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Thermal biology of temperate and high-latitude arachnids

Susan E. Anthony
The University of Western Ontario

Supervisor
Sinclair, Brent J.
The University of Western Ontario

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Abstract

Temperate and high latitude terrestrial ecosystems have high thermal variability, and the ectotherms that inhabit these regions must have thermal tolerances that mirror these temperatures. However, the thermal limits of many high-latitude arachnids are unknown, as well as any underlying mechanisms of seasonal plasticity for any arachnid. The objective of my thesis is to measure the thermal tolerances of temperate, Arctic, and sub-Arctic arachnids, and identify if they have thermal plasticity, either seasonally or following acclimation. I collected the high-latitude pseudoscorpion *Wyochernes asiaticus* streamside from the Yukon Territory, where besides large thermal variability, they are also inundated with spring flooding. I also collected a variety of wolf spiders (Genus *Pardosa*) in the Yukon, Greenland, and Norway, where they are abundant and active on the tundra in the Arctic summer. In the lab, half of the of the air-exposed and low oxygen water-submerged pseudoscorpions survived for 17 days; showing that they are likely adapted to seasonal flooding. The pseudoscorpions and spiders I collected in the summer have thermal tolerances (the low and high temperatures at which activity stops) that range from -6°C in both pseudoscorpions and spiders, to 37.8°C (in pseudoscorpions) and 45°C (in spiders). Following 4°C-acclimation, the spiders did not show an ecologically significant change in their thermal tolerance breadths (T_{br}, the difference between their low- and high-temperature tolerance), potentially because their T_{br} is large enough to remain active during summer temperatures. I collected the temperate and freeze-tolerant red velvet mite in late fall, mid-winter, and early spring to compare their lower lethal temperature, and potential mechanisms associated with cold-tolerance. In mid-winter, the hemolymph osmolality and glycerol content increases, and water content decreases: all likely cryoprotectant mechanisms. Temperate red velvet mites show seasonal acclimatization resulting in freeze-tolerance, the first evidence of freeze-tolerance in microarthropods.

Keywords

Co-Authorship Statement

Chapter 2 (modified for format) is published in *Polar Biology* with Dr. Christopher Buddle and Dr. Brent Sinclair as co-authors (Anthony et al., 2016). Chris contributed by introducing me to the study organism and inspired the study design. Brent contributed to animal collection and contributed to the conception of the study design as well as leading the final drafts of the manuscript. I contributed to the animal collection, study design, and led the data collection and manuscript research and writing.

Chapter 3 (modified for format) is submitted for publication in *Polar Biology* with Dr. Christopher Buddle, Dr. Toke Høye, and Dr. Brent Sinclair. Chris and Toke introduced me to the polar environment and species and contributed ideas to the study design. Brent contributed extensively to animal collection, study design, analysis, and discussion as well as providing extensive edits of the manuscript. I led the collection of the study species and study design. I further conducted the experiments and analysis and wrote the manuscript.

Chapter 4 was designed and carried out by me, Dr. Christopher Buddle, Dr. Toke Høye, Dr. Nils Hein, and Dr. Brent Sinclair. Chris and Toke contributed by introducing me to the polar environment and species and contributed ideas to the study design. Nils provided the Norway-collected specimens. Brent contributed to animal collection, study design, analysis, and discussion as well as providing extensive edits of the manuscript. I collected the animals and designed the study. I further conducted the experiments and analysis and wrote the manuscript.

Chapter 5 (modified for format) is published in *Physiological and Biochemical Zoology* with Dr. Brent Sinclair as co-author. Brent contributed to the study design as well as providing statistical help and manuscript editing. I discovered that this species was freeze-tolerant, designed the study, conducted the experiments, and wrote the initial draft of the manuscript.

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List of Abbreviations

ANOVA – analysis of variance

BAH – beneficial acclimation hypothesis

CT_{max} – critical thermal maximum

CT_{min} – critical thermal minimum

LLt – lower lethal time

LLT – lower lethal temperature

LLT_{50} – lower lethal temperature of 50% survival

Q_{10} – metabolic difference in an ectotherm between two temperature separated by 10 °C

SCP – supercooling point

SE – standard error

SEM – standard error of the mean

T_{br} – thermal breadth

T_{br}-A – absolute thermal breadth

T_{br}-F – functional thermal breadth

THP – thermal hysteresis proteins

TPC – thermal performance curve

Chapter 1

1 Introduction

Thermal biologists study the impact of the environmental temperature on an individual level which ultimately affects performance and fitness (Clarke, 2017). Species are limited in their poleward expansion by many biotic and abiotic factors, including temperature (Sexton et al., 2009; Stevens, 1989): species that have ranges that extend to high latitudes have therefore evolved mechanisms to increase their tolerance in response to abiotic stressors they are likely to encounter at these higher latitudes through physiological and behavioural means. However, climate change will cause an increase in average global temperatures, and lead to more frequent thermal extremes (IPCC, 2014; Kattsov and Källén, 2005; Post et al., 2009). Additionally, acclimation resulting from prolonged pre-exposure to a change in temperature can shift the thermal limits, expanding the thermal breadth of activity and survival. By studying the thermal biology of organisms, we can learn how an organism survives in its environment and provide information that leads to predictions about the survival, distribution, and adaptability of species to climate change (e.g. Sinclair et al., 2016).

Arachnids are a Class of arthropods in the subphylum Chelicerata. Of the 12 Orders of Arachnids, five are distributed as far north as the Arctic: the Araneae (spiders), Pseudoscorpiones (pseudoscorpions), Actinotrichida (mites), Anactinotrichida (ticks), and Opiliones (harvestmen) (Figure 1.1). Two orders of arachnids (mites and ticks) are found on the Antarctic continent (Barbosa et al., 2011; Block, 1994; Leech, 1966). Arachnids are also present at high altitudes such as salticid spiders from Mt. Everest at 6700 m (Swan, 1992). Arachnids play ecological roles as predators, detritivores, and herbivores. Some negatively impact humans as crop pests (e.g. the spider mite *Tetranychus urticae*; Attia et al., 2013), disease vectors (e.g. the tick *Ixodes dammini*; Burgdorfer et al., 1982), or by biting and stinging (e.g. some spiders and scorpions) with nuisance or health impacts (e.g. Diaz, 2004; Isbister and Bawaskar, 2014).
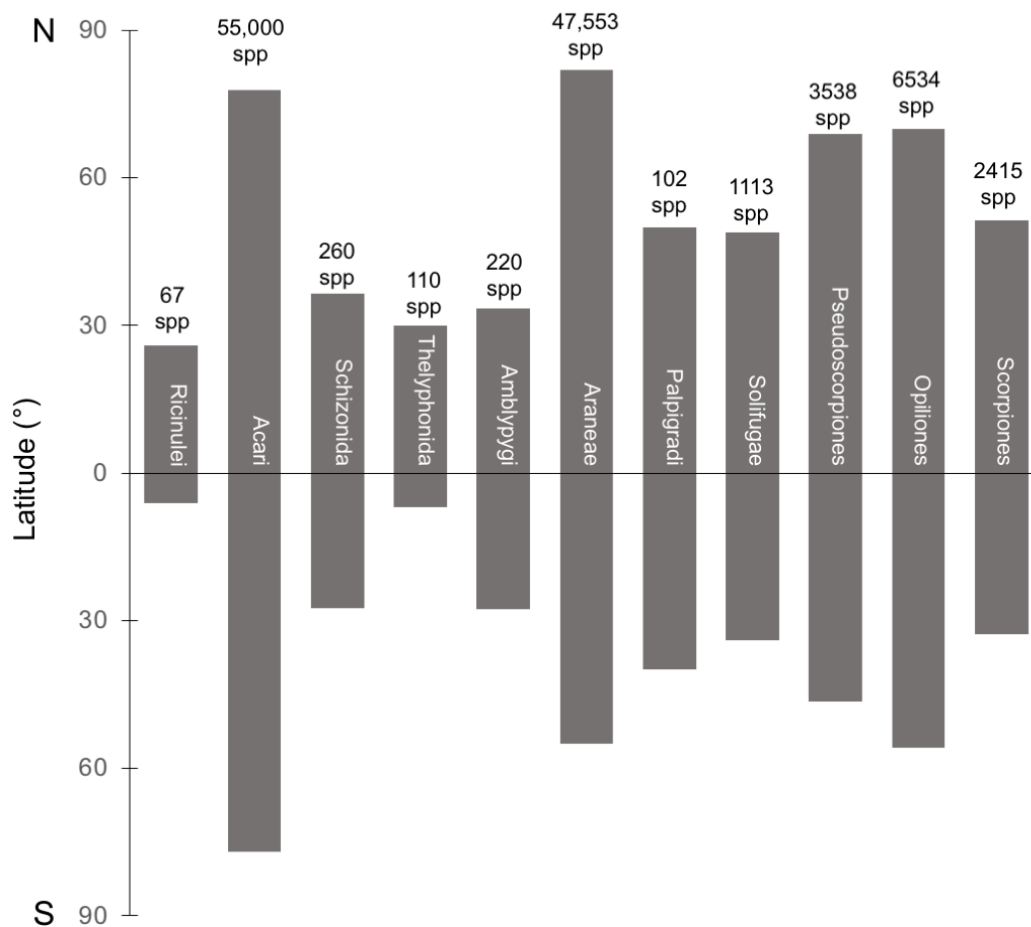
Figure 1.1 Latitudinal distribution of arachnid orders. Orders Actinotrichida (mites) and Anactinotrichida (ticks) were recently separated into two orders, but I present them together here as Subclass Acari. References for distribution and number of known species within each order in Table 1.1.

Table 1.1 References for latitudinal distribution and species number for arachnid orders (Figure 1.1).

Order	Northern Distribution	Southern Distribution	Species number
Ricinulei	Ratnasingham and Hebert (2007)	Ratnasingham and Hebert (2007)	Tourinho et al. (2010)
Acari	Gwiazdowicz and Coulson (2011)	Sjursen and Sinclair (2002)	Beccaloni (2009)
Schizonida	Cokendolpher and Lanfranco (1985)	Ratnasingham and Hebert (2007)	Manzanilla et al. (2008)
Thelyphonida	Beron (2018)	Beron (2018)	Harvey (2013)
Amblypygi	Beron (2018)	Beron (2018)	de Miranda et al. (2018)
Araneae	Marusik et al. (2005)	Davies et al. (1997)	World spider catalog (2019)
Palpigradi	Harvey et al. (2006)	Harvey et al. (2006)	Beron (2018)
Solifugae	Beron (2018)	Beron (2018)	Prendini (2011)
Pseudoscorpiones	Buddle (2015)	Vitali-di-Castri (1968)	Harvey (2011)
Opiliones	Stol (2003)	Beron (2018)	Kury (2013)
Scorpiones	Fet (2010) cited in Beron (2018)	Acosta (2005)	Rein (2017)

Arachnids are one of the top terrestrial arthropod predators in Arctic and temperate habitats (Wirta et al., 2015), however, they are often neglected in global macrophysiological studies (e.g. Bennett et al., 2018; Sunday et al., 2011). The absence of high-latitude arachnid data in these studies is problematic. The predicted changes to climate will not only result in a larger increase in average air temperature in high latitudes compared to lower latitudes, but more extreme weather events (Duarte et al., 2012). Changes in temperature, and also hydrology (e.g. snowfall and water run-off) could negatively impact high-latitude arachnids, therefore there is an urgent need to understand physiological boundaries to abiotic influences. The aim of my thesis is to measure the thermal tolerances of some temperate, sub-Arctic, and Arctic arachnids, determine the capacity for plasticity in response to acclimation in high-latitude spiders, and seasonal acclimatization in a temperate mite. I conducted this research on the summer-collected pseudoscorpion *Wyochernes asiaticus* from the Yukon Territory and on wolf spiders of the genus *Pardosa* from the Yukon Territory, Greenland, and Norway. I further measured seasonal acclimatization in the red velvet mite *Allothrombium* sp. from Southwestern Ontario, Canada. I selected the pseudoscorpions because of their unique distribution (Beringia) and the abiotic stressors, both temperature and flooding, that they likely experience. I selected the *Pardosa* spiders because they are active and

abundant on the tundra through the short summer season. I selected the red velvet mite, later identified as *Allothrombium* sp., because it was the first known freeze-tolerant microarthropod, and the third freeze-tolerant arachnid described.

1.1 Biology of Arachnids

1.1.1 Diversity and Habitat

Of the 12 orders of arachnids (Dunlop, 2010), the commonest include mites and ticks, spiders, scorpions, and harvestmen, which are more widely distributed and speciose than the other orders: for example, there are over 47,000 named species of spider (World Spider Catalog, 2019), but only 67 species of Ricinulei (Tourinho et al., 2010; Figure 1.1) (Figure 1.1). At high latitudes, temperatures vary greatly by season (e.g. from 30.4 °C in summer to -39.7 °C in winter) (Government of Yukon; RWIS, 2019); and in desert ecosystems, daily surface temperatures can vary: for example the Israeli desert can have a daily surface temperature range from 15.8 to 50.2 °C (Gutterman, 1997). Arachnids in these environments have evolved behavioural and physiological and anatomical adaptations to survive the variable environments.

1.1.2 General Anatomy and Gas Exchange

The anatomy and physiology of arachnids vary among and within the orders, although they share some similar features. Most arachnids have eight walking legs as adults; spiders, amblypygids, mites, ticks, uropygids, palpigrades, and ricinulids extend these legs by forcing the hemolymph into the extremities, whereas the harvestmen, scorpions, pseudoscorpions, and solifuges have extensor muscles (Schultz, 1989). Some arachnids produce silk for prey capture, reproduction, and/or shelter; some arachnids produce venom, primarily for prey capture (Coddington and Colwell, 2001).

There are several mechanisms by which arachnids oxygenate their cells. Some spiders (the infraorder Mygalomorphae) have book lungs as a site of gas exchange, or a combination of book lungs and an oxygen transport system using hemocyanin as the carrier molecule (Burmester, 2002). However, the other orders of arachnids make use of

their small size for simple diffusion (e.g. palpigrades and some mites), and others (such as pseudoscorpions, Ricinulei, solifuges, harvestmen, mites, ticks, and araneomorph spiders) use the same spiracle and tracheal system employed by insects (Beccaloni, 2009; Foelix, 2011).

1.2 Thermal Biology

Previous reviews on the subject of arachnid thermal biology focus on a few key species, such as the Antarctic mites (e.g. Block and Convey, 1995), general desert adaptations (e.g. Cloudsley-Thompson, 1993), or are represented under a small sub-heading in general arachnid reviews (Canals et al., 2015). There is very little information on low-temperature tolerance of high latitude species and pre-adult arachnids, other than in mites (e.g. Cannon and Block, 1988). Temperature is also very important in the early life-stages: for example, spiderlings likely experience very low temperatures when dispersing by ballooning; ballooning spiderlings were collected by plane at 4500 m (Glick, 1939).

1.2.1 Thermal Tolerances

Arachnids are ectotherms, and therefore their internal body temperature mirrors that of the environment. Their activity is influenced by temperature, best described by a thermal performance curve (Huey and Stevenson, 1979; Sinclair et al., 2016), which is bounded by the critical thermal limits (minima CT_{min} and maxima CT_{max}), where activity ceases (Figure 1.2). The temperature range between the CT_{min} and CT_{max} is the thermal breadth (T_{br}). When activity means the ability to hunt, feed, defend, and reproduce, the effects of temperature can have repercussions on fitness (Huey and Kingsolver, 1989).
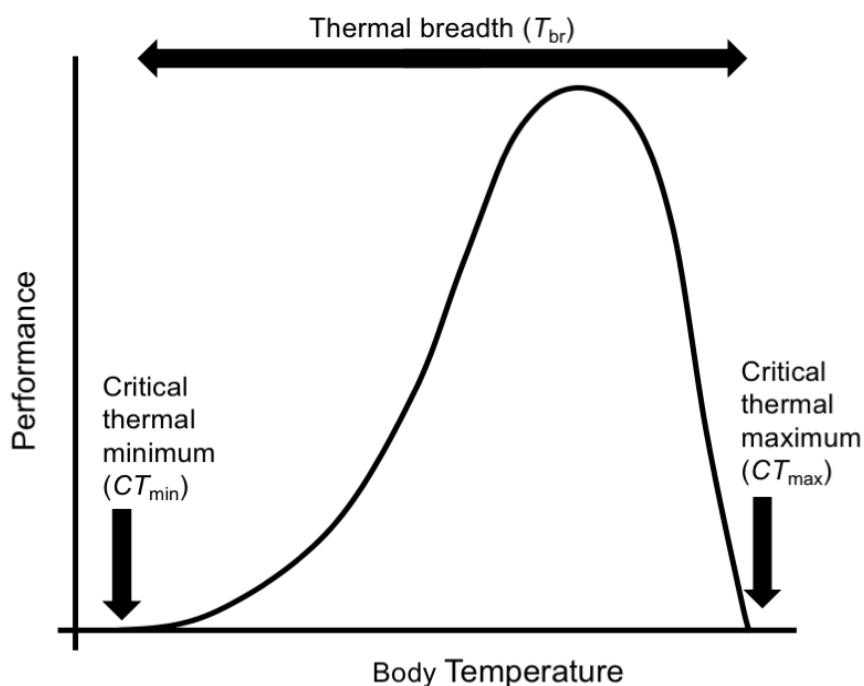
Figure 1.2 A hypothetical Thermal Performance Curve (TPC). The performance is zero at the critical thermal limits (CT_{min} and CT_{max}). Adapted and redrawn from Huey and Stevenson (1979).

Cold tolerance strategies of ectotherms include freeze-tolerance, where the organism can survive internal ice formation; and freeze-avoidance, where the animal survives until they freeze; chill susceptible animals die at low temperatures unrelated to freezing (Lee, 2010). The abiotic environments that could favour the evolution of freeze-tolerance include environments with long periods of low temperatures, those with unpredictable freeze-thaw cycles and environments with high humidity in conjunction with low temperature, where external ice inoculation is less avoidable (Sinclair et al., 2003; Toxopeus and Sinclair, 2018). The supercooling point (SCP) is the temperature at which the animal freezes. The lower lethal temperature (LLT), or lower lethal temperature where 50% survive (LLT_{50}), in freeze tolerant and chill susceptible species provides information on the lower survival temperature; the equivalent to SCP in freeze-avoidant organisms (Sinclair et al., 2015). Both LLT and LLT_{50} are time-dependent, meaning the hold time at the low temperature, and the rate of cooling and warming can affect the

outcome: slower rates and longer time held at low temperatures can result in high LLT/LLT_{50} (Sinclair et al., 2015). At the CT_{min}, sometimes referred to as the chill coma onset (Sinclair et al., 2015), ectotherms cease to respond to tactile stimuli. The theorised cause of this is the inactivation of membrane proteins which ultimately cause the loss of ion homeostasis, resulting in a reversible state of paralysis (Overgaard and MacMillan, 2017).

Low temperatures can cause lethal injury by a number of mechanisms, and in terrestrial ectotherms, these mechanisms have been described primarily in insects. At low temperatures, ion and water homeostasis are lost in crickets (MacMillan and Sinclair, 2011) causing death in chill-susceptible ectotherms. Low temperatures also decrease membrane fluidity, further disrupting normal cell activity and membrane permeability (Somero et al., 2017). At low temperatures, there is also a risk of freezing, and ice can physically damage cell membranes, cause dehydration stress by concentrating cellular fluids, and cause hypoxia stress by reducing gas diffusion (Toxopeus and Sinclair, 2018).

At high temperatures, ectotherms reach a point where their bodies spasm (Lutterschmidt and Hutchison, 1997) and controlled activity ceases (CT_{max}). The processes that result in CT_{max} in insects can be caused by the destabilization or denaturation of macromolecules, or increase in membrane fluidity which prevents cellular communication, disrupts ion balance, decreases pH, and increases desiccation (Neven, 2000; Somero et al., 2017). Insects can protect their cells at both high and low temperatures, and mitigate the impact of these temperatures by mechanisms such as heat shock proteins, desiccation resistance, alteration in membrane composition, and changes to enzyme number and type (see section 1.2.2).

1.2.2   Plasticity of Thermal Tolerances

There are many mechanisms that ectotherms have evolved to counteract the negative consequences of their ambient temperature, especially if it exceeds their thermal tolerance limits. Some arachnids regulate their temperature through behaviour, where they will move away from temperatures that are detrimental, either too high or too low (e.g. burrowing in desert arachnids; Cloudsley-Thompson, 1993; Lubin and Henschel,

1990). Others have shown physiological plasticity in their thermal tolerance in response to changing seasons or temperature acclimation: the supercooling point of mite *Halozetes marionensis* from Antarctica is -5 °C when acclimated at 15 °C, and -20.3 when acclimated at 0 °C (Deere et al., 2006).

Acclimation to different temperatures and seasonal changes can alter low-temperature tolerance, such as SCP. The supercooling point is often lowered in the winter, correlated with empty guts, and/or the production of cryoprotectant polyols, antifreeze proteins, or by cryoprotective dehydration (Wharton, 2003). Gut contents (food) can act as a site of ice nucleation, and therefore the absence of any gut nucleators will result in a lower SCP (Block and Sømme, 1982; Clark and Worland, 2008; Salt, 1961). The increased concentration of cryoprotectants, such as low molecular weight polyols and free amino acids, protect organisms from the effects of low temperatures and freezing, by several hypothesised processes (outlined in Toxopeus and Sinclair, 2018; Zachariassen, 1985). For example, the cryoprotectants may act colligatively to concentrate the hemolymph, reducing the amount of free water for freezing. Dehydration acts in the same manner, and leads to an increase in hemolymph concentration, also suppressing the freezing point (Elnitsky et al., 2008; Toxopeus and Sinclair, 2018). Thermal hysteresis factors, such as thermal hysteresis proteins (THPs, or antifreeze proteins) protect freeze-avoidant organisms from ice damage by preventing ice crystal spread, or controlling ice formation in freeze-tolerant insects (Duman, 2001). These molecular correlates commonly found in insects that lower their SCP in preparation for low winter temperatures are also accumulated by overwintering or low-temperature acclimated arachnids, such as mites (e.g. Block and Duman, 1989) (Table 1.2).

Table 1.2 Evidence of cryoprotectants and thermal hysteresis factors in arachnids and seasonality of their synthesis. (-)
indicates that this was not investigated.

Order	Species	Cryoprotectant molecules	Thermal hysteresis factors	Seasonality	Ecosystem	Reference
Actinotrichida	*Stereotydeus mollis*	Glucose, myo-inositol, trehalose, trace glycerol	absent	-	Polar	Sinclair and Sjursen (2001)
Actinotrichida	*Alaskozetes antarcticus*	glucose, ribitol, glycerol	present	Yes	Sub-Polar	Block and Sømme (1982); Block and Duman (1989)
Actinotrichida	*Phauloppia* spp.		present	Yes	Sub-Polar	Sjursen and Sømme (2000)
Actinotrichida	*Allothrombium* sp.	Glycerol	absent	Yes	Temperate	Anthony and Sinclair (2019)
Araneae	*Euathlus condorito*	Glycerol and trehalose	present	Yes	Alpine	Cubillos et al. (2018)
Araneae	*Ceraticelus laetus*	Glycogen, trehalose, fructose, glucose, mannitol, glycerol, and other unidentified free sugars and polyols	-	Yes	Prairie	Aitchison and Hegdekar (1982)
Araneae	*Diplocephalus cuneatus* (winter)	Glycogen, mannitol, and other free sugars and polyols	-	-	Prairie	Aitchison and Hegdekar (1982)
Araneae	*Oxyptila sincera canadensis* (winter)	Glycogen, trehalose, glucose, mannitol, glycerol	-	Yes	Prairie	Aitchison and Hegdekar (1982)
Araneae	*Parasteatoda tepidariorum*	Glycogen, sorbitol, scyllo-inositol, myo-inositol	-	Yes	Temperate	Tanaka (1995)
Araneae	*Erigone arctica*		present	-	Arctic	Aunaas et al. (1983)
Araneae	*Araneus cavaticus*	Proline and alanine increase in diapause	-	Yes	Temperate	Tillinghast and Townley (2008)
Scorpionida	*Paruroctonus aquilonalis*	No glycerol or sorbitol	-	No	Desert	Riddle and Pugach (1976)
Scorpionida	*Centruroides vittatus*	Trehalose and trace amounts of glycerol	-	Yes	Desert	Whitmore et al. (1985)

Changes in CT_{min} can also occur as a response to acclimation: the CT_{min} decreases with low acclimation temperature in the mite *Alaskozetes antarcticus* (Everatt et al., 2013). To alter CT_{min}, and therefore to remain active at lower temperatures, there is evidence of increased ion-transport proteins in *Drosophila* (Yerushalmi et al., 2018) and increased activity of those ion-transport proteins at low temperatures in the cricket *Gryllus pennsylvanicus* (Des Marteaux et al., 2018).

