Survivorship and longevity of *Diamesa mendotae* Muttkowski (Diptera: Chironomidae) under snow

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Abstract

Diamesa mendotae Muttkowski commonly grow and emerge from groundwater dominated streams in winter. Previous estimates of longevity for adults of *D. mendotae* collected from the snow surface averaged 18.6 days post-collection, with 76.9% of individuals dying between Day 10 and Day 30 post-collection and 4.4% surviving over 40 days. Maximum longevities for males and females were 48 and 54 days, respectively. In this paper, we report survivorship and longevity of field-collected adults of *D. mendotae* kept at ambient snow temperature conditions. Adults (n=140) were collected in February from snow along groundwater-dominated sections of the Kinnickinnic River (Wisconsin, USA). All individuals were placed in vials, buried in snow, and retrieved in batches of 10 males and 10 females at 4-day intervals for 28 days. Once retrieved, adults were maintained at 6°C in controlled environmental chambers to determine survivorship and longevity. All individuals survived snow burial treatment, indicating they are capable of surviving sub-freezing field conditions for at least 28 days. Estimates of adult maximum longevity were as high as 66 days, higher than previous estimates for this genus. Our results suggest adults of *D. mendotae* can persist under snow cover, with high survivorship and longevity, potentially increasing their probability of successful reproduction in regions where lethal winter air temperatures occur.

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Introduction

Cold climates seasonally restrict activity and development of most aquatic insect species (Danks, 2007) which has led many to overlook winter emergence of aquatic insects or interpret this phenomenon as having relatively little ecological impact or life-history significance (Bouchard, 2007). However, insects living in habitats that do not freeze during the winter, such as the groundwater dominated trout streams in southeastern Minnesota, USA, often show significant emergence throughout the coldest months of the year. This lack of ice cover, due to the temperature-moderating influence of groundwater input, permits growth, development and winter emergence of cold-adapted aquatic insects.

Chironomidae (Diptera), commonly known as non-biting midges, are among the few aquatic insect families with species able to grow, develop, and emerge as mature and fecund adults throughout the winter months. Some chironomid species in the subfamilies Diamesinae, Orthocladiinae and Podonominae are recognized as dependable indicators of cold environments (Lencioni, 2004; Eggermont & Jeiri, 2012). Recent winter field work has demonstrated that over 50 chironomid species emerge from streams in Kansas, USA (Ferrington, 2000, 2007; Anderson *et al.*, 2011), and Anderson and Ferrington (2012) recorded 39 species of Chironomidae emerging from trout streams in southeast Minnesota during winter.

In lotic systems of north temperate ecoregions, members of the subfamily Diamesinae are recognized as among the most cold-adapted Chironomidae, with representative species that consistently emerge during winter and early spring (Hansen & Cook, 1976; Nolte & Hoffman, 1992; Armitage, 1995; Ferrington, 2000, 2007; Bouchard & Ferrington, 2009; Ferrington *et al.,* 2010; Anderson *et al.,* 2011; Eggermont & Heiri, 2012). Adult flight and mating activity has been witnessed on stream banks at air temperatures well below 0°C. Young (1969) noted flight and breeding activity of *Diamesa arctica* (Boheman) at an air temperature of –0.4°C near Smithfield, Utah, USA. Hågvar and Østbye (1973) recorded adults of five chironomid species, including three in the genus *Diamesa*, flying and mating on the snow surface surrounding various Norwegian brooks throughout winter. Their study demonstrated that adults of *Diamesa permacra* (Walker) are capable of surviving temperatures at least as low as –13.5°C, and can regain active movement at a temperature of –4.5°C (Hågvar & Østbye, 1973).

