

# Thermal biology of the alien ground beetle *Merizodus soledadinus* introduced to the Kerguelen Islands

L. Lalouette · C. M. Williams · M. Cottin ·  
B. J. Sinclair · D. Renault

Received: 25 April 2011 / Revised: 3 August 2011 / Accepted: 17 August 2011 / Published online: 4 September 2011  
© Springer-Verlag 2011

**Abstract** Thermal tolerance is one of the major determinants of successful establishment and spread of invasive aliens. *Merizodus soledadinus* (Coleoptera, Carabidae) was accidentally introduced to Kerguelen from the Falkland Islands in 1913. On Kerguelen, the climate is cooler than the Falklands Islands but has been getting warmer since the 1990s, in synchrony with the rapid expansion of *M. soledadinus*. We aimed to investigate the thermal sensitivity in adults of *M. soledadinus* and hypothesised that climate warming has assisted the colonisation process of *M. soledadinus*. We examined (1) survival of constant low temperatures and at fluctuating thermal regimes, (2) the critical thermal limits ( $CT_{min}$  and  $CT_{max}$ ) of acclimated individuals (4, 8 and 16°C), (3) the metabolic rates of acclimated adults at temperatures from 0 to 16°C. The FTRs moderately increased the duration of survival compared to constant cold exposure. *M. soledadinus* exhibited an activity window ranged from  $-5.5 \pm 0.3$  to  $38 \pm 0.5^\circ\text{C}$ . The  $Q_{10}$  after acclimation to temperatures ranging from 0 to 16°C was 2.49. Our work shows that this species is only moderately cold tolerant with little thermal plasticity. The  $CT_{min}$  of *M. soledadinus* are close to the low

temperatures experienced in winter on Kerguelen Islands, but the  $CT_{max}$  are well above summer conditions, suggesting that this species has abundant scope to deal with current climate change.

**Keywords** Sub-Antarctic island · Insect · Critical thermal limit · Survival · Fluctuating thermal regime · Metabolic rate

## Introduction

Anthropogenic activities are resulting in shifts in species distributions (Parmesan and Yohe 2003; Parmesan 2006), with especially strong effects on the geographical distribution of plants and insects (Bergstrom and Chown 1999; Frenot et al. 2005). Temperature is an important determinant of successful establishment and spread of invasive aliens (Vitousek et al. 1997; Thuiller et al. 2007). The importance of thermal conditions on insects' life cycle is particularly manifest in polar regions, where air and microclimate temperatures are consistently cool but are increasing at the fastest rates on the planet (Smith 2002; Bergstrom et al. 2006; Convey 2006). Overall, an increase in winter temperature is expected to have a more significant effect on the population dynamics of alien insects in cool sub-Antarctic regions than in moderately warm temperate ones, as these insects likely live close to their limit of low temperature tolerance (Convey 2001; Convey et al. 2002). Studying the low temperature biology of alien insects is thus particularly relevant as it determines their overwintering success and predicts whether the species could survive through seasonal climatic fluctuations and winter cold stress particularly in a changing climate context (Bale and Hayward 2010).

L. Lalouette (✉) · D. Renault  
Université de Rennes 1, UMR CNRS 6553,  
263 Avenue du Gal Leclerc, CS 74205,  
35042 Rennes Cedex, France  
e-mail: lisa.lalouette@gmail.com

C. M. Williams · B. J. Sinclair  
Department of Biology, The University of Western Ontario,  
1151 Richmond Street, London, ON N6A 5B7, Canada

M. Cottin  
Université de Strasbourg IPHC, UMR CNRS 7178,  
23 Rue Becquerel, 67087 Strasbourg, France

Thermal biology has been studied in a wide variety of insect species exposed to constant (Bale 2002; Sinclair et al. 2003) and fluctuating temperatures (Renault et al. 2004; Terblanche et al. 2010; Fischer et al. 2011). Several studies have demonstrated that fluctuating thermal regimes (FTRs), i.e. cold exposure interrupted by periodic short warming pulses, increase the duration of survival in several insect species (Coulson and Bale 1996; Nedvěd et al. 1998; Hanc and Nedved 1999; Renault et al. 2004; Colinet et al. 2006; Košťál et al. 2007; Colinet and Hance 2010; Renault 2011), potentially through physiological repair of accumulated injuries during warm bouts that are involved in the re-establishment of ion-gradient homeostasis (Košťál et al. 2007), the metabolism of compatible solutes (Wang et al. 2006; Colinet et al. 2007a, Lalouette et al. 2007), the expression of heat shock proteins (Hsps) (Wang et al. 2006; Tollarová-Borovanská et al. 2009; Colinet et al. 2007b, 2010), the activation of antioxidant system (Lalouette et al. 2011), and the upregulation of various proteins (Colinet et al. 2007b). Another potential explanation is that under FTRs, the daily cold dose accumulated by the insects is reduced compared to those exposed at constant low temperatures (Hanc and Nedved 1999; Renault et al. 2004; Colinet et al. 2006, 2011). However, if the total amount of cold exposure is controlled for there are still survival advantages to FTRs, though fitness tradeoffs may be induced (Marshall and Sinclair 2010). However, although this repair of accumulated injuries and the subsequent increased duration of survival have been demonstrated in several insect taxa from temperate regions (Coulson and Bale 1996; Hanc and Nedvěd 1999; Renault et al. 2004; Colinet et al. 2006), it has rarely been examined in those inhabiting Polar or alpine regions.

*Merizodus soledadinus* Guérin-Méneville (Coleoptera: Carabidae) [previously misnamed *Ooapterus soledadinus*] is an opportunistic predator whose native range is restricted to the cold temperate zone of south America including islands off the southern coast of Cape Horn (Johns 1974). This species was introduced from the Falkland Islands to Port-Couvreux (Kerguelen Islands) in 1913 (Jeannel 1940), and more recently to South Georgia in 1963 (Convey et al. 2011; Darlington 1970). For the past 60 years (1941–2010) in the Falkland Islands (in Port Stanley and the Mount Pleasant airports), mean monthly temperatures range from  $9.6 \pm 1.1^\circ\text{C}$  in February to  $2.3 \pm 1.3^\circ\text{C}$  in July, with an annual mean of  $6.6 \pm 0.5^\circ\text{C}$  (Steve Colwell, British Antarctic Survey, personal communication). Like thermal tolerance and performance are generally related to the temperatures, an organism experiences in its native habitat (Stevens 1989; Addo-Bediako et al. 2000; Ghalambor et al. 2006; Pörtner et al. 2006) *M. soledadinus* would a priori be expected to be stenothermal and cold tolerant.