Ectotherms may alter their low-temperature tolerance seasonally, such as a decrease in SCP in winter months. Seasonal thermal plasticity evolves most often in geographic regions with predictable seasonal changes in temperature and reliable cues indicating the onset of temperature change (Auld et al., 2010; Whitman and Agrawal, 2009). For example, seasonal plasticity of SCP occurs in the mite *Phauloppia* spp. from Norway, where their SCP decreases from -9.4 °C in the summer to -35.5 in the winter (Sjursen and Sømme, 2000). Low molecular weight cryoprotectants such as glycerol and trehalose increase in freeze-tolerant insects (e.g. *Gryllus veletis*) potentially to reduce ice content or to protect cells (Toxopeus, 2018); the same molecules that are increased in freeze-avoidant species, putatively to decrease SCP. In the winter freeze-tolerant goldenrod gall fly (*Eurosta solidaginis*), unsaturated membrane fatty acids increased relative to saturated fatty acids in preparation for winter, potentially to increase the membrane fluidity (Bennett et al., 1997). Further, ice nucleating molecules are produced to initiate freezing and prevent supercooling. Antifreeze proteins are also produced when becoming freeze-tolerant, potentially to prevent intercellular ice growth or recrystallization (Lee, 2010). Although these findings are from insect species, there is some evidence that arachnids may also use the same mechanisms for low-temperature tolerance, such as the winter production of cryoprotectants, and evidence of thermal hysteresis factors (Table 1.2).

SCP varies within arachnid species such as among life stages, and feeding status. Both the adults and larvae of the seal and seabird ectoparasitic tick *Ixodes uriae* live in the same environment (Frenot et al., 2001; Murray and Vestjens, 1967), however the SCP is 13.8 °C lower as a larva than as an adult (Lee and Baust, 1987). Food acts as an ice nucleator (Salt, 1968) and can therefore increase SCP. The Antarctic mite *Alaskozetes*

antarcticus (Block and Sømme, 1982) and Japanese populations of the spider *Parasteatoda tepidariorum* spiders (Tanaka and Watanabe, 2003) have higher SCPs when fed *vs.* unfed. However, SCP is not significantly different between blood-engorged and non-engorged female *Ixodes scapularis* (ticks) from Nova Scotia, Canada at 44.6°N (Curry et al., 2017), therefore the nucleating effect of food is likely unrelated to the water that is ingested with the food, and may instead be initiated by the bacteria that accompany food (Tanaka and Watanabe, 2003). Because of the differences that can occur in SCP within species, it is important to clearly identify the conditions (age, feeding status, water content) of any organism when measuring SCP.

At high temperatures, proteins can denature, membrane fluidity can increase, and ion homeostasis can be lost (Williams et al., 2016) all of which may contribute to the loss of activity (at the CT_{max}) and ultimately result in death (Somero, 2002). Acclimation does not often change CT_{max} (Sørensen et al., 2016); however, when it does, pre-exposure to short and long-term high temperatures (heat shock and acclimation, respectively) will increase the expression of certain genes, including heat shock proteins, which act to chaperone damaged macromolecules to lysosomes for degradation (Chiang et al., 1989) or to protect macromolecules and thereby increase CT_{max} in *Drosophila* (Colinet et al., 2013; Williams et al., 2016). Further, species adapted to high-temperatures may produce protein orthologs that retain their native conformation at high temperatures, increasing the high-temperature tolerance (Somero et al., 2017). High-temperature acclimation improved survival at 45 °C in the mite *Neoseiulus barkeri* (Zhang et al., 2018). However, the sub-Antarctic spider *Myro kerguelenensis* had a small but significantly higher CT_{max} when acclimated at 15 °C, than those acclimated at 0 °C (Jumbam et al., 2008).

Thermal tolerance is often not fixed within a species, a population, or even an individual. With the plasticity in thermal tolerance, it is important to determine the full potential of acclimation or acclimatisation in ectotherms to temperature, before concluding their thermal niches and their response to future climate change (Somero, 2010).

1.3   Arachnids at Low Temperatures

1.3.1   Winter Ecology

Temperate, high-latitude, and high-altitude ectotherms can spend many months at temperatures below zero. At low temperatures, ectotherms are at a risk of freezing, have low metabolic activity, and reduced locomotion. Arachnids show evidence of overwintering at all life stages (Table 1.3); some may remain active through the winter, often in the subnivean habitat (Aitchison, 1978), while others enter diapause or quiescence (e.g. the spider *Parasteatoda tepidariorum* overwinters in diapause; Tanaka, 1991). High-latitude arachnids, such as *Alaskozetes antarcticus* from 60°S and *Pardosa glacialis* from 81°N may overwinter at any life stage (except as eggs for both and adults for *P. glacialis*) and for more than five times before reaching sexual maturity (Convey, 1994; Leech, 1966). However, lower latitude populations (c. 49.5° to 54.0°N) of *Pardosa* (such as *P. moesta*, *P. groenlandica*, *P. hyperborea*, and *P. fuscula*) overwinter only once or twice, and only as juveniles and sub-adults (Buddle, 2000; Pickavance, 2001), therefore overwintering strategies in some high latitude arachnids may not be fixed within the species.

Table 1.3 Examples of the life-stages which overwinter, categorised by arachnid order and the ecosystem in which they are found.

Order and Species	Overwintering life stage	Ecosystem	Reference
Opiliones			
Trachyrhinus marmoratus	Eggs	Desert	Cokendolpher et al. 1993
Leiobunum paessleri	Adults	Alpine	Holmberg et al. 1984
Leiobunum japanense	Nymphs	Temperate	Tsurusaki 2003
Araneae			
Floronia bucculenta	Eggs	Temperate	Schaefer 1976
Argiope aurantia	Spiderlings (nymphs)	Temperate	Riddle and Markezich 1981
Centromerus sylvaticus	Adults	Temperate	Aitchison 1978
Pseudoscorpiones			
Chthonius ischnocheles	Adults and nymphs	Temperate	Wood and Gabbutt 1978
Actinotrichida			
Bryobia cristata	Eggs	Temperate	Hallas and Gudleifsson 2004
Phytoptus avellanae	Nymphs	Mediterranean	Ozman 2000
Alaskozetes antarcticus	Adults and nymphs	Polar	Convey 1994

In winter, some arachnids remain active while others enter a state of dormancy, such as diapause or quiescence (e.g. Cloudsley-Thompson, 1978; Convey, 1994; Tanaka, 1991). The arachnids that live in the subnivean environment (e.g. pseudoscorpions and spiders in Manitoba, Canada; Aitchison, 1978) experience temperatures buffered by the snow (Convey et al., 2018). Some winter-active spiders feed through the winter (Aitchison, 1987a; Korenko et al., 2010) although at a lower rate (Aitchison, 1984; Gunnarsson, 1983); others may live off of reserve lipids (Aitchison, 1984). Food in the gut can nucleate ice (Salt, 1968), therefore mechanisms such as the production of cryoprotectant molecules may have evolved which reduce the risk of freezing in feeding species. Winter-inactive arachnids, including some mites, pseudoscorpions, harvestmen, and spiders, are often found in buffered microhabitats, such as tree bark (e.g. the spider *Phiddipus audax*; Bower and Snetsinger, 1985), caves (e.g. harvestmen *Leiobunum paessleri*; Holmberg et al., 1984), and soil; they also likely rely on physiological adjustments to survive the winter.

1.3.2 Arachnids at Low-Temperatures

Among the arachnids, cold tolerance strategy has not often been identified. Mostly it has been noted only whether or not they survived measurements of SCP (i.e. freezing), without differentiating between those that die from freezing from those that do not survive low temperatures above freezing (e.g. Lee and Baust, 1987; Riddle and Pugach, 1976). There are two scorpion species that survive freezing: *Centroides vittatus* from the Texas desert (Whitmore et al., 1985) and *Diplocentrus spitzeri* from the SW New Mexico desert (Crawford and Riddle, 1975). A low-latitude alpine tarantula (*Euathlus condorito*) and lab-reared *Amblyseius californicus* predatory mites do not survive freezing (Cubillos et al., 2018; Hart et al., 2002); however, it was not determined if they died from freezing (i.e. were freeze-avoidant), or at temperatures above freezing (i.e. were chill-susceptible). Freeze-avoidance and chill susceptibility has been distinguished in the lab-bred mite *Typhlodromus montdorensis* (Hatherly et al., 2004) (Figure 1.3). There have been few studies testing specifically the seasonal changes in cold tolerance strategy in arachnids: the scorpion *Paruroctonus aquilonalis*, which does not survive freezing at any time of year in New Mexico, USA (Riddle and Pugach, 1976); and the mite *Alaskozetes antarcticus* which also remains freeze-avoidant through the year (Young and Block, 1980).

Supercooling point is the commonest measurement of low-temperature tolerance in arachnids because it is easy to measure and is an indication of physiological changes in response to changing seasons. The majority of these measurements of SCP are from mid to high latitude species (50 to 70°N and S), where the risk of freezing is higher. The SCPs of arachnids range from -35.3 °C in the Norwegian mite *Phauloppia* spp. (collected in winter; Sjursen and Sømme, 2000) to -2.2 °C in the freeze-tolerant desert scorpion *Centruroides vittatus* (Whitmore et al., 1985). Although a decreased low-temperature tolerance, such as a low SCP, would be advantageous to species from higher latitudes or altitudes, there is a great variation in SCP among species within a small latitudinal range (Figure 1.3). For example, the spiders *Pachygnatha clerki* and *Larinioides* (*Araneus*) *cornutus* from Germany both overwinter in hollow plant stems, however their SCPs differ by 17 °C (-5.8 and -22.8 °C, respectively) (Kirchner, 1973). Further, individuals of

the high-latitude mite *Gamasellus racovitzai* from Galindez Island, Antarctica (65.3°S) have a mean SCP of -6.6 °C, higher than other Antarctic microarthropods (e.g. Block, 1982) (Figure 1.3). Therefore, variation in SCP is not simply a result of the organism's environment; and is likely a combination of the species genotype, environment, and adaptive potential (Sinclair et al., 2012).
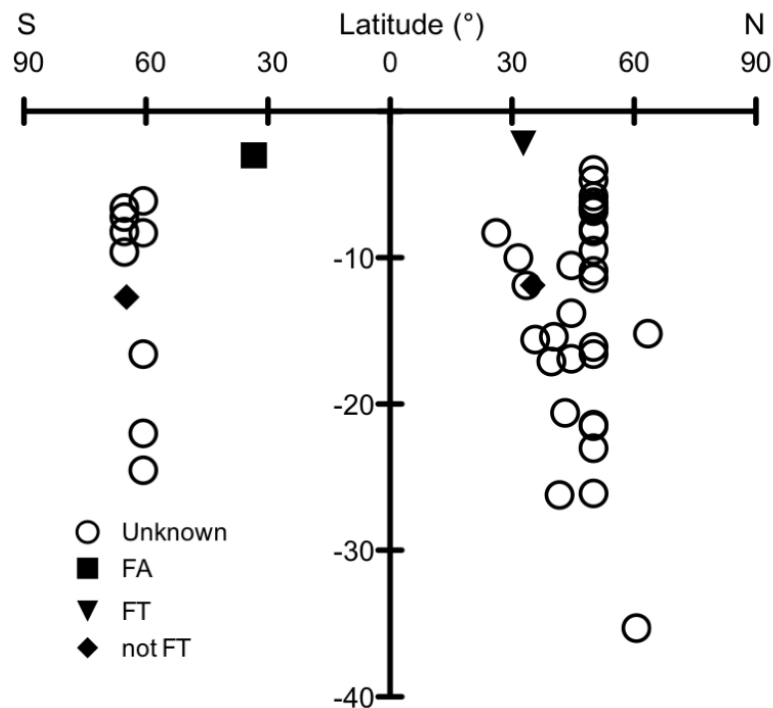
Figure 1.3 Supercooling points and the cold tolerance strategies of arachnids from various latitudes. Open circles are where no cold tolerance strategy was determined; closed squares indicate species that are freeze avoidant; downward-pointed triangles indicate those that were determined to be freeze-tolerant; diamonds indicate those who did not survive freezing, however the distinction between chill-susceptible and freeze-avoidant was not made. References are in Table 1.4.

Table 1.4 References for the supercooling points of arachnids, by latitude (Figure 1.3).

Order	Species	Mean SCP (°C)	Latitude	Reference
Araneae	*Bolyphantes index* (adult)	-15.2	63.4°	Husby and Zachariassen (1980)
Acari	*Phauloppia* spp. (winter)	-35.3	60.6°	Sjursen and Sømme (2000)
Araneae	*Theridion tepidariorum* (winter)	-8.2	50°	Kirchner (1973)
Araneae	*Theridion notatum* (winter)	-26.1	50°	Kirchner (1973)
Araneae	*Theridion deuticulatum* (winter)	-11.4	50°	Kirchner (1973)
Araneae	*Tentana triangulosa* (winter)	-10.9	50°	Kirchner (1973)
Araneae	*Tentana castanea* (winter)	-9.5	50°	Kirchner (1973)
Araneae	*Tegenaria* sp. (winter)	-8	50°	Kirchner (1973)
Araneae	*Singa nitidula* (winter)	-21.4	50°	Kirchner (1973)
Araneae	*Philodromus* sp. (winter)	-21.5	50°	Kirchner (1973)
Araneae	*Pardosa lugubris* (winter)	-6.8	50°	Kirchner (1973)
Araneae	*Pachygnatha clercki* (winter)	-5.8	50°	Kirchner (1973)
Araneae	*Nesticus cellulanus* (winter)	-4.7	50°	Kirchner (1973)
Araneae	*Meta menardi* (winter)	-4	50°	Kirchner (1973)
Araneae	*Histopona torpida* (winter)	-6.5	50°	Kirchner (1973)
Araneae	*Eresus niger* (winter)	-16.6	50°	Kirchner (1973)
Araneae	*Coelotes terestris* (winter)	-6.2	50°	Kirchner (1973)
Araneae	*Clubiona phragmitis* (winter)	-16.1	50°	Kirchner (1973)
Araneae	*Cicurina cicurea* (winter)	-6.7	50°	Kirchner (1973)
Araneae	*Araneus cornutus* (winter)	-23	50°	Kirchner (1973)
Araneae	*Amaurobius fenestralis* (winter)	-6.6	50°	Kirchner (1973)
Araneae	*Parasteatoda tepidariorum*	-8.3	26.2°	Tanaka (1996)
Araneae	*Pardosa groenlandica*	-10.54	44.6°	Murphy et al. (2008)
Acari	*Ixodes scapularis*	-16.9	44.6°	Curry et al. (2017)
Acari	*Dermacentor variabilis*	-13.8	44.6°	El Nabbout et al. (2017)
Araneae	*Parasteatoda tepidariorum*	-20.6	43.1°	Tanaka (1996)
Araneae	*Philodromus* sp. (winter, immature)	-26.2	41.7°	Duman (1979)
Araneae	*Clubiona* sp. (winter, immature)	-15.4	40.3°	Duman (1979)
Araneae	*Parasteatoda tepidariorum*	-17.1	39.7°	Tanaka (1996)
Araneae	*Parasteatoda tepidariorum*	-15.6	35.7°	Tanaka (1996)
Scorpionida	*Paruroctonus aquilonalis* (winter)	-11.9	35.1°	Riddle and Pugach (1976)
Araneae	*Parasteatoda tepidariorum*	-11.9	33.6°	Tanaka (1996)
Scorp	*Centruroides vittatus*	-2.2	32.8°	Whitmore et al. (1985)
Araneae	*Parasteatoda tepidariorum*	-10.0	31.6°	Tanaka (1996)
Araneae	*Euathlus condorito*	-3.0	-33.4°	Cubillos et al. (2018)
Acari	*Gamasellus racovitzai* (summer)	-6.1	-60.7°	Block and Sømme (1982)
Acari	*Nanorchestes antarctica*	-22	-60.7°	Block and Sømme (1982)
Acari	*Stereotydeus villosus* (summer)	-8.3	-60.7°	Block and Sømme (1982)
Acari	*Halozetes littoralis*	-16.6	-60.7°	Pugh (1994)
Acari	*Alaskozetes antarcticus* (adult, summer, starved)	-24.5	-60.7°	Block and Sømme (1982)
Acari	*Ixodes uriae* (adult, summer)	-12.7	-64.8°	Lee and Baust (1987)
Acari	*Oppia loxolineata*	-9.6	-65.3°	Block (1982)
Acari	*Gamasellus racovitzai*	-6.6	-65.3°	Block (1982)
Acari	*Eupodes minutus*	-8.2	-65.3°	Block (1982)
Acari	*Rhagidia gerlachei*	-7.2	-65.3°	Block (1982)

Lower lethal temperature (LLT) is the thermal limit for chill-susceptible and freeze-tolerant organisms; whereas SCP approximates LLT in freeze-avoidant organisms. The commonest measurement among arachnid studies is LLT_{50}, the lowest temperature at which 50 % of the arachnids survive. The LLT_{50} of chill-susceptible desert whipscorpion (Uropygi) *Mastigoproctus giganteus* was from +3.3 to +4.9 °C, depending upon life stage (Punzo and Olsen, 2005). The LLT_{50} of 12 species of spiders collected from the same region in Sweden ranged from -6.7 °C in *Agroeca proxima* (in winter) to -20.9 °C in *Clubiona similis* (winter; Almquist, 1970), using the same cooling and rewarming rates and low-temperature holding time. The results of these two studies provide further evidence that latitude is not the only factor affecting the evolution of cold-tolerance.

The CT_{min} of mites, pseudoscorpions, and spiders ranges from -9.3 °C in the winter-active and feeding spider *Bolyphantes index* (Oslo, Norway; Hågvar, 1973) to +10.3 °C in the lab-reared mite *Tetranychus urticae* (overwintering strategy unknown, some naturalized populations undergo diapause; Coombs and Bale, 2014) (Figure 1.4). The majority of the CT_{min} measurements in arachnids are in high latitude mites (e.g. Block and Sømme, 1982), high altitude spiders (e.g. Alfaro et al., 2013) and lab-reared strains of mites (Allen, 2009; Coombs and Bale, 2014) (Figure 1.4). These measurements are only a small representation of all arachnid species, and I have only found measurements of CT_{min} for spiders, mites, and one uropygid species. There is much room for further examination of the thermal tolerance in arachnids.
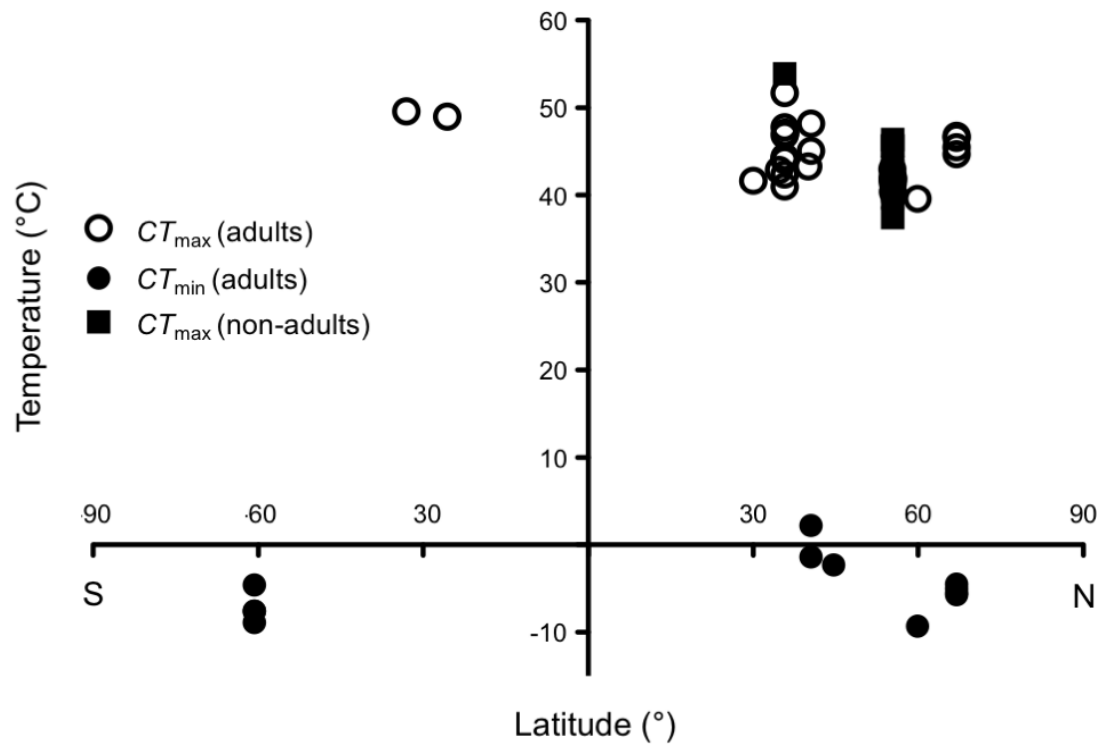
Figure 1.4 Critical thermal limits of arachnids by latitude. Critical thermal minima (CT_{min}) of adult arachnids are designated filled circles, critical thermal maxima (CT_{max}) of adults (open circles) and juvenile life-stage (solid squares). References are in Table 1.5.

Table 1.5 References for and values of the critical thermal minima and critical thermal maxima of arachnids by latitude (Figure 1.4).

Order and Species	CT_{min}, adult (°C)	CT_{max}, juvenile (°C)	CT_{max}, adult (°C)	Latitude (°)	Reference
Araneae					
Bolyphantes index	-9.3			59.9	Hågvar (1973)
Zora spinimana			41.8	55.4	Almquist (1970)
Zelotes serotinus		46.4		55.4	Almquist (1970)
Tibellus oblongus		42.8		55.4	Almquist (1970)
Stemonyphantes lineatus		40.5		55.4	Almquist (1970)
Scotina gracilipes		41.4		55.4	Almquist (1970)
Phrurolithus festivus		45.6		55.4	Almquist (1970)
Philodromus aureolus		42.1		55.4	Almquist (1970)
Pardosa nigriceps		39.7		55.4	Almquist (1970)
Oedothorax apicatus			40.4	55.4	Almquist (1970)
Euophrys frontalis		45.7		55.4	Almquist (1970)
Dipoena inornata		43.2		55.4	Almquist (1970)
Crustulina guttata			41.8	55.4	Almquist (1970)
Clubiona trivialis			42.1	55.4	Almquist (1970)
Clubiona similis			42.0	55.4	Almquist (1970)
Clubiona diversa			41.4	55.4	Almquist (1970)
Agroeca proxima		37.4		55.4	Almquist (1970)
Lycosa pullata			43.0	55.4	Almquist (1970)
Pisaurina mira			39.6	45.0	Barton (2011)
Pardosa groenlandica	-2.3			44.6	Murphy et al. (2008)
Misumenops asperatus	-1.4		45.1	40.5	Schmalhofer (1999)
Misumenoides formosipes	2.2		48.2	40.5	Schmalhofer (1999)
Pisaurina mira			43.3	40.0	Barton (2011)
Peucetia viridans			47.2	35.8	Hanna and Cobb (2007)
Neriene longipedella			44.2	35.7	Kato et al. (2008)
Neriene brongersmai			41.0	35.7	Kato et al. (2008)
Leucauge subblanda			42.4	35.7	Kato et al. (2008)
Laucauge blanda			44.0	35.7	Kato et al. (2008)
Argyrodes kumadai			44.4	35.7	Kato et al. (2008)
Argyrodes bonadea			46.9	35.7	Kato et al. (2008)
Argiope minuta			47.8	35.7	Kato et al. (2008)
Argiope bruennichii			51.7	35.7	Kato et al. (2008)
Rabidosa rabida			42.9	34.7	Stork (2012)
Nephila clavipes			41.65	30	Krakauer (1972)
Seothyra sp.			49.0	-25.6	Lubin and Henschel (1990)

Table 1.5 (continued).

Order and Species	CT_{min}, adult (°C)	CT_{max}, juvenile (°C)	CT_{max}, adult (°C)	Latitude (°)	Reference
Araneae			49.6	-33.0	van den Berg et al. (2015)
Morebilus plagusius (summer)					
Acari					
Stereotydeus villosus	-7.6			-60.7	Block and Sømme (1982)
Nanorchestes antarctica	-8.9			-60.7	Block and Sømme (1982)
Gamasellus racovitzai	-7.6			-60.7	Block and Sømme (1982)
Alaskozetes antarcticus	-4.6			-60.7	Block and Sømme (1982)

1.3.3　Mechanisms that may Mitigate the Effects of Low Temperatures in Arachnids

Some arachnids produce putative cryoprotectants in winter, increasing hemolymph osmolality, and potentially improving cold tolerance. Spiders (e.g. *Ceraticelus laetus*; Aitchison and Hegdekar, 1982) and mites (e.g. *Alaskozetes antarcticus*; Young and Block, 1980) produce free sugars and polyols in winter, and a SCP decrease in the mite *Alaskozetes antarcticus* is correlated with an increase in the cryoprotectant glycerol (Lee and Baust, 1981) (Table 1.2). Further, free amino acids are found in higher concentrations during diapause in the orb-weaving spider *Araneus cavaticus* (Tillinghast and Townley, 2008) (Table 1.2). Seasonal increases in glycerol in the wolf spider *Pardosa astrigera* increases in lower lethal time at 0 °C, without modifying the SCP (Tanaka and Ito, 2015). In the freeze-tolerant scorpion *Centruroides vittatus*, the concentration of the putative SCP suppressant trehalose increased from zero in summer to c. 18 µg/mg wet weight in winter – in result, a negligible increase of 0.07 mmol/L –with no corresponding change in SCP (Whitmore et al., 1985). In general, the increase in cryoprotectants in arachnids increases hemolymph osmolality, concentrating the hemolymph, and increasing its viscosity, which Aitchison (1987a) speculated could interfere with locomotion. Thermal hysteresis activity has been identified in some arachnids (Table 1.2), which may be a means of suppressing SCP without impacting

viscosity. In insects, thermal hysteresis molecules, such as proteins (THPs) are likely to stabilize ice crystals, to prevent ice growth and allow the hemolymph to supercool without freezing (Husby and Zachariassen, 1980).

