Are the Antarctic dipteran, *Eretmoptera murphyi*, and Arctic collembolan, *Megaphorura arctica*, vulnerable to rising temperatures?

M. J. Everatt[a*], P. Convey[b, c, d], M. R. Worland[b], J. S. Bale[a] and S. A. L. Hayward[a]

[a] *School of Biosciences, University of Birmingham, Edgbaston, Birmingham B15 2TT, UK*

[b] *British Antarctic Survey, Natural Environment Research Council, High Cross, Madingley Road, Cambridge, CB3 0ET, UK*

[c] *National Antarctic Research Center, IPS Building, University Malaya, 50603 Kuala Lumpur, Malaysia*

[d] *Gateway Antarctica, University of Canterbury, Private Bag 4800, Christchurch 8140, New Zealand*

*Corresponding author. Tel.: + 44 789 620 1770. *Email address: mxe746@bham.ac.uk (M. J. Everatt).*

Abstract

Polar terrestrial invertebrates are suggested as being vulnerable to temperature change relative to lower latitude species, and hence possibly also to climate warming. Previous studies have shown Antarctic and Arctic Collembola and Acari to possess good heat tolerance and survive temperature exposures above 30°C. To test this feature further, the heat tolerance and physiological plasticity of heat stress were explored in the Arctic collembolan, *Megaphorura arctica*, from Svalbard, and the Antarctic midge, *Eretmoptera murphyi*, from Signy Island. The data obtained demonstrate considerable heat tolerance in both species, with upper lethal temperatures ≥ 35 °C (1 h exposures), and tolerance of exposure to 10 and 15°C exceeding 56 d. This tolerance is far beyond that required in their current environment. Average microhabitat temperatures in August 2011 ranged between 5.1 and 8.1°C, and rarely rose above 10°C, in Ny-Ålesund, Svalbard. Summer soil microhabitat temperatures on Signy Island have previously been shown to range between 0 and 10°C. There was also evidence to suggest that *E. murphyi* can recover from high temperature exposure and that *M. arctica* is capable of rapid heat hardening. *Megaphorura arctica* and *E. murphyi* therefore have the physiological capacity to tolerate current environmental conditions, as well as future warming. If the features they express are characteristic more generally, such polar terrestrial invertebrates will likely fare well under climate warming scenarios.

Keywords: Rapid heat hardening; Acclimation; Thermal sensitivity; Recovery; Diptera; Collembola

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1. Introduction

It is becoming increasingly clear that many terrestrial invertebrates resident in the Antarctic and Arctic are remarkably heat tolerant. Block *et al.* (1994), Hodkinson *et al.* (1996) Deere *et al.* (2006), Everatt *et al.* (2013), Sinclair *et al.* (2006) and Slabber *et al.* (2007) have shown survival above 30°C in a number of polar Collembola and Acari, including 'model' polar species, such as *Cryptopygus antarcticus*, *Megaphorura arctica* (Tullberg) and *Alaskozetes antarcticus*. In the Antarctic, typical summer microhabitat temperatures range between 0 and 10°C, whereas in the Arctic, the temperature range is slightly higher (Davey *et al.*, 1992; Coulson *et al.*, 1996; Hodkinson *et al.*, 1996; Block *et al.*, 2009). Temperatures above 30°C have been recorded, but are rare, occurring only in certain microhabitats for brief periods of minutes to hours and not consistently between years (Smith, 1988; Convey, 1996; Hodkinson *et al.*, 1996; Everatt *et al.*, 2013). It is generally assumed that invertebrates respond behaviourally to such temperatures, and rapidly relocate if or when they become stressful

(Hayward *et al.*, 2003). Polar Collembola and Acari therefore have ample capacity to tolerate current conditions. Annual mean temperatures have risen by over 2°C in parts of the polar regions in the last 50 years and similar, possibly more extreme, increases are predicted to occur over the next half century (Convey *et al.*, 2009; Turner *et al.*, 2009). Such warming is within the physiological thresholds of the resident Collembola and Acari (Block *et al.*, 1994; Hodkinson *et al.*, 1996; Deere *et al.*, 2006; Sinclair *et al.*, 2006; Slabber *et al.*, 2007; Everatt *et al.*, 2013).

The capacity of Antarctic invertebrates to tolerate future warming is in line with Deutsch *et al.* (2008), who suggested that the sensitivity of terrestrial invertebrates to a temperature change decreases with increasing latitude (see also Addo-Bediako *et al.*, 2000). It has even been suggested that climate warming might alleviate the stresses of living in a low temperature environment and benefit some polar species (Convey, 2006, 2011; Bale & Hayward, 2010). This proposal is consistent with the results of some climate manipulation studies which have shown warming to increase populations of invertebrates in Antarctic communities (Convey *et al.*, 2002; Convey & Wynn-Williams, 2002; Day *et al.*, 2009). Convey *et al.* (2002) and Day *et al.* (2009) , however, highlighted that continued water availability during warming is crucial , and some Arctic studies have shown declines or no change following artificial increases in temperature alone (Coulson *et al.*, 1996; Webb *et al.*, 1998). Manipulation studies should therefore be treated with care for they are complex in their effects and often inconsistent in the consequences identified, emphasising that the changes observed are strongly influenced by the specific microhabitat characteristics and invertebrate populations investigated, as well as the seasonal timing and duration of the study (Convey *et al.*, 2002, 2003; Bokhorst *et al.*, 2011, 2013). Climate manipulation studies also lack an assessment of the potential impact of possible new colonists as a result of climate change.

The first studies investigating heat tolerance in polar terrestrial invertebrates concentrated on Arctic species, including three species of Collembola (*M. arctica*, *Onychiurus groenlandicus* and *Hypogastrura tullbergi*) and four species of mite (*Camisia anomia*, *Diapterobates notatus*, *Hermannia reticulata* and *Ceratoppia hoeli*) (Block *et al.*, 1994; Hodkinson *et al.*, 1996). The current study also uses *M. arctica* and, although it revisits this collembolan's short-term and long-term tolerance to heat, the methods used here take into account more ecologically relevant rates of warming and cooling. The ability of the collembolan to acclimate using rapid heat hardening (RHH) is also investigated for the first time. *Megaphorura arctica* (formerly *Onychiurus arcticus*) is a pale yellow collembolan found in the palaearctic regions (Fjellberg, 1994). This collembolan is common under rocks and within moss beneath bird cliffs, where it commonly aggregates in groups of 100 or more individuals (Worland, 1996). Partly because of its ability to cryoprotectively dehydrate, *M. arctica* is considered a "model" in Arctic terrestrial invertebrate ecophysiological research (Worland *et al.*, 1998).

