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3   **Geographical variation in thermal tolerance within Southern Ocean marine ectotherms.**

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13   Short title: geographic thermal tolerance

14

15   **Abstract**

16   Latitudinal comparisons of the Southern Ocean limpet, *Nacella concinna*, and clam, *Laternula*

17   *elliptica*, acclimated to $0.0°C$, were used to assess differences in thermal response to two

18   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,

19   tissue energy status was measured through a combination of O_2 consumption, intracellular pH,

20   cCO_2, citrate synthase (CS) activity, organic acids (succinate, acetate, propionate), andelylates

21   (ATP, ADP, AMP, ITP, PLA (phospho-L-arginine)) and heart rate. *L. elliptica* from Signy

22   (60ºS) and Rothera (67ºS) had the same lethal limits (7.5-10.0ºC), but small yet consistent

23   indicators (O2, CS activity, PLA, ATP, organic acids and cCO_2) suggest that Rothera *L. elliptica*

24   had lower critical and *pejus* (=getting worse) limits than Signy *L. elliptica. N. concinna,* which

25   experiences a wider thermal regime, had higher lethal limits (10.0-12.5ºC). However, at their

26   Northern geographic limit *N. concinna* living in a warmer environment (South Georgia, 54ºS),

27   had a lower critical limit (5.1-10.0ºC; O2, PLA and organic acids) than Rothera and Signy *N.*

28   *concinna* (10.0-12.5ºC). This lower limit indicates that South Georgia *N. concinna* have different

29   biochemical responses to temperatures close to their thermal limit, which may make them more

30   vulnerable to future warming trends.

31

32 **Key words**: *Nacella concinna*; *Leternula elliptica*; Southern Ocean; tissue biochemistry; *pejus*

33 limits; critical limits; lethal limits; latitudinal comparisons;

34

35 **1. Introduction**

36 Applying the principles of symmorphosis (Weibel, 2000) to the oxygen supply cascade

37 provides a conceptual framework to explain how temperature affects oxygen availability to

38 tissues, and hence tissue energy status. This has developed towards a unifying principle that the

39 physiological thermal limits of aquatic ectotherms are defined by oxygen availability, the

40 balance between supply and demand (Pörtner, 2002; Pörtner, 2006; Peck et al., 2007). Within

41 their optimal range ectotherms are expected to have the physiological flexibility to cope with

42 normally experienced seasonal variation and tissue energy status should be maximal. However,

43 in the face of extreme conditions, or towards range limits, aerobic scope is reduced, as more of

44 the available energy is required to cope with routine metabolic processes and oxygen supply

45 mechanisms become sub-optimal. There is growing evidence that the effects of thermal stress act

46 hierarchically, from high to low complexity, i.e. whole body processes are affected before

47 biochemical reactions and cells (Pörtner, 2002; Pörtner and Knust, 2007; Pörtner et al., 2007).

48 Improving our understanding of how functionality is lost and energy is partitioned between

49 processes will improve our understanding of the likely impact of climate change on species

50 distributions and resulting ecosystem shifts.

51 Thermal sensitivity can be assessed at a number of levels from whole animal activity

52 through to tissue energy status and finally mortality. Characterising changes in tissue

53 biochemistry has been developed as a test for *critical* thermal limits in aquatic ectotherms which

54 indicate the transition to anaerobic metabolism (*cf* Pörtner, 2002). Within the window bounded

55 by critical temperatures, an even narrower window is bordered by *pejus* limits (pejus: getting

56 worse) which indicate when temperatures move beyond the optimal range and functionality starts

57 to be lost, which is paralleled by a shortfall in oxygen supply and demand until critical limits are

58 reached and anaerobic metabolic pathways are recruited. Species tested this way include the

59 boreal freshwater gadoid, *Lota lota* (Hardewig et al., 2004), the worms, *Sipunculus nudus*

60 (Zielinski and Pörtner, 1996), several populations of *Arenicola marina* (Sommer et al., 1997),

61 and several Antarctic species (e.g. *Limopsis marionensis*; Pörtner et al., 1999) including both *L.*

62 *elliptica* (Peck et al., 2002) and *N. concinna* (Pörtner et al., 1999). Biochemical parameters were

63   selected that measure the integrity of cellular systems and identify the temperature at which there

64   is a departure from homeostasis (pHi and cCO_2), with the main focus on those that measure the

65   aerobic status of the animal, circulation (heart rate), aerobic metabolism (O_2 consumption and

66   citrate synthase activity), tissue energy status (balance of adenylates) and recruitment of

67   anaerobic pathways (build up of organic acids). We aim to utilise these techniques to compare

68   *pejus* limits, *critical* and *lethal* limits of *N. concinna* and *L. elliptica* from different locations

69   within the Antarctic. These measures will be used to look for adaptive differences between

70   locations that relate to experienced thermal environment.