More recently, research on *Diamesa mendotae* Muttkowski, has shown that larvae are able to survive freezing, with a lower-lethal temperature with 50% mortality (LLT_{50}) of –17.4°C (Bouchard *et al.*, 2006b). The LLT_{50} values increase as larvae near pupation, and this species undergoes physiological changes to become a freeze-intolerant adult. Adults survive sub-zero temperatures by enhancing their capacity to supercool to temperatures below –20°C (Carrillo *et al.*, 2004; Bouchard *et al.*, 2006a). Adults can be collected from snow banks around groundwater dominated streams in Minnesota and Wisconsin from September through May (Hansen & Cook, 1976; Bouchard & Ferrington, 2009), emerging at water temperatures ranging from 0°C to +10°C (Ferrington *et al.*, 2010), and are commonly observed walking, flying or mating on snow-covered stream banks at air temperatures as low as –8°C (L.C. Ferrington *et al.*, personal observation, 2010). Large emergences often occur on winter days with maximum air temperatures around 0°C (RW Bouchard & LC Ferrington, personal observation, 2009), and adults seem to be most common after several days of bright, sunny weather (Ferrington *et al.*, 2010). *Diamesa mendotae* can develop from egg to adult in as little as 63 days and have the potential to undergo multiple generations per winter (Bouchard & Ferrington, 2009). Fast development, from egg to adult in as little as 39-43 days, has also been reported for the cold-tolerant species *D. incallida* (Nolte & Hoffman, 1992).

Previous estimates of longevity for adults of *D. mendotae* collected from the snow and maintained in the laboratory at 6°C indicated that 76.9% of mortality occurred between Day 10 and Day 30 post-collection. Only 4.4% adults survived over 40 days, with maximum longevities for males and females being 48 and 54 days, respectively (Ferrington *et al.*, 2010). This exceeds the previously recorded maximum longevity of the Himalayan *Diamesa* sp. where females were reported to survive for at least one month and maintain activity at temperatures as low as -16°C, leading to speculation that they survive winter as gravid females (Kohshima, 1984). Based on these findings, and the known life cycle details of *D. mendotae* and *D. incallida*, Ferrington *et al*. (2010) proposed a new *labile life cycle hypothesis* that may apply more broadly to species that emerge in winter. However, little else is known about cold-tolerance aspects and life cycle dynamics of winter-emerging Diamesinae. Therefore, the primary aim of this research was to further explore survivorship and longevity of field-collected adults of *D. mendotae* at sub-zero temperatures, part of a continuing attempt to understand the autecology of adults of this cold-adapted species. In addition, we tested for differences in longevity and survivorship between males and females.

Materials and methods

Adults of *D. mendotae* (n=140) were collected from the surface of snow banks along groundwater-dominated sections of the Kinnickinnic River, at River Falls, Wisconsin, USA (N 44°55', W 92°38') on February 16th 2004. Once located on snow banks, specimens were scooped individually into 3.7-mL snap-top vials along with a small amount of snow and vials were stored in coolers filled with snow to keep them cool. After collection, all adults were inspected and individuals that were damaged or showed uncoordinated movements in the vials were set aside. Seventy males and seventy females were selected from the remaining adults, and then randomly assigned to batches of 10 males and 10 females. Batches of males and females were placed into plastic re-sealable zipper bags and buried in a snow pile at an approximate depth of 0.4 m at 8:00 pm on 16th February. Most males selected for the study had antennal whorl-setae that were closely appressed to the flagellum indicating they were teneral and possibly emerged on the day of collection. However, with females there was no obvious indication as to whether they were teneral or not; but none had withered abdomens which would suggest they had oviposited prior to collection.

One batch of males and one batch of females were retrieved from the snow pile at 8:00 pm on 20th February after being in the snow bank for 96 h. Retrieval time for the second batch was shifted to 8:00 am on 26th February (total 206 h in snow bank) to allow for easier post-retrieval assessment. All remaining batches were retrieved at 8:00 am, resulting in consistent 4-day treatment intervals for up to 28 days. All batches not retrieved on a particular date were left undisturbed. Snow pile temperature data for each treatment interval are presented in Table 1.

Following retrieval of batches, field survivorship was assessed when specimens were brought to the laboratory and maintained at 6°C in a controlled environmental chamber. Specimens were checked daily until dying to determine longevity from collection date. Specimens were held at 6°C to maintain continuity of test conditions with previous *D. mendotae* longevity research (Ferrington *et al.*, 2010). The longevities we report here only include the lifespan of the adult and do not include length of other life cycle stages. Adults of this species are non-feeding, thus no food sources were provided; however, water from the melted snow prevented dehydration as the flies aged. Upon death, specimens were preserved in 70% ethanol and deposited in the University of Minnesota Insect Collection (UMSP). A two-way analysis of variance (ANOVA) was performed using SigmaPlot© software (version 12.3) to compare average longevity between sex and time within the snow pile. Normality assumptions were met by squaring the data before analysis. When a significant effect was detected using ANOVA, a Tukey's Studentized range test was performed to determine which groups were significantly different.

