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SHORT NOTE

Thermal Biology and immersion tolerance of the Beringian pseudoscorpion *Wyochernes asiaticus*

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Abstract

Wyochernes asiaticus (Arachnida: Pseudoscorpiones: Chernetidae) is a pseudoscorpion distributed across Beringia, the areas of Yukon, Alaska and Siberia that remained unglaciated at the last glacial maximum. Along with low temperatures, its streamside habitat suggests that submergence during flood events is an important physiological challenge for this species. We collected *W. asiaticus* in midsummer from 66.8°N Yukon Territory, Canada, and measured thermal and immersion tolerance. *Wyochernes asiaticus* is freeze avoidant, with a mean supercooling point of -6.9 °C. It remains active at low temperatures (mean critical thermal minimum, CT_{min}, is -3.6 °C), and has a critical thermal maximum (CT_{max}) of 37.8 °C, which is lower than other arachnids, and consistent with its restriction to high latitudes. Fifty percent of *W. asiaticus* individuals survived immersion in oxygen-depleted water for 17 days, suggesting that this species has high tolerance to immersion during flooding events. To our knowledge, these are the first data on the environmental physiology of any pseudoscorpion, and a new addition to our understanding of the biology of polar microarthropods.

Keywords: Pseudoscorpion, microarthropod, cold tolerance, critical thermal limits, immersion

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. 2008). 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 our 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) Holarctic 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, we measured the critical thermal limits and supercooling points of adult and sub-adult *W. asiaticus* shortly after mid-summer collections. We also measured immersion tolerance to explore the capacity of this species to withstand submergence during seasonal flooding events. To our 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.

Methods

We 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. We 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 nine days elapsed between collection and use in experiments. During this period, females which had been carrying egg sacs dropped them, but only five of 200 animals died during transport, and no controls died during the experiments.

Thermal Biology

We measured critical thermal minima (CT_{min}) and maxima (CT_{max}) using an approach similar to that described by Sinclair et al. (2006). Briefly, we 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. We observed them using a dissecting microscope during cooling or heating. For CT_{min}, we cooled the pseudoscorpions from 12 °C at 0.25 °C min^{-1}, periodically poked them with a fine paintbrush; we 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$_{max}$, we heated the pseudoscorpions at 0.25 °C min^{-1} from 12 °C; we defined the CTmax as the temperature where they jerked briefly and no longer responded to stimulus from the paintbrush. We report mean ± SEM for CT$_{min}$ and CT$_{max}$.

To measure the supercooling point (SCP), we 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). We recorded the temperature every 0.5 s using Picolog software (v 5.24.2 Picotech). We 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). We 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, we placed ten pseudoscorpions in pipette tips in contact with thermocouples in a cooled aluminium block, as described above. We cooled them from 12 °C at 0.1 °C min^{-1} until five of the ten pseudoscorpions had frozen. At this point, we 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, we would define that as chill susceptibility, if only individuals that froze died, we would define that as freeze avoidance, while if individuals that froze survived, we would define that as freeze tolerance.

Immersion tolerance

To explore the ability of *W. asiaticus* to survive long periods immersed, we 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 one 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. We 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, we 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), we 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 14 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 1 week, and every 1-3 days thereafter until 50 % of the immersed animals had died (no sign of movement following agitation of the tube).

Results & 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 we 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 for survival of 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, we 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 our 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, 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} we observed is consistent with low temperature activity (at least extending the active season), we 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. We do note that the CT_{max} of *W. asiaticus* we 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 (S.E. Anthony, unpublished observations). 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 our first experiment, we observed no mortality in control animals, and survival of 9/10 individuals held submerged. We 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; we 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), we repeated this experiment with deoxygenated water. After one week, mortality was the same as in oxygenated water (no mortality in control, 1/10 in submerged), but the time to 50 % mortality was seventeen days for both treatment and control, suggesting that factors other than immersion were responsible for mortality. Historical river flow data from Eagle Creek (2.5°S of our collections) suggest that flood events in this part of Yukon Territory generally last 2-7 days, with occasional high discharges persisting for 10 days (Environment Canada: www.wateroffice.ec.gc.ca, station 09FB002).