Critical thermal minima respond to acclimation in the Chilean spider *Scytodes globula* (Alfaro et al., 2013); and in sub-Antarctic spiders *Myro kerguelenensis* and *Prinerigone vagans* (Jumbam et al., 2008) and mites *Halozetes marinus*, *Halozetes fulvus*, *Podacarus auberti*, and *Halozetes belgicae* (Deere et al., 2006). However, there have been no investigations into the mechanisms that result in changes to CT_{min} in arachnids.

The mechanisms surrounding changes to thermal tolerances and cold tolerance strategy are being investigated in insects. Arachnids differ anatomically and physiologically from insects, and the avenues of research into thermal plasticity in arachnids have only followed those discovered in insects, such as identifying the same cryoprotectants already identified in insects.

1.4 Arachnids at High Temperatures

1.4.1 Summer Ecology

Arachnids from all latitudes could experience surface temperatures that are higher than air temperatures due to the emission of trapped solar radiation from the surface. At mid-latitudes, surface temperatures can exceed 60 °C (Wu and Wright, 2015), and many low latitude species have evolved anatomical, physiological, and behavioural adaptations to cope with or avoid extreme high temperatures. Ectotherms at higher latitudes have a short summer for growing, feeding, and reproducing. Although the ground temperatures are not as high as those at low latitudes, they can reach 34.5 °C in the high-Arctic shrub tundra (Convey et al., 2018).

High-temperature tolerance is investigated mostly in desert arachnids. In the desert ecosystem, summer is a time of food scarcity and extreme high temperatures. Some arachnids enter a state of summer dormancy called estivation where activity and feeding are reduced, and time between moults extends (Punzo, 2000). Estivation has evolved in

scorpions (Stockmann, 2015), spiders (Kotzman, 1990), ticks (Belozerov, 2008, 2009), and possibly opiliones (Belozerov, 2012); and in egg (Schaefer, 1976), juvenile, and adult life-stages (e.g. desert scorpions; Cloudsley-Thompson, 1991). In August and September, the Chihuahuan desert tarantula *Aphonopelma hentzi* retreat into their burrows (Punzo and Henderson, 1999). Summer estivation has also evolved in non-desert species, such as the mite *Metarhombognathus armatrus* from Germany (Belozerov, 2009) and the winter tick *Dermacentor albipictus* from New Hampshire, USA (43.2°N), likely to conserve water while off its moose host in the latter (Yoder et al., 2016). Therefore, estivation is potentially a means to avoid temporary unfavourable abiotic situations.

1.4.2 High-Temperature Physiology and Mechanisms to Mitigate the Effect of High Temperatures in Arachnids

Critical thermal maxima (CT_{max}) in arachnids differs significantly among species, and within species by population, age, thermal history, and potentially by season. The CT_{max} of field-fresh arachnids range from 37.4 °C in the spider *Agroeca proxima* from Sweden, 55.4°N (Almquist, 1970) to 58.2 °C in the spider *Argiope trifasciata* from 35.7°N (Tolbert, 1979), and possibly higher: CT_{max} in the mite *Paratoarsotomus macropalpis* from Southern California shows "heat coma" at temperatures of 58 °C and above (Wu and Wright, 2015) (Figure 1.4). Among arachnids, CT_{max} can also vary among species from similar latitudes, within species (by age, sex), or as a result of temperature acclimation or seasonally. For example, the spiders *Pardosa nigriceps* and *Euophrys frontalis* from Sweden (55.4°N) differ in CT_{max} by 6 °C (39.7 and 45.7 °C, respectively; Almquist, 1970). CT_{max} does not differ greatly between seasons in the arachnids investigated: for example, the CT_{max} of the spider *Morebilus plagusius* from Australia is c. 1 °C higher in summer than spring (van den Berg et al., 2015). CT_{max} is often related to the thermal maximum of the environment of which the species or population is found.

At high temperatures, metabolism and water loss due to respiration increases. Low-latitude and desert arachnids cope with high temperatures and desiccation with a thick, waxy epicuticle; guanine crystals in the exoskeleton; and controlled spiracle opening (Cloudsley-Thompson, 1991; Gefen, 2005; Hadley, 1974). However, arachnids living at

higher latitudes also experience high temperatures (Convey et al., 2018), and these desert-species adaptations, such as nocturnality and summer aestivation, are maladaptive in regions with short growing seasons and nights, by missing the only opportunity to grow, feed, and reproduce. Therefore, summer arachnids from high latitudes will likely have high-temperature tolerances that allow them to remain active as much as possible.

1.5 Thesis Overview

The thermal biology of high latitude ectotherms is important natural history information that may be useful for assessing the effect of climate change on the physiology, ecology, and distribution of these species. Arachnids are globally distributed, however they have been omitted from global macrophysiological studies (e.g. Sunday et al., 2011). In my thesis, I provide important thermal biology of the often forgotten high-latitude arachnids. To address the gaps in high-latitude and arachnid thermal biology, I have selected three representative arachnid groups (pseudoscorpions, wolf spiders, and red velvet mites) to study the thermal tolerances and immersion tolerance, plasticity, and seasonality in arachnids.

Objective 1: Describe the thermal and immersion tolerances of a high-latitude pseudoscorpion (Chapter 2), and the thermal tolerances of high-latitude spiders from different elevations and at different life-stages (Chapter 3)

For my first objective (Chapter 2: "Thermal biology and immersion tolerance of the Beringian pseudoscorpion *Wyochernes asiaticus*"), I collected the pseudoscorpion *Wyochernes asiaticus* stream-side in the Yukon Territory, and measured its thermal and immersion tolerance at our laboratory at the University of Western Ontario. I found that the pseudoscorpions have both low- and high-temperature tolerances that would allow survival in summer conditions in their microenvironment under rocks. Further, they survive as long under low-oxygen water as they did in air (at 4 °C), with a 50 % mortality after 17 days; long enough to survive their yearly spring habitat flooding. The high-latitude spiders (Chapter 3: "Thermal limits of summer-collected wolf spiders from the Yukon Territory, Canada and Greenland") were similarly tolerant to summer ground

temperatures at high latitudes, with only minor differences between high and low altitude populations and adult and juvenile life-stages; spiderlings, however, have exceptionally low SCPs.

Objective 2: Test for plasticity in the thermal tolerances of high-latitude spiders following low-temperature acclimation (Chapter 4)

My second objective was to test for plasticity in high-latitude spiders (Chapter 4: "Effect of low temperature acclimation on thermal tolerances in summer-collected Arctic wolf spiders"), which I conducted by acclimating spiders from the Yukon Territory, Canada, Greenland, and Norway to low and high temperatures prior to testing low and high-temperature tolerance. I found that in one of the Yukon-collected spiders, which were fed during acclimation, CT_{max} increased with warm acclimation. However, the majority of the unfed Greenland- and Norway-collected spiders showed a small decrease in CT_{max} following warm acclimation, and a small increase in SCP following cold acclimation; these results are likely the result of increased metabolism and dehydration in the warm-acclimated group, rather than plasticity.

Objective 3: Measure the seasonal changes to low-temperature tolerance and underlying molecular mechanisms in a tractable mite species from a seasonally variable temperate environment (Chapter 5)

For my third objective, I found a red velvet mite in Southwestern Ontario that is freeze-tolerant, and which I was able to collect in large numbers to hold in field cages for winter retrieval (Chapter 5: "Overwintering red velvet mites are freeze-tolerant"). I determined cold tolerance strategy and measured SCP and LLT_{50} as well as osmolality, glycerol content (a common cryoprotectant), and water content in autumn-, winter-, and spring-collected mites to correlate any changes in cold tolerance to changes in hemolymph composition. I found that SCP did not change through the seasons, however LLT_{50} decreased in mid-winter, co-occurring with an increase in osmolality and glycerol content, and decrease in water content.

1.6   An Introduction to the Study Species

The study species that were the focus of my thesis were chosen because of their high-latitude distributions (the pseudoscorpion *Wyochernes asiaticus* and the *Pardosa* spiders), or for their abundance, tractability, and freeze-tolerance (the red velvet mite *Allothrombium* sp.). I used the species most appropriate to each of my objectives. The high-latitude arachnids from the Yukon Territory, Greenland, and Norway were abundant and easy to collect in the summer, however I was unable to return in winter for collections, and these species were not amenable to lab rearing using the protocols available. The temperate red velvet mite was easy to collect in autumn and retrieve from field-cages in the winter, allowing me to track its seasonal changes to cold tolerance strategy. I present here the current knowledge of the ecology, life-history, and thermal biology of my study species.

1.6.1   *Wyochernes asiaticus*

Of the roughly 3000 species of pseudoscorpions (Harvey, 2002), 30 species are found in Canada (Buddle, 2010). The pseudoscorpion *Wyochernes asiaticus* (Figure 1.5) is a "Beringian relict" (Buddle, 2015) – survived the last ice age – and is found in Central Asia, Siberia, and NW North America. The Yukon latitude distribution is from 64.3°N (Buddle, 2015) to 69°N (Buddle, 2015; Muchmore, 1990) (Figure 1.6). In the Yukon, *W. asiaticus* lives under rocks in seasonally flooded streamside habitat (Buddle, 2010; Buddle, 2015) (Figure 1.6). Very little is known about their natural history except that all life stages are found in the summer, including females with brood sacs, and therefore the species has a multiyear lifespan (Buddle, 2010). *Wyochernes asiaticus'* habitat has high seasonal temperature variation (Convey et al., 2018) and annual spring flooding; however, their thermal and immersion tolerance is not known.
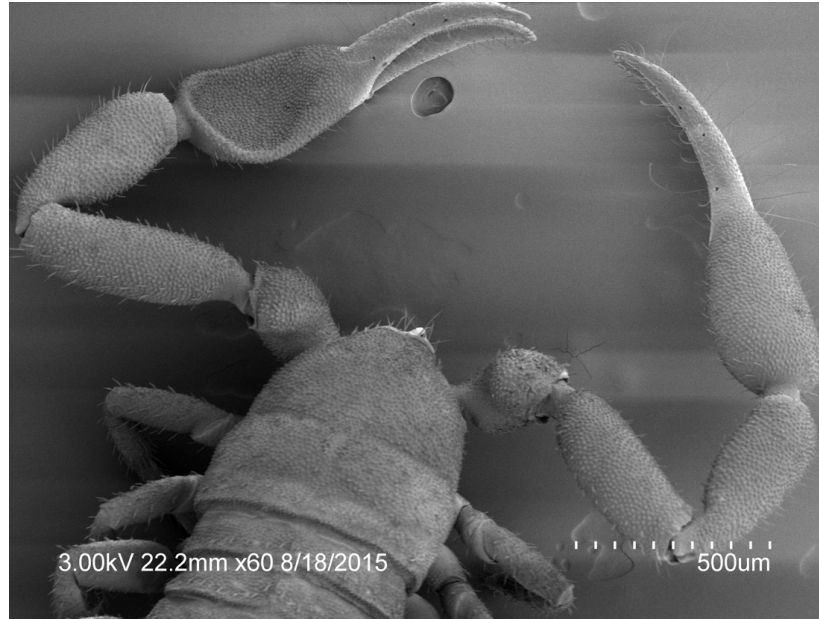
Figure 1.5 Scanning electron micrograph image of pseudoscorpion *Wyochernes asiaticus*. The image was produced at the Biotron Imaging Facility at Western University.
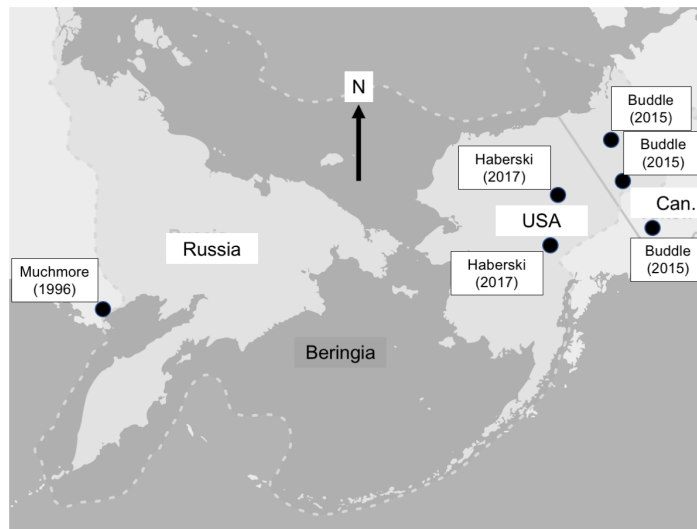
Figure 1.6 Collection locations of pseudoscorpion *Wyochernes asiaticus* in Asia and North America. Dashed line indicates the extent of Beringia, the region that was not glaciated in the last ice age. Illustration from Mark Garrison, data from Buddle (2015), Muchmore (1996), and (Haberski, 2017).

1.6.2 *Pardosa* spp.

Although spiders of the genus *Pardosa* are globally distributed, this introduction will be limited to those found at high-latitudes. There are 46 species of North American *Pardosa* (Sim et al., 2014), and a subset of these are also found in Northern Europe and Asia. For example, *P. hyperborea* (Koponen, 2002), *P. glacialis* (Cotton, 1979), *P. lapponica* (Sim et al., 2014), *P. furcifera* (Ameline et al., 2017; Dondale et al., 1997), *P. sodalis* (Bowden and Buddle, 2012b; Kronestedt, 1986), and *P. groenlandica* (Dondale and Redner, 1987; Murphy et al., 2008). *Pardosa moesta* is found in Canada and the USA, from NW Yukon and Alaska to Newfoundland and south to Utah and Tennessee where they live in beach drift, forest, and tundra (Dondale and Redner, 1987). *Pardosa* spp. are wandering spiders and generalist predators. They are top predators of the other arthropods in the tundra and in turn are food for shrews (Aitchison, 1987b) and migratory birds (Jansson and von Bromssen, 1981). In the Yukon, their eggs are often parastised by the wasp *Gelis* sp. (Bowden and Buddle, 2012a).

High latitude *Pardosa* spp. often have multi-year lifespans, whereas lower latitude conspecifics are univoltine (Pickavance, 2001) (Figure 1.7). Most *Pardosa* overwinter as young and subadult juveniles and none are known to overwinter as adults (Schmoller, 1970). The young emerge in the spring for growing and eating, and the penultimate juveniles moult into sexually mature adults that mate and produce offspring (Eason, 1964). The high-latitude growing season is short, and the number of snow-free days is correlated with the size of female *P. glacialis*, and with males, only to a lesser extent (Høye et al., 2009). Body size (as measured as prosoma width) increases with elevation in *P. hyperborea* in Narsarsuaq, Greenland, and *P. palustris* in Iceland and the Faroe Islands; however this does not result in a significant change to clutch size or egg volume (Ameline et al., 2018).
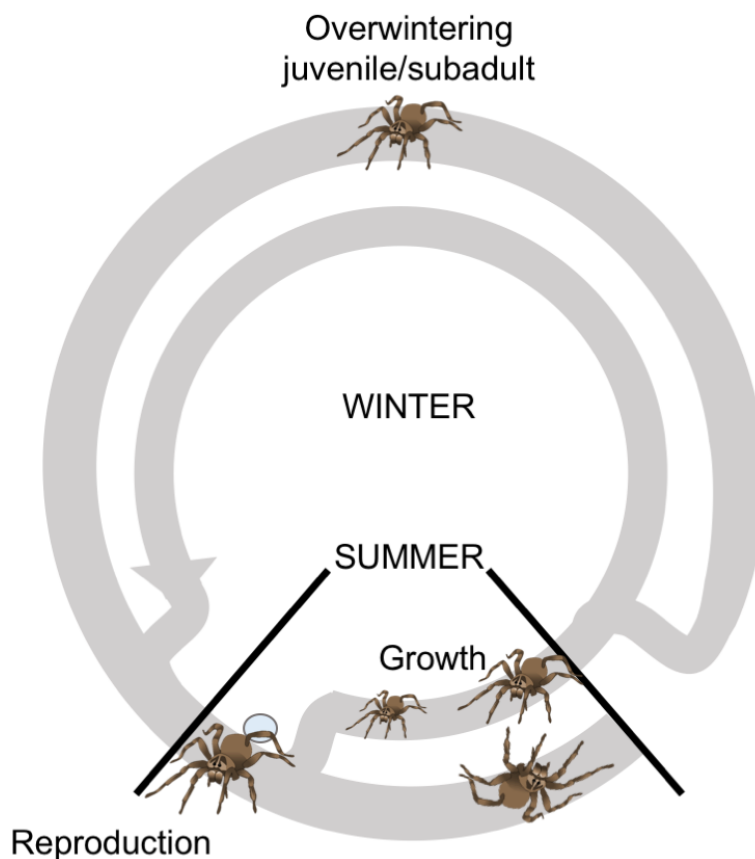
Figure 1.7 Estimated life cycle of a *Pardosa glacialis* from Hazen Camp, Ellesmere Island, North-West Territories, Canada. Information from Leech (1966).

Growth and reproduction occur in the short summer season, and the spiders overwinter as juveniles or subadults (non-reproductive). Males and females emerge in the summer, complete their final moult and reproduce, producing egg sacs which the females carry attached to their spinnerets. The spiderlings emerge from the egg sacs and mount on the mother's abdomen for up to two weeks, before the juveniles disperse. The spiders may overwinter up to seven times Leech (1966).

In Arctic and sub-Arctic Yukon, Canada, Lycosidae (to which *Pardosa* belong) are present at densities from between 0.6 and 3.4 individuals m^{-2} (Turney et al., 2018), and I estimate that they were as abundant in Narsarsuaq and Disko Island, Greenland and Norway. At such high densities and distribution within my study sites, I could capture them by hand in high numbers, allowing multiple individuals per experiment, and I could

categorise them according to collection sites (separated by elevation or latitude), by age class (spiderling, juvenile, adults) and by sex as adults. They were also able to survive at least one week without food, which allowed me to acclimate them at different temperature in the field lab.

1.6.3   Red Velvet Mites

The red velvet mites (Actinotrichida: Trombidiidae) (Dunlop, 2010), are large mites (2-4 mm) (Moss, 1961), and are distributed globally, from tropical to Nearctic regions (Moss, 1961). As adults, the red velvet mites live in soil (Zhang 1998) and are predators of eggs and emerged arthropods (Moss, 1961); as larvae, they are ectoparasites of other arthropods. They have been investigated for their use as biocontrol agents for pest arthropods (e.g. on *Aphis pomi*; Wiggins et al., 2001; Zhang and Xin, 1992) and have no known predators, although they are known to cannibalise or parasitise each other (Zhang, 1998).

Red velvet mites are usually univoltine and overwinter as adults: eggs are mostly laid in spring, however some can be laid in autumn and this generation will overwinter twice (Zhang, 1998). The red velvet mite *Allothrombium* sp. (identified by W. C. Welbourn 2016), lives in Southern Ontario, Canada (c. 43°N) and I have only observed them overwintering as adults. This species is amenable to overwintering in field cages. Therefore, I am able to track the cold tolerance and potential mechanisms to changes in cold tolerance in this species through winter.

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Chapter 2

2 Thermal biology and immersion tolerance of the Beringian pseudoscorpion *Wyochernes asiaticus*

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2.1 Introduction

At high latitudes, microarthropods (small-bodied arthropods, including Collembola, and mites and other arachnids) can dominate soil and tundra ecosystems (Bale et al., 1997; Block, 1994; Convey and Stevens, 2007; Hodkinson and Coulson, 2004; Hodkinson et al., 1996; Hodkinson et al., 1998). In the Antarctic and, to a lesser extent, the Arctic, the environmental physiology of mites and springtails has received considerable attention (e.g. Cannon and Block, 1988; Coulson et al., 1995; Sømme, 1981). Polar springtails and mites are almost universally freeze-avoidant and are killed by the formation of internal ice. They avoid freezing by depressing the supercooling point (SCP, the temperature at which their bodies freeze) by some combination of polyol and proteinaceous cryoprotectants, or (more rarely) via cryoprotective dehydration (Cannon and Block, 1988; Coulson et al., 1995; Holmstrup and Sømme, 1998; Sinclair et al., 2006; Worland et al., 1998). The activity ranges of arthropods are usually delimited by the critical thermal maximum (CT_{max}, the high temperature at which coordinated movement is lost and spasms begin) and critical thermal minimum (CT_{min}, the low temperature at which ability to move is lost; Sinclair et al., 2015). Polar and sub-polar mites and springtails usually show some evidence of cold adaptation, with relatively low CT_{max} and CT_{min} (Addo-Bediako et al., 2000; Sinclair et al., 2006; Slabber et al., 2007).

In addition to low temperatures, polar organisms must withstand other environmental stressors (Convey, 2011; Sømme, 1995). Because of their small size and dependence on soil structure, soil disturbance and flooding can also cause significant physiological stress in any season, whether it is from ice-cover-induced hypoxia (Coulson et al., 2000) or long-term immersion in water (Hertzberg and Leinaas, 1998). This is particularly the case in riparian zones, where seasonal snowmelt can cause significant flooding. Some animals such as Collembola appear to survive inundation by being hydrophobic and rafting on the surface of water (Coulson et al., 2002; Hawes et al., 2007). Alternately, microarthropods may survive inundation either through anaerobiosis (Sømme and Conradi-Larsen, 1977), or perhaps via adaptations that allow oxygen to be stored (Burmester, 2004) or extracted from the surrounding water (Seymour and Matthews, 2013).

Although mites and springtails are the only microarthropods in Antarctica, pseudoscorpions (Arachnida: Pseudoscorpiones) are present in the sub-Arctic and the Arctic (Buddle, 2015; Koponen, 1994; Koponen and Sharkey, 1988; Muchmore, 1990). Pseudoscorpions are small predators, and some species in alpine Europe and Manitoba, Canada, are active under the snow during winter (Aitchison, 1979; Vanin and Turchetto, 2007). Although there is evidence that extreme high temperatures may decrease reproductive success of tropical pseudoscorpions (Zeh et al., 2012), to my knowledge there have been no investigations of the environmental physiology of any pseudoscorpions, including those of northern latitudes.

Wyochernes asiaticus Redikorzev 1922 (Arachnida: Pseudoscorpiones: Chernetidae) is a large (female body length 2–2.5 mm) pseudoscorpion, whose distribution in Northern Yukon, Alaska, and Eastern Siberia suggests it is a Beringian relict (Buddle, 2015). In the Yukon Territory of Canada, *W. asiaticus* lives under rocks on seasonally flooded stream beds north of 64.28°N. Because all life stages were present in all collections, Buddle (2015) inferred that this species has a multi-year life cycle; although this remains to be confirmed with winter collections, it seems likely that adults and juveniles both overwinter. Here, I measured the critical thermal limits and supercooling points of adult and sub-adult *W. asiaticus* shortly after midsummer collections. I also

measured immersion tolerance to explore the capacity of this species to withstand submergence during seasonal flooding events. To my knowledge, this represents the first ecophysiological study on a pseudoscorpion and an extension of our understanding of the ecophysiology of polar microarthropods beyond mites and springtails.

2.2 Methods

I collected c. 200 *W. asiaticus* by hand from beneath stones on the gravel banks of Sheep Creek, Yukon Territory, Canada (66.8°N, 136.3°W, 562 m elevation). The pseudoscorpions were separated into individual perforated 1.5-mL microcentrifuge tubes and kept together in a plastic bag (with humidity maintained via wet cotton wool in a perforated 15-mL plastic centrifuge tube) in an insulated container. I returned them to Western University and held them at a constant 12 °C under 24 h light (consistent with summer conditions during the collection period). A maximum of 9 days elapsed between collection and use in experiments. During this period, females who had been carrying egg sacs dropped them, but only five of 200 animals died during transport, and no controls died during the experiments.

I measured critical thermal minima (CT_{min}) and maxima (CT_{max}) using an approach similar to that described by Sinclair et al. (2006). Briefly, I placed individual pseudoscorpions into depressions (1.9 mm diameter, 2 mm depth) milled into an aluminium block cooled by 50 % ethylene glycol circulated from a VWR 1157P recirculating chiller (VWR, Mississauga, ON, Canada), and covered with a glass microscope slide to prevent escape. I observed them using a dissecting microscope during cooling or heating. For CT_{min}, I cooled the pseudoscorpions from 12 °C at 0.25 °C·min^{-1}, and I periodically poked them with a fine paintbrush; I defined the CT_{min} as the temperature where an individual's legs curled, and it no longer moved in response to stimulation from the paintbrush. For CT_{max}, I heated the pseudoscorpions at 0.25 °C·min^{-1} from 12 °C; I defined the CT_{max} as the temperature where they jerked briefly and no longer responded to stimulus from the paintbrush. I report mean ± SEM for CT_{min} and CT_{max}.