Previous Antarctic studies have examined heat tolerance in Collembola and Acari (Deere *et al.*, 2006; Sinclair *et al.*, 2006; Slabber *et al.*, 2007; Everatt *et al.*, 2013), but have given little attention to Antarctic Diptera. In this study, the capacity of the midge, *Eretmoptera murphyi* (Schaeffer), to respond to high temperature is investigated, including an assessment of its CTmax, and its ability to recover from heat stress. *Eretmoptera murphyi* is native and endemic to the sub-Antarctic island of South Georgia (55^{o}S 37^{o}W). Likely as a result of plant transplant experiments in the 1960s, this midge was accidentally transferred to maritime Antarctic Signy Island (60^{o}S 45^{o}W) and is now established as a non-native species there (Block *et al.*, 1984; Convey & Block, 1996). The species has since spread to cover an area > 2000 m^{2} and is now having a significant impact on the local environment (Hughes *et al.*, 2013). *Eretmoptera murphyi* is closely related to the endemic *Belgica antarctica* of the maritime Antarctic (Allegrucci *et al.*, 2012). While heat tolerance has received some attention in the latter species, the subject has not been explored in detail (Hayward *et al.*, 2007; Benoit *et al.*, 2009).

~~This study determined the capacity of *M. arctica* and *E. murphyi* to cope with continued climate warming by measuring both short (hours) and long term (weeks) heat tolerance, the plasticity of tolerance, thermal activity thresholds and the role of recovery. This will help to predict winners and losers under different environmental scenarios, and thus how community composition may change in a warming climate.~~

2. Materials and methods

2.1. Invertebrate collection and storage conditions

Summer-acclimatised individuals of *M. arctica* were collected from moss-covered slopes at Krykkefjellet and Stuphallet, near Ny-Ålesund, Spitsbergen, Svalbard (78°55’N, 11°56’E) between 14th and 24th August 2011. Summer acclimatised larvae of *E. murphyi* were collected from soil and moss on Signy Island (60°S, 45°W) near to the British Antarctic Survey Signy Research Station between January and March 2012. These were subsequently transported to the University of Birmingham under refrigerated conditions and held in plastic boxes containing substratum from the site of collection at 4-5°C (0:24 L:D). The duration of travel was approximately 2 d from the Arctic and two months from the Antarctic. Numbers of *M. arctica* were limited, and as a result this species was not assessed for the effect of recovery or heat coma (sub-sections 2.4 and 2.6).

2.2. Microhabitat temperatures

The thermal regime experienced by *M. arctica* during the summer was measured at four different sheltered sites (laid on surface, but covered by rocks), two at Krykkefjellet and two at Stuphallet, between 17th and 24th August 2011. Temperature was measured at each site using a Tinytag Transit 2 Datalogger, and data were uploaded using Tinytag Explorer Software (Gemini Data Loggers, Chichester, U.K.). Fieldwork was not conducted on Signy Island as part of this study and microhabitat temperature data for *E. murphyi* are inferred from previous studies.

2.3. Upper Lethal Temperatures (ULTs)

The upper temperature at which a species is no longer able to survive (ULT) was determined for *M. arctica* and *E. murphyi* by warming individuals at 0.2°C min^{-1} from 4°C (rearing temperature) to progressively higher temperatures (30 to 36°C for *M. arctica*, 35 to 39°C for *E. murphyi*). Individuals were subsequently held at the target temperature for 1 h, before being cooled back to 4°C at the same rate. Three replicates of 10 individuals of each species were placed in Eppendorf tubes, inserted into glass test tubes that were then plugged with sponges, and placed inside an alcohol bath (Haake Phoenix II C50P, Fisher Scientific UK Ltd, Loughborough, U.K.), prior to each experimental treatment. Control groups were handled, and exposed, in the same way at 4°C. The temperature experienced by the invertebrates was measured by placing a thermocouple within an identical Eppendorf tube into one of the glass test tubes. Humidity typically remains high within this experimental set-up, and is assumed not to impact survival based on previous findings (Everatt *et al.*, 2013). At the end of experimental treatments, individuals were rapidly transferred (over ice) from the Eppendorf tubes into plastic universal tubes containing substratum, and returned to the rearing conditions (see also Everatt *et al.* 2013). Survival, defined by individuals moving either spontaneously or in response to gentle contact stimulus, was assessed 72 h after treatment.

2.4. Activity thresholds

Activity thresholds were assessed for *E. murphyi* only, within an aluminium block arena. The temperature within the arena was regulated using an alcohol bath, and activity monitored using a digital video camera with a macro lens (see Hazell *et al.*, 2008). Thirty larvae in groups of 10 were transferred into the arena and allowed to settle before video recording (Studio Capture DT, Studio86Designs, Lutterworth, UK) and the alcohol bath programme began. The temperature of the arena was raised from 4 to 40°C at two different rates, 0.2 and 0.1°C min^{-1}. The temperature at which each individual larva last moved its body was recorded.

2.5. Long-term heat tolerance

Five replicates of 10 individuals of *M. arctica* and *E. murphyi* were transferred to either 4, 9 or 15°C for up to 210 d. Individuals were held in universal tubes with a base of moist Plaster of Paris and a small amount of substratum within an incubator or temperature controlled room (9°C). The temperature inside the incubators and room was checked using a Tinytag Transit 2 Datalogger. Survival was assessed every 7 d (see also Everatt *et al.*, 2013).

2.6. The effect of recovery on heat tolerance

To test the effect of recovery at cooler temperatures on heat tolerance, three replicates of 10 individuals were exposed to one of three treatments: i) 25°C for 10 d, ii) ten 24 h exposure periods at 25°C, each separated by 1 h recovery at 4°C and iii) ten 24 h exposure periods at 25°C, each separated by 2 h recovery at 4°C. Larvae were kept in plastic universal tubes with a base of moist Plaster of Paris and substratum. Transfer from and to 25°C was followed and preceded by 1 h at 15°C to avoid cold and heat shock. Survival was assessed after each day (treatment i) or 24 h exposure period (treatment ii and iii).

2.7. Rapid Heat Hardening (RHH)

2.7.1. Determination of the discriminating temperature

The discriminating temperature is defined as the temperature at which there is 10-20% survival (Lee *et al.*, 1987). Three replicates of 10 individuals of *M. arctica* were exposed directly (without ramping at 4°C) to progressively higher temperatures (30 – 36°C) for 1 h, before cooling to 4°C at 0.2°C min^{-1}. Invertebrate collection and handling, controls, thermocouple use, recovery and survival assessment were as described in sub-section 2.3. Preliminary trials on *E. murphyi* suggested that the midge did not show RHH (data not shown) and so RHH was only assessed in *M. arctica*.

