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Geographical variation in thermal tolerance within Southern Ocean marine ectotherms.

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Short title: geographic thermal tolerance

Abstract

Latitudinal comparisons of the Southern Ocean limpet, *Nacella concinna*, and clam, *Laternula elliptica*, acclimated to 0.0°C, were used to assess differences in thermal response to two regimes, 0.0, 5.1 to 10.0°C and 2.5, 7.5 to 12.5°C, raised at 5.0°C per week. At each temperature, tissue energy status was measured through a combination of O_2 consumption, intracellular pH, cCO_2, citrate synthase (CS) activity, organic acids (succinate, acetate, propionate), andelylates (ATP, ADP, AMP, ITP, PLA (phospho-L-arginine)) and heart rate. *L. elliptica* from Signy (60ºS) and Rothera (67ºS) had the same lethal limits (7.5-10.0ºC), but small yet consistent indicators (O2, CS activity, PLA, ATP, organic acids and cCO_2) suggest that Rothera *L. elliptica* had lower critical and *pejus* (=getting worse) limits than Signy *L. elliptica*. *N. concinna*, which experiences a wider thermal regime, had higher lethal limits (10.0-12.5ºC). However, at their Northern geographic limit *N. concinna* living in a warmer environment (South Georgia, 54ºS), had a lower critical limit (5.1-10.0ºC; O2, PLA and organic acids) than Rothera and Signy *N. concinna* (10.0-12.5ºC). This lower limit indicates that South Georgia *N. concinna* have different biochemical responses to temperatures close to their thermal limit, which may make them more vulnerable to future warming trends.

Key words: *Nacella concinna*; *Leternula elliptica*; Southern Ocean; tissue biochemistry; *pejus* limits; critical limits; lethal limits; latitudinal comparisons;

1. Introduction

Applying the principles of symmorphosis (Weibel, 2000) to the oxygen supply cascade provides a conceptual framework to explain how temperature affects oxygen availability to tissues, and hence tissue energy status. This has developed towards a unifying principle that the physiological thermal limits of aquatic ectotherms are defined by oxygen availability, the balance between supply and demand (Pörtner, 2002; Pörtner, 2006; Peck et al., 2007). Within their optimal range ectotherms are expected to have the physiological flexibility to cope with normally experienced seasonal variation and tissue energy status should be maximal. However, in the face of extreme conditions, or towards range limits, aerobic scope is reduced, as more of the available energy is required to cope with routine metabolic processes and oxygen supply mechanisms become sub-optimal. There is growing evidence that the effects of thermal stress act hierarchically, from high to low complexity, i.e. whole body processes are affected before biochemical reactions and cells (Pörtner, 2002; Pörtner and Knust, 2007; Pörtner et al., 2007). Improving our understanding of how functionality is lost and energy is partitioned between processes will improve our understanding of the likely impact of climate change on species distributions and resulting ecosystem shifts.

Thermal sensitivity can be assessed at a number of levels from whole animal activity through to tissue energy status and finally mortality. Characterising changes in tissue biochemistry has been developed as a test for *critical* thermal limits in aquatic ectotherms which indicate the transition to anaerobic metabolism (*cf* Pörtner, 2002). Within the window bounded by critical temperatures, an even narrower window is bordered by *pejus* limits (pejus: getting worse) which indicate when temperatures move beyond the optimal range and functionality starts to be lost, which is paralleled by a shortfall in oxygen supply and demand until critical limits are reached and anaerobic metabolic pathways are recruited. Species tested this way include the boreal freshwater gadoid, *Lota lota* (Hardewig et al., 2004), the worms, *Sipunculus nudus* (Zielinski and Pörtner, 1996), several populations of *Arenicola marina* (Sommer et al., 1997), and several Antarctic species (e.g. *Limopsis marionensis*; Pörtner et al., 1999) including both *L. elliptica* (Peck et al., 2002) and *N. concinna* (Pörtner et al., 1999). Biochemical parameters were

selected that measure the integrity of cellular systems and identify the temperature at which there is a departure from homeostasis (pHi and cCO_2), with the main focus on those that measure the aerobic status of the animal, circulation (heart rate), aerobic metabolism (O_2 consumption and citrate synthase activity), tissue energy status (balance of adenylates) and recruitment of anaerobic pathways (build up of organic acids). We aim to utilise these techniques to compare *pejus* limits, *critical* and *lethal* limits of *N. concinna* and *L. elliptica* from different locations within the Antarctic. These measures will be used to look for adaptive differences between locations that relate to experienced thermal environment.

We exploit access to a unique latitudinal gradient within the Southern Ocean, Rothera (67°S), Signy (60°S) and South Georgia, (54°S) which enable us to separate latitude and temperature (Barnes et al. 2006). Our highest latitude site at Rothera Point, Adelaide Island, has an annual seawater temperature ranging from –2°C in winter to +1°C in the height of summer (at 15m depth; Barnes et al., 2006). Signy Island, within the South Orkney Islands (60°C) is on the edge of the Weddell Sea gyre and despite its more northerly location has the same annual seawater temperature range to that of Rothera (Clarke and Leakey, 1996). However the sub-Antarctic Island of South Georgia (54°S), although still within the Southern Ocean (south of the Antarctic circum-polar current) and therefore the biological Antarctic (Barnes et al., 2005), has almost twice the annual range due to a higher summer maximum (–1 to +4°C at 10-20m; Barnes et al., 2006). In response to the constant cold at Rothera benthic marine ectotherms are generally stenothermal, they have a limited temperature range, upper lethal limits typically occurring between 5 and 10ºC (Peck, 2005) but for some species are considerably lower than these, e.g. the brittle star *Ophionotus victoriae* which cannot survive even 1 month at 2°C (Peck et al, *in press*). Furthermore, limits for critical activities are often considerably lower than the lethal limits (2 to 4ºC; Peck et al., 2004). The +4°C maximum summer seawater temperature at South Georgia therefore has the potential to impact these species, unless they have adaptive capacity to cope with this thermal range. It is interesting to note that the range of *O. victoriae* does not stretch as far as South Georgia.

Symmorphosis (Weibel, 2000) and the climate variability hypothesis (Stevens, 1989) both predict that species, which experience a wider range of temperature, will have greater thermal flexibility. From this, sub-littoral species living at South Georgia should be able to cope with higher temperatures than the same species from Rothera. Habitat temperatures would also

predict that the thermal response of individuals from Signy would be closer to those from Rothera than South Georgia. Although littoral species will experience a much higher range of temperatures during low water emersion, habitat temperatures still predict the warmest environment at South Georgia. The highest recorded littoral temperature is 12.3°C at Rothera (Waller et al., 2006), 10.7°C at Signy (Barnes et al., 1996) and 15.8°C at South Georgia (Davenport, 1997). The littoral limpet, *Nacella concinna* (Strebel 1908), would therefore be expected to have a higher thermal capacity than the sub-littoral clam, *Laternula elliptica* (King and Broderip 1831) and higher thermal limits in *N. concinna* and *L. elliptica* would be predicted from South Georgia, than Signy and Rothera. However, species living at their range limit, where they experience temperatures close to their maxima may have a reduced ability to cope with further temperature increases (Tomanek, 2005; Deutsch et al., 2008); adaptations that enable ectotherms to live in warmer environments may occur at the expense of further acclimatory capacity (Stillman, 2003).

