Thermal tolerance patterns of a carabid beetle sampled along invasion and altitudinal gradients at a sub-Antarctic island

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1. Introduction

The ectothermic nature of insects implies that all aspects of their life are shaped by temperature and its variation. As a result, thermal tolerance (the degree of tolerance of an individual to thermal extremes), in parallel with the plasticity of this trait (the capacity to adjust thermal tolerance through acclimatization), are important predictors of the geographic distribution and sensitivity to global warming of organisms (Andersen et al., 2015a, 2015b; Buckley and Huey, 2016; García-Robledo et al., 2016; Hoffmann et al., 2013). In addition, considering variation in temperature tolerance between populations within species can be used as an indicator of potential abundance and distribution in response to climate change (Sorte et al., 2011). Indeed, thermal tolerance, and the underlying physiological mechanisms permitting adaptation to temperature changes and extremes, represent important traits under selection (Angilletta et al., 2002), particularly for range-shifting and invasive species.

Cold and warm thermal extremes determine the temperature tolerance range over which the performance of an insect can be measured, the so-called thermal performance curve. Using mathematical models, Hillaert et al. (2015) concluded that thermal performance curves of invasive insect populations matched local thermal conditions. Most importantly, they reported that the thermal ranges of individuals were increased at the expansion front, thus sorting the population according to the value of this trait along the invasion gradient; an invasion gradient refers here to increased residence time of the individuals among different colonized localities. However, despite the numerous examples of rapid changes in individual traits along an invasion gradient (Fronhofer and Altermatt, 2015; Laparie et al., 2013; Ochocki and Miller, 2017), few authors have examined potential changes in thermal tolerance in range expanding alien species (Renault et al., 2018).

Thermal biology of insects may vary greatly among ecotypes (individual x habitat characteristics) (Diamond et al., 2017; Sinclair et al., 2012; Tougeron et al., 2016), as the level of thermal tolerance is not only dependent on environmental temperature exposures, but is also influenced by the capacity of individuals to tolerate other environmental factors (Gotcha et al., 2018; Renault et al., 2015) and by the quality of available trophic resources (Colinet et al., 2013). At cellular and molecular levels, a certain uniformity of stress responses can be observed in organisms facing different abiotic stressors (Sulmon et al., 2015), also showing that activating responses against one stress can enhance the ability to cope with other stresses (cross-tolerance; Sinclair et al., 2013; Everatt et al., 2014).

The carabid beetle, *Merizodus soledadinus*, is an invasive insect in the sub-Antarctic Kerguelen Islands in the Indian Ocean. It was introduced from the Falkland Islands to Port-Couvreux (Kerguelen Islands) in 1913 (Arnaud and Beurois, 1996; Jeannel, 1940), from where it has now colonized several locations, creating populations with different times of origin, from 1913 to 2015 (Fig. 1), and phenotypes (Laparie et al., 2013, 2010; Lebouvier et al., 2011). During its continuing range expansion in the Kerguelen Islands, populations of *M. soledadinus* have occupied a variety of habitats, ranging from the seashore to herbfields and bare soil (Renault et al., 2015), whose native species richness and environmental conditions vary markedly. The species has also increased its altitudinal range: no individuals were observed above 110 m above sea level (m a.s. l.) in 1997 (Chevrier et al., 1997), whereas they can now be found up to 400 m a.s.l. ("Subantarctic fauna and flora database, Program IPEV 136 'SUBANTECO'). This recent colonisation of higher altitude habitats has likely been favoured by the clear warming trends documented at the Kerguelen Islands (Lebouvier et al., 2011).

Few studies have examined the temperature sensitivity and thermal tolerance of *M. soledadinus* (Block and Sømme, 1983; Lalouette et al.,

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2012; Laparie and Renault, 2016; Ottesen, 1990). Lalouette et al. (2012) examined the thermal sensitivity of adult *M. soledadinus* sampled at the research station (Port-aux-Français) on the Kerguelen Islands. They concluded that the insect has moderate cold tolerance (in the context of the austral origin of the species), with critical thermal minimum (CT_{min}) around −6.5 °C. Conversely, heat tolerance of the insect greatly exceeded any temperature recorded at the Kerguelen Islands, with a CT_{max} of about 37 °C.

The aim of this study was to compare temperature tolerance among populations of the carabid beetle, *Merizodus soledadinus* Guérin-Méneville, 1832, sampled along invasion (time of colonization) and altitudinal gradients at the French sub-Antarctic Kerguelen Islands. We assumed that temperature average and variation were similar across non-altitudinal sampling locations, and that temperature average decreases by 0.65 °C with each 100 m elevation (Ahrens, 2009; Dillon et al., 2006). We predicted that this temperature difference along a small altitudinal transect should result in the collection of more cold tolerant insects when sampling at higher altitudes. Furthermore, we collected individuals along a transect inland from the coast, in an area where the elevation was low, to investigate if there was a separate effect of distance to the sea. To compare temperature tolerance among insect populations, we used the time to recover after cold or heat exposure (Hoffmann et al., 2003). By investigating responses to both thermal extremes in populations of *M. soledadinus*, we hypothesized that a trade-off may be found in certain populations, tolerant to heat stress and more sensitive to cold stress, and *vice versa*, whereas some populations may show reasonable performance at both extremes, without excelling under any conditions. This expectation is typical of species distributed across environmental gradients (Addo-Bediako et al., 2000). More specifically, we hypothesized, first, that the spatial sorting of populations of *M. soledadinus* would result in different levels of thermal tolerance, with individuals from the invasion front exhibiting a higher temperature tolerance range as a part of a dispersal syndrome. Second, we hypothesized that the colonization of higher altitude habitats has been favoured by global warming, and has resulted in the selection of the most cold-tolerant individuals, that then further acclimatised and/or adapted to cooler habitats.

