

1 Heat tolerance and physiological plasticity in the
2 Antarctic collembolan, *Cryptopygus antarcticus*, and
3 mite, *Alaskozetes antarcticus*

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12
13 **Abstract**

14 Polar amplification of global warming has led to an average 2°C rise in air temperatures in parts of the
15 polar regions in the last 50 years. Poikilothermic ectotherms that are found in these regions, such as
16 Collembola and mites, may therefore be put under pressure by changing environmental conditions.
17 However, it has also been suggested that the thermal sensitivity of invertebrates declines with higher
18 latitudes and, therefore, that polar ectotherms may not be at risk. In the current study, the heat
19 tolerance and physiological plasticity to heat stress of two well-studied Antarctic invertebrates, the
20 collembolan, *Cryptopygus antarcticus*, and the mite, *Alaskozetes antarcticus*, were investigated. Both
21 species showed considerable heat tolerance, with each having an Upper Lethal Temperature (ULT)
22 above 35°C (1 hour exposure). These species were also able to survive for over 43 d at 10°C and for
23 periods of 5-20 min at 40°C. Across all experimental procedures, *A. antarcticus* possessed a
24 somewhat greater level of heat tolerance than *C. antarcticus*. Water loss during short duration
25 exposures did not differ between the two species at 30, 35 and 40°C, suggesting that the greater
26 tolerance of *A. antarcticus* over this timescale was not due to higher desiccation resistance.
27 Physiological plasticity was investigated by testing for Rapid Heat Hardening (RHH) and long-term
28 acclimation. RHH was observed to a small degree in both species at a warming rate of 0.5°C min⁻¹,
29 and also 0.2°C min⁻¹ in *A. antarcticus* alone. Longer-term acclimation (1 week at 10°C) did not
30 enhance the heat tolerance of either species. Even with this limited physiological plasticity, the results
31 of this study indicate that *C. antarcticus* and *A. antarcticus* have capacity in their heat tolerance to
32 cope with current and future environmental extremes of high temperature.

33 *Keywords:* Global warming, Rapid Heat Hardening, Acclimation, Thermal sensitivity, Invertebrate

34
35 **1. Introduction**

36 Over the last century, the mean surface temperature of the Earth has increased by 0.6°C (IPCC 2001).
37 However, the rate of warming has been amplified at higher latitudes, with an average 2°C rise in parts
38 of the polar regions in the last 50 years (Arctic Council 2005; Convey *et al.* 2009; Turner *et al.* 2009).
39 The northern and western parts of the Antarctic Peninsula have been particularly affected; over the

40 period 1951-2006, data from Vernadsky (Faraday) station in the Argentine Islands recorded an 0.53°C
41 rise in temperature per decade. A further consequence of this warming at a global scale has been a
42 decrease in snow and ice cover of over 10% since the 1960s (Walther *et al.* 2002). These trends are
43 set to continue, with general circulation models predicting further warming across the planet, and
44 especially rapid warming in the polar regions.

45 Invertebrates are poikilothermic ectotherms, meaning that their body temperature is highly influenced
46 by, and varies markedly with, the external environment (Speight *et al.* 2008). In essence, they are
47 unable to regulate their body temperature as do birds and mammals, and are therefore susceptible to
48 injuries, and developmental and reproductive impairment, resulting from temperature changes (Bale
49 and Hayward 2010). Invertebrates can respond to these changes through alterations in their behaviour,
50 phenology, physiology and genetic make-up, with these responses acting within or between
51 generations (Lachenicht *et al.* 2010). Behaviourally, they can track favourable temperatures by
52 moving towards either higher latitudes or altitudes (Walther *et al.* 2002; Sinclair *et al.* 2003; Gobbi *et al.*
53 *et al.* 2006). Several alpine spiders, for instance, have been shown to remain in their preferred
54 temperature range by tracking the recession of the Forni Glacier in Italy (Gobbi *et al.* 2006).
55 Invertebrates can also adapt behaviourally on a smaller scale, via microhabitat selection. Habitats,
56 such as the Antarctic fellfields, are host to a diversity of microclimates and invertebrates select those
57 which are the least stressful (Hodkinson *et al.* 1999; Holmstrup and Zachariassen 1996; Hoshikawa *et al.*
58 *et al.* 1988; Spaul 1973). Hayward *et al.* (2000, 2003, 2004) have gone on to show thermal and hygric
59 preferences that are suggestive of this type of behavioural selection in a laboratory setting. A further
60 response identified is a shift of spring and autumn phenology with the changing of the growing season
61 (Ibanez *et al.* 2010; Walther *et al.* 2002).

62 Within generations, physiological adaptation is demonstrated through experimental acclimation or
63 natural acclimatisation - permitting an organism to adapt to changing conditions via a change in form,
64 movement or rate of physiological activity (Lachenicht *et al.* 2010). In the context of climate change,
65 acclimatisation may involve the improvement of heat tolerance and upper thermal sub-lethal
66 characteristics, such as physical activity, as temperatures rise. This form of adaptation has been shown
67 in a number of organisms, including plants (Meyer and Santarius 1998), nematodes (Jagdale and
68 Grewal 2003) and insects (Lachenicht *et al.* 2008). Over generations, invertebrates can adapt their
69 physiology through the process of natural selection (Somero 2010).

70 The thermal sensitivity of terrestrial invertebrates to temperature change has been reported to decline
71 from the tropics to the poles (Addo-Bediako *et al.* 2000; Deutsch *et al.* 2008). Some tropical species
72 live very close to their upper thermal limits and, in some cases, at temperatures that exceed their
73 physiological optima (Somero 2010). Polar species, in contrast, may live chronically below their
74 temperature optima, and are suggested to have sufficient scope to tolerate higher temperatures.
75 Warming might even help to alleviate the stress associated with low temperatures in the polar regions.
76 Climate warming simulation studies using screens, solar domes and other controlled environmental
77 systems (Bokhorst *et al.* 2008; Bale and Hayward 2010) suggest a rise in temperature will indeed lead
78 to greater invertebrate numbers in Antarctic communities (Convey *et al.* 2002; Convey & Wynn-
79 Williams 2002; Day *et al.* 2009). However, some manipulation studies also suggest the opposite
80 outcome, with responses depending both on the detailed changes at micro-environmental level
81 associated with the manipulation, and also on the group of invertebrates being considered (Convey *et al.*
82 *et al.* 2002, 2003; Bokhorst *et al.* 2011). Studies into upper thermal thresholds are also used in
83 conjunction with climate manipulation studies and support the view that polar terrestrial invertebrates
84 have low sensitivity to temperature change. Slabber *et al.* (2007), for example, showed that five
85 Collembola species from a sub-Antarctic island, including *Cryptopygus antarcticus*, possessed Upper

86 Lethal Temperatures (ULT_{50s}) above 30°C, far higher than the mean summer temperature in the
87 Antarctic.

88 In the current study, the capacity of the collembolan, *Cryptopygus antarcticus*, and the mite,
89 *Alaskozetes antarcticus*, to tolerate exposure to high temperatures was investigated, and their
90 physiological plasticity to heat stress explored. In particular, this study addressed the ability of each
91 species to respond to rapid increases in temperature, as might occur as a result of solar insolation of
92 their microhabitats during diurnal cycles, and their tolerance to more prolonged exposures to high
93 temperatures based on climate warming predictions. These species were selected as they represent
94 two of the most successful arthropod groups in the maritime Antarctic and are considered 'model'
95 organisms in polar research (Block and Convey 1995; Block *et al.* 2009), reaching numbers of up to
96 1.5×10^6 individuals m⁻² (Burn 1986; Convey and Smith 1997; Tilbrook 1967). Consequently, any
97 effect warming may have on them will likely be reflected throughout the community.

