Metabolic Demand, Oxygen Supply, and Critical Temperatures in the Antarctic Bivalve *Laternula elliptica*

Lloyd S. Peck1,2,*
Hans O. Pörtner1
Iris Hardewig2†

1National Environment Research Council British Antarctic Survey, High Cross, Madingley Road, Cambridge CB3 0ET, United Kingdom; 2Alfred Wegener Institut für Polar und Meeresforschung, Biologie I/Ökophysiologie, D-27515 Bremerhaven, Germany

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ABSTRACT

Oxygen consumption (M0), heartbeat rate and form, and circulating hemolymph oxygen content were measured in relation to temperature in the large Antarctic infaunal bivalve *Laternula elliptica*. After elevations in temperature from 0°C to 3°C, 6°C, and then 9°C, M0 and heartbeat rate rose to new levels, whereas maximum circulating hemolymph oxygen content fell. At 0°C, M0 was 19.6 μmol O2 h⁻¹ for a standard animal of 2-g tissue ash-free dry mass, which equates to a 8.95-g tissue dry-mass or 58.4-g tissue wet-mass animal. Elevation of metabolism following temperature change had acute Q10 values between 4.1 and 5, whereas acclimated figures declined from 3.4 (between 0°C and 3°C) to 2.2 (3°C–6°C) and 1.9 (6°C–9°C). Heartbeat rate showed no acclimation following temperature elevations, with Q10 values of 3.9, 3.2, and 4.3, respectively. Circulating hemolymph oxygen content declined from 0°C to 3°C and 6°C but stayed at a constant Po2 (73–78 mmHg) and constant proportion (~50%) of the oxygen content of the ambient water. At 9°C, M0 and heartbeat rate both peaked at values 3.3 times those measured at 0°C, which may indicate aerobic scope in this species. After these peaks, both measures declined rapidly over the ensuing 5 d to the lowest measured in the study, and the bivalves began to die. Hemolymph oxygen content fell dramatically at 9°C to values between 2% and 12% of ambient water O2 content and had a maximum Po2 of around 20 mmHg. These data indicate an experimental upper lethal temperature of 9°C and a critical temperature, where long-term switch to anaerobic metabolism probably occurs, of around 6°C for *L. elliptica*. Concurrent measures of mitochondrial function in the same species had indicated strong thermal sensitivity in proton leakage costs, and our data support the hypothesis that as temperature rises, mitochondrial maintenance costs rapidly outstrip oxygen supply mechanisms in cold stenothermal marine species.

Introduction

Polar marine ectotherms are at one end of the temperature continuum for life in the oceans. They can generally only survive at low temperatures, and within small temperature ranges, they are highly stenothermal (Somero et al. 1996, 1998; Peck and Conway 2000). This trait has come from evolution to a very stable temperature environment and carries with it many specific biochemical and physiological adaptations. These include the possession of enzymes with increased activity at low temperatures, larger quantities of key enzymes, and mitochondrial proliferation (Clarke 1998).

Recently, there has been significant interest in physiological adaptation to low temperatures (e.g., Johnston and Bennet 1996; Pörtner and Playle 1998) and the mechanisms limiting tolerance to elevated or reduced temperatures. As lethal temperature limits are approached, a transfer to anaerobic metabolism has been identified in temperate and polar species, and this transfer has been termed the “critical temperature” (Zielinski and Pörtner 1996; Pörtner et al. 1998, 2000). These critical temperatures probably indicate long-term lethal limits. In a study of the Weddell Sea bivalve *Limopsis marionensis*, Pörtner et al. (1999b) found a critical temperature around 2°C, indicating transition to a time-limited situation. Fifty percent mortality occurred within 10 d of exposure at 4.0°C. At temperatures of 2°C and above, the anaerobic end products succinate and acetate accumulated in mantle tissue and were not reduced to control levels even after 14 d of exposure. ATP and levels of the Gibbs free-energy change of ATP hydrolysis also decreased and did not recover over this time. The long-term temperature envelope where *L. marionensis* are capable of physiological homeostasis is, therefore, between –2°C and +2°C, making this possibly the most stenothermal marine invertebrate so far reported.

Later investigations centered on other mechanisms associated with setting temperature limits for polar ectotherms, with a
focus on mitochondrial contributions to metabolic costs in the large infaunal Antarctic bivalve *Laternula elliptica* (Pörtner et al. 1999b). Maximum mitochondrial capacity, state 3 mitochondrial respiration, and the costs of proton leakage in mitochondria were assessed in relation to temperature. Costs of proton leakage through inner mitochondrial membranes were assessed as the rate of oxygen consumption by mitochondria during the blockage of oxidative phosphorylation at high phosphorylation potentials by oligomycin (state 4+ respiration). State 3 respiration and proton leakage costs rose exponentially with temperature, and an unusually high sensitivity to temperature was found for proton leakage. This high thermal sensitivity of proton leakage caused a progressively increasing mitochondrial oxygen demand, which was thought to set upper temperature limits when it exceeded the whole-animal capacity for oxygen uptake and supply.

