

Emergence, survival, and longevity of adult *Diamesa mendotae* Muttkowski
(Diptera: Chironomidae) in groundwater-fed streams

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Abstract

Groundwater-fed streams, which remain cold in summer but ice-free in winter, provide ideal habitat for ultra-cold stenotherm insects. *Diamesa mendotae* Muttkowski (Diptera: Chironomidae) is a winter-active species common to groundwater-fed streams in Minnesota. In order to improve the understanding of the winter dynamics of this species, we studied the influence of temperature on its emergence, survival, and longevity. The winter emergence dynamics of *D. mendotae* and other winter-active chironomids were documented by collecting surface-floating pupal exuviae samples from 24 groundwater-fed streams in southeastern Minnesota. Early, mid, and late winter samples were collected from each stream, and mean water temperatures during the week preceding sample collection were estimated using air-water temperature regressions. The results of this assessment indicate that *D. mendotae* are influenced by both thermal stability and water temperature. Abundance of *D. mendotae* was positively related to air-water temperature regression slope in early and mid-winter; emergence was negatively related to water temperature in late-winter. Emergence patterns of other genera were related to estimated water temperatures, showing significant thermal partitioning within the chironomid community. Field collections of adult *D. mendotae* were used to determine survivorship under long-term exposure to controlled sub-freezing conditions. Batches of specimens were placed into a controlled treatment chamber at -5°C for between 7 and 70 days. Survivorship at constant sub-freezing temperatures was negatively related to treatment length, although some individuals survived sub-freezing temperatures for 70 days. Additionally, male *D. mendotae* had a significantly higher rate of survivorship than

females within the same treatment. Post-treatment longevity decreased with increased exposure to sub-freezing temperatures; however, total longevity increased with treatment time. These studies indicate that *D. mendotae* is well adapted to the cold winter-weather conditions across southeastern Minnesota, suggesting that adults may be able to survive long periods of extreme temperature conditions in the winter to increase their ability to successfully reproduce. Groundwater inputs not only influence the thermal regime of streams in southeastern Minnesota's karst landscape, but also significantly impact chironomid community dynamics, which may play a significant role in the broader invertebrate and fish communities of these streams.

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CHAPTER I

OVERWINTER EMERGENCE DYNAMICS OF ADULT CHIRONOMIDAE IN GROUNDWATER-FED STREAMS OF SOUTHEASTERN MINNESOTA

Introduction

Southeastern Minnesota's karst geology is characterized by surface and sub-surface groundwater flows through a carbonate-sandstone landscape. Groundwater flows remain near mean annual air temperature (approximately 9°C) throughout the year, with little thermal variability. Consequently, constant-temperature groundwater inputs into southeastern Minnesota streams moderate the diurnal and seasonal water temperature fluctuations present in surface-water dominated streams.

Complete groundwater system mapping is complex and rarely achieved. Krider *et al.* (2013) and O'Driscoll & DeWalle (2004) have shown that air-water temperature regressions can be used to model the influence of groundwater on stream thermal regime. The slope of the linear regressions between average weekly air and water temperatures can be used as a surrogate for the volume of groundwater entering a stream reach. In streams with the most groundwater influence, fluctuations in air temperature have minimal impact on water temperature. As a result, regression models for groundwater-fed streams with more groundwater input have lower slopes and higher intercepts than those of streams with less groundwater input, reflecting the mitigating thermal influence of groundwater (Krider *et al.* 2013).

Groundwater-fed streams, which remain cold in summer but ice-free in winter, provide ideal habitat for cold stenotherm chironomids such as *Diamesa mendotae* Muttkowski, which grow, develop, and emerge over the winter. Although cold stenotherm species are regulated by a thermal maximum, other chironomid species require sufficient degree days above a thermal minimum to complete their development and life cycle. Thus, chironomid emergence is often thermally partitioned among differently-adapted genera within the same stream reach (Bouchard 2007).

Previous studies have corroborated the influence of groundwater on the abundance and richness of aquatic invertebrates. Chironomid taxa richness has been shown to be correlated with stream thermal variability. Bouchard (2007) found that six surface-water dominated streams contained significantly more chironomid taxa than six groundwater-dominated streams within the same area of eastern Minnesota. Streams with less groundwater input would be expected to have more niche space available, due to their thermal heterogeneity (Ward & Stanford 1982). In contrast, groundwater-fed streams would be expected to be dominated by few taxa – those which are most adapted to the narrow thermal range of the stream (Ward 1976).

We used surface-floating pupal exuviae samples to examine the winter emergence patterns of chironomids in southeastern Minnesota and understand the influences of groundwater input and water temperature on winter-emergent species such as *D. mendotae*. Additionally, we evaluated the ability of air-water temperature regressions to

accurately estimate mean weekly stream water temperatures.

Methods

Study sites and field methods

A total of 24 groundwater-fed stream reaches were assessed in this study (Appendix A).

The selected sites are relatively small, wadeable stream reaches located in the southeastern corner of Minnesota (Fillmore, Goodhue, Houston, Wabasha, and Winona counties).

Surface-floating pupal exuviae (SFPE) samples were collected from each of the 24 sample sites during the winters of 2010-2011 and 2011-2012 using standard protocol (Ferrington *et al.* 1991). Half of the sites were visited each winter, and each site was visited on 3 occasions: early winter (mid-November through early-January); mid-winter (mid-January to mid-February); and late winter (March). Samples consisted of a timed 10-minute sampling period within a 150-meter sample reach. Working upstream, SFPE were scooped from the snags and eddies on the water surface where they collect, and poured into a 125-um sieve. Samples were transferred to 60-ml jars and preserved in 70% ethanol. All pupal exuviae were picked from the samples in the laboratory and identified to genus. Whole pupae and adults were not counted; however, adults were identified to confirm species-level identifications.

Instream temperature data were collected using HOBO® water temperature data loggers. Data loggers were placed during the first sample event for each site, and water temperature data was collected at 30-minute intervals over the sampling period.

Prediction of water temperatures

Linear air-water temperature regressions and weather stations for each stream were obtained from Krider *et al.* (2013). Air temperatures for the week preceding each sample event were obtained from their respective weather stations, via NOAA's National Climatic Data Center. For each sample event, daily maximum and minimum air temperatures were averaged over the week preceding data collection to produce mean weekly air temperatures. The air-water temperature regressions were used to predict mean weekly water temperatures for each sample event from the mean weekly air temperatures (Appendix A). Slope and intercept values from the air-water regressions were used as measures of relative groundwater input to each stream (Appendix A).

Analysis of data

All statistical analyses were calculated using the program R (version 2.15.1). A paired t-test was used to compare estimated and actual mean weekly water temperatures for each sample event (Figure 1.1). Multiple regression models were used to evaluate generic richness among sample sites and collection periods; t-tests were used to test for significant differences between collection periods (Figure 1.2). Poisson-family generalized linear models and Wald tests (z-values) were used to evaluate generic

abundance and composition (Figures 1.3 & 1.4). In all models, regression slope, collection period, and estimated water temperature were evaluated as potential explanatory variables. Thermal partitioning among genera was determined by identifying the observed thermal maximum and minimum of emergence for each genus.

Results

Estimation of water temperature from air-water regressions

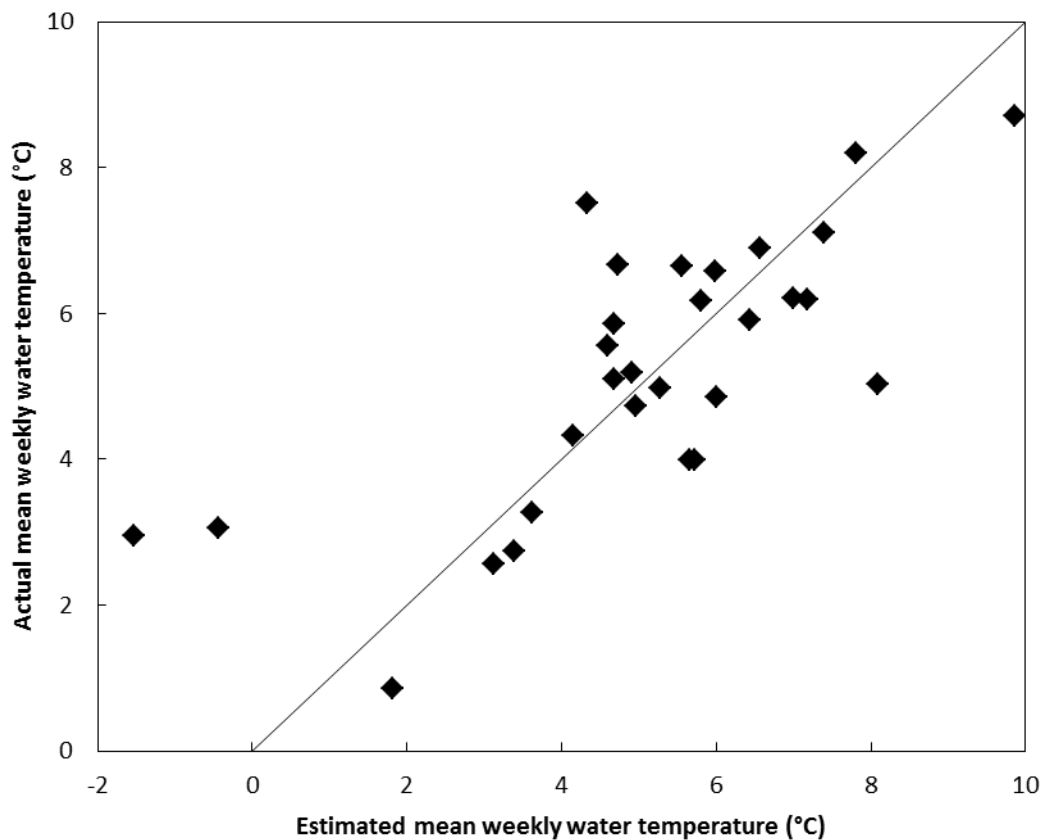


Figure 1.1: Estimated mean weekly water temperature in relation to actual mean weekly water temperature for the week preceding sample collection. Estimated mean weekly water temperatures were calculated using air temperature and air-water regression data. The regression line indicates values where actual and estimated mean weekly water temperatures are equal.

