment whereas the site of origin had a clear effect on larval FM and DM as well as on C and N contents (Tables 1 and 2). Larvae from SC exhibited

Table 1. Results of the ANOVAs conducted to assess the effect of temperature and site of origin (SC *versus* CC) on larval fresh mass (FM), dry mass (DM), carbon content (C), nitrogen content (N) (total mass and as a percentage of larval dry mass) and C:N ratio

Response variable	Units	Site effect			Temperature effect			Interaction		
		F	d.f.	*P*	*F*	d.f.	*P*	*F*	d.f.	*P*
FM	μg individual^{-1}	268.43	1,66	**<0.0001**	1.86	6,66	0.11	0.79	6,66	0.59
DM	μg individual^{-1}	129.6	1,66	**<0.0001**	0.24	6,66	0.96	0.48	6,66	0.82
	% FM	1.90	1,66	0.17	1.42	6,66	0.22	0.39	6,66	0.88
C	μg individual^{-1}	149.1	1,66	**<0.0001**	0.36	6,66	0.90	0.40	6,66	0.87
N	μg individual^{-1}	88.9	1,66	**<0.0001**	0.17	6,66	0.98	0.70	6,66	0.64
C:N	–	0.43	1,66	0.51	0.44	6,66	0.85	0.53	6,66	0.78

SC, southern Chile; CC, central Chile.

higher FM, DM and higher C and N contents (P always<0.05). It should be noted that no difference between sites was found for the % DM of FM and the C:N ratio (Tables 1 and 2). In all cases, the interaction terms were not significant (Table 1).

DISCUSSION

Thermal tolerance patterns observed in zoea I of *T. dentatus* from both populations corroborate the hypothesis (developed for adults) that thermal tolerance is oxygen and capacity limited (Frederich and Pörtner, 2000; Pörtner et al., 2005), resulting in narrow temperature tolerance windows for maxilliped beat rates. Temperature-dependent oxygen consumption rates (indicating aerobic oxygen demand) were reflected in $\dot{Q}$ (indicating a key role for oxygen delivery capacities). V_S and mass were insensitive to acute temperature changes but showed differences between populations. By contrast, elemental composition was insensitive to both acute and long-term temperature changes.

The increase in oxygen consumption rates with rising temperature is typically exponential within the passive thermal tolerance window set by upper and lower critical temperatures (Pörtner et al., 2005; Wittmann et al., 2008). Such an exponential increase was not observed in larval *T. dentatus*, indicating that animals were not displaying standard metabolic rate. Non-exponential temperature-dependent oxygen consumption has previously been detected in larvae of *Cancer irroratus* (Sastry, 1979). Planktonic zoea of *T. dentatus* and *C. irroratus* swim actively in the water column. Therefore, oxygen consumption comprises the energy demand for both maintenance and swimming activity and thus includes elements of performance or aerobic scope that are crucial in setting zoea l thermal tolerance at ecosystem level (Pörtner and Knust, 2007; Pörtner and Farrell, 2008). The decrease in aerobic scope affects higher functions such as activity and behavior and sets the lower and upper so-called pejus temperatures for long-term survival of a species (Pörtner and Knust, 2007; Wang and Overgaard, 2007). This was observed in zoea I of *T. dentatus*; constantly low maxilliped beat rates as oxygen consumption rates increase at temperature >15°C suggest that increasing baseline oxygen demand constrains aerobic scope and limits the level of maxilliped activity.

A further decrease in the capacity of zoea to perform aerobically is indicated by the levelling-off in oxygen consumption at 19°C. Such a pattern has been shown to denote the critical temperature beyond which anaerobic metabolism sets in and supports only short-term survival (Frederich and Pörtner, 2000; Mark et al., 2002). Oxygen consumption remained constant even though $\dot{Q}$ and f_H continued to increase beyond 19°C. This suggests that oxygen supply through the cardio-respiratory system becomes insufficient beyond critical temperatures when oxygen concentration in the hemolymph has fallen to critically low levels no longer fully supporting aerobic metabolism (Frederich and Pörtner, 2000). As a corollary, our finding in zoea I supports our hypothesis and demonstrates that thermal tolerance is oxygen- and capacity-limited in larvae of marine decapod crustaceans.

At first sight, lower temperature sensitivity of abdominal activity in comparison with maxilliped beat rates seems to contradict the hypothesis. However, in addition to eliciting locomotion in the water column, the abdomen also serves to provide additional oxygen to the larvae as demonstrated for zoea from *Nephrops norwegicus* (Spicer and Eriksson, 2003). Movements of the abdomen might improve diffusion gradients for oxygen and may therefore be maintained beyond high and low pejus temperatures, when maxilliped activity is already constrained.

