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Exposure of cold-adapted *Diamesa mendotae* Muttkowski, 1915 (Diptera: Chironomidae) to short-term high temperature reduces longevity and reproduction

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ABSTRACT
Climate change is increasing mean winter temperatures and the frequency of short-term high temperatures. Winter-emerging aquatic insects require an extended cold period to develop and may be negatively impacted by high winter air temperatures. *Diamesa mendotae* Muttkowski, 1915 is a cold-adapted, winter-emerging chironomid common in groundwater-dominated streams in Minnesota. Previous studies have found constant exposure to high air temperatures reduced adult *D. mendotae* survivorship, but not how short-term high temperature exposure may affect *D. mