



# Thermal adaptations of adults and eggs in the Arctic seed bug *Nysius groenlandicus* (Insecta: Hemiptera) from South Greenland

S. Bahrndorff<sup>1</sup> · T. Alemu<sup>2</sup> · T. N. Kristensen<sup>1</sup> · M. H. Sørensen<sup>1</sup> · T. T. Høye<sup>3,5</sup> · M. Holmstrup<sup>4,5</sup>

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## Abstract

Temperatures in Arctic and sub-Arctic regions are highly variable, with long, and cold winters, and short summers. Studies on thermal biology of ectotherms from these latitudes have mostly focused on how organisms cope with cold temperatures. However, temperatures in microhabitats such as south-facing slopes can occasionally reach high and potentially stressful temperatures. Such microclimatic conditions are challenging for organisms, since they need to time their development to coincide with the short summer, while coping with thermal extremes during summer and winter. In the present study, we investigate upper and lower thermal limits of adults and eggs in *Nysius groenlandicus*, a univoltine seed bug widely distributed in Greenland and alpine areas of Scandinavia and with preference for dry and sunny habitats. Secondly, we investigate the survival strategy of the overwintering egg stage during cold exposure. We found a clear association between the microhabitat temperatures experienced by each specific life stage and their heat and cold tolerance. Thus, adults and eggs, which are both exposed to high summer temperatures, showed a high heat tolerance. Adults which do not overwinter had very poor cold tolerance, whereas eggs produced in autumn were very cold hardy (with some survival at  $-21\text{ °C}$ ). Cold tolerance of the eggs of *N. groenlandicus* was based on freeze avoidance through extensive supercooling and by conserving water. These results suggest life stage-specific adaptation to heat and cold stress in Arctic and sub-Arctic insects, and highlight new research avenues addressing adaptation to higher and more variable temperatures in Arctic and sub-Arctic regions.

**Keywords** Life cycle · Insects · Arctic · Cold stress · Univoltine · Seed bug · Heat stress

## Introduction

Temperatures in Arctic and sub-Arctic regions are highly variable, and large temperature fluctuations can occur both within and across seasons (IPCC 2014; Convey et al. 2018). In winter, species will experience severe cold conditions, where temperatures can go below  $-30\text{ °C}$ , and depending

on microhabitat, many species will regularly be exposed to temperatures below  $-20\text{ °C}$  (Danks et al. 1994). In contrast, although summers are generally short and cool, microhabitat temperatures vary substantially and on sun exposed slopes may reach  $40\text{ °C}$  or more (Danks et al. 1994; Böcher and Nachman 2001; Graham et al. 2012; Convey et al. 2018).

Conditions suitable for insect growth and development in the Arctic are limited to short summers, which means that many species need more than 1 year to complete their life cycle (Danks et al. 1994; Denlinger and Lee 2010). Similarly, across species, it varies which life stage overwinters and thus also which stage(s) is exposed to the extreme cold conditions during winter (Danks et al. 1994). The life cycle timing of Arctic and sub-Arctic insects thus depends as much on the need to coincide development with short summers as on the need to resist thermal extremes during winter and summer. Life stage-specific abilities to cope with heat and cold stress are well investigated in temperate and tropical insects (Bowler and Terblanche 2008; MacLean et al. 2017). However, we have limited information on life

✉ S. Bahrndorff  
sba@bio.aau.dk

<sup>1</sup> Department of Chemistry and Bioscience, Aalborg University, Fredrik Bajers Vej 7H, 9220 Aalborg, Denmark

<sup>2</sup> Department of Natural Resource Management, Jimma University, Jimma, Ethiopia

<sup>3</sup> Section for Biodiversity, Department of Bioscience, Aarhus University, Grenåvej 14, 8410 Rønne, Denmark

<sup>4</sup> Section for Terrestrial Ecology, Department of Bioscience, Aarhus University, Vejlshøjvej 25, 8600 Silkeborg, Denmark

<sup>5</sup> Arctic Research Center, Aarhus University, Ny Munkegade 114, 8000 Aarhus C, Denmark

stage-specific abilities to cope with heat in Arctic or sub-Arctic species (but see Jaco and Chown 1997 and Anthony et al. 2019).

For the study of thermal limits across life stages of Arctic and sub-Arctic insects, the seed bug, *Nysius groenlandicus* Zetterstedt, is a well-suited model. *N. groenlandicus* is widely distributed in Arctic and sub-Arctic regions including Greenland and can reach numbers exceeding 100 individuals per square meter. Results suggest that, in Greenland, the species is univoltine, generally hatching from overwintering eggs in June, and then develop through its five nymph stages to reach the adult stage in July before laying eggs throughout August (Böcher 1976; Böcher and Nachman 2001). Temperatures in habitats where *N. groenlandicus* is found are often high during summertime and with correspondingly low levels of humidity (Böcher 1972; Böcher and Nachman 2001; Sørensen et al. 2019), although low temperatures can also be experienced during summer (Coulson et al. 1995). For example, temperature during summer can range from 5 to 45 °C in one day (Sørensen et al. 2019). In accordance with this, both nymphs and adults prefer temperatures of about 30 °C (Böcher and Nachman 2001). During winter, the same areas may have shallow snow cover with soil surface temperatures similar to air temperatures that occasionally can drop below – 35 °C in northern parts of the range of *N. groenlandicus*. Insects in polar regions, including *N. groenlandicus*, are living at temperatures below their optimal temperature for development and reproduction. They are, therefore, expected to benefit from higher temperatures (Deutsch et al. 2008). However, the mechanisms which enable insects to cope with heat stress or benefit from occasional high summer temperatures in the Arctic and sub-Arctic regions are virtually unknown (but see Hodkinson et al. 1996; Høye and Culler 2018; Sørensen et al. 2019).

The eggs of *N. groenlandicus* are laid in litter on the soil surface and the species overwinters in the diapausing egg stage, which is exposed to microhabitat temperatures of – 20 °C or lower during winter (Böcher 1976; Böcher and Nachman 2011). No data indicate that eggs spend more than a single winter before hatching and nymphs or adults never overwinter. The winter survival strategy employed by *N. groenlandicus* eggs is not known, although freeze avoidance by supercooling is the predominant strategy in many Arctic insects including other Hemiptera species (Sømme 1982; Sinclair et al. 2003). The effects of sub-zero temperatures depend on exposure duration and severity as well as life stage or acclimation status of the organism (Denlinger and Lee 2010). The physiological adaptations of insects to survive these sub-zero winter temperatures in polar habitats are relatively well understood. Species which inhabit cold environments generally use one of two main survival strategies known as *freeze avoidance* and *freeze tolerance*, respectively (Denlinger and Lee 2010; Teets and Denlinger 2013).

Many insects exposed to sub-zero temperatures are freeze avoiding and will in winter increase cold tolerance by promoting their ability to supercool. The capacity to supercool increases with the shedding of ice nucleators from hemolymph and gut, accumulation of low-molecular-mass solutes in hemolymph, and as water content decreases (Denlinger and Lee 2010). Freeze tolerant insects are able to tolerate freezing of their extracellular body fluids and most species avoid extensive supercooling by accumulation of ice nucleating molecules in their guts or in hemolymph (Denlinger and Lee 2010). Finally, small and highly permeable invertebrates use cryoprotective dehydration, where they do not base their winter survival on supercooling, but instead dehydrate and equilibrate their body fluid melting point to the ambient temperature (Holmstrup et al. 2002).

In the present study, we investigated the critical thermal limits in adults and eggs of *N. groenlandicus*. We expected, given an assumed univoltine life cycle, that the adults of *N. groenlandicus* would be adapted to the thermal conditions experienced during summer, whereas the eggs would show thermal adaptations to both summer and winter conditions. Since eggs of *N. groenlandicus* are small and exposed to low sub-zero temperatures for long periods of time during winter, they are prone to become dehydrated during winter by the low water vapor pressure of ice in their habitat (Holmstrup 2014). We therefore also investigated if the cold tolerance strategy of the eggs of *N. groenlandicus* is based on cryoprotective dehydration or supercooling.