Through sampling these two species from 3 locations within the Southern Ocean, at the same time of year, we aim to conduct the first latitudinal comparison of thermal tolerance within the Southern Ocean. Environmental history determines thermal response (e.g.. Osovitz and Hofmann, 2005) and so all animals were acclimated to the same temperature (0.0°C) and maintained under as near identical conditions as possible for 2 months before thermal responses were tested. Any differences in thermal response should therefore indicate adaptive differences between locations.

2. Materials and Methods

2.1. Collection and culture

Limpets, *Nacella concinna* and the soft shelled clam, *Laternula elliptica* were collected during cruise JR109 on the RRS James Clark Ross and concurrently at Rothera, the British Antarctic Survey Research Station at Adeleide Island on Antarctic Peninsula, during early summer as detailed in table 1. Except for *N. concinna* from Signy, which were collected from the lowest part of the intertidal, all animals were collected by SCUBA divers.

After collection all animals were maintained in recirculating aquaria at 0.0±0.3°C, for 63 days until the start of experiments. Water quality was maintained through biological filtration, U.V. sterilisation, protein skimmers and daily water changes. During this period animals were

not fed to reduce any metabolic increment due to feeding (SDA), which can last up to 25 days in Antarctic benthic ectotherms (Peck, 1998). Nonetheless, *N. concinna* were observed grazing on biofilms on the tank walls and *L. elliptica* were seen with siphons open, so both species are likely to have continued low level feeding.

At the start of incubation experiments animals were transferred to two 200 litre jacketed acrylic tanks (Engineering Design and Plastics, Cambridge, Cambs.) attached to LTD20G thermocirculators (Grant instruments Ltd, Shepreth, Cambs.), which maintained temperatures to an accuracy of ±0.2°C. The method was similar to that that used by Morley et al. (2007) and Peck et al. (2002; 2004; 2007 and 2008). Animals were allowed 48 hours to recover from movement stress before temperatures were altered or measurements taken. Temperature was then raised at approximately 0.1°C per hour until the required temperatures were reached and then animals were allowed 48 hours to recover from any metabolic overshoot (Peck et al., 2002). Due to time constraints animals were separated into two groups, which were taken through different 5.0°C temperature steps, tank 1) 0.0, 5.1 and 10.0°C, tank 2) 2.5, 7.5 and 12.5°C. Following this protocol animals were tested over approximately 3 weeks in each tank.

2.2. Oxygen consumption

Routine metabolic rate was measured in closed cell respirometers, following the methodology of Peck (1989), except that oxygen concentration was measured with a Fibox-3 oxygen meter (Presens GmbH, Regensberg, Germany; e.g. Morley et al., 2007). Oxygen sensitive foils were calibrated before each measurement using 5% w/w sodium diothionite for 0% and fully aerated water for 100%. During trials oxygen concentration was not allowed to fall below 70% of air saturation, which is above the threshold for oxy-regulation for *L. elliptica* (Peck et al., 2002). Two empty chambers (controls) were run with each trial to account for background oxygen consumption, which was routinely less than 10% of the animal's consumption. The basal metabolic rate of 4-5 individuals was measured for three consecutive days after which data were analysed for significant differences in oxygen consumption between days. When significant differences were found, daily measurements continued until three stable readings were obtained and then temperature was raised. The volume of each animal was measured through displacement and subtracted from the volume of water in the respirometer. At the end of the experiment dry weight (constant weights at 60°C) and ash free dry weight (dry

156 weight minus the weight of ash remaining after ignition at 475°C for 24h) of each whole animal
157 was measured.
158 At each temperature the circulatory system of *L. elliptica* was monitored with the
159 impedance-based heart rate monitoring system (Buchan et al., 1988). Impedance systems record
160 the frequency of heart rate through variations in resistance between two electrodes. Methods
161 used were similar to those of Peck et al. (2002). Two 1-mm holes were drilled through the shell
162 either side of the heart, and 2mm of tinned 18 standard gauge copper wire electrodes, with the
163 coating removed from the tips, were inserted and glued in place with cyanocrylate gel adhesive.
164 4 Signy and 4 Rothera *L. elliptica* had electrodes implanted in each of the two temperature tanks.
165 At the end of each temperature step the foot muscle from 4 animals was freeze clamped
166 in liquid nitrogen and stored at -80°C for biochemical analysis at the Alfred Wegener Institute in
167 Germany (except South Georgia and Signy *N. concinna* at 10°C for which only 3 animals were
168 available). Foot muscle was the chosen tissue as it performs a similar function in both *N.*
169 *concinna* and *L. elliptica*. Tissue was ground in a pestle and mortar, under liquid nitrogen, and a
170 number of extracts produced for biochemical analysis.
171 Temperature steps continued until 50% mortality occurred in the tanks. A further set of
172 20 *N. concinna* and *L. elliptica* from each location was maintained in the flow through aquarium,
173 at ambient temperature, for the duration of the experiment, with no mortalities.
174
175 *2.3. Biochemical analysis*
176 Intracellular pH (pH_i) and cCO_2 could only be measured in tissues of *L. elliptica* as *N.*
177 *concinna* foot muscle has large calcium carbonate stores, which would interfere with
178 homogenate acidity (Pörtner et al., 1999). Extracts were prepared in a media containing
179 160mmol.l^{-1} potassium fluoride and 1.0 mmol.l^{-1} nitrilotriacetic acid following the methods of
180 Pörtner et al. (1990). pH_i was measured in the supernatant of the centrifuged homogenates at
181 animal incubation temperature, using needle type fibre optic pH sensors attached to a pH1 micro
182 pH meter (Presens GmbH. Regensburg, Germany) whose analogue output signal was boosted
183 and digitised through a Powerlab system (AD instruments). Due to the high sensitivity of the
184 optode system to solution ionic strength, KCl was added to each calibration buffer to match *L.*
185 *elliptica* tissue ionic strength of 0.16mol L^{-1}. Due to the small change in tissue pH, calibration
186 was conducted using three buffers covering the pH range 6.865 to 7.413 (at 25°C) whose pH

change with temperature had already been calculated. pH sensors were re-calibrated using buffers cooled to each incubation temperature.

To measure cCO_2 100µl of this supernatant extract was injected into a 10ml sealed sample vial, containing 1.5ml of nitrogen gassed HCl (0.05M). The sample was rotated for 10 minutes and then 50µl of this gas phase was injected into an Agilent technologies 6890N Network gas chromatogram through a G1888 network headspace sampler. cCO_2 was measured with a HP-PLOTQ column and a TCD detector. Triplicate peak areas were converted to cCO_2 by plotting a calibration curve for standard samples between 0.5 mM and 10.0 mM dissolved inorganic carbon (r^2 =0.998).

Tissue citrate synthase activity was measured from tissue extract at a ratio of 1g of tissue to 9ml of extraction buffer, following the methods of Sidell et al. (1987). Enzyme activity was measured at animal incubation temperature with a Beckman UV-DU 7400 spectrophometer fitted with a temperature controlled cuvette holder and diode array detector.