2. Methods

2.1. Insect collection

Adults of *M. soledadinus* were hand-collected from nine distinct locations in the eastern part of the Kerguelen Islands: Cataractes, Isthme Bas, Molloy, (Anse de) Papous, Port Couvreux, Port Elizabeth, Ratmanoff, Saint Malo and Val Studer (Fig. 1). A brief habitat description was made at each site before insects were collected. Insect collection was carried out using aspirators, and insects were directly transferred into plastic containers (L: 17.5 cm, W: 11.5 cm, H: 7 cm), with a piece of paper tissue (approx. 20 cm × 20 cm) provided for shelter. The insects were then returned to the research station at Port-aux-Français. They were supplied with water in a small tube (approx. 3 mL) with cotton wool, and placed for 16–36 h in a cabinet at a constant 4 °C before commencement of experiments. This was considered sufficient for the insects to recover from the transport phase, but not too long for them to acclimate to the laboratory conditions.

2.1.1. Invasion gradient

Six of the distinct collection locations for *M. soledadinus* populations were characterized by different duration of residence since colonisation: Port Couvreux (introduced in 1913), Port Elizabeth (colonized between 1939 and 1983), Cataractes (colonized between 1983 and 1990), Isthme Bas (colonized between 2000 and 2005), Val Studer (colonized between 2014 and 2016) and Ratmanoff (colonized in 2016). There is no evidence that *M. soledadinus* has been introduced to the Kerguelen Islands on several distinct occasions, and therefore we consider that all contemporary populations of *M. soledadinus* have originated from the Port Couvreux population. For ease of overview of the residence times of the different populations, each location name was given a number corresponding to their minimum residence time (years) at the time of collection of the insects (2018): Port Couvreux 105, Port Elizabeth 35, Cataractes 28, Isthme Bas 13, Val Studer 2, and Ratmanoff 2.

2.1.2. Altitudinal and lowland gradients

At Saint Malo and Molloy, insects were collected along an altitudinal gradient up to 250 m a.s.l.. At Saint Malo, three populations were
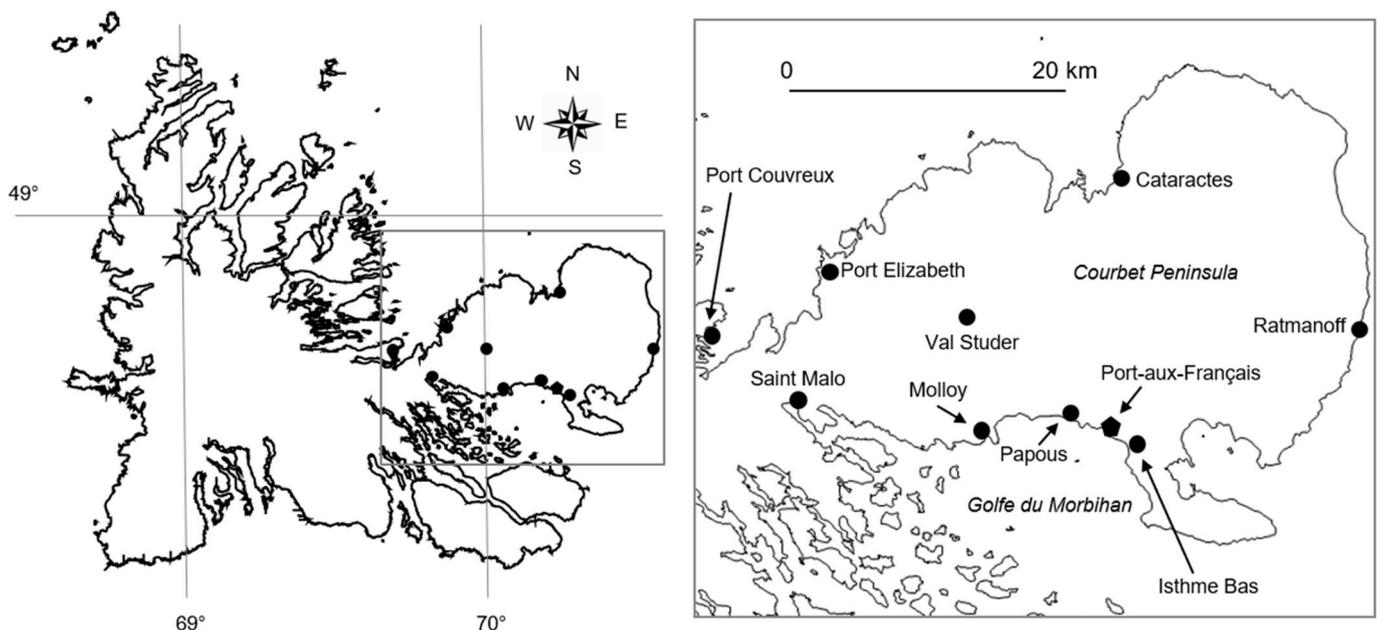