98 **2. Materials and methods**

99 *2.1. Invertebrate collection and storage conditions*

100 Naturally occurring summer-acclimatised individuals of *C. antarcticus* and *A. antarcticus* were
101 collected from algae, moss and rocks on Léonie Island (67°S, 68°W), near to the British Antarctic
102 Survey's Rothera Research Station, Adelaide Island between January and March 2012. Samples were
103 stored at 4°C (24:0 L:D) in plastic buckets containing substratum from the site of collection. For water
104 loss experiments (sub-section 2.2.1.), samples were transported to the University of Birmingham
105 under cool conditions (4 to 6°C), taking approximately two months, before being stored at 4°C (0:24
106 L:D). All other experiments described were carried out at Rothera Research Station.

107 *2.2. Microhabitat temperatures*

108 The temperature range on Léonie Island on the soil surface underneath a rock was measured between
109 24 January and 12 March 2012. To illustrate the extremes of temperature potentially experienced by
110 an animal on an exposed surface, temperature was also recorded every 5 min on a rock between 5 and
111 21 February 2012 at Rothera Research Station, using a Tinytag Transit 2 Datalogger (Gemini Data
112 Loggers, Chichester, UK). Data were uploaded using Tinytag Explorer Software (Gemini Data
113 Loggers, Chichester, UK).

114 *2.3. Upper Lethal Temperatures (ULTs)*

115 The upper temperature at which invertebrates no longer survived was determined by warming
116 individuals of *C. antarcticus* and *A. antarcticus* at 0.2°C min⁻¹ from 4°C to progressively higher
117 temperatures (30 to 37°C for *C. antarcticus* and 30 to 40°C for *A. antarcticus*). Individuals were
118 subsequently held at the target temperature for 1 h, before being cooled back to 4°C at the same rate.
119 Three replicates of 10 individuals of each species were placed in Eppendorf tubes, which were packed
120 inside glass test tubes plugged with sponge and placed in an alcohol bath (Haake Phoenix II C50P,
121 Thermo Electron Corporation), prior to each experimental treatment. Control groups were handled,
122 and exposed, in the same way at 4°C. The temperature experienced by the invertebrate was measured
123 by placing a thermocouple within an identical Eppendorf tube into one of the glass test tubes. At the
124 end of experimental treatments, individuals were rapidly transferred (over ice) from the Eppendorf
125 tubes into plastic universal tubes containing moist Plaster of Paris, and returned to the rearing
126 conditions (4°C, 0:24 L:D). Survival, defined by individuals moving either spontaneously or in
127 response to gentle contact stimulus, was assessed 24 and 72 h after treatment. Replicate collection,

128 controls, thermocouple use, recovery and survival assessment were the same for all following
129 experimental procedures unless stated otherwise.

130 2.3.1. Water loss following high temperature exposure

131 For both species, five replicates of 10 individuals were exposed to three temperatures (30, 35 and
132 40°C) as described in sub-section 2.2. Individuals were weighed prior to and upon removal from each
133 treatment, then following drying to constant mass at 60°C for 24 h. From these values, initial water
134 content and percentage water loss or gain were calculated (cf. Hayward *et al.* 2007).

135 2.4. Rapid Heat Hardening (RHH)

136 2.4.1. Determination of the discriminating temperature

137 In rapid cold and heat hardening experiments the discriminating temperature is defined as the
138 temperature at which there is 10-20% survival after an exposure time of e.g. 1 h (Lee *et al.* 1987).
139 This temperature was determined here by exposing individuals (three replicates of 10 individuals) of
140 *C. antarcticus* and *A. antarcticus* directly (i.e. without ramping from 4°C) to progressively higher
141 temperatures (30 to 36°C for *C. antarcticus* and 36 to 40°C for *A. antarcticus*) for 1 h, before
142 returning to the rearing temperature (4°C) at 0.2°C min⁻¹.

143 2.4.2. Induction of RHH

144 To investigate the RHH response, individuals of *C. antarcticus* and *A. antarcticus* (3 replicates of 10
145 individuals for each species) were warmed to the discriminating temperature at three different rates
146 (0.5°C min⁻¹, 0.2°C min⁻¹ and 0.1°C min⁻¹). As before, individuals were held for 1 h at the
147 discriminating temperature and then cooled back to the rearing temperature (4°C) at 0.2°C min⁻¹.

148 2.5. Long-term heat tolerance

149 Five replicates of 10 individuals of *C. antarcticus* and *A. antarcticus* were transferred to either 4 or
150 10°C for up to 49 d. Individuals were held in universal tubes with a base of moist Plaster of Paris and
151 a small amount of substratum within an incubator. Survival was assessed every 7 d for the first four
152 weeks and then every 3 d thereafter. The temperature inside the incubator was measured using a
153 Tinytag Transit 2 Datalogger.

154 2.6. Acute heat exposure

155 Three replicates of 10 individuals of *C. antarcticus* and *A. antarcticus* were exposed directly to three
156 temperatures: 40, 45 and 50°C. At each temperature, individuals were held for 5, 10 or 20 min.
157 Following high temperature treatment, they were transferred directly to recovery conditions (4°C,
158 24:0 L:D).

159 2.7. Effect of acclimation on heat tolerance

160 Stock cultures of *C. antarcticus* and *A. antarcticus* were held for one week at 10°C prior to
161 experimental treatments. Three replicates of 10 individuals of each species were subsequently warmed
162 at 0.2°C min⁻¹ to three temperatures (33, 34 and 35°C for *C. antarcticus* and 39, 39.5 and 40°C for *A.*
163 *antarcticus*), and held there for 1 h, before being cooled to the rearing temperature (4°C) at 0.2°C min⁻¹.
164

165 2.8. Statistical analysis

166 The Kolmogorov-Smirnov test was used to check for normal distribution of survival and percentage
167 water loss data. Normally distributed data were analysed using analysis of variance (ANOVA) and
168 Tukey's multiple range test; data that were not normally distributed were analysed using the Kruskal-
169 Wallis test.