To measure both the build up of the end products of anaerobic metabolism (organic acids) and tissue energy status (the adenylates) tissue homogenate was prepared using 0.6mol.l^{-1} perchloric acid (PCA) (modified from Hardewig et al., 1998). Diluted PCA extracts were injected into an ICS 2000 ion chromatography system fitted with a Gilson Diluter 401 autosampler. Organic acids were separated on an ion exclusion column (Dionex ICE-AS 1) at 60°C fitted with an AMMS-ICE 4mm micro-membrane suppressor cell, with 0.20mM heptafluorobutyric acid (HFBA) as eluent at a flow rate of 1 ml.min^{-1} and 5mM tetrabutylammonium hydroxide (TBAOH) as the regenerant. The signal was detected on a DS6 conductivity detector. Duplicate sample peaks were converted into concentrations using calibration curves with standard solutions, succinate (12 µM to 212 µM, r^2 =0.997) acetate (14µM to 296µM, r^2 =0.997) and propionate (12 µM to 189µM, r^2 = 0.999).

Adenylates were measured on a Beckman PACE/MDQ capillary electropheris system following the adapted methods of Casey et al. (1999). The capillary was uncoated fused silica, 50µm ID, 50 cm long. The pH of homogenates was set to 9.5, diluted in separation buffer (40mM tetraborate buffer and 10mM sodium chloride (pH 9.7)) and 50µl of 4 mM uric acid was added to 200µl of each standard and sample as an internal standard. The absorbance signals of separation were simultaneously detected at different wavelengths with a photo diode array detector: at 254 nm, adenosine, AMP, ATP, ADP and ITP; at 200 nm, arginine and PLA; at 290

nm, internal standard uric acid. Duplicate peaks were converted into concentrations using the following calibration curves: adenosine (71.5 µM to 572 µM, r^2 =0.995), AMP (70.5 µM to 564 µM, r^2 =0.988), ATP (80.4 µM to 643 µM, r^2 =0.995), ADP (71.3 µM to 570 µM, r^2 =0.966), ITP (70.2 µM to 561 µM, r^2 =0.992), arginine (82.0 µM to 656 µM, r^2 =0.986) and PLA (68.8 µM to 550 µM, r^2 =0.996).

2.4. Analysis

Conducting the experiment in two tanks simultaneously created a mixed design where, within tanks, some individuals were sampled more than once (e.g. for oxygen consumption), but different individuals were sampled between tanks. As there was a small, non significant, effect of individual (as a random factor in a GLM analysis) a simple analyses was conducted, ignoring repeated observations on some individuals (P. Rothery, pers. coms.). ANOVAS with Tukey post-hoc tests were used to test for differences between locations and temperatures for each species. Where ANOVAS indicated only significant differences between locations a separate analysis was conducted for each location. Signficant differences was accepted as $P<0.05$ throughout. Oxygen consumption was standardised to that of a 0.373g dry mass *N. concinna* and a 10.6g dry mass *L. elliptica* (the mean mass of animal used) using scaling coefficients of 0.82 (Fraser et al., 2002) and 0.73 (Peck et al., 2002) respectively.

3. Results

3.1. Animal size

The shell length of *L. elliptica* sampled for biochemistry was not significantly different between locations, table 2. However, due to logistic constraints on collections by SCUBA divers, limpets from Rothera were smaller than those from Signy and South Georgia. *N. concinna* collected inter-tidally from Signy were not significantly different in size than *N. concinna* collected sub-tidally from South Georgia.

3.2. L. elliptica

More than 50% of *L. elliptica* died during the transition from 5.0 to 10°C and from 7.5 to 12.5°C so the experiment was stopped at this stage and the upper thermal limit recorded as between 7.5 and 10.0°C. Although the sample size was too small to detect significant effects,

heart rate increased with temperature between 0.0 and 5.1°C. 50% of animals with implanted impedance wires died at 7.5°C so heart rate was not measured at this temperature.

Temperature had a significant effect on every biochemical parameter, there were a few significant differences between Rothera and Signy *L. elliptica* (Table 3) and some consistent patterns that indicate differences between *L. elliptica* from the two locations. Although generally not significant, at 0.0°C, Rothera *L. elliptica* had higher oxygen consumption, CS activity and higher concentrations of high energy phosphates (PLA, ITP and ATP) than Signy *L. elliptica* suggesting an overall higher metabolism at 0.0°C. Between 0.0 and 2.5ºC there was also a, non-significant, but consistently greater magnitude change in PLA and ATP (reduction), cCO_2 (increase) and organic acids (increase of succinate and acetate) in Rothera than Signy *L. elliptica* (Fig 1, Table 3).

The oxygen consumption of *L. elliptica* rose between 0.0 and 2.5°C for specimens from both sites, but no oxygen consumption was significantly different from that at 0.0°C. There was, however, a significant drop in oxygen consumption between 2.5 and 7.5°C for both Signy and Rothera *L. elliptica*. Citrate synthase activity at 2.5, 5.1 and 7.5°C was not significantly different from that at 0.0°C. However, in a similar fashion to oxygen consumption, *L. elliptica* from both locations had a peak in enzyme activity at 2.5°C, which was significantly higher than activity at both 5.1 and 7.5°C.

The energy status of the high energy phosphates fell as the concentration of their low energy forms, arginine, AMP and ADP, increased at 7.5°C in *L. elliptica* from both locations. PLA the buffer that allows rapid rephosphorylation of ADP yielding ATP was also reduced at 7.5ºC. The response of the triphosphates, ITP and ATP, to temperature was slightly different between *L. elliptica* from Signy and Rothera. The ATP levels of Rothera *L. elliptica* were lower at both 5.1 and 7.5°C than at 0.0°C, whilst the ATP levels of Signy *L. elliptica* were only reduced at 7.5°C. The ITP concentration of Rothera *L. elliptica* generally fell upon warming but was only significantly lower than 0.0°C at 7.5°C, whilst the ITP concentration of Signy *L. elliptica* was lower at both 0.0°C and 7.5°C than 5.1°C.

The concentration of all three organic acids, succinate, acetate and propionate was elevated at 2.5°C in Rothera *L. elliptica* and at 5.0°C in both, but was only significantly higher at 7.5°C. There were no significant differences in acetate and propionate accumulation patterns

between locations except that succinate rose to a significantly higher level in Signy than Rothera *L. elliptica* at 7.5°C.

Intracellular pH was significantly higher at 0°C than at 2.5, 5.1 or 7.5°C for *L. elliptica* from both locations. There was also a significant drop in pHi between 5.1 and 7.5°C. Tissue cCO_2 was highly variable and there was only one significantly elevated value, in Rothera *L. elliptica* at 5.1°C.