Fig. 1. Map of the Kerguelen Islands indicating the localities where populations of the carabid beetle Merizodus soledadinus were sampled. Port Couvreux (introduction site of M. soledadinus in 1913), Port Elizabeth (colonized by M. soledadinus in 1939–1983), Cataractes (colonized in 1983–1990), Saint Malo (colonized in 1991–1995), (Anse de) Papous (colonized in 1995–2000), Isthme Bas (colonized in 2000–2005), Molloy (colonized in 1995–2005), Val Studer (colonized in 2014–2016) and Ratmanoff (colonized in 2016).

collected from different elevations (0–5 m, 95–110 m and 190–210 m a. s.l.) and given the corresponding names: Saint Malo 0, Saint Malo 100 and Saint Malo 200. At Molloy, four populations were collected at three different elevations (0–5 m, 110–130 m and 245–250 m a.s.l.), with two distinct habitats being sampled at around 125 m a.s.l., one having less vegetation (Molloy 125) than the other (Molloy 125 Vegetation).

At Papous, a horizontal seashore – inland transect was completed, with sampling at different distances from the coast (approximately 2–15 m, 185–215 m, 385–415 m and 785–815 m): Papous 0, Papous 200, Papous 400 and Papous 800 respectively.

2.2. Measurement of the duration of recovery from cold and heat exposures

Empty Petri dishes lined with paper were placed into an incubator. After approximately 10 min, five individuals were added into each of 4-5 Petri dishes, and covered with lids; the cabinet was closed and a timer started. After 15 min exposure to a given temperature, the dishes were removed from the incubator, rapidly transferred into a walk-in chamber at $9 \pm 1.5\,°C$, and a new timer was started. The number of insects in coma, and number of recovered insects (walking ability restored) were subsequently noted every minute, over 30 min (The recovery period was monitored for up to 120 min in a preliminary test, and no change in the number of recovered beetles was observed as compared with 30 min). Any individual that had not recovered after 30 min was considered dead. This procedure was repeated for each population and temperature treatment. All populations were exposed to −6, −7 or 37 °C for 15 min. The duration of exposure of 15 min was selected after having performed preliminary tests, with insects being exposed at these three temperatures for different durations (from 5 to 120 min; data not shown). Depending on the number of insects available for each population, the remaining adults were exposed to up to eight distinct temperatures (−5, −6, −7, −8, 35, 36, 37 and 38 °C) for 15 min; this additional experiment ensured that we investigated temperatures that were close to the thermal limits of the species, and that any difference in temperature tolerance would be identified.

To test for the effect of prolonged duration of exposure on the subsequent recovery, further assays conducted at −6 °C were completed with duration of exposure of the adult *M. soledadinus* of 1 or 2 h in addition to the 15 min (0.25 h) treatment for the populations of the altitudinal (Molloy, St Malo) and inland-shore (Papous) gradients. During the experiments, each insect was only used once (*i.e.* exposed to one temperature and duration combination on a single occasion).

2.3. Statistical analysis

All curves were generated based on Kaplan Meier estimates computed in R version 3.5.2, using the Survminer package (Kassambara and Kosinski, 2018; R Core Team, 2018). The curves were estimated based on the time of recovery of 20–25 individuals for each population. Pairwise comparisons with adjusted P-values (Benjamini & Hochberg) were performed *post-hoc*, with a significance value of 0.05.

3. Results

3.1. Recovery of adult M. soledadinus sampled along an invasion gradient transect

The recovery dynamics of 20 adult *M. soledadinus* from six different populations after exposure to −7 °C or 37 °C for 15 min are presented in Fig. 2A, B. There were significant differences in recovery dynamics and temperature tolerance among insects from the different populations (−7 °C: P < 0.0001; 37 °C: P = 0.0018), but these differences were not related to residence times of the different populations. The populations from Isthme Bas 13 and Cataractes 28 were more sensitive to cold than individuals from the four other populations (Val Studer 2, Port Elizabeth 35, Ratmanoff 2 and Port Couvreux 105), whose levels of cold tolerance were similar (Fig. 2A).

Adults of *M. soledadinus* from Ratmanoff 2 and Val Studer 2 were significantly more sensitive to heat exposure (*i.e.* insects exhibited more limited ability to recover after 15 min exposure at 37 °C) compared to those collected from Cataractes 28 (Fig. 2B) (Ratmanoff:Cataractes, P = 0.002; Val Studer:Cataractes, P = 0.047). At the end of the recovery period, the proportion of insects from Ratmanoff 2 that recovered was relatively low after heat shock (*ca.* 20% recovery after 30 min), whereas *ca.* 60% of the insects from Port Elizabeth 35 and Port Couvreux 105 recovered, and *ca.* 42% from Isthme Bas 13 and Val Studer 2 (Fig. 2B).