Air-water regression slopes varied between 0.2038 and 0.6112 (Appendix A). There were no significant differences between estimated and actual average weekly water temperatures for 14 sample sites that had both estimated and instream data available ($T_{18}=-0.5175$, $p=0.6089$; Figure 1.1). Therefore, I assumed that estimated weekly water temperatures were a sufficient measure of actual water temperature. Estimated weekly water temperatures were used in all subsequent analyses (Appendix A).

Taxa richness and total abundance

A total of 5,144 SFPE were picked from the samples collected from the 24 sites, ranging from 0 to 501 individuals per sample. From these individuals, 29 taxa were identified (Appendix B). The greatest number of taxa collected in one sample event was 15, found in late-winter samples from both Trout Run and the Middle Branch of the Whitewater River. No emergence was found in four of the 72 SFPE samples.

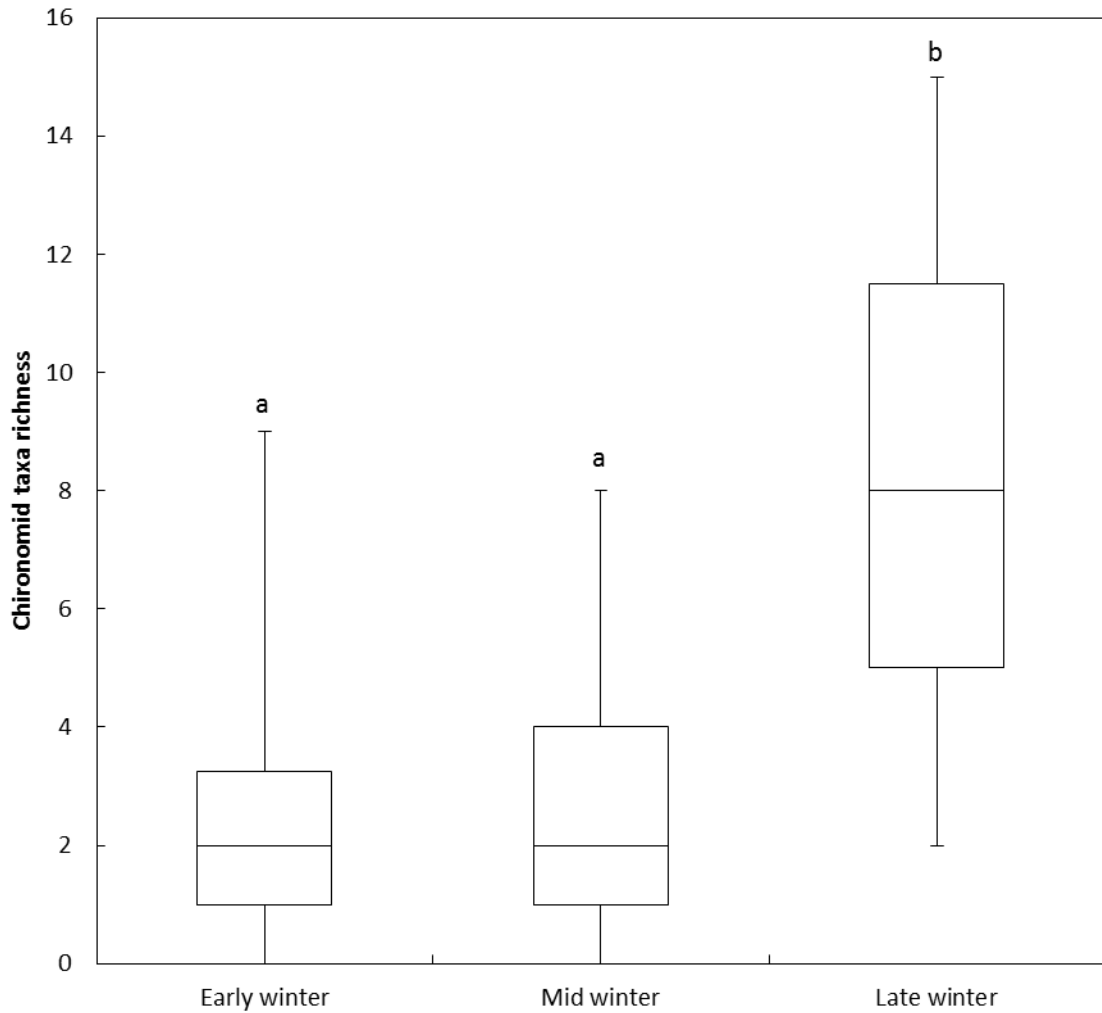


Figure 1.2: Box plot of mean chironomid taxa richness in SFPE samples in early, mid, and late winter. The upper and lower boxes contain the 2nd and 3rd quartiles of taxa richness, respectively. Endpoints indicate maximum and minimum taxa richness. Letters indicate significant differences between groups.

Taxa richness was significantly higher in late-winter SFPE samples than those collected in early or mid-winter ($F_{1,70}=37.98$, $p<0.001$; Figure 1.2). Mean taxa richness in late winter was 8.3 taxa (maximum of 15 taxa). In contrast, mean taxa richness in both early and mid-winter was 2.6 taxa (maxima of 9 and 8 taxa, respectively). More than 60% of identified taxa were only collected in the late winter.

Seasonal and thermal partitioning

Table 1.1: Seasonal and thermal partitioning of sub-families of Chironomidae. Thermal range of emergence indicates the minimum and maximum estimated water temperatures at which emergence was recorded. * Indicates that emergence was recorded at the minimum or maximum sampled temperature, respectively.

Sub-family	Number of sites where found			Thermal range of emergence (°C)	
	Early	Mid	Late	Minimum	Maximum
Diamesinae	18	20	19	-0.43	8.25
Prodiamesinae	8	9	17	-0.43	9.86 *
Orthoclaadiinae	14	14	24	1.37	9.86 *
Chironominae	5	5	16	3.06	9.86 *

All taxa identified represented only 4 sub-families of Chironomidae: Chironominae, Diamesinae, Orthoclaadiinae, and Prodiamesinae (Table 1.1). The sub-families Diamesinae and Prodiamesinae were first collected at a minimum estimated water temperature of -0.43°C; emergence did not occur during the two sample events below that temperature. The thermal minima of Orthoclaadiinae and Chironominae were 1.37°C and 3.06°C, respectively. Diamesinae emergence was not found above 8.25°C. However, the remaining 3 sub-families were all collected at the highest estimated water temperature of 9.86°C. Additional seasonal and thermal partitioning occurred among genera within each sub-family (Appendix C).

Emergence patterns of D. mendotae

D. mendotae was the most common taxon found in SPFE samples, with emergence occurring in 55 of 72 sample events and at all sample sites. Additionally, over 35% of all collected individuals were identified as *D. mendotae*. The highest abundance of *D.*

mendotae was 288 exuviae, which were collected in the early-winter sample from Pickwick Creek (Appendix B).