3.3. N. concinna

More than 50% of *N. concinna* died during the transition from 7.5 to 12.5°C and 10.0 to 15.0°C so the experiment was stopped at this point. There was no significant effect of location or temperature on arginine but there were complex, significant effects on the other adenylates, with higher concentrations of both low (AMP and ADP) and high (ATP) energy adenylates at 10.0°C (Fig. 2). At 10.0°C AMP was significantly higher in Rothera *N. concinna* than all treatments except Signy *N. concinna* at 2.5°C and South Georgia at 10.0°C. At 10.0°C ADP was higher in *N. concinna* from all locations. Although there was no significant difference in ATP concentration from that at 0.0°C, at 10.0°C the concentration of ATP was higher at all locations than at 2.5°C. However, despite this significant increase in ATP for all locations, the increase for South Georgia *N. concinna* appeared to be less (Fig. 2). The PLA concentration of South Georgia *N. concinna* was also lower at 10.0°C than at either 0.0 or 5.1°C.

Despite a general increase in the organic acids with increasing temperature, succinate, acetate and proprionate were only significantly elevated in South Georgia *N. concinna* at 10.0°C (Fig.3). The large inter-individual variation in organic acids at 10°C is typical of biochemical responses around physiological limits as some individuals reach their limits before others. For *N. concinna* from all locations, citrate synthase activity determined in tissue samples collected at each temperature generally increased upon warming but was only significantly higher at 7.5 and 10.0°C (Fig.4).

Oxygen consumption was very different between *N. concinna* from different locations. Oxygen consumption increased in a linear fashion with temperature (T) for Rothera (O_2cons = 0.14T +1.58, R^2=0.27, $p<0.05$, F=6.8) and Signy (O_2cons = 0.10T +0.92, R^2=0.57, $p<0.01$, F=22.9) *N. concinna* (Fig 4). Oxygen consumption for South Georgia *N. concinna* reached a similar maximum value but was best described by a curvilinear relationship (O_2cons = 0.64T –

310 $0.06T^2 + 0.76$, R^2=0.45, p<0.01, F=6.6). The calculated maximum (the point of inflexion of the
311 differential equation) occurred at 5.3ºC, but the highest oxygen consumption was recorded at
312 2.5ºC (Fig. 4).
313
314 **4. Discussion**
315 Measuring a suite of physiological and biochemical parameters gave a comprehensive
316 picture of how tissue energy status changed in response to a 5.0°C per week temperature
317 elevation. It also allowed comparisons of the responses of *L. elliptica*, and *N. concinna*, collected
318 from different geographic locations. As all animals were collected at the same time, were held
319 under nearly identical conditions for approximately 2 months before thermal sensitivity was
320 tested and they were tested over a maximum of 3 weeks, any consistent differences between
321 locations are most likely due differences in response between animals from these locations.
322
323 *4.1. L. elliptica*
324 The critical limit, which was confirmed by a drop in oxygen consumption, a drop in
325 energy status of the adenylates, an increase in anaerobic end products of metabolism (the organic
326 acids) and a drop in intracellular pH, occurred between 5.1 and 7.5°C, which is comparable to
327 that measured previously, over a similar rate of temperature change (6°C; Peck et al., 2002).
328 Anaerobic metabolism is generally considered one of the protective mechanisms that allow time-
329 limited survival above *critical* temperatures (Pörtner, 2006). However, molluscs in particular can
330 have high anaerobic capacities that can be utilised to survive hypoxia for days or weeks,
331 particularly when accompanied by metabolic depression (e.g. Holmes and Miller, 2006; Morley
332 et al., 2007; Long et al., 2008). Reductions in citrate synthase activity and metabolic rate suggest
333 that thermal limitation may have started at a lower temperature, with *pejus* limits below 5.1°C.
334 Succinate and volatile fatty acid formation set in somewhat earlier in Rothera than the Signy *L.*
335 *elliptica*. This would indicate that, as a consequence of severe oxygen limitation, the critical
336 temperature is lower in the Rothera than the Signy population. The greater, but non-significant,
337 biochemical changes between 0.0 and 2.5 ºC for Rothera *L. elliptica*, coupled with significant
338 accumulation of carbon dioxide in the haemolymph, which shows that Rothera *L. elliptica* have a
339 lower capacity for gas exchange at 5.0 ºC (cf Peck et al., 2002), also suggest that *pejus* limits are
340 lower in Rothera than Signy *L. elliptica*. After 2 months at 0.0 ºC, there were also signs of

differences in cold acclimation of; CS activity, oxygen consumption and the turnover of high energy phosphates were consistently (but not significantly) higher in Rothera than Signy *L. elliptica* at 0.0 ºC.

The lethal limit in the current study also occurred at a similar temperature, between 7.5 and 10.0°C (within 15 days), to that recorded previously (9°C within 25 days; Peck et al., 2002). The similarity of lethal limits between Rothera and Signy *L. elliptica*, is consistent with the hypothesis that thermal capacities will be matched to the experienced environment (both -2 to +1°C), although the lower *pejus* limits of Rothera *L. elliptica* compared to Signy indicate subtle differences in thermal tolerance between locations. Clearly, studies incorporating *L. elliptica* from South Georgia, which is at the northern limit of its range, are needed to quantify how close it is living to its upper thermal limits and therefore with reduced capacity to cope with further temperature increases (Stillman, 2003). Such studies are crucial to our understanding of species wide thermal capacity and therefore the ability of species to cope with environmental change. Acclimations of *L. elliptica* at 3-4°C would indicate if South Georgia *L. elliptica* have adaptations to cope with higher maximum experienced summer temperature.

4.2. N. concinna

Due to the constraints on geographical sampling within the Southern Ocean, there were sampling differences between *N. concinna* collected at different locations. *N. concinna* were collected from the bottom of the inter-tidal from Signy, compared to sub-tidal animals collected from South Georgia and Rothera, however, all *N. concinna* were collected from depths shallower than 6m. A recent study found small, non-consistent, genetic differences between inter-tidal and 15m *N. concinna* from Potter Cove, King George Island, but not between inter-tidal and 6m *N. concinna* (de Aranzamendi et al., 2008). Different biochemical responses to emersion were also evident between 15m and intertidal *N. concinna*, also from King George Island (Weihe and Abele, *In Press*) was also evident This suggests that comparisons of 6m (Rothera), 2-3m (South Georgia) and inter-tidal (Signy) *N. concinna* will not be affected by population differences between animals collected from different depths. *N. concinna* collected in the middle of summer and tested within days of collection had no significant ($t=0.57$, $p=0.57$) differences in upper lethal thermal limits (1°C per day) between those collected in the inter-tidal (11.6°C) or sub-tidal (6m, 10.8°C; SAM, unpublished data). The lack of any clear differences between Rothera