The recovery dynamics of adult *M. soledadinus* that were exposed to other temperature conditions (−5, −6, 35, or 36 °C) are presented in Supp Fig. 1.

3.2. Recovery of adult M. soledadinus sampled along an altitudinal gradient

The ability to recover from cold or heat exposure in insects sampled along an altitudinal gradient at Saint Malo and Molloy is presented in Figs. 3 and 4, respectively. All populations collected from Saint Malo exhibited a similar ability to recover from cold or heat exposure (Fig. 3A,
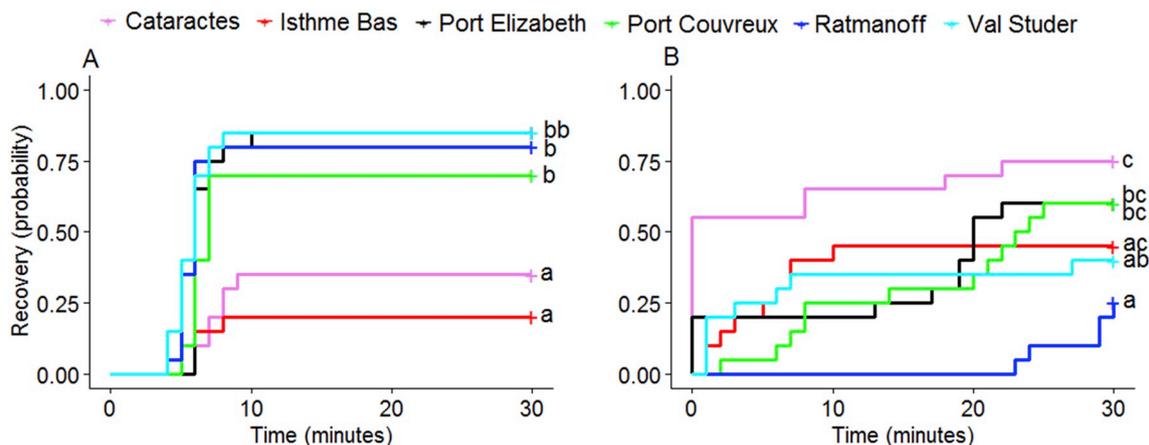

Fig. 2. Recovery curves of adult *Merizodus soledadinus* from six different populations sampled along an invasion gradient at the Kerguelen Islands after they were exposed to **A)** −7 °C for 15 min or **B)** 37 °C for 15 min. Recovery curves were estimated using the Kaplan Meier method; vertical tick-marks represent insects that did not recover at the end of the 30 min recovery period ("right censored data"). Distinct letters indicate different recovery dynamics among the populations (log rank test with adjusted P-values (Benjamini & Hochberg) for pairwise comparisons.

B) (−7 °C: P = 0.056; 37 °C: P = 0.17). A trend was observed in the cold treatment recovery curves (Fig. 3A) where, the higher altitude the population was collected from, the more individuals were able to recover.

As shown in Fig. 4A, B, there were no significant differences in the ability to recover from extreme low or high temperature exposure in any of the Molloy populations (−7 °C: P = 0.13 (4A; 37 °C: P = 0.063). The recovery dynamics of the individuals from Saint Malo that were exposed to other temperature conditions (−5, −6, 36 or 38 °C) are presented in Supp Fig. 2. The recovery dynamics of the individuals from Molloy that were exposed to other temperature conditions (−5, −6, −8, 35, 36 or 38 °C) are presented in Supp Fig. 3.

3.3. Duration of recovery of adult M. soledadinus exposed for different time periods

The recovery times of 25 individuals from the Molloy populations after they were exposed to −6 °C for 0.25 h, 1 h or 2 h are shown in Fig. 5. For populations sampled at Molloy 0 (Fig. 5A), Molloy 125 (Fig. 5B) and Molloy 125 Vegetation (Fig. 5C), the recovery dynamics of the insects exposed to cold for the three durations did not differ significantly (0 m: P = 0.17; 125 m rocky habitat: P = 0.076; 125 m vegetation: P = 0.19). However, there was a significant difference between recovery curves from the Molloy 250 population after 1 h exposure as compared with those exposed for 0.25 h at −6 °C (P = 0.002). This latter population was not exposed to −6 °C for 2 h, due to lack of available individuals. No significant difference was observed among the recovery curves when the insects from the altitudinal transect at St Malo and the seashore-inland transect at Papous were exposed to - 6 °C for different durations (Supp Figs 4 and 5) (0 m [Supp Fig. 4A]: P = 0.29; 100 m [Supp Fig. 4B]: P = 0.08; 200 m [Supp Fig. 4C]: P = 0.56; 0 m [Supp Fig. 5A]: P = 0.2; 200 m [Supp Fig. 5B]: P = 0.48; 400 m [Supp Fig. 5C]: P = 0.24; 800 m [Supp Fig. 5D]: P = 0.44).