The eastern part of the Kerguelen Islands, where *M. soledadinus* is widely distributed, is characterised by cool temperatures ranging from  $7.7 \pm 0.9^\circ\text{C}$  in February to  $2.0 \pm 0.7^\circ\text{C}$  in July with an annual mean of  $4.6 \pm 0.4^\circ\text{C}$  (Meteo France data from 1951 to 2009). During the coldest month (July), mean minimum temperature is  $-5.0 \pm 1.6^\circ\text{C}$  (Meteo France data from 1951 to 2009). The population size and geographical distribution of *M. soledadinus* were relatively small at the Kerguelen Islands until the 1980s, with a rapid expansion in the 1990s at the expense of native invertebrate species (Chevrier 1996; Lebouvier et al. 2011). Temperatures on Kerguelen have warmed consistently since the 1980s: e.g. mean annual temperatures were  $4.3 \pm 0.4^\circ\text{C}$  over the period 1951–1975 and  $4.8 \pm 0.3^\circ\text{C}$  over the period 1976–2008 with a more significant increase during winter periods (Lebouvier et al. 2011). Thus, a changing climate may have improved winter survival of *M. soledadinus* over this time period. Despite this warming, temperatures at the Kerguelen Islands so far remain slightly cooler than the Falklands Islands.

In the present work, we aimed to describe the thermal sensitivity and responses to thermal fluctuations in *M. soledadinus*, as a first step towards predicting responses to climate change in the field. We measured survival at constant low temperatures and FTRs. We also examined the critical thermal limits (lower and upper) and the  $\text{CO}_2$  release in adults acclimated at several temperatures to examine the capacity of this species to compensate their metabolic rate to the range of temperatures experienced across the life cycle under warming conditions.

## Materials and methods

### Rearing and acclimation conditions

Wild imagoes of *M. soledadinus* (Coleoptera: Carabidae) were hand-collected in coastal areas at Port-aux-Français at the Kerguelen Islands ( $49^\circ 21'\text{S}$ ,  $70^\circ 13'\text{E}$ ) in January 2008 and 2009. Imagoes were sampled under stones in a habitat where *Acaena magellanica* was dominant. The insects were then maintained under controlled conditions at  $+8^\circ\text{C}$  (R.H. of  $70 \pm 5\%$ ), with a 15 h:9 h light/dark cycle, for 1 week before use in critical thermal limits and duration of survival at constant and FTRs, or up to 8 months before respirometry. They were supplied ad libitum with water and food consisting of larvae of *Calliphora* sp., *Anatalanta aptera* and *Drosophila melanogaster*.

### Chronic low temperature survival

To avoid cold shock, adults of *M. soledadinus* were pre-acclimated at  $+4^\circ\text{C}$  for 24 h prior to being used for the

survival experiments. Groups of ten beetles were then directly transferred to Petri dishes which were randomly assigned to each one of the following low-temperature treatments: (1) Constant temperature (CT):  $-8$ ,  $-6$ ,  $-4$ ,  $-2$  and  $+4^{\circ}\text{C}$ ; (2) FTRs:  $-6$  or  $-4^{\circ}\text{C}$  for 22 h cycling with 0, 4, 8 or  $12^{\circ}\text{C}$  for 2 h, i.e.  $-6/0$ ,  $-6/4$ ,  $-6/8$ ,  $-6/12$ ,  $-4/0$ ,  $-4/4$ ,  $-4/8$  and  $-4/12^{\circ}\text{C}$ . During the chronic low-temperature exposure, beetles were food and water deprived and beetles held at constant  $+4^{\circ}\text{C}$  were used as the controls. One Petri dish per thermal treatment was transferred to optimum conditions ( $+8^{\circ}\text{C}$  with water ad libitum) at daily intervals to observe recovery. Survival was scored as the proportion of active insects (that exhibited coordinated walking activities) after 2 days of recovery at  $+8^{\circ}\text{C}$  (preliminary tests demonstrated that survival then remained similar from 48 h onwards).

### Critical thermal limits

Critical thermal limits ( $\text{CT}_{\min}$  and  $\text{CT}_{\max}$ ) of individual adult *M. soledadinus* were assessed after 7 days acclimation at  $+4$ ,  $+8$  (control beetles) and  $+16^{\circ}\text{C}$  with water and food supplied ad libitum.  $\text{CT}_{\min}$  ( $N = 30$  per treatment except for control, for which  $N = 26$ ) and  $\text{CT}_{\max}$  ( $N = 26$  per treatment) of beetles was measured by knockdown using a method adapted from Powell and Bale (2006). Beetles were placed in a  $35 \times 5\text{-cm}$  jacketed glass column cooled with ethylene glycol circulated from a programmable alcohol bath (Haake F8-C50 Thermo Electron, Karlsruhe, Germany), through the outer chamber. The opening of the inner chamber was then closed with insulation foam. The air temperature in the inner chamber was monitored by thermocouples ( $\pm 0.1^{\circ}\text{C}$ ) at the top and bottom of the column. As the beetles could not cling or climb up on the glass of the column, a  $20 \times 2\text{-cm}$  piece of white paper ( $90\text{ g m}^{-2}$ ) was positioned within the inner chamber. Preliminary experiments demonstrated that the beetles very often fell when the column was vertical; a fall that was stressful and that increased the locomotor activity of the insects as they quickly tried to stand on their legs again. The column was thus inclined to  $21^{\circ}$ , an angle that solved the problem. Each insect was placed individually in the column and allowed 10 min to acclimatise to the new environment. Temperature in the column was ramped from  $+8^{\circ}\text{C}$  to the  $\text{CT}_{\min}$  or to the  $\text{CT}_{\max}$  at a rate of  $0.5^{\circ}\text{C}\cdot\text{min}^{-1}$ .  $\text{CT}_{\min}$  and  $\text{CT}_{\max}$  were determined as the temperatures at which the beetles lost the ability to cling to or climb the paper, and thus became motionless and fell out at the bottom of the column. The temperature was recorded, the insulation foam removed, and the insect was collected in a Petri dish. Timing of the duration of recovery from coma (both heat- and cold-induced) was then recorded at  $10^{\circ}\text{C}$  and began the moment the insect fell out of the

column; the end point being the time when the insect exhibited coordinated walking abilities (i.e. the ability to stand on their legs and take a first step). This end point (rather than the commonly used measure of the first coordinated movement of the legs) was chosen as having more ecological relevance to the insect.

After determination of critical thermal limits and recovery times, beetles were kept individually in Petri dishes at  $+8^{\circ}\text{C}$  with water and food ad libitum for 10 days and survival was scored every day as the number of beetles exhibiting coordinated walking activities, after which the beetles were sexed, and weighed (Sartorius M4 microbalance,  $d = 1\text{ }\mu\text{g}$ ) after 6 days at  $60^{\circ}\text{C}$  (dry mass).