Results

Survivorship

All males and females survived their respective sub-zero treatment intervals in the snow. In addition, all but 3 of the individuals placed into the snow showed co-ordinated movement, climbing and fluttering of wings in the vials within 5 min of being retrieved from the snow bank. In the final batch (28 days in snow), 2 males and one female were not able to walk in a co-ordinated fashion and did not attempt to climb the sides of the vial or fly. These individuals died within two days of retrieval.

Table 1. Maximum and minimum snow pile temperatures. Maximum and minimum temperatures were recorded for each of the treatment intervals corresponding to retrieval of batches. Snow pile temperature (°C) was measured with a max-min thermometer placed adjacent to the batches within the snow pile (45°02'N; 93°6'W).

Snow temperature	16-20 Feb	21-25 Feb	26-29 Feb	1-4 Mar	5-8 Mar	9-12 Mar	13-16 Mar
Max	–3.89	–2.78	–2.22	–2.78	–2.22	–2.78	–2.22
Min	–18.33	–7.78	–6.67	–5.56	–6.67	–12.78	–8.33

Longevity

Average longevity of males ranged from 40.3 to 55 days, while female average longevities ranged from 42.5 to 50.5 days (Table 2). Minimum longevity of males ranged from 13 to 47 days and of females ranged from 20 to 33 days. Maximum male longevity ranged from 48 to 66 days and females ranged from 57 to 66 days. The maximum longevities range between 48 and 57 days for batches remaining in the snow pile for 4, 8 or 12 days and maximum longevities range from 60 to 66 for batches in the snow pile 16 or more days.

The two-way ANOVA indicated no significant effect of sex ($F_{1,\ 139}$=0.438, P=0.509), but a significant effect of time in snow pile ($F_{6,\ 139}$=2.796, P=0.014) was identified. Subsequently, Tukey's Studentized range test indicated a significant difference between batches 1 and 6, but there were no statistically significant differences between other batches. Despite the lack of a consistent significant pattern, there does appear to be a trend of increasing longevity with increasing time held in snow (Figure 1).

Discussion and conclusions

Adults of *D. mendotae* are often observed aggregating and walking on snow banks adjacent to groundwater-fed streams, which we interpret as a mechanism for mate location and reproductive activities. When conditions are not suitable for aggregation activities (*i.e.* during cold, windy or otherwise inclement winter weather) we have observed adults in shielded areas under snow drifts overhanging the stream margins, and within crevices and melt spaces in the snow around trees, rocks, or brush and dried plants. We have interpreted this movement to shielded areas as a mechanism to insulate adults from rapid changes in air temperature during inclement weather conditions, but were unsure if adults survived periods at sub-freezing conditions when surrounded by snow. Our results show that *D. mendotae* adults have the potential to survive at least up to 28 days in vials at fluctuating sub-zero temperatures (–18.3°C to –2.2°C). Similar conditions could occur naturally within the shielded areas we describe. Therefore, these types of microhabitats could serve as an area of refuge for individuals awaiting more favorable conditions to commence reproductive activities such as mate location, copulation and oviposition.

The data reported here build upon previous knowledge of survivorship and longevity of *Diamesa* in two important ways. First, in contrast to the design used by Ferrington *et al.* (2010), the adults in our study were maintained in a snow pile at close-to-natural field conditions from a minimum of four to a maximum of 28 days before transfer to constant-temperature chambers in the laboratory. Since no previous survivorship data were available for *D. mendotae* held in natural conditions, we assumed the 28-day baseline would be a reasonable starting point, particularly in light of research by Kohshima (1984) indicating month-long survivorship of *Diamesa* sp. females when held at natural conditions. However, in the present study, all male and female adults survived the entire duration of all field treatments. Consequently, the factor limiting our ability to determine survivorship patterns under sub-freezing conditions was the lack of additional adults to continue the study beyond 28 days. This should be expanded upon in future studies, using data presented here as a baseline for survivorship.