## Materials and methods

### Sample collection and maintenance

Collection of adult individuals was performed using a fine mesh sweeping net and seed bugs were subsequently transferred to plastic tubes with screw caps (100 mL, 76 × 62 mm) containing various grass seeds collected in the field for food and structure. Eggs for the experiments were collected either on grass seeds from the field or eggs laid by individuals in the laboratory using a fine brush. Seed bugs were collected in August, 2016 and 2017 in the area of Narsarsuaq in South Greenland (Lat: 61.18, Long: – 45.38). The habitat in which the eggs were collected is dominated by large fluctuations in temperature during summer and can reach temperatures up to 45 °C (Sørensen et al. 2019). In the laboratory, the seed bugs were maintained in large petri dishes (145 × 20 mm) in a climate room at 20 °C (± 0.5) and a relative humidity of 51.6% (± 4.4) with 12:12 light/dark cycle. Individuals had access to water from tubes (11.5 mL, 100 × 15.7 mm) closed with cotton balls. The seed bugs were fed raw and unsalted sunflower seeds.

## Study design

### Thermal tolerance of adults

Field obtained adults collected in 2016 were quickly sexed under CO<sub>2</sub> anesthesia (less than 5 min exposure) and then returned to 20 °C for 24 h before tests of critical thermal limits (CT<sub>min</sub> ( $N=7-8$ ) and CT<sub>max</sub> ( $N=9$ )) following procedures in Overgaard et al. (2012) and Sørensen et al. (2019). In short, adults were placed in individual 5 mL glass vials and placed in a circulating water/glycol bath at 20 °C and subsequently exposed to a slow ramp down or up of temperature (rate of 0.1 °C min<sup>-1</sup>) for assessment of critical thermal minimum (CT<sub>min</sub>) or maximum (CT<sub>max</sub>), respectively. The temperature at which all movement ceased was recorded as the thermal limit (CT<sub>min</sub> or CT<sub>max</sub>) for that individual (Terblanche et al. 2011). Controls were kept in similar vials and conditions, but at constant 20 °C.

### Thermal tolerance of eggs

Field studies suggest that *N. groenlandicus* is hibernating in an obligatory diapause egg stage. However, laboratory studies show that some individuals will develop without diapause and that the percentage of hatching eggs will increase after exposure to cold periods (Böcher 1975). Eggs collected in the field in 2016 (see above) were thus kept at 0 °C for 21 days to terminate diapause. Subsequently, eggs were exposed to either heat or cold ramping (0.1 °C min<sup>-1</sup>). When lower temperatures (− 10, − 15, − 20, and − 21 °C) or upper temperatures (30, 35, 40, and 50 °C) were reached six-to-seven replicates of 10 eggs per temperature were removed from the water bath and placed under control conditions (20 °C). Subsequently, the number of hatched eggs (first instar nymphs) was recorded daily.

### Supercooling points, hemolymph melting point, water content, and survival during long-term cold exposure

Eggs laid by the adults collected in the field in August 2017 were used for Differential Scanning Calorimetry analysis (DSC) following cold exposure. In this experiment, eggs were placed in vials (1.6 cm diameter and 3 cm high), which were subsequently placed in 200 mL plastic beakers (7 cm diameter and 4 cm high) containing crushed ice to simulate overwintering in frozen litter. The beakers were closed with parafilm and a fitting lid, so that the eggs were exposed to the vapor pressure of ice (Holmstrup 2014). The beakers containing ice and sample vials were placed in custom-made programmable freezing cabinets where temperature was gradually lowered. Initially, the beakers were held at − 4 °C for 19 days, then 14 days at − 6 °C, 14 days at − 9 °C, and finally 35 days at − 12 °C and at each of these temperatures

eggs were taken for DSC analysis to establish supercooling point ( $N=14-26$ ), water content ( $N=4-5$ ), and melting temperature ( $N=4-5$ ) of eggs. Furthermore, hatchability of eggs ( $N=4-5$ ) was scored following cold exposure after exposure to temperatures down to − 6 °C and − 12 °C. Adults kept at 20 °C were included in DSC analysis for comparison.