This study was aimed at evaluating changes in whole-animal oxygen consumption and in mechanisms of physiological oxygen supply in relation to temperature in *L. elliptica*. Experiments were conducted at the same time on specimens from the same population as for the assessment of mitochondrial capacity and costs. Whole-animal oxygen consumption, heart-beat rate and form, and circulating hemolymph oxygen content were measured to provide a detailed description of oxygen supply in relation to temperature stress.

*Laternula elliptica* was chosen because it is a common, large infaunal bivalve with easily accessible nearshore populations. It has a circum-Antarctic distribution, and much of its general ecology, including feeding, routine metabolism, and growth rate, have been previously investigated (Ralph and Maxwell 1977a; Ahn 1994, 1997; Brey and Mackensen 1997; Ahn and Shim 1998). Also, tissue volumes are large enough to allow extraction of sufficient mitochondria to allow accurate measurement of mitochondrial function, and the animal is large enough to facilitate the collection of hemolymph samples for oxygen analysis.

**Material and Methods**

*Laternula elliptica* specimens were collected from North Bay, Rothera Point, Adelaide Island (67°34'S, 68°08'W), from a soft-sediment site at a depth of 29–33 m by scuba divers in November 1997. The sea temperature during the preceding 2 wk had been in the range of −1.0° to −0.2°C. The specimens were transferred to buckets in the sea and transported 0.8 km to the Bonner Laboratory (Rothera station) marine aquarium, underwater at all times. In the aquarium, they were held in a throughflow seawater system at temperatures between −0.6° and +0.4°C (temperatures were continuously logged) for between 5 and 8 d before the initiation of experiments.

The experimental system was comprised of a jacketed water bath connected to a thermostatically controlled water heater/chiller. Animals were placed in the inner 175-L compartment, and temperature-controlled water passed through the outer jacket. The whole system was held in a controlled temperature room. Using this system, seawater temperature in the experiments was controlled to a maximum variation of ±0.2°C. Initially, 19 specimens were placed in the system, and three were replaced after 5 d; five were used to measure oxygen consumption, four for heartbeat assessments, and 13 for hemolymph oxygen content. A control group of 15 specimens was held without food in the main aquarium system for over 50 d with no mortality. It is therefore unlikely that starvation had a large effect in these experiments. That cold-water species can survive long periods of starvation, over 100 d, with little obvious effect has been demonstrated for the limpet *Nacella concinna* and the brachiopod *Liothyrella uva* (Peck 1989), and it has been demonstrated for over 120 d for the urchin *Stereochinus nemayeri* (Brockington and Clarke 2001) and for over 60 d for the bivalve *Limopsis marionensis* (Pörtner et al. 1999b). However, starvation tends to reduce metabolic rates while elevated temperature raises them. Peck (1989) showed that starvation may continue to reduce metabolic rates for at least 25–30 d before standard levels are reached in *L. uva* and *N. concinna*. Measured metabolic rates at a given temperature may therefore be slightly different from those reported here in recently fed animals.

**Oxygen Consumption and Hemolymph Oxygen Measures**

Closed-bottle respirometry methods were used. Experimental animals were carefully placed inside 2-L glass chambers within the controlled temperature system, keeping the *Laternula* underwater at all times. Chambers were then sealed, and the animals were incubated for variable amounts of time. Incubations were ended when oxygen content of the chambers had been depleted by between 10% and 20%. Oxygen consumption of the bivalves was calculated by comparison with parallel incubations of identical control chambers with no animal. The oxygen content of the water in chambers was measured using 25-μL samples injected into a couloxiometer (Peck and Uglow 1990). The syringe was fitted with a Chaney adaptor and was calibrated weekly from samples (*N* = 10) weighed on a microbalance. At the end of the respirometry trials, animal volume was measured by displacement, and this was allowed for in the calculation of respirometer volume. The same five specimens were used throughout the temperature trials. Oxygen consumption was monitored following a temperature rise, and a further rise in temperature was implemented only when metabolism had stabilized following the previous rise. The experiment was ended at 9°C when over 50% of the *L. elliptica* had died (three out of five). Other specimens of *L. elliptica* collected at the same time and held without food in the station aquarium suffered no mortality in 50 d. It is unlikely, therefore, that starvation played a significant role in the results obtained.