The thermal tolerance window of zoea from SC was found to be shifted to lower temperatures when compared with those from CC. Larval functions in zoea from SC displayed cold compensation indicated by higher maxilliped beat rates, oxygen consumption rates and better cardiac performance at the same temperatures. Oxygen demand for maintenance and activity at low temperatures (3–11°C) was covered by enhanced oxygen delivery evidenced by increased

Table 2. Mean fresh mass (FM), mean dry mass (DM), carbon (C), nitrogen (N) and C:N mass ratios in zoea I of *T. dentatus* from southern Chile (SC) and central Chile (CC)

Response variable	Units	SC			CC		
		Means	±s.d.	*N*	Means	±s.d.	*N*
FM	μg individual^{-1}	430	21	63	328	19	63
DM	μg individual^{-1}	98	8	63	74	1	63
	% FM	21,9	2,0	–	21,2	2,0	–
C	μg individual^{-1}	31,5	2,9	63	22,2	2,7	63
N	μg individual^{-1}	5,7	0,7	63	4,1	0,6	63
C:N	–	5,6	0,5	63	5,5	0,4	63

s.d. indicates standard deviation; *N* indicates number of replicates.

f_H, V_S and $\dot{Q}$ in larvae from SC. High cardiac performance at 3°C may enable larvae from SC to remain as active as larvae from CC at 11°C. In contrast to zoea from SC, zoea from CC showed increased maxilliped beating when temperature was reduced from 11°C to 7°C. This suggests that larvae are stimulated by temperature change, which might serve to escape unfavourably low temperatures and to return to warmer physiologically tolerable water masses. These observed activity responses in zoea of *T. dentatus* resulted in comparable oxygen consumption rates at 7°C and 11°C. The oxygen demand for swimming at 7°C is supported by maintaining f_H and $\dot{Q}$ as high as under control conditions at 11°C.

Interestingly, at 7°C significantly higher mass-specific oxygen consumption rates at similar activity rates (maxilliped and abdominal) were found in larvae from SC than from CC. This suggests that tissue oxygen demand for maintenance in the southern population is higher, which is characteristic of metabolic cold adaptation. Higher FM and DM and similar C:N ratios suggest that the composition (water content:DM) and the lipid:protein ratio (C:N) of the larvae remain unchanged whereas body size increases in the cold (D.S., K.C. and M.F., unpublished data) providing more space for mitochondrial proliferation. A rise in mitochondrial densities and increased mitochondrial capacities to compensate for the cold-induced slowing of metabolism increases the costs of mitochondrial maintenance and whole organism oxygen demand (Sommer and Pörtner, 2004; Tschischka et al., 2000). It would also explain the observed increase in oxygen consumption of cold eurythermal zoea from the south.

At high temperatures, zoea from CC perform slightly better than zoea from SC suggested by: (1) higher maxilliped beat rates in zoea from CC, which can be related to higher capacities to perform aerobically, and (2) constant maxilliped beating of zoea from CC between 11°C and 15°C, which contrasts the drastic decrease in zoea from SC. Improved cardiac performance of zoea from SC compared with zoea from CC at low temperatures cannot be preserved at higher temperatures, resulting in the same cardiac outputs at high temperatures in larvae from both populations. This represents a decrease in aerobic performance for larvae in the southern population to similar values as found in the central population.

As a corollary, we have demonstrated how zoea I from the temperate and southern *T. dentatus* populations responded to acute experimental and latitudinal temperature variations. Thermal limits in zoea of *T. dentatus* become visible at the highest organizational level and are seen in decreasing activity followed by a reduction in cardio-respiratory performance. On evolutionary timescales, larvae from different populations adjust not only the cardio-respiratory system but they also adjust mass and size to adapt to their prevailing environmental temperature regime (D.S., unpublished observations). Adaptive plasticity in maintaining fitness according to climate is reflected in constant C:N ratios between populations. The small but clear shift between thermal tolerance windows between populations suggests an optimization of reaction norms and local adaptation in larvae of *T. dentatus*. Lower mean annual seawater temperatures and lower extreme temperatures match the shifted temperature tolerance windows reported in the present study for larvae from the SC site. Therefore, this differentiation allows the species to cover a wider range of distribution than when restricted to one and the same thermal window for all populations. This also means that larvae of *T. dentatus* from one population, although having a great potential for dispersal by wind-driven transport along the Chilean coast, seem to be restricted to the environmental thermal regime they developed and hatched in. Further comparative studies of thermal tolerance of crustacean larvae within and between populations are needed to confirm this observation as a unifying principle.

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