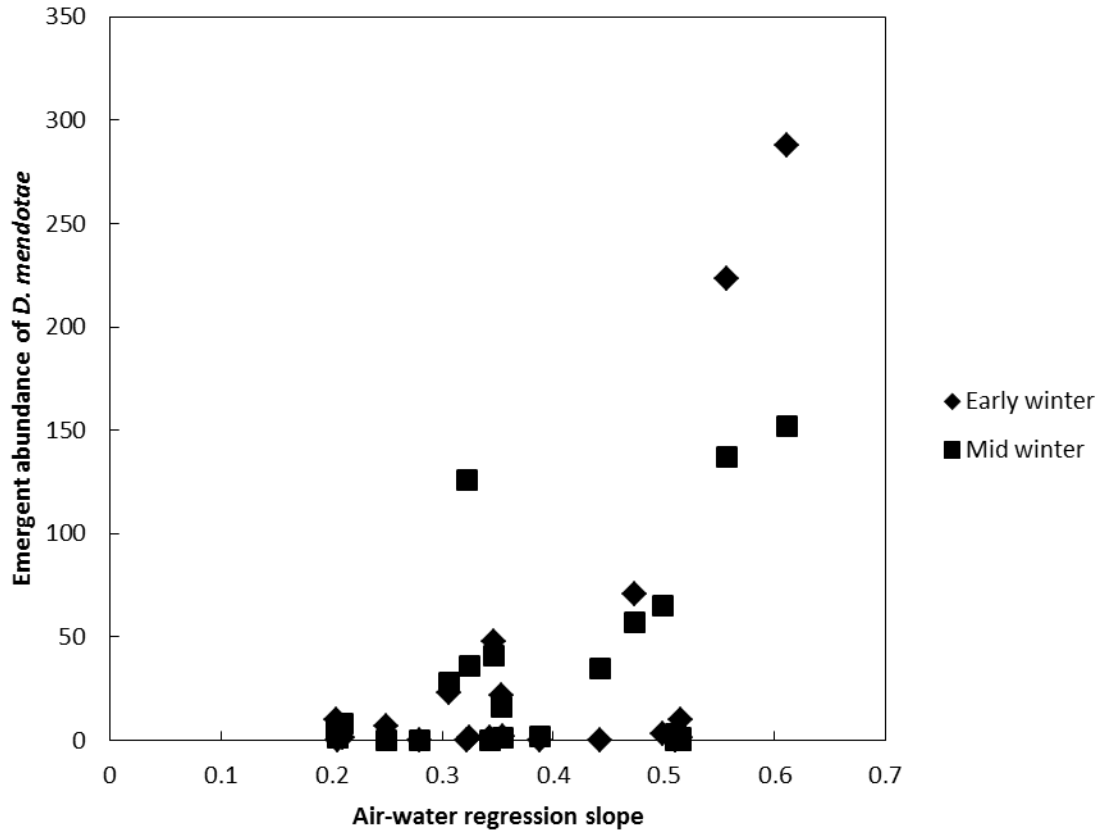


Figure 1.3: Abundance of *D. mendotae* exuviae in early and mid-winter SFPE samples as a function of air-water regression slope.

Abundance of *D. mendotae* exuviae was positively related to air-water regression slope in both early winter ($z=29.3$, $p<0.001$; Figure 1.3) and mid-winter ($z=18.97$, $p<0.001$; Figure 1.3). However, late winter abundance was not significantly related to the amount of groundwater input at the site.

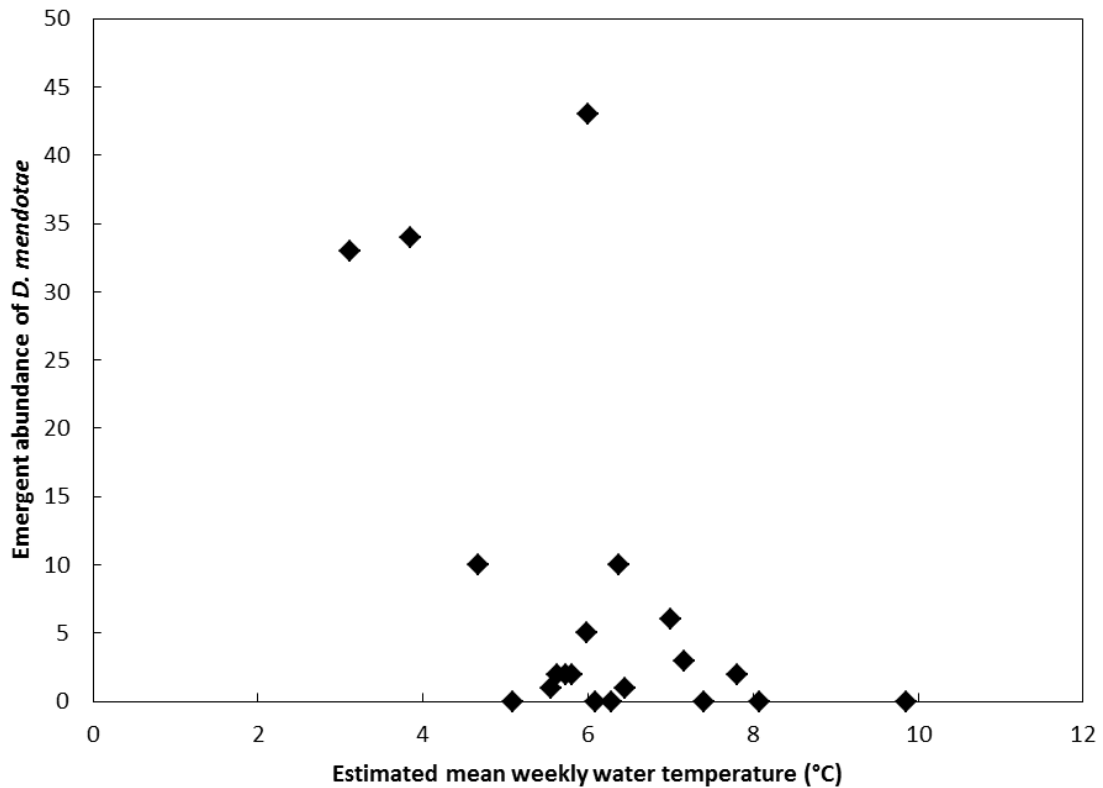


Figure 1.4: Abundance of *D. mendotae* exuviae in late winter as a function of estimated mean weekly water temperature for the week preceding sample collection.

Late-winter abundance of *D. mendotae* was significantly and negatively related to estimated weekly water temperature ($z=-10.74$, $p<0.001$; Figure 1.4).

Discussion

Taxa richness and thermal partitioning

Previous research on chironomid emergence has shown that taxa richness is seasonally and thermally variable in both groundwater and surface-water dominated streams.

Chironomid taxa richness began to decrease in August, corresponding to seasonal temperature declines, in a study of 12 eastern Minnesota streams (Bouchard 2007).

However, taxa richness was not significantly different between early and mid-winter

emergence events in our study, indicating that taxa richness reaches a minimum plateau by mid-November. Genera that stop emerging with decreasing fall water temperatures have reached their thermal minimum and no longer emerge; the primary genera that continue to emerge are likely cold-stenotherms. These genera, such as *D. mendotae*, are adapted to emerge throughout the winter and are thus present in both early and mid-winter samples.

D. mendotae is one of two winter-emergent genera collected from the sub-family Diamesinae. Diamesinae and Prodiamesinae are the two most cold-adapted sub-families of Chironomidae (Table 1.1). These two sub-families contained 4 genera; the remaining 28 genera are from less cold-adapted sub-families (Chironominae and Orthocladiinae). Consequently, 60% of identified taxa were only found in late-winter samples. Bouchard (2007) found that taxa richness increased in the spring with water temperature. Thus, this significant increase in taxa richness (Figure 1.2) indicates that spring emergence patterns begin in late March to early April in these streams.

Emergence patterns of D. mendotae

Abundance of *D. mendotae* exuviae was positively related to air-water regression slope during both early and mid-winter; thus, streams with less groundwater input had higher emergence abundances (Figure 1.3). This relationship is likely a function of the life cycle of *D. mendotae*. Bouchard & Ferrington (2009) estimated that this species has a minimum development time of 63 days. Consequently, there may be a time gap of up to

two months between the time a stream reaches the thermal maximum of *D. mendotae* and the first emergence events. In streams with relatively low groundwater input, fall water temperatures can more quickly drop into the preferred thermal range of *D. mendotae* as a consequence of air-temperature fluctuations, and thus may trigger the development cycle of *D. mendotae*. Because groundwater-dominated streams mitigate air temperature effects, development may begin at a later date. By early and mid-winter, differences in growth and development time may be reflected in the number of individuals that emerge within a stream.

In contrast, late-winter abundance of *D. mendotae* was significantly and negatively related to estimated weekly water temperature, rather than groundwater input (Figure 1.4). As spring water temperatures near 10°C, populations of *D. mendotae* are characterized by a lack of emergence and high mortality (Bouchard & Ferrington 2009). Thus, we expect that *D. mendotae* may reach their maximum growth rate at water temperatures between 6°C and 8°C.