A second major finding of our research is that in contrast to the study by Kohshima (1984), all males and females of *D. mendotae* survived the snow pile treatment. Comparatively, although over 90% of the Himalayan *Diamesa* females survived snow treatment for 35 days, no males survived the treatment (Kohshima, 1984). This observed male mortality was another factor that influenced our study design, prompting us to check survivorship every four days so that the mortality patterns could be better resolved if males of *D. mendotae* had shorter longevities than females, as exhibited by the Himalayan males of *Diamesa* sp.

We placed adults in glass vials that were left in the snow pile to provide nearly natural exposure to fluctuating sub-freezing conditions. This ensured that the test adults would be easy to locate and not damaged during retrieval. However, it is unclear whether this field protocol produced any artifacts in estimating the actual field survivorship and longevity within or on snow. A potential consequence of holding specimens in glass vials is that individuals were not directly experiencing the natural desiccating conditions inside the snow pile, since conditions in the vial may have deviated slightly from those outside. For example, we expect the humidity to be slightly higher and less variable in the glass vial compared to the snow pile. Additionally, the vial serves to block any convection that would occur due to wind. Therefore, it is possible that the sub-freezing survivorship and longevities that we

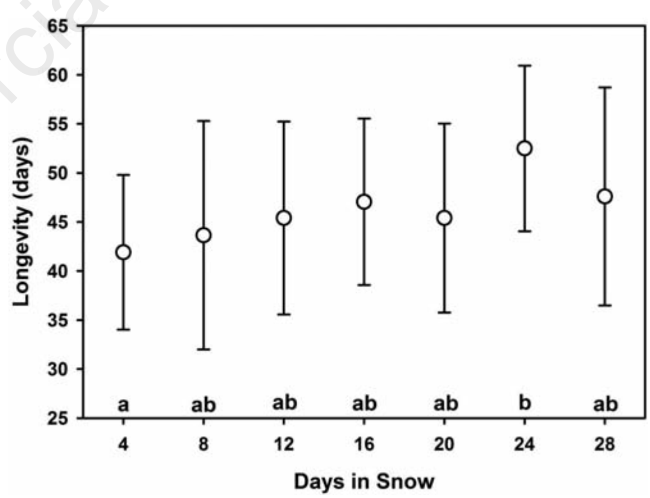

Figure 1. Mean (±SE) longevity of male and female *Diamesa mendotae* following retrieval from snow. Batches with significantly different (P<0.05) mean longevities are indicated by different letters below each mean as determined by Tukey's Studentized range test.

Table 2. Average, minimum, and maximum longevities of male and female *Diamesa mendotae* versus days in the snow pile. Within each column, the left number represents male longevity in days and the right number represents female longevity in days.

	Days in snow pile						
	4	8	12	16	20	24	28
Average	41.3/42.5	40.3/47	42.4/48.4	48.3/45.8	47.4/43.4	55/50	44.7/50.5
Minimum	35/20	13/33	24/33	34/32	36/32	47/28	29/29
Maximum	48/57	56/57	51/58	66/61	62/66	65/61	60/61

have reported in this study could be an overestimate of longevity of *D. mendotae* adults in the environment.

Alternatively, field collection of *D. mendotae* adults could have resulted in an underestimation of the longevities under natural conditions. Based on the results of this experiment, we now know that it is possible that some of the adults we collected from snow could have emerged days to weeks prior to collection. We think this is less likely for males because the condition of the whorl-setae on the antennae suggested they were teneral and presumably emerged from the stream on the day of capture. However, we are not as confident about females, particularly in the light of our results that indicate that adults of this species can persist at sub-freezing temperatures for at least 28 days. Thus there is a potential that test organisms were much older when collected if they were residing in the snow pack or along stream margins during the days prior to collection. Therefore, an alternative interpretation of our estimates of survivorship and longevity reported here is that they reflect the minimum estimate of maximum longevity and minimum estimate of average longevity, especially for females. This interpretation has implications for earlier work (*e.g.* Ferrington *et al.*, 2010) where it was assumed that the test individuals had emerged within the 24-48 h prior to collection. In the future, a study design that allows more accurate assessment of survivorship and longevity should be employed, such as using emergence traps placed over the stream to collect recently emerged adults.