### Differential scanning calorimetry analysis

Differential scanning calorimetry analysis provided the supercooling point (SCP) during cooling scan, and the melt onset temperature during the heating scan. The melt onset temperature of intact insects and eggs provides an indication of the melting point of hemolymph or egg contents (Holmstrup 2018).

DSC analysis was conducted using a DSC4000 calorimeter (Perkin Elmer, Waltham, MA, USA). Three-to-five eggs per replicate were transferred to a preweighed 50 µL aluminum DSC-pan, and their combined fresh weight was determined using a Sartorius SC 2 microbalance (Sartorius AG, Goettingen, Germany) accurate to 1 µg. The pan was immediately hermetically sealed and the combined weight of the eggs and sealed pan was determined. After the DSC analysis, the pan was punctured and dried for 48 h at 60 °C. The combined weight of pan and dried individual was determined and water content calculated as mg water mg<sup>-1</sup> dry weight. Samples were subjected to a DSC program consisting of four steps: (1) hold for 1 min at 5 °C; (2) cool to − 50 °C at a rate of 2 °C min<sup>-1</sup>; (3) hold for 1 min at − 50 °C; (4) heat to 5 °C at a rate of 1 °C min<sup>-1</sup>. The melt endotherm (enthalpy change during melting of ice formed in the animal) of the heating scan curve was analyzed and the melt onset temperature estimated using Pyris Software (Perkin Elmer, Waltham, MA, USA) as described by Block (1994).

### Statistics

Prior to analysis, all data were tested for normality and homogeneity of variance. Percentage hatching was arcsin sqrt transformed to improve normality. For analysis of differences between treatment groups, we performed one-way analyses of variance (ANOVA) or a non-parametric (Kruskal–Wallis) test in cases where the assumption of normality was not met. Comparisons of individual samples were done using Tukey's pairwise tests or Dunn's post hoc test. IBM SPSS Statistics (v. 25) and SigmaPlot (v. 11) were used for statistical analyses and visualization.

## Results

### Thermal tolerance of adults and eggs

For adults of *N. groenlandicus*  $CT_{min}$  for females and males were  $-3.4 \pm 0.2$  °C and  $-3.2 \pm 0.1$  °C (mean  $\pm$  SE), respectively, whereas  $CT_{max}$  for females and males were  $48.5 \pm 0.1$  °C and  $48.6 \pm 0.2$  °C, respectively. There were no significant differences between sexes in upper and lower thermal limits ( $CT_{min}$ ,  $t = 0.569$ ,  $P = 0.579$ ;  $CT_{max}$ ,  $t = 0.100$ ,  $P = 0.921$ ).

For eggs kept at 0 °C for 21 days and subsequently at 20 °C, the percentage of eggs hatching was 31%. When exposed to a cold ramping treatment, decreasing temperatures resulted in a lower hatchability, with 21% at  $-10$  °C and 7% at  $-21$  °C (Fig. 1a). There was a significant effect of temperature treatment on hatchability ( $df = 4$ ,  $MS = 0.330$ ,  $P < 0.01$ ) and pairwise multiple comparison showed that hatchability was significantly lower at  $-15$  °C,  $-20$  °C, and  $-21$  °C compared to that of 20 °C ( $P < 0.05$ ).