### Metabolic rate

To examine the metabolic rate under various constant thermal conditions, fed insects were first cold-acclimated for 7 days at 0, 4, 8 (control beetles), 12 and  $16^{\circ}\text{C}$  ( $N = 8$  insects for each thermal condition) and the metabolic rate measured at the temperatures at which the insects were acclimated. Body mass of the adults were measured prior to and immediately after each trial (Mettler-Toledo MX-5 microbalance (Mettler-Toledo, Columbus, OH, USA;  $d = 0.1\text{ }\mu\text{g}$ ).

$\text{CO}_2$  production was measured using a flow-through method modified from Williams et al. (2010) and described in Lalouette et al. (2011). Briefly, beetles were placed individually in a glass cylindrical respiration chamber ( $4\text{ cm}^3$ ) through which dry,  $\text{CO}_2$ -free air was passed through the chamber at a rate of  $25\text{ mL min}^{-1}$  controlled by a mass flow controller (Sable Systems International [SSI], Las Vegas, NV, USA) connected to mass flow valves (Sierra Instruments, CA, USA). Dry  $\text{CO}_2$ -free air was supplied by a Purge Gas Generator (Parker-Balston, OH, USA) and further scrubbed of remaining  $\text{CO}_2$  and water vapour by passage through a Drierite–ascarite–drierite column. Temperature was controlled ( $\pm 0.1^{\circ}\text{C}$ ) by SSI PELT-4 cooling cabinets and monitored by thermistors inside the unit.  $\text{CO}_2$  concentration of excurrent air was determined with a Li7000 infra-red gas analyser (LiCor, Lincoln, NE, USA). After 10 min equilibration,  $\text{CO}_2$  release was recorded every second over a 2.5 h period for each beetle and the analyses were done during the second half hour of the recording (in the time range 0.5–1 h). Data were baselined to an empty chamber recorded at the beginning and end of each run to correct for any instrument drift. Activity of beetles was monitored using infra-red activity detectors (AD-2, SSI), and the absolute difference sum over the relevant time period was calculated. The resulting slope was compared amongst temperatures as an index of activity (Lighton 2008). Data were captured and processed using a UI2 interface and Expedata software (SSI), averaged over 5 s and converted into  $\text{mL CO}_2\text{ min}^{-1}$

using Expedata. We converted CO<sub>2</sub> release (mL min<sup>-1</sup>) in O<sub>2</sub> consumption (mL min<sup>-1</sup>), and then in Watts (J s<sup>-1</sup>) in assuming a Respiratory Quotient of 0.8 through the following formula:  $\frac{\text{CO}_2 \text{ release} \times 1.25 \times 20.1312}{60}$  (Lighton 2008).

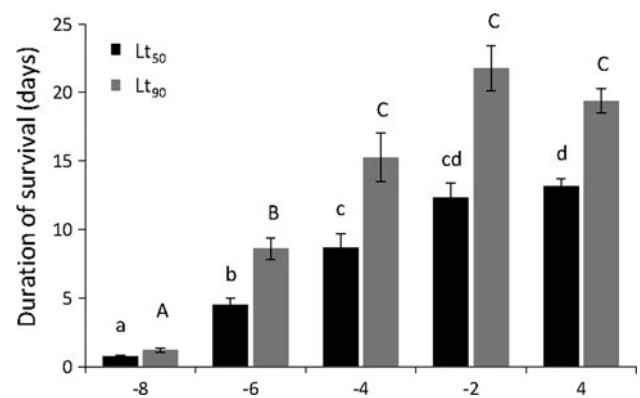
### Statistical analysis

Data are expressed as means  $\pm$  SEM. Survival data were expressed as lethal times for 50 and 90% of the samples, Lt<sub>50</sub> and Lt<sub>90</sub>, respectively calculated by probit analysis using MINITAB Statistical Software Release 13 (MINITAB Inc., State College, PA, USA), where time of exposure was an expression of dosage level at a particular temperature. Pearson correlations were used to test relationship amongst critical thermal limits, dry mass, and duration of recovery from chill coma, or amongst the duration of recovery from chill coma, dry mass, and the time spent in the column. Kruskal–Wallis tests (*H* test) and Mann–Whitney (*W* test) were performed to compare the temperature at the onset of chill coma and the time necessary to recover. Survival curves after the onset of chill coma were compared using log-rank (Mantel–Cox) test.  $\chi^2$  contingency tests were used to compare mortality using GRAPHPAD PRISM Software 5.02 (San Diego California USA, <http://www.graphpad.com>) with  $\alpha = 0.05$ . The CO<sub>2</sub> release ( $\dot{V}\text{CO}_2$ ) data were log<sub>10</sub>-transformed to improve normality and homoscedasticity. We calculated the  $Q_{10}$  over the range of temperatures measured using the equation  $Q_{10} = 10^{(10a)}$ ; where ‘*a*’ is the slope of the regression of log<sub>10</sub>  $\dot{V}\text{CO}_2$  plotted against temperature. A general linear model with mass as covariate was performed on insects exposed at constant temperatures to determine effects of acclimation temperature on  $\dot{V}\text{CO}_2$ . Pairwise post hoc comparisons were conducted to identify significant differences (Tukey’s tests). A linear regression was performed to determine whether the slopes of the absolute difference sums for activity (an index of the magnitude of signal in the activity channel) were correlated with temperature using R software 2.13.0.

## Results

### Survival at chronic low temperatures

Survival at constant low temperatures was significantly increased with increasing temperatures. The lowest survival duration was obtained at  $-10^\circ\text{C}$  (data not shown on the Fig. 1, mortality occurred in less than 5 min and 100% mortality after 2.4 h). The longest duration of survival was obtained at  $-2^\circ\text{C}$  (Lt<sub>50</sub> =  $12.6 \pm 1.0$  days) and was similar to that of the control ( $+4^\circ\text{C}$ ) (Fig. 1).