To measure the supercooling point (SCP), I chased an individual into the narrow end of a 10-µL pipette tip and used cotton wool to hold it in contact with a 36 AWG type-T thermocouple (copper–constantan, Omega, Laval, QC, Canada) interfaced to a computer via a TC-08 thermocouple interface (Pico Technology, Cambridge, UK). I recorded the temperature every 0.5 s using Picolog software (v 5.24.2 Picotech). I placed the pipette tips containing pseudoscorpions in holes milled in an aluminium block cooled by 50 % methanol circulating from a Lauda Proline RP855 circulator (Lauda, Würzburg, Germany). I cooled them at 0.1 °C min^{-1} from 12 °C and recorded the SCP as the lowest temperature reached before the exotherm indicating ice formation (Lee, 2010).

To determine the cold tolerance strategy, I placed ten pseudoscorpions in pipette tips in contact with thermocouples in a cooled aluminium block, as described above. I cooled them from 12 °C at 0.1 °C·min^{-1} until five of the ten pseudoscorpions had frozen as indicated by an exotherm. At this point, I removed all of the individuals rapidly to room temperature and removed the cotton wool and thermocouple; survivors resumed movement after a few seconds. If all of the individuals died regardless of whether they had frozen, I would define that as chill susceptibility; if only individuals that froze died, I would define that as freeze avoidance; while if individuals that froze survived, I would define that as freeze tolerance (see also Sinclair et al., 2015).

To explore the ability of *W. asiaticus* to survive long periods immersed in water, I first submerged *n* = 10 individuals in 0.7 mL microcentrifuge tubes filled to overflowing with distilled, deionised water and sealed with Parafilm (Bemis Flexible Packaging, Neenah, WI, USA). These tubes were kept in an incubator at 4 °C, 24 h light, and the pseudoscorpions were observed under a dissecting microscope for movement after 1 week. As a control, an equal number of individuals were placed in dry, perforated vials in the same incubator and observed at the same interval as the immersed animals. I weighed each animal before and after the experiment (blotted dry on tissue paper for the immersed individuals) on a Mettler MX-5 microbalance (Mettler-Toledo, Columbus, OH, USA).

In the first immersion experiment, I observed a silvery film of air on the ventral abdomen that could be consistent with a plastron or other physical gill (Seymour and Matthews, 2013), and I repeated the immersion experiment, but this time with water that had been depleted of oxygen by bubbling dry N_2 gas through it for 2 h prior to use in the experiment (Tamburri et al., 2002). This decreased the oxygen saturation of the water from 70.0 to 27.4 % (YSI 600 Q-S dissolved oxygen meter, Yellow Springs, OH, USA). The tubes were again sealed with parafilm and held at 4 °C under 24 h daylight. A control again consisted of pseudoscorpions in similar-sized microcentrifuge tubes but that were perforated and dry, giving them full access to air. The pseudoscorpions were checked for survival after one week and every one to three days thereafter until 50 % of the immersed animals had died (no sign of movement following agitation of the tube).

2.3   Results and Discussion

Only pseudoscorpions that froze died, suggesting that they are freeze-avoidant, in keeping with other polar microarthropods (Cannon and Block, 1988). The mean SCP was −6.9 ± 0.7 °C (mean ± SE; range −5.6 to −10.7, $n = 7$), which is relatively high for a small (0.62 ± 0.02 mg, $n = 80$) microarthropod, suggesting the presence of ice-nucleating agents. Antarctic springtails and mites generally have SCPs below −20 °C (Cannon and Block, 1988), although springtails that are feeding can have SCPs similar to those I report for *W. asiaticus* (e.g. Sinclair et al., 2003; Worland et al., 2000), and feeding can also increase SCP in spiders (Tanaka, 1994; Tanaka and Watanabe, 1996). This SCP is likely too high to survive Yukon's winter conditions, even under snow cover; in Fairbanks, Alaska, temperatures beneath snow can reach at least −13 °C (Barnes et al., 1996). Thus, I would expect substantial seasonal plasticity in cold tolerance, as has been observed in other microarthropods (e.g. van der Woude, 1987). Alternately, it is possible that the moist under-rock habitat of the pseudoscorpions might be conducive to cryoprotective dehydration, as has been observed for the Arctic springtail *Megaphorura arctica* (Holmstrup and Sømme, 1998; Worland et al., 1998), which has a similar SCP to *W. asiaticus*. However, pseudoscorpions exposed to air for one week as controls in my immersion experiments lost relatively little mass (see

below), suggesting that they may not be permeable enough to use this strategy (Holmstrup et al., 2002).

The CT_{min} of *W. asiaticus* was -3.6 ± 0.5 °C (range -0.7 to -4.8 °C, $n = 9$), and the CT_{max} was 37.8 ± 1.1 °C (range 33.3 to 43.6 °C, $n = 10$). Pseudoscorpions have been reported active beneath the snow in Southern Manitoba (Aitchison, 1979), and although the CT_{min} I observed is consistent with low-temperature activity (at least extending the active season), I expect that these animals will spend much of the winter inactive, assuming that under-snow temperatures are similar to those reported by Barnes et al. (1996). Both the CT_{min} and CT_{max} are broadly similar to those reported for oribatid mites from the maritime Antarctic (Everatt et al., 2013). Thus, the CT_{min} of *W. asiaticus* is consistent with that of other polar microarthropods and would likely extend activity during the otherwise short growing season well into the spring and autumn. I do note that the CT_{max} of *W. asiaticus* I report here is lower than the c. 45 °C recorded for wolf spiders from the same region, even though the CT_{min} for *W. asiaticus* is broadly similar to that of these spiders (Chapter 3). This may indicate that spiders and *W. asiaticus* experience different selection pressures on CT_{max}, even in nearby habitats.

The near-stream riparian habitat of *W. asiaticus* is regularly flooded in the spring, leading us to explore the capacity of this species to tolerate immersion in water. In my first experiment, I observed no mortality in control animals, and survival of nine out of ten individuals held submerged. I observed a silvery film of air on the abdomen of the submerged individuals, and most individuals clung to the vial wall, trapping a larger bubble between their body and the vial; I agitated the vials to remove this large bubble at the beginning of the experiment. To test the hypothesis that the trapped air on the abdomen acts as a gill (Seymour and Matthews, 2013), I repeated this experiment with low oxygen water. After one week, mortality was the same as in oxygenated water (no mortality in control, one out of ten in submerged), but the time to 50 % mortality was 17 days for both low-oxygen submerged and control pseudoscorpions, suggesting that factors other than immersion were responsible for mortality. Historical river flow data from Eagle Creek (2.5°S of my collections) suggest that flood events in this part of Yukon Territory generally last two to seven days, with occasional high discharges

persisting for ten days (Environment Canada: www.wateroffice.ec.gc.ca, station 09FB002).

During the first immersion experiment, I also observed changes in mass (assumed to be due to change in water content); while the air-exposed controls lost 4.6 ± 0.6 % (range 1.3–8.2 %) of their body water over this time, the surviving immersed individuals gained 6.2 ± 1.3 % (range 1.8–13 %) of their body water. The individual that died gained 14.9 % mass. It is possible that the pseudoscorpions were slightly dehydrated at the start of the experiment (they did not have access to liquid water) and that the mass gain I observed was a function of rehydration by drinking. However, the submerged individuals did appear engorged, suggesting that this may instead be a case of 'overhydration' (cf. Lopez-Martinez et al., 2009), which might imply that long periods of immersion eventually lead to osmotic stress. Under this scenario, I hypothesise that mortality of the control and immersed individuals in my second immersion experiment could be from different causes: desiccation in the air-exposed controls, but overhydration in the submerged individuals. Given the significant variation in water availability between summer and winter, and during flooding, water balance of this species merits future attention.

In conclusion, *W. asiaticus* appears to be relatively cold-adapted, with a low CT_{min} and CT_{max}, but I predict it will show significant seasonal plasticity in cold hardiness. It can easily withstand immersion for one week and does not appear to rely on oxygen from the water for this survival. These are the first direct measures of environmental physiology for any pseudoscorpion and an important taxonomic extension of our understanding of the physiology of Arctic microarthropods. Given the relative accessibility of this species, it may be a useful model for understanding pseudoscorpion physiology in general.

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Chapter 3

3   Thermal limits of summer-collected wolf spiders from the Yukon Territory, Canada and Greenland

This chapter has been submitted for publication in *Polar Biology*.

3.1   Introduction

Terrestrial ectotherms in the Arctic experience substantial seasonal temperature fluctuations. For example, air temperature extremes in Eagle Plains, Yukon, Canada (66.4°N) between 2010 and 2016 ranged from +30.4 °C in the summer to -39.7 °C in the winter (RWIS, 2019). Mean temperatures in the Arctic are expected to increase by as much as 9 °C as a result of climate change (IPCC, 2014; Kattsov and Källén, 2005), with additional impacts from predicted increases in the frequency of extreme weather events (IPCC, 2014; Kattsov and Källén, 2005; Post et al., 2009). The altered Arctic climate has already resulted in changes in ectotherm body size (Bowden et al., 2015a; Bowden et al., 2013; Høye et al., 2009) and distribution (Jepsen et al., 2011; Parmesan et al., 1999); as well as phenological mismatch between herbivores and plants (Høye and Forchhammer, 2008; Post and Forchhammer, 2008).

The need to predict the responses of terrestrial ectotherms to climate change has motivated a range of macrophysiological studies relating environmental conditions to thermal performance and fitness (Bennett et al., 2018; Deutsch et al., 2008; Sinclair et al., 2016; Sunday et al., 2011). A key conclusion of this work has been that tropical ectotherms are more vulnerable to climate change than their polar counterparts. However, these macrophysiological studies depend on the quality of input data and often include only a limited Arctic dataset. For example, Deutsch et al. (2008) exclude locations beyond 60°N and 60°S, while Sunday et al. (2011) include only marine organisms north of 60°N. Although Holarctic spiders are speciose (Marusik and Koponen, 2005) and abundant (Coulson, 2000), they have not been included in recent macrophysiological datasets, likely because thermal biology data are not available.

The cosmopolitan wolf spider genus *Pardosa* (Lycosidae) reaches high densities in the Arctic and sub-Arctic; for example, there are 3.4 individuals·m^{-2} in the Ogilvie Mountain area of the Yukon Territory (Turney et al., 2018). At high latitudes, *Pardosa* emerge as juveniles in late spring to feed and grow; older individuals mature into adults, while others overwinter again as juveniles (Buddle, 2000; Pickavance, 2001). After mating, the females produce one or two egg sacs per summer. After hatching, the spiderlings grow and develop through the remainder of the summer and overwinter as juveniles and subadults. These high-latitude *Pardosa* will encounter both high and low temperatures during the summer growth and reproductive season (e.g., in Narsarsuaq, Greenland, on June 21, 2016, the temperature ranged from +2.1 to +20.4 °C within 24 h; TTH, unpublished data).

In ectotherms, activity is temperature-dependent, bounded by the critical thermal minimum (CT_{min}) and maximum (CT_{max}), the temperatures at which the ectotherm can no longer move (Huey and Kingsolver, 1989). Critical thermal limits vary among both species and populations (Sinclair et al., 2012); in many cases, arthropods living at higher latitudes have broader thermal tolerances (measured as the range between CT_{min} and CT_{max}), presumably reflecting a latitudinal increase in temperature variance (Calosi et al., 2010). Generally, this broadening thermal tolerance range is driven by greater cold tolerance (Addo-Bediako et al., 2000). The CT_{min} of spiders can range as low as -8 °C in the sub-Antarctic linyphiid *Prinerigone vagans* (Jumbam et al., 2008), and the only CT_{min} reported for a lycosid is -2.3 °C in *Pardosa groenlandica* (Lycosidae) from 44.6°N in Nova Scotia, Canada (Murphy et al., 2008). The CT_{max} of subadult *Pardosa nigriceps* from Southern Sweden (55.4°N) was +39.7 °C (Almquist, 1970), and *Rabidosa rabida* from Arkansas, USA (34.7°N) is +42.9 °C (Stork, 2012); I am not aware of any other reported CT_{max} for lycosids.

Below the CT_{min}, arthropods use two main strategies to survive low temperatures: freeze-tolerance and freeze-avoidance. Freeze-tolerant species can survive internal ice formation (Sinclair et al., 2015), whereas freeze-avoidant species are killed by ice formation at their supercooling point (SCP), the temperature at which they freeze, however, they will survive low temperatures if unfrozen (Sinclair et al., 2015). By contrast, chill-susceptible

species die from low temperatures unrelated to freezing. Spiders that have been studied are either chill-susceptible (Kirchner, 1973) or freeze-avoidant (Lee and Baust, 1985). To my knowledge, cold tolerance strategy has not been determined for any lycosid, however Stork (2012) reports that *Rabidosa rabida* did not survive freezing, implying that it is either freeze-avoidant or chill-susceptible.

Although the ecology of arctic spiders has received some attention (Bowden and Buddle, 2012; Bowden et al., 2015b; Hodkinson et al., 2001; Høye and Culler, 2018), there is relatively little information about their thermal biology. This lack of data could account for the absence of polar spiders from macrophysiological studies. Here I report the SCP, cold tolerance strategy, CT_{min}, and CT_{max} of seven species of *Pardosa* wolf spiders collected from sub-Arctic and Arctic (c. 61°N - 70°N) habitats in the Yukon Territory (Canada) and Greenland (c. 61° - 70°N), contributing to the data available to study global patterns of ectotherm thermal biology.

3.2   Materials and Methods

3.2.1   Animal collections

I collected active *Pardosa* spiders by hand into 30 ml plastic containers at nine locations during the boreal summers of 2015 and 2016 (Table 3.1). Because of the timing of collections, a majority of spiders I collected were female or juvenile (see Table 3.1); males were included in my analyses only where sample size allowed. I collected adult *P. lapponica*, *P. sodalis*, *P. glacialis*, and *P. moesta* from tundra in Yukon Territory (Yukon Science and Explorer's License 15-15S&E) at three sites along the Dempster Highway, between 2 and 10 July, 2015 (Table 3.1). The spiders were identified with the assistance of C.M. Buddle. The Yukon sites were mostly moist tundra, dominated by low-lying vegetation, with the exception of *P. glacialis*, which I collected from a scree field at the Yukon-North West Territories border (YT-NWT). I maintained the spiders in their collection containers, with damp moss and food (flightless *Drosophila melanogaster*) and returned them in insulated containers (to moderate temperature variation) to Western University in London, ON, where they were housed at 12 °C under 24 h light, mimicking

Northern Yukon summer conditions. I made all the measurements between four and 17 days of collection.

Table 3.1 Collection details for *Pardosa* spiders in the Yukon Territory (Summer 2015), and Greenland (Summer 2016). F = adult females; M = adult males; J = juveniles; S = spiderlings, Low = low elevation; High = high elevation.

Location name	Latitude, Longitude	Elevation (m)	Species, sexes, and life stages
Yukon Territory			
Tombstone Mtns	64.6°N, 138.3°W	1150	*P. lapponica* (F), *P. sodalis* (F)
Ogilvie Mtns	65.8°N, 137.8°W	837	*P. moesta* (F)
YT-NWT border	67.0°N, 136.2°W	647-1000	*P. lapponica* (F), *P. sodalis* (F), *P. moesta* (F), *P. glacialis* (adult, sex not recorded)
Greenland			
Narsarsuaq			
Low elevation	61.2°N, 45.4°W	s.l.-80	*P. furcifera* (J, F, M), *P. groenlandica* (S, J, F), *P. hyperborea* (J, F, M)
High elevation	61.1°N, 45.4°W	c. 450	*P. furcifera* (J, F, M), *P. groenlandica* (S, J, F, M), *P. hyperborea* (S, J, F)
Disko Island			
Low elevation	69.3°N, 53.5°W	50	*P. glacialis* (F)
Hot Springs	69.3°N, 53.5°W	50	*P. glacialis* (F)

I collected *P. furcifera*, *P. groenlandica*, and *P. hyperborea* within c. 2 km of Narsarsuaq in Southern Greenland (Table 3.1) between 21 June and 2 August, 2016 (Greenland Survey License G16-042). Low elevation sites ranged from sea level to c. 80 m a.s.l. and included rocky cobble riverbed (*P. groenlandica*), and moss- and lichen-dominated fen (*P. furcifera* and *P. hyperborea*). Air temperatures during the collection period (1.5 m above ground) ranged from 2 to 22 °C (using a HOBO U30-NRC Weather Station, Onset Computer Corporation, Bourne, MA). The high elevation site was c. 450 m a.s.l. and the *P. furcifera* and *P. hyperborea* collection habitat was similar to the low elevation site, albeit drier and windier, whereas I collected *P. groenlandica* from dried lake beds. After returning to the laboratory in Narsarsuaq, I held the animals in their collection containers in a clear plastic container in a shaded outdoor area. I separated the Narsarsuaq-collected spiders by age and sex (spiderling, juvenile, sexually mature female, sexually mature male); with the exception of the spiderlings, which were still carried on their mother's abdomen (n=3 *P. hyperborea* mothers; n=4 *P. groenlandica* mothers), until I removed them with a paintbrush immediately before the experiments. I measured the thermal

limits of the Narsarsuaq-collected *Pardosa* within three days of collection; I did not feed them during that period. Adult female *Pardosa glacialis* were collected by hand at two sites on Disko Island, West Greenland (Table 3.1): at low elevation (50 m a.s.l.) in Blæsedalen, and in a nearby areas with hot springs (50 m a.s.l.) with constant stream temperatures around 10 °C. The low elevation site was in dry heath vegetation dominated by *Dryas integrifolia* and *Cassiope tetragona*. The areas surrounding the hot springs were moss-dominated. The summer temperatures at Disko Island are about 5 °C cooler than at the collection sites in Narsarsuaq (DMI Is Centralen, www.dmi.dk). The spiders were shipped from Disko Island to Narsarsuaq in 3 mL plastic tubes and kept moist with water-soaked cotton wool. I did not feed the Disko spiders and measured thermal limits approximately one week after collection.

3.2.2   Measurements of thermal biology

I measured SCP by putting the spiders in 1.7 mL microcentrifuge tubes in contact with a 36-AWG type-T copper-constantan thermocouple (OMEGA, Laval, Quebec, Canada), held in place with cotton wool. I recorded temperature every 0.5 s using a TC-08 interface and PicoLog software (Pico Technology, Cambridge, UK). The tubes containing the Yukon-collected spiders were placed in holes milled in metal blocks cooled from 12 °C at 0.1 °C·min^{-1} by a refrigerated 50% methanol blend (Lauda Proline 3530, Würzburg, Germany) (Sinclair et al., 2015). I cooled the Greenland-collected spiders from 15 °C to 0 °C, at -1.0 °C·min^{-1}, then at -0.5 °C·min^{-1} in a custom-built Peltier-effect cooled copper block. SCP was defined as the lowest temperature immediately before the freezing exotherm (Sinclair et al., 2015). I determined the cold tolerance strategy as outlined by Sinclair et al. (2015). Dead individuals were those that did not move or right themselves 24 h post-chill. For SCP determination, I attached the spiderlings each to a thermocouple using silicone vacuum grease; because it is hard to remove the spiderlings from the grease without damaging them, I did not formally determine their cold tolerance strategy.

I measured CT_{min} by cooling the animals using an approach described by Sinclair et al. (2015). For Yukon-collected spiders, I used jacketed glass chambers cooled by 50%

ethylene glycol flowing from a refrigerated circulator (Model 1157P, VWR International, Mississauga, ON, Canada) as described by MacMillan and Sinclair (2011). For Greenland-collected spiders, I housed the spiders in a custom-built copper arena with temperature controlled by a Peltier-effect device. In both cases, I monitored the chamber temperatures with a 36-AWG type-T copper-constantan thermocouple (OMEGA, Laval, Quebec, Canada) connected to a TC-08 interface and PicoLog software (Pico Technology, Cambridge, UK). I cooled the Greenland-collected spiders from 15 °C to 0 °C at 1.0 °C·min^{-1}, followed by 0.25 °C·min^{-1} until they reached their CT_{min}; I cooled the Yukon spiders starting at 12 °C by 0.25°C·min^{-1}. I determined CT_{min} as the temperature at which the animals no longer responded to touch from a paintbrush, and their legs curled without apparent control (Sinclair et al., 2015). I observed that adult low elevation *P. groenlandica* and *P. furcifera* responded to touch until they froze at their SCP (SEA, pers. obs.), suggesting that the CT_{min} and SCP coincided, allowing us to measure SCP as a proxy for lower lethal temperature in these cases.

To determine CT_{max}, I heated the spiders in the apparatus previously described for cooling. I warmed the Greenland-collected spiders at 1.0 °C·min^{-1} from 15 °C to 30 °C, then +0.25 °C·min^{-1} above 30 °C, and the Yukon-collected spiders at 0.25 °C·min^{-1} from 12 °C until the animals spasmed, and their legs curled (Lutterschmidt and Hutchison, 1997). I weighed the *Pardosa* spiders after the measurements of thermal tolerance.

3.2.3   Statistical Analyses

I compared the SCP and CT_{max} within *Pardosa* species among age group (spiderling, juvenile, and adult females), elevation (high and low), and the age × elevation interaction by two-way ANOVA and Tukey's *post hoc* test. I tested the interaction between juveniles and adults because they may respond differently to their microclimates separated by elevation. Where sufficient males were available to measure a trait, I compared adult males and females using Welch's t-test. I compared the CT_{max} in *P. lapponica* and *P. sodalis* between populations separated by latitude in the Yukon by Welch's t-test; I did not collect sufficient *Pardosa* from each collection site in the Yukon to allow latitudinal comparisons within species of SCP and CT_{min}, nor were there enough species to test for

correlation between mean body size and mean SCP or CT_{max} among species. I performed all analyses using R version 3.2.2 (R Development Core Team, 2016).

3.3   Results

None of the 275 spiders I froze as part of SCP or cold tolerance strategy determinations survived ice formation. Cold tolerance strategies are summarised in Table 3.2. All juvenile spiders I measured were freeze avoidant, as were all adult females from the Yukon and adult female *P. furcifera* and *P. groenlandica* from high elevations in Greenland. Adult female *P. groenlandica* from low elevation were chill-susceptible, as were all the *P. hyperborea* adult females. *Pardosa groenlandica* (high and low elevation) and *P. hyperborea* spiderlings appeared to be chill-susceptible, however, much mortality was likely due to the difficulty of removing the spiders from the adhering grease, making it difficult to separate handling- and cold-related mortality.

Table 3.2 Survival of frozen and unfrozen Arctic *Pardosa* exposed to low temperatures. The Greenland low elevation spiders were collected from 0 to c. 80 m a.s.l., and the high elevation were collected at c. 450 m a.s.l. *Pardosa* were cooled until c. 50% froze and allowed to recover for 24 h at room temperature. Dead individuals were those that did not move or right themselves. CS – chill susceptible, FA – freeze-avoidant.