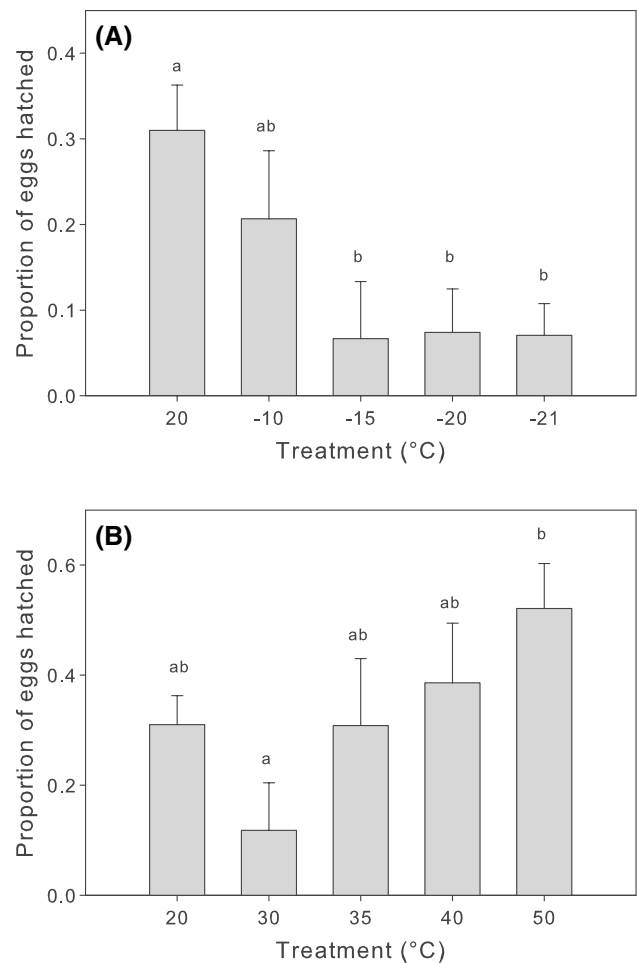
When exposed to a heat ramping treatment, increasing temperatures resulted in higher egg hatchability, with 12% after brief exposure to 30 °C and 52% after exposure to 50 °C (Fig. 1b). There was a significant effect of temperature treatment on hatchability ( $df = 4$ ,  $MS = 0.312$ ,  $P < 0.05$ ) and pairwise multiple comparison showed that hatchability after exposure to 50 °C was significantly higher compared to those exposed to 30 °C ( $P < 0.05$ ).

### Supercooling points, hemolymph melt onset temperature, water content, and survival during long-term cold exposure of eggs

The supercooling point of eggs ranged between  $-30.4$  °C and  $-32.6$  °C throughout the experiment (Fig. 2a). There was no significant effect of acclimation time/temperature on supercooling point ( $H = 4.951$ ,  $df = 4$ ,  $P = 0.292$ ). Mean supercooling point of adults was  $-9.5$  °C ( $N = 8$ ).

The water content of the eggs did not differ significantly across timepoints ( $H = 1.252$ ,  $df = 4$ ,  $P = 0.869$ ). The eggs had a mean water content of  $2.20 \pm 0.21$  mg  $mg^{-1}$  dry weight at the beginning of the experiment and  $1.97 \pm 0.20$  mg  $mg^{-1}$  at day 81 (Fig. 2b).

The melt onset temperature was  $-1.85 \pm 0.08$  °C at day 3, and subsequently decreasing until reaching  $-2.11 \pm 0.04$  °C at day 81 (Fig. 2c). There was a significant effect of exposure time/temperature on melt onset temperature ( $H = 14.18$ ,  $df = 4$ ,  $P < 0.01$ ), where melting point at day 3 was significantly higher compared to the melting point at day 35 and 49 ( $P < 0.05$ ).