**Fig. 1** Lt<sub>50</sub> (black bars) and Lt<sub>90</sub> (grey bars) (mean  $\pm$  SE) in adults of *M. soledadinus* kept at constant  $-8$ ,  $-6$ ,  $-4$ ,  $-2$  and  $4^\circ\text{C}$ . Bars with distinct letters are significantly different ( $P < 0.05$ )—lower case for Lt<sub>50</sub> and upper case for Lt<sub>90</sub>

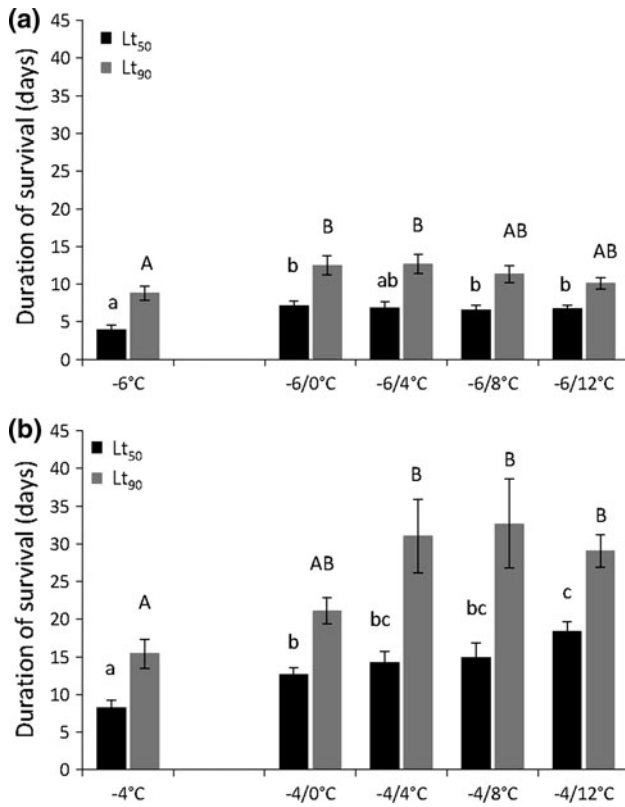
### Survival at fluctuating temperatures

Overall, survival of the ground beetles was significantly higher in the FTRs using  $-6^\circ\text{C}$  as the low temperature than in the constantly cold exposed to  $-6^\circ\text{C}$  (Fig. 2a). Within the different FTRs using  $-6^\circ\text{C}$  as the low temperature, increasing the warmer temperatures (from 0 to  $12^\circ\text{C}$ ) during the daily pulses had no more beneficial effects on the duration of survival (Fig. 2a). For exposure to the less severe cold temperature ( $-4^\circ\text{C}$ ): duration of survival at the Lt<sub>50</sub> was increased by exposure to pulses of either 0 or  $+12^\circ\text{C}$ , whilst survival duration at the Lt<sub>90</sub> was sensitive only to pulses above  $+4^\circ\text{C}$  (Fig. 2b).

### Critical thermal limits

There was no significant difference between male and female critical thermal limits (Males:  $\text{CT}_{\min} = -4.20 \pm 0.96^\circ\text{C}$ ,  $N = 69$  and Females:  $\text{CT}_{\min} = -4.49 \pm 1.07^\circ\text{C}$ ,  $N = 51$ ;  $T_{102} = 1.42$ ;  $P > 0.05$ ; Males:  $\text{CT}_{\max} = 37.63 \pm 1.98^\circ\text{C}$ ,  $N = 33$  and Females:  $\text{CT}_{\max} = 37.75 \pm 0.82^\circ\text{C}$ ,  $N = 43$ ;  $T_{40} = 0.35$ ;  $P > 0.05$ ), so sexes were pooled for subsequent analyses. Neither  $\text{CT}_{\max}$  nor  $\text{CT}_{\min}$  were correlated with dry mass ( $\text{CT}_{\min}$ :  $r_{90} = 0.004$ ,  $P > 0.05$ ;  $\text{CT}_{\max}$ :  $r_{68} = 0.167$ ,  $P > 0.05$ ), nor to the duration of recovery from chill coma ( $\text{CT}_{\min}$ :  $r_{90} = 0.000$ ;  $P > 0.05$ ;  $\text{CT}_{\max}$ :  $r_{68} = 0.218$ ;  $P > 0.05$ ). The recovery of coordinated walking was neither correlated to dry mass ( $\text{CT}_{\min}$ :  $r_{90} = -0.104$ ;  $P > 0.05$ ;  $\text{CT}_{\max}$ :  $r_{68} = 0.236$ ;  $P > 0.05$ ) nor to the duration the insects stayed in the column ( $\text{CT}_{\min}$ :  $r_{90} = -0.000$ ;  $P > 0.05$ ;  $\text{CT}_{\max}$ :  $r_{68} = 0.238$ ;  $P > 0.05$ ).

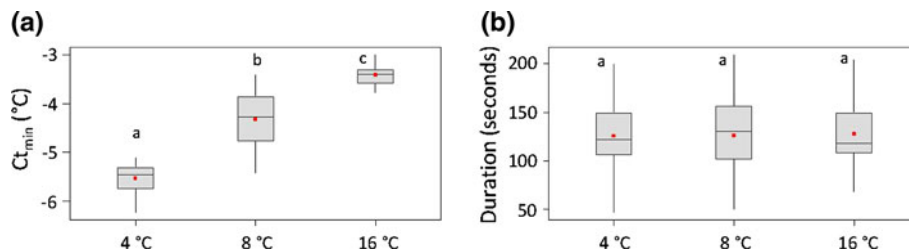
Low temperature acclimation significantly decreased  $\text{CT}_{\min}$  in adults of *M. soledadinus* ( $H_2 = 64.46$ ;  $P < 0.01$ ) (Fig. 3a) but had no effect on the duration from chill coma recovery (CCR,  $H_2 = 0.09$ ;  $P > 0.05$ ; Fig. 3b). There was



**Fig. 2** Lt<sub>50</sub> (black bars) and Lt<sub>90</sub> (grey bars) (mean ± SE) in adults of *M. soledadinus* kept at constant (CT) -6 (a) and -4°C (b); and cycling thermal regimes (FTR, -4 or -6°C for 22 h with a daily return at 0, 4, 8 or 12°C for 2 h. Bars with distinct letters are significantly different ( $P < 0.05$ )—lower case for Lt<sub>50</sub> and upper case for Lt<sub>90</sub>

no mortality in the 10 days after the experiment in any of the groups.

Conversely, acclimation had no effect on CT<sub>max</sub> ( $H_2 = 3.00$ ;  $P > 0.05$ ) (Fig. 4a) but the time needed to fully recover walking activities decreased significantly (heat coma recovery HCR  $H_2 = 7.88$ ;  $P < 0.05$ ) with increased acclimation temperature (Fig. 4b), indeed 16°C-acclimated beetles had the shortest duration of recovery ( $W = 492.5$ ;  $P < 0.05$ ) and the highest longevity after 10 days of the experiments ( $\chi^2 = 12.06$ ;  $P < 0.01$ ) (Fig. 5).