170 **3. Results**

171 *3.1. Microhabitat temperatures*

172 Soil surface temperatures beneath a rock on Léonie Island ranged from 13.5 to -6.1°C, and averaged
173 1.9°C, between 24 January and 12 March 2012 (Fig. 1), whereas the temperature on the rock surface
174 ranged between 31.2 and -8.7°C (Fig. 2). The diurnal temperature range on the rock surface was high,
175 regularly exceeding 20°C (with temperature changing at rates > 2.5°C/h), and on seven occasions the
176 temperature ranged from below 0°C to above 20°C within 12 h.

177 *3.2. Upper Lethal Temperatures (ULTs)*

178 Survival declined dramatically at temperatures close to the ULT for both species (Fig. 3). After 1h at
179 34°C, almost 90% of *C. antarcticus* survived, while only 3% survived 1 h at 36°C, and none survived
180 at 37°C. *Alaskozetes antarcticus* had greater heat tolerance than *C. antarcticus*, with 100% survival of
181 1 h at 37°C, 81% survival at 39°C, but 0% survival at 40°C. The difference between species was not
182 significant at 35, 36 and 37°C, according to the Kruskal-Wallis test ($P > 0.05$ Kruskal-Wallis test).

183 *3.2.1. Water loss following high temperature exposure*

184 Water loss was minimal following a 1 h exposure to 30, 35 and 40°C in both species (Table 1). The
185 amount lost did not differ significantly from the control (1 h at 4°C) in all treatments, except for a 1 h
186 exposure at 40°C in *C. antarcticus* ($P < 0.05$ Tukey's multiple range test). There was no significant
187 difference between the amount of water lost in *C. antarcticus* and *A. antarcticus* across each of the
188 three treatments ($P > 0.05$ Tukey's multiple range test).

189 *3.3. Rapid Heat Hardening (RHH)*

190 *3.3.1. Determination of the discriminating temperature*

191 The discriminating temperature was determined to be 35°C for *C. antarcticus* (10% survival), and
192 39.5°C for *A. antarcticus*, a temperature which although resulting in 0% survival, was chosen because
193 it was closer to the 10-20% survival required than the 37% value obtained at 39°C (Fig. 4).

194 *3.3.2. RHH induction*

195 In both species, all three warming treatments (0.5, 0.2 and 0.1°C min⁻¹) gave greater survival
196 compared to direct exposure to the discriminating temperature (Fig. 5). The increase in survivorship
197 was significant for 0.5°C min⁻¹ in *C. antarcticus* ($P < 0.05$ Tukey's multiple range test), and for 0.5
198 and 0.2°C min⁻¹ in *A. antarcticus* ($P < 0.05$ Tukey's multiple range test). For *A. antarcticus*, survival
199 declined as the rate of warming was lowered, from 73% at 0.5°C min⁻¹ to 30% at 0.1°C min⁻¹. The rate
200 of 0.5°C min⁻¹ also gave the greatest survival in *C. antarcticus*.

201 *3.4. Long-term heat tolerance*

202 *C. antarcticus* was more susceptible at both 4 and 10°C than *A. antarcticus* (Fig. 6). Survival of *C.*
203 *antarcticus* decreased significantly at 4°C to 70% after 46 d ($P < 0.05$ Tukey's multiple range test),

204 and to 0% at 10°C ($P < 0.05$ Kruskal-Wallis test) (Fig. 6). *Alaskozetes antarcticus* survival also
205 decreased significantly at 10°C ($P < 0.05$ one-way ANOVA), but only to 63% after 49 d, and was not
206 significantly different at 4°C (80% survival, $P > 0.05$ Kruskal-Wallis test).

207 3.5. Acute heat exposure

208 At 40°C, *A. antarcticus* outperformed *C. antarcticus* in all treatments (5, 10 and 20 min, Fig. 7), but
209 this was not significant ($P > 0.05$ Mann-Whitney U test; one-way ANOVA). At 45 and 50°C, both *C.*
210 *antarcticus* and *A. antarcticus* survived poorly (Fig. 7).

211 3.6. Effect of acclimation on heat tolerance

212 Acclimation at 10°C did not significantly enhance the heat tolerance of *C. antarcticus* or *A.*
213 *antarcticus* at any of the temperatures tested ($P > 0.05$ Mann-Whitney U test; one-way ANOVA, Fig.
214 8).

215 4. Discussion

216 The Antarctic environment is unable to support large biological communities and, in extreme cases,
217 may only support a food web of less than five animal species (Block *et al.* 2009; Hodgson *et al.*
218 2010). The few terrestrial invertebrates that inhabit these communities play an important role in
219 processes such as soil conditioning and nutrient cycling (Bokhorst *et al.* 2007). In contrast to the
220 temperate and tropical regions, which have greater species diversity and subsequently greater
221 functional redundancy, polar communities will struggle to compensate for the loss of species and their
222 associated services. Changing environmental conditions as a result of climate warming may put
223 pressure on polar species. However, the thermal sensitivity of polar invertebrates to temperature
224 increase has been suggested to be low, and warming may even result in more optimal conditions and a
225 reduction in environmental constraints on invertebrate physiology (Addo-Bediako *et al.* 2000; Convey
226 *et al.* 2009; Deutsch *et al.* 2008). The acute and chronic tolerances, as well as the physiological
227 plasticity, of the collembolan, *C. antarcticus*, and the mite, *A. antarcticus*, are discussed here in the
228 context of their ability to respond to climate warming.

229 4.1. Basal heat tolerance

230 The collembolan, *C. antarcticus*, and the mite, *A. antarcticus*, demonstrated considerable heat
231 tolerance, with each having a ULT of over 35°C (Fig. 3). In two sub-Antarctic studies on Marion
232 Island (Deere *et al.* 2006; Slabber *et al.* 2007) and one study at Cape Hallet, North Victoria Land
233 (Sinclair *et al.* 2006), several mites and Collembola, including *C. antarcticus* on Marion Island, were
234 also shown to possess ULTs above 30°C. While this level of tolerance is somewhat lower than found
235 in temperate or tropical species, such as the Asian brown planthopper, *Nilaparvata lugens*, which has
236 a ULT₅₀ of 41.8 to 42.5°C (Piyaphongkul *et al.* 2012), this nevertheless demonstrates a considerable
237 capacity to cope with current conditions (Convey 1996a). Indeed ULTs above 35°C are high when
238 considering the temperatures these Antarctic species typically experience during the summer. Tinytag
239 measurements on Léonie Island through February and March did not show surface temperatures
240 exceeding 15°C (Fig. 1). Likewise, temperatures recorded between 2002 and 2008 on nearby
241 Anchorage Island did not rise higher than 20°C. However, it should be noted that diurnal fluctuations
242 in some microhabitats and years can exceed 30°C for short periods of minutes to hours (Fig. 3; Smith
243 1988; Convey 1996a). Both *C. antarcticus* and *A. antarcticus* were also able to survive for over 43 d
244 at 10°C (Fig. 6) and showed survival at 40°C over periods of 5-20 min (Fig. 7). These two species are

245 therefore well adapted to survive the summer on Léonie Island and have some capacity to tolerate
246 higher temperatures than those that are currently experienced (Day *et al.* 2009; Convey *et al.* 2009).