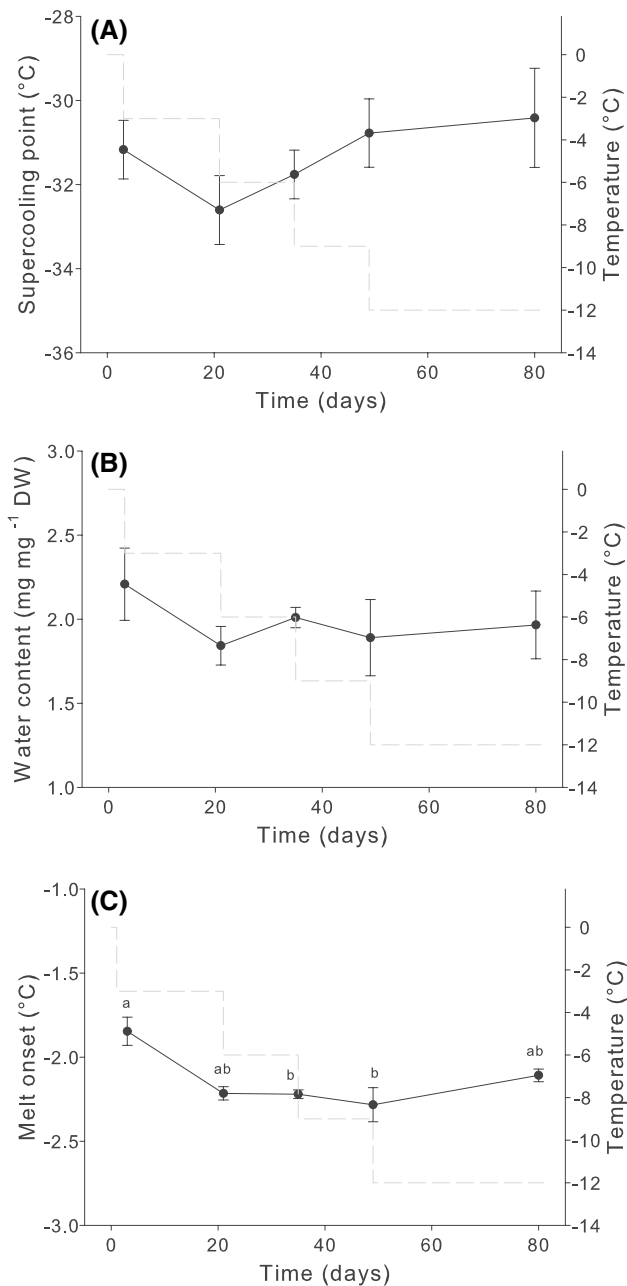


**Fig. 1** Mean ( $\pm$ SE) percentage of eggs of *Nysius groenlandicus* hatching following **a** cold exposure and **b** heat exposure. Eggs were exposed to heat or cold ramping ( $0.1 \text{ min}^{-1}$ ), and when lower temperatures ( $-5$  °C,  $-10$  °C,  $-15$  °C,  $-20$  °C, and  $-25$  °C) or upper temperatures (25 °C, 30 °C, 35 °C, 40 °C, and 45 °C) were reached, six-to-seven replicates of 10 eggs per temperature were removed from the water bath and placed under control conditions (20 °C) and number of hatched eggs scored

Survival of eggs following exposure to cold temperatures down to  $-6$  °C was 40.7% whereas at  $-12$  °C, survival had increased to 79.1%, and there was a significant difference between timepoints ( $T = 30$ ,  $P < 0.05$ ).

## Discussion

In Arctic and sub-Arctic regions, insects are exposed to extreme cold conditions during winter. However, in summer, both adults and eggs of *N. groenlandicus* can be exposed to stressful high temperatures (Danks et al. 1994; Danks 2004; Sørensen et al. 2019). We found that the upper thermal limit, measured as  $CT_{max}$ , of summer acclimated adults, was on average 48.5 °C. This may seem surprisingly high



**Fig. 2** Mean ( $\pm$ SE): **a** supercooling point, **b** water content, and **c** melting point of eggs of *Nysius groenlandicus* following cold exposure (left y-axis). The gray dashed line in each plot indicates thermal exposure conditions ( $-3^{\circ}\text{C}$ ,  $-6^{\circ}\text{C}$ ,  $-9^{\circ}\text{C}$ , and  $12^{\circ}\text{C}$ ) (right y-axis) and the timepoints where samples were taken to evaluate the effects of on survival, supercooling point ( $N=14\text{--}26$ ), water content, and melting temperature ( $N=4\text{--}5$ ) of eggs of *Nysius groenlandicus*

for an Arctic insect, but is in accordance with the microhabitat temperatures experienced in their natural habitat and in agreement with what has been found in the other studies (Sørensen et al. 2019). Results suggest that this species can respond plastically to high temperatures, but it is currently unclear to what extent adults and nymphs of *N.*