**Fig. 3** Critical thermal minima (CT<sub>min</sub>) (a) in *M. soledadinus* acclimated for one week at 4, 8 or 16°C, and the duration of recovery from chill coma (b). Circles represent mean values, lines within box are the medians. Different letters indicate significant differences ( $P < 0.05$ )

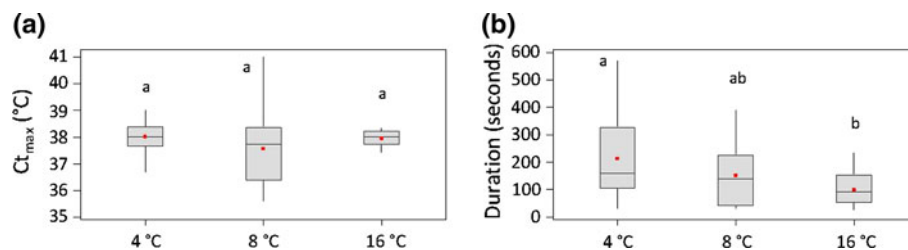
### Respirometry

The fresh masses did not differ between temperature acclimation ( $F_4 = 0.33$ ;  $P > 0.05$ ) and were included in the analysis as a covariate of  $\dot{V}CO_2$  (Table 1).  $\dot{V}CO_2$  of adult *M. soledadinus* increased with increasing acclimation temperature ( $F_4 = 39.52$ ;  $P < 0.001$ ) (Table 1). The  $Q_{10}$  after acclimation to temperatures ranging from 0 to 16°C was 2.49 (Fig. 6). There was no relationship between activity and temperature ( $F_{1,40} = 2.776$ ,  $P = 0.601$ ).

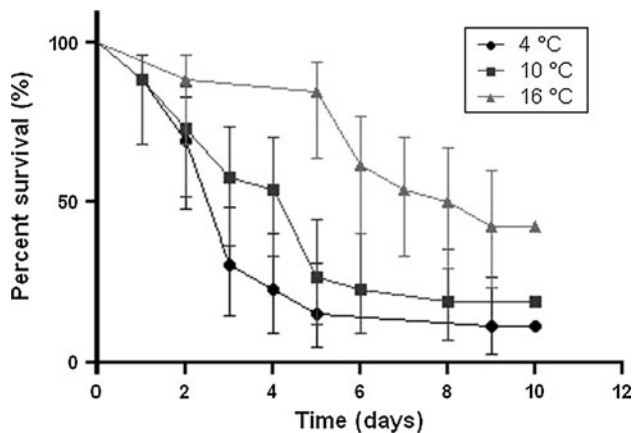
### Discussion

We found that adult *M. soledadinus* have the ability to survive cold exposures to temperatures as low as -6°C for a few days, and that acute cold injuries start to accumulate below -8°C. These results are consistent with populations from South Georgia, where adult *M. soledadinus* have supercooling points of -5 to -6°C (Block and Somme 1983; Ottesen 1990), and which suggests that the threshold in mortality that we observed between -6 and -8°C may be accompanied by freezing. The chronic cold tolerance of adults of this ground beetle was moderate (ca. 2 weeks at -2°C) compared to some other arctic or sub-Antarctic species, for example, the carabid *Pelophila borealis* from South Georgia, which survived 7 weeks at -5°C and 7 months at -3°C (Sømme 1974). However, the similar survival duration obtained at -2 and +4°C suggests that adult *M. soledadinus* mortality at -2°C was related to water deprivation rather than to cold exposure. Adult *M. soledadinus* supplied with water ad libitum can survive about 60 days at -4°C (D. Renault, unpublished data), it thus is unlikely that mortality occurred as a result of the exhaustion of energy reserves in any of the survival experiments in the present study.

Work on temperate and tropical insect species has demonstrated a strong relationship between duration of chilling exposure and the amount of accumulated injuries (Rojas and Leopold 1996; Košťál et al. 2004, 2007) and the beneficial impact of FTRs on adults' survival (Renault et al. 2004; Colinet et al. 2006; Renault 2011). By contrast,

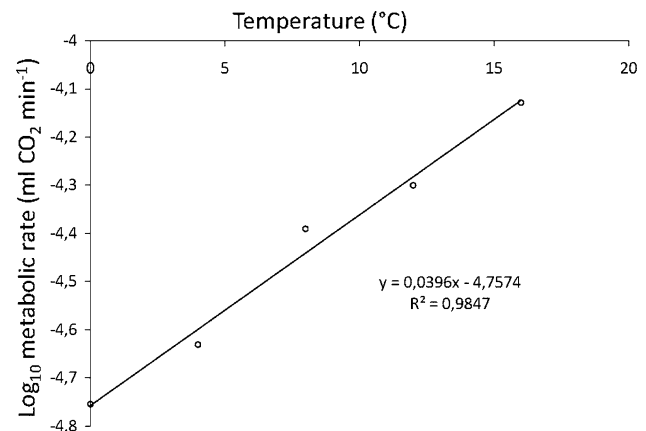


**Fig. 4** Critical thermal maxima ( $CT_{max}$ ) (a) in *M. soledadinus* acclimated for 1 week at 4, 8 or 16°C, and the duration of recovery from heat coma (b). Circles represent mean values, lines within box are the medians. Different letters indicate significant differences ( $P < 0.05$ )



**Fig. 5** Survival after recovery from heat coma in adult *M. soledadinus* that were acclimated 1 week at 4, 8 and 16°C.  $N = 26$  for each experimental condition

we found only a small benefit of FTR on chronic cold survival of adult *M. soledadinus*. Under the temperature cycles, adult *M. soledadinus* survived for longer than constantly cold-exposed individuals. Although there was a slight effect of the high temperature on survival at an FTR to  $-4^{\circ}\text{C}$ , modulating the highest temperature applied during the pulses for the  $-6^{\circ}\text{C}$  FTR had no effect on survival, suggesting that at this latter temperature there is a limited ability to repair cold injuries. This weak ability to exploit intermittent periods of favourable thermal conditions in our experimented cycles matches with the geographical distribution of *M. soledadinus* in cold temperate but not extreme and sub-Antarctic habitats where thermal variation is of low amplitude (Bergstrom and Chown 1999). Indeed organisms' flexibility in thermal tolerance and performance



**Fig. 6** The relationship between temperature (in  $^{\circ}\text{C}$ ) and  $\log_{10}$  standard (resting) metabolic rate (in  $\text{mL CO}_2 \text{ min}^{-1}$ ) in adults of *M. soledadinus* ( $N = 8$  for each temperature), after a 1 week acclimation to the measurement temperature. All values were obtained using flow-through respirometry

are thought to be proportional to the range of the thermal fluctuations they undergo in their living environment (Stevens 1989; Addo-Bediako et al. 2000; Ghalambor et al. 2006; Pörtner et al. 2006). FTRs with cold exposures of  $-4^{\circ}\text{C}$  increased survival to levels comparable to that seen at  $+4^{\circ}\text{C}$ , likely resulted from water deprivation. Todd and Block (1997) have previously described low desiccation tolerance in adult *M. soledadinus*, and this species is largely nocturnal, possibly to prevent desiccation (Ottesen 1990).