247 Survival alone is not an accurate measure of fitness. Success is also influenced by the sub-lethal
248 characteristics of a species, such as the effects of heat stress on reproduction and development. In
249 many species, survival is possible at extremes of temperature, but they are then unable to fully
250 develop and reproduce once usual temperatures are restored (Shreve *et al.* 2004). Invertebrates are
251 also hampered during temperature extremes (Piyaphongkul *et al.* 2012; Powell and Bale 2006; Shreve
252 *et al.* 2004; Wang and Kang 2003). Uncoordinated movement 72 h after high temperature treatment in
253 the current study (> 30°C, data not shown) indicates that permanent damage might have been incurred
254 as a result of high temperature exposure, which could subsequently result in impaired development
255 and reproduction. Thus, whilst *C. antarcticus* and *A. antarcticus* can survive above 35°C, negative
256 effects on them and their communities might be seen at much lower temperatures.

257 4.2. Interspecific comparisons

258 *Alaskozetes antarcticus* showed significantly greater heat tolerance than *C. antarcticus*. This capacity
259 was demonstrated across all experimental procedures; *A. antarcticus* had a higher ULT (Fig. 3),
260 exhibited higher survival of acute heat exposure (Fig. 7) and survived for longer at 10°C (Fig. 6).
261 Previous studies also show that mite species tend to have higher heat tolerance than Collembola
262 (Deere *et al.* 2006; Sinclair *et al.* 2006). It was initially hypothesised that higher desiccation resistance
263 accounted for the greater heat tolerance in *A. antarcticus*. This is because *C. antarcticus* is a hygric
264 species, with little or no control of water loss (Convey *et al.* 2003; Worland and Block 1986, 2003),
265 whereas *A. antarcticus* is a mesic species and has good control over its water content (Benoit *et al.*
266 2007; Worland and Block 1986). However, there was little difference in water loss with temperature
267 and no significant difference in the water lost between the two species over the experimental durations
268 under all temperature treatments (Table 1). It seems, therefore, that *A. antarcticus* possesses a more
269 adaptive heat tolerance physiology than *C. antarcticus*. Possible physiological adaptations capable of
270 operating over these experimental timescales include the activation of heat shock proteins (Schill *et al.*
271 *et al.* 2004; Rinehart *et al.* 2006; Michaud *et al.* 2008) and membrane remodelling (Hazel 1995).

272 The results of this study suggest that, in a rapidly warming Antarctic, *A. antarcticus* would have some
273 advantage over *C. antarcticus*. Climate manipulation studies also suggest that mites will be favoured
274 over Collembola under warming. In both the Arctic (Coulson *et al.* 1996) and the Antarctic (Bokhorst
275 *et al.* 2008; Convey *et al.* 2002), Collembola numbers decreased significantly under artificially
276 warmed conditions over three years, while mite numbers remained largely unchanged. However,
277 Webb *et al.* (1998) proposed that oribatid mite populations are slow to show a response to short-term
278 environmental changes and that manipulations longer than those used in the aforementioned studies
279 are required to identify any effect. A further consideration is how the heat tolerance of these species
280 relates to their behaviour. Collembola are more mobile than oribatid mites, and so may be better able
281 to relocate to habitats in their preferred temperature range. Consequently, the more rapid movement of
282 *C. antarcticus* could compensate for reduced heat tolerance in this species. It is therefore only in a
283 uniform thermal environment where *A. antarcticus* would be favoured (see also Hayward *et al.* 2003).

284 4.3. Physiological plasticity

285 The Antarctic hosts a diversity of microclimates. In some of these, the daily temperature can fluctuate
286 by as much as 50°C (Convey 1996a). In the current study, measurements on a rock surface showed
287 temperature variation approaching or exceeding 30°C on a diurnal timescale (Fig. 2). Similar patterns

288 have been reported in other microhabitats; temperatures within the moss cushion, *Schistidium*
289 *antarctici*, were shown to cycle between -9.2°C and 42.8°C over 24 h (Smith 1988). It could,
290 therefore, be to an invertebrate's advantage to adapt quickly to changes in temperature. One means of
291 tracking temperature changes is via a process termed Rapid Heat Hardening (RHH), which is the
292 rapid induction of heat tolerance over minutes to hours (Benoit *et al.* 2009). Both *C. antarcticus* and
293 *A. antarcticus* showed evidence of RHH, with enhanced survival at their discriminating temperatures
294 following warming at the three rates of 0.1, 0.2 and 0.5°C min⁻¹ (Fig. 5). The rate of 0.5°C min⁻¹ gave
295 the greatest increase in survival for both species, and was likely due to the reduced time spent at
296 harmful temperatures. Overall, the RHH response was small, however, giving an average rise in
297 survivorship of only 38% across all treatments. It is possible that RHH has more of an influence on
298 the sub-lethal characteristics of *C. antarcticus* and *A. antarcticus*. Although there is as yet little
299 support for this occurring in other species, there is ample evidence of a sub-lethal influence during
300 Rapid Cold Hardening (RCH) (Denlinger and Lee 2010). For example, courting, reproduction, and the
301 Critical Thermal minimum (CT_{min} – loss of coordination at low temperatures) were all improved in
302 *D. melanogaster* following RCH (Shreve *et al.* 2004; Kelty and Lee 1999).

303 Physiological plasticity can also be seen over longer timescales in the form of experimental
304 acclimation (Lachenicht *et al.* 2010). The nematodes, *Steinernema carpocapsae* and *Steinernema*
305 *feltiae*, for instance, showed enhanced heat tolerance, and higher virulence under heat stress, when
306 reared at higher, and thus acclimatory, temperatures (Jagdale and Grewal 2002). Similarly, heightened
307 heat tolerance following time at higher rearing temperatures was exhibited in both marine and
308 terrestrial mites found on Marion Island (Deere *et al.* 2006). In the current study, a one week
309 acclimation at 10°C had no significant impact on survivorship in either *C. antarcticus* or *A.*
310 *antarcticus* (Fig. 8). A null response in the sub-Antarctic collembolan *Tullbergia bisetosa*, and a
311 decline in heat tolerance in *C. antarcticus*, was also shown following acclimation at 15°C (Slabber *et*
312 *al.* 2007).