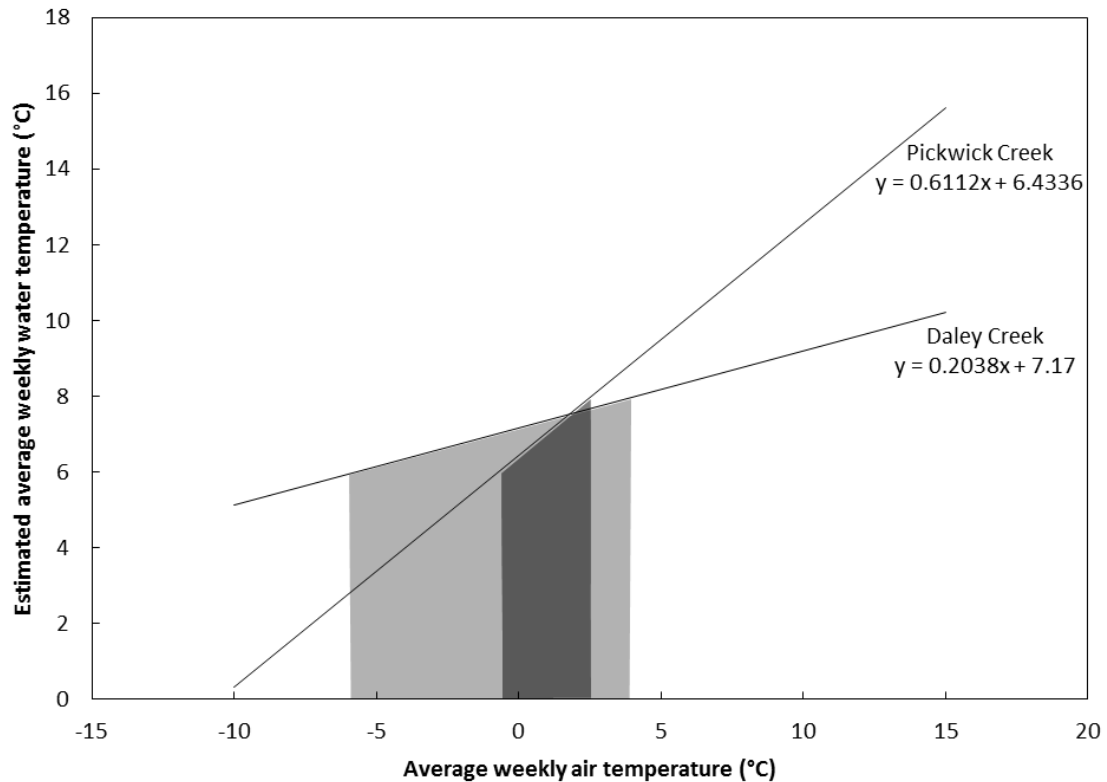


Figure 1.5: Air-water temperature regressions for Daley Creek (most groundwater input) and Pickwick Creek (least groundwater input) (Krider *et al.* 2013). Dark and light shaded areas indicate air temperatures which correspond to estimated water temperatures within the thermal optimum of *D. mendotae*, for Pickwick Creek and Daley Creek, respectively.

Groundwater inputs may significantly influence *D. mendotae* growth rates in individual stream reaches (Figure 1.5). As a result of their thermal buffering capacity, streams with high volumes of groundwater input are more thermally stable throughout the winter.

Water temperatures in such streams remain within the thermal optimum of *D. mendotae* for longer periods of time (Figure 1.5). Thus, we expect that *D. mendotae* may have shorter life cycles in highly groundwater-fed streams. Additionally, these streams remain below the 10°C thermal maximum of *D. mendotae* under a wider range of air temperatures. In these streams, *D. mendotae* may be able to continue their life cycles longer into the spring, as water temperatures do not increase as quickly due to

groundwater influence. Together, higher growth rates and longer growth seasons may allow *D. mendotae* to complete additional life cycles in highly groundwater-fed streams.

Conclusions

Winter chironomid emergence varied both seasonally and thermally in groundwater-buffered streams in southeastern Minnesota. Taxa richness was highest during late winter; over 60% of genera were only collected in late-winter samples. In contrast, early and mid-winter samples were dominated by select cold-adapted genera, such as *D. mendotae*, which were inversely related to groundwater input. Emergence patterns of other sub-families and genera were also related to estimated water temperatures, indicating thermal partitioning. These results suggest that groundwater inputs not only influence the thermal regime of streams in southeastern Minnesota's karst landscape, but also significantly impact chironomid community dynamics.

CHAPTER II
SURVIVORSHIP AND LONGEVITY
OF ADULT *DIAMESA MENDOTAE* (DIPTERA: CHIRONOMIDAE)
AT CONTROLLED SUB-FREEZING TEMPERATURES

Introduction

Diamesa mendotae Muttkowski (Diptera: Chironomidae) is an ultra-cold stenotherm species common to groundwater-buffered streams in Minnesota and Wisconsin. As with other chironomids within the sub-family Diamesinae, *D. mendotae* are cold-adapted (Kohshima 1984; Nolte & Hoffmann 1992; Lindegaard & Broderson 1995; Oliver & Dillon 1997; Willassen & Cranston 1986). Emergence of adult *D. mendotae* occurs throughout the winter, at water temperatures below their thermal maximum near 10°C (Bouchard & Ferrington 2009). Larvae of *D. mendotae* are freeze-tolerant; however, adults are freeze-intolerant. Although unable to survive freezing, adult *D. mendotae* are able to minimize mortality from freezing by a low supercooling point (SCP) of -19.7°C (Bouchard *et al.* 2006).

Previous studies have shown that adult *D. mendotae* are able to maintain their SCP for at least 17 days (Carrillo *et al.* 2004). Although daily minimum temperatures in our study area may dip below the SCP of adult *D. mendotae*, monthly average minimum air temperatures remain above the SCP throughout the winter. As a result of this adaptation to winter temperatures, emergent adults can be found walking, flying, mating, and ovipositing at sub-freezing air temperatures (Bouchard *et al.* 2006). These observations suggest that *D. mendotae* may be chill tolerant as defined by Bale (1996) (i.e., having the

ability to survive subfreezing temperatures above SCP, though mortality may increase with exposure). A previous study of *D. mendotae* by Anderson *et al.* (2013) found that all individuals survived sub-freezing treatment under snow cover for up to 28 days. Thus, if *D. mendotae* are chill tolerant, we hypothesize that mortality will increase under treatment lengths longer than 28 days.

Under the labile life cycle hypothesis, as proposed by Ferrington *et al.* (2010), larval development rates may be variable among individual *D. mendotae*, resulting in multiple bursts of asynchronous emergence during winter. Consequently, we hypothesize that long-term survivorship at sub-freezing temperatures may be a mechanism to increase the potential for adult *D. mendotae* to successfully mate and oviposit (Anderson *et al.* 2013; Bouchard *et al.* 2006).

Adult *D. mendotae* have a mean longevity of 18.6 days when held under controlled conditions at 6 C (Ferrington *et al.* 2010). Maximum reported longevity ranges from 25 to 54 days under the same conditions (Ferrington *et al.* 2010). Another cold-tolerant species of *Diamesa*, found in Himalayan glacial habitats, is active to -16°C, and adult females of the species are capable of surviving more than 30 days (Kohshima 1984). Adult *D. mendotae* have increased longevity under sub-freezing conditions. Maximum total longevities ranged from 48 to 66 days when individuals were placed in a snowbank after collection for between 4 and 28 days (Anderson *et al.* 2013).

We examined the effect of extended, constant, sub-freezing temperatures on the survivorship of field-collected adults to further understand the chill tolerance of *D. mendotae*. Additionally, we wanted to determine whether adult longevity was influenced by exposure time, to further understand the autecology of adult *D. mendotae*.

Materials and Methods

Collection and treatment of test specimens

Adult male (n=59) and female (n=67) *D. mendotae* were collected from Valley Creek on 3 March 2010. Valley Creek is a groundwater-fed stream located in Washington County, Minnesota. Individuals were removed from snowbanks adjacent to the stream, placed into snap-top vials in a snow-filled cooler, and returned to the lab. The age and reproductive status of individuals in the study were not assessed.

All individuals were subsequently placed into a climate-controlled chamber at -5°C. Specimens were initially provided with adequate water from snowmelt but no food; *D. mendotae* are not known to feed as adults (Bouchard & Ferrington 2009). Batches of 10 adults (5 males and 5 females) were removed at weekly intervals and held at 6°C under controlled conditions to determine survivorship and longevity. All remaining individuals were removed after 70 days of sub-freezing treatment and held at 6°C.

Determination of mortality

Upon removal from treatment, specimens were checked daily until all died. Mortality was determined by a lack of response to vial disturbance and confirmed under 12x magnification (Ferrington *et al.* 2010). We found that most specimens remained active until 1-2 days preceding death, at which time individuals reduced wing movement and began to remain in the water film.

Analysis of data

All statistical analyses were calculated using the program R (version 2.15.1). A binomial-family generalized linear model and Wald test were used to compare survival of treatment among individuals of different treatment length and sex. Multiple regression models were used to compare longevities of individuals. Mean post-treatment longevities were compared to previously published survivorship and longevity data for *D. mendotae* (Anderson *et al.* 2013; Ferrington *et al.* 2010).