71   We exploit access to a unique latitudinal gradient within the Southern Ocean, Rothera

72   (67°S), Signy (60°S) and South Georgia, (54°S) which enable us to separate latitude and

73   temperature (Barnes et al. 2006). Our highest latitude site at Rothera Point, Adelaide Island, has

74   an annual seawater temperature ranging from –2°C in winter to +1°C in the height of summer (at

75   15m depth; Barnes et al., 2006). Signy Island, within the South Orkney Islands (60°C) is on the

76   edge of the Weddell Sea gyre and despite its more northerly location has the same annual

77   seawater temperature range to that of Rothera (Clarke and Leakey, 1996). However the sub-

78   Antarctic Island of South Georgia (54°S), although still within the Southern Ocean (south of the

79   Antarctic circum-polar current) and therefore the biological Antarctic (Barnes et al., 2005), has

80   almost twice the annual range due to a higher summer maximum (–1 to +4°C at 10-20m; Barnes

81   et al., 2006). In response to the constant cold at Rothera benthic marine ectotherms are generally

82   stenothermal, they have a limited temperature range, upper lethal limits typically occurring

83   between 5 and 10ºC (Peck, 2005) but for some species are considerably lower than these, e.g. the

84   brittle star *Ophionotus victoriae* which cannot survive even 1 month at 2°C (Peck et al, *in press*).

85   Furthermore, limits for critical activities are often considerably lower than the lethal limits (2 to

86   4ºC; Peck et al., 2004). The +4°C maximum summer seawater temperature at South Georgia

87   therefore has the potential to impact these species, unless they have adaptive capacity to cope

88   with this thermal range. It is interesting to note that the range of *O. victoriae* does not stretch as

89   far as South Georgia.

90   Symmorphosis (Weibel, 2000) and the climate variability hypothesis (Stevens, 1989)

91   both predict that species, which experience a wider range of temperature, will have greater

92   thermal flexibility. From this, sub-littoral species living at South Georgia should be able to cope

93   with higher temperatures than the same species from Rothera. Habitat temperatures would also

94 predict that the thermal response of individuals from Signy would be closer to those from

95 Rothera than South Georgia. Although littoral species will experience a much higher range of

96 temperatures during low water emersion, habitat temperatures still predict the warmest

97 environment at South Georgia. The highest recorded littoral temperature is 12.3°C at Rothera

98 (Waller et al., 2006), 10.7°C at Signy (Barnes et al., 1996) and 15.8°C at South Georgia

99 (Davenport, 1997). The littoral limpet, *Nacella concinna* (Strebel 1908), would therefore be

100 expected to have a higher thermal capacity than the sub-littoral clam, *Laternula elliptica* (King

101 and Broderip 1831) and higher thermal limits in *N. concinna* and *L. elliptica* would be predicted

102 from South Georgia, than Signy and Rothera. However, species living at their range limit, where

103 they experience temperatures close to their maxima may have a reduced ability to cope with

104 further temperature increases (Tomanek, 2005; Deutsch et al., 2008); adaptations that enable

105 ectotherms to live in warmer environments may occur at the expense of further acclimatory

106 capacity (Stillman, 2003).

107 Through sampling these two species from 3 locations within the Southern Ocean, at the

108 same time of year, we aim to conduct the first latitudinal comparison of thermal tolerance within

109 the Southern Ocean. Environmental history determines thermal response (e.g.. Osovitz and

110 Hofmann, 2005) and so all animals were acclimated to the same temperature (0.0°C) and

111 maintained under as near identical conditions as possible for 2 months before thermal responses

112 were tested. Any differences in thermal response should therefore indicate adaptive differences

113 between locations.

114

115 **2. Materials and Methods**

116 *2.1. Collection and culture*

117 Limpets, *Nacella concinna* and the soft shelled clam, *Laternula elliptica* were collected

118 during cruise JR109 on the RRS James Clark Ross and concurrently at Rothera, the British

119 Antarctic Survey Research Station at Adeleide Island on Antarctic Peninsula, during early

120 summer as detailed in table 1. Except for *N. concinna* from Signy, which were collected from the

121 lowest part of the intertidal, all animals were collected by SCUBA divers.

122 After collection all animals were maintained in recirculating aquaria at 0.0±0.3°C, for 63

123 days until the start of experiments. Water quality was maintained through biological filtration,

124 U.V. sterilisation, protein skimmers and daily water changes. During this period animals were

125 not fed to reduce any metabolic increment due to feeding (SDA), which can last up to 25 days in

126 Antarctic benthic ectotherms (Peck, 1998). Nonetheless, *N. concinna* were observed grazing on

127 biofilms on the tank walls and *L. elliptica* were seen with siphons open, so both species are likely

128 to have continued low level feeding.

129   At the start of incubation experiments animals were transferred to two 200 litre jacketed

130 acrylic tanks (Engineering Design and Plastics, Cambridge, Cambs.) attached to LTD20G

131 thermocirculators (Grant instruments Ltd, Shepreth, Cambs.), which maintained temperatures to

132 an accuracy of ±0.2°C. The method was similar to that that used by Morley et al. (2007) and

133 Peck et al. (2002; 2004; 2007 and 2008). Animals were allowed 48 hours to recover from

134 movement stress before temperatures were altered or measurements taken. Temperature was then

135 raised at approximately 0.1°C per hour until the required temperatures were reached and then

136 animals were allowed 48 hours to recover from any metabolic overshoot (Peck et al., 2002). Due

137 to time constraints animals were separated into two groups, which were taken through different

138 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

139 protocol animals were tested over approximately 3 weeks in each tank.