313 Physiological plasticity across generations may also be important; species with sufficient genetic
314 variation that produce progeny with higher physiological thermal optima may end up as the 'winners'
315 in scenarios of climate warming (Somero 2010). In a number of species, life at low temperatures has
316 resulted in the loss of physiology suited to warming conditions (Somero 2010). The polar marine
317 ectotherms of the Southern Ocean provide a particularly good illustration. These species are
318 stenothermal and have experienced a narrow range of low temperatures for millions of years (at
319 present -1.9 to +1.8°C or much less) (Somero 2010). As a result, many have lost their ability to initiate
320 a heat shock response (Clark *et al.* 2009). The same might be true of polar terrestrial invertebrates
321 with regard to their physiological plasticity, and if so these will therefore become less successful as
322 climate change intensifies. However, it has also been suggested that the greater thermal variability
323 typical of polar terrestrial environments will preserve heat tolerance adaptation (Peck *et al.* 2006).
324 Indeed, the climatic variability hypothesis (Stevens 1989) suggests that the greater thermal variability
325 at higher latitudes means that invertebrates must have a greater physiological range and subsequently
326 retain physiological plasticity at higher temperatures. Also of note are the long generation times of
327 these animals, which frequently extend to five years or more, and therefore limit their ability to adapt
328 across generations (Convey 1994, 1996b).

329 **5. Conclusion**

330 It has been suggested that the thermal sensitivity of invertebrates to temperature change decreases
331 from the tropics to the poles (Deutsch *et al.* 2008). This statement is supported by the current study,
332 which shows that both *C. antarcticus* and *A. antarcticus* have scope with which to tolerate current and

333 future conditions. Warming may even alleviate the stresses experienced by these invertebrates and
334 provide an opportunity for population growth. If these species are assumed to be characteristic of
335 other Collembola and Acari in the maritime Antarctic, a positive impact on the community and on
336 ecosystem functions such as nutrient cycling, may also be seen.

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Figure and Table legends

Fig. 1. Surface temperature beneath a rock on Léonie Island, near Rothera Research Station, Adelaide Island, between 24th January and 12th March 2012.

Fig. 2. Temperature on a rock surface outside the Bonner Laboratory at Rothera Research Station, Adelaide Island, between 5th and 21st February 2012.

Fig. 3. Mean percentage survival of *C. antarcticus* and *A. antarcticus*, following exposure to progressively higher temperatures (30 to 37°C – *C. antarcticus*, 30 to 40°C – *A. antarcticus*) for 1h, before cooling at 0.2°C min⁻¹ to 4°C. Means ± S.E.M. are presented for three replicates of 10 individuals. Survival was assessed 72 h after treatment. Means with the same letter (*A. antarcticus*) and same number of * symbols (*C. antarcticus*) are not significantly different within each species group at $P < 0.05$ (Kruskal-Wallis test and Tukey's multiple range test, respectively). *A. antarcticus* was not tested at 33 or 34°C.

Fig. 4. Mean percentage survival of *C. antarcticus* and *A. antarcticus*, following direct exposure to progressively higher temperatures (30 to 36°C for *C. antarcticus* and 36 to 40°C for *A. antarcticus*) for 1 h, before cooling at 0.2°C min⁻¹ to 4°C. Means ± S.E.M. are presented for three replicates of 10 individuals. Survival was assessed 72 h after treatment. Means with the same letter (*A. antarcticus*) and same number of * symbols (*C. antarcticus*) are not significantly different within each species group at $P < 0.05$ (Kruskal-Wallis test).

Fig. 5. Mean percentage survival of *C. antarcticus* and *A. antarcticus*, following exposure to the discriminating temperature (35°C – *C. antarcticus*, 39.5°C – *A. antarcticus*) for 1 h, after being warmed to the discriminating temperature at one of three rates (0.5, 0.2 or 0.1°C min⁻¹). Means ± S.E.M. are presented for three replicates of 10 individuals. Survival was assessed 72 h after treatment. Means with the same letter (*A. antarcticus*) and same number of * symbols (*C. antarcticus*) are not significantly different within each species group at $P < 0.05$ (Tukey's multiple range test).

Fig. 6. Mean percentage survival of *C. antarcticus* and *A. antarcticus* at +4 and +10°C over a period of 46 (*C. antarcticus*) and 49 d (*A. antarcticus*). Means ± S.E.M. are presented for five replicates of 10 individuals. Means with the same letter (*A. antarcticus*) and same number of * symbols (*C. antarcticus*) are not significantly different within each species group at $P < 0.05$ (Kruskal-Wallis test).

Fig. 7. Mean percentage survival of *C. antarcticus* and *A. antarcticus* following exposure to 40°C for 5, 10 or 20 min. Means ± S.E.M. are presented for three replicates of 10 individuals. Survival was assessed 72 h after treatment. Means with the same letter (*A. antarcticus*) and same number of * symbols (*C. antarcticus*) are not significantly different within each species group at $P < 0.05$ (Kruskal-Wallis test).

Fig. 8. Mean percentage survival, following exposure to 33, 34 and 35°C – *C. antarcticus*, and 39, 39.5 and 40°C – *A. antarcticus*) for 1 h, before cooling at 0.2°C min⁻¹ to 4°C. Both species were held at 10°C for one week prior to experimentation. Means ± S.E.M. are presented for three replicates of 10 individuals. Survival was assessed 72 h after treatment. Means with the same letter (*A. antarcticus*) and same number of * symbols (*C. antarcticus*) are not significantly different within each species group at $P < 0.05$ (Tukey's multiple range test).

Table 1. Mean percentage water loss of *C. antarcticus* and *A. antarcticus*, following exposure to 30, 35 and 40°C for 1 h, prior to cooling at 0.2°C min⁻¹ to 4°C. Water content of control sample held at 4°C for 1 h also given. Means ± S.E.M. are presented for five replicates of 10 individuals.