We believe that the preliminary patterns in our results here are valuable and warrant discussion related to the life cycle hypotheses proposed for *D. mendotae*, *D. incallida,* and the *Diamesa* species studied by Kohshima (1984). Our findings show that there is at least one species of *Diamesa* in the Holarctic region that exhibits survivorship patterns at sub-freezing temperatures similar to the females of the high alpine species occurring on glacial ice and snow in the Himalayas. Assuming that all of our test specimens had recently emerged, there were no significant differences in longevities between males and females. Therefore, males of *D. mendotae* appear to survive better at sub-zero temperatures than the species investigated by Kohshima (1984). However, the field design used by Kohshima (1984) does not conclusively rule out protandry (Holzapfel & Bradshaw, 2002). Thus, the demonstrated differences in survivorship among males and females could have resulted from protandry, if males emerged much earlier than females, thereby giving the impression of reduced cold tolerance in males as evidenced by mortality during the test duration.

Kohshima (1985) attributed female longevity of the Himalayan *Diamesa* sp. as a possible mechanism permitting migration and overwintering survival in glacial snowmelt streams that may meander or have reduced flow under snow cover in winter. This interpretation is novel for chironomids, where many species persist through winter as larvae. Here, we attribute the male and female survivorship under snow and longevity after collection as a fecundity-enhancing mechanism. We believe this allows males and females of *D. mendotae* that have emerged over a range of dates the opportunity to delay reproductive activities until environmental conditions are more suitable for energetically expensive activities such as mate location and copulation on snow, followed by a return to the stream for oviposition.

Our results also suggest that oviposition by one generation can be separated by 28 or more days, particularly if weather conditions change during the emergence period of a single cohort. We have observed teneral males mating with females on the snow that then fly back to the water to oviposit. However, if weather conditions (*e.g.* air temperature, wind speed) fluctuate over short time intervals, recently emerged adults may discontinue mating activities and move to shielded areas before mating to wait for favorable environmental conditions before returning to the snow surface for mating. Consequently, our results show that adults may successfully survive under snow banks or within snow crevasses for more than one half of the minimal time of 63 days required for larvae to development from egg to adult as shown by Bouchard and Ferrington (2009). Delays in mating and oviposition could potentially result in some of the next generational cohort differing substantially in development and growth. The effects of this type of cohort splitting would be even more significant if *D. incallida* has similar capability to survive sub-freezing temperatures, given that only 39-43 days are estimated for its development from eggs to adults.

Another important finding of our work was that across all treatments and both sexes, 72.1% of individuals had longevities that surpassed 40 days. This contrasts with Ferrington *et al.* (2010) who found that only 4.4% of laboratory-maintained individuals were found to have longevities exceeding 40 days. Additionally, our estimates of total maximum longevity exceed those presented by Ferrington *et al.* (2010), with maximum male and female longevity reaching or surpassing 48 and 57 days, respectively (Table 2). Consequently, there appears to be a trend among individuals held in the sub-freezing conditions of the snow pile for longer periods to have greater maximum longevities, with 4-day exposure to sub-freezing conditions nearly doubling the estimate of average longevities. The effect of sub-freezing conditions on longevity appears even more pronounced among individuals exposed to longer sub-freezing periods. For example, maximum longevities for individuals in batches buried in snow for 16-28 days were in excess of 60 days (Table 2). We recognize that these may represent minimum estimates based on our relatively small batch sizes, or the artificial laboratory temperature (6°), which may have led to a higher metabolism and consequently a reduced longevity. However, we believe the data demonstrate an important trend, and should be developed further. Consequently, we suggest that future studies include larger batch sizes, a greater number of total batches, and additional laboratory incubation air temperatures.

Our findings suggest that adults of *D. mendotae* survive well under snow cover, exhibiting high survivorship and longevity, thereby increasing their probability of successful reproduction during periods of lethal low air temperatures in the northern temperate zone. The survivorship and longevity patterns documented in this paper have direct implications supporting the labile life cycle hypothesis proposed for *D. mendotae* by Ferrington *et al.* (2010). This hypothesis states the *sporadic winter emergence can be viewed as an integral, highly adapted strategy for spreading emergence over a longer period of time when air temperature conditions and the consequent probabilities for successful mating and ovipositional flight back to the stream cannot be predictably determined* (Ferrington *et al.*, 2010). This hypothesis was originally proposed based on potential dynamics of larval development and metamorphosis, but our findings now demonstrate that adults have the potential to delay mating activities, which can then produce additional asynchrony of development among larvae of a single generation, resulting in more sporadic bursts of emergence throughout winter.

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