2.7.2. Induction of RHH

To test for the RHH response, three replicates of 10 individuals were warmed to the discriminating temperature at three different rates, 0.5, 0.2 and 0.1^{o}C min^{-1}. As before, samples were held for 1 h at the discriminating temperature and then cooled back to 4^{o}C at 0.2^{o}C min^{-1}.

2.8. Statistical analyses

The Kolmogorov-Smirnov test was used to confirm whether survival and heat coma data were normally distributed. Normally distributed data were analysed using analysis of variance (ANOVA) and Tukey's multiple range test, and non-normally distributed data were analysed using either the Mann-Whitney U test or the Kruskal-Wallis test.

3. Results

3.1. Arctic site microhabitat temperatures

Temperatures remained above 3^{o}C throughout the period 17th - 24th August 2011 (Fig. 1) at both locations. At Stuphallet, temperatures averaged 6.6^{o}C when combining data from both Tinytag sites, and at Krykkefjellet, 7.8^{o}C. Temperatures deviated considerably from these averages, rising as high as 16^{o}C at Krykkefjellet. The first 3 d were noticeably warmer, averaging 0.8 and 1.3^{o}C higher than over the whole period in Stuphallet and Krykkefjellet, respectively. The time at which these temperatures were recorded also coincided with the warmest period on Svalbard to date (Coulson, S. J., personal communication).

3.2. Upper Lethal Temperatures (ULTs)

Individuals of *M. arctica* survived up to 35^{o}C, while larvae of *E. murphyi* survived up to 39^{o}C (Fig. 2). The difference in survival between the two species at 35^{o}C was significant ($F_{1,4} = 841.000$, $P <$

0.05 one-way ANOVA, variances not equal). Survival in both species declined rapidly, falling by > 80%, within 2-3^{o}C as they approached the ULT.

3.3. Heat coma

The point at which *E. murphyi* larvae no longer showed signs of movement (heat coma) occurred above 31^{o}C under two different rates of warming, 0.1 (31.4 ± 0.14^{o}C) and 0.2^{o}C min^{-1} (32.3 ± 0.18^{o}C). The heat coma temperature was significantly higher under faster warming ($F_{1, 52} = 18.523$, $P < 0.05$ one-way ANOVA).

3.4. Long-term heat tolerance

Survival of both species was greatest at 4°C (Fig. 3). *Megaphorura arctica* tolerated 9°C for 91 d, while survival of *E. murphyi* was still above 75% following 56 d, when the experiment finished. Both species tolerated a 15^{o}C exposure for at least 56 d (Fig. 3), at which point survival was greater in *E. murphyi* (32%) than in *M. arctica* (13%). Survival of *E. murphyi* larvae at all temperatures was not significantly different after 35 d ($P > 0.05$ Tukey's multiple range test, variances not equal in some cases). However, survival after 56 d was significantly lower for larvae exposed to 15°C compared to 4 or 9°C ($P < 0.05$ Tukey's multiple range test). Survival of *E. murphyi* at 9 or 4°C did not differ significantly for any of the durations tested ($P > 0.05$ Tukey's multiple range test).

3.5. Effect of recovery on heat tolerance

Constant exposure to 25°C was lethal after 8 days, but survival increased with the introduction of daily recovery periods of 1 or 2 h at 4^{o}C (Fig. 4). This was significant overall ($F_2 = 9.064$, $P < 0.05$ two-way ANOVA), but the interaction between time and recovery was not significant ($F_{14} = 1.849$, $P > 0.05$ two-way ANOVA). Survival following a daily 2 h recovery period at 4^{o}C was greater than survival without recovery over the course of the entire experiment (day 2 to day 8), though the difference in survival was only significant after 6 d ($P < 0.05$ Tukey's multiple range test). A 1 h recovery period also gave greater survival for days 3-5 and day 8, but none of these differences were significant when analysed individually.

3.6. Rapid Heat Hardening (RHH)

3.6.1. Determination of the discriminating temperature

The discriminating temperature was determined to be 34.5°C for *M. arctica* (17% survival, Fig. 5).

3.6.2. RHH induction

Mean survival was significantly higher following warming at a rate of 0.1°C min^{-1} (73%), compared with survival after direct transfer (17%) to the discriminating temperature ($P < 0.05$ Tukey's multiple range test, variances not equal, Fig. 6). Survival was also raised following warming at a rate of 0.2 and 0.5°C min^{-1}, but this was not significant ($P > 0.05$ Tukey's multiple range test, variances not equal).

4. Discussion

As poikilothermic ectotherms, invertebrate body temperatures are determined by, and vary with, the external environment (Speight *et al.*, 2008). Invertebrates are therefore susceptible to injuries, and impaired development and reproduction, resulting from exposure to temperature alterations, such as those that may result from climate change (Bale & Hayward, 2010). Changes in temperatures due to climate warming are already known to affect invertebrate population dynamics and distribution (Parmesan, 1996; Walther *et al.*, 2002). For example, climate warming has led to the occurrence of extreme heat events, which have resulted in the mass mortality of tropical species, such as corals (Walther *et al.*, 2002). Tropical species are particularly vulnerable to temperature change as the upper temperatures they are able to tolerate lie very close to the upper temperatures experienced in their environment (Somero, 2010). Indeed, in some cases, tropical species live at temperatures which exceed their physiological optima (Somero, 2010). The current study considers whether polar species are also vulnerable to climate warming, by examining the heat tolerance and activity thresholds of the dipteran, *E. murphyi*, from the Antarctic, and further examining the heat tolerance capacity of the Arctic collembolan, *M. arctica*.

4.1. Basal tolerance

Both study species demonstrated considerable heat tolerance and showed survival above 34^{o}C for a period of 1 h (Fig. 2.). The heat coma temperature of *E. murphyi* was also very high, averaging above 31^{o}C following warming at 0.1 or 0.2^{o}C min^{-1}. Correspondingly, Everatt *et al.*, (2013) demonstrated survival up to 37^{o}C in the collembolan, *C. antarcticus*, and survival up to 40^{o}C in the mite, *A. antarcticus*, with similar results also being demonstrated in other Antarctic species (Deere *et al.*, 2006; Sinclair *et al.*, 2006; Slabber *et al.*, 2007). Block *et al.* (1994) and Hodkinson *et al.* (1996) likewise demonstrated high temperature survival in Arctic Acari and Collembola, including in *M. arctica*. The survival of *M. arctica* in this study was almost identical to that found by both Block et al. (1994) and Hodkinson *et al.* (1996), with all three studies showing virtually 100% survival after a 1 h exposure at 30^{o}C and an upper lethal temperature of 35^{o}C. Extending the exposure time to 3 h shifted survival downwards, but still gave survivorship above 30 ^{o}C (Block *et al.*, 1994; Hodkinson *et al.*, 1996). These temperatures are considerably higher than the temperatures experienced throughout the year in both the Antarctic and Arctic, including in summer and short duration extreme maxima. Temperature conditions varied across small spatial scales at both the Stuphallet and Krykkefjellet sites (Fig. 1), and microhabitat buffering would further protect terrestrial invertebrates from temperature extremes. *Megaphorura arctica* and *E. murphyi* therefore have considerable capacity to tolerate current summer conditions, including conditions that are unusually warm. These species also have the capacity to tolerate the much higher temperatures that will likely occur as a result of climate warming (Arctic Council, 2005; Convey *et al.*, 2009; Turner *et al.*, 2009), further consolidating the hypothesis set out by Deutsch *et al.* (2008).