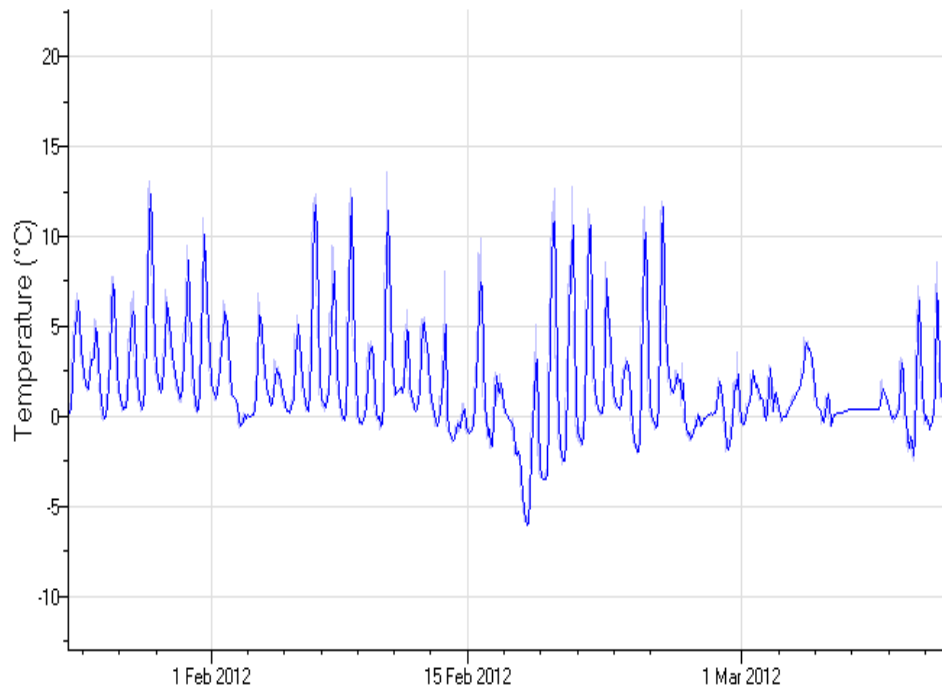


Figure 1

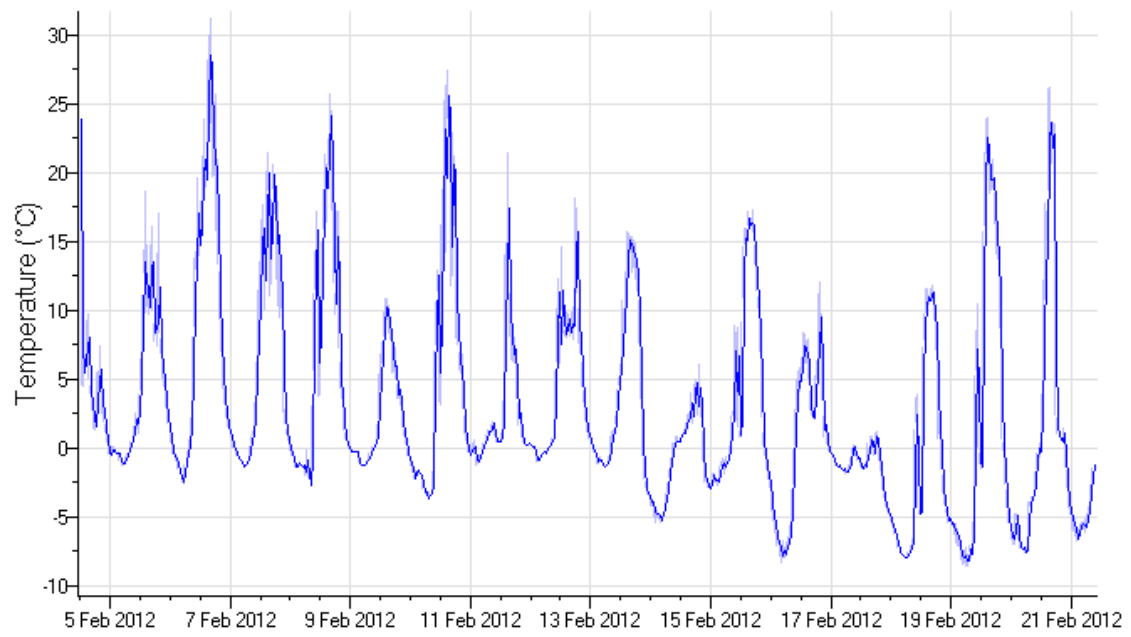
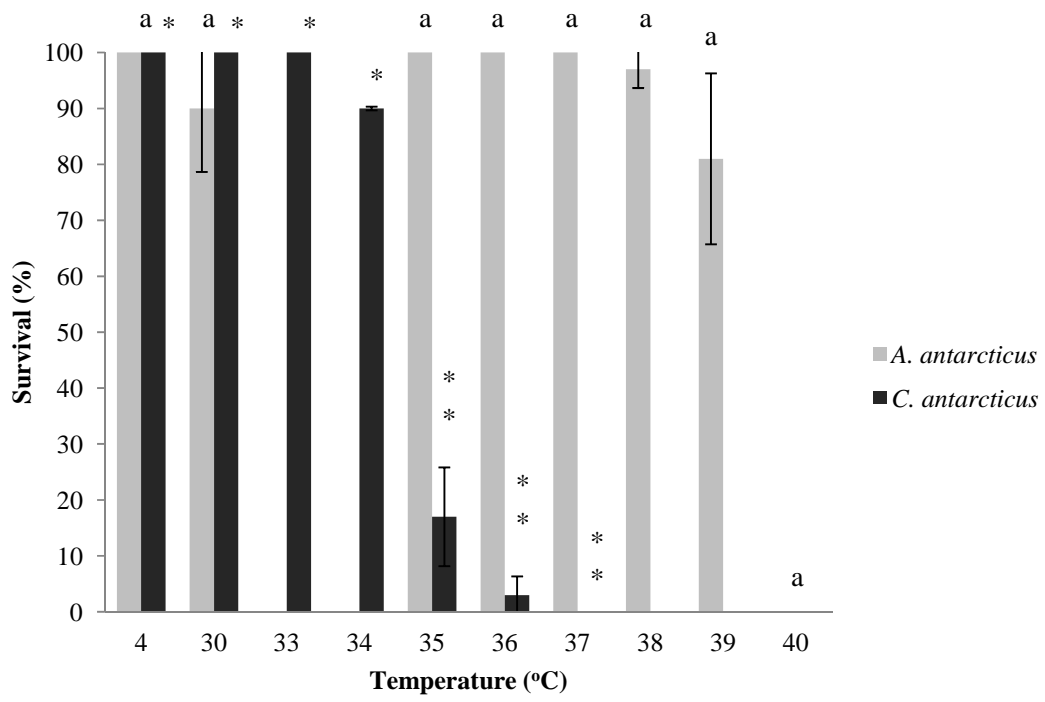