*groenlandicus* can behaviorally avoid thermal extremes (Sørensen et al. 2019). Similarly, eggs exposed to rapid and brief heat treatments and subsequently left to hatch at benign temperatures showed increasing hatchability with increasing heat exposure. Together, this suggests that both the adults and eggs show adaptations to extreme heat stress and highlight the need for studies addressing adaptations to heat stress in cold environments (Hodkinson et al. 1996; Everatt et al. 2013). Before upper thermal limit was determined, individuals were exposed to  $0^{\circ}\text{C}$ . It is likely that such cold exposure of the eggs will affect thermal tolerances due to acclimation effects (e.g., Bahrndorff et al. 2016; MacLean et al. 2017; Noer et al. 2020). For example, in *Drosophila*, brief exposure to cold during the egg and larval stage can affect thermal performance of adults (using activity measurements, but not  $CT_{\text{max}}$  and  $CT_{\text{min}}$ ) (MacLean et al. 2017). The percentage of eggs hatching at control conditions was 31%, which is relatively low, but comparable to results by Böcher (1975). It is possible that the remaining eggs were infertile or did not break the diapause. However, future studies may improve percentage egg hatching focusing on the effect of photoperiod and importance of temperature (Böcher 1975).

It is also unclear why brief exposure to  $50^{\circ}\text{C}$  before incubation at  $20^{\circ}\text{C}$  improved hatchability compared with eggs exposed to constant  $30^{\circ}\text{C}$ . It may be that this species depends on high-temperature spells for successful egg development and that increasing temperatures will be advantageous for *N. groenlandicus*. Alternatively, this may simply be a stress response, which could have costs affecting subsequent life-history traits negatively (Loeschke and Hoffmann 2007; Marshall and Sinclair 2010; Alemu et al. 2017; Jensen et al. 2019). Several studies have suggested a link between diapause, thermal tolerance, and expression of heat shock proteins. For example, Goto et al. (1998) found that diapausing individuals of *Drosophila triauraria* were significantly more tolerant to heat, but there were no differences in Hsp70 expression between diapausing and non-diapausing individuals. Other studies have found that transcription of Hsp23 and Hsp70 in pupae in diapause is highly upregulated and that expression is reduced when diapause is terminated (Yocum et al. 1998; Rinehart et al. 2000). The involvement of heat shock proteins could explain the increase in hatchability with increasing temperature, but may also explain the low hatchability at moderate ramping end temperature. It would thus be relevant in future studies to address the role of diapause on thermal tolerance and expression of heat shock proteins, and the costs and benefits of diapause.

Generally, increasing temperature ramping rates (e.g., ranging from  $0.01$  to  $0.4^{\circ}\text{C min}^{-1}$ ) used to reach maximum and minimum thermal exposure limits will increase the thermal tolerance limits (Sørensen et al. 2013; Allen et al. 2016; Alemu et al. 2017). In the present study, individuals were

exposed to a temperature ramping rate of  $0.1\text{ }^{\circ}\text{C min}^{-1}$ . This is a slow temperature ramping rate compared to some other studies, but is more likely to reflect ecologically relevant situations. However, the investigation of heating and cooling rate effects on thermal tolerance limits was outside the scope of the present study.

Lower thermal tolerance limits of adults, measured as  $\text{CT}_{\text{min}}$ , were  $-3.15\text{ }^{\circ}\text{C}$  and the SCP was  $-9.5\text{ }^{\circ}\text{C}$ . We did not conduct mortality assays on adults exposed to sub-zero temperatures. However, a limited number of individuals were removed during a cold ramping exposure and their survival scored after 24 h of recovery. These individuals survived exposure to  $-6.0\text{ }^{\circ}\text{C}$ , but died after cooling to  $-10.0\text{ }^{\circ}\text{C}$  (data not shown). This suggests that summer acclimated adults can survive brief periods of cold exposure, but it remains to be understood if longer cold exposures (e.g., freezing spells during summer nights) would be lethal to nymphs and adults. We can, however, establish that adults did not survive freezing of body fluids. When summer-collected, but cold acclimated eggs were exposed to a brief ramping cold treatment, the hatchability was less than 20% and decreased with increasing cold exposures. However, the egg hatchability after being exposed to a more gradual cold treatment (resembling seasonal acclimatization) was relatively high, and surprisingly was highest (80% hatchability) after 80 days of exposure to temperatures down to  $-12\text{ }^{\circ}\text{C}$ . The fact that long-term storage at freezing temperatures clearly stimulated hatchability suggests that termination of egg diapause is triggered by long-term cold chilling followed by warming (Kostal 2006). The difference in the proportion of eggs hatching after a rapid or slow cold exposure observed for *N. groenlandicus* is in agreement with observations in other insect species (Strathdee et al. 1995; Hao and Kang 2014) and conforms with the general notion that gradual physiological adjustments during cold exposure are important for survival during winter (Teets and Denlinger 2013). These results also suggest that eggs may be susceptible to brief cold snaps during summer.