372 sub-tidal and Signy inter-tidal *N. concinna*, in the present study suggests that they respond
373 similarly to temperature, after a 2 month acclimation to 0.0°C.
374 Rothera *N. concinna* were also smaller than those from the other two locations, which
375 could introduce complicating factors due to growth and age differences as well as wider thermal
376 windows of small specimens (e.g. Pörtner 2002; Peck et al., 2007; Gsottbauer et al., 2007).
377 However, broad scale metabolic differences (see below), in the response to temperature, were
378 found between South Georgia and both Signy and Rothera *N. concinna*, which are unlikely to be
379 caused by size differences. Accepting these caveats, the present geographic comparisons within
380 the Southern Ocean, show for the first time specific responses of the adenylates, organic acids
381 and oxygen consumption to temperature, which suggest differences in critical limits and the
382 metabolic mechanisms employed to cope with acute temperatures close to these limits. There
383 was little evidence to suggest that Rothera or Signy *N. concinna* were temperature limited over
384 the range 0.0 to 10.0°C with measures of aerobic metabolism (oxygen consumption, citrate
385 synthase activity and ATP concentration) all increasing with temperature above 2.5°C. This
386 contrasts strongly with the pattern in *L. elliptica* which didn't show a reduction in these metrics
387 at temperatures above 2.5°C. This inicates they were suffering heat stress and have a narrower
388 thermal window than *N. concinna*.
389 In South Georgia *N. concinna*, citrate synthase activity and therefore the capacity of
390 aerobic metabolism in the mitochondria, increased with temperature in a similar fashion to
391 limpets from the other locations. However, in contrast to Rothera and Signy *N.* concinna, two
392 indicators of metabolism decreased in South Georgia *N. concinna* at higher temperatures.
393 Oxygen consumption peaked somewhere between 2.5 and 5.3°C and then declined whilst the
394 PLA pool, for buffering rapid ATP production, also declined above 5.1°C. Both of these suggest
395 a lower critical limit between 5.1 and 10.0°C (but perhaps as low as 2.5°C) for South Georgia *N.*
396 *concinna*, which was supported by the sharp increase in organic acids between 7.5 and 10.0°C
397 and therefore the recruitment of anaerobic pathways.
398 The wider thermal tolerance of *N. concinna* compared to *L. elliptica* is not surprising
399 considering it ranges into the intertidal and can therefore be exposed to a more variable thermal
400 environment. However, the difference in maximum littoral temperatures recorded so far, 12.3°C
401 at Rothera (Waller et al., 2006) 10.7°C at Signy (Barnes et al., submitted) and 15.8°C at South
402 Georgia (Davenport, 1997) was not reflected in any difference in the upper lethal limits for *N.*

concinna from different locations after 2 months acclimation to 0.0°C (10.0 to 12.5°C). Davenport reported a short term survival limit of 15.6°C (12 hour exposure) for *N. concinna* at South Georgia, higher than our medium term, 22 day, temperature limit of between 10.0 and 12.5°C. This is not unexpected as different thermal limits and protective mechanisms might be expected if different rates of heating or cooling are used (Terblanche et al., 2007; Barnes et al., 2008; Peck et al., *in press*). This difference may also have been because *N. concinna* tested by Davenport were acclimatised to summer conditions which could have raised their lethal limit above that of animals acclimated to 0.0°C. However, it should also be noted that due to behavioural adaptations, such as utilisation of cryptic habitats and evaporative cooling (Branch, 1981), body temperatures of limpets in the field are unlikely to reach these temperatures. The maximum foot temperature of a Rothera *N. concinna* exposed on a sunny day has been shown to be 8.8°C, although the average was much lower, 3.3 to 3.8°C, ranging from 1.5 to 9.1°C below air temperature (Clark et al., 2008). If this relationship holds for South Georgia *N. concinna* then foot temperature is unlikely to exceed their short-term (daily exposure) lethal limit during low water emersion but might approach their medium term (weekly exposure) limit. Although *N. concinna* are regularly exposed in the intertidal zone at Rothera and Signy, at South Georgia, it is mainly a sub-tidal species (Davenport, 1997) and only rarely found above mean low water spring tide level, reducing the risk of exposure to damaging high temperatures. The few inter-tidal individuals found at South Georgia inhabit sheltered microhabitats and not exposed surfaces, which are colonised by a siphonariid limpet, *Kerguelenella lateralis*. At its northern geographic limit differences in habitat utilisation may be key to reducing the likelihood of *N. concinna* being exposed to air temperatures close to its upper limit. They may therefore have evolved a high anaerobic capacity to allow short-term, passive, survival during less frequent exposure (Pörtner, 2002). This fits with a growing body of evidence which shows that adaptation to life at range limits may actually make animals less able to cope with further temperature increases (Stillman, 2003, Tomanek, 2005, Deutsch et al., 2008).

The passive survival strategy of *N. conccina* appears to be effective over several days, but is unlikely to be effective over longer time scales (Pörtner, 2006). After initial shock, animals may switch from acute mechanisms that allow short-term “passive” survival to longer-term acclimatory mechanisms with associated changes in tissue biochemistry and structure. The greater thermal flexibility of *N. concinna* than *L. elliptica* was further evidenced by the increase

in adenylate concentration with temperature which suggests that there was an acclimatory increase of the adenylates over the 2 week duration of the experiment, in an attempt to meet the increase in energy demand with temperature.

Two month long incubations of *N. concinna* from South Georgia, Signy and Rothera, long enough for seasonal acclimation, led to a reduction in the surface area of mitochondrial cristae per unit muscle fibre volume (Morley et al., submitted). Latitudinal comparisons showed that acclimated *N. concinna* from South Georgia had a higher citrate synthase activity than Rothera *N. concinna*, which was negatively correlated with mitochondrial density across locations. This strongly suggested an adaptation for fewer, more efficient mitochondria in acclimated *N. concinna* from the northern limit of their range (Morley et al., submitted). Tissue samples of *N. concinna* acclimated to 3.0°C are currently being analysed to investigate if changes in aerobic pathways were accompanied by a change of anaerobic scope. These differences in the mechanisms underlying, and the limits of, thermal tolerance between *N. concinna* from South Georgia and both Signy and Rothera *N. concinna* are supported by genetic differences, which suggest South Georgia *N. concinna* are a separate population (Beaumont and Wei, 1991).

The Scotia arc region of the Southern Ocean has one of the fastest warming climates on the planet (Meredith and King, 2005). If this rate of warming is faster than the rate of evolutionary adaptation, then the acclimatory ability of individuals, populations and species is likely to be a key determinate of their survival (e.g. Peck, 2008). Species with wider geographic ranges are thought to be more resistant to extinction (Jablonski, 1986), which may in part be due to the wider range of environments they experience. This should in turn lead to wider genetic variation and therefore an increased physiological capacity to cope with environmental perturbations (the climate variability hypothesis; Stevens, 1989). The current study has found differences in thermal response of marine ectotherms between locations on the Scotia Arc and Antarctic Peninsula. Investigations of a wider range of species will confirm if these differences are an adaptive response to differences in experienced environment. Latitudinal comparisons testing for differences in capacity between animals from different locations within the Southern Ocean could be a key tool for predicting the vulnerability of species to the current trend of warming.

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581

582 Table 1. Site details where limpets, *N. concinna* and the soft shelled clam *L. elliptica* were
583 collected during cruise JR109 and at Rothera research station.
584

Species	Site	Depth/m	Co-ordinates	Location
N. concinna	South Cove	6	67°34.25'S, 68°08.00'W	Rothera Point, Adelaide Island
	Borge Bay	0-1	60°42.16'S, 45°35.45'W	Signy, South Orkney Islands
	King Edward Point	2-3	54°17.03'S, 36°39.30'W	South Georgia
L. elliptica	Hangar Cove	10-20	67°33.92'S, 68°07.67'W	Rothera Point, Adelaide Island
	Borge Bay	10-20	60°42.16'S, 45°35.45'W	Signy, South Orkney Islands

585
586

586 Table 2. Shell lengths for limpets, *N. concinna* and the mud clam *L. elliptica* sampled for tissue
587 biochemistry. Mean ± sem (number of individuals in brackets). Within each species shell lengths
588 not sharing a common superscript are significantly different ($p<0.05$).