Figure 2



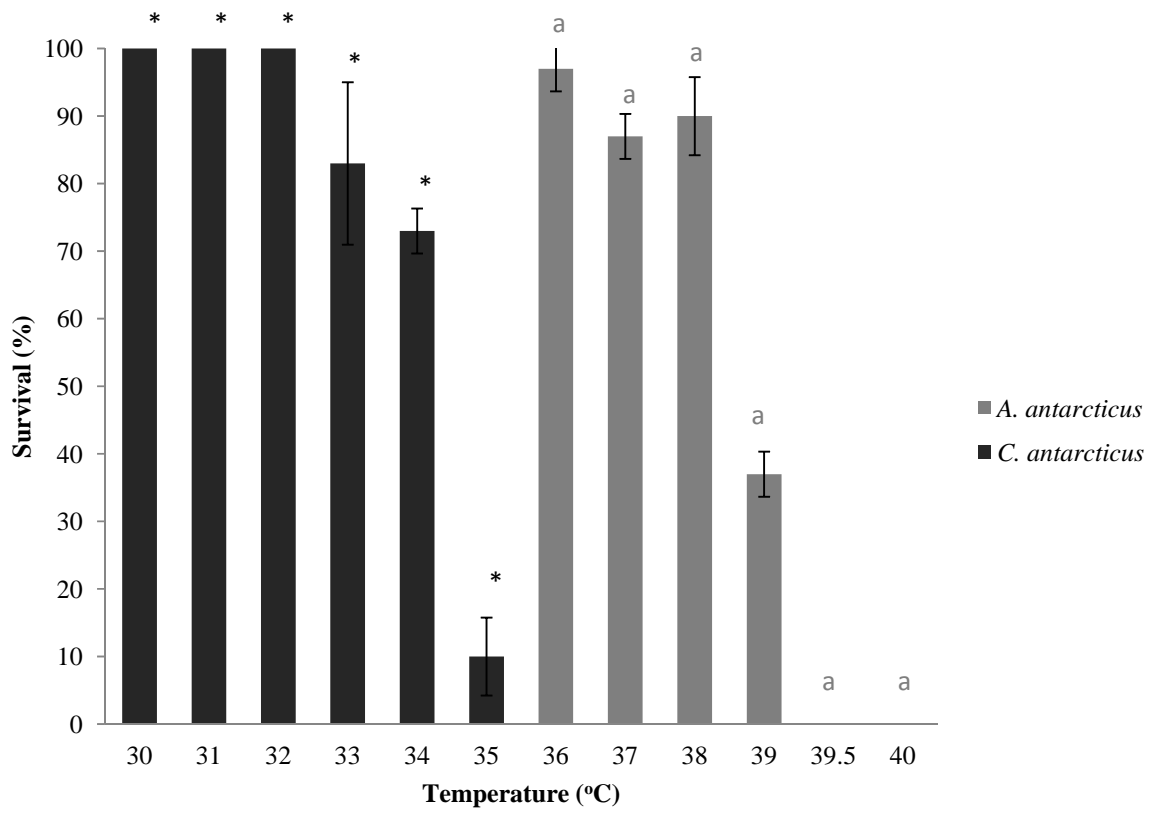


Figure 4

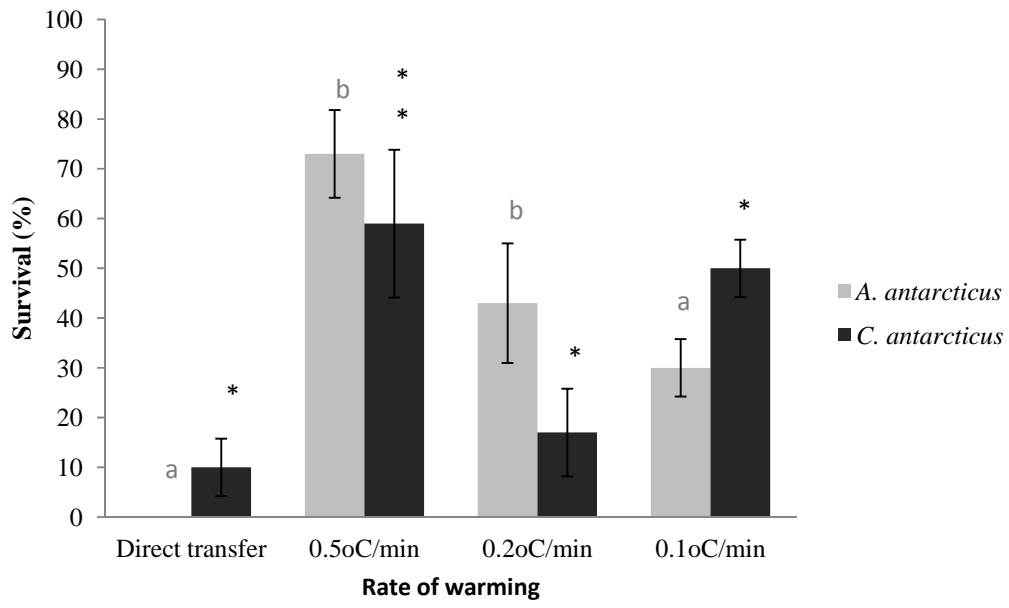


Figure 5

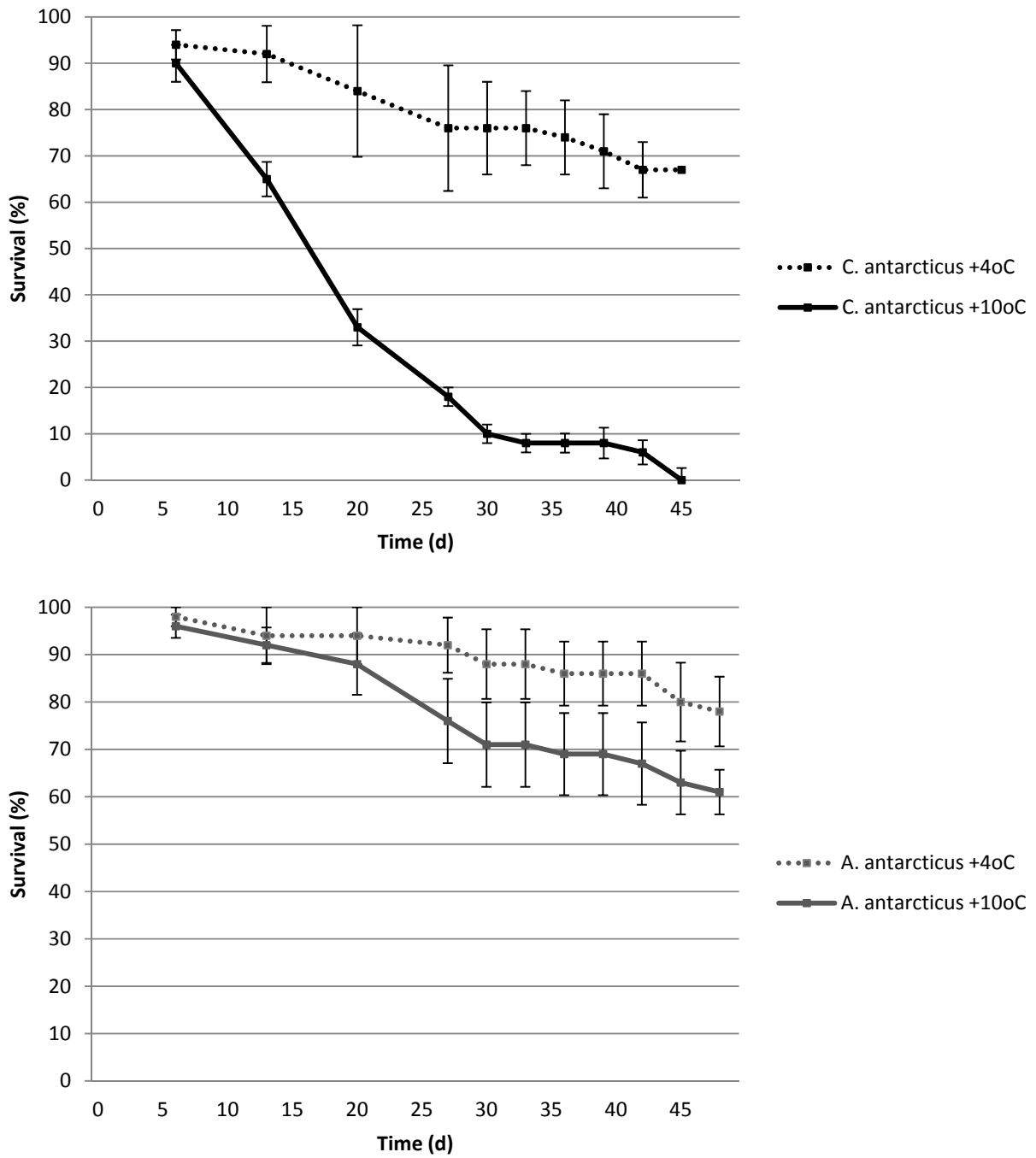


Figure 6

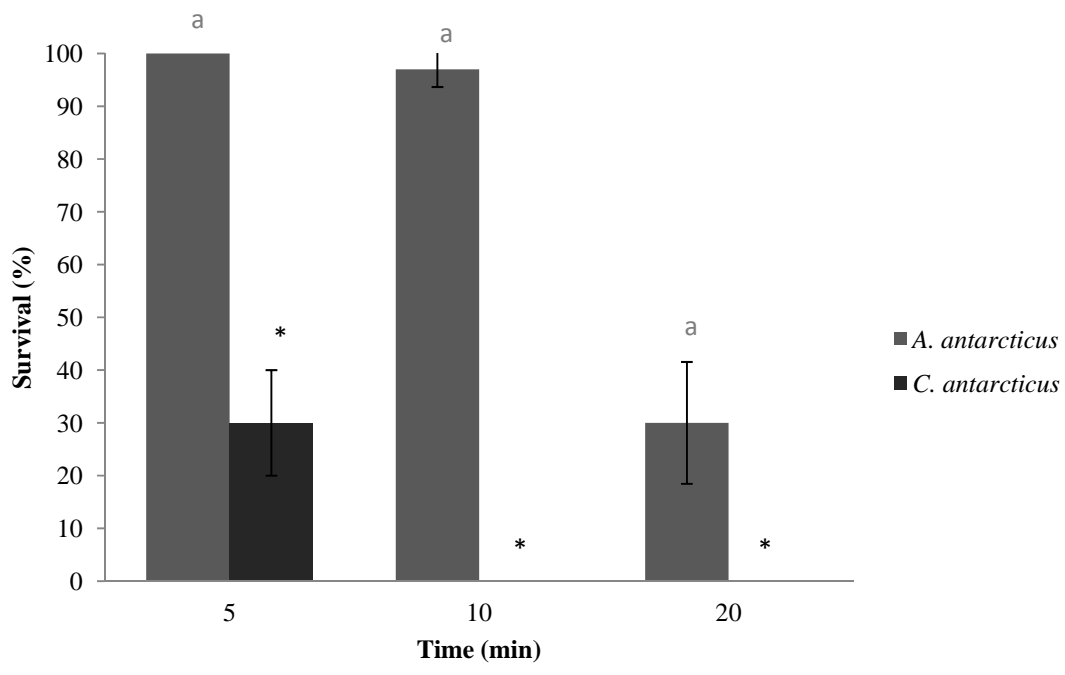


Figure 7

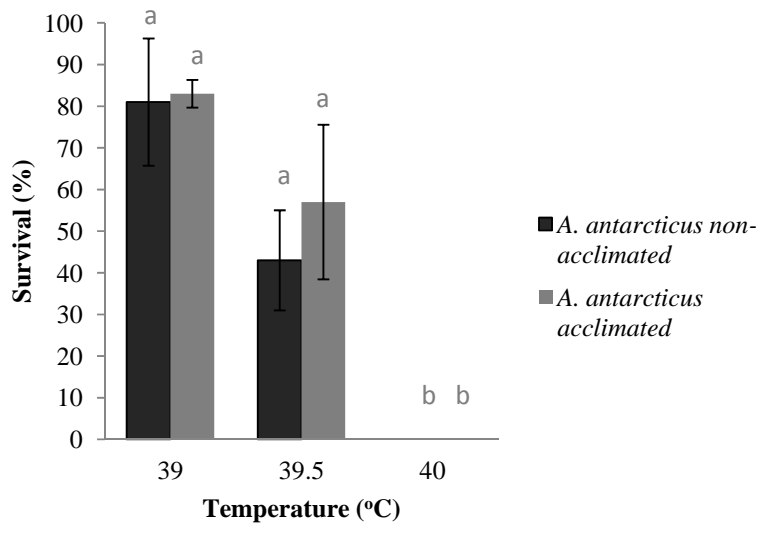