In addition to the well characterised cellular damage inflicted during acute exposure to temperature extremes, injury can also occur following long-term exposure to more moderate temperatures (e.g. Czajka, 1990). To assess this, in the current study, both *M. arctica* and *E. murphyi* were exposed to 9 and 15^{o}C for several weeks. Although mortality occurred at these temperatures, both species survived well for the first 4 weeks, particularly at 9^{o}C (Fig. 3). The collembolan survived until 91 d at 9^{o}C and 56 d at 15^{o}C and, while the experiment was only carried out over 56 d for *E. murphyi*, mean survival at 9°C was still above 70%. Hodkinson *et al.* (1996) showed similarly good survival in *M. arctica* at 10^{o}C, with the collembolan surviving up to 196 d, with less than 50% mortality after 140 d, in that instance. Some individuals were also able to survive up to 68 d at 25^{o}C. Such tolerance is notable when compared with their Arctic microhabitat temperatures where, for only a few periods of no more than 24 hours, did temperatures exceed 9^{o}C, and at only one point did they exceed 15^{o}C (Fig. 1). Likewise, maximum temperatures 3 cm below the soil surface recorded between 1991 and 1993 did not exceed 14^{o}C (Hodkinson *et al.*, 1996). Temperatures above 9^{o}C are even more unusual on Signy Island or more generally in the maritime Antarctic (Davey *et al.*, 1992; Bokhorst *et al.*, 2008).

4.2. Physiological plasticity

Polar terrestrial invertebrates are exposed to a highly variable climate. At the extreme, temperatures can vary seasonally by up to 100^{o}C and daily by as much as 50^{o}C (Convey, 1996). Even in buffered microhabitats, there can be considerable variation. Terrestrial invertebrates will therefore not be exposed to either constant low or high temperatures, and will also be exposed to milder transitional temperatures, giving them an opportunity to recover from thermal injuries. It has already been shown in a number of invertebrates, including the firebug, *Pyrrhocoris apterus* (Kostal *et al.*, 2007), the beetle, *Alphitobius diaperinus* (Renault *et al.*, 2004; Kostal *et al.*, 2007; Colinet, 2011; Lalouette *et al.*, 2011), the parasitic wasp, *Aphidius colemani* (Colinet *et al.*, 2007), and the flesh fly, *Sarcophaga crassipalpis* (Dollo *et al.*, 2010), that pulses at warmer temperatures allow recovery from chilling injury. However, few studies have looked at analogous recovery from higher temperatures. In the current study, larvae of *E. murphyi* exhibited improved survival following daily recovery of 1 h, but particularly following 2 h, at 4^{o}C (Fig. 4). Greater survival with increasing duration of recovery has also been demonstrated in *A. diaperinus* (Colinet *et al.*, 2011). The lethal time (LT_{50}) of the beetle increased significantly from a 0.5 to 4 h recovery period. We speculate that longer recovery times than used in the current study would further enhance survival of *E. murphyi* larvae. Recovery from, and repair of, chilling injury has been shown to involve ion gradient homeostasis (Kostal *et al.*, 2007), induction of antioxidants (Lalouette *et al.*, 2011), and the up-regulation of key proteins (Colinet *et al.*, 2007). Analogous responses during recovery from high temperature injury may also occur. The up-regulation of heat shock proteins (HSPs), for example, is a common response to stressful conditions and is known as the ‘heat shock response’ because of its role in repair of heat shock injuries (Clark & Worland, 2008). Heat shock proteins help refold and stabilise proteins and other macromolecules during stress (Clark & Worland, 2008), and may also be involved with the recovery of microfilament dynamics (Tammariello *et al.*, 1999) and the regulation of apoptosis (Yi *et al.*, 2007). However, continuous HSP expression can be detrimental to cell cycle regulation (Hayward *et al.*, 2005), and so recovery periods between stress events are important. ~~An ability of polar terrestrial invertebrates to recover from high temperature conditions adds to their capacity to tolerate future warming.~~

A further means by which terrestrial invertebrates show physiological plasticity to high temperatures is through acclimation. However, the benefits of long-term acclimation (weeks to months) have so far been shown to be slight in polar terrestrial invertebrates. Following long-term acclimation, the widespread collembolan, *C. antarcticus*, and mite, *A. antarcticus*, were shown to either exhibit no improvement in their survival, or reduced survival, at high temperatures (Slabber *et al.*, 2007; Everatt *et al.*, 2013). Acclimation to higher temperatures can also occur over shorter timescales in the form of rapid heat hardening (RHH), which is defined as the rapid induction of heat tolerance over minutes to hours (Benoit *et al.*, 2009). Unlike rapid cold hardening, which has now been demonstrated in an increasing number of species (e.g. Kelty and Lee, 1999; Powell and Bale, 2004; Lee *et al.*, 2006; Owen *et al.*, 2013) including *E. murphyi* (Everatt *et al.*, 2012), RHH has been little explored. In polar terrestrial invertebrates, there is evidence for the effect only in *C. antarcticus* and *A. antarcticus* (Everatt *et al.*, 2013). The current study also showed an RHH response in *M. arctica* (Fig. 6). Following a warming rate of 0.1^{o}C min^{-1}, survival of *M. arctica* at 34.5^{o}C was increased by 56%, compared with survival after a direct transfer to the same temperature. However, survival was not raised at 34.5^{o}C following a rate of 0.2 or 0.5^{o}C min^{-1}. Greater survival at a rate of 0.1^{o}C min^{-1} can be explained by an increased time being available for *M. arctica* to respond physiologically. Greater time at protection-inducing temperatures has also been shown to give greater survival at lower temperatures, including in the western flower thrips, *Frankliniella occidentalis* (McDonald *et al.*, 1997). While 0.1^{o}C min^{-1} is a slow rate compared with other studies, rates will be slower still in nature

(Convey & Worland, 2000, also see Fig. 1). It is therefore speculated that, with more time to acclimate, *M. arctica* will show an even greater RHH response and thereby possess an additional mechanism improving its tolerance of temperature change.