Species	Location	Shell length/mm
N. concinna	Rothera	27.7±0.7 (20) [a]
	Signy	33.9±0.8 (20) [b]
	South Georgia	33.2±0.7 (20) [b]
L. elliptica	Rothera	74.0±3.9 (16) [c]
	Signy	84.2±3.7 (16) [c]

589
590
591 Table 3 The concentration of selected biochemical measures in *Laternula elliptica* foot muscle
592 as temperature was increased in 5.0°C steps, after 1-week incubation at each temperature. Mean
593 ±sem. Within each parameter, means not sharing a common superscript were significantly
594 different ($p<0.05$).
595

Location	Parameter	Temperature			
		0.0	2.5	5.1	7.5
Rothera	O_2 consumed	73.3±12.1^{ab}	83.4±16.4^{a}	85.4±16.0^{ab}	45.8±3.6^{b}
Signy	O_2 consumed	49.6±4.8^{ab}	94.9±21.7^{a}	64.2±14.3^{ab}	34.2±2.6^{b}
Rothera	Heart rate	4.5±0.4	5.1	6.1	-
Signy	Heart rate	4.1±0.4	5.3±0.9	7.2	-
Rothera	CS activity	0.57±0.05^{ab}	0.65±0.06^{a}	0.40±0.14^{b}	0.44±0.10^{b}
Signy	CS activity	0.38±0.07^{ab}	0.61±0.05^{a}	0.37±0.08^{b}	0.30±0.02^{b}
Rothera	Arginine	3.0±1.2	3.1±0.4	1.5±0.2	8.3±1.2^{a}

Signy	Arginine	1.5±2.6	1.4±0.2	3.1±0.7	7.4±0.6[a]
Rothera	AMP	0.08±0.01	0.07±0.005	0.03±0.006	0.22±0.07[a]
Signy	AMP	0.04±0.007	0.03±0.003	0.07±0.02	0.53±0.22[a]
Rothera	ADP	0.60±0.02	0.53±0.02	0.41±0.02	0.90±0.2[a]
Signy	ADP	0.44±0.03	0.42±0.02	0.56±0.03	0.79±0.07[a]
Rothera	ITP	0.11±0.007[a]	0.10±0.00[ac]	0.090±0.008[ab]	0.067±0.006[bc]
Signy	ITP	0.06±0.01[b]	0.08±0.004[ab]	0.10±0.005[ac]	0.06±0.007[b]
Rothera	Acetate	0.0±0.0	87.0±48	62.1±27.2	1182.7±324[a]
Signy	Acetate	0.0±0.0	0.0±0.0	52.9±15	1251±267[a]
Rothera	Propionate	0.0±0.0	0.0±0.0	0.0±0.0	540.7±141[a]
Signy	Propionate	0.0±0.0	0.0±0.0	0.0±0.0	586±45[a]
Rothera	pHi	7.6±0.02[a]	7.4±0.01[b]	7.5±0.03[b]	7.1±0.03[c]
Signy	pHi	7.6±0.02[a]	7.4±0.02[b]	7.5±0.01[b]	7.0±0.09[c]

Figure Legends

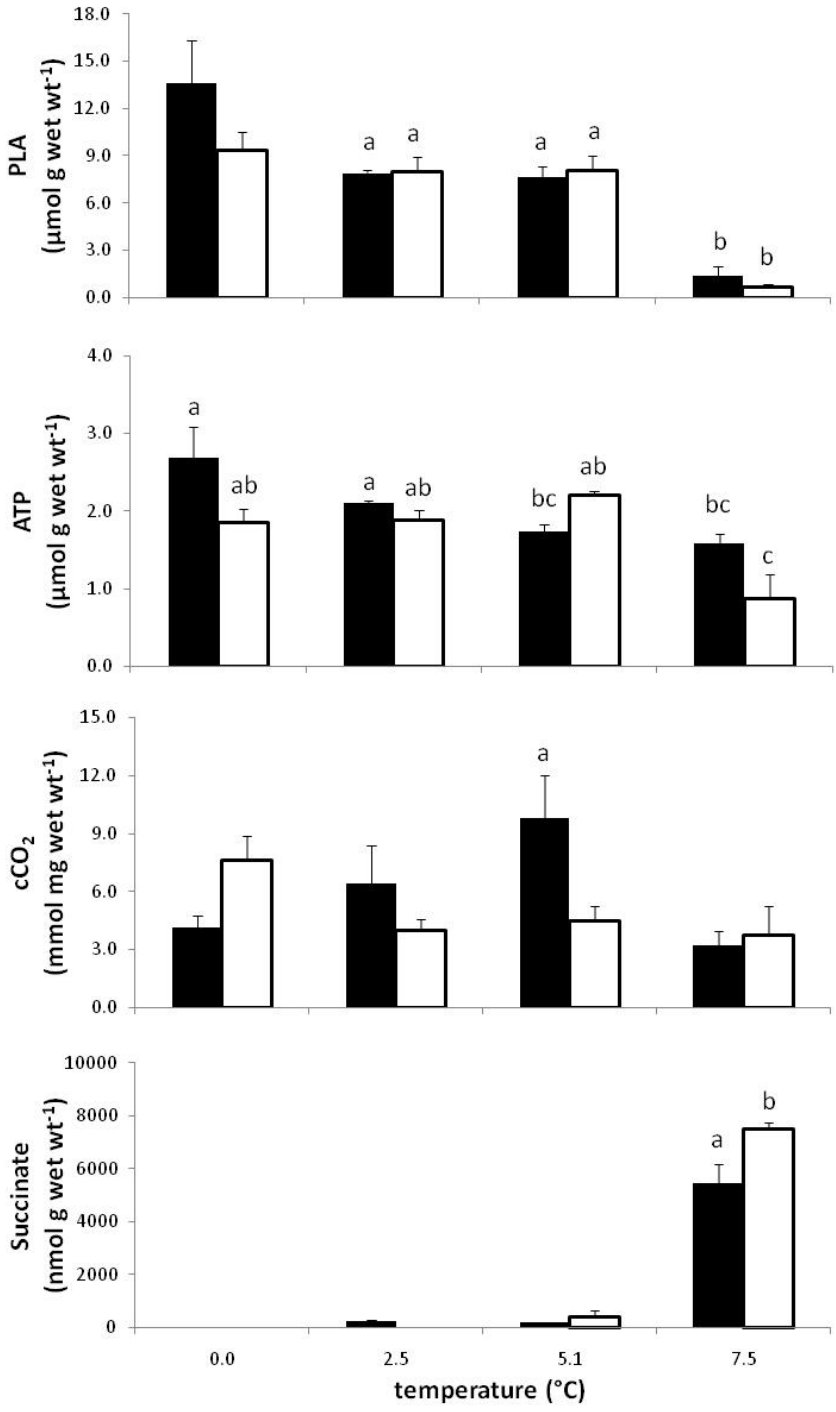
Fig. 1. The concentration of selected biochemical measures in *Laternula elliptica* foot muscle as temperature was increased in 5.0°C steps, after 1-week incubation at each temperature; PLA (phospho-L-arginine), ATP, cCO_2, (tissue carbon dioxide) and succinate. Filled bars, Rothera; Open bars, Signy. Within each parameter, means not sharing a common superscript were significantly different ($p<0.05$).