140

141 *2.2. Oxygen consumption*

142   Routine metabolic rate was measured in closed cell respirometers, following the

143 methodology of Peck (1989), except that oxygen concentration was measured with a Fibox-3

144 oxygen meter (Presens GmbH, Regensberg, Germany; e.g. Morley et al., 2007). Oxygen

145 sensitive foils were calibrated before each measurement using 5% w/w sodium diothionite for

146 0% and fully aerated water for 100%. During trials oxygen concentration was not allowed to fall

147 below 70% of air saturation, which is above the threshold for oxy-regulation for *L. elliptica*

148 (Peck et al., 2002). Two empty chambers (controls) were run with each trial to account for

149 background oxygen consumption, which was routinely less than 10% of the animal's

150 consumption. The basal metabolic rate of 4-5 individuals was measured for three consecutive

151 days after which data were analysed for significant differences in oxygen consumption between

152 days. When significant differences were found, daily measurements continued until three stable

153 readings were obtained and then temperature was raised. The volume of each animal was

154 measured through displacement and subtracted from the volume of water in the respirometer. At

155 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

187 change with temperature had already been calculated. pH sensors were re-calibrated using

188 buffers cooled to each incubation temperature.

189   To measure cCO_2 100µl of this supernatant extract was injected into a 10ml sealed

190 sample vial, containing 1.5ml of nitrogen gassed HCl (0.05M). The sample was rotated for 10

191 minutes and then 50µl of this gas phase was injected into an Agilent technologies 6890N

192 Network gas chromatogram through a G1888 network headspace sampler. cCO_2 was measured

193 with a HP-PLOTQ column and a TCD detector. Triplicate peak areas were converted to cCO_2 by

194 plotting a calibration curve for standard samples between 0.5 mM and 10.0 mM dissolved

195 inorganic carbon ($r^2 = 0.998$).

196   Tissue citrate synthase activity was measured from tissue extract at a ratio of 1g of tissue

197 to 9ml of extraction buffer, following the methods of Sidell et al. (1987). Enzyme activity was

198 measured at animal incubation temperature with a Beckman UV-DU 7400 spectrophometer

199 fitted with a temperature controlled cuvette holder and diode array detector.

200   To measure both the build up of the end products of anaerobic metabolism (organic

201 acids) and tissue energy status (the adenylates) tissue homogenate was prepared using $0.6mol.l^{-1}$

202 perchloric acid (PCA) (modified from Hardewig et al., 1998). Diluted PCA extracts were

203 injected into an ICS 2000 ion chromatography system fitted with a Gilson Diluter 401

204 autosampler. Organic acids were separated on an ion exclusion column (Dionex ICE-AS 1) at

205 60°C fitted with an AMMS-ICE 4mm micro-membrane suppressor cell, with 0.20mM

206 heptafluorobutyric acid (HFBA) as eluent at a flow rate of $1\ ml.min^{-1}$ and 5mM

207 tetrabutylammonium hydroxide (TBAOH) as the regenerant. The signal was detected on a DS6

208 conductivity detector. Duplicate sample peaks were converted into concentrations using

209 calibration curves with standard solutions, succinate (12 µM to 212 µM, $r^2 = 0.997$) acetate

210 (14µM to 296µM, $r^2 = 0.997$) and propionate (12 µM to 189µM, $r^2 = 0.999$).

211   Adenylates were measured on a Beckman PACE/MDQ capillary electropheris system

212 following the adapted methods of Casey et al. (1999). The capillary was uncoated fused silica,

213 50µm ID, 50 cm long. The pH of homogenates was set to 9.5, diluted in separation buffer

214 (40mM tetraborate buffer and 10mM sodium chloride (pH 9.7)) and 50µl of 4 mM uric acid was

215 added to 200µl of each standard and sample as an internal standard. The absorbance signals of

216 separation were simultaneously detected at different wavelengths with a photo diode array

217 detector: at 254 nm, adenosine, AMP, ATP, ADP and ITP; at 200 nm, arginine and PLA; at 290

218   nm, internal standard uric acid. Duplicate peaks were converted into concentrations using the

219   following calibration curves: adenosine (71.5 µM to 572 µM, r^2 =0.995), AMP (70.5 µM to 564

220   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),

221   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

222   µM to 550 µM, r^2 =0.996).

223

224   *2.4. Analysis*

225   Conducting the experiment in two tanks simultaneously created a mixed design where,

226   within tanks, some individuals were sampled more than once (e.g. for oxygen consumption), but

227   different individuals were sampled between tanks. As there was a small, non significant, effect of

228   individual (as a random factor in a GLM analysis) a simple analyses was conducted, ignoring

229   repeated observations on some individuals (P. Rothery, pers. coms.). ANOVAS with Tukey post-

230   hoc tests were used to test for differences between locations and temperatures for each species.

231   Where ANOVAS indicated only significant differences between locations a separate analysis

232   was conducted for each location. Signficant differences was accepted as $P<0.05$ throughout.

233   Oxygen consumption was standardised to that of a 0.373g dry mass *N. concinna* and a 10.6g dry

234   mass *L. elliptica* (the mean mass of animal used) using scaling coefficients of 0.82 (Fraser et al.,

235   2002) and 0.73 (Peck et al., 2002) respectively.

236

237   **3. Results**

238   *3.1. Animal size*

239   The shell length of *L. elliptica* sampled for biochemistry was not significantly different

240   between locations, table 2. However, due to logistic constraints on collections by SCUBA divers,

241   limpets from Rothera were smaller than those from Signy and South Georgia. *N. concinna*

242   collected inter-tidally from Signy were not significantly different in size than *N. concinna*

243   collected sub-tidally from South Georgia.

244

245   *3.2. L. elliptica*

246   More than 50% of *L. elliptica* died during the transition from 5.0 to 10°C and from 7.5 to

247   12.5°C so the experiment was stopped at this stage and the upper thermal limit recorded as

248   between 7.5 and 10.0°C. Although the sample size was too small to detect significant effects,

249 heart rate increased with temperature between 0.0 and 5.1°C. 50% of animals with implanted

250 impedance wires died at 7.5°C so heart rate was not measured at this temperature.