4.3. Water availability and alien species in an era of climate warming

Although the direct impacts of high temperature are important, climate warming in the polar regions is also associated with changes in water availability and a heightened threat of alien species establishment. As climate warming intensifies, precipitation is predicted to increase at mid-high latitudes (Walther *et al.*, 2002; Ávila-Jiménez *et al.*, 2010). Under conditions of increased water availability, Antarctic invertebrates have been shown to thrive under warming, with increases in both Collembola and mite numbers (Convey *et al.*, 2002; Schulte *et al.*, 2008; Day *et al.*, 2009). However, rising temperatures are also expected to reduce snow cover and thaw ice earlier in the season, in turn resulting in the earlier evaporation of meltwater during the summer, which may instead leave invertebrates susceptible to desiccation (Callaghan *et al.*, 1992; Walther *et al.*, 2002; Ávila-Jiménez *et al.*, 2010). Under this scenario, polar terrestrial invertebrates have been shown to fare less well. Block *et al.* (1994) and Hodkinson *et al.* (1996) demonstrated the heat tolerance of collembola, including *M. arctica*, to be reduced when desiccated, as compared to those which were hydrated, while Coulson *et al.* (1996), Convey *et al.* (2002) and Day *et al.* (2009) showed decreasing numbers of Collembola under field conditions. Even so, because the heat tolerance of polar terrestrial invertebrates far exceeds buffered microhabitat temperatures, as shown in the current study, and because their heat tolerance still remains high under desiccation (Block *et al.*, 1994; Hodkinson *et al.*, 1996), we speculate that changes associated with climate warming will result in a positive change to the invertebrate fauna.

The probability of alien species establishment is also predicted to increase with climate warming. As temperatures rise, areas which were previously too stressful for invading organisms are beginning to open up (Frenot *et al.*, 2005; Chwedorzewska, 2009). Increasing human activity, as a result of scientific research and, more recently, tourism is also aiding the transfer of alien species by allowing them to bypass geographical and environmental barriers, particularly in the Antarctic (Frenot *et al.*, 2005; Chown *et al.*, 2012). Events in the sub-Antarctic provide a glimpse into what might happen, with native invertebrates of many islands suffering in the presence of invasive alien species (Frenot *et al.*, 2005; Chwedorzewska, 2009).

5. Conclusion

As with the polar Collembola and Acari that have been studied to date, the Antarctic midge, *E. murphyi*, possesses considerable heat tolerance that equips it to survive current and predicted future environmental conditions. This species and the Arctic collembolan, *M. arctica*, also demonstrate physiological plasticity with respect to recovery from high temperature, and RHH, respectively. Polar terrestrial invertebrates may therefore be protected from the harmful consequences of a temperature rise that may result from climate change, at least at a physiological level (Addo-Bediako *et al.*, 2000; Deutsch *et al.*, 2008). However, to identify likely consequences at community level, it is imperative that this is also balanced with other factors, including changes in water availability and competition from alien species, and that the sub-lethal characteristics of invertebrates, including development and reproduction, are also considered.

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Figure and Table Legends

Fig. 1. Surface temperature at four sites, two at Stuphallet (A) and two at Krykkefjellet (B), near Ny-Ålesund, Svalbard, between 17th and 24th August 2011.

Fig. 2. Survival (%) of *M. arctica* and *E. murphyi* following exposure to progressively higher temperatures (30-35°C for *M. arctica*, 35-40°C for *E. murphyi*) for 1 h. Controls (4 °C) are represented by dashed (*M. arctica*) and diagonally lined bars (*E. murphyi*). Means ± S.E.M. are presented for three replicates of 10 individuals. Survival was assessed 72 h after treatment. Means with the same letter are not significantly different within each species group at $P < 0.05$ (Tukey's multiple range test, variance not equal for *M. arctica*).

Fig. 3. Survival (%) of *M. arctica* (A) and *E. murphyi* (B) at 4, 10 and 15°C over a period of up to 210 d. Means ± S.E.M. are presented for five replicates of 10 individuals.

Fig. 4. Survival (%) of *E. murphyi* at 25°C over a period of 9 d. Larvae were either given no recovery period, 1 h at 4°C after each 24 h period or 2 h at 4°C after each 24 h period. Means ± S.E.M. are presented for three replicates of 10 individuals. Asterisks indicate a recovery treatment significantly different from the constant treatment $P < 0.05$ (Tukey's multiple range test, variances not equal).

Fig. 5. Survival (%) of *M. arctica* following direct exposure (without ramping) to progressively higher temperatures (30-35°C) for 1 h. Means ± S.E.M. are presented for three replicates of 10 individuals. Survival was assessed 72 h after treatment. Means with the same letter are not significantly different at $P < 0.05$ (Tukey's multiple range test).

Fig. 6. Survival (%) of *M. arctica*, following exposure to the discriminating temperature (34.5°C) 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}). Survival following these three rates is shown in comparison to direct transfer, which is denoted as 34.5. Means ± S.E.M. are presented for three replicates of 10 individuals. Survival was assessed 72 h after treatment. Means with the same letter are not significantly different at $P < 0.05$ (Tukey's multiple range test).

Figure and Table Legends

Fig. 1. Surface temperature at four sites, two at Stuphallet (A and B) and two at Krykkefjellet (C and D), near Ny-Ålesund, Svalbard, between 17th and 24th August 2011.