*Merizodus soledadinus* enters chill coma between  $-3$  and  $-5^{\circ}\text{C}$ , which is similar to other sub-Antarctic insects (e.g. Klok and Chown 2003), however due to concerns

**Table 1**  $\text{CO}_2$  release, metabolic activity and fresh mass (mean  $\pm$  SD) in adults of *M. soledadinus* after 1 week acclimation at 0, 4, 8, 12 or 16°C

	0°C	4°C	8°C	12°C	16°C
$\text{CO}_2$ release ( $10^{-6} \text{ mL min}^{-1}$ )	17.58 $\pm$ 1.03 a	23.43 $\pm$ 0.75 a	40.69 $\pm$ 0.85 b	50.10 $\pm$ 1.15 b	74.46 $\pm$ 1.03 c
Metabolic activity ( $10^{-6}$ Watt)	7.37 $\pm$ 0.43 a	9.83 $\pm$ 0.31 a	17.06 $\pm$ 0.35 b	21.01 $\pm$ 0.48 b	31.23 $\pm$ 0.43 c
Fresh mass (mg)	8.11 $\pm$ 0.96 a	8.27 $\pm$ 0.50 a	8.05 $\pm$ 0.91 a	7.82 $\pm$ 1.06 a	8.23 $\pm$ 1.09 a

Different letters indicate significant differences ( $P < 0.05$ )

about the outcome of differences in the methodological procedures for measuring critical thermal limits from one study to the next, inter-study comparisons must be taken with care (Terblanche et al. 2007). The  $CT_{max}$  of about 37°C is relatively low compared to temperate insects (Terblanche et al. 2006), but is considerably higher than the thermal conditions likely to be encountered by *M. soledadinus* in the field, where maximum temperatures rarely exceed 25°C (Lebouvier et al. 2011). In the present work, acclimation had a significant effect on  $CT_{min}$  and on the duration of recovery from  $CT_{max}$  in adult *M. Soledadinus*. Our results support the idea that physiological adjustments to cold and heat stress are at least partially decoupled in terrestrial arthropods (Klok and Chown 2003).

Recovery from thermal coma in *M. soledadinus* showed large individual variation (as has been reported in other species, e.g. David et al. 1998), and whilst warm acclimation significantly reduced recovery time from heat coma, cold acclimation had no effect on recovery from chill coma, which supports the hypothesis that recovery and onset of coma have different underlying mechanisms (Sinclair and Roberts 2005). Our observations are also consistent with observed decoupling of low and high temperature tolerances in insects (Addo-Bediako et al. 2000). Although we did not observe an effect of acclimation on CCR time, such an effect has been observed in many other species, including *Acheta domesticus* (Orthoptera: Gryllidae) and *Drosophila melanogaster* (Diptera: Drosophilidae) (Anderson et al. 2005; Lachenicht et al. 2010). We did not find an acclimation effect on heat knockdown time, but in the duration of recovery time from heat coma. This underlines the fact that recovery time and knockdown temperature are not necessarily interchangeable measures, but may give very different interpretations of thermal adaptation. A comprehensive exploration of the relationship between the two measures is called for.

Despite a 1 week acclimation period, respiration in *M. soledadinus* increased strongly with increasing temperature. The relationship between  $\dot{V}CO_2$  and temperature is roughly linear on a log scale, indicating that the measurement temperatures lie within the physiological temperature range (Knies and Kingsolver 2010), and that the highest measurement temperature is not physiologically stressful for these beetles. The  $Q_{10}$  of 2.49 is comparable to typical acute temperature sensitivities of ectotherms, both at the whole organism and enzyme level (e.g. Glanville and Seebacher 2006) due to the exponential relationship between the rate of biochemical reactions and temperature (Arrhenius 1915). Because the metabolic rate of *M. soledadinus* conforms to Arrhenius kinetics in spite of acclimation, it appears that the response of this species does not show metabolic compensation. The metabolic rate of

*M. soledadinus* is lower than that of similar-sized Coleoptera from sub-Antarctic climates (Sømme et al. 1989; Crafford and Chown 1993), which in turn is lower than that of temperate beetles (Keister and Buck 1974). Todd (1997) also found a higher respiratory metabolism in *Trechisibus antarcticus* than in *M. soledadinus* at any given temperature, both being introduced on South Georgia. These relatively low baseline metabolic costs may come at the expense of decreased maximal performance (Pörtner 2006), and are a hallmark of relatively stenothermic organisms.

Our study shows that the invasive beetle *M. soledadinus* on Kerguelen Island is only moderately cold tolerant, and exhibits relatively little thermal plasticity. The lower thermal limits of *M. soledadinus* are relatively close to the low temperatures experienced in winter on Kerguelen Island, but the upper thermal limits are well above summer conditions, suggesting that this species has abundant scope to deal with current climate change (Lebouvier et al. 2011). Furthermore, the thermal sensitivity we observe, alongside a relatively steep increase in metabolic rate with warm acclimation, suggest that recent warming on Kerguelen Island may be releasing this species from abiotic constraints. Indeed population size and geographical distribution of *M. soledadinus* on the island was relatively constrained until the 1980s, with a rapid expansion in the 1990s (Chevrier 1996). Recent field observations (2003–2010, D. Renault, personal observation) suggest that the densities of *M. soledadinus* are also considerably higher than that reported for 1992–1993 by Chevrier (1996). Given that *M. soledadinus* is an opportunistic predator, continued release from thermal constraints could allow considerable spread and increased impact on indigenous insect populations.

**Acknowledgments** The authors thank the French Ministry of Foreign Affairs that funded the travel of D. Renault from France to Canada (French–Canada scientific cooperation). The work in Canada was funded by NSERC and Canadian Foundation for Innovation grants to BJS. This research was supported by the Institut Polaire Français (IPEV, programme 136 coordinated by Marc Lebouvier), the CNRS (Zone-Atelier de Recherches sur l'Environnement Antarctique et Subantarctique), and the Agence Nationale de la Recherche (ANR-07-VULN-004, Vulnerability of native communities to invasive insects and climate change in sub-Antarctic Islands, EVINCE). We also thank Peter Convey, Steve Colwell and Thomas Bracegirdle (British Antarctic Survey, Cambridge UK) for the meteorological data from the Falklands Islands. This research is linked with the SCAR *Evolution and Biodiversity in the Antarctic* research programme. We thank Jeff Bale for the jacketed glass cylinder and Roger Worland and an anonymous referee for comments that improved the manuscript.