3.4. Duration of recovery of adult M. soledadinus sampled along a seashore-inland transect

Populations collected from Papous were exposed to −7 °C (Fig. 6A) and 37 °C (Fig. 6B) for 15 min. Insects from the Papous 800 population were characterized by a significantly higher recovery after exposure to −7 °C for 15 min than those from Papous 200 (Fig. 6A) (P = 0.02). There were no significant differences among the recovery curves from different heat exposures (Fig. 6B) (P = 0.19). The recovery dynamics of adult M. soledadinus that were exposed to other temperature conditions (−5, −6, −8, 35, 36 or 38 °C) are presented in Supp Fig. 6.

4. Discussion

In the present study, we examined the thermal sensitivity (to cold and heat) of the carabid beetle M. soledadinus, which is invasive to the Kerguelen sub-Antarctic Islands. In the Kerguelen archipelago, the progressive range expansion of M. soledadinus has generated invasion, residence time, altitudinal, and seashore – inland gradients, allowing examination of whether the invasion process (dispersal syndrome) and colonisation of higher altitude and inland locations are associated with shifts in thermal sensitivity.

4.1. Thermal sensitivity of M. soledadinus adults sampled along an invasion gradient

A large range of habitats have been colonized by M. soledadinus in the Kerguelen Islands, and it has been hypothesized that this invasion success is facilitated by the trophic and physiological plasticity of the insect (Hidalgo et al., 2013; Laparie et al., 2012). In this study, measurement of the thermal sensitivity of adult M. soledadinus revealed two main recovering groups after cold exposure, and a wide range in ability to recover after heat exposure, among populations. The differences in thermal tolerance among geographically distinct populations have likely been shaped by the different biotic and abiotic filters encountered by the insects during range expansion, and by genetic drift, as small insect groups are likely to have split off and established new colonies when M. soledadinus spread across the island.

We infer that the degree of stress resistance, including thermal tolerance, of the insects has increased along the invasion gradient, as this would enhance the probability of successfully reaching new habitats in heterogeneous environments. Consistent with this, individuals sampled at the invasion front (Val Studer 2 and Ratmanoff 2) exhibited high ability to recover from cold exposure, even though their cold tolerance levels were similar to those of individuals from the longer-established populations at Port Elizabeth 35 and Port Couvreux 105. The colder winter conditions that were typical until late 1970s may have shaped the thermal sensitivity of the populations from habitats that have been colonized longer, in turn explaining the comparatively high recovery abilities in individuals from the populations of Port Couvreux 105 and Port Elizabeth 35. The mean minimum winter temperature was −5.9 °C over the years 1951-69, with frequent occurrence of temperatures lower than – 7 °C. Since then, climatic conditions have progressively changed at the Kerguelen Islands (see also Lebouvier et al., 2011), and mean minimum temperatures increased to −4.6 °C from 1970 to 2017, and even higher (−3.6 °C) in the period 1990–2004 coinciding with when the geographic expansion of M. soledadinus greatly increased (Météo France data, https://donneespubliques.meteofrance.fr/, records from Port-aux-Français, Kerguelen Islands).
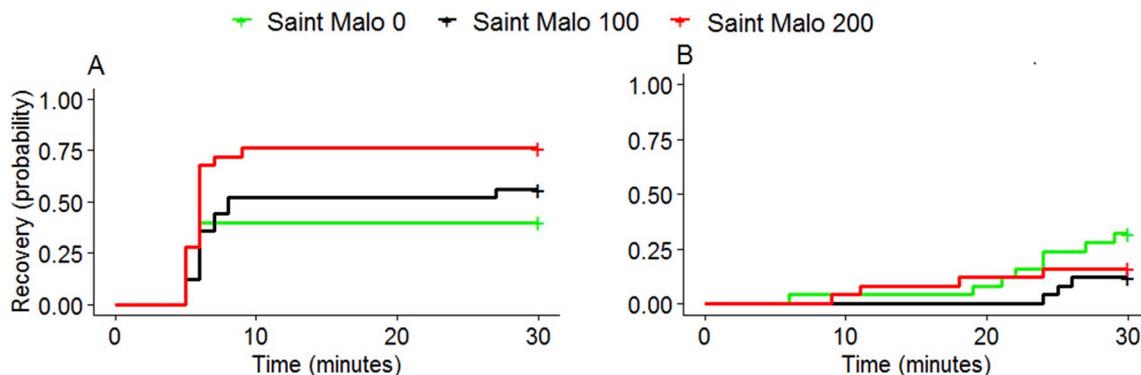