251 Temperature had a significant effect on every biochemical parameter, there were a few

252 significant differences between Rothera and Signy *L. elliptica* (Table 3) and some consistent

253 patterns that indicate differences between *L. elliptica* from the two locations. Although generally

254 not significant, at 0.0°C, Rothera *L. elliptica* had higher oxygen consumption, CS activity and

255 higher concentrations of high energy phosphates (PLA, ITP and ATP) than Signy *L. elliptica*

256 suggesting an overall higher metabolism at 0.0°C. Between 0.0 and 2.5°C there was also a, non-

257 significant, but consistently greater magnitude change in PLA and ATP (reduction), cCO_2

258 (increase) and organic acids (increase of succinate and acetate) in Rothera than Signy *L. elliptica*

259 (Fig 1, Table 3).

260 The oxygen consumption of *L. elliptica* rose between 0.0 and 2.5°C for specimens from

261 both sites, but no oxygen consumption was significantly different from that at 0.0°C. There was,

262 however, a significant drop in oxygen consumption between 2.5 and 7.5°C for both Signy and

263 Rothera *L. elliptica*. Citrate synthase activity at 2.5, 5.1 and 7.5°C was not significantly different

264 from that at 0.0°C. However, in a similar fashion to oxygen consumption, *L. elliptica* from both

265 locations had a peak in enzyme activity at 2.5°C, which was significantly higher than activity at

266 both 5.1 and 7.5°C.

267 The energy status of the high energy phosphates fell as the concentration of their low

268 energy forms, arginine, AMP and ADP, increased at 7.5°C in *L. elliptica* from both locations.

269 PLA the buffer that allows rapid rephosphorylation of ADP yielding ATP was also reduced at

270 7.5°C. The response of the triphosphates, ITP and ATP, to temperature was slightly different

271 between *L. elliptica* from Signy and Rothera. The ATP levels of Rothera *L. elliptica* were lower

272 at both 5.1 and 7.5°C than at 0.0°C, whilst the ATP levels of Signy *L. elliptica* were only

273 reduced at 7.5°C. The ITP concentration of Rothera *L. elliptica* generally fell upon warming but

274 was only significantly lower than 0.0°C at 7.5°C, whilst the ITP concentration of Signy *L.*

275 *elliptica* was lower at both 0.0°C and 7.5°C than 5.1°C.

276 The concentration of all three organic acids, succinate, acetate and propionate was

277 elevated at 2.5°C in Rothera *L. elliptica* and at 5.0°C in both, but was only significantly higher at

278 7.5°C. There were no significant differences in acetate and propionate accumulation patterns

279 between locations except that succinate rose to a significantly higher level in Signy than Rothera

280 *L. elliptica* at 7.5°C.

281   Intracellular pH was significantly higher at 0°C than at 2.5, 5.1 or 7.5°C for *L. elliptica*

282 from both locations. There was also a significant drop in pHi between 5.1 and 7.5°C. Tissue

283 cCO_2 was highly variable and there was only one significantly elevated value, in Rothera *L.*

284 *elliptica* at 5.1°C.

285

286 *3.3. N. concinna*

287   More than 50% of *N. concinna* died during the transition from 7.5 to 12.5°C and 10.0 to

288 15.0°C so the experiment was stopped at this point. There was no significant effect of location or

289 temperature on arginine but there were complex, significant effects on the other adenylates, with

290 higher concentrations of both low (AMP and ADP) and high (ATP) energy adenylates at 10.0°C

291 (Fig. 2). At 10.0°C AMP was significantly higher in Rothera *N. concinna* than all treatments

292 except Signy *N. concinna* at 2.5°C and South Georgia at 10.0°C. At 10.0°C ADP was higher in

293 *N. concinna* from all locations. Although there was no significant difference in ATP

294 concentration from that at 0.0°C, at 10.0°C the concentration of ATP was higher at all locations

295 than at 2.5°C. However, despite this significant increase in ATP for all locations, the increase for

296 South Georgia *N. concinna* appeared to be less (Fig. 2). The PLA concentration of South Georgia

297 *N. concinna* was also lower at 10.0°C than at either 0.0 or 5.1°C.

298   Despite a general increase in the organic acids with increasing temperature, succinate,

299 acetate and proprionate were only significantly elevated in South Georgia *N. concinna* at 10.0°C

300 (Fig.3). The large inter-individual variation in organic acids at 10°C is typical of biochemical

301 responses around physiological limits as some individuals reach their limits before others. For *N.*

302 *concinna* from all locations, citrate synthase activity determined in tissue samples collected at

303 each temperature generally increased upon warming but was only significantly higher at 7.5 and

304 10.0°C (Fig.4).

305   Oxygen consumption was very different between *N. concinna* from different locations.

306 Oxygen consumption increased in a linear fashion with temperature (T) for Rothera (O_2cons =

307 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,

308 F=22.9) *N. concinna* (Fig 4). Oxygen consumption for South Georgia *N. concinna* reached a

309 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

341 differences in cold acclimation of; CS activity, oxygen consumption and the turnover of high

342 energy phosphates were consistently (but not significantly) higher in Rothera than Signy *L.*

343 *elliptica* at 0.0 ºC.

344   The lethal limit in the current study also occurred at a similar temperature, between 7.5

345 and 10.0°C (within 15 days), to that recorded previously (9°C within 25 days; Peck et al., 2002).