	Frozen		Unfrozen		Strategy
	No. Alive	No. Dead	No. alive	No. Dead	
Greenland					
Pardosa furcifera					
Juvenile, low elevation	0	9	7	0	FA
Adult female, low elevation	0	5	5	0	FA
Pardosa groenlandica					
Juvenile, low elevation	0	5	5	0	FA
Juvenile, high elevation	0	8	6	0	FA
Adult female, low elevation	0	8	5	3	CS
Adult female, high elevation	0	6	2	1	CS
Pardosa hyperborea					
Juvenile, low elevation	0	10	6	0	FA
Adult female, low elevation	0	9	4	3	CS
Adult female, high elevation	0	8	5	2	CS
Yukon					
Pardosa lapponica, adult female	0	5	4	1	FA
Pardosa moesta, adult female	0	6	3	1	FA
Pardosa sodalis, adult female	0	5	4	1	FA

The SCPs of Arctic *Pardosa* ranged from -23.3 °C in a low elevation *P. groenlandica* spiderling, to -4.5 °C in two *P. glacialis* adult females collected near hot springs on Disko Island, Greenland. The mean SCPs of Yukon-collected *Pardosa* ranged from -5.4 °C ± 0.2 (female *P. lapponica*) to -6.9 °C ± 0.3 (female *P. glacialis*), and in adult Yukon females, the SCP appears to be higher in larger species, however this could not be tested statistically (Figure 3.1). Among *P. glacialis*, the SCP of the low elevation Disko Island population was significantly lower than the hot springs location; the SCP of both the Disko Island populations were similar to those collected from the Yukon Territory (Figure 3.5).
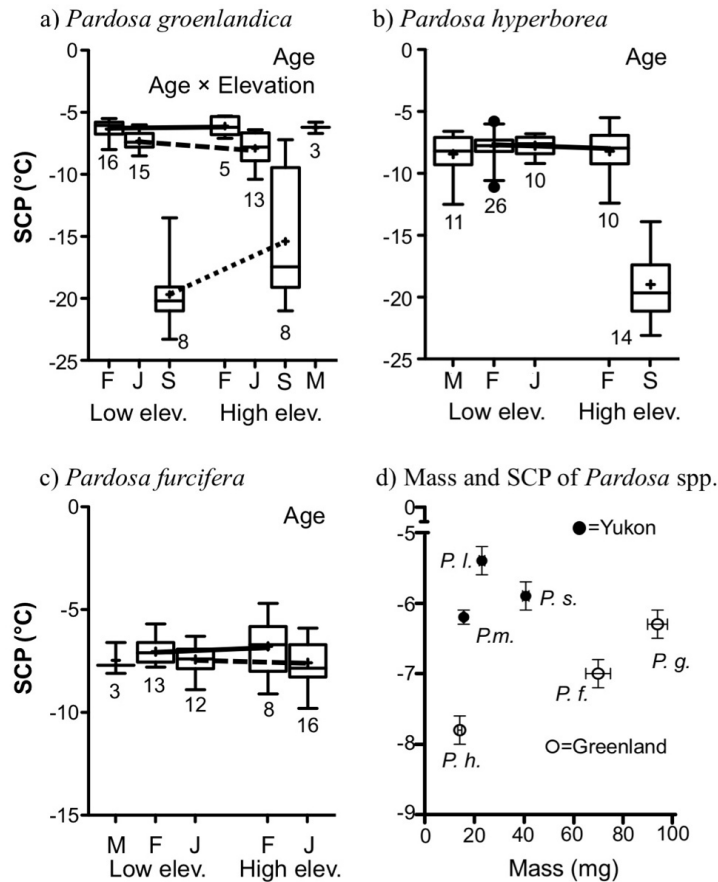
Figure 3.1 Supercooling points (SCP) of *Pardosa* sp. spiders from various high latitude locations, and the relationship between mass and SCP. Spiderling (S), juvenile (J), adult female (F), and adult male (M) a) *Pardosa groenlandica*, b) *P. hyperborea*, and c) *P. furcifera* from both low (below 80 m a.s.l.) and high elevations (c. 450 m a.s.l.). d) Mean body mass (mg) and SCP for Yukon-collected female *P. lapponica* (*P. l.*), *P. sodalis* (*P. s.*), and *P. moesta* (*P. m.*) from the Yukon-North West Territories border, and for Greenland-collected female *P. groenlandica* (*P. g.*), *P. furcifera* (*P. f.*), and *P. hyperborea* (*P. h.*) females from low elevation. Terms in the upper right-hand corner of the graphs indicate statistically significant effects on SCP within species (two-way ANOVA). There was no difference between female and male *P. groenlandica* ($t_{6.0}$=0.312, p=0.766), *P. hyperborea* ($t_{13.4}$=1.210, p=0.247), or *P. furcifera* ($t_{2.3}$=0.679, p=0.558). Boxes indicate quartiles and whiskers the 95th percentile, crosses indicate means. Error bars (SE) in d) may be obscured by symbols, numbers indicate samples sizes.

Table 3.3 Statistical comparisons of supercooling points and critical thermal maxima (CT_{max}) of Greenland- and Yukon-collected *Pardosa* within species. Results are comparing within species among age groups (spiderling, juvenile, adult females) and among collection locations separated by elevation (0-80 m a.s.l., c. 450m a.s.l.). Results are from two-way ANOVA; df = degrees of freedom. Asterisks indicate significant terms.

	Age			Elevation			Age × Elevation		
	df	*F*-ratio	*p* value	df	*F*-ratio	*p* value	df	*F*-ratio	*p* value
SCP									
P. groenlandica	2,59	139.6	<0.001*	1,59	2.8	0.101	2,59	6.5	0.003*
(omitting spiderlings)	1,45	24.1	<0.001*	1,45	1.1	0.307	1,45	2.0	0.165
P. hyperborea	2,56	228.0	<0.001*	1,56	0.4	0.509	-	-	-
(omitting spiderlings)	1,43	0.1	0.739	1,43	0.9	0.357	-	-	-
P. furcifera	1,45	4.3	0.043*	1,45	<0.1	0.969	1,45	0.5	0.468
CT_{max}									
P. groenlandica	1,30	0.02	0.898	1,30	2.2	0.150	-	-	-
P. hyperborea	1,38	11.9	0.001*	1,38	91.0	<0.001*	1,38	17.3	<0.001*
P. furcifera	1,22	22.0	<0.001*	1,22	2.5	0.128	-	-	-

Within the Greenland-collected species, the SCP of *P. furcifera* juveniles was significantly lower than that of the adult females, however there was no effect of elevation on SCP nor an age × elevation interaction (Figure 3.1; Table 3.3). *Pardosa groenlandica* and *P. hyperborea* spiderlings had lower mean SCPs than juveniles and adult females of the same species; the SCP of the *P. groenlandica* spiderlings was higher in the high elevation site, and there was an age × elevation interaction (Figure 3.1; Table 3.3). Eliminating the spiderlings from the analysis, the SCP of *P. hyperborea* juveniles and adult females did not differ, however there was a difference in *P. groenlandica*: there was no difference in SCP between collection elevations, or an age × elevation interaction for either species (Table 3.3). The SCPs of adult male and female *P. furcifera* (low elevation), *P. groenlandica* (high elevation), and *P. hyperborea* (low elevation) did not differ significantly (Figure 3.2; Table 3.3).

The lowest CT_{min} I measured was -6.9 °C in an adult female *P. glacialis* from the YT-NWT Border, while the highest was +1.7 °C in an adult female *P. lapponica* from Tombstone Mountains in the Yukon Territory. There was no difference in CT_{min} among the Yukon-collected *Pardosa* species (Figure 3.1). The mean CT_{min} of the Yukon-collected *Pardosa* fell within a 1.4 °C range; from -4.5 ±0.3 °C (adult female *P. moesta*) to -5.7 ± 0.3 °C (adult female *P. glacialis*; Figure 3.1). The CT_{min} of the juvenile, Greenland-collected *P. groenlandica* from low elevations was -2.2 ±0.2 °C (Figure 3.1); I did not compare the CT_{min} with those from the Yukon-collected individuals because the transport differences between the Yukon-collected spiders (transported from the Yukon to the laboratory in London, Ontario) and the Greenland-collected spiders (I did not transport the animals before measurement).
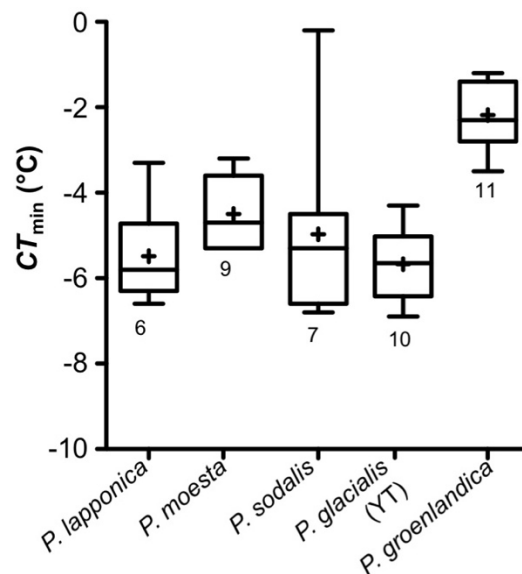
Figure 3.2 Critical thermal minima (CT_{min}) of Yukon- and Greenland-collected
Pardosa **spp.** I collected the adult female *P. lapponica*, *P. moesta*, and *P. sodalis* and
unsexed adult *P. glacialis* at the YT-NWT border; juvenile, low elevation *P.*
groenlandica were collected in Greenland. Boxes indicate quartiles and whiskers the 95th
percentile, crosses indicate means; numbers indicate sample size.

The CT_{max} of Arctic *Pardosa* ranged from +30.5 °C in an adult female *P. lapponica* from
the YT-NWT border, to +48.7 °C in an adult female *P. glacialis* from the Ogilvie
Mountains. The CT_{max} of Greenland-collected *Pardosa* fell between 42.3 and 46.6 °C,
and CT_{max} was generally higher in larger species (Figure 3.3).
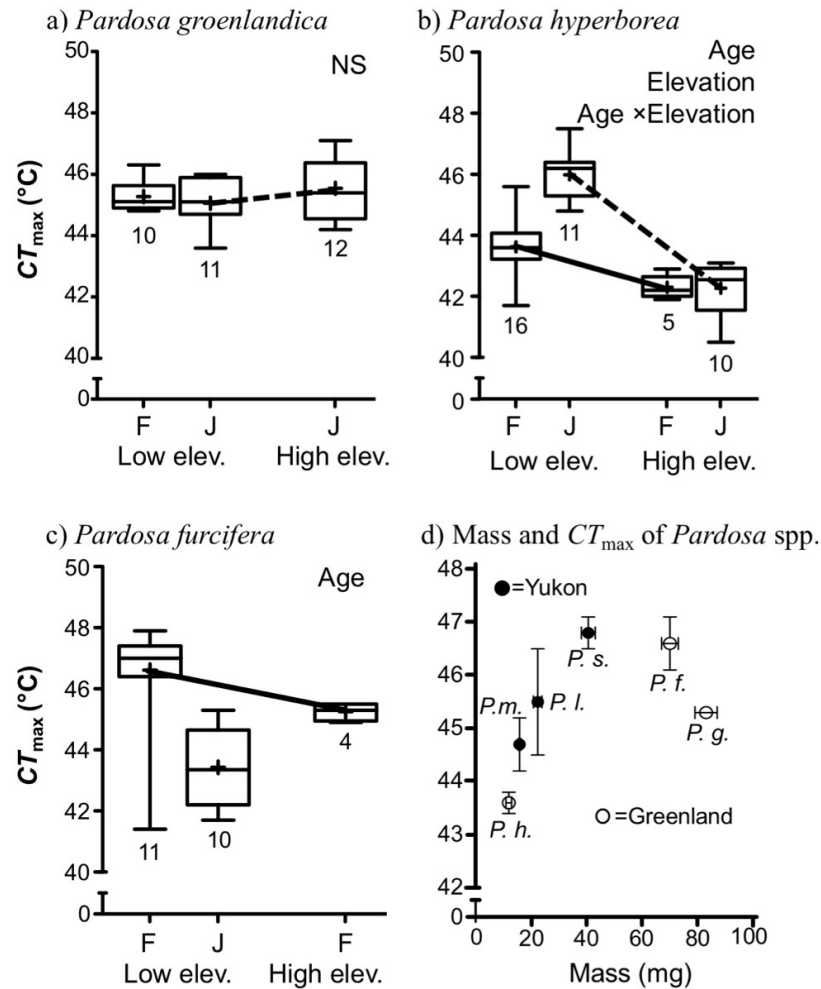
Figure 3.3 Critical thermal maxima (CT_{max}) of summer field-fresh *Pardosa* spp. collected from high latitudes, with the relationship between body mass and CT_{max}. The juvenile (J) and adult female (F) Greenland-collected a) *Pardosa furcifera*, b) *P. groenlandica*, and c) *P. hyperborea* spiders were collected from low and high elevations (see text for details). d) The mean spider body mass (mg) and mean CT_{max} for Yukon-collected female *P. lapponica* (*P. l.*), *P. sodalis* (*P. s.*), and *P. moesta* (*P. m.*) from Yukon-North West Territories border, and for Greenland-collected female *P. groenlandica* (*P. g.*), *P. furcifera* (*P. f.*), and *P. hyperborea* (*P. h.*) from low elevation. Terms in the upper right-hand corner of graphs a), b), and c) are those with statistically significant effects on SCP within species (two-way ANOVA). Boxes indicate quartiles and whiskers the 95th percentile, crosses indicate means; numbers indicate sample size, error bars (SE) in d) may be obscured by symbols.

Within species, there was no relationship between CT_{max} and latitude within the Yukon-collected *P. lapponica* and *P. sodalis* (Figure 3.4; Table 3.3). Within the Greenland-collected *Pardosa*, the CT_{max} of juvenile *P. furcifera* was significantly lower than female adults, but there was no difference between the high and low elevation populations (Figure 3.5, Table 3.3). The juvenile *P. hyperborea* had higher CT_{max} than the female adults: the CT_{max}es of all *P. hyperborea* from high elevation populations were significantly lower than that of spiders from low elevations. There was also an age × elevation interaction: the juveniles had a lower CT_{max} in high elevation populations, the adults also had a lower CT_{max} at high elevation (Figure 3.5; Table 3.3). The CT_{max} of *P. glacialis* collected from the Yukon was similar to that of the Disko Island hot spring population, and they were both c. 2 °C higher than that of the Disko Island low elevation population (Figure 3.5).
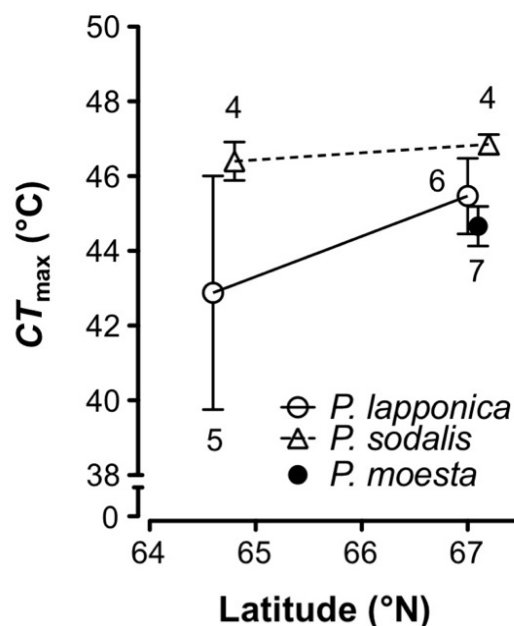
Figure 3.4 Critical thermal maxima (CT_{max}) of *Pardosa* adult females from different latitudes in the Yukon Territory. The CT_{max} of *P. lapponica* and *P. sodalis* did not differ by latitude (*P. lapponica*: $t_{4.8}$=0.788, p=0.468; *P. sodalis*: $t_{4.7}$=0.681, p=0.528). Error bars (SE) may be obscured by symbols, data points are slightly offset for clarity. Sample sizes are given for each point.

a)   b)
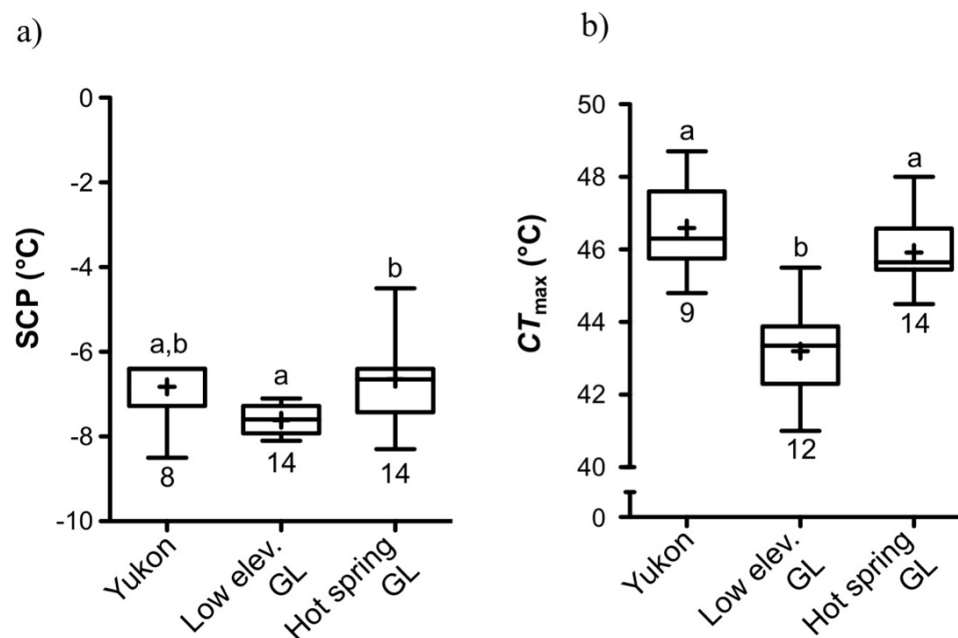
Figure 3.5 Supercooling points (a) and critical thermal maxima (b) of adult female
Pardosa glacialis **populations.** The spiders were collected from Disko Island, Greenland
(GL) at either a low elevation or near a hot spring, and from the Ogilvie Mountains in the
Yukon Territory (Yukon). Different letters denote statistically significant differences in
SCP (one-way ANOVA $F_{2,32}$ = 5.46, p = 0.009) or CT_{max} (one-way ANOVA $F_{2,32}$ =
31.56, p <0.001). Boxes indicate quartiles and whiskers the 95th percentile, crosses
indicate means; numbers indicate sample size.

3.4 Discussion

Spiders are abundant in Arctic and sub-Arctic terrestrial ecosystems (Hodkinson et al.,
2001), yet because their thermal biology has received only limited attention they are
poorly represented in global-scale meta-analyses relating thermal tolerances to latitude
(e.g. Deutsch et al., 2008; Sunday et al., 2011). Here, I show that the CT_{min} and SCP of
Pardosa from Greenland and Yukon are slightly lower than those of their lower-latitude
congeners, whereas their CT_{max} is similar to that of lower-latitude spiders (e.g.
Schmalhofer, 1999). This pattern is consistent with observations for insects (Addo-
Bediako et al., 2000), in which species are more cold-tolerant towards the poles, but there
is little latitudinal variation in upper thermal limits.

In keeping with reports for other spiders (Kirchner, 1973, 1987), I found that summer-collected Greenland and Yukon *Pardosa* were largely freeze avoidant. I note that in almost all cases, the CT_{min} and SCP appeared to be very close together. For example, the freeze-avoidant Yukon-collected adult female *P. lapponica* has a mean CT_{min} of -5.5 °C and a mean SCP of -5.4 °C (Figure 3.1). I use the proximity of CT_{min}, SCP, and lethal temperature to justify using SCP as a convenient metric of cold tolerance in these species in this study. I acknowledge that I have based these measurements on brief exposures to cold, and that the profile of low-temperature survival can depend on both the temperature and duration of exposure (Sinclair et al., 2015); thus, experiments exploring longer cold exposures could reveal mortality at higher temperatures. However, I note that many freeze-avoidant and chill-susceptible arthropods can withstand long cold exposures (Bale, 1993), and that I can reasonably expect spiders from these Arctic and sub-Arctic habitats to tolerate long exposures to the relatively mild sub-zero temperatures I explore here.

Spiderlings had much lower SCPs than other life stages. This could be because of their small size and lack of feeding, both of which will favour low SCPs (Salt, 1968; Zachariassen et al., 2004), and may also be consistent with this life stage encountering sub-zero temperatures at high altitude when dispersing by ballooning (Decae, 1987; Foelix, 2011). Unlike in other life stages, I did observe some variation in SCP among *P. groenlandica* spiderlings which was roughly consistent with expectations based on collection locality: high elevation-collected individuals had lower SCPs than their low-elevation counterparts. In general, *Pardosa* spp. with lower body mass tended to have lower SCP and CT_{max}. This suggests that future comparisons of SCP and CT_{max}, among species should account for body size, especially since small animals tend to have lower SCPs (Salt, 1961; Zachariassen et al., 2004), and also dehydrate more quickly when exposed to high temperatures, which may affect critical thermal limits and SCP (Harrison et al., 2012).

Arctic *Pardosa* reach sexual maturity in the early to mid-summer and die at the end of the breeding season. It is the juveniles that overwinter, in some cases more than once (Buddle, 2000). For this reason, I expected greater low-temperature tolerance in the juveniles compared to the adults, but found no evidence of this, which implies that the

juveniles likely significantly increase their cold tolerance (and possibly even change cold tolerance strategy) prior to winter. I suggest that laboratory acclimation and seasonal comparisons will be necessary to extrapolate this work to allow cold tolerance-based estimates of overwintering capacity.

The CT_{max} of the Yukon and Greenland *Pardosa* were consistent with those reported for other spiders: for example, +45.1 °C in *Misumenops asperatus* (Thomisidae) from New Jersey (Schmalhofer, 1999) and +46.9 °C in *Peucietia viridans* (Oxyopidae) from Tennessee (Hanna and Cobb, 2007). Surface temperatures in tundra can exceed 30 °C (6 July 2015 at 65.8°N in YT), so this high tolerance to high temperatures means that *Pardosa* will likely not encounter temperatures close to their CT_{max} and will therefore be able to take full advantage of the summer growing season. I observed some variation in CT_{max} among populations that is consistent with the expectation that lower elevation (and latitude) populations of *P. hyperborea* have higher CT_{max} (and therefore presumably high-temperature tolerance) than their high elevation counterparts.

My data are useful for comparison with other studies reporting or comparing thermal tolerances of arthropods measured during the growing season. However, the most significant latitudinal differences in climate occur in winter (Williams et al., 2015). Like the Beringian pseudoscorpion, also collected in Yukon (Chapter 2), the cold tolerance I measured for these juvenile polar *Pardosa* would be insufficient to survive the temperatures I expect they would encounter in the winter. In the absence of a capacity for substantial thermal buffering (underlying permafrost will yield low soil temperatures), I expect that juveniles of these species exhibit significant seasonal variation in cold tolerance. Future work could explore seasonal variation in thermal biology to understand the limits of thermal tolerance and overwintering capacity in polar spiders.

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Chapter 4

4 Effect of low temperature acclimation on thermal tolerances in summer-collected Arctic wolf spiders

I have prepared this chapter as a manuscript for submission.

4.1 Introduction

Ambient temperatures impact the performance and survival of ectothermic animals (Huey and Kingsolver, 1989). Polar and sub-polar ectotherms, in particular, face short growing seasons and large seasonal temperature fluctuations (Convey et al., 2018). Ectotherms can adapt to such thermal fluctuation through phenotypic plasticity, which allows them to shift their performance to match changing conditions (Kingsolver, 2009). For example, plasticity has been linked to improved foraging efficacy (Kristensen et al., 2008), and better cold tolerance (Slabber and Chown, 2004). Species with the ability to alter their phenotype to optimise performance to changing environmental conditions will most likely be resilient to climate change (Somero, 2010).

The thermal tolerances of terrestrial arthropods are bounded by thermal limits within which temperatures permit activity and survival. The critical thermal limits – critical thermal maximum (CT_{max}) and minimum (CT_{min}) – are the temperatures beyond which the animals cannot move; the temperature at which they freeze is their supercooling point (SCP). Plasticity of these upper and lower thermal limits can extend the range of temperatures over which an animal can remain active, the thermal breadth (T_{br}). The fundamental thermal breadth (T_{br}-F) is the T_{br} at any one time, whereas the absolute T_{br} (T_{br}-A) is the difference between the lowest and highest thermal limits following acclimation to either high or low temperatures and describes the potential range of thermal tolerance after acclimation (Calosi et al., 2010; Spicer and Gaston, 1999).

Organisms living in thermally variable environments can have either a broad T_{br}-F, or change their thermal tolerances via acclimation. We would expect that following

acclimation, an organism would improve thermal tolerances in tune with its acclimation temperature, according to the 'beneficial acclimation hypothesis' (BAH; Kingsolver, 2009; Leroi et al., 1994). In the sub-Antarctic spider *Myro kerguelenesis*, low-temperature acclimation decreases the CT_{min} by c. 1 °C, and high-temperature acclimation increases CT_{max} by c. 0.3 °C (Jumbam et al., 2008). However, T_{br}-F does not change with acclimation, but is broader in species that have larger distributions, such as in the European diving beetle (Calosi et al., 2008). Thus, a general prediction for high-latitude arthropods is that T_{br}-F will either be broad enough to survive the large summer temperature range of high-latitudes, or that the thermal tolerances will shift with acclimation (T_{br}-A > T_{br}-F).

Many polar and sub-polar terrestrial arthropods show pronounced seasonal thermal plasticity, which allows them to survive winter low temperatures, while avoiding the costs of maintaining year-round low-temperature tolerance (Sinclair et al., 2003b). For example, the spider *Parasteatoda* (*Arachaearanea*) *tepidariorum* from Japan (Tanaka, 1996) and the mite *Alaskozetes antarcticus* from Antarctica (Lee and Baust, 1981) decrease their SCP in winter. Decreases in SCP are associated with an accumulation of cryoprotectants (Tanaka, 1995; Young and Block, 1980), and/or starvation to avoid ice nucleation in the gut (Block and Sømme, 1982). However, temperatures in polar and sub-polar habitats also fluctuate markedly during the growing season, and there is evidence in other species of phenotypic plasticity at these timescales. For example, SCP shifts diurnally in Antarctic Collembola, with lower SCP at night compared to midday (Sinclair et al., 2003a; Worland and Convey, 2001), and Antarctic mites from more thermally-stable (on daily and seasonal timescales) marine environments show less plasticity than terrestrial mites from thermally variable environments (Deere et al., 2006). Thus, we expect the thermal tolerances of terrestrial polar and sub-polar arthropods to be plastic, even in the summer, because of the selective pressure to fine-tune performance during a short growing season.

Wolf spiders of the genus *Pardosa* are abundant in the Arctic and sub-Arctic (Turney et al., 2018). Because summers are short, *Pardosa* spiders have limited time to feed, mate, and rear eggs and spiderlings. Their summer thermal limits are the boundaries within

which the organism can perform. We collected *Pardosa* spp. over two summers from the Yukon, Greenland, and sub-Arctic Norway to measure the extent to which these high latitude species have plastic thermal limits. I previously measured the SCP, CT_{min}, and CT_{max} of *Pardosa* collected in summer from 64.6 to 67.0°N in the Yukon and 61.2 to 69.8°N in Greenland (Chapter 3). The CT_{max} of these animals ranged from 30.5 to 48.7 °C, higher than the surface temperatures reported from the Arctic (Convey et al., 2018). The CT_{min} of Yukon-collected spiders ranged from -0.2 to -6.9 °C and SCP from -4.5 to -23.3 °C (Chapter 3), which approximates the majority of low summer temperatures, not including brief summer cold spells (Convey et al., 2018). Here, my objective was to determine whether these summer thermal limits are plastic over the short term in high-latitude summer-collected spiders from Yukon, Greenland, and northern Scandinavia.