Results

Survivorship of sub-freezing treatment

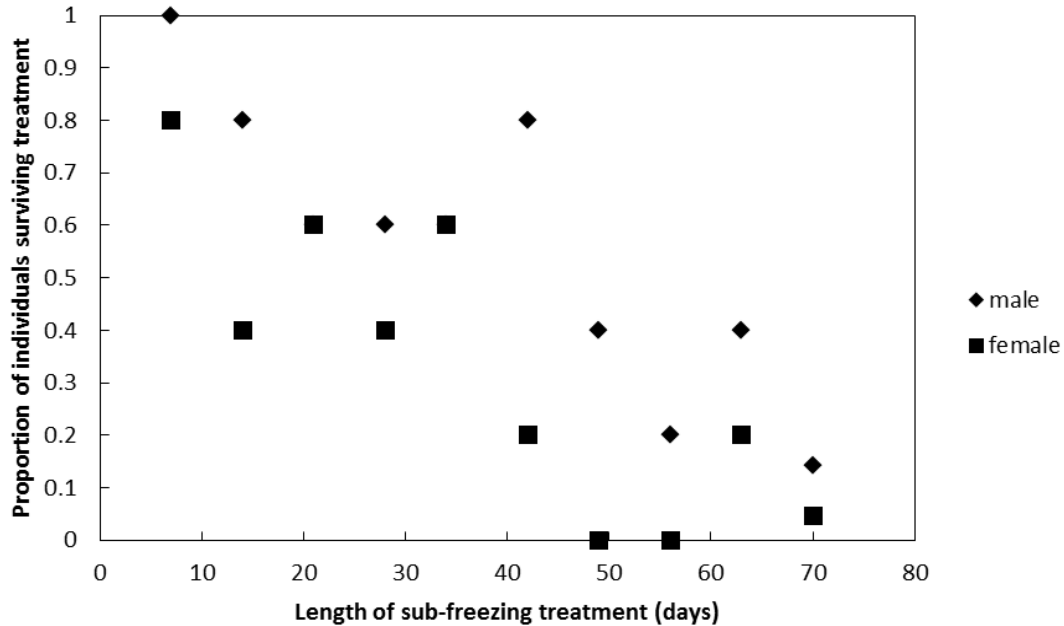


Figure 2.1: Proportion of male and female adult *D. mendotae* surviving sub-freezing treatments from 7 to 70 days in length.

Survival was significantly and negatively related to length of treatment for both males and females between 7 and 70 days ($z=-5.155$, $p<0.001$; Figure 2.1). Although both male and female adult *D. mendotae* show a decrease in survivorship as treatment length increases, individual male and female both survived the longest sub-freezing treatment of 70 days (Figure 2.1).

Within each treatment, males averaged a rate of survivorship of treatment that was significantly higher than that of females ($z=2.706$, $p=0.007$). Male survivorship was 100 percent after a sub-freezing treatment length of 7 days, whereas female survivorship was only 80 percent. For each treatment length, there were individual adult males that

survived treatment. However, no females survived the 49 or 56 day sub-freezing treatments.

Post-treatment longevity

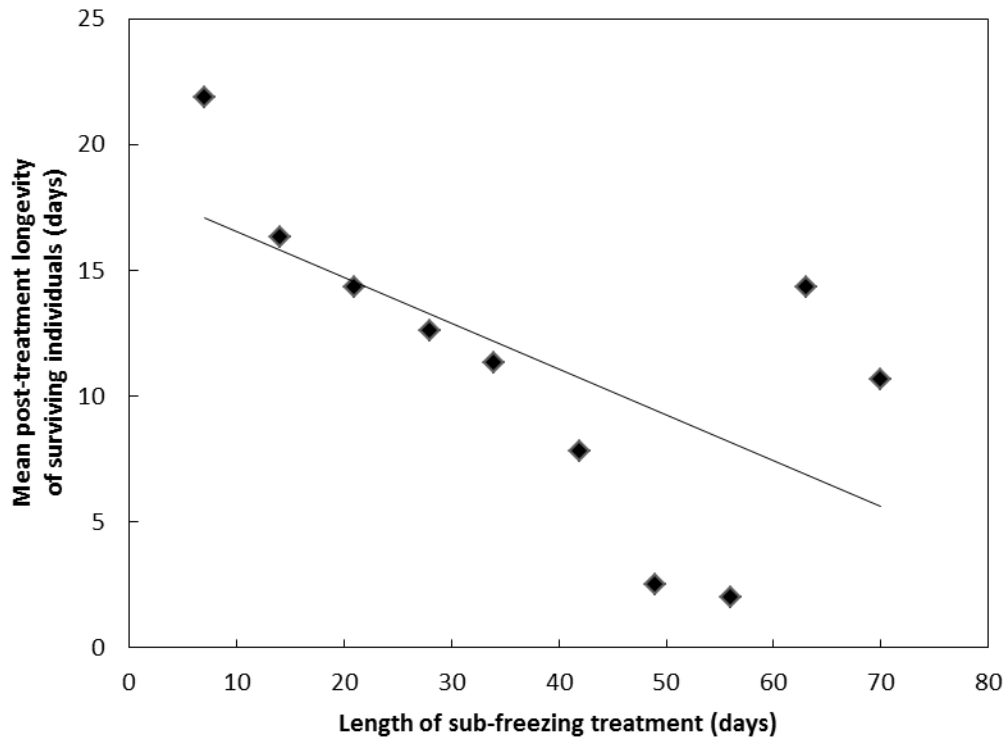


Figure 2.2: Mean post-treatment longevity of surviving adult *D. mendotae* as a function of sub-freezing treatment length ($y = -0.1998x + 19.754$, $R^2 = 0.182$).

Post-treatment longevity of surviving adult *D. mendotae* were negatively related to treatment length ($F_{1,44} = 9.674$, $p = 0.003$; Figure 2.2). However, there was no significant difference between the post-treatment longevity of male and female adults. Maximum post-treatment longevity occurred at the shortest treatment length of 7 days. Mean post-treatment longevity after 7 days treatment was 21.9 days, with a maximum of 35 days, whereas mean post-treatment longevity after 70 days treatment was 10.7 days, with a

maximum of 22 days. Average post-treatment longevity decreased an average of 1.4 days per week of controlled sub-freezing treatment.

Total longevity from collection

Table 2.1: Average, minimum, and maximum total longevity for male and female adult *Diamesa mendotae* surviving treatment. NA indicates that no individuals survived treatment.

Treatment length (days)	Average longevity (days)		Minimum longevity (days)		Maximum longevity (days)	
	Males	Females	Males	Females	Males	Females
7	28.4	29.5	25	18	35	42
14	29.2	32.5	22	27	32	38
21	33.3	37.3	28	25	43	35
28	44.7	34.5	31	31	58	38
34	44.3	46.3	40	35	50	56
42	51.5	43	45	43	64	43
49	51.5	NA	50	NA	53	NA
56	58	NA	58	NA	58	NA
63	79.5	73	78	73	81	73
70	83	76	74	76	92	76

Total longevity after collection increased with treatment length ($F_{1,44}=156.7$, $p<0.001$).

Average total longevity for survivors of the 7-day treatment was 28.9 days (maximum 42 days), whereas average total longevity for survivors of the 70-day treatment was 90.7 days (maximum 92 days; Figure 2.1). Average total longevity of surviving male and female adult *D. mendotae* was not significantly different within a given treatment group.

Discussion

Total and post-treatment longevities

Previous studies of adult *D. mendotae* have generally focused on short-term longevity. Ferrington *et al.* (2010) measured the longevity of adults held at a constant temperature of 6°C. Average longevities of individuals from different sites ranged between 12.1 and 33 days, with an overall average of 18.6 days (Ferrington *et al.* 2010). Valley Creek, the location used for field collection in our study, was included in Ferrington *et al.* (2010). In our study, all individuals were exposed to sub-freezing temperatures for a minimum of 7 days; however, these data can be extrapolated to successfully estimate the longevity of untreated adult *D. mendotae* as found by Ferrington *et al.* (2010). Mean post-treatment and total longevities (Figure 2.2; Table 2.1) were significantly and linearly related to treatment length. Extrapolation of these regressions predicts an average longevity of 19.75 days for a treatment length of 0 days, which is similar to the average longevity of 18.6 days previously observed by Ferrington *et al.* (2010). This close agreement supports the linear relationship between longevity and length of exposure to sub-freezing temperatures.

Adult *D. mendotae* demonstrate increased longevity when held under sub-freezing temperatures. When buried in snow for up to 28 days, 72.1% of individuals survived longer than 40 days (Anderson *et al.* 2013). Maximum total longevity ranged from 48-66 days in the same study, with a trend of increasing longevity with treatment length (Anderson *et al.* 2013). Our study addressed the implications of longer-term exposure to

controlled sub-freezing conditions (-5°C). Maximum longevities were higher than those found by Anderson *et al.* (2013), ranging from 42-92 days. Thus, we confirm that the total longevity of adult *D. mendotae* is positively related to treatment length.

Total longevity after collection increases with sub-freezing treatment length; however; average post-treatment longevity of adult *D. mendotae* decreases with sub-freezing treatment length. The relationship between treatment length and total longevity reflects the expected physiological effects of sub-freezing treatment. Decreases in temperature reduce the metabolic rate of an individual; thus, an individual undergoing sub-freezing treatment would be expected to use less energy than an untreated individual over the same period of time. At the same time, individuals undergoing longer treatment have less energy remaining at the end of their respective treatment, and thus we would expect a shorter average post-treatment longevity for individuals in the longest treatment groups.

Differences in survivorship between males and females

Both male and female adult *Diamesa mendotae* had decreased survival rates in response to increasing treatment length. This response contrasts with previous studies where all individuals survived sub-freezing treatments of up to 28 days (Anderson *et al.* 2013). Additionally, male and female adult *D. mendotae* had significantly different survival rates to constant temperature sub-freezing treatment, although survivors showed no differences in post-treatment longevity. Thus, we hypothesize that some of the female *D.*

mendotae that did not survive treatment may have had a significantly lower cold-tolerance than surviving females and males.