During the first immersion experiment, we 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 to 8.2 %) of their body water over this time, the surviving immersed individuals gained 6.2 ± 1.3 % (range: 1.8 to 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 we observed was a function of rehydration by drinking. However, the submerged individuals did appear engorged (S.E. Anthony pers. Observations), 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, we hypothesise that mortality of the control and immersed individuals in our 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 we predict it will show significant seasonal plasticity in cold hardiness. It can easily withstand immersion for one week, and does not appear to be reliant 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.

References

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

Aitchison CW (1979) Low temperature activity of pseudoscorpions and phalangids in southern Manitoba. J Arachnol. 7:85-86

Bale JS, Hodkinson ID, Block W, Webb NR, Coulson SC, Strathdee AT (1997) Life strategies of Arctic terrestrial arthropods. In: Woodin SJ, Marquiss M (eds). British Ecological Society Special Publication No. 13, pp 137-165

Barnes BM, Barger JL, Seares J, Tacquard PC, Zuercher GL (1996) Overwintering in Yellowjacket queens *(Vespula vulgaris)* and green stinkbugs *(Elasmostethus interstinctus)* in subarctic Alaska. Physiol Zool. 69:1469-1480

Block W (1994) Terrestrial Ecosystems: Antarctica. Polar Biol. 14:293-300

Buddle CM (2015) Life history and distribution of the Arctic pseudoscorpion, *Wyochernes asiaticus* (Chernetidae). Can Field Nat. 129:134-138

Burmester T (2004) Evolutionary history and diversity of arthropod hemocyanins. Micron. 35:121-122

Cannon RJC, Block W (1988) Cold tolerance of microarthropods. Biol Rev. 63:23-77

Convey P (2011) Antarctic terrestrial biodiversity in a changing world. Polar Biol. 34:1629-1641

Convey P, Stevens MI (2007) Antarctic biodiversity. Science. 317:1877-1878

Coulson SJ, Hodkinson ID, Block W, Webb NR, Worland MR (1995) Low summer temperatures: a potential mortality factor for high arctic soil microarthropods? J Insect Physiol. 41:783-792

Coulson SJ, Hodkinson ID, Webb NR, Harrison JA (2002) Survival of terrestrial soil-dwelling arthropods on and in seawater: implications for trans-oceanic dispersal. Funct Ecol. 16:353-356

Coulson SJ, Leinaas HP, Ims RA, Søvik G (2000) Experimental manipulation of the winter surface ice layer: the effects on a High Arctic soil microarthropod community. Ecography. 23:299-306

Everatt MJ, Bale JS, Convey P, Worland MR, Hayward SAL (2013) The effect of acclimation temperature on thermal activity thresholds in polar terrestrial invertebrates. J Insect Physiol. 59:1057-1064

Hawes TC, Worland MR, Bale JS, Convey P (2008) Rafting in Antarctic Collembola. J Zool. 274:44-50

Hertzberg K, Leinaas HP (1998) Drought stress as a mortality factor in two pairs of sympatric species of Collembola at Spitsbergen, Svalbard. Polar Biol. 19:302-306

Hodkinson ID, Coulson SJ (2004) Are high Arctic terrestrial food chains really that simple? The Bear Island food web revisited. Oikos. 106:427-431

Hodkinson ID, Coulson SJ, Webb NR, Block W (1996) Can high Arctic soil microarthropods survive elevated summer temperatures? Funct Ecol. 10:314-321

Hodkinson ID, Webb NR, Bale JS, Block W, Coulson SJ, Strathdee AT (1998) Global change and Arctic ecosystems: Conclusions and predictions from experiments with terrestrial invertebrates on Spitsbergen. Arct Alp Res. 30:306-313

Holmstrup M, Bayley M, Ramløv H (2002) Supercool or dehydrate? An experimental analysis of overwintering strategies in small permeable Arctic invertebrates. Proc Natl Acad Sci USA. 99:5716-5720