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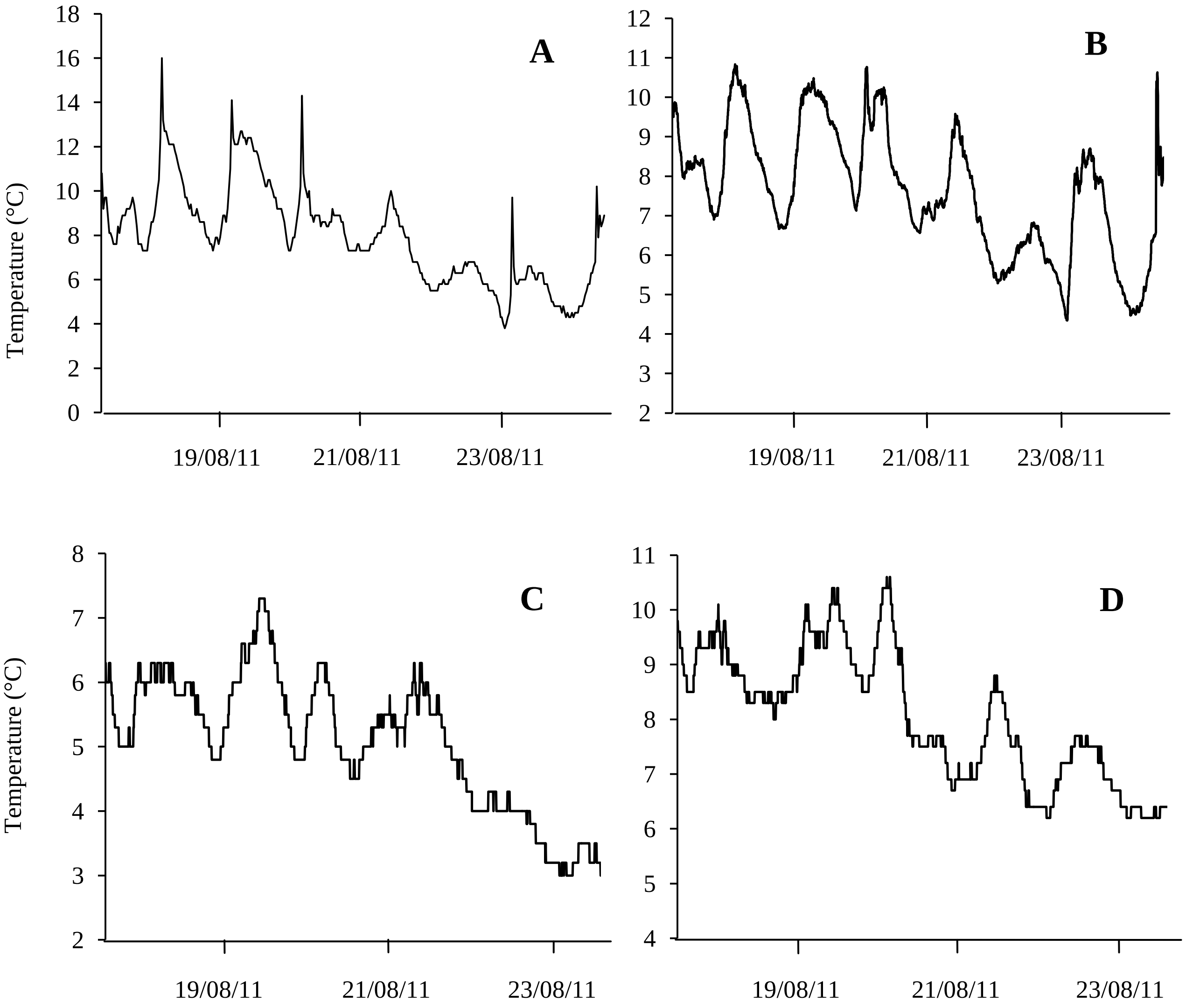
A
B
C
D
Temperature (°C)
Temperature (°C)
0
2
4
6
8
10
12
14
16
18
2
3
4
5
6
7
8
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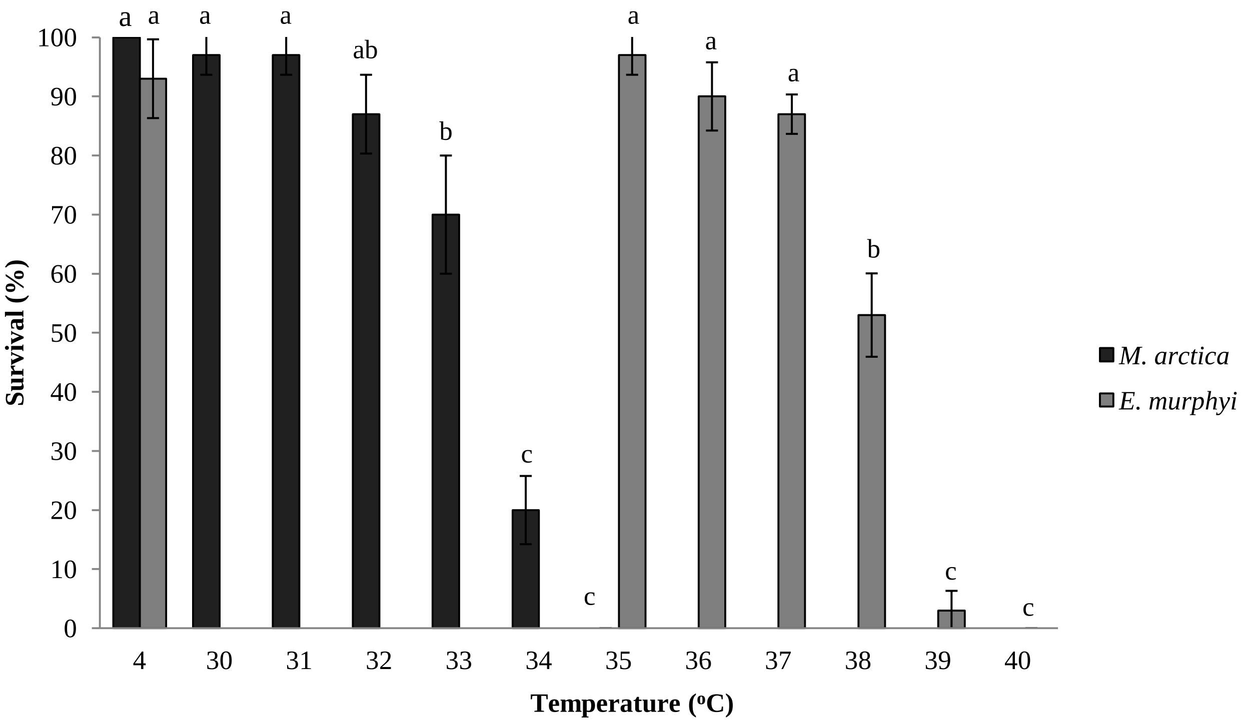
Figure 1



Survival (%)
100
90
80
70
60
50
40
30
20
10
0
a
a
a
a
ab
b
c
c
a
a
a
b
c
c
4
30
31
32
33
34
35
36
37
38
39
40
Temperature (ºC)
■ *M. arctica*
■ *E. murphyi*