Fig. 2. The concentration of adenylates in *Nacella concinna* foot muscle as temperature was increased in 5.0°C steps, after 1-week incubation at each temperature. * indicates that AMP concentrations for 10.0°C Rothera *N. concinna* were significantly different from all locations except Signy *N. concinna* at 2.5°C and South Georgia *N. concinna* at 10.0°C ($p<0.05$). a indicates that ADP levels were higher at 10.0°C than at other temperatures. Superscripts a and b indicate that the PLA (phospho-L-arginine) concentration for South Georgia *N. concinna* was lower at 10.0°C than either 0.0 or 5.1°C. Superscript c indicates that ATP concentration was higher at 10.0 than 2.5°C. Mean ±SEM.

Fig 3. The concentration of organic acids in *Nacella concinna* foot muscle as temperature was increased in 5.0°C steps, after 1 week incubation at each temperature. Points with superscript a are significantly different from all other treatments ($p < 0.05$). * indicates that the succinate concentration of South Georgia *N. concinna* at 10.0°C was significantly higher than all treatments except Rothera 7.5°C. Mean±SEM.

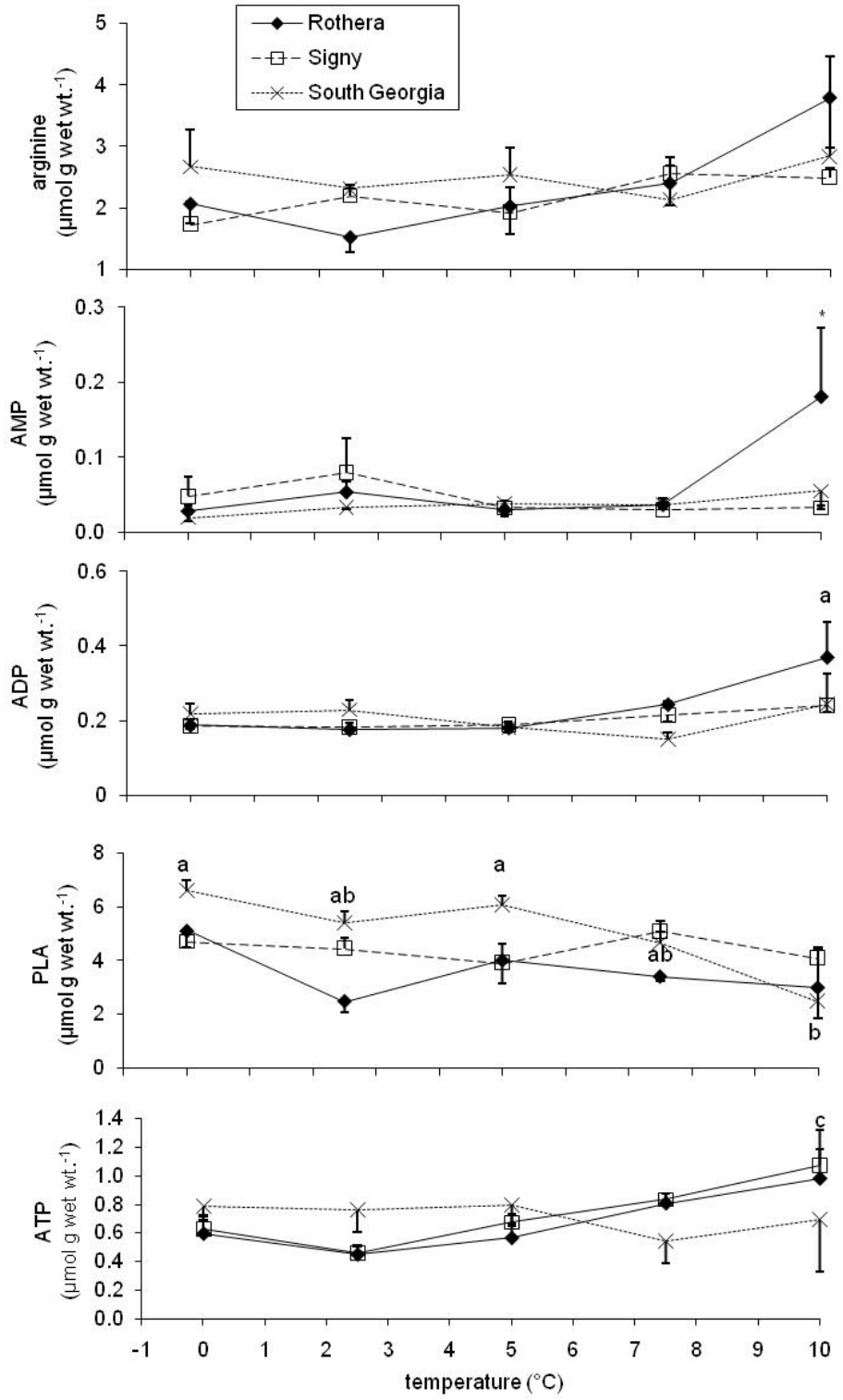
Fig. 4. The CS activity of foot muscle and the oxygen consumption of *Nacella concinna* as temperature were increased in 5.0°C steps, after 1 week incubation at each temperature. Superscripts indicate that for each location CS activity was significantly higher at 7.5 and 10.0°C than at lower temperatures ($p<0.05$). The complex significant differences in oxygen consumption between temperatures and locations are explained in the text. Mean ± SEM.

625 Fig. 1


PLA
(µmol g wet wt-1)
18.0
15.0
12.0
9.0
6.0
3.0
0.0
a a a a b b
ATP
(µmol g wet wt-1)
4.0
3.0
2.0
1.0
0.0
a ab a ab bc ab bc c
cCO2
(mmol mg wet wt-1)
15.0
12.0
9.0
6.0
3.0
0.0
a
Succinate
(nmol g wet wt-1)
10000
8000
6000
4000
2000
0
a b
0.0 2.5 5.1 7.5
temperature (°C)