Oxygen content of the hemolymph was measured from sam-
and the samples were freeze clamped in liquid nitrogen. These samples were held in liquid nitrogen for transport to Germany, where they were later analyzed for succinate content by ion-exchange chromatography as described in Pörtner et al. (1999b).

Animal Biometrics

At the end of the trials, or immediately upon death at 9°C, shell length was measured to the nearest 0.1 mm using vernier callipers. Specimen dry mass was obtained by drying to constant weight at 60°C, and AFDM is the difference between dry mass and the material remaining following ignition at 475°C for 24 h. Data are presented for a standard animal of 2-g tissue AFDM, which was close to the mean for the group studied. Tissue-mass values are given in Table 1.

Results

Oxygen Consumption

In initial trials to establish the relationship between oxygen consumption (M\textsubscript{O\textsubscript{2}}) and ambient water oxygen partial pressure (P\textsubscript{O\textsubscript{2}}) at 0°C, an ability to regulate M\textsubscript{O\textsubscript{2}} down to P\textsubscript{O\textsubscript{2}} values around 60–70 mmHg was found (Fig. 1). Thus, in later trials measuring M\textsubscript{O\textsubscript{2}} in relation to temperature, there was no experimental influence of reduced P\textsubscript{O\textsubscript{2}} because values were not allowed to fall below 120 mmHg.

After 5–10 d acclimation at 0°C, standard animal M\textsubscript{O\textsubscript{2}} stabilized at 19–20 μmol O\textsubscript{2} h\textsuperscript{-1} (Fig. 2). Following elevations in temperature to 3°C and 6°C, metabolism rose to new levels. At 3°C, M\textsubscript{O\textsubscript{2}} simply rose to a new level, whereas at 6°C, M\textsubscript{O\textsubscript{2}} rose to a peak after 3 d (48 μmol O\textsubscript{2} animal\textsuperscript{-1} h\textsuperscript{-1}); there was then a decline to a lower M\textsubscript{O\textsubscript{2}} rate. At 9°C, M\textsubscript{O\textsubscript{2}} rose to a high value of 63 μmol O\textsubscript{2} animal\textsuperscript{-1} h\textsuperscript{-1} within 24 h of the rise in temperature. On successive days, M\textsubscript{O\textsubscript{2}} fell rapidly until, 4 d later, standard animal M\textsubscript{O\textsubscript{2}} was 15–18 μmol O\textsubscript{2} animal\textsuperscript{-1} h\textsuperscript{-1} and was lower than the lowest rates previously recorded (19–20 μmol O\textsubscript{2} animal\textsuperscript{-1} h\textsuperscript{-1} at 0°C). These low M\textsubscript{O\textsubscript{2}} rates were recorded for 3 d before the experiment was terminated when mortality levels passed 50%. Overall temperature had a significant effect on M\textsubscript{O\textsubscript{2}} (repeated measures ANOVA: F = 8.82, P = 0.002,

### Table 1: Shell length and mass data for the experimental *Laternula elliptica* group studied

<table>
<thead>
<tr>
<th>Tissue Measured</th>
<th>Mean Length (mm) or Mass (g) ± SE</th>
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</thead>
<tbody>
<tr>
<td>Shell length</td>
<td>74.6 ± 2.76</td>
</tr>
<tr>
<td>Whole animal (mantle cavity drained, wet mass)</td>
<td>107.5 ± 14.50</td>
</tr>
<tr>
<td>Tissue wet mass</td>
<td>57.2 ± 9.46</td>
</tr>
<tr>
<td>Tissue dry mass</td>
<td>8.78 ± 1.52</td>
</tr>
<tr>
<td>Tissue ash-free dry mass</td>
<td>1.96 ± 0.27</td>
</tr>
</tbody>
</table>

Note. N = 5.
Acute Q10 values, comparing previously acclimated activity between temperatures were all between 1.9 and 3.4 (Table 2). The acute value for metabolically inactive periods of low and high amplitude (Fig. 3) were higher than at 3°C (excluding the initial peak value). At 0°C, the acclimated rate for metabolically inactive periods of low and high amplitude (Fig. 3) was significantly higher than at 0°C (paired t-test: t = 8.81, P < 0.001, N = 66), and values at 6°C were higher than at 3°C (paired t-test: t = 7.45, P < 0.001, N = 70).

Using all data for 0°C and 3°C, the acclimated Mo2 rate for 6°C, and Mo2 data for 9°C (excluding the initial peak value and the values after metabolic collapse), Q10 values for metabolic change between temperatures were all between 1.9 and 3.4 (Table 2). Acute Q10 values, comparing previously acclimated Mo2 with peaks following a temperature rise, were all between 4 and 5.