346 The similarity of lethal limits between Rothera and Signy *L. elliptica*, is consistent with the

347 hypothesis that thermal capacities will be matched to the experienced environment (both -2 to

348 +1°C), although the lower *pejus* limits of Rothera *L. elliptica* compared to Signy indicate subtle

349 differences in thermal tolerance between locations. Clearly, studies incorporating *L. elliptica*

350 from South Georgia, which is at the northern limit of its range, are needed to quantify how close

351 it is living to its upper thermal limits and therefore with reduced capacity to cope with further

352 temperature increases (Stillman, 2003). Such studies are crucial to our understanding of species

353 wide thermal capacity and therefore the ability of species to cope with environmental change.

354 Acclimations of *L. elliptica* at 3-4°C would indicate if South Georgia *L. elliptica* have

355 adaptations to cope with higher maximum experienced summer temperature.

356

357 *4.2. N. concinna*

358   Due to the constraints on geographical sampling within the Southern Ocean, there were

359 sampling differences between *N. concinna* collected at different locations. *N. concinna* were

360 collected from the bottom of the inter-tidal from Signy, compared to sub-tidal animals collected

361 from South Georgia and Rothera, however, all *N. concinna* were collected from depths shallower

362 than 6m. A recent study found small, non-consistent, genetic differences between inter-tidal and

363 15m *N. concinna* from Potter Cove, King George Island, but not between inter-tidal and 6m *N.*

364 *concinna* (de Aranzamendi et al., 2008). Different biochemical responses to emersion were also

365 evident between 15m and intertidal *N. concinna*, also from King George Island (Weihe and

366 Abele, *In Press*) was also evident This suggests that comparisons of 6m (Rothera), 2-3m (South

367 Georgia) and inter-tidal (Signy) *N. concinna* will not be affected by population differences

368 between animals collected from different depths. *N. concinna* collected in the middle of summer

369 and tested within days of collection had no significant (t=0.57, *p*=0.57) differences in upper

370 lethal thermal limits (1°C per day) between those collected in the inter-tidal (11.6°C) or sub-

371 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.*

403 *concinna* from different locations after 2 months acclimation to 0.0°C (10.0 to 12.5°C).

404 Davenport reported a short term survival limit of 15.6°C (12 hour exposure) for *N. concinna* at

405 South Georgia, higher than our medium term, 22 day, temperature limit of between 10.0 and

406 12.5°C. This is not unexpected as different thermal limits and protective mechanisms might be

407 expected if different rates of heating or cooling are used (Terblanche et al., 2007; Barnes et al.,

408 2008; Peck et al., *in press*). This difference may also have been because *N. concinna* tested by

409 Davenport were acclimatised to summer conditions which could have raised their lethal limit

410 above that of animals acclimated to 0.0°C. However, it should also be noted that due to

411 behavioural adaptations, such as utilisation of cryptic habitats and evaporative cooling (Branch,

412 1981), body temperatures of limpets in the field are unlikely to reach these temperatures. The

413 maximum foot temperature of a Rothera *N. concinna* exposed on a sunny day has been shown to

414 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

415 air temperature (Clark et al., 2008). If this relationship holds for South Georgia *N. concinna* then

416 foot temperature is unlikely to exceed their short-term (daily exposure) lethal limit during low

417 water emersion but might approach their medium term (weekly exposure) limit. Although *N.*

418 *concinna* are regularly exposed in the intertidal zone at Rothera and Signy, at South Georgia, it is

419 mainly a sub-tidal species (Davenport, 1997) and only rarely found above mean low water spring

420 tide level, reducing the risk of exposure to damaging high temperatures. The few inter-tidal

421 individuals found at South Georgia inhabit sheltered microhabitats and not exposed surfaces,

422 which are colonised by a siphonariid limpet, *Kerguelenella lateralis*. At its northern geographic

423 limit differences in habitat utilisation may be key to reducing the likelihood of *N. concinna* being

424 exposed to air temperatures close to its upper limit. They may therefore have evolved a high

425 anaerobic capacity to allow short-term, passive, survival during less frequent exposure (Pörtner,

426 2002). This fits with a growing body of evidence which shows that adaptation to life at range

427 limits may actually make animals less able to cope with further temperature increases (Stillman,

428 2003, Tomanek, 2005, Deutsch et al., 2008).

429   The passive survival strategy of *N. conccina* appears to be effective over several days, but

430 is unlikely to be effective over longer time scales (Pörtner, 2006). After initial shock, animals

431 may switch from acute mechanisms that allow short-term "passive" survival to longer-term

432 acclimatory mechanisms with associated changes in tissue biochemistry and structure. The

433 greater thermal flexibility of *N. concinna* than *L. elliptica* was further evidenced by the increase

434   in adenylate concentration with temperature which suggests that there was an acclimatory

435   increase of the adenylates over the 2 week duration of the experiment, in an attempt to meet the

436   increase in energy demand with temperature.