Fig. 3. Recovery curves of adult *Merizodus soledadinus* from three different populations sampled along an altitudinal gradient at the Kerguelen Islands after the insects were exposed to **A)** −7 °C for 15 min (The recovery curves were not significantly different, P = 0.056). **B)** 37 °C for 15 min (The recovery curves were not significantly different, P = 0.17). Recovery curves were estimated using the Kaplan Meier method; vertical tick-marks represent insects that did not recover at the end of the 30 min recovery period ("right censored data").
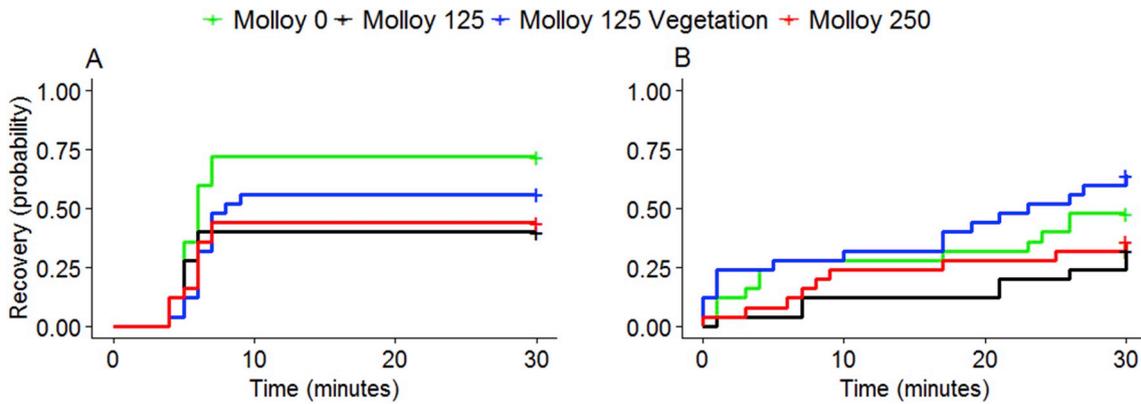
Fig. 4. Recovery curves of adult *Merizodus soledadinus* from four different populations sampled along an altitudinal gradient at the Kerguelen Islands after they were exposed to **A)** −7 °C for 15 min (The recovery curves were not significantly different, P = 0.13). **B)** 37 °C for 15 min (The recovery curves were not significantly different, P = 0.063). Recovery curves were estimated using the Kaplan Meier method; vertical tick-marks represent insects that did not recover at the end of the 30 min recovery period ("right censored data").
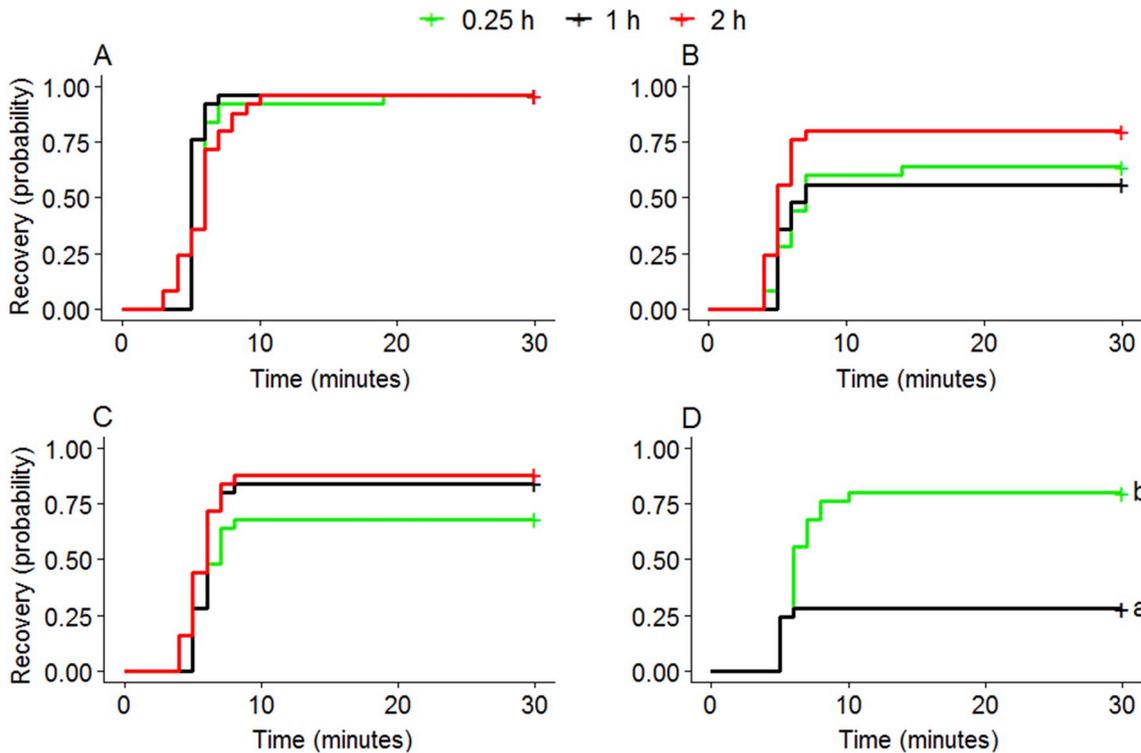

Fig. 5. Recovery curves of adult *Merizodus soledadinus* from four populations sampled along an altitudinal gradient at Molloy (Kerguelen Islands) after they were exposed for increasing periods (0.25 h, 1 h, 2 h) to −6 °C **A)** Individuals sampled at 0 m a.s.l.; the recovery curves were not significantly different (P = 0.17). **B)** Individuals sampled at 125 m a.s.l. in a rocky habitat; again, no significant difference between the recovery curves (P = 0.076). **C)** Individuals sampled at 125 m a.s.l in a vegetated habitat; again, no significant difference between the recovery curves (P = 0.19). **D)** Individuals sampled at 250 m a.s.l.; distinct letters indicate significant differences between recovery curves (log rank test with Benjamini & Hochberg adjusted P-values for pairwise comparisons). Recovery curves were estimated using the Kaplan Meier method; vertical tick-marks represent insects that did not recover at the end of the 30 min recovery period ("right censored data").