Heartbeat Rate

At 0°C, which is within the normally experienced temperature range for this species at Rothera, impedance heartbeat traces were typified by long periods (10–75 min) of regular oscillation interspersed with blocks of irregular activity (Fig. 3A). These irregularities were observed to be associated with other activity including siphon contraction and protrusion of the foot associated with attempted burrowing movements. Two specimens often exhibited cyclical periodicity in heartbeat traces, with blocks of 8–15 normal heartbeat cycles separated by one or two beats of significantly lower amplitude (Fig. 3B). It is not known if these cycles were associated with other possible cyclical activity such as cycles in ventilation. One preparation exhibited a distinct biphasic contraction cycle (Fig. 3C). When temperatures were raised, heartbeat traces alternated between periods of low and high amplitude (Fig. 3D), indicating that the animals attempt to regulate hemolymph flow and respond to enhanced temperature-induced metabolic stress. After the move to 3°C, heartbeat traces returned to normal in 12–24 h; after the move to 6°C, the return took around 24 h, and no return to normal occurred at 9°C.

Heartbeat rate showed a similar response to Mo2, when temperatures were raised (Fig. 4). However, no pattern of peak followed by acclimation was apparent at any temperature. Values rose from approximately 4 min−1 at 0°C to around 6 min−1 at 3°C, 8 min−1 at 6°C, and to a peak of 13 min−1 at 9°C. At 0°C, 3°C, and 6°C, heartbeats stabilized at each new temperature after a period of fluctuation lasting 3–4 d. At 9°C, heartbeat rate fell continuously for the 5 d after the peak to low levels, around 2 min−1, when animals began to die.

Q10 values were 3.80 and 3.31 for the temperature rises from 0°C to 3°C and 5°C to 6°C, respectively (Table 2). The acute value for 6°C to 9°C was 4.46, and there was no acclimated value.

Hemolymph Oxygen Content

This method measures total oxygen content, both bound to pigment and unbound, because the Po2 in the desorber (the sample chamber) is close to 0 under normal conditions. Levels of total oxygen content of the hemolymph at 0°C, 3°C, and 6°C ranged from 0.01 μmol cm−3 to 0.17 μmol cm−3 (Fig. 5). The relationship between the maximum observed hemolymph oxygen content and temperature was investigated by regressing the highest two oxygen concentration values against temperature for assessments between 0°C and 6°C. The highest two
values at each temperature were taken as a measure of maximum hemolymph oxygen content at the sample site. Other values were taken to represent blood at lower oxygen content ranging from partially oxygenated to deoxygenated hemolymph. Maximum hemolymph oxygen content declined linearly with temperature from 0°C to 6°C as described by the equation

$$[O_2]_{\text{max}} = 0.16 \text{ to } 0.0037 T,$$

where oxygen concentration ([O_2]_{\text{max}}) is in μmol cm⁻³, temperature (T) is in °C, $$r^2 = 0.80, F = 21.16, P = 0.01,$$ and $$N = 6.$$ Thus, for every 1°C rise in temperature, maximum oxygen content fell by 0.367 μmol cm⁻³. The decline in maximum hemolymph oxygen content with temperature paralleled the decline in saturated seawater oxygen content and remained between 47.5%–49.4% of saturated seawater at these temperatures. Levels of [O_2]_{\text{max}} in holding tanks and control chambers were around 95% calculated maximum saturation, so maximum hemolymph concentration from samples taken near the heart was around 50% that of the seawater they were held in at temperatures between 0°C and 6°C. Low values were always around 2%–3% of saturated seawater.

In terms of partial pressures, maximum levels were relatively constant in the hemolymph between 0°C and 6°C at values between 51 and 78 mmHg (Table 3), showing that the decline in concentration is due to the maintenance of a constant P_o₂ gradient at sites of oxygen uptake. Minimum hemolymph P_o₂ values were always between 3.3 and 7.4 mmHg.

At 9°C, hemolymph oxygen content collapsed such that no high values were recorded. Maximum levels obtained were under 12% saturated seawater. Minimum values were similar to those recorded at 0°C, 3°C, and 6°C. The same effect was observed when data are expressed as hemolymph P_o₂ with maximum levels declining to around 20 mmHg at 9°C and with minimum values at 3.5 mmHg.