437   Two month long incubations of *N. concinna* from South Georgia, Signy and Rothera,

438   long enough for seasonal acclimation, led to a reduction in the surface area of mitochondrial

439   cristae per unit muscle fibre volume (Morley et al., submitted). Latitudinal comparisons showed

440   that acclimated *N. concinna* from South Georgia had a higher citrate synthase activity than

441   Rothera *N. concinna*, which was negatively correlated with mitochondrial density across

442   locations. This strongly suggested an adaptation for fewer, more efficient mitochondria in

443   acclimated *N. concinna* from the northern limit of their range (Morley et al., submitted). Tissue

444   samples of *N. concinna* acclimated to 3.0°C are currently being analysed to investigate if

445   changes in aerobic pathways were accompanied by a change of anaerobic scope. These

446   differences in the mechanisms underlying, and the limits of, thermal tolerance between *N.*

447   *concinna* from South Georgia and both Signy and Rothera *N. concinna* are supported by genetic

448   differences, which suggest South Georgia *N. concinna* are a separate population (Beaumont and

449   Wei, 1991).

450   The Scotia arc region of the Southern Ocean has one of the fastest warming climates on

451   the planet (Meredith and King, 2005). If this rate of warming is faster than the rate of

452   evolutionary adaptation, then the acclimatory ability of individuals, populations and species is

453   likely to be a key determinate of their survival (e.g. Peck, 2008). Species with wider geographic

454   ranges are thought to be more resistant to extinction (Jablonski, 1986), which may in part be due

455   to the wider range of environments they experience. This should in turn lead to wider genetic

456   variation and therefore an increased physiological capacity to cope with environmental

457   perturbations (the climate variability hypothesis; Stevens, 1989). The current study has found

458   differences in thermal response of marine ectotherms between locations on the Scotia Arc and

459   Antarctic Peninsula. Investigations of a wider range of species will confirm if these differences

460   are an adaptive response to differences in experienced environment. Latitudinal comparisons

461   testing for differences in capacity between animals from different locations within the Southern

462   Ocean could be a key tool for predicting the vulnerability of species to the current trend of

463   warming.

464

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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

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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]

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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]