4.2   Methods

4.2.1   Animal collections and acclimation

I collected the spiders by hand from tundra ecosystems in the Yukon Territory (summer of 2015), Greenland, and Norway (both summer of 2016) into c. 30 mL plastic containers.

I collected *Pardosa lapponica* and *P. moesta* between 1 July and 6 July 2015, at 67.1°N, on the tundra in the Yukon Territory (Yukon Science and Explorer's License 15-15S&E). I transported all Yukon-collected spiders in insulated containers to the University of Western Ontario in London, Ontario, Canada, and kept them for three to seven days at 12 °C and 24 h light, conditions comparable to summer at those collection sites. The CT_{min}, CT_{max}, and SCP of these individuals are presented in Chapter 3. These spiders were called "field-fresh" in Chapter 3, but here I refer to them as "warm-acclimated" for consistency with the other data presented herein. A subset of each species collected from the Yukon was cold-acclimated at 4 °C, 24 h light (Conviron CMP 3244, Controlled Environments Inc., Winnipeg, MB, Canada), a temperature they would experience during summer. I fed the Yukon-collected spiders flightless *Drosophila melanogaster* every two to three days during collection, travel, and acclimation.

Collaborators in Norway collected *P. hyperborea* from Vågå, Oppland, Norway (61.9°N, 9.1°E). They were shipped in an insulated carrier on 20 July 2016, three to four days post-capture, to the University of Western Ontario, and I haphazardly assigned the spiders to an acclimation treatment of either 4 °C and 24 h light (Conviron CMP 3244, Controlled Environments Inc., Winnipeg, MB), or 20 °C and 24 h light for one week (MIR153, Sanyo, Bensenville, Illinois, USA), mimicking the photoperiod that they would encounter in the field. I fed these spiders *D. melanogaster* every three to four days during acclimation.

I collected *P. furcifera*, *P. groenlandica*, and *P. hyperborea* between June 21 and July 24, 2016, near Narsarsuaq, Greenland (61.2°N, 45.4°W; Greenland Survey License G16-042) from two elevations - low (sea level to c. 80 m a.s.l.), and high (c. 450 m a.s.l.). I returned them to the temporary laboratory within 4 h and acclimated them for one week without food in a domestic refrigerator for cold acclimation (c. 4 °C) or a PTC-1 portable incubator set to 20 °C for warm acclimation (Sable Systems International, Las Vegas, NV, USA), both with 24 h light (summer photoperiod). To determine the effect of feeding on SCP, I fed 45 adult, low elevation *P. hyperborea D. melanogaster* at Day 0. I measured the SCP of a subset of these spiders every two days for one week while housing them at 20 °C and 24 h light.

4.2.2   Supercooling points and critical thermal limits

I measured CT_{min} and CT_{max} in the Yukon-collected spiders, and SCP and CT_{max} in the Greenland-collected spiders following Sinclair et al. (2015) and Chapter 3. Briefly, I cooled the Yukon-collected spiders in capped 250 mL beakers surrounded by ethylene glycol cooled at -0.25 °C·min^{-1} by a circulating bath (Model 1157P, VWR International, Mississauga, ON, Canada) as described by MacMillan and Sinclair (2011) until the spiders were unable to respond to a touch from a paintbrush. I measured CT_{max} on different individual spiders in the same set-up as CT_{min}, however, the ethylene glycol was heated at +0.25 °C·min^{-1}, and I recorded CT_{max} at the onset of spasms (Lutterschmidt and Hutchison, 1997). I recorded the ambient temperature by 36-AWG type-T copper-constantan thermocouple (OMEGA, Laval, Quebec, Canada) in the beakers as a proxy

for the spiders' body temperature and sampled every 0.5 s using a TC-08 interface and PicoLog software (Pico Technology, Cambridge, UK).

I measured SCP and CT_{max} in the Greenland- and Norway-collected spiders using a custom-built Peltier-controlled heating and cooling system. To measure SCP, I placed the spiders in a 1.7 mL Eppendorf tube, with a 36-AWG type-T copper-constantan thermocouple (OMEGA, Laval, Quebec, Canada) held in place with cotton wool. I cooled the spiders at -1°C·min^{-1} from acclimation temperature to 0 °C, held at 0 °C for 5 min, then cooled at -0.5 °C·min^{-1} until an exotherm was detected indicating the spider had frozen. I used SCP as a proxy for low-temperature tolerance rather than CT_{min} (see Chapter 3 for justification). We measured CT_{max} by placing the spiders in a Peltier-effect temperature-controlled copper arena separated into six regions with aluminum foil. We monitored the arena temperature as a proxy for body temperature with a 36-AWG type-T copper-constantan thermocouple (OMEGA, Laval, Quebec, Canada) connected to a TC-08 interface and PicoLog software (Pico Technology, Cambridge, UK). The arena temperature was heated at 1.0 °C·min^{-1} from 15 °C to 30 °C, then +0.25 °C·min^{-1} above 30 °C, until we noted the spiders spasming and curling their legs (Lutterschmidt and Hutchison, 1997). The Norway-collected spiders were measured the same way as the Greenland-collected *Pardosa*. None of the spiders survived the SCP or CT_{max} measurements. I weighed all the spiders after they were used in the experiments.

4.2.3   Data analyses

For the Yukon-collected spiders, I calculated the T_{br}-F as the difference between the CT_{min} and the CT_{max} after cold- and warm-acclimation and the T_{br} -A as the difference between CT_{min} of the cold-acclimated spiders and the CT_{max} of the warm-acclimated spiders. I calculated the T_{br}-F and T_{br}-A in the Greenland- and Norway-collected spiders in a similar manner, except I used SCP instead of CT_{min} as the low-temperature limit.

Within species, I compared CT_{min} or CT_{max} between acclimation treatments in the Yukon-collected spiders, and SCP or CT_{max} within Greenland and Norway-collected spiders. Where there was only one comparison within species, I tested for differences by t-test;

when the comparison within species included age or elevation, I used a two-way ANOVA. I compared the SCP among unfed *P. hyperborea* by days since fed by ANOVA, followed by a Tukey's *post hoc* test. All statistics were conducted with R version 3.2.2 (R Core Team, 2015).

4.3 Results

The highest and lowest CT_{min} of the Yukon-collected *Pardosa* were from the previously-reported warm-acclimated *P. lapponica* (Chapter 3). The mean CT_{min} of the cold-acclimated, adult female *P. lapponica* was -4.2 ± 0.3 °C, which was significantly higher than their warm-acclimated counterparts (-4.6 ± 0.8 °C; Figure 4.1, Table 4.1); however, the CT_{min} of the cold-acclimated and warm-acclimated *P. moesta* did not differ (Figure 4.1, Table 4.1).

Table 4.1 Comparisons between warm- and cold-acclimated high-latitude *Pardosa* spp. Model terms that are statistically significant between cold- and warm-acclimated individuals are in bold (from Welch's t-test). Data from warm-acclimated Yukon-collected spiders are from Chapter 3 and are presented for comparison with the cold-acclimated *P. lapponica* and *P. moesta*.

	N cold, warm	df	t	p
CT_{min}				
P. lapponica (female, Yukon)	10, 6	9.05	2.73	**0.023**
P. moesta (female, Yukon)	11, 9	17.30	1.66	0.116
SCP				
P. hyperborea				
(female, low elevation, Greenland)	10, 10	17.74	2.80	**0.012**
(adult, Norway)	19, 12	16.96	1.63	0.122
CT_{max}				
P. lapponica (female, Yukon)	6, 11	15.00	0.02	0.982
P. moesta (female, Yukon)	10, 5	12.40	3.28	**0.006**
P. hyperborea (adult, Norway)	20, 12	25.20	1.88	0.072
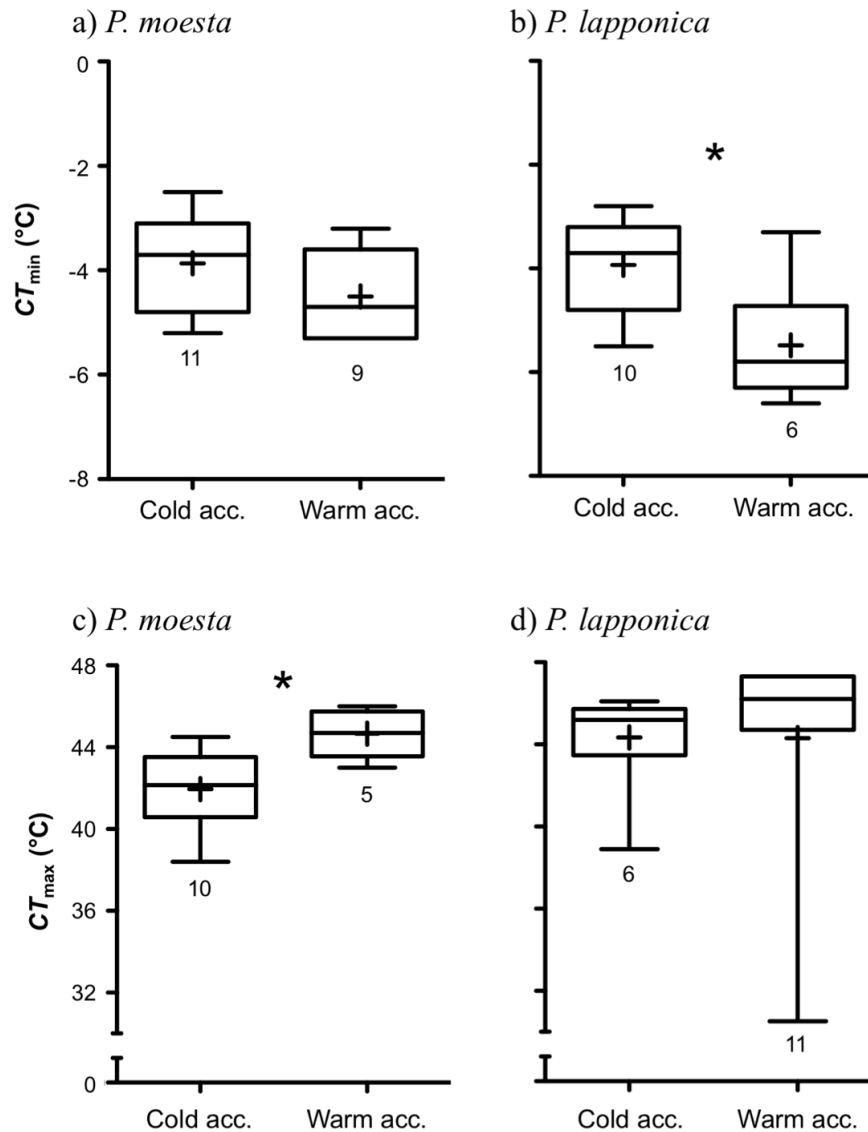
Figure 4.1 The critical thermal minima (CT_{min}; a,b) and critical thermal maxima (CT_{max}; c,d) of Yukon-collected adult female *Pardosa* spiders. *Pardosa moesta* (a,c) and *P. lapponica* (b,d) were cold-acclimated at 4 °C (Cold acc.) or warm-acclimated at 12 °C (Warm acc.). Boxplots are 25th and 75th percentiles, whiskers are 5th and 95th percentiles, the cross represents mean, and numbers indicate sample size. Asterisks indicate statistically significant differences (Table 4.1).

The SCP of Greenland- and Norway-collected spiders ranged from -17.8 °C (in a warm-acclimated juvenile, high elevation *P. furcifera* from Greenland) to -5.2 °C (a cold-

acclimated, adult female, low elevation *P. furcifera* from Greenland). There was a significant effect of age and/or sex and acclimation on SCP in *P. groenlandica* and *P. furcifera* such that warm-acclimated spiders had lower SCP than those cold-acclimated; (Figure 4.2; Table 4.2). The SCP was significantly lower in warm-acclimated than cold-acclimated Greenland-collected *P. hyperborea* (female, low elevation), but not in the cold- and warm-acclimated Norway-collected *P. hyperborea* (Figure 4.3; Table 4.1).
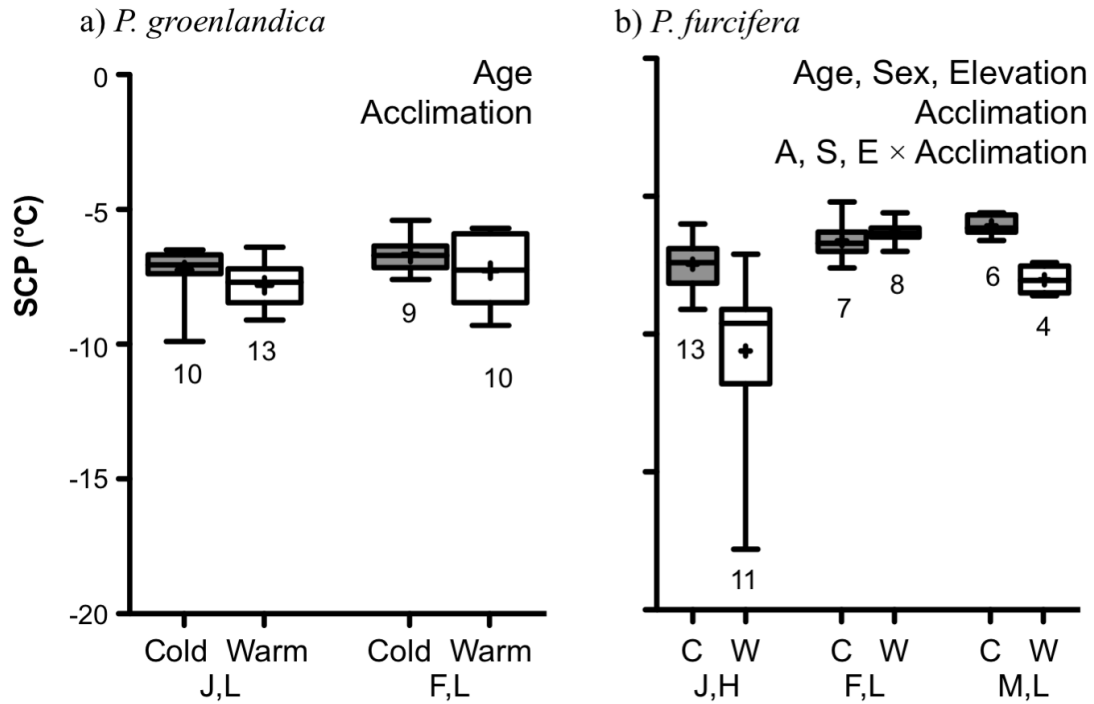
Figure 4.2 Supercooling points (SCP) of acclimated *Pardosa* spp. from Greenland.
We collected *Pardosa groenlandica* and *P. furcifera* juveniles (J), adult females (F), and
adult males (M) from Greenland at low (L) and high (H) elevation sites and acclimated
them in the cold (C; 4 °C; grey) or warm (W; 20 °C; white). The cold-acclimated spiders
were held for one week at 4 °C (Cold, C), and the warm-acclimated spiders were held at
20 °C for one week (Warm, W). Boxplots are 25th and 75th percentiles, whiskers are 5th
and 95th percentiles, the crosses are means, and the numbers indicate sample size. Terms
in the upper right-hand corner of the graphs indicate statistically significant effects on
SCP within species (two-way ANOVA, Table 4.2).

Table 4.2 The differences between cold- and warm- acclimated Greenland-collected *Pardosa* separated by life stage, sex (juvenile, adult female, and adult male) and elevation (low or high elevation). Statistically significant differences between the cold- and warm-acclimated spiders (two-way ANOVA) are in bold.

	By Life-stage or Sex and Elevation			By Acclimation			By Life-stage or Sex and Elevation × Acclimation		
	df	*F*-ratio	*p* value	df	*F*-ratio	*p* value	df	*F*-ratio	*p* value
SCP									
P. groenlandica (low-elevation juveniles and low-elevation adult females)	**1,38**	**4.5**	**0.040**	**1,38**	**4.5**	**0.042**	1,38	0.1	0.758
P. furcifera (low elevation females, and males; high elevation juveniles)	**2,43**	**11.9**	**<0.001**	**1,43**	**15.3**	**<0.001**	**2,43**	**5.0**	**0.011**
CT$_{max}$									
P. groenlandica (low elevation juveniles and adult females)	1,33	3.9	0.055	**1,35**	**10.9**	**0.002**	**1,35**	**15.6**	**<0.001**
P. hyperborea (low elevation adult females, high elevation adult females)	1,37	<0.0	0.993	**1,37**	**4.5**	**0.041**	**1,37**	**4.2**	**0.048**
P. furcifera (low elevation juveniles, females, and males; high elevation juveniles)	3,60	1.1	0.357	1,33	0.6	0.459	3,60	1.0	0.398
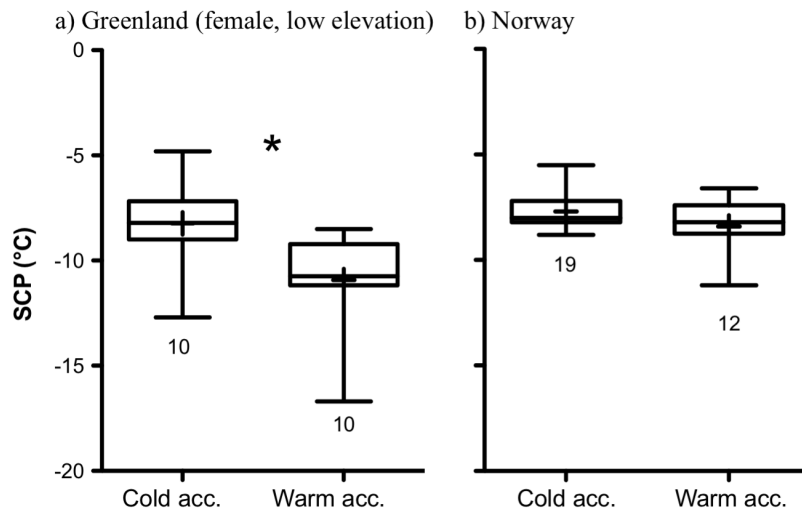
Figure 4.3 Supercooling points of cold (4 °C)- and warm (20 °C)-acclimated *Pardosa hyperborea*. The *P. hyperborea* were a) female, collected from low elevation in Greenland, and b) adults collected from Norway. Boxplots are 25th and 75th percentiles, whiskers are 5th and 95th percentiles, crosses are the means, and the numbers indicate sample size. Asterisks indicate statistically significant difference between acclimation groups (Table 4.1).

The lowest and highest CT_{max} were from the Yukon-collected warm-acclimated *P. lapponica* (+30.5 and +47.3 °C, respectively; Chapter 3). There was no difference between the mean CT_{max} of cold- and warm-acclimated *P. lapponica* (Figure 4.1; Table 4.1), although the mean CT_{max} of the cold-acclimated *P. moesta* was significantly lower than the warm-acclimated individuals (Figure 4.1; Table 4.1). The CT_{max} of the Greenland- and Norway-collected spiders ranged from +39.7 (warm-acclimated adult female, low elevation *P. hyperborea*) to +47.4 °C (cold-acclimated juvenile, low elevation *P. groenlandica*). In the Greenland-collected *P. groenlandica* and *P. hyperborea*, there was a significant effect of acclimation and an interaction between acclimation and age, sex, or elevation (Figure 4.4; Table 4.2), with the warm-acclimated individuals having a lower CT_{max} than the cold-acclimated individuals (Figure 4.4; Table

4.2). The CT_{max} of the Greenland-collected *P. furcifera* did not differ within species or by acclimation (Figure 4.4; Table 4.2). Finally, the mean CT_{max} of the cold- and warm-acclimated *P. hyperborea* from Norway did not differ (Figure 4.5; Table 4.1).

4.3.1 Thermal breadth

In the Yukon-collected spiders, the functional T_{br} is larger in warm-acclimated than cold-acclimated individuals by 1.0 °C (*P. lapponica*) and 3.3 °C (*P. moesta*) (Table 4.3). Within the Greenland-collected spiders, the differences in T_{br}-F between the cold- and warm-acclimated spiders are also small: less than 1.8 °C, with the exception of juvenile *P. furcifera* from high elevation, which was 3.2 °C (Table 4.3). Among the species acclimation yielded significant differences in either SCP or CT_{max}, the T_{br}-A is smaller than the warm-acclimated T_{br}-F; and in three out of four instances, T_{br}-A was smaller than the T_{br}-Fs of both warm- and cold-acclimated groups (Table 4.3).
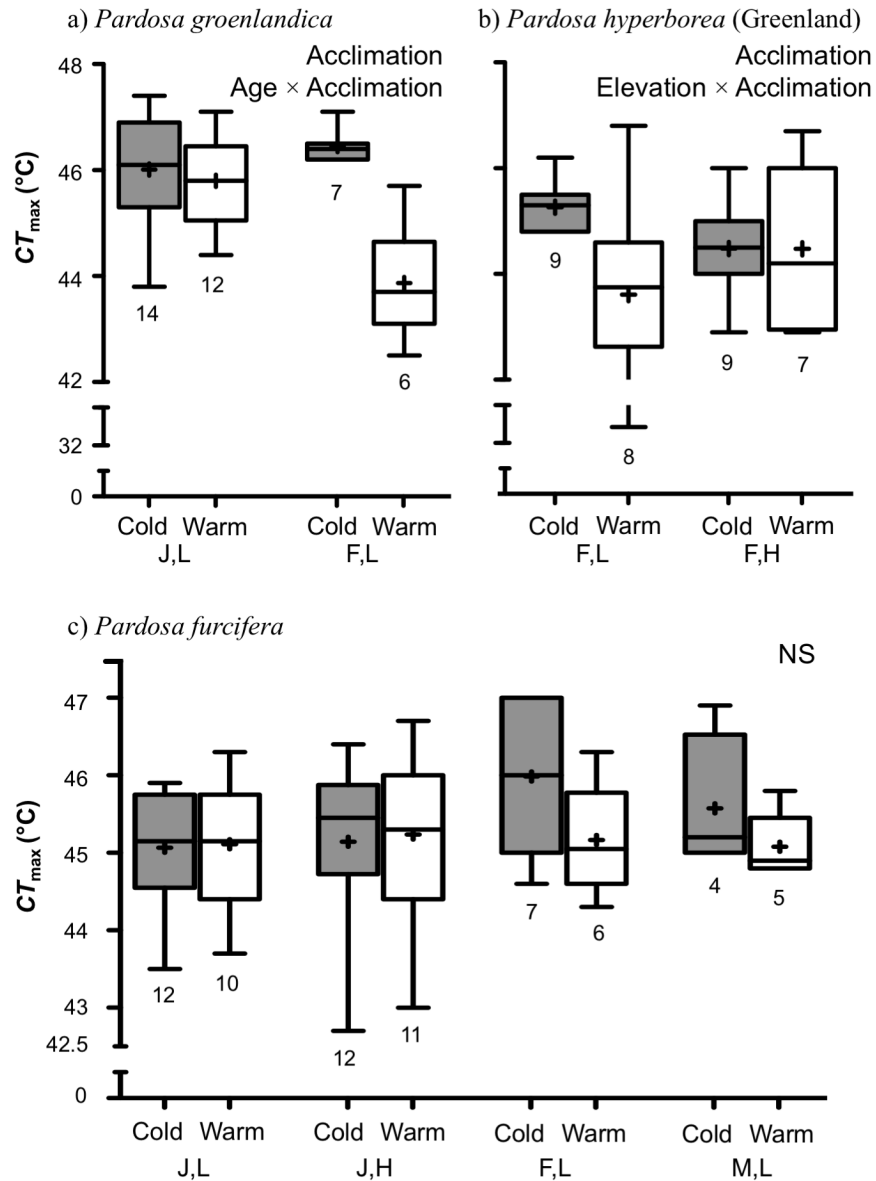
Figure 4.4 The critical thermal maxima (CT_{max}) of acclimated *Pardosa* spp. from Greenland. The juveniles (J), adult females (F), and adult males (M) a) P. groenlandica, b) P. hyperborea, and c) P. furcifera were collected from low (L) and high (H) elevation sites and acclimated to cold (4 °C; grey) and warm (20 °C; white). Boxplots are 25th and 75th percentiles, whiskers are 5th and 95th percentiles, crosses are the means, and numbers indicate sample size. Terms in the upper right-hand corner of the graphs indicate statistically significant effects on SCP within species (two-way ANOVA, Table 4.1).
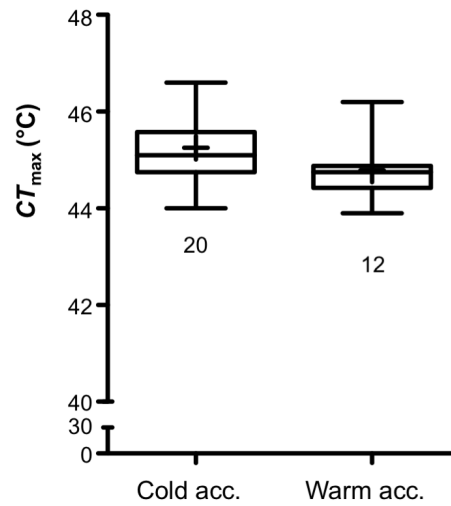
Figure 4.5 Critical thermal maximum (CT_{max}) of cold (4 °C)- and warm (20 °C)-acclimated adult *Pardosa hyperborea* collected in Norway. Boxplots are 25th and 75th percentiles, whiskers are 5th and 95th percentiles, numbers indicate sample size. There was no significant difference in CT_{max} between acclimation groups (Table 4.1).