### Tissue Succinate Content

Succinate contents of mantle tissues were elevated at 6°C (~2.9 μmol g⁻¹ wet weight), compared with 0°C and 3°C (1–1.5 μmol g⁻¹ wet weight; Fig. 6). In the siphon, succinate accumulation occurred with a smaller temperature elevation and was elevated at 3°C and 6°C compared to 0°C. In both tissues, succinate levels fell from 6°C to 9°C. In the siphon, succinate levels rose after 3 d at that temperature, but in the group elevated to 10.5°C, succinate levels were low in both tissues after 2 d of exposure.

### Discussion

*Laternula elliptica* regulates its rate of M_o₂ down to P_o₂ levels around 60–70 mmHg in the inspired seawater. This indicates that none of the measurements of M_o₂ here were affected by low P_o₂ because P_o₂ values in these trials were not allowed to fall below 120 mmHg. Bivalve molluscs show a wide range of metabolic responses to reduced external P_o₂ (Bayne and Newell 1983), and the ability to regulate M_o₂ exhibited by *L. elliptica* is not unusual.

The routine metabolic rate of *L. elliptica* at 0°C, 19.6 μmol O₂ h⁻¹ for a 2-g tissue AFDM individual (6.42-g tissue dry mass, 58.4-g tissue wet mass), is within the range but low compared to other Antarctic bivalves and gastropods, most of which have M_o₂ values between 4 and 10.7 μmol O₂ g⁻¹ dry tissue mass h (Table 4). The data here are around three times lower than values for the same species measured by Peck and Conway (2000). This is probably because Peck and Conway (2000) measured M_o₂ after only allowing the bivalves 10–24 h to acclimate to the holding systems, which is likely to have produced somewhat elevated rates. They also measured routine rates, which would have been elevated by feeding. In Antarctic marine ectotherms, feeding usually raises metabolism between 1.5 and 2.5 times standard or basal rates (Peck 1998). Another factor that may account for some of the difference is that they measured tissue dry mass by drying tissues to constant weight at 60°C, which took between 36 and 60 h. In this investigation, tissues were dried for a standard 24 h. The difference in weights produced with these slightly different methods will account for some of the differences seen in tissue-mass-specific M_o₂. Peck and Conway (2000) also recently compared metabolic rates of 41 species of bivalve molluscs across temperature regimes from 0°C–30°C. They compared routine metabolic rates measured at normal habitat temperatures and found that there was no ev-

<table>
<thead>
<tr>
<th>Table 2: Acute and acclimated Q₁₀ values for <em>Laternula elliptica</em> M₀₂ and heartbeat rate in response to temperature elevations</th>
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<tbody>
<tr>
<td>Temperature (°C)</td>
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<tr>
<td></td>
</tr>
<tr>
<td>0–3</td>
</tr>
<tr>
<td>3–6</td>
</tr>
<tr>
<td>6–9</td>
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</table>

Note. N/A indicates that no stable acclimated rate was obtained after a temperature increment. Q₁₀ values were calculated for each individual, and figures shown are group means ± SE.
Figure 3. Impedance heartbeat traces for *Laternula elliptica* specimens held in the experimental regime. 

A, Typical trace at 0°C showing long periods of steady oscillation punctuated with short periods of disruption caused by bouts of activity (e.g., siphon contraction). Heartbeat rate was around 4 min⁻¹ in this specimen. 

B, Trace for a specimen held at 0°C showing heartbeat contractions grouped into cycles of 2–3-min duration. 

C, Trace for one specimen at 0°C showing biphasic contraction. 

D, Trace for a specimen during the period when temperature was raised from 0° to 3°C.
Figure 4. *Laternula elliptica* heartbeat rate (beats min\(^{-1}\)) in relation to temperature. Values are means ± SE for a group of four specimens. Bars indicate periods when temperatures were held at the values indicated above.

idence for elevated metabolism in polar species (whole-animal metabolic cold adaptation). Indeed, their data suggest that if there is a difference in the relationship between bivalve metabolism and temperature at low ambient temperatures, then polar species have lower \(\dot{M}_{O_2}\) rates than would be expected, although differences from the trend were only marginally significant. Their findings are similar to those for Antarctic fish by Clarke and Johnston (1999) who, in a comparison of all available metabolic-rate data for Perciform species, also showed no elevation of whole-animal metabolic rate at polar temperatures.