## References

- Addo-Bediako A, Chown SL, Gaston KJ (2000) Thermal tolerance, climatic variability and latitude. *Proc R Soc Lond B* 267:739–745

- Anderson AR, Hoffmann AA, McKechnie SW (2005) Response to selection for rapid chill-coma recovery in *Drosophila melanogaster*: physiology and life-history traits. *Genet Res* 85:15–22
- Arrhenius S (1915) Quantitative laws in biological chemistry. Bell, London
- Bale JS (2002) Insects and low temperatures: from molecular biology to distributions and abundance. *Philos Trans R Soc Lond B* 357:849–861
- Bale JS, Hayward SAL (2010) Insect overwintering in a changing climate. *J Exp Biol* 213:980–994
- Bergstrom DM, Chown SL (1999) Life at the front, ecology and change on southern ocean islands. *Trends Ecol Evol* 14:472–477
- Bergstrom DM, Convey P, Huiskes AHL (2006) Trends in Antarctic terrestrial and limnetic ecosystems. Springer, The Netherlands
- Block W, Somme L (1983) Low temperature adaptations in beetles from the sub-Antarctic Islands of South Georgia. *Polar Biol* 2:109–114
- Chevrier M (1996) Introduction de deux espèces d'insectes aux Iles Kerguelen: processus de colonisation et exemples d'interactions. Thèse de Doctorat d'Université, Université de Rennes 1
- Colinet H, Hance T (2010) Inter-specific variation in the response to low temperature storage in different aphid parasitoids. *Ann Appl Biol* 156:147–156
- Colinet H, Renault D, Hance T, Vernon P (2006) The impact of fluctuating thermal regimes on the survival of a cold-exposed parasitic wasp, *Aphidius colemani*. *Physiol Entomol* 31:234–240
- Colinet H, Hance T, Vernon P, Bouchereau A, Renault D (2007a) Does fluctuating thermal regime trigger free amino acid production in the parasitic wasp *Aphidius Colemani* (Hymenoptera: Aphidiinae)? *Comp Biochem Physiol A* 147:484–492
- Colinet H, Nguyen TTA, Cloutier C, Michaud D, Hance T (2007b) Proteomic profiling of a parasitic wasp exposed to constant and fluctuating cold exposure. *Insect Biochem Mol Biol* 37:1177–1188
- Colinet H, Lee SF, Hoffmann A (2010) Temporal expression of heat shock genes during cold stress and recovery from chill coma in adult *Drosophila melanogaster*. *FEBS J* 277:174–185
- Colinet H, Lalouette L, Renault D (2011) A model for the time-temperature-mortality relationship in the chill-susceptible beetle, *Alphitobius diaperinus*, exposed to fluctuating thermal regimes. *J Therm Biol* (in press). doi:10.1016/j.jtherbio.2011.07.004
- Convey P (2001) Terrestrial ecosystem response to climate changes in the Antarctic. In: Walther GR, Burga CA, Edwards PJ (eds) "Fingerprints" of climate change-adapted behaviour and shifting species ranges. Kluwer, New York, pp 17–42
- Convey P (2006) Antarctic climate change and its influences on terrestrial ecosystems. In: Bergstrom DM, Convey P, Huiskes AHL (eds) Trends in Antarctic terrestrial and limnetic ecosystems. Springer, Dordrecht, pp 235–272
- Convey P, Pugh PJA, Jackson C, Murray AW, Ruhland CT, Xiong FS, Day TA (2002) Response of Antarctic terrestrial arthropods to multifactorial climate manipulation over a four year period. *Ecology* 83:3130–3140
- Convey P, Key RS, Key RJD, Belchier M, Waller CL (2011) Recent range expansions in non-native predatory beetles on sub-Antarctic South Georgia. *Polar Biol* 34:597–602
- Coulson SJ, Bale JS (1996) Supercooling and survival of the beech leaf mining weevil *Rhynchaenus fagi* L. (Coleoptera: Curculionidae). *J Insect Physiol* 42:617–623
- Crafford JE, Chown SL (1993) Respiratory metabolism of sub-Antarctic insects from different habitats on Marion Island. *Polar Biol* 13:411–415
- Darlington PJ (1970) Coleoptera: Carabidae of South Georgia. *Pac Insects Monogr* 23:234
- David JR, Gibert P, Pla E, Petavy G, Karan D, Moreteau B (1998) Cold stress tolerance in *Drosophila*: analysis of chill coma recovery in *D. melanogaster*. *J Therm Biol* 23:291–299
- Fischer K, Kölzow N, Höltje H, Karl I (2011) Assay conditions in laboratory experiments: is the use of constant rather than fluctuating temperatures justified when investigating temperature-induced plasticity? *Oecologia* 166:23–33
- Frenot Y, Chown SL, Whinam J, Selkirk M, Convey P, Skotnicki M, Bergstrom M (2005) Biological invasions in the Antarctic: extent, impacts and implications. *Biol Rev* 80:45–72
- Ghalambor C, Huey RB, Martin PR, Tewksbury JJ, Wang G (2006) Are mountain passes higher in the tropics? Janzen's hypothesis revisited. *Integr Comp Biol* 46:5–17
- Glanville EJ, Seebacher F (2006) Compensation for environmental change by complementary shifts of thermal sensitivity and thermoregulatory behaviour in an ectotherm. *J Exp Biol* 209:4869–4877
- Hanč Z, Nedvěd O (1999) Chill injury at alternating temperatures in *Orchesella cincta* (Collembola: Entomobryidae) and *Pyrrhocoris apterus* (heteroptera: Pyrrhocoridae). *Eur J Entomol* 96:165–168
- Jeannel R (1940) Croisière du Bougainville aux îles australes françaises. III. Coléoptères. *Memoires du Muséum National d'Histoire Naturelle, France, Série A* 14:63–202
- Johns PM (1974) Arthropoda of the subantarctic islands of New Zealand. I. Coleoptera: Carabidae. Southern New Zealand, Patagonian and Falkland Islands insular Carabidae. *J R Soc N Z* 4:283–302
- Keister M, Buck J (1974) Respiration: some exogenous and endogenous effects on rate of respiration. The physiology of the Insecta. Academic Press, New York, pp 469–509
- Klok CJ, Chown SL (2003) Resistance to temperature extremes in sub-Antarctic weevils: interspecific variation, population differentiation and acclimation. *Biol J Linn Soc* 78:401–414
- Knies JL, Kingsolver JG (2010) Erroneous Arrhenius: modified Arrhenius model best explains the temperature dependence of ectotherm fitness. *Am Nat* 176:227–233
- Košťál V, Vambera J, Bastl J (2004) On the nature of pre-freeze mortality in insects: water balance, ion homeostasis and energy charge in the adults of *Pyrrhocoris apterus*. *J Exp Biol* 207:1509–1521
- Košťál V, Renault D, Mehrabianová A, Bastl J (2007) Insect cold tolerance and repair of chill-injury at fluctuating thermal regimes: role of ion homeostasis. *Comp Biochem Physiol A* 147:231–238
- Lachenicht MW, Clusella-Trullas S, Boardman L, Le Roux C, Terblanche JS (2010) Effects of acclimation temperature on thermal tolerance, locomotion performance and respiratory metabolism in *Acheta domesticus* L. (Orthoptera: Gryllidae). *J Insect Physiol* 56:822–830
- Lalouette L, Košťál V, Colinet H, Gagneul D, Renault D (2007) Cold exposure and associated metabolic changes in adult tropical beetles exposed to fluctuating thermal regimes. *FEBS J* 274:1759–1767
- Lalouette L, Williams CM, Hervant F, Sinclair BJ, Renault D (2011) Metabolic rate and oxidative stress in insects exposed to low temperature thermal fluctuations. *Comp Biochem Physiol A* 158:229–234
- Lebouvier M, Laparie M, Hullé M, Marais A, Cozic Y, Lalouette L, Vernon P, Candresse T, Frenot Y, Renault D (2011) The significance of the sub-Antarctic Kerguelen Islands to assess the vulnerability of native communities to climate change, alien insect invasions and plant viruses. *Biol Invasions* 13:1195–1208
- Lighton JRB (2008) Measuring metabolic rates: a manual for scientists. Oxford University Press, New York