Table 4.3 The functional and absolute thermal breadths (T_{br}-F and T_{br}-A, respectively) of acclimated *Pardosa* spiders collected from the Yukon Territory, Canada and Narsarsuaq, Greenland. Asterisks indicate species where there was a statistical difference in either CT_{max}, CT_{min}, and/or SCP between the warm- and cold-acclimated groups (Tables 4.1 & 4.2), indicating a difference between the T_{br}-Fs. J= juvenile, A=adult, L=low elevation, H=high elevation. Warm-acclimated data from the Yukon-collected spiders are from Chapter 3 and are presented for comparison purposes.

	Mean functional T_{br} (°C) of acclimated *Pardosa*		Absolute T_{br} (°C)
Species	**Cold**	**Warm**	
Yukon			
P. lapponica (A)*	47.9	48.9	48.5
P. moesta (A)*	45.8	49.1	47.9
Greenland			
P. furcifera			
(J,H)*	52.6	55.8	52.7
(F,L)	52.6	51.5	51.8
(M,L)*	51.6	53.1	51.1
P. groenlandica			
(J,L)	53.2	53.6	53.0
(F,L)*	53.0	51.1	50.4
P. hyperborea			
(F,L)*	53.5	54.5	51.8
(A,N)	52.9	53.2	52.5

4.3.2   Feeding status

The SCP of the unfed Greenland-collected low elevation, adult *P. hyperborea* decreased significantly in the days since fed (ANOVA $F_{3,41}$ = 3.53, p = 0.023), however the differences between each day were not significant, according to the Tukey's *post hoc* test. We observed a threshold decrease in SCP between Day 3 and Day 5, from -7.8 to -9.0 °C (Figure 4.6).
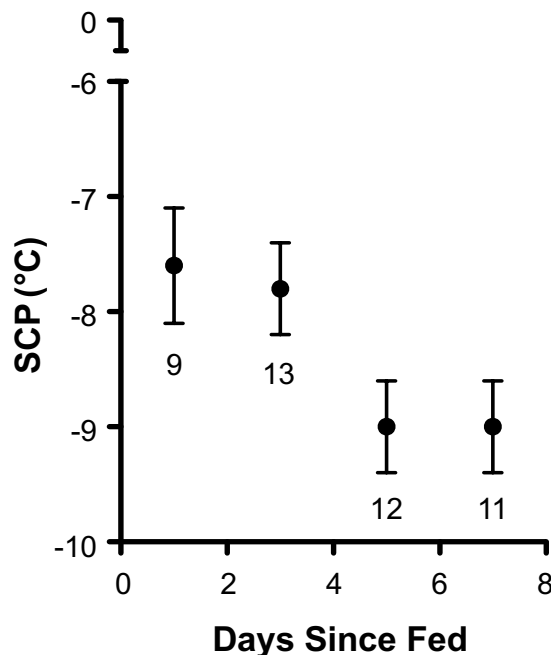
Figure 4.6 The change in SCP (mean ± SE) after feeding in *Pardosa hyperborea* adult females collected from the low elevation in Greenland. There was a significant difference in SCP among days since fed, however those differences were non-significant with the Tukey's *post hoc* test; numbers indicate sample sizes.

4.3.3   Body mass

In the Greenland- and Norway-collected spiders, the mean mass of the spiders was mostly lower in the warm-acclimated spiders than those cold-acclimated (Table 4.4). The exceptions include a large (45.5 %) increase in mean mass between the cold- and warm-acclimation *P. groenlandica* (juveniles, from the low-elevation, Greenland site); and a 2.4 % increase in the male, low-elevation (Greenland) *P. furcifera*. The mean masses of warm-acclimated, Yukon-collected spiders decreased in those used to measure CT_{max} (in *P. lapponica*) and CT_{min} (in *P. moesta*) and increased in CT_{min} (in *P. lapponica*) and CT_{max} (in *P. moesta*).

Table 4.4 The mass of *Pardosa* spiders high-latitude following one-week acclimation.
The cold-acclimated spiders were held at 4 °C; the warm-acclimated Greenland- and Norway-collected spiders were held at 20 °C; the warm-acclimated Yukon-collected spiders were held at 12 °C. J= juvenile, F= female, M= male, Y= Yukon, GL= Greenland, low elevation, GH= Greenland, high elevation, N= Norway.

Species, life stage, and collection location	Measurement	Mass (mg) (cold acclimation)	Mass (mg) (warm acclimation)	Percent difference between warm and cold acclimation
P. lapponica				
F, Y	CT_{min}	24.953 ± 1.813	25.674 ± 1.234	-2.9 %
F, Y	CT_{max}	24.578 ± 1.421	22.019 ± 1.160	10.4 %
P. moesta				
F, Y	CT_{min}	16.553 ± 0.619	14.789 ± 0.725	10.7 %
F, Y	CT_{max}	15.097 ± 0.622	15.523 ±0.479	-2.8 %
P. hyperborea				
F, GH	CT_{max}	11. ± 0	10. ± 1	10.3 %
F, GL	SCP	14. ± 2	12. ± 1	14.3 %
F, GL	CT_{max}	10. ± 1	9 ± 0	10.9 %
A, N	SCP	13.031 ± 0.347	11.547 ± 0.506	11.4 %
A, N	CT_{max}	12.026 ± 0.351	11.190 ± 0.426	7.0 %
P. groenlandica				
J, GL	SCP	11. ± 2	16. ± 3	-45.5 %
J, GL	CT_{max}	17. ± 4	15. ± 2	11.8 %
F, GL	SCP	101. ± 7	90. ± 6	11.1 %
F, GL	CT_{max}	86. ± 7	81. ± 4	6.3 %
P. furcifera				
J, GH	SCP	25. ± 4	18. ± 4	28.0 %
J, GH	CT_{max}	17. ± 1	15. ± 1	11.8 %
J, GL	CT_{max}	26. ± 4	21. ± 3	19.2 %
M, GL	SCP	42. ± 5	42. ± 3	0 %
M, GL	CT_{max}	42. ± 4	43. ± 2	-2.4 %
F, GL	SCP	67. ± 9	60. ± 6	10.4 %
F, GL	CT_{max}	70. ± 5	66. ± 6	5.7 %

4.4   Discussion

In the short and thermally-variable Arctic summer, I predicted that acclimation benefits high latitude ectotherms by maximising their time to eat, mate, and rear offspring (Auld et al., 2010). However, acclimation to low (4 °C) temperatures did not improve low-temperature tolerance in the *Pardosa* spp. I studied; and in some cases, low-temperature tolerance was diminished. This is contrary to sub-Antarctic spiders (*Myro kerguelenensis*

and *Prinerigone vagans*) where low-temperature tolerance improves when acclimated to low-temperatures (Jumbam et al., 2008).

Among the Yukon, Greenland, and Norway-collected spiders, warm acclimation decreased both low- and high- temperature tolerance compared to cold-acclimation; however, the changes in thermal tolerances after acclimation were small (maximum 3.2 °C). The maximum difference between the T_{br}-F of the low-temperature-acclimated spiders and the high-temperature-acclimated spiders (within species, age group, and location) was 3.3 °C. In *P. moesta* from the Yukon, the difference between the T_{br}-F of the treatment groups (3.3 °C) is driven by the decrease in CT_{max} in the cold-acclimated spiders; in *P. furcifera*, the difference (3.2 °C) is driven by a decrease in SCP in the warm-acclimated group. In *Drosophila melanogaster*, CT_{min} is more responsive to acclimation than CT_{max} (Sørensen et al., 2016); and in the tarantula *Aphonopelma* and sub-Antarctic spiders, CT_{max} has little acclimatory capacity (Jumbam et al., 2008; Seymour and Vinegar, 1973). The rest of the spiders from this study had a <1.8 °C difference between their T_{br}-Fs between warm and cold acclimation groups, an ecologically irrelevant difference in this environment.

I cannot explain why there is a small decrease in SCP and CT_{max} in some of the warm- *vs* cold-acclimated Greenland- and Norway-collected spiders, but suggest that the change I detected may not be the result of plasticity, but as a result of faster gut clearance and dehydration at higher *vs* lower temperatures. At increased temperatures, metabolic rate increases in spiders (Q_{10} of 1.5 – 2.5 Humphreys, 1974; Riddle and Markezich, 1981; Stork, 2012), resulting in an increase in the rate of food clearance and dehydration. Gut clearance voids the organism of potential ice nucleators, thereby depressing SCP (Block and Sømme, 1982; Nyamukondiwa and Terblanche, 2009), as has been demonstrated in the mites *Alaskozetes antarticus* (Block and Sømme, 1982) and the spider *Parasteatoda tepidariorum* (Tanaka and Watanabe, 2003). IThe SCP decreased three to five days post-feeding in *P. hyperborean*. I speculate that the gut is cleared during this window, lowering the SCP. During the acclimations, I did not feed the spiders, so it is likely that SCP was lower post-acclimation because of an empty gut (especially in warm acclimations, which presumably have higher metabolic rates). Evaporation also increases

at higher temperatures, and desiccation may lower CT_{max} (Rezende et al., 2011), as well as concentrate the hemolymph, further decreasing SCP (Zachariassen, 1985). Although I did not measure water loss in the spiders, the mean mass of the unfed Greenland and Norway-collected spiders after SCP and CT_{max} determination was more often lower (from a 6 % to 28 % decrease, depending upon species and measurement; Table 4.4) in the warm-acclimated spiders than the cold-acclimated spiders (except juvenile, low elevation *P. groenlandica* and male, low-elevation *P. furcifera* from Greenland; Table 4.4). However, there was no relationship between the mass of the fed warm- and cold-acclimated Yukon-collected spiders. The increased rate of food clearance and dehydration in starved, warm-acclimated spiders more likely explains the small differences in SCP and CT_{max} between acclimation groups, rather than any molecular changes with acclimation.

Animals in environments with high-temperature variability may have either plastic thermal limits, or a broad T_{br}-F that encompass their thermal environment (Calosi et al., 2008). Organisms that do not show plasticity are often those from thermally stable environments (Somero, 2010): such as the tropics (Tewksbury et al., 2008), and marine environments (e.g. Antarctic marine fish; Somero and DeVries, 1967) where their T_{br} are adequate to encompass their thermal environments. I speculate that I did not detect plasticity in the thermal tolerances of our high-latitude spiders either because they have: 1) broad T_{br}-Fs; 2) that they have seasonal plasticity requiring additional cues to trigger; or 3) they have plasticity, however the plasticity acts on their performance between thermal limits, and not the limits themselves.

I speculate that the acclimation regime I implemented on the high-latitude wolf spiders did not result in a change to their thermal limits because their T_{br}-F (c. 47 °C), is likely sufficient to survive their environment within summer (Convey et al., 2018; Huey and Hertz, 1984; Kingsolver, 2009). Thermal tolerance plasticity may still occur seasonally within these species, however with the addition of more reliable cues of seasonal change (Danks, 2006), such as changes to photoperiod, food quality and quantity, or fluctuating thermal regimes. Shortened photoperiod reliably indicates seasonal timing, regardless of uncommon temperature fluctuations within season, such as summer cold snaps and

winter thaws: low temperature and reduced photoperiod are both required to induce diapause in *Parasteatoda* (*Archaearanea*) *tepidariorum* (Tanaka, 1992). Reduced photoperiod and reduced and fluctuating temperatures are necessary to induce freeze-tolerance in the spring field cricket (McKinnon, 2015), and fluctuating temperatures, as occur in nature, can benefit species (reviewed by Colinet et al., 2015) by increasing thermal tolerance range (e.g., Oliver and Palumbi, 2011). Food quality and quantity induce overwintering physiology in various insects such as the increase in cold tolerance (e.g. Duman and Horwath, 1983), likely because it is a proxy for current abiotic conditions (e.g. light levels, temperature, water availability; Danks, 2007). However, the acclimation I imposed may have induced plasticity in our spiders that I did not measure, such as performance within the thermal limits. Within the thermal limits, performance – a proxy for fitness, and often measured as activity (Huey and Stevenson, 1979) – can also change in response to acclimation by reducing or increasing overall performance (Sinclair et al., 2012). Acclimation is expected to improve performance at the acclimation temperature, according to the Beneficial Acclimation Hypothesis (Leroi et al., 1994), and I would recommend measuring activity in the spiders at temperatures within their thermal limits.

In conclusion, a one-week acclimation to 4 °C did not improve low-temperature tolerance and instead increased low- and high-temperature tolerance in non-fed Greenland and fed Norway-collected *Pardosa*. I speculate that the changes in thermal tolerance (SCP and CT_{max}) may be an artefact of increased metabolic rates at higher temperatures which expedited gut-clearance in Greenland-collected spiders. I further speculate that I do not see thermal tolerance plasticity in the spiders because they either have a broad T_{br}-F, their acclimation results in effects that I did not measure, or because plasticity is triggered by other cues regardless of or in addition to, temperature change.

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Chapter 5

5 Overwintering red velvet mites are freeze tolerant

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5.1 Introduction

Winter temperatures drop below 0 °C in terrestrial temperate, polar, and alpine ecosystems. Arthropods in these habitats generally adopt one of two cold tolerance strategies: freeze avoidance, preventing ice formation by depressing the temperature at which they freeze (the supercooling point, SCP), or freeze tolerance, withstanding internal ice formation (Lee, 2010). Freeze-tolerant insects accumulate low molecular weight cryoprotectants, such as glycerol or proline (Lee, 1991). They have high SCPs that likely facilitate control of the site or rate of ice formation (Toxopeus and Sinclair, 2018). Finally, freeze tolerant insects sometimes produce ice-binding proteins that initiate freezing at high temperatures (ice nucleating proteins) or control the growth and distribution of ice crystals (thermal hysteresis and recrystallisation-inhibiting proteins; Zachariassen and Kristiansen, 2000).

Freeze tolerance appears to be a derived trait that has evolved multiple times in arthropods (Sinclair et al., 2003; Toxopeus and Sinclair, 2018), including many Insecta, Chilopoda, Crustacea, and in two Arachnids (both scorpions; Crawford and Riddle, 1975; Whitmore et al., 1985). Small arthropods contain a small volume of water and therefore supercool easily (Sinclair et al., 2003). This propensity to supercool probably predisposes them towards freeze avoidance (Cannon and Block, 1988). In particular, there has been extensive work on the cold tolerance of mites (Acari) and springtails (Collembola; collectively 'microarthropods') in the Antarctic and Arctic (as well as non-polar regions; Cannon and Block, 1988; Sjursen and Sømme, 2000), and thus far all have been freeze-

avoidant or chill-susceptible. This is despite many of those species having soft, permeable bodies and occupying the ice-rich microhabitats likely to promote inoculation by external ice and therefore freeze tolerance (Sinclair et al., 2003). Thus, the general conclusion is that – whether due to their small size or phylogenetic constraints – microarthropods do not evolve freeze tolerance (Cannon and Block, 1988; Sinclair et al., 2003; Toxopeus and Sinclair, 2018).

Here I report that overwintering red velvet mites are freeze tolerant, extending the taxonomic breadth of incidence of this strategy and upending the expectation that all microarthropods are freeze avoidant.

5.2 Methods

I hand-collected a total of 340 adult red velvet mites [*Allothrombium* sp. (Trombidiidae), voucher CNC871154, Canadian National Collection], mean ± SEM fresh mass 4.3 ± 0.2 mg, from the soil surface and leaves of *Cirsium arvense* at the Environmental Sciences Western farm in Ilderton, Ontario (43.1°N, 81.3°W) between March 2016 and May 2017. To access mites under snow in winter, I collected c.100 individuals in November 2016 and buried them in 'field cages' – 600 mL plastic containers containing 2 cm of soil and *C. arvense* leaves, such that the surface of the soil in the containers was level with the surrounding soil. After collecting them from the field or removing them from the field cages (the mites always remained on the surface of the soil), I housed mites in 600 mL containers at 4 °C (winter - January, February, March) or room temperature c. 21 °C (spring - April, May and autumn – September, November) for up to five days. I was unable to find mites between May and September, likely because juvenile trombindinid red velvet mites are ectoparasitic on other arthropods (Zhang, 1998). I determined the SCP, cold tolerance strategy, and low temperature at which 50% of individuals are killed (LLT_{50}) using methods outlined elsewhere (Sinclair et al., 2015).

I measured SCP in March, April, September, October, and November 2016, and January, February, March, and May 2017. I placed mites individually in 1.7mL microcentrifuge tubes in contact with a 36-AWG type-T thermocouple and recorded temperature at 2 Hz

via a TC-08 interface and PicoLog software (Pico Technology, Cambridge, UK). I placed tubes in an aluminium block cooled by fluid circulated from a Proline RP855 bath (Lauda, Würzburg, Germany; before July 2016) or by a custom-built Peltier-effect device. I cooled mites from 4 °C (winter) or 20 °C (spring and autumn) to 0°C at 1.0 °C·min^{-1}, and 0.5 °C·min^{-1} (or 0.25 °C·min^{-1} before July 2016) thereafter.

To determine cold tolerance strategy in March, September, October, and November 2016, and January 2017, I cooled groups of mites until half the individuals had frozen (i.e. produced exotherms), then removed all of them to 4 °C (winter) or ~20 °C (spring and autumn). After 24h, mites that were upright and moving were recorded as alive.

I estimated LLT$_{50}$ in March and November 2016, and January 2017 in groups of four mites exposed for 1 h to a range of temperatures below the SCP. I began cooling at 21 °C (for March measurements), 15 °C (November), or 4 °C (January; I based these temperatures on approximate maximum air temperatures for each month), and cooled and rewarmed at 0.25 °C·min^{-1} (March measurements), or to 0 °C at 1°C·min^{-1}, and 0.5 °C·min^{-1} thereafter, and rewarmed at 0.5 °C·min^{-1}. I assessed survival after 24 h at 20 °C (March, November) or 4 °C (January).

I examined survival after prolonged freezing at temperatures below the SCP (but that yielded 100 % survival for brief exposures) in April 2016 and March 2017. I cooled groups of 7-10 mites from 21 °C to -8.6 °C at 0.25 °C·min^{-1}, held them for 1, 8, and 24 h, rewarmed at 0.25 °C·min^{-1} and assessed survival (as above). In March 2017, I cooled three groups of four mites from 0 °C to -9.0 ± 0.7 °C at 0.5 °C·min^{-1}, and held one group for each of 1 h, 12 h, and one week, before rewarming at 0.5 °C·min^{-1}.

To measure hemolymph osmolality, I amputated the front right leg under immersion oil, extracted c. 20 nL of hemolymph, and determined osmolality and thermal hysteresis (Otago Osmometers, Dunedin, New Zealand) as previously described (Crosthwaite et al., 2011). I measured whole-body water content gravimetrically, as the difference between fresh mass and mass after drying to a constant mass at 60 °C (Sjursen and Sømme, 2000).

I rehydrated and crushed these mites in 0.05 % Tween 20, and measured glycerol concentration spectrophotometrically (details in Crosthwaite et al., 2011).

I compared SCP, osmolality, water content, and glycerol content using ANOVA in R (version 3.2.2). I calculated LLT_{50} from a generalised linear model in R and used non-overlapping 95 % confidence intervals to compare months.

5.3 Results

All mites collected between November and March survived internal ice formation; a smaller proportion survived freezing at other times (Table 5.1). SCP ranged from -3.9 °C (March 2016) to -9.2 °C (March 2017), and mean SCPs ranged from -6.2 ± 0.2 °C in March 2016 to -8.4 ± 0.2 °C in January 2017 (Figure 5.1A). SCP differed significantly among sampling points (Figure 5.1A), but not in a manner that was associated with freeze tolerant mites having consistently higher or lower SCPs than their freeze intolerant counterparts. LLT_{50} (± 95 % CI) was lower in January (-20.0 ± 2.7 °C), than in March (-7.4 ± 3.2) or November (-12.1 ± 1.8 °C; Figure 5.1B). All mites in April and March survived being frozen at -8.6 °C or -9.0 ± 0.7 °C for 24 h, and 8/12 of March-collected mites survived frozen for one week at -9.0 °C.

Table 5.1 Freeze tolerance in *Allothrombium* sp. I cooled mites until c. 50% froze, then removed them from the cold for recovery.

	Frozen		Unfrozen	
Date collected	No. alive	No. dead	No. alive	No. dead
March 2016	9	0	8	1
September 2016	5	3	8	0
October 2016	2	2	4	0
November 2016	8	0	8	0
January 2017	7	0	3	0
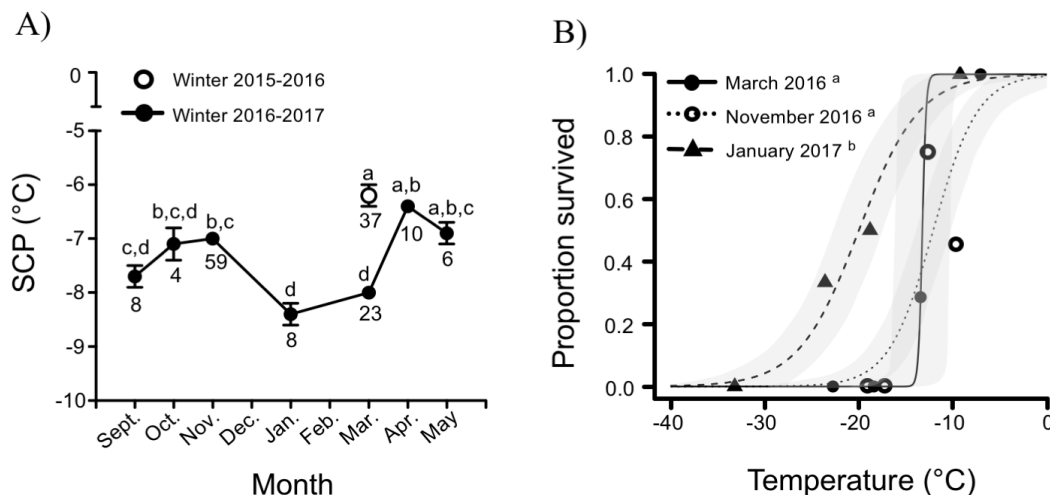
Figure 5.1 Cold tolerance of field-collected red velvet mites, *Allothrombium* sp. (A) Mean ± SEM supercooling point (SCP; numbers indicate sample size; different letters indicate points that are significantly different, $F_{7,147}= 22.07$, $p<0.01$). (B) Survival after 1 h cold exposure; curves are the result of a generalised linear model with 95% confidence intervals shown in grey. Note that cooling rates in the 2015-2016 winter were $0.25°C·min^{-1}$, but $0.5°C·min^{-1}$ in the 2016-17 winter for both datasets.

Hemolymph osmolality ranged from 462 (March 2017) to 1997 mOsm (February 2017; Figure 5.2A). Mean osmolality was highest in mites collected in January and February (Figure 5.2A; Table 5.2). Water content was significantly lower in February 2017 than at other times of the year, except November 2016 (Figure 5.2A). I did not observe thermal hysteresis in any hemolymph sample and saw no spicular ice crystal growth suggestive of ice-binding activity (Crosthwaite et al., 2011). Glycerol concentration was highest in midwinter (Figure 5.2B).
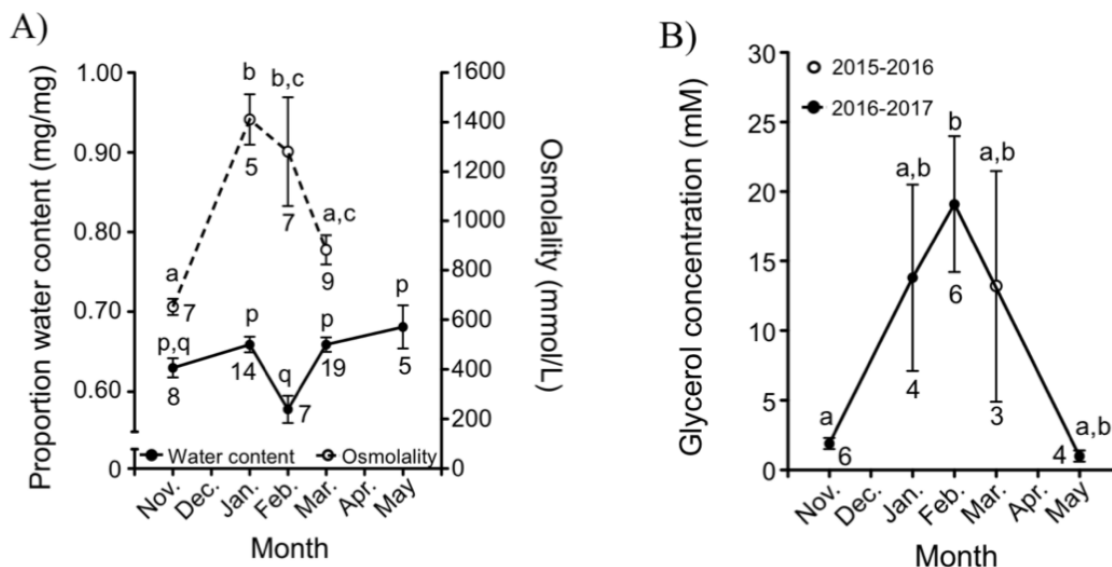
Figure 5.2 Hemolymph composition of overwintering red velvet mites,
***Allothrombium* sp.** (A) Water content (mg water/mg of wet mass), osmolality; (B)
glycerol concentration. Different letters indicate points that are significantly different
(Water content: $F_{4,48}$=6.33, p<0.01; Osmolality: $F_{3,24}$=21.88, p<0.001; [glycerol]:
$F_{4,21}$=3.52, P=0.03), numbers indicate sample sizes; mean ±SEM shown throughout.