This poses an interesting conundrum. Recent considerations of cellular metabolic cold adaptation indicate whole-animal metabolism should be elevated at low temperatures (Pörtner et al. 2000). Maximum mitochondrial function rates show no temperature compensation between warm and cold species (Johnston et al. 1994, 1998; Guderley 1998; Pörtner et al. 1999a, 2000), although this character may differ between stenotherms and eurytherms (Pörtner et al. 2000). In fish, mitochondrial densities are elevated in low-temperature aerobic muscle to maintain whole-animal capacities for activity (Dunn 1988; Johnston et al. 1998). There is a cost associated with the maintenance of the intramitochondrial environment, because of the passive influx of protons through the mitochondrial membrane, driven by the proton gradient (Pörtner et al. 2000). Homeo-

Elevated Temperature and Metabolism

As temperatures were increased progressively above 0\(^\circ\)C to 3\(^\circ\)C and then 6\(^\circ\)C and 9\(^\circ\)C, *L. elliptica* metabolism increased to new levels. For \(\dot{M}_{O_2}\), acute Q\(_{10}\) values were all between 4 and 5,

Figure 5. *Laternula elliptica* hemolymph oxygen content (\(\mu\)mol cm\(^{-3}\)) in relation to temperature. High values at 0\(^\circ\), 3\(^\circ\), and 6\(^\circ\)C are all ~50% the level of oxygen content of the ambient seawater. Maximum values at 9\(^\circ\)C were 12% of the content of ambient seawater.
was evident for the response from *L. elliptica* evident in (temperature change, which were followed by partial acclimation *Liothyrella uva*, Peck (1989) found distinct peaks following a mitochondrial proton leakage in a few species (e.g., Forster et al. 1991). This is because mitochondrial proton leakage in *L. elliptica* has a high-temperature sensitivity (Pörtner et al. 1999a), and the limited data available suggest that high-temperature sensitivity of proton leakage may be characteristic of polar ectotherms (Hardewig et al. 1999). The limitations posed by high-temperature sensitivity in mitochondria of low-temperature species pose problems irrespective of whether densities are enhanced.  

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Maximum PO₂</th>
<th>Minimum PO₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>78.4</td>
<td>7.4</td>
</tr>
<tr>
<td>3</td>
<td>73.1</td>
<td>6.9</td>
</tr>
<tr>
<td>6</td>
<td>75.6</td>
<td>3.3</td>
</tr>
<tr>
<td>9</td>
<td>20.3</td>
<td>3.5</td>
</tr>
</tbody>
</table>

Note. Values quoted are in mmHg and are the maximum and minimum figures obtained at each temperature.

whereas acclimated values were lower, between 2.2 and 3.4. The acclimated values are all well within the normal range for temperature effects on ectotherm metabolic rate (Clarke 1998; Peck, in press) and indicate no difference in temperature sensitivity of metabolism compared to temperate species. This finding is similar to that found for other Antarctic bivalves (Davenport 1988; Pörtner et al. 1999b), gastropods, and brachiopods (Peck 1989). It does, however, run contrary to the expectations concomitant with enhanced numbers of mitochondria, and the observations of high-temperature sensitivity in a few species (e.g., Forster et al. 1991). This is because mitochondrial proton leakage in *L. elliptica* has a high-temperature sensitivity (Pörtner et al. 1999a), and the limited data available suggest that high-temperature sensitivity of proton leakage may be characteristic of polar ectotherms (Hardewig et al. 1999). The limitations posed by high-temperature sensitivity in mitochondria of low-temperature species pose problems irrespective of whether densities are enhanced.  

Q₁₀ values for heartbeat rate were similar to those for M₀₂, ranging between 3.2 and 4.5. It was not possible to differentiate acute and acclimated responses in heartbeat rate. This, combined with the dramatic variations in heartbeat amplitude when temperatures were raised (Fig. 3), suggests that large effort was required to maintain physiological condition under temperature stress. This may be an indication that circulation and oxygen supply via the gills and the hemolymph may be limiting factors in tolerance to elevated temperature.

In an investigation of metabolic rate in response to raised temperature in the limpet *Nacella concinna* and the brachiopod *Liothyrella uva*, Peck (1989) found distinct peaks following a temperature change, which were followed by partial acclimation (sensu Precht et al. 1955) to new levels. A similar response was evident in *L. elliptica* for the temperature elevation from 3°C to 6°C and also possibly from 6°C to 9°C. However, no acclimation was evident for the M₀₂ response from 0°C to 3°C. No peak followed by acclimation was observed in heartbeat rate at any temperature.

At 0°C, 3°C, and 6°C, hemolymph oxygen content varied widely from values around 3 mmHg to over 75 mmHg. High values indicate the maximum oxygen content *L. elliptica* can attain at this site in these conditions. Low values are associated with periods of siphon closure (H. O. Pörtner, unpublished observation) and may be associated with a strategy for reducing costs of pumping water. With rising temperature, maximum oxygen content of the circulating hemolymph declined as a constant proportion of dissolved oxygen in the ambient seawater, approximately 50%. This suggests that the *L. elliptica* were not capable of increasing the quantity of oxygen transferred across the gills, and it may indicate the limitation imposed by maximum transfer efficiency, although it should be noted that samples were collected from near the heart and not immediately after the hemolymph had passed through the gills.