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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.
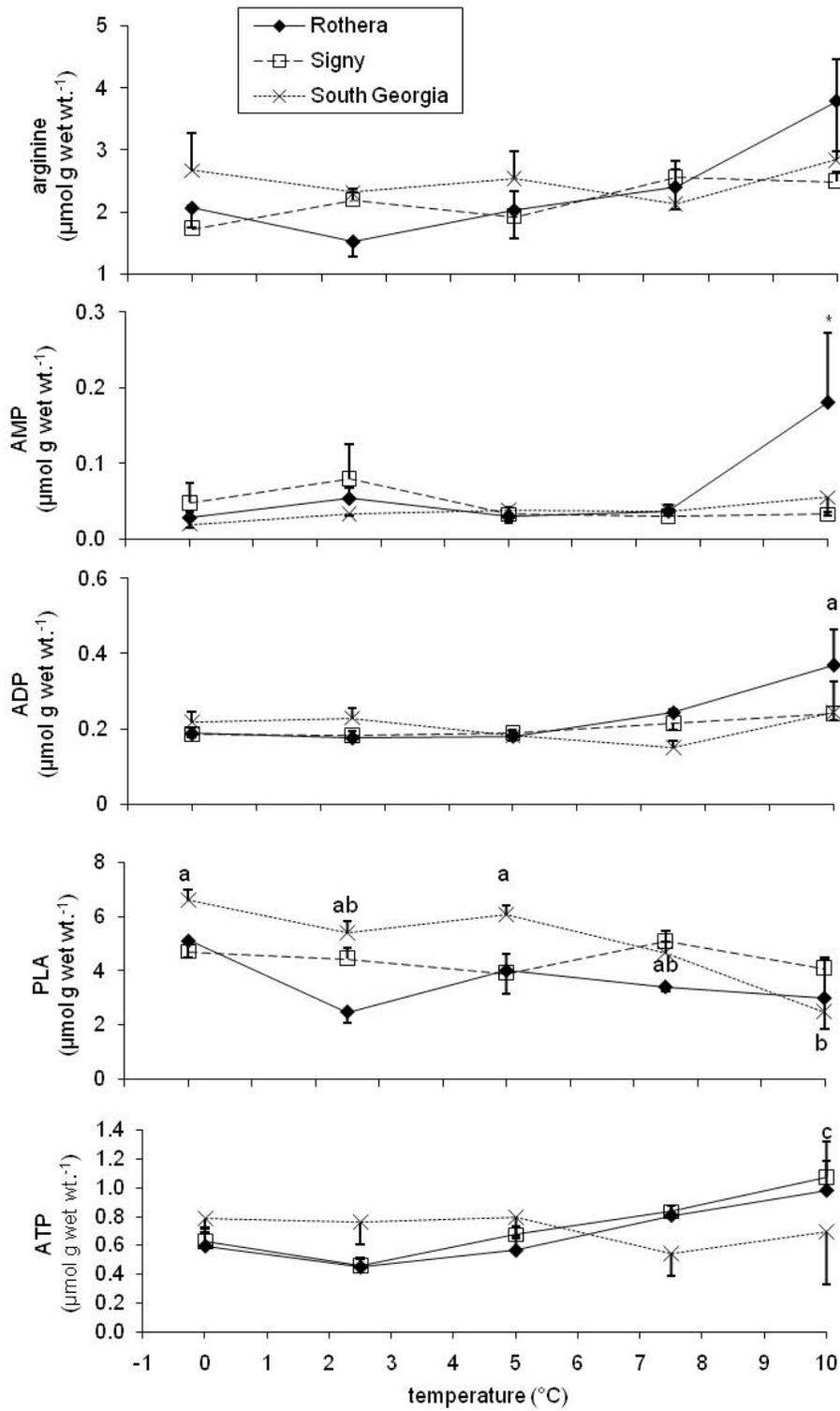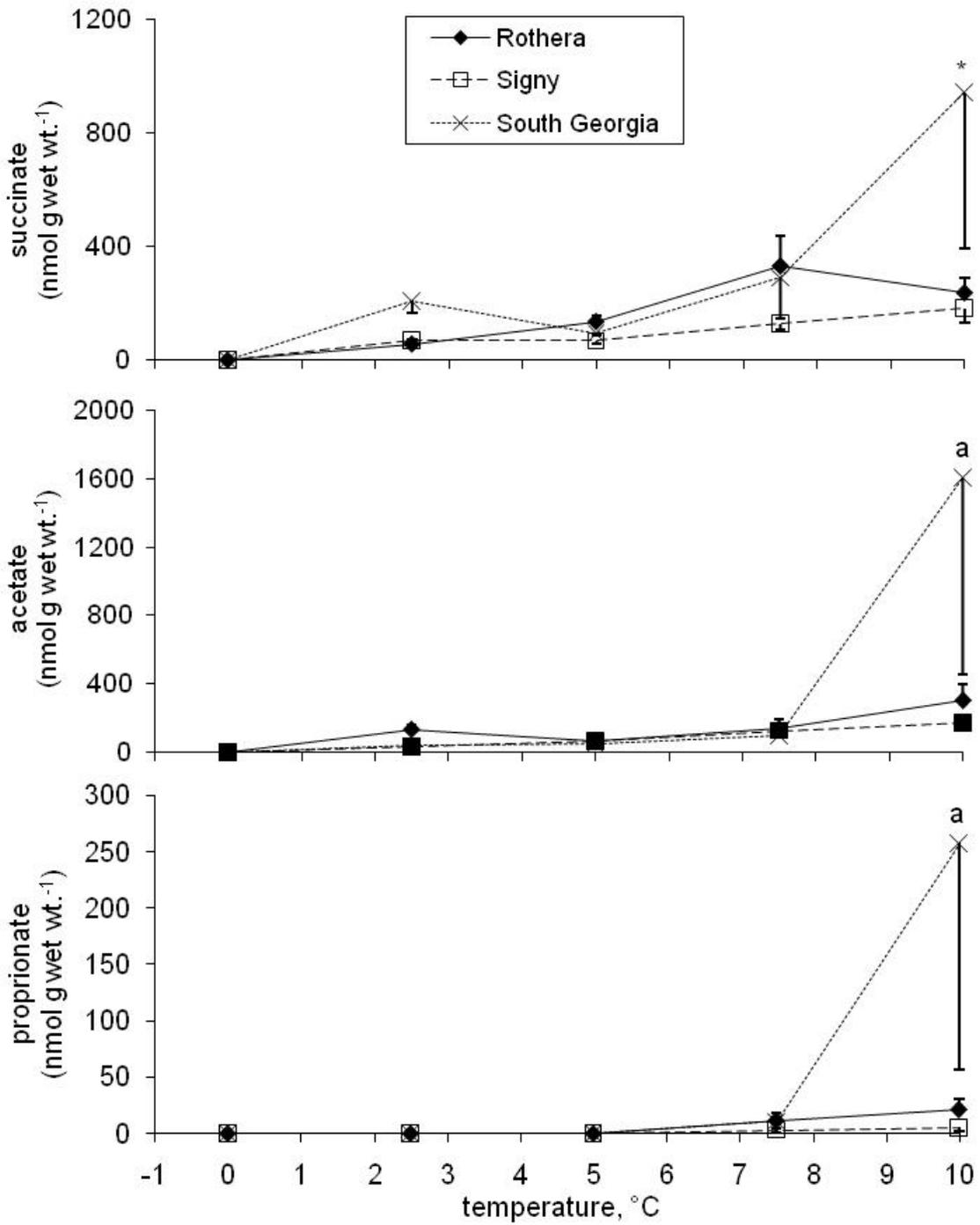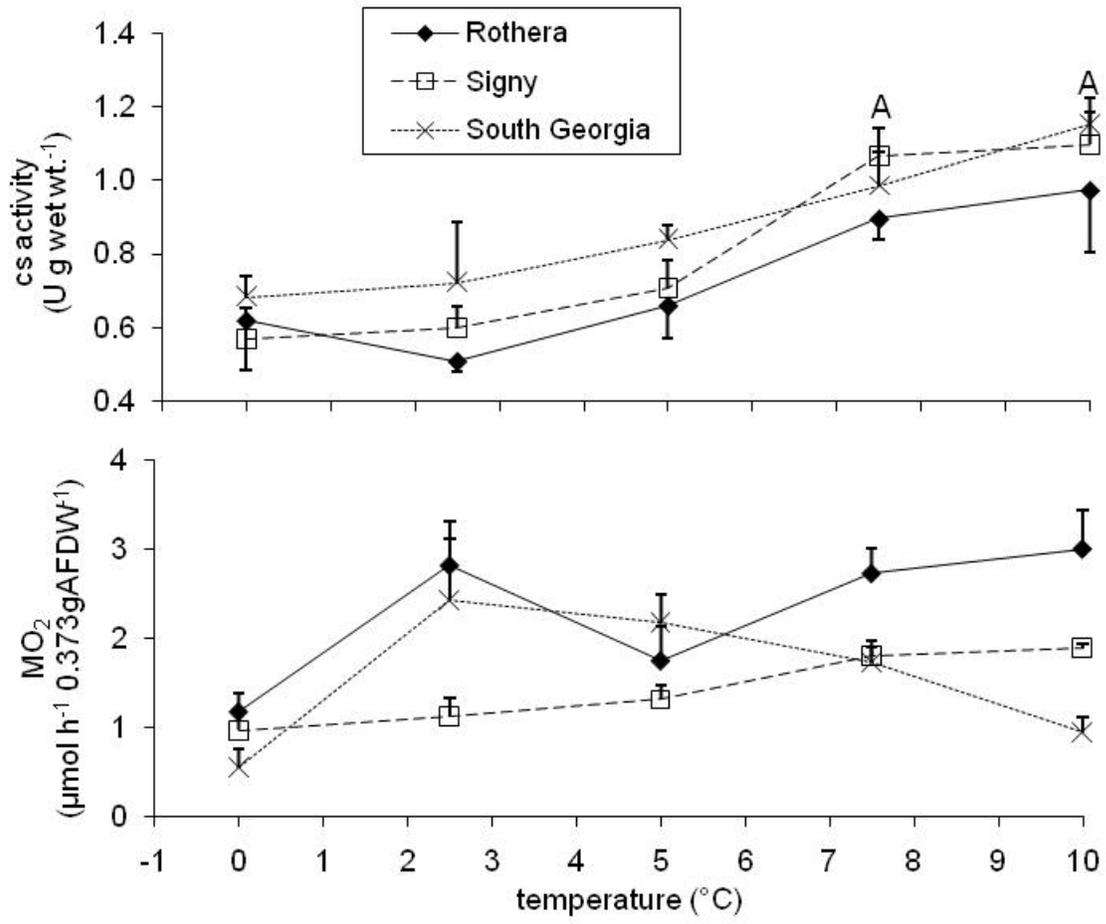
625   Fig. 1
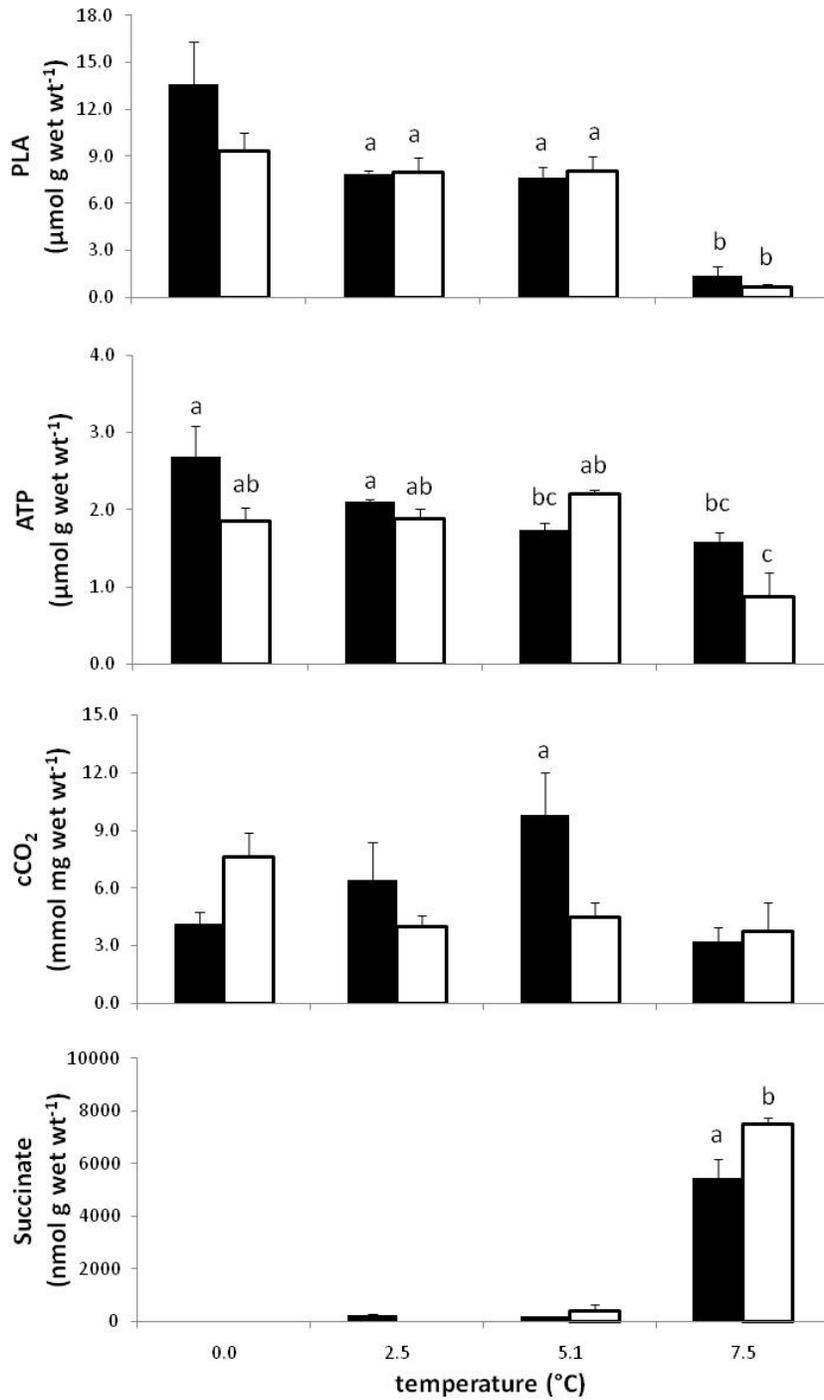
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627 Fig. 2

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629   Fig. 3

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631   Fig.4
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