Reduced cold-tolerance may be due to differences in reproductive status among individual females (Bowler & Terblanche 2008). Carrillo *et al.* (2004) noted that a female adult *D. mendotae* which had oviposited twice had a SCP of -6.02°C . In comparison, females from the same treatment that had oviposited only once had SCPs ranging from -25.55°C to -18.27°C (Carrillo *et al.* 2004). Apparently, a second oviposition event can reduce energy reserves and increase the SCP of female adult *D. mendotae* (Carey 2003; Carrillo *et al.* 2004). Because *D. mendotae* are not known to feed as adults, females have no method of regaining energy stores lost via oviposition. Our sub-freezing treatment of -5°C is similar to the -6.02°C SCP recorded for the twice-ovipositing female reported by Carrillo *et al.* (2004). It is possible that some of our females had oviposited before collection, and thus may not have been capable of surviving the constant sub-freezing treatment in our lab. However, because the reproductive status of individuals in the study was not assessed, we cannot confirm that hypothesis.

Another potential explanation for differences in male and female survivorship is potential variation in the ages of individuals at the time of collection. Adult *D. mendotae* are known to maintain their SCP of -19.7°C for a minimum of 17 days (Bouchard *et al.* 2006; Carillo *et al.* 2004). Longer term data are not available; however, older individuals would be expected to exhibit increased mortality. If age at collection was significantly

higher in our females than males, average survival rates of those individuals would be expected to be lower.

Individual versus population-level implications

Population-level survival of adult *D. mendotae* decreases with increased exposure to sub-freezing temperatures (Figure 2.1). In contrast, the average total longevity of individuals surviving treatment increases with treatment length (Table 2.1). These increases in individual longevity are significant on a population level; average total longevity increases with treatment length even when decreases in survival are taken into account ($F_{1,124}=20.41$, $p<0.001$). Consequently, we expect that exposure to sub-freezing winter temperatures may increase the reproductive success of populations of *D. mendotae*. Average longevity of non-surviving individuals was estimated at half the treatment length, based on the linear relationship between treatment and survivorship (Figure 2.1). Average total longevity within each treatment also significantly differed between male and female adult *D. mendotae* ($F_{2,123}=14.38$, $p=0.008$), reflecting the differences in survivorship by sex.

Conclusions

Winter-emergent adult *Diamesa mendotae* Muttkowski were found to be capable of surviving sub-freezing laboratory treatments at a constant temperature of -5°C for at least 70 days, although survivorship rates were negatively related to treatment length. Based on that finding, we confirm that adult *D. mendotae* are a chill tolerant species (Bale

1996). Although male adults had significantly higher survival rates to constant sub-freezing treatment than females, there were no differences in longevity as a function of sex. Average post-treatment longevity was inversely related to treatment time, whereas total longevity from collection was positively related, likely as a result of reduced metabolic function during treatment. Maximum total longevity from collection was 92 days, including 70 days in treatment held at -5°C . These results suggest that populations of adult *D. mendotae* may increase their reproductive success through their ability to survive unfavorable conditions for extended periods of time before mating.

References Cited

- Anderson, A. M., P. Kranzfelder, R. W. Bouchard Jr., & L. C. Ferrington Jr. 2013. Survivorship and longevity of *Diamesa mendotae* Muttkowski (Diptera: Chironomidae) under snow. *Journal of Entomological and Acarological Research* 45(e6):22-26.
- Bale, J. S. 1996. Insect cold hardiness: a matter of life and death. *European Journal of Entomology* 93:369-382.
- Bouchard Jr., R. W., M. A. Carrillo, & L. C. Ferrington Jr. 2006. Lower lethal temperature for adult male *Diamesa mendotae* Muttkowski (Diptera: Chironomidae), a winter-emerging aquatic insect. *Aquatic Insects* 28(1):57-66.
- Bouchard Jr., R. W. 2007. Richness, taxonomic composition, and phenology of Chironomidae in northern temperate streams with contrasting thermal regimes. In: Bouchard Jr. R. W., Phenology and taxonomic composition of lotic Chironomidae (Diptera) communities in contrasting thermal regimes. (Doctoral Dissertation).
- Bouchard Jr., R. W. & L. C. Ferrington Jr. 2009. Winter growth, development, and emergence of *Diamesa mendotae* (Diptera: Chironomidae) in Minnesota streams. *Environmental Entomology* 38(1):250-259.
- Bowler, K. & J. S. Terblanche. 2008. Insect thermal tolerance: what is the role of ontogeny, ageing, and senescence? *Biological Reviews* 83:339-355.
- Carey, J. R. 2003. Longevity: the biology and demography of lifespan. Princeton University Press, Princeton. pp. 70-71,150-157.

- Carrillo, M. A., C. A. Cannon, L. C. Ferrington Jr. 2004. Effect of sex and age on the supercooling point of the winter-active *Diamesa mendotae* Muttkowski (Diptera: Chironomidae). *Aquatic Insects* 26:243-251.
- Ferrington Jr., L. C., M. A. Blackwood, C. A. Wright, N. H. Crisp, J. L. Kavanaugh, & F. J. Schmidt. 1991. A protocol for using surface-floating pupal exuviae of Chironomidae for rapid bioassessment of changing water quality. *Proceedings of the Vienna Symposium* 181-190.
- Ferrington Jr., L. C., R. W. Bouchard Jr., & B. Karns. 2010. Longevities of *Diamesa mendotae* Muttkowski, a hibernal emerging species of Chironomidae (Diptera). *Proceedings of the XV International Symposium on Chironomidae*, pp. 22-29.
- Kohshima, S. 1984. A novel cold-tolerant insect found in a Himalayan glacier. *Nature* 310:225-227.
- Krider, L. A., J. A. Magner, J. Perry, B. Vondracek, & L. C. Ferrington Jr. 2013. Air-water temperature relationships in the trout streams of southeastern Minnesota's carbonate-sandstone landscape. *Journal of the American Water Resources Association* 00(0): 1-12.
- Lindegaard, C., & K. P. Broderson. Distribution of Chironomidae in the river continuum. In: Cranston, P. (Ed.), *Chironomids: From Genes to Ecosystems*. CSIRO Australia, East Melbourne. pp. 257-272.
- Nolte, U. & T. Hoffmann. 1992. Fast life in cold water: *Diamesa incallida* (Chironomidae). *Ecography* 15(1):25-30.

- O'Driscoll, M. A. & D. R. DeWalle. 2004. Stream-air temperature relationships as indicators of groundwater inputs. *Journal of the American Resources Association Watershed Update* 2 (6).
- Ward, J. V. 1976. Effects of thermal constancy and seasonal temperature displacement on community structure of stream macroinvertebrates. In: Esch, G. W. & R. W. McFarlane (Eds.), *Thermal Ecology II*, Vol. 404. Technical Information Center, Energy Research and Development Administration, Oak Ridge, TN.
- Ward, J. V. & J. A. Stanford. 1982. Thermal responses in the evolutionary ecology of aquatic insects. *Annual Review of Entomology* 27: 97-117.
- Oliver, D. R., & M. E. Dillon. 1997. Chironomids (Diptera: Chironomidae) of the Yukon Arctic North Slope and Herschel Island. In: Danks, H. V. and J. A. Downes (Eds.), *Insects of the Yukon. Biological Survey of Canada (Terrestrial Arthropods)*, Ottawa. pp. 615-635.
- Willassen, E. & P. S. Cranston. 1986. Afrotropical montane midges (Diptera, Chironomidae, *Diamesa*). *Zoological Journal of the Linnean Society* 87:91-123.

Appendix A: Location and groundwater input of study sites

Sites visited in year 1 (2010-2011). Visit 1=early winter, 2=mid-winter, 3=late winter.

Site (County)	Coordinates	Slope	Intercept	Visit	Date	Est. water temp.
Beaver Creek (Winona Co.)	44.1514° N 92.0365° W	0.4429	6.1095	1	11/12/2010	9.0105° C
				2	1/11/2011	-0.4296° C
				3	3/16/2011	4.6764° C
Daley Creek (Houston Co.)	43.7528° N 91.6884° W	0.2038	7.1705	1	1/6/2011	4.9374° C
				2	2/5/2011	4.5924° C
				3	3/26/2011	7.3976° C
Forestville Creek (Fillmore Co.)	43.6389° N 92.2412° W	0.2053	8.2874	1	12/9/2010	6.0218° C
				2	1/27/2011	5.2812° C
				3	4/1/2011	8.0836° C
Garvin Brook (Winona Co.)	44.0070° N 91.8078° W	0.3539	6.3711	1	12/5/2010	4.7179° C
				2	1/12/2011	2.1749° C
				3	3/3/2011	3.8407° C
Gibbon Creek (Fillmore Co.)	43.7076° N 91.9128° W	0.2499	7.0373	1	12/17/2010	3.7486° C
				2	2/4/2011	4.6757° C
				3	3/26/2011	7.1730° C
Hay Creek (Goodhue Co.)	44.4780° N 92.5875° W	0.3881	6.4663	1	12/1/2010	4.7892° C
				2	1/14/2011	1.5568° C
				3	3/30/2011	5.0941° C
Middle Br. Whitewater (Winona Co.)	44.0366° N 92.1002° W	0.5149	6.1346	1	12/29/2010	2.2692° C
				2	2/18/2011	5.6565° C
				3	4/2/2011	5.8073° C
Rush Creek (Winona Co.)	43.9372° N 91.8609° W	0.5107	5.9286	1	1/7/2011	-0.3567° C
				2	2/11/2011	-1.5313° C
				3	4/2/2011	5.5498° C
South Br. Whitewater (Winona Co.)	44.07° N 91.98° W	0.4994	6.5962	1	12/28/2010	3.1432° C
				2	2/17/2011	4.5665° C
				3	4/2/2011	6.2787° C
Trout Run (Winona Co.)	43.8703° N 92.0684° W	0.3057	6.4061	1	12/8/2010	3.3491° C
				2	1/28/2011	2.6329° C
				3	4/2/2011	6.3712° C
W. Indian Creek (Wabasha Co.)	44.2493° N 92.1511° W	0.3247	6.2724	1	11/20/2010	6.5623° C
				2	1/13/2011	1.8124° C
				3	3/4/2011	3.1205° C
Winnebago Creek (Houston Co.)	43.5557° N 91.4505° W	0.3424	6.93	1	12/10/2010	3.4008° C
				2	2/3/2011	4.1566° C
				3	3/15/2011	5.9811° C