### Temperature Limits and Critical Temperatures

At 9°C, where 50% *L. elliptica* mortality was observed within 7 d, M₀₂ and heartbeat rate both peaked within 24 h of the temperature elevation, at values 3.3 times higher than the acclimated rates at 0°C. On succeeding days, both measures collapsed such that between 5 and 6 d after moving to 9°C, M₀₂ and heartbeat rate were below the same measures at 0°C. Similar collapses of M₀₂ have been observed at upper lethal temperatures in other Antarctic gastropods and brachiopods (Peck 1989), sea urchins (Brockington and Peck, in press), and starfish (T. Hill and L. S. Peck, unpublished observation). Measures of hemolymph oxygen content collapsed above 6°C (Fig. 5), indicating that oxygen limitation was the likely mechanism setting the upper limit. On this basis, the long-term upper lethal temperature would be expected to be around 6°C.

Previous studies have implicated oxygen supply as important in setting upper lethal limits, from observations of a transfer to anaerobic metabolism and the accumulation of anaerobic end products such as acetate, succinate, and octopine in tissues in both polar and temperate marine invertebrates (Zielinski and Pörtner 1996; Sommer et al. 1997; Pörtner et al. 1999b; Sommer and Pörtner 1999; van Dijk et al., in press). This transfer to anaerobic metabolism occurs close to the experimental upper lethal limit and has been termed the “critical temperature” (Pörtner et al. 1998, 2000). It identifies a physiological limit where oxygen supply and energy balance can no longer be maintained and indicates the maximum temperature for long-term survival in terms of weeks or months. Critical temperatures with transfers to anaerobic states have been observed to define both upper and lower limits for temperate marine species (Sommer et al. 1997; Pörtner et al. 1998).

Succinate measures here indicated that some tissues began accumulating anaerobic end products at 3°C, and both tissues studied accumulated succinate at 6°C. The reduced levels of succinate at 9°C and 10.5°C probably indicate a transfer to accumulation of other anaerobic end products of metabolism such as acetate. These data support the contention that the upper critical temperature for *L. elliptica* is around, or slightly below, 6°C.

It has been suggested that the maximum metabolic rates
measured in studies such as this are set by limitations to oxygen supply mechanisms, which may indicate maximum physiological capacities. If this is so, the difference between this and acclimated resting metabolism in the normal temperature range is a measure of maximum aerobic potential that may be analogous to maximum aerobic capacity (Peck 1998, in press). The maximum elevations of $\text{Mo}_2^-$ and heartbeat rate of 3.3 and 3.4 times, respectively, values at $0^\circ\text{C}$ fall within the range seen for Antarctic marine ectotherms but are high (Peck 1998). Values are usually in the range of 1.5 to 2.5, and the figures for $L. \text{elliptica}$ suggest it may have more metabolic flexibility than many other polar species. This assertion is also supported by the finding of an upper lethal temperature of $9^\circ\text{C}$, which is one of the highest so far reported for an Antarctic species (Peck and Conway 2000).

The upper lethal temperatures defined here indicate short-term survival limits, in terms of days, and animals may be able to survive shorter-term exposures to higher temperatures, although the value of such observations is questionable. Critical temperatures define the long-term survival limits for a species. Within this envelope, there will be functional and ecological limits, which will define more closely the temperature regimes and environments a given species can colonize. Thus, if the difference between standard metabolism at $0^\circ\text{C}$ and maximum metabolism seen here at $9^\circ\text{C}$ can be taken as an indication of maximum possible metabolic elevation, $L. \text{elliptica}$ has a potential metabolic scope of 3.3 times values at $0^\circ\text{C}$, which would equate to an actual scope of around 45 $\mu\text{mol O}_2$ animal$^{-1}$ h$^{-1}$ for a standard 2-g tissue AFDM animal. From Figure 2, it is clear that this potential scope would be reduced to 35–40 $\mu\text{mol}.