Holmstrup M, Sømme L (1998) Dehydration and cold hardiness in the Arctic collembolan *Onychiurus arcticus* Tullberg 1876. J Comp Physiol B. 168:197-203

Koponen S (1994) Ground-living spiders, opilionids, and pseudoscorpions of peatlands in Quebec. Mem Entomol Soc Can:41-60

Koponen S, Sharkey MJ (1988) Northern records of *Microbisium brunneum* (Pseudoscorpionida, Neobisiidae) form Eastren Canada. J Arachnol. 16:388-390

Lee RE (2010) A primer on insect cold tolerance. In: Denlinger DL, Lee RE (eds) Low Temperature Biology of Insects. Cambridge University Press, Cambridge, pp 3-34

Lopez-Martinez G, Benoit JB, Rinehart JP, Elnitsky MA, Lee RE, Denlinger DL (2009) Dehydration, rehydration, and overhydration alter patterns of gene expression in the Antarctic midge, *Belgica antarctica.* J Comp Physiol B. 179:481-491

Muchmore WB (1990) A pseudoscorpion from Arctic Canada (Pseudoscorpionida, Chernetidae). Can J Zool. 68:389-390

Seymour RS, Matthews PGD (2013) Physical gills in diving insects and spiders: theory and experiment. J Exp Biol. 216:164-170

Sinclair BJ, Coello Alvarado LE, Ferguson LV (2015) An invitation to measure insect cold tolerance: Methods, approaches, and workflow. J Therm Biol. 53:180-197

Sinclair BJ, Klok CJ, Scott MB, Terblanche JS, Chown SL (2003) Diurnal variation in supercooling points of three species of Collembola from Cape Hallett, Antarctica. J Insect Physiol. 49:1049-1061

Sinclair BJ, Terblanche JS, Scott MB, Blatch G, Klok CJ, Chown SL (2006) Environmental physiology of three species of Collembola at Cape Hallett, North Victoria Land, Antarctica. J Insect Physiol. 52:29-50

Slabber S, Worland MR, Leinaas HP, Chown SL (2007) Acclimation effects on thermal tolerances of springtails from sub-Antarctic Marion Island: Indigenous and invasive species. J Insect Physiol. 53:113-125

Sømme L (1981) Cold tolerance of Alpine, Arctic and Antarctic Collembola and Mites. Cryobiol. 18:212-220

Sømme L (1995) Invertebrates in Hot and Cold Arid Environments. Springer-Verlag, Berlin.

Sømme L, Conradi-Larsen E-M (1977) Anaerobiosis in overwintering collembolans and oribatid mites from windswept mountain ridges. Oikos. 29:127-132

Tamburri MN, Wasson K, Matsuda M (2002) Ballast water deoxygenation can prevent aquatic introductions while reducing ship corrosion. Biol Conserv. 103:331-341

Tanaka K (1994) The effect of feeding and gut contents on supercooling in the house spider, *Achaearana tepidariorum* (Araneae: Theridiidae). Cryo-Lett. 15:361-366

Tanaka K, Watanabe M (1996) Influence of prey species on the supercooling ability of the Redback Spider, *Latrodectus hasseltii* (Araneae: Theridiidae). Acta Arachnol. 45:147-150

van der Woude HA (1987) Seasonal changes in cold hardiness of temperate Collembola. Oikos. 50:231-238

Vanin S, Turchetto M (2007) Winter activity of spiders and pseudoscorpions in the South-Eastern Alps (Italy). Ital J Zool. 74:31-38

Worland MR, Convey P, Lukešová A (2000) Rapid cold hardening: a gut feeling. CryoLetters. 21:315-324

Worland MR, Grubor-Lajsic G, Montiel PO (1998) Partial desiccation induced by sub-zero temperatures as a component of the survival strategy of the Arctic collembolan *Onychiurus arcticus* (Tullberg). J Insect Physiol. 44:211-219

Zeh JA, Bonilla MM, Su EJ, Padua MV, Anderson RV, Kaur D, Yang D-s, Zeh DW (2012) Degrees of disruption: projected temperature increase has catastrophic consequences for reproduction in a tropical ectotherm. Global Change Biol. 18:1833-1842