5.4 Discussion

To my knowledge, this is the first report of freeze tolerance in a microarthropod. Not all
individuals survive internal ice formation in autumn, but by midwinter (January and
February), *Allothrombium* sp. can survive at least one week in a frozen state.

The supercooling point was lowest in midwinter, but there did not appear to be a strong
association between SCP and cold tolerance, unlike in many freeze-tolerant insects, and
the total range of mean SCP is only c. 2 °C in *Allothrombium* sp., compared to seasonal
shifts of 10 °C or more in other freeze tolerant species (Duman, 2001). The supercooling
point was c. 1.5 °C higher in March 2016 than in March 2017 (Figure 5.1A). I identify
two possible explanations for this that may not simply be due to inter-annual variation.
First, the March 2016 sample was collected on March 26th, much later in the month than
the March 2017 sample (collected on March 6th); the 2016 sample may perhaps be better
reflective of SCP in the spring (I note it does not differ from the April and May

timepoints). Second, the March and April 2016 SCPs were measured by cooling the mites at 0.25 °C·min^{-1}, whereas the 2017 mites were cooled at 0.5 °C·min^{-1}. Slower cooling rates do lead to higher SCPs (Salt, 1966), so this could explain the discrepancy, although the likely presence of ice nucleating agents would be expected to at least partially mitigate this effect. Either way, a difference in mean SCP of a few °C is unlikely to be biologically significant and reflects the overall small range of SCPs in adult *Allothrombium* sp. and suggests selection for a consistently high supercooling point.

Allothrombium sp. is a large mite (fresh mass 4.3 ± 0.2 mg in the animals in my study), about twenty times the size of the c. 0.2 mg *Alaskozetes antarcticus* (Block, 1977). This relatively large size does not explain the high SCP nor imply an inability to depress the SCP in the winter; non-cold-hardy insects of comparable size to *Allothrombium* sp., such as *Drosophila*, have SCPs below -15 °C without special adaptations (Strachan et al., 2011), and much larger insects can maintain very low SCPs (e.g. the 70-100 mg emerald ash borer *Agrilus planipennis* has a mean SCP below -30 °C in winter; Crosthwaite et al., 2011). SCP depression may not be possible for soft-bodied species that (like *Allothrombium* sp.) are routinely exposed to ice nucleators from the habitat. I speculate that this high probability of freezing (alongside year-round activity) could have favoured the evolution of freeze tolerance in this species, as has been postulated for freeze tolerance more generally (Toxopeus and Sinclair, 2018). Interestingly, these mites appear to have adopted a divergent strategy to the cryoprotective dehydration used by Collembola in similar habitats to avoid freezing (Sorensen and Holmstrup, 2011).

Hemolymph osmolality was highest in midwinter. Decreased water content accounts for c. 215 mOsm of that increase, with another 15 mOsm from increased glycerol concentration. Glycerol has been reported as a cryoprotectant in other arachnids (Aitchison and Hegdekar, 1982; Kirchner and Kestler, 1969; Young and Block, 1980) and is thought to enhance freeze tolerance by stabilising macromolecules and reducing ice content and minimum cell volume (Lee, 2010). Although my small sample sizes (and consequently high variance) mean that I probably lack statistical power to detect small differences in glycerol concentration, the magnitude of the change from summer to winter (~15 mM) is substantially lower than the large changes observed in other mites;

for example, the freeze-avoidant *A. antarcticus* accumulates c. 0.5 M glycerol (Young and Block, 1980). Approximately 530 mOsm remain unaccounted for; I hypothesise that other osmotically-active agents (such as other polyols or amino acids, see Toxopeus and Sinclair, 2018) that may contribute to the increase of hemolymph osmolality could also act as cryoprotectants. Possibly, the small decrease in SCP in midwinter results from this increased osmolality.

In arachnids, freeze tolerance has evolved in two desert scorpions (Crawford and Riddle, 1975; Whitmore et al., 1985), but not in any other mites as far as I am aware (Cannon and Block, 1988). Winter temperatures in southwestern Ontario can be highly variable (see, e.g., Marshall and Sinclair, 2012), and I suggest that this thermal variability coupled with winter activity (which likely precludes the accumulation of very high cryoprotectant concentrations), and extensive environmental moisture (promoting inoculative freezing) has favoured freeze tolerance in this species. Thus, I show that neither small size nor a phylogenetic tendency towards freeze avoidance in mites prevents them from evolving freeze tolerance, and I speculate that other mites in similar circumstances may also be freeze tolerant.

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Chapter 6

6 General Discussion

Although arachnids are globally distributed, highly speciose, and ecologically important, they are under-represented in thermal physiological research. Thermal physiology has focused on insects (e.g. Denlinger and Lee, 2010) and vertebrates (e.g. Huey and Stevenson, 1979) and in global macrophysiological studies, there are no high-latitude terrestrial ectotherms, and arachnids are omitted (Bennett et al., 2018; Sunday et al., 2011; Žagar et al., 2018). The purpose of my thesis was to expand upon the few thermal tolerance investigations in high-latitude arachnids (e.g. the Antarctic mite *Alaskozetes antarcticus*; Convey, 1994) with the pseudoscorpion *Wyochernes asiaticus* (Chapter 2) and the wolf spiders of the genus *Pardosa* (Chapter 3), identify whether the thermal tolerances are plastic (Chapter 4), and measure seasonal changes and describe freeze-tolerance in a temperate arachnid (Chapter 5).

6.1 Contributions to the Field

My thesis provides information about the low- and high- temperature tolerances for sub-Arctic and Arctic pseudoscorpions (Chapter 2), and spiders (Chapter 3), and the plasticity of those thermal tolerances in the high-latitude spiders (Chapter 4) and temperate mite (Chapter 5). At high latitudes, ground temperatures are low, especially in the winter (e.g. -38.2 C in the Antarctic polar desert; Convey et al., 2018). However, I have found that the spiders and the pseudoscorpion have poor low-temperature tolerance for species that live at high-latitudes (Chapters 2 & 3). I therefore predicted that these arachnids would have thermal tolerance plasticity, which I tested in summer-collected *Pardosa* spiders from the Yukon, Greenland, and Norway. A one-week acclimation altered the thermal tolerances of some *Pardosa* species: both low- and high- temperature tolerance decreased in the Greenland-collected spiders acclimated at 20 °C, compared to those cold-acclimated (4 °C; Chapter 4). I concluded that the small changes I saw in both high- and low-temperature tolerance were a product of desiccation and clearance of gut contents in the warm-acclimated group. My research provides evidence that thermal tolerance in

high-latitude *Pardosa* spp. does not change in response to temperature acclimation. However, my results do agree with the theory that species that live in highly thermally variable environments (such as in high latitudes; Convey et al., 2018) have been selected for large thermal breadths (T_{br}) or eurythermy (Sunday et al., 2011), rather than plasticity (Moore, 1940; Pörtner, 2002; Pörtner et al., 2000).

To explore changes in thermal tolerance associated with longer time scales such as season, I found a tractable temperate arachnid species that I could access throughout the winter. I collected the temperate red velvet mite *Allothrombium* sp. in the autumn and contained them in a field-cages for winter retrieval. The mite is therefore likely acclimatized to the large seasonal temperature, moisture, and insolation conditions. I show that this mite seasonally switches from freeze-avoidant to freeze-tolerant and decreases its LLT_{50}, concomitant with increases in hemolymph osmolality and glycerol, and a decrease in water content (Chapter 5). Glycerol is found in other freeze-tolerant arthropods and identified as a possible cryoprotectant (Lee et al., 1993), and there is correlative evidence that it does the same in this arachnid (Chapter 5), and in the spider *Parasteatoda tepidariorum* (Tanaka, 1995). This species provided me with measurable changes in response to seasonal acclimatisation.

6.1.1   Implications

Research into thermal physiology is especially important now because the thermal environment is being affected by climate change: average temperatures are increasing, so are extreme weather events; further, there are expected shifts in hydroclimate (IPCC, 2014; Somero, 2010). High latitudes are predicted to be especially affected by climate change (Post et al., 2009), with an average temperature increase of 10 °C in the next 100 years (IPCC, 2014). It is now therefore important to get baseline thermal biology measurements of organisms that will be affected by climate change, to allow monitoring of physiology, and understand the drivers of changes in species distributions and ecosystem function.

Extreme weather events can result in early frosts and early snowmelts. Early cold snaps can catch species physiologically or behaviourally unprepared for low temperatures. As

this thesis shows, the summer-collected, high-latitude *W. asiaticus* and *Pardosa* spp. have poor low-temperature tolerance (Chapters 2 and 3) and could be physiologically unprepared by these cold snaps. Even after low-temperature acclimation, low-temperature tolerance does not decrease in *Pardosa* spp. (Chapter 4). Therefore, *Pardosa* spp., and *W. asiaticus* may be vulnerable to extreme cold events during summer or autumn.

Climate change is also predicted to change precipitation and hydrology (IPCC, 2014). Streamside-dwelling *W. asiaticus* can survive total submergence of water for the expected high-water timing of their habitat (50% survival after 17 days, stream runoff c. 14 days Environment Canada: www.wateroffice.ec.gc.ca, station 09FB002; Chapter 2). The potential water runoff increase, combined with the increased metabolism that accompanies higher temperatures, could reduce its survival. Therefore, my results of thermal and submergence tolerance can be used to infer the effects of temperature and precipitation changes on the survival of *W. asiaticus*.

Climate change may not directly affect survival in ectotherms such as arachnids, but may alter species distributions (see Pearson and Dawson, 2003), fecundity (such as a reduction in fecundity in wasps at higher winter temperatures; Williams et al., 2003), and multitrophic ecosystem phenologies (e.g. Høye et al., 2014; Post and Forchhammer, 2008). Further, the female *Pardosa glacialis*, a study species of this thesis, is increasing in size which is correlated with earlier spring snowmelt; however, the males are not increasing in size at the same rate, leading to an increase in sexual size dimorphism within this species (Høye et al., 2009). Although the previous study does not conclude any negative impact of the increased sexual size dimorphism, it is important to note that the downstream ecological effects of climate change on high latitude ecosystems are as yet unpredictable.

6.2   Overwintering and Cold Tolerance in Arachnids: Future Research

Although the high-latitude spiders I studied did not show the expected effect of acclimation, their low-temperature tolerance is not low enough to survive winter at their latitudes. How do high-latitude arachnids survive winter, and will alterations in normal thermal regimes as a result of climate change impact their ability to survive winter? Winter field collections and/or more extensive laboratory acclimation are required to better understand the overwintering thermal biology of these organisms. With these winter-acclimatised arachnids, mechanisms underlying overwintering success in arachnids can be investigated.

6.3   How to Study Overwintering Arachnids

There are many challenges with research into overwintering animals, such as low temperatures and short daylength. However, I present some suggestions for investigating thermal biology in overwintering arachnids and inducing winter-like conditions in the lab.

Winter-inactive arachnids with easy-to-access winter hibernacula would be most appropriate for winter studies, because individuals could be retrieved at different points throughout the winter for thermal physiology measurements. Winter-inactive arachnids are also amenable to field cages, if the overwintering habitat is known, including appropriate thermal buffering to prevent death from low temperatures and avoid depleting energetic reserves if too warm (Sinclair et al., 2003). The animals can be trapped in autumn and retrieved at will over winter. This method also allows you to bury the appropriate number of individuals required for winter retrieval: in wild-collected arachnids, you may not find the number you require. Examples of arachnids amenable to field collection followed by field cage housing include the red velvet mite *Allothrombium* sp. from Chapter 5 and the spider *Phiddipus audax* (Salticidae; Bower and Snetsinger, 1985) from the NE United States, which overwinter in tree bark.

Winter-active spiders may be live-collected by pitfall trapping if subnivean. Although usual pitfall traps use ethylene glycol or other preservative to kill and preserve organisms that fall in (e.g. Aitchison, 1984) and need only weekly monitoring, live pitfall traps require constant monitoring because when an organism is trapped it may risk freezing or become prey to other winter-active organisms (e.g. arthropod-eating shrews; Aitchison, 1987). Housing winter-active arachnids in field-cages for collection through the winter is also a possibility, however this requires information about their overwintering environment and ecology. If the species is a predator, will they continue to eat over the winter? If so, the prey items will need to be added. If they behaviourally thermoregulate, the field cage will have to provide the appropriate thermal buffering areas. Other winter-active spiders that live on the snow or in snow-free areas can be collected by hand using the reflective properties of their eyes. A flashlight shining along the ground on a dark night will reflect back to the collector, allowing for hand-collecting (Whitney et al., 2014). This collection method can be used to collect large numbers of wolf spiders for studies of thermal biology.

Alternatively, arachnids can be collected in the summer or autumn, and winter field conditions can be induced in the lab. Prior to this, you would have to know the overwintering activity, habitat, and ecology of the species, and further knowledge of the cues that trigger overwintering physiology, which can be gained through laboratory experiments. This would require field research on the species in question, or extensive trial-and-error attempts to induce overwintering conditions, as well as wild-caught overwintering individuals to compare the lab-induced overwintering individuals (e.g. *Gryllus veletis*; McKinnon, 2015). The red velvet mite *Allothrombium* sp. is an example of an arachnid that I have shown to be amenable to field cages and winter retrieval (Chapter 3).

6.3.1 Directions to Identifying Mechanisms Related to Overwintering Success in Arachnids

In Chapter 5, I described the low-temperature tolerance and associated physiological changes in the red velvet mite. However, there are still more areas of investigation in this

freeze-tolerant mite, as well as other overwintering arachnids. With the increased use of transcriptomics and metabolomics, and the addition of chelicerates and arachnids in the genome and transcriptome libraries, seasonal changes at the transcriptome-level within a model arachnid could be identified. An example of such a comparison exists for different stages of chilling in the cricket *Gryllus pennsylvanicus* (Des Marteaux et al., 2017) and in the freeze-tolerant *Gryllus veletis* (Toxopeus et al., 2018). This avenue of research can also be used to compare the mechanisms of cold tolerance between arachnids and insects. Arachnids and insects share a lot of similarities, including the production of known cryoprotectants (i.e. glycerol, inositol, trehalose; Sjursen and Sinclair, 2002; Tanaka, 1995) (Chapter 5). However, the cryoprotectant molecules identified in arachnids were likely targeted searches, having been previously identified in insects. This leaves room for exploration into cryoprotectant molecules unique to arachnids.

Another avenue of research is into the imbalance of ions that are known to occur within the gut and hemolymph of chilled insects (Overgaard and MacMillan, 2017). Insects lose ion and water homeostasis, that with prolonged cooling can lead to chilling injury and death. However, the mechanisms resulting in chill coma are unexplored in arachnids. Chilled arachnids, such as *Wyochernes asiaticus* (Chapter 2), enter a state of chill coma similar to *Drosophila* and *Gryllus pennsylvanicus* (MacMillan and Sinclair, 2011). To test whether spiders also lose ion balance in arachnids, the crop pest *Tetranychus urticae* which can be purchased from a lab supplier, is easy to rear in the lab, and enters a reversible state of chill-coma at 10.3 °C (Coombs and Bale, 2014). However, its small size may make hemolymph extraction difficult. The large arachnid *Mastigoproctus giganteus* (Thelyphonidae) has a CT_{min} of between 4.7 and 7.5 °C and therefore hemolymph can be easily extracted, and there are rearing protocols in place (Punzo and Olsen, 2005)

6.4 Conclusion

The natural history and thermal biology I described in this thesis provide useful baseline measurements of important high latitude predators. There is much more to know about the thermal biology of temperate, sub-Arctic, and Arctic arachnids. I now know that

summer-collected arachnids are not adequately cold tolerant to survive winter and therefore must have adaptations that allow winter survival; and that at least one species (the red velvet mite) has hemolymph changes that correlate with increased cold-tolerance and switching to freeze tolerance. I have also discovered that there is no plasticity to the thermal tolerances on Arctic and sub-Arctic wolf spiders (*Pardosa* spp.) in response to temperature acclimation alone. This lack of short-term plasticity could be detrimental for these spiders if encountering early or late extreme warm or cold snaps, a potential reality with climate change. It is my hope that this research will inspire exciting new research in thermal physiology of arachnids from all latitudes, and the discovery of new mechanisms that lead to changes in such tolerances in arachnids.

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Appendices

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LICENSE

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Dr. Susan Anthony (University of Western Ontario)

to enter the Yukon Territory to conduct scientific research with respect to:

Cold Tolerance of the Wolf Spider (*Pardosa moesta*).

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1. A complete, final report of the research conducted under this license shall be submitted, in duplicate, within one year of completion or termination of the project.
   a) A field or progress report, including descriptions or catalogues of collections made (where applicable) shall be submitted in duplicate on, or before, the expiry date written below.
   b) The Licensee shall provide a copy of any report or article published on the research conducted under this license to Heritage Resources Unit.

2. All camps shall be established according to the provisions of the Territorial Land Use Regulations.

3. All steps shall be taken to avoid unnecessary disturbance of wildlife.
   a) No camp site shall be established within 2 km of an active raptor nest.
   b) When using aircraft, maintain a minimum of 1,000 feet over wildlife such as sheep, raptor nests and migrating caribou.
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5. The Licensee shall strictly observe all applicable Territorial and Federal legislation and regulations.

OTHER CONDITIONS:

1. Acquisition of Park Use Permit for any research in a Tombstone Territorial Park.

THIS License is valid for the period **June 28th** to **July 21st** , 2015.

DATED at the City of Whitehorse, in the Yukon Territory, this 27th day of March, A.D., 2015.

Manager, Heritage Resources Unit
Cultural Services Branch
Tourism and Culture

Appendix B The Government of Greenland Survey License.

Inuussutissarsiornermut, Suliffeqarnermut Niuernermullu Naalakkersuisoqarfik

Ministry of Industry, Labour and Trade

NAALAKKERSUISUT
GOVERNMENT OF GREENLAND

Survey Licence
- without allowance for commercial utilisation

Date: 16-02-2016

Survey Licence number: G16-042

This survey licence is issued under authority of the Government of Greenland Act no. 20 of 20 November 2006 on the use of biological resources for commercial and research purposes (in the following: Act on Biological Resources).

This licence has been assigned the reference number **G16-042** which must all ways be cited in communication with authorities.

Title of the Project: Cold tolerance of Greenland spiders

Principal Research Leader: Brent Sinclair

Period of Research Project: June 15 – December 2016
(The period in which the survey licence is valid)

Concerning collection of biological material
The Government of Greenland will notify the local police district of where the survey is to take place, when it is expected to begin, how long it is expected to last and when it is expected to end.

Geographical Area for Fieldwork:
Narsarsuaq, Greenland, within the local community and in surrounding hills. 61.2° N, 45.4°W

Type of Biological Material:
Wolf spiders, *Pardosa lapponica, P. glacialis, P. hyperborea, P. groenlandica*, size: up to 1cm, collection amount: 500 each
Opilionid, daddy long-legs, *Mitopus morio*, body size: c. 5mm, collection amount: 500

Dates of Field Periods: June 15 – August 15

Source/ Institute: University of Western Ontario

Terms and conditions

1. Legislation
This survey licence is issued under authority of the Act on Biological Resources, and the regulations on commercial utilisation of genetic resources in the Biodiversity Convention.
All surveys must be carried out taking due consideration to Greenland's rights to its biological resources. Researchers must comply with the Greenlandic legislation.

2. Expiration of the survey licence
The survey licence must cover the period in which research is carried out on collected and/or acquired biological resources.
The licences are issued with a time limit. The expiration date of the survey licence is stated on the survey licence. It is possible to apply for an extension of the survey licence by sending an application to the Ministry of Industry, Labour and Trade. Use the form for "application for survey licence for collection and/or acquisition of biological resources for research purposes".

3. Liability
The holder of the licence is responsible for ensuring that anyone involved in the survey complies with the terms and conditions of the survey licence, the Act on Biological Resources and any other relevant legislation.

4. Other licences
The holder of the licence must have obtained all relevant licences and authorisations in connection with the survey.

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Any kind of transfer to or communication to a third party of the survey licence, all or parts of the survey materials or survey results must be approved in advance by the Government of Greenland. "Third party" includes university groups, private individuals and enterprises, etc. that are affiliated with the holder of the survey licence. Everyone involved in the survey must hold a copy of the survey licence while carrying out work related to the survey.

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2) collection location or
3) type of biological resources.

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8. Duty of information

Publication and other disclosure of results must be effected with due respect for Greenland's rights to biological resources and in accordance with the provisions of the Convention on Biological Diversity. A copy of publications concerning Greenland biological resources must be submitted to the Government of Greenland before the publication is published. Publications must state that the material is from Greenland.

9. Patenting

The duty of information also entails that the holders of the licence report whether they are applying for a patent or any other form of protection of the survey results, and inventions of parts of these that are directly or indirectly based on biological resources collected and/or acquired in Greenland. This is to be reported by submitting a copy of the patent application as well as stating which legal person will hold the rights. This copy is to be submitted to the Ministry of Industry, Labour and Trade before the application is submitted. Patenting itself does not require a commercial licence. However, commercial utilisation of a patent based on biological resources acquired and/or collected in Greenland requires a commercial licence.

10. Commercial utilisation

The survey licence does not allow for commercial utilisation. Commercial utilisation of collected and/or acquired biological resources (including, but not limited to organisms, enzymes, genetic material, seeds and other biotic material) or results hereof, require a commercial licence from the Government of Greenland. If the holder of the survey licence wishes to utilise commercially a patent that has been achieved based on results generated in the survey (via e.g. sub-licencing or sale of intellectual property rights to a third party), a commercial licence is also needed.

11. Transferral of rights

In the event that the holder of the licence does not wish to utilise commercially the knowledge obtained on the basis of the survey licence, the rights to this knowledge can be transferred to the Government of Greenland. The holder of the licence must report this upon completion of the project

12. Revocation

In the event of violation of the terms and agreements, the Government of Greenland may revoke the survey licence, cf. section 13 of the Act on Biological Resources.

Concerning Field Work

13. Tagging and field equipment

The holder of the licence must remove all tagging and equipment from the field when the survey is complete, or while the survey licence is still valid. The Government of Greenland reserves the right to insert more restrictive terms concerning this in the terms and conditions of the survey licence.

14. Local commercial activity

The survey must be carried out with respect for local commercial activity such as mining, hydro-power activities, hunting, fishing, agriculture, tourism etc.

15. Environmental sustainability

Collection of biological resources must be done in a sustainable manner and in a way that protects the environment and nature. Collection methods may not cause unnecessary damage or destruction, or impact the environment and other resources, e.g. historic sites.

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Curriculum Vitae

Name: Susan Anthony

Post-secondary Education and Degrees:
The University of Western Ontario
London, Ontario, Canada
2014-2019 Ph.D. Biology

University of Alberta
Edmonton, Alberta, Canada
2011-2014 M.Sc. Biological Sciences

University of Victoria
Victoria, British Columbia, Canada
2009-2012 B.Sc. Biology

Honours and Awards:
University of Western Ontario J. D. Detwiler Alumni Award, 2017
Natural Sciences and Research Council of Canada CGS-D, 2016-2018
Queen Elizabeth II Scholarship (declined), 2016
Natural Sciences and Research Council of Canada CGS-M, 2013-2014
Bamfield Marine Sciences Centre John Boom Memorial Scholarship, 2013
Queen Elizabeth II Scholarship, 2013
Bamfield Marine Sciences Centre Undergraduate Scholarship, 2011
UVic University Transfer Scholarship, 2009
Tony Pletcher Memorial Scholarship, 2009

Related Work Experience:
Course Co-instructor
Ontario Universities Field Courses
2017, 2018

Teaching Assistant
University of Western Ontario
2014, 2015, 2016

Teaching Assistant
Bamfield Marine Sciences Centre
2012, 2013

Teaching Assistant
University of Alberta
2012, 2013, 2014

Publications:

Anthony, S.E. & Sinclair, B.J. (2019) Overwintering red velvet mites are freeze-tolerant. Physiological and Biochemical Zoology 92(2): 201 – 205.

Anthony, S.E., Buddle, C.M. & Sinclair, B.J. (2016) Thermal biology and immersion tolerance of the Beringian pseudoscorpion *Wyochernes asiaticus*. Polar Biology 39: 1351 – 1355.