Since the 1970s, the progressively warming climate across the sub-Antarctic islands (Berthier et al., 2009; Frenot et al., 1993) may have facilitated the geographic expansion of *M. soledadinus* in the Kerguelen Islands. Selection for cold tolerance may have become less intense, at least at low altitudes, whereas other environmental factors, such as salinity in coastal habitats and desiccation resistance (see for instance Hidalgo et al. (2013), Ouisse et al. (2016) and Yerushalmi et al. (2016)) may have driven the physiological performance of the range-expanding populations of *M. soledadinus*. It is noteworthy that both the well-established populations of Cataractes 28 and Isthme Bas 13 are relatively sensitive to cold compared to the other populations, and are

thus not following a pattern of increased cold tolerance along the invasion gradient. In parallel, individuals from these two populations are equally or more resistant to heat exposure when compared to individuals from Port Couvreux 105 and Port Elizabeth 35. Studies that have investigated temperature acclimation under field conditions provide evidence that enhanced thermal tolerance could lead to clear benefits for individuals, but also costs in the case of exposure to high temperatures (Chidawanyika and Terblanche, 2010; Kristensen et al., 2007; Loeschcke and Hoffmann, 2007; Sørensen et al., 2013). In addition, Dewitt et al. (1998) and Auld et al. (2010) described how fitness costs can be associated with the level of adaptive phenotypic plasticity
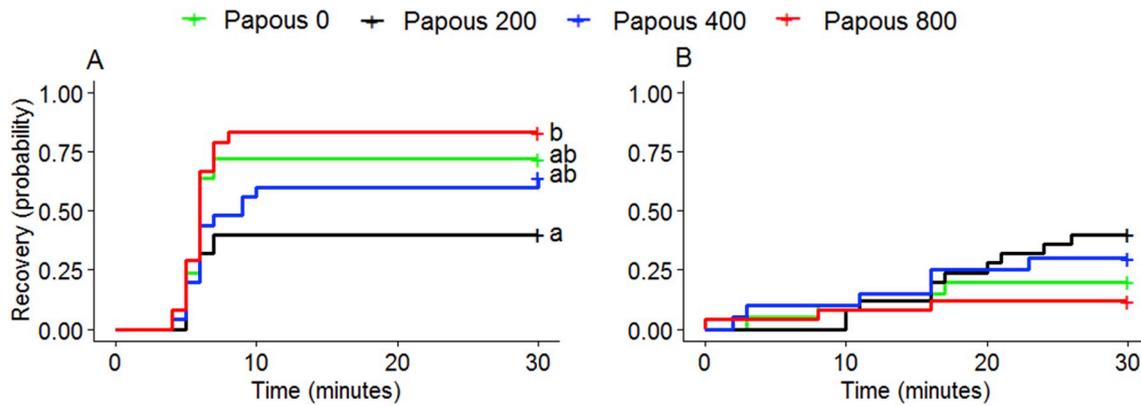
Fig. 6. Recovery curves of adult *Merizodus soledadinus* from four different populations sampled along an inland - seashore transect at the Kerguelen Islands after they were exposed to **A)** −7 °C for 15 min. Distinct letters indicate different recovery dynamics among the populations (log rank test with adjusted P-values (Benjamini & Hochberg) for pairwise comparisons. **B)** 37 °C for 15 min (The recovery curves were not significantly different, P = 0.19). Curves were estimated using the Kaplan Meier method; vertical tick-marks represent insects that did not recover at the end of the 30 min recovery period ("right censored data").

for a certain trait. If such costs apply to the thermal tolerance of *M. soledadinus* in the Kerguelen Islands (the higher the plasticity of thermal tolerance, the higher the fitness cost), this would explain the observation that many populations showing somewhat higher ability to recover from cold also have lower ability to recover from heat, and *vice versa*. However, no such trade-off between cold and heat tolerance was found in a winter acclimatized Danish *Drosophila subobscura* population (Givskov Sørensen et al., 2015), and the absence of this trade-off in populations of *M. soledadinus* from Port Couvreux 105 and Port Elizabeth 35 suggests that additional variables shape the thermal breadth of these insects.

4.2. Adult M. soledadinus sampled along an altitudinal gradient

The colonization of higher altitude habitats may have been favoured by recent climate change. However, the climatic conditions encountered at moderate elevations at the Kerguelen Islands may remain sub-optimal for adult *M. soledadinus*. We thus hypothesized that populations from higher altitudes would be more cold tolerant than those collected closer to sea level. Although no statistically significant difference was detected among the recovery curves of the different populations sampled along the altitudinal gradient at Saint Malo, the curves fall in sequence from the highest location (Saint Malo 200) to the location closest to sea level (Saint Malo 0) in terms of recovery ability from cold exposure. In bumble bees, the critical thermal minimum was lower and the insects recovered at lower ambient temperatures when they were sampled at high altitudes (Oyen et al., 2016), as also reported in the copper butterfly *Lycaena tityrus* (Karl et al., 2008); however, these two studies compared the thermal tolerance of specimens collected from low to high altitudes on a larger altitudinal gradient than in the present work.