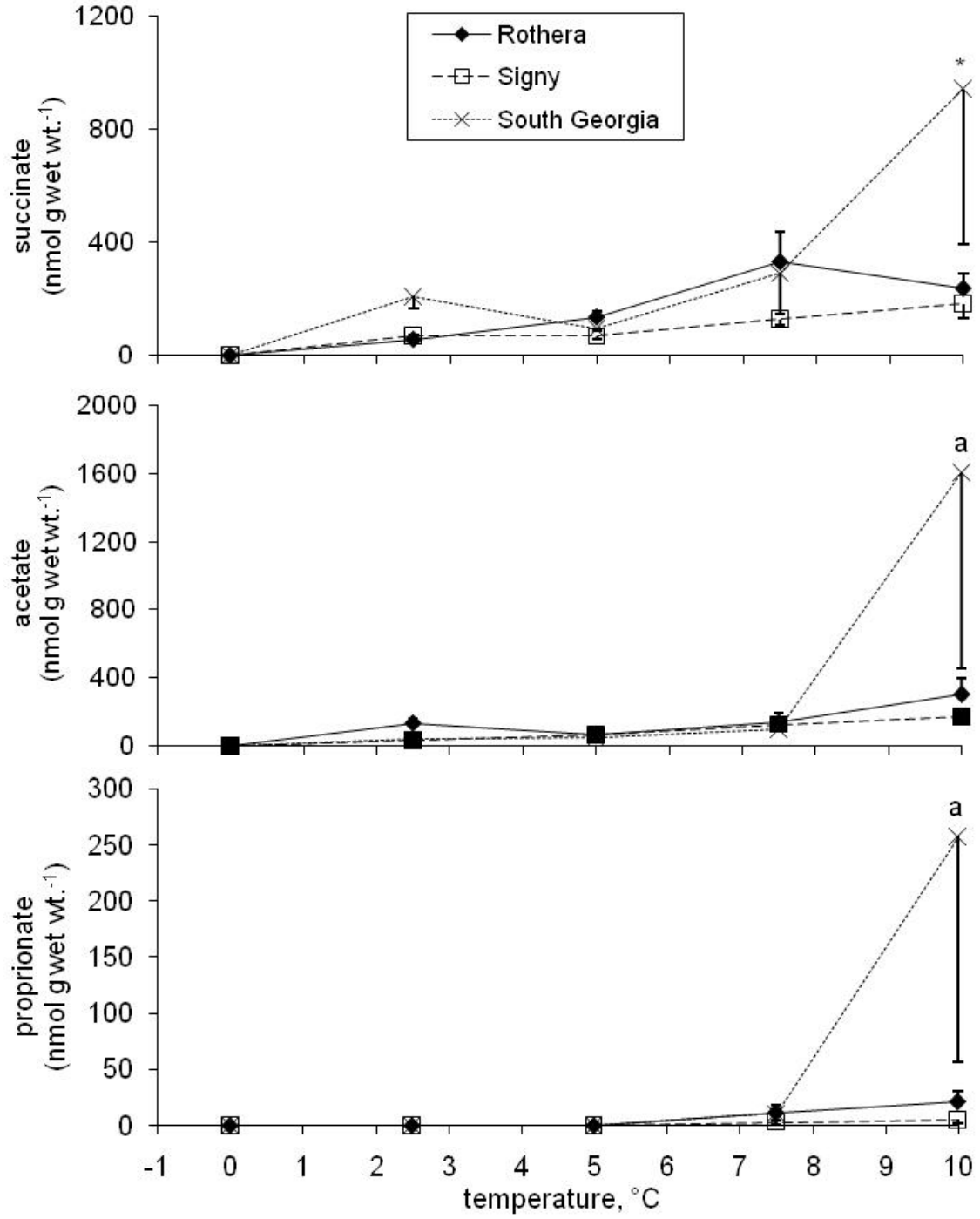
626

627 Fig. 2


Rothera
Signy
South Georgia
arginine (µmol g wet wt.-1)
AMP (µmol g wet wt.-1)
ADP (µmol g wet wt.-1)
PLA (µmol g wet wt.-1)
ATP (µmol g wet wt.-1)
a
ab
a
ab
b
c
*
temperature (°C)

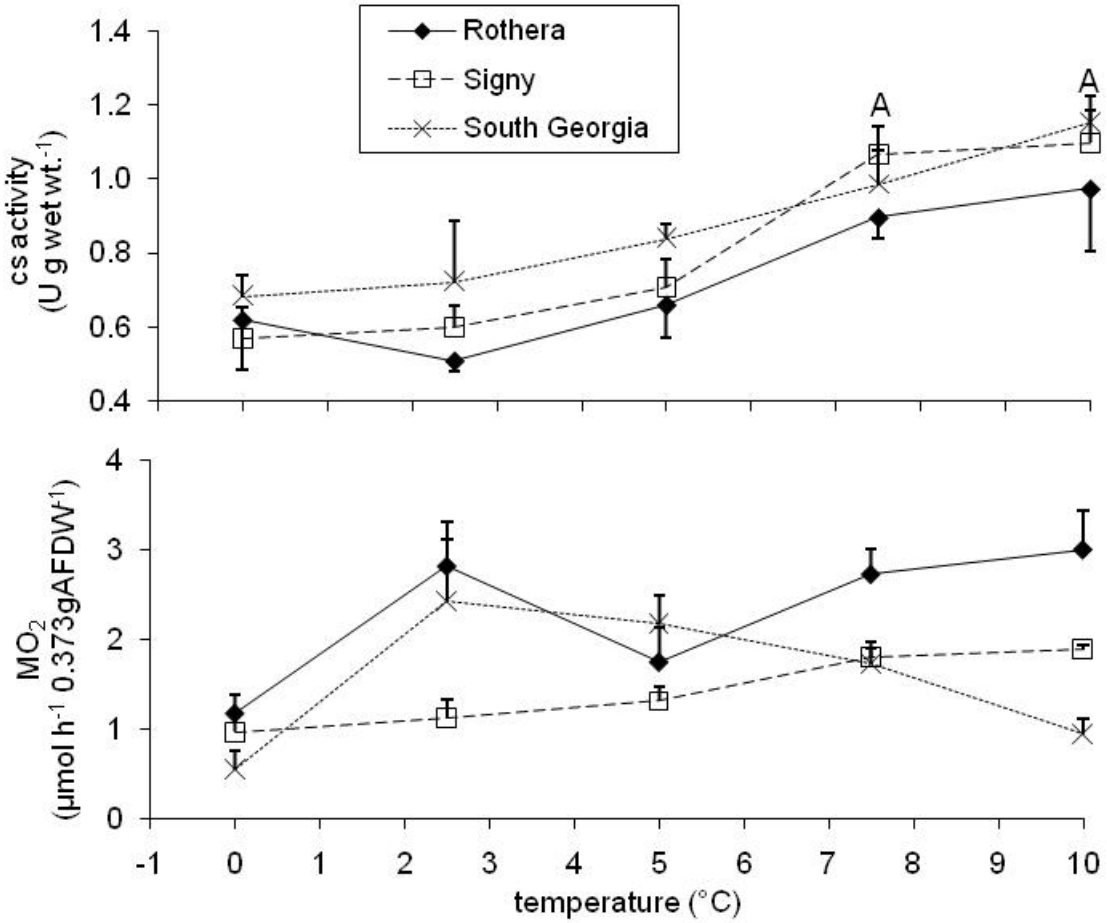
628

629 Fig. 3


Rothera
Signy
South Georgia
succinate (nmol g wet wt.-1)
acetate (nmol g wet wt.-1)
proprionate (nmol g wet wt.-1)
*
a
a
temperature, °C

630

631 Fig.4
632



633