- Marshall KE, Sinclair BJ (2010) Repeated stress exposure results in a survival-reproduction trade off in *Drosophila melanogaster*. *Proc R Soc Lond* 277:963–969
- Nedv ed O, Lavy D, Verhoef HA (1998) Modelling the time–temperature relationship in cold injury and effect of high-temperature interruptions on survival in a chill-sensitive collembolan. *Funct Ecol* 12:816–824
- Ottesen PS (1990) Diel activity patterns of Carabidae, Staphylinidae and Perimylopidae (Coleoptera) at South Georgia, Sub-Antarctic. *Polar Biol* 10:515–519
- Parmesan C (2006) Ecological and evolutionary responses to recent climate change. *Annu Rev Ecol Syst* 37:637–669
- Parmesan C, Yohe G (2003) A globally coherent fingerprint of climate change impacts across natural systems. *Nature* 421:37–42
- P ortner HO (2006) Climate-dependent evolution of Antarctic ectotherms: an integrative analysis. *Deep Sea Res Part II* 53:1071–1104
- P ortner HO, Bennett AF, Bozinovic F, Clarke A, Lardies MA, Lucassen M, Pelster B, Schiemer F, Stillman JH (2006) Trade-offs in thermal adaptation: the need for a molecular to ecological integration. *Physiol Biochem Zool* 79:295–313
- Powell SJ, Bale JS (2006) Effect of long-term and rapid cold hardening on the cold torpor temperature of an aphid. *Physiol Entomol* 31:348–352
- Renault D (2011) Long-term after-effects of cold exposure in adult *Alphitobius diaperinus* (Tenebrionidae): the need to link survival ability with subsequent reproductive success. *Ecol Entomol* 36:36–42
- Renault D, Nedv ed O, Hervant F, Vernon P (2004) The importance of fluctuating thermal regimes for repairing chill injuries in the tropical beetle *Alphitobius diaperinus* (Coleoptera: Tenebrionidae) during exposure to low temperature. *Physiol Entomol* 29:139–145
- Rojas RR, Leopold RA (1996) Chilling injury in the housefly: evidence for the role of oxidative stress between pupariation and emergence. *Cryobiology* 33:447–458
- Sinclair BJ, Roberts SP (2005) Acclimation, shock and hardening in the cold. *J Therm Biol* 30:557–562
- Sinclair BJ, Vernon P, Klok CJ, Chown SL (2003) Insects at low temperatures: an ecological perspective. *Trends Ecol Evol* 18:257–262
- Smith VR (2002) Climate change in the sub-Antarctic: an illustration from Marion Island. *Clim Change* 52:345–357
- S omme L (1974) Anaerobiosis in some alpine Coleoptera. *Norsk Entomologisk Tidsskrift* 21:155–158
- S omme L, Ring RA, Block W, Worland MR (1989) Respiratory metabolism of *Hydromedion sparsutum* and *Perimylops antarcticus* (Coleoptera, Perimylopidae) from South Georgia. *Polar Biol* 10:135–139
- Stevens GC (1989) The latitudinal gradient in geographical range: how so many species coexist in the tropics. *Am Nat* 133:240–256
- Terblanche JS, Klok CJ, Krafur ES, Chown SL (2006) Phenotypic plasticity and geographic variation in thermal tolerance and water loss of the tsetse *Glossina pallidipes* (Diptera: Glossinidae): implications for distribution modeling. *Am J Trop Med Hyg* 74:786–794
- Terblanche JS, Deere JA, Clusella-Trullas S, Janion C, Chown SL (2007) Critical thermal limits depend on methodological context. *Proc R Soc B* 274:2935–2942
- Terblanche JS, Nyamukondiwa C, Kleynhans E (2010) Thermal variability alters climatic stress resistance and plastic responses in a globally invasive pest, the Mediterranean fruit fly (*Carattitis capitata*). *Entomol Exp Appl* 137:304–315
- Thuiller W, Richardson DM, Midgley GF (2007) Will climate change promote alien plant invasions? *Biol Invasions* 193:197–211
- Todd CM (1997) Respiratory metabolism in two species of carabid beetle from the sub-Antarctic island of South Georgia. *Polar Biol* 18:66–171
- Todd CM, Block W (1997) Responses to desiccation in four coleopterans from sub-Antarctic South Georgia. *J Insect Physiol* 43:905–913
- Tollarova-Borovanska M, Lalouette L, Košťal V (2009) Insect cold tolerance and repair of chill-injury at fluctuating thermal regimes: role of 70 kDa heat shock protein expression. *Cryo-Letters* 30:312–319
- Vitousek PM, D’Antonio CM, Loope LL, Rejmanek M, Westbrooks R (1997) Introduced species: a significant component of human-caused global change. *New Zeal J Ecol* 21:1–16
- Wang HS, Zhou CS, Guo W, Kang L (2006) Thermoperiodic acclimations enhance cold hardiness of the eggs of the migratory locust. *Cryobiology* 53:206–217
- Williams CM, Pelini SL, Hellmann JJ, Sinclair BJ (2010) Intra-individual variation allows an explicit test of the hygric hypothesis for discontinuous gas exchange in insects. *Biol Lett* 6:274–277