Sites visited in year 2 (2011-2012). Visit 1=early winter, 2=mid-winter, 3=late winter.
 NA indicates no available data.

Site (County)	Coordinates	Slope	Intercept	Visit	Date	Est. water temp.
Badger Creek (Houston Co.)	43.6950° N 91.5509° W	0.3543	7.3462	1	11/19/2011	8.2471
				2	1/27/2012	4.3245
				3	3/15/2012	9.8617
Cedar Valley (Winona Co.)	43.9467° N 91.5662° W	0.4738	5.9978	1	12/17/2011	4.7930
				2	1/28/2012	3.3851
				3	3/3/2012	5.7271
Cold Spring Brook (Wabasha Co.)	44.2918° N 92.4335° W	0.2095	6.5848	1	1/12/2012	6.3805
				2	1/27/2012	4.7292
				3	3/14/2012	7.8156
Gilmore Creek (Winona Co.)	44.0294° N 91.7042° W	0.347	6.874	1	1/7/2012	5.7318
				2	1/28/2012	4.9605
				3	3/10/2012	6.9955
Long Creek (Wabasha Co.)	44.2291° N 92.2360° W	NA	NA	1	1/11/2012	NA
				2	1/27/2012	NA
				3	3/16/2012	NA
Money Creek (Winona Co.)	43.9038° N 91.6858° W	NA	NA	1	1/8/2012	NA
				2	1/28/2012	NA
				3	3/9/2012	NA
North Br. Whitewater (Winona Co.)	44.0868° N 92.0249° W	0.5569	6.3026	1	1/7/2012	4.6916
				2	1/27/2012	1.3701
				3	3/9/2012	6.0958
Pickwick Creek (Winona Co.)	43.9628° N 91.5023° W	0.6112	6.4336	1	12/16/2012	4.0281
				2	1/28/2012	3.0633
				3	3/2/2012	5.6216
Pine Creek (Winona Co.)	43.8910° N 91.4721° W	NA	NA	1	12/3/2012	NA
				2	1/28/2012	NA
				3	2/25/2012	NA
Swede Bottom Creek (Houston Co.)	43.7424° N 91.5426° W	0.2799	6.7204	1	12/2/2011	6.7544
				2	1/28/2012	4.9110
				3	2/24/2012	6.4425
Torkelson Creek (Fillmore Co.)	43.7738° N 91.9829° W	0.3223	6.3347	1	11/19/2011	7.0990
				2	1/27/2012	3.6205
				3	2/24/2012	6.0009
Wells Creek (Goodhue Co.)	44.4868° N 92.4329° W	NA	NA	1	1/13/2012	NA
				2	1/27/2012	NA
				3	3/17/2012	NA

Appendix B: Abundances of pupal exuviae

Genera in the sub-families Chironominae, Diamesinae, and Prodiamesinae.

	Genus	<i>Dicrotendipes</i>	<i>Micropsectra</i>	<i>Paratanytarsus</i>	<i>Stictoichironomus</i>	<i>Tanytarsus</i>	Unknown sp.	<i>Diamesa</i>	<i>Pagastia</i>	<i>Odontomesa</i>	<i>Prodiamesa</i>
Site (County)	Visit	Chironominae						Diamesinae		Pro-diamesinae	
Beaver Creek (Winona Co.)	1	0	0	0	0	0	0	0	0	3	0
	2	0	0	0	0	0	0	35	0	1	0
	3	0	1	0	0	0	0	10	1	4	0
Daley Creek (Houston Co.)	1	0	0	0	0	0	0	10	0	0	0
	2	0	0	0	0	0	0	5	0	24	0
	3	1	2	0	4	1	0	0	1	16	3
Forestville Creek (Fillmore Co.)	1	0	0	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	1	0	0	0
	3	0	1	0	0	0	0	0	0	0	0
Garvin Brook (Winona Co.)	1	0	0	0	0	0	0	22	0	0	0
	2	0	0	0	0	0	0	16	0	0	0
	3	0	0	0	0	0	0	34	0	0	0
Gribbon Creek (Fillmore Co.)	1	0	0	0	0	0	0	7	0	0	0
	2	0	0	0	0	0	0	0	0	0	0
	3	0	114	0	0	0	0	3	2	1	5
Hay Creek (Goodhue Co.)	1	0	0	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	2	0	0	0
	3	0	24	0	0	0	0	0	16	11	0
Middle Br. Whitewater (Winona Co.)	1	0	0	0	0	0	0	10	0	0	0
	2	0	0	0	0	0	0	1	0	0	0
	3	0	6	0	0	0	0	2	26	33	1
Rush Creek (Winona Co.)	1	0	0	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0	0	0
	3	0	9	1	0	4	0	1	1	1	0
South Br. Whitewater (Winona Co.)	1	0	0	0	0	0	0	3	0	0	0
	2	0	0	0	0	0	0	65	0	0	0
	3	0	0	0	0	0	0	0	0	0	0

	Genus	<i>Dicrotendipes</i>	<i>Micropsectra</i>	<i>Paratanytarsus</i>	<i>Stictoironomus</i>	<i>Tanytarsus</i>	Unknown sp.	<i>Diamesa</i>	<i>Pagastia</i>	<i>Odontomesa</i>	<i>Prodiamesa</i>
Site (County)	Visit	Chironominae						Diamesinae		Pro-diamesinae	
Trout Run (Winona Co.)	1	0	0	0	0	0	0	23	0	0	0
	2	0	0	0	0	0	0	28	0	0	0
	3	0	3	0	0	1	0	10	6	18	1
W. Indian Creek (Wabasha Co.)	1	0	0	0	0	0	0	1	0	5	0
	2	0	0	0	0	0	0	36	0	0	0
	3	0	0	0	0	0	0	33	0	8	0
Winnebago Creek (Houston Co.)	1	0	0	0	0	0	0	1	0	0	0
	2	0	0	0	0	0	0	0	0	0	0
	3	0	55	0	0	0	0	5	0	12	0
Badger Creek (Houston Co.)	1	0	0	0	0	0	0	2	1	0	1
	2	0	0	0	0	0	0	1	0	0	0
	3	0	2	0	0	0	0	0	0	1	0
Cedar Valley (Winona Co.)	1	0	1	0	0	0	0	71	0	0	0
	2	0	1	0	0	0	0	57	0	0	0
	3	0	0	0	0	0	0	2	0	0	0
Cold Spring Brook (Wabasha Co.)	1	0	0	0	0	0	0	1	0	0	0
	2	0	0	0	0	0	0	8	0	0	0
	3	0	2	0	0	0	0	2	0	1	0
Gilmore Creek (Winona Co.)	1	0	1	0	0	0	0	48	0	5	0
	2	0	0	0	0	0	0	41	0	5	0
	3	0	1	0	0	0	0	6	0	6	0
Long Creek (Wabasha Co.)	1	0	4	0	0	0	0	18	0	0	0
	2	0	4	0	0	0	0	16	0	3	0
	3	0	34	0	0	0	0	0	0	14	1
Money Creek (Winona Co.)	1	0	0	0	0	0	0	41	0	1	0
	2	0	0	0	0	0	0	2	0	0	0
	3	0	0	0	0	0	0	3	0	1	0
North Br. Whitewater (Winona Co.)	1	0	0	0	0	0	0	223	0	4	0
	2	0	0	0	0	0	0	137	0	1	0
	3	0	0	0	0	0	0	0	0	0	0

	Genus	<i>Dicrotendipes</i>	<i>Micropsectra</i>	<i>Paratanytarsus</i>	<i>Stictoironomus</i>	<i>Tanytarsus</i>	Unknown sp.	<i>Diamesa</i>	<i>Pagastia</i>	<i>Odontomesa</i>	<i>Prodiamesa</i>
Site (County)	Visit	Chironominae						Diamesinae		Pro-diamesinae	
Pickwick Creek (Winona Co.)	1	0	0	0	0	0	0	288	0	0	0
	2	0	2	0	0	0	1	152	0	2	0
	3	0	2	0	0	0	0	2	0	5	0
Pine Creek (Winona Co.)	1	0	0	0	0	0	0	97	0	2	0
	2	0	1	0	0	0	0	44	0	2	0
	3	0	1	0	0	0	0	13	0	0	0
Swede Bottom Creek (Houston Co.)	1	0	1	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	1	0	0	0
Torkelson Creek (Fillmore Co.)	1	0	1	0	0	0	0	0	0	3	0
	2	0	1	0	0	0	0	126	0	2	0
	3	0	0	0	0	0	0	43	0	1	0
Wells Creek (Goodhue Co.)	1	0	0	0	0	0	0	2	0	0	0
	2	0	0	0	0	0	0	4	0	1	0
	3	0	5	0	0	0	0	1	0	59	0
TOTAL		1	279	1	4	7	1	3632	54	256	12

Common genera in the sub-family Orthocladiinae (found in more than 2 samples).