Figure 2

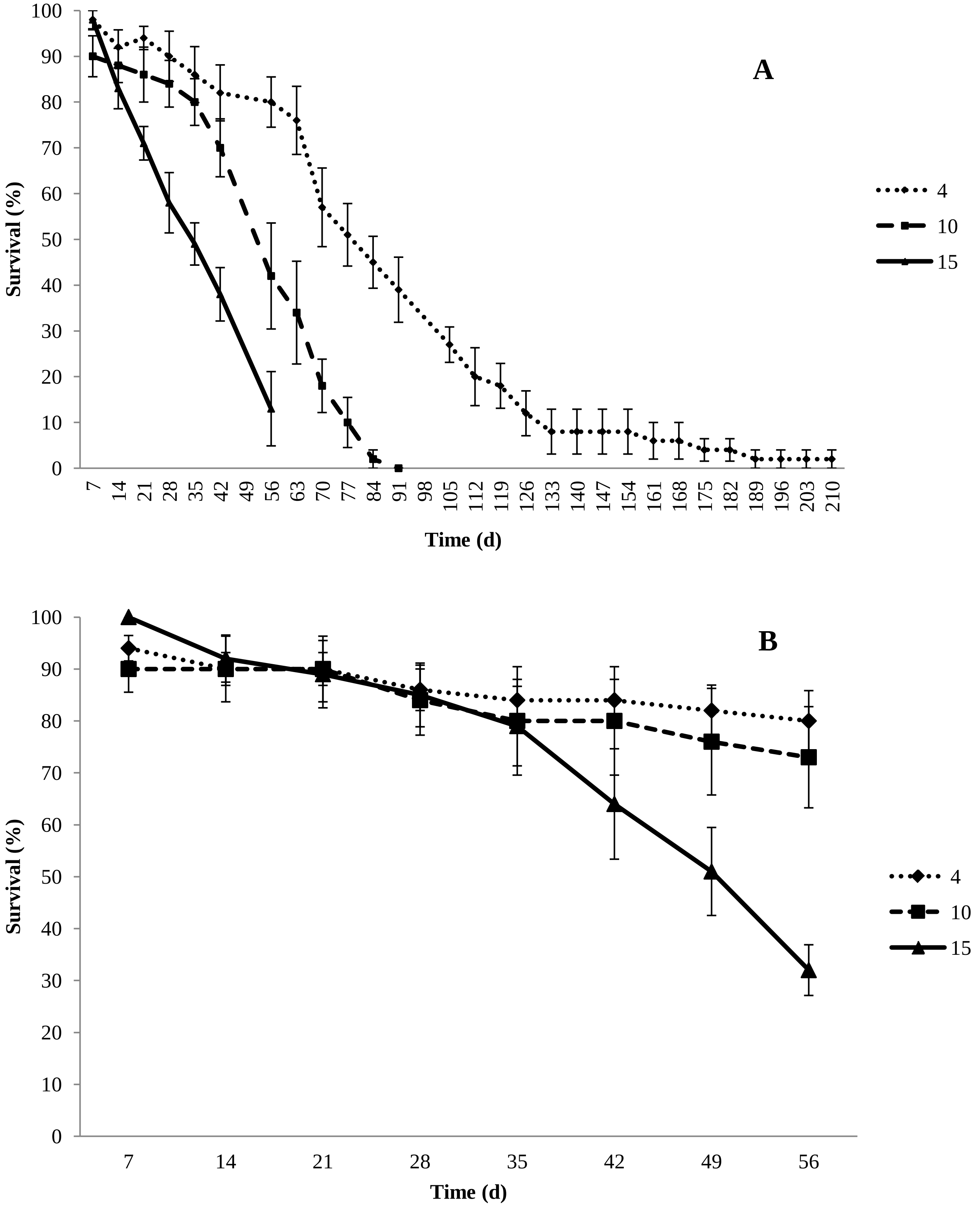

A
Survival (%)
100
90
80
70
60
50
40
30
20
10
0
Time (d)
7
14
21
28
35
42
49
56
63
70
77
84
91
98
105
112
119
126
133
140
147
154
161
168
175
182
189
196
203
210
4
10
15
B
Survival (%)
100
90
80
70
60
50
40
30
20
10
0
Time (d)
7
14
21
28
35
42
49
56
4
10
15