A different recovery pattern was observed for the individuals collected from the second altitudinal transect (Molloy), with fewer individuals from Molloy 250 and Molloy 125 recovering from exposure to either high or low temperature compared with those collected at Molloy 0 and Molloy 125 vegetation. These findings suggest that habitat quality influenced temperature tolerance in these populations as distinct from elevation *per se*. The locations where Molloy 0 and Molloy 125 Vegetation were collected had a larger proportion of vegetation, and can therefore also be expected to give access to more nutrient-rich and varied habitats. Thermal tolerance physiology can be influenced by the quality of trophic resources as reported by Colinet et al. (2013), who found that sugar-enriched diets increased chill coma recovery ability in the fruit fly *Drosophila melanogaster*. In other studies, low diet quality had no effect on the critical thermal maximum of the chrysomelid *Cephaloleia belti* (Garcia-Robledo et al., 2018), whereas the feeding status (fed *versus* starved insects) of the fly *Ceratitis capitata*

(Nyamukondiwa and Terblanche, 2009) affected their thermal tolerance, and starvation lowered the supercooling point - and increased cold tolerance - in the beetle *Alphitobius diaperinus* (Salin et al., 2000). The overall quality of the environment can contribute to temperature tolerance in insects, either directly, or by eliminating some level of multi-stress exposure occurring when factors such as starvation or dehydration coincide. Finally, vegetation cover makes ground temperature more similar to air temperature than in rocky habitats where insects may be more exposed to heat stress because of high level of radiation (Buckley et al., 2013).

4.3. Adult M. soledadinus sampled along a seashore-inland transect and exposed for different time periods

The seashore-inland transect was established in order to identify any effects of the distance to the sea on the thermal tolerance of adult *M. soledadinus*. The distance to the coast did not systematically affect temperature tolerance: the four populations sampled at Papous had very similar recovery curves after exposure to high temperature, but the pattern of the graphs could suggest a trade-off with cold tolerance. As the different populations sampled at Papous were physically closer to each other compared to the other studied populations, and the ground between them apparently without obstruction, it is possible that movement of individuals between populations at Papous 0, Papous 200 and Papous 400 was sufficient to obscure any differences between samples collected from the different locations. Moreover, while landscape composition has been reported to affect the thermal tolerance of parasitic wasps (Tougeron et al., 2016), micro-scale characteristics of the sampled habitats may have remained under oceanic influence, and thus under salinity gradients (Herbst, 2001) that constrain the thermal tolerance of adult *M. soledadinus*. Similarly, Warren et al. (2018) reported that the thermal tolerance of ants from coastal areas was lower as compared with the specimens collected further inland. It is thus possible that insects from Papous 800 were sufficiently well separated from the other populations and far away for the coast to lead to different recovery patterns after extreme cold exposure.

As differences in thermal tolerance sometimes become apparent when animals are exposed to high stress levels, it is interesting to note that increasing the duration of exposure to −6 °C did not have a significant effect on the recovery dynamics of all but one of the tested populations. In insects, chill coma results from the progressive depolarization muscle resting membrane potential (MacMillan et al., 2012; Andersen et al., 2015a, 2015b). Our results suggest that the 15 min exposure at cold temperature was long enough for altering the membrane potential over the critical threshold associated with the onset of chill coma, as the recovery dynamics and number of recovered beetles

did not change significantly with increasing exposure duration. The exception to this generalisation was provided by the population from the highest elevation at Molloy (Molloy 250), which showed a significant reduction in tolerance to exposure to −6 °C when the duration was increased from 15 min to 1 h.

5. Conclusions

In this study, carabid beetles sampled from populations at their invasion front in the Kerguelen Islands were characterized by a high level of cold tolerance, but this was also the case for populations with the longest residence times. Conversely, two populations with intermediate residence times exhibited lower cold tolerance, confirming that the expression of this trait is not systematically enhanced along the invasion gradient. A pattern indicating a trade-off between cold and heat tolerance was observed for many of the tested populations collected along the invasion gradient. Temperature tolerance of insects from the two altitudinal transects was not systematically enhanced with elevation, illustrating that other factors in addition to environmental temperature, including trophic resources, determine the temperature tolerance of the animals. Similarly, the thermal tolerance varied among insects sampled from the horizontal seashore-inland transect. In future studies, the influence of quality and quantity of available trophic resources, humidity, and biotic interactions on the thermal tolerance of adult *M. soledadinus* should be investigated in order to better characterise the drivers of this trait along environmental gradients. Moreover, the experimental design could be amended by increasing the duration of laboratory acclimation in order to better disentangle possible adaptive patterns from the effects of environmental conditions experienced by the insects in their respective habitats before they were sampled. Finally, the similar recovery curves of the insects exposed to longer duration of cold exposure suggest that any damage inflicted by cold exposure in the treatment used here occurs within the first 15 min of exposure in this species.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jtherbio.2019.102447.

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