	Genus	<i>Chaetocladius</i>	<i>Corynoneura</i>	<i>Cricotopus</i>	<i>Eukiefferiella</i>	<i>Heterotrissocladius</i>	<i>Limnophyes</i>	<i>Orthocladius</i>	<i>Euorthocladius</i>	<i>O. Orthocladius</i>	<i>Parakiefferiella</i>	<i>Parametriochenus</i>	<i>Synorthocladius</i>	<i>Thienemaniella</i>	<i>Tvetenia</i>
Site (County)	Visit	Orthocladiinae													
Beaver Creek (Winona Co.)	1	0	0	0	0	0	0	0	2	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	3	2	0	31	0	1	0	6	241	0	0	0	0	0	7
Daley Creek (Houston Co.)	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	62	0	0	0	0	0	0
	3	0	1	1	0	0	0	0	7	0	0	1	0	0	0
Forestville Creek (Fillmore Co.)	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0	2	0	0	0	0	0	0
Garvin Brook (Winona Co.)	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0	117	0	1	0	0	0	0
Gibbon Creek (Fillmore Co.)	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	2	1	0	0	0	0	0	0	1	0	0	0	0	0	0
	3	0	17	13	21	0	1	0	6	0	0	0	3	5	0
Hay Creek (Goodhue Co.)	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	3	0	46	96	6	2	0	1	35	1	0	1	6	16	0
Middle Br. Whitewater (Winona Co.)	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	3	0	3	4	25	2	0	0	249	27	0	0	2	10	0
Rush Creek (Winona Co.)	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	3	0	6	1	2	6	0	0	3	2	1	0	0	0	0
South Br. Whitewater (Winona Co.)	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0
	2	1	0	0	0	0	0	44	0	0	0	0	0	0	0
	3	0	0	1	4	0	0	0	33	15	0	0	0	11	0

	Genus	<i>Chaetocladius</i>	<i>Corynoneura</i>	<i>Cricotopus</i>	<i>Eukiefferiella</i>	<i>Heterotrissocladius</i>	<i>Limnophyes</i>	<i>Orthocladius</i>	<i>Euorthocladius</i>	<i>O. Orthocladius</i>	<i>Parakiefferiella</i>	<i>Parametriochenus</i>	<i>Synorthocladius</i>	<i>Thienemaniella</i>	<i>Tvetenia</i>
Site (County)	Visit	Orthoclaadiinae													
Trout Run (Winona Co.)	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	2	1	0	0	0	0	0	0	0	0	0	0	0	0	0
	3	0	31	227	17	0	0	3	127	4	1	0	9	0	
W. Indian Creek (Wabasha Co.)	1	0	1	7	1	0	0	0	16	6	0	0	0	0	
	2	0	0	0	0	0	0	0	0	0	0	0	0	0	
	3	1	0	0	0	0	0	0	23	0	0	0	0	0	
Winnebago Creek (Houston Co.)	1	0	0	0	0	0	0	0	0	0	0	0	0	0	
	2	0	0	0	0	0	0	0	0	0	0	0	0	0	
	3	0	0	29	6	0	1	0	38	0	0	0	0	8	
Badger Creek (Houston Co.)	1	0	0	3	0	0	0	0	3	0	0	3	0	16	
	2	0	0	0	0	0	0	0	2	0	0	0	0	1	
	3	0	0	1	1	0	0	0	0	0	1	0	0	4	
Cedar Valley (Winona Co.)	1	0	0	0	0	0	0	0	0	0	0	0	0	0	
	2	44	0	0	0	0	0	0	0	0	0	0	0	1	
	3	0	0	0	0	0	0	0	4	0	0	0	0	1	
Cold Spring Brook (Wabasha Co.)	1	0	0	0	0	0	0	0	0	0	0	0	0	0	
	2	0	0	0	0	0	0	0	0	0	0	0	0	1	
	3	0	1	9	0	0	1	0	3	0	0	0	0	7	
Gilmore Creek (Winona Co.)	1	0	0	0	0	0	0	0	0	0	0	0	0	0	
	2	4	0	0	0	0	0	0	0	0	0	0	0	0	
	3	1	0	9	1	0	0	0	14	0	0	0	1	2	
Long Creek (Wabasha Co.)	1	0	0	0	0	1	0	0	0	0	0	0	0	3	
	2	8	0	0	0	2	0	0	1	0	0	0	0	1	
	3	3	0	234	111	0	1	4	7	1	0	0	0	47	
Money Creek (Winona Co.)	1	0	0	0	0	0	0	0	0	1	0	0	0	1	
	2	1	0	0	0	0	0	0	0	0	0	0	0	0	
	3	0	0	0	0	0	0	0	9	2	0	0	0	1	
North Br. Whitewater (Winona Co.)	1	0	0	1	0	0	0	0	1	0	0	0	0	0	
	2	1	0	0	0	0	0	1	1	1	0	0	0	0	
	3	0	0	0	1	0	0	1	2	2	0	0	0	0	

	Genus	<i>Chaetocladius</i>	<i>Corynoneura</i>	<i>Cricotopus</i>	<i>Eukiefferiella</i>	<i>Heterotrissocladius</i>	<i>Limnophyes</i>	<i>Orthocladius</i> <i>Euorthocladius</i>	<i>O. Orthocladius</i>	<i>Parakiefferiella</i>	<i>Parametriochenus</i>	<i>Synorthocladius</i>	<i>Thienemaniella</i>	<i>Tvetenia</i>
Site (County)	Visit	Orthoclaadiinae												
Pickwick Creek (Winona Co.)	1	0	0	1	0	0	0	0	0	0	0	0	0	0
	2	5	0	2	0	0	0	0	1	0	0	0	0	0
	3	0	0	0	4	0	0	1	16	1	0	0	0	4
Pine Creek (Winona Co.)	1	0	0	1	0	0	0	0	5	0	0	0	0	3
	2	7	0	0	0	0	0	0	0	0	0	0	0	0
	3	7	0	0	0	0	0	0	10	0	0	0	0	2
Swede Bottom Creek (Houston Co.)	1	0	0	0	0	0	0	0	2	0	0	0	0	0
	2	0	0	0	0	0	0	0	0	0	0	0	0	0
	3	3	0	0	0	0	1	0	4	0	0	0	0	1
Torkelson Creek (Fillmore Co.)	1	0	0	5	1	0	0	0	0	1	0	0	0	0
	2	11	0	0	0	0	0	0	0	0	0	0	0	0
	3	9	0	2	0	0	0	0	20	0	0	0	0	1
Wells Creek (Goodhue Co.)	1	0	0	0	0	0	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0	0	0	0	0	0
	3	0	0	3	1	0	0	1	49	81	0	0	0	33
	TOTAL	110	106	681	202	14	5	65	1118	145	4	5	21	187

Uncommon genera in the sub-family Orthoclaadiinae (found in less than 3 samples). Sites without uncommon genera are omitted.

	<i>Genus</i>	<i>Brillia</i>	<i>Diplocladius</i>	<i>Hydrobaenus</i>	<i>Nanocladius</i>	<i>Orthocladus</i> (<i>Symposiocladus</i>)	<i>Parachaetocladus</i>	<i>Paracladius</i>
Site (County)	Visit	Orthoclaadiinae						
Gibbon Creek (Fillmore Co.)	1	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0
	3	1	0	0	0	0	0	0
Middle Br. Whitewater (Winona Co.)	1	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0
	3	0	0	1	0	0	0	0
Rush Creek (Winona Co.)	1	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0
	3	1	0	0	0	0	0	0
Trout Run (Winona Co.)	1	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0
	3	0	0	0	2	0	0	0
W. Indian Creek (Wabasha Co.)	1	0	0	0	0	0	0	1
	2	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0
Badger Creek (Houston Co.)	1	0	0	0	0	1	0	0
	2	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0
Long Creek (Wabasha Co.)	1	0	0	0	0	0	0	0
	2	0	0	0	1	0	0	0
	3	0	41	0	0	0	1	0
TOTAL		2	41	1	3	1	1	1

Appendix C: Thermal partitioning of winter-emerging chironomid sub-families and genera. Only genera occurring in more than one emergence event are included.