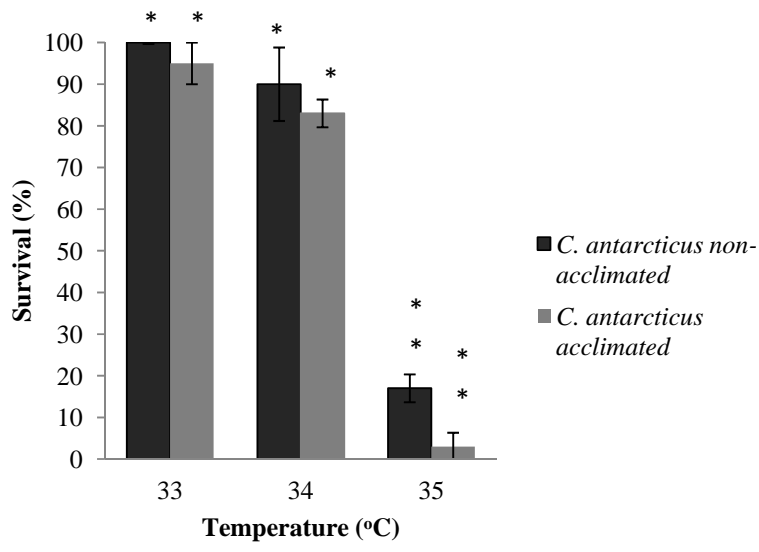


Figure 8

Table

Temperature (°C)	Water Content change (%)	
	<i>C. antarcticus</i>	<i>A. antarcticus</i>
4	3.19 ± 2.86	-0.02 ± 1.82
30	-1.58 ± 1.76	0.12 ± 0.38
35	0.88 ± 3.65	-3.82 ± 1.61
40	-6.68 ± 0.81	-2.08 ± 0.45

Table 1