Figure 3

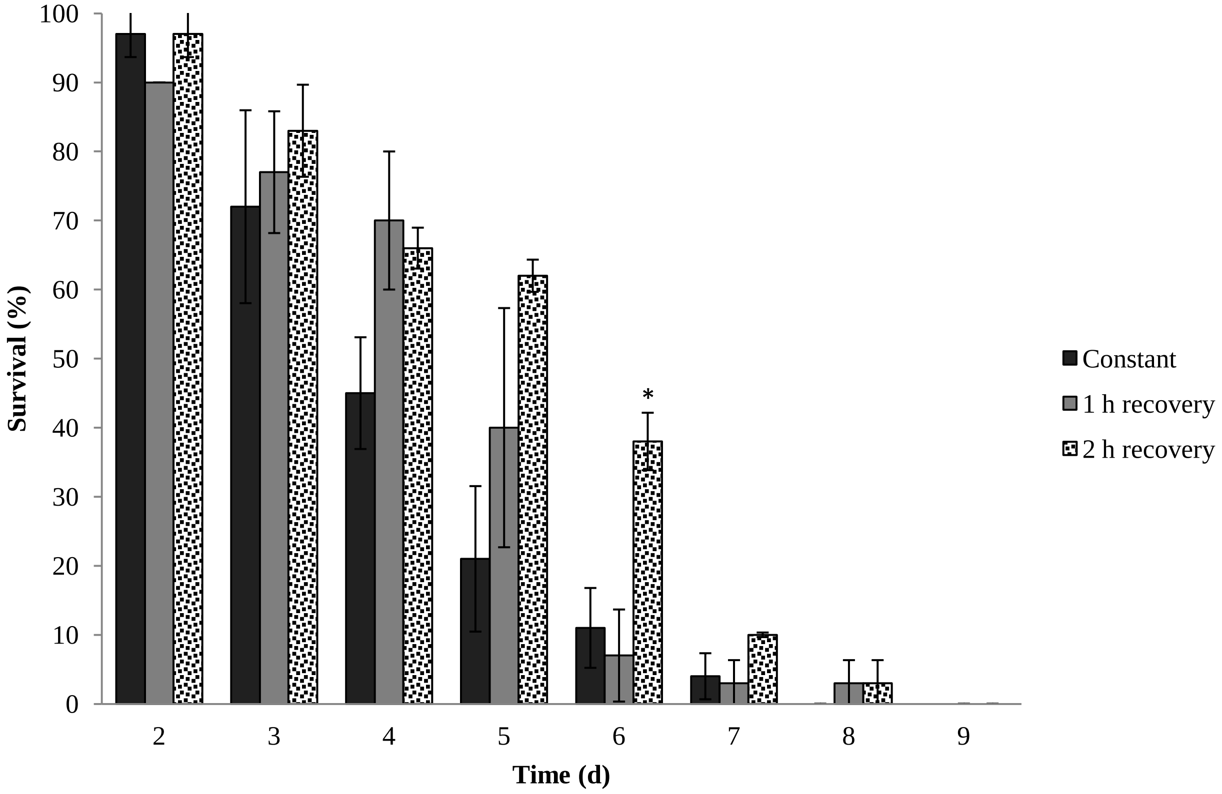
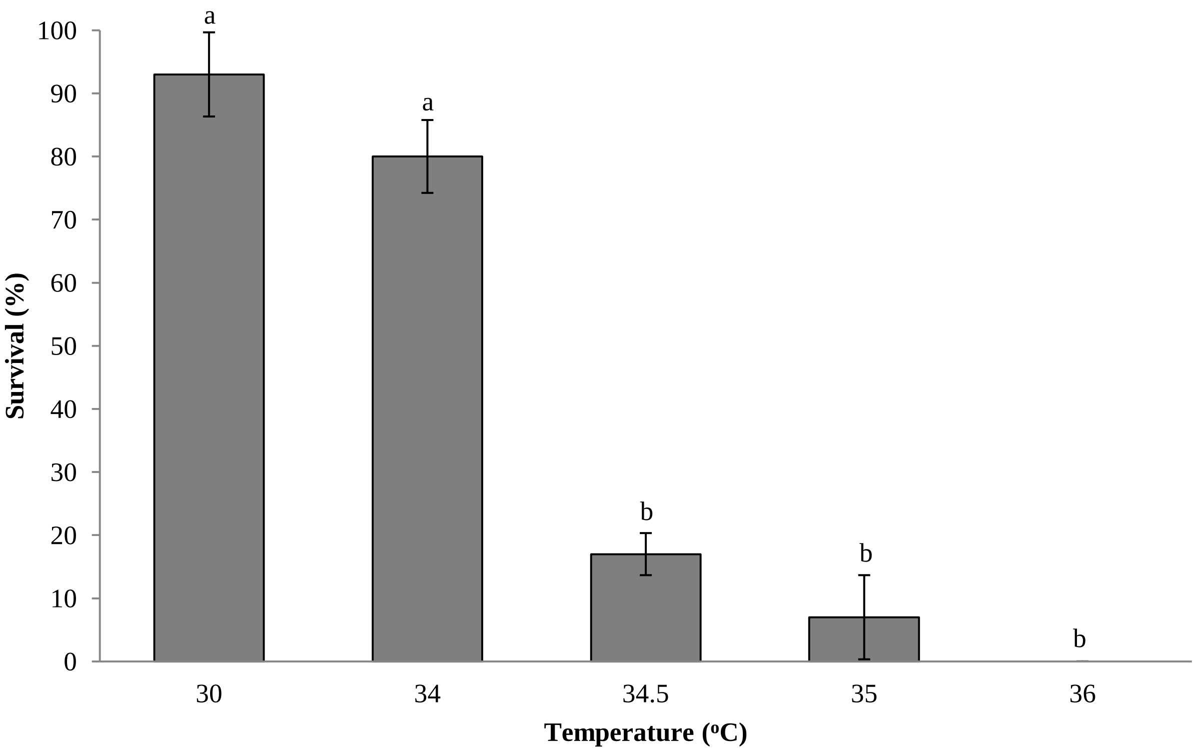
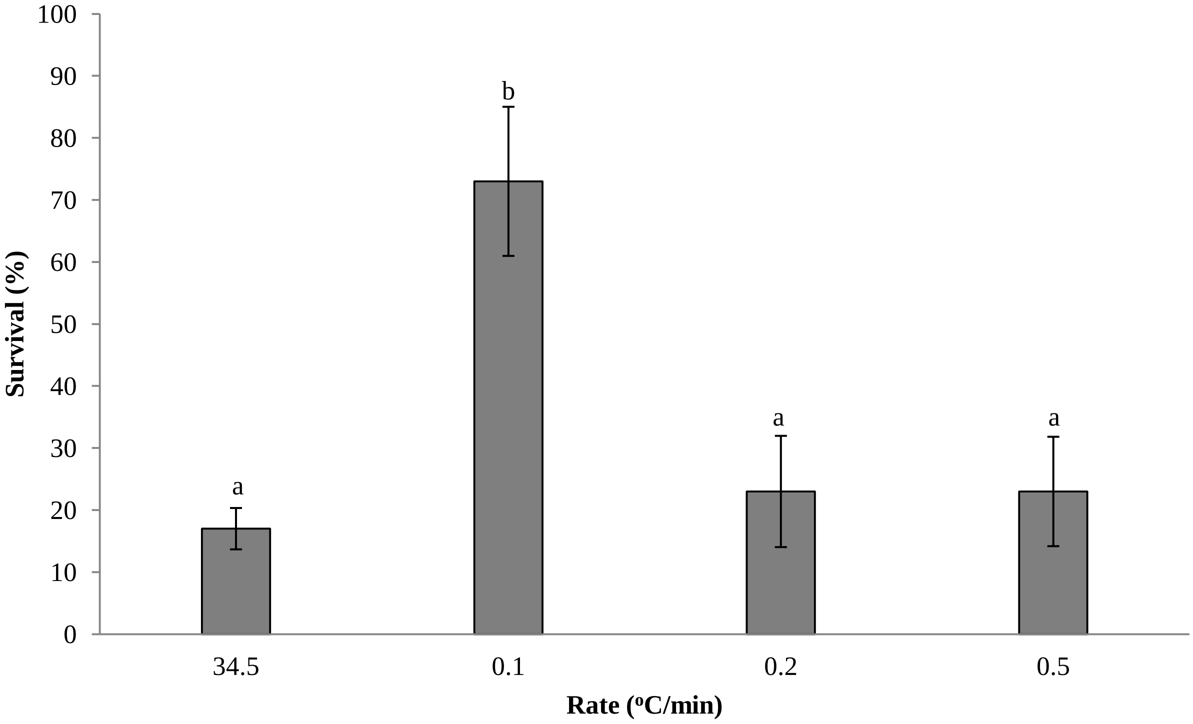

100
90
80
70
60
50
40
30
20
10
0
Survival (%)
*
2
3
4
5
6
7
8
9
Time (d)
Constant
1 h recovery
2 h recovery

Figure 4



a
a
b
b
b
100
90
80
70
60
50
40
30
20
10
0
Survival (%)
30
34
34.5
35
36
Temperature (°C)

Figure 5



100
90
80
70
60
50
40
30
20
10
0
Survival (%)
a
b
a
a
34.5
0.1
0.2
0.5
Rate (°C/min)

Figure 6