The low supercooling points of eggs agree with field observations, indicating that *N. groenlandicus* survive the Arctic and sub-Arctic winter temperatures in the egg stage. Water vapor pressure of supercooled water is higher than that of ice and will cause a substantial water loss in an organism with a high integumental permeability (Holmstrup et al. 2002; Holmstrup 2014). However, the eggs displayed unaltered water content during the almost 3-month exposure to freezing conditions. This observation demonstrates that eggs were extremely resistant to desiccation, since the difference in water vapor pressure between the unfrozen eggs and surrounding ice in the beakers have exerted a considerable vapor pressure deficit that would cause dehydration in many other small organisms (Holmstrup et al. 2002). Hence, despite their small

size, the eggs of *N. groenlandicus* do not base their cold tolerance on cryoprotective dehydration, but on the ability to supercool and conserve water for extended periods during winters. These results are in agreement with other studies, showing that eggs of different insect species from cold regions supercool to survive harsh winter conditions (Strathdee et al. 1995; Ávila-Jiménez et al. 2010; Hao and Kang 2014). Within the insects, freeze avoidance appears to predominate, although freeze tolerance has evolved many times within different taxa (Sinclair et al. 2003). The reasons why one strategy or the other is adopted remain unclear, but it has been proposed that certain environmental conditions may select for freeze tolerance. This seems to be the case when individuals are exposed to sub-zero temperatures for long time as when supercooled to low sub-zero temperatures, the probability of freezing is proportional to time (Sømme 1982), or when exposed to mild and unpredictable cold snaps (Sinclair et al. 2003). Supercooling permits survival over a broader temperature range, but freezing can occur spontaneously at any temperature below freezing/melting point (Costanzo and Lee 2013). For small and highly permeable soil organisms, supercooling is of minor importance and these organisms often use cryoprotective dehydration (Holmstrup 2014).

Adults and eggs of *N. groenlandicus* are exposed to variable and occasionally very high temperatures during summer, and eggs to long and harsh winters in Arctic and sub-Arctic regions. Our results show a clear association between the microhabitat temperatures experienced by the specific life stage and its heat and cold tolerance. Thus, the eggs which constitute the life stage being exposed to the coldest periods throughout the year in the natural habitat of *N. groenlandicus* were clearly also more cold hardy than adults. Still, they were able to tolerate high temperatures, which is likely an adaptation to the hot summers that the eggs are also experiencing. Furthermore, we reveal the mechanistic basis for the ability of *N. groenlandicus* eggs to survive extreme cold temperatures, which depend on freeze avoidance through extensive supercooling and by conserving water. Our results demonstrate that *N. groenlandicus* and likely other polar ectotherms have evolved life stage-specific adaptations enabling them to survive in harsh and extreme variable thermal environments.

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**Author contributions** S.B., T.N.K., A.T., M.H.S., and M.H. designed the experiments. S.B., T.N.K., M.H., A.T., T.T.H., and M.H.S. performed the experiments. S.B. and M.H. analyzed the data and interpreted the results. S.B. wrote the first version of the manuscript. All authors contributed to revision of the manuscript and approved the final version. All authors agree to be held accountable for the content of this manuscript. We thank Michael Elnitsky and two anonymous reviewers for invaluable suggestions and help in improving the review.

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**Availability of data and materials** Data are available from authors on request.

## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no competing interests.

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