Figure 6. Succinate contents (\(\mu\text{mol g}^{-1}\) wet weight) of $L. \text{elliptica}$ mantle and siphon tissues at temperatures between $0^\circ$ and $10.5^\circ\text{C}$. Values in the siphon were elevated at $3^\circ\text{C}$ and were elevated in both tissues at $6^\circ\text{C}$. The upper lethal temperatures defined here indicate short-term survival limits, in terms of days, and animals may be able to survive shorter-term exposures to higher temperatures, although the value of such observations is questionable. Critical temperatures define the long-term survival limits for a species. Within this envelope, there will be functional and ecological limits, which will define more closely the temperature regimes and environments a given species can colonize. Thus, if the difference between standard metabolism at $0^\circ\text{C}$ and maximum metabolism seen here at $9^\circ\text{C}$ can be taken as an indication of maximum possible metabolic elevation, $L. \text{elliptica}$ has a potential metabolic scope of 3.3 times values at $0^\circ\text{C}$, which would equate to an actual scope of around 45 $\mu\text{mol O}_2$ animal$^{-1}$ h$^{-1}$ for a standard 2-g tissue AFDM animal. From Figure 2, it is clear that this potential scope would be reduced to 35–40 $\mu\text{mol}. 
for polar ectotherms are small, usually between 5
and 10°C. The physiological temperature envelopes
for the species. Such limits will be within the envelope set by
adequate to support either a critical function, or a combination
of functions when temperature rises, the remaining aerobic scope will be insufficient
support either a critical function, or a combination
of functions, and this will define the functional limit
for the species. Such limits will be within the envelope set by
critical temperatures. The physiological temperature envelopes
for polar ectotherms are small, usually between 5°
and 10°C (Somero and DeVries 1967; Peck and Conway 2000), and the
species living there are termed “stenothermal.” Clearly, their
functional survival windows are even smaller, making them
highly susceptible in the face of environmental change.

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manuscript.

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**Table 4: Oxygen consumption rates (M_o) for Antarctic bivalve molluscs**

<table>
<thead>
<tr>
<th>Species</th>
<th>M_o</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Cyclocardia astartoides</em></td>
<td>1.87</td>
<td>Peck and Conway 2000</td>
</tr>
<tr>
<td><em>Gaimardia trapesina</em></td>
<td>9.07</td>
<td>Ralph and Maxwell 1977b</td>
</tr>
<tr>
<td><em>Laternula elliptica</em></td>
<td>6.44</td>
<td>Ahn and Shim 1998</td>
</tr>
<tr>
<td><em>L. elliptica</em></td>
<td>10.70</td>
<td>Peck and Conway 2000</td>
</tr>
<tr>
<td><em>L. elliptica</em></td>
<td>3.05</td>
<td>This study</td>
</tr>
<tr>
<td><em>Limopsis marionensis</em></td>
<td>4.56</td>
<td>Pörtner et al. 1999b</td>
</tr>
<tr>
<td><em>Yoldia eightsii</em></td>
<td>4.46</td>
<td>Davenport 1988</td>
</tr>
</tbody>
</table>

Gastropod molluscs:

<table>
<thead>
<tr>
<th>Species</th>
<th>M_o</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Nacella concinna</em></td>
<td>6.86</td>
<td>Houlihan and Allan 1982</td>
</tr>
<tr>
<td><em>N. concinna</em></td>
<td>2.09</td>
<td>Peck 1989</td>
</tr>
<tr>
<td><em>Pellilitorina setosa</em></td>
<td>7.89</td>
<td>Houlihan and Allan 1982</td>
</tr>
<tr>
<td><em>Trophon sp.</em></td>
<td>5.23</td>
<td>Houlihan and Allan 1982</td>
</tr>
</tbody>
</table>

Brachiopods:

<table>
<thead>
<tr>
<th>Species</th>
<th>M_o</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Liothyrella uva</em></td>
<td>1.37</td>
<td>Peck et al. 1987</td>
</tr>
<tr>
<td><em>L. uva</em></td>
<td>1.80</td>
<td>Peck 1989</td>
</tr>
</tbody>
</table>

Note. M_o = μmol O_2 g~1~ dry tissue mass h~1~. Values shown are for measures
made at temperatures between 0° and 1°C. Where conversions have been made
from data expressed as tissue wet mass or AFDM, the values obtained for those
measures in this study have been used.

O_2 animal~1~ h~1~ at 3°C and 25–30 μmol O_2 animal~1~ h~1~ at
6°C. Aerobic capacity is required for work. At some stage as
temperature rises, the remaining aerobic scope will be insufficient
to support either a critical function, or a combination
of critical functions, and this will define the functional limit
for the species. Such limits will be within the envelope set by
critical temperatures. The physiological temperature envelopes
for polar ectotherms are small, usually between 5° and 10°C
(Somero and DeVries 1967; Peck and Conway 2000), and the
species living there are termed “stenothermal.” Clearly, their
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Critical Temperatures and Oxygen Supply in an Antarctic Bivalve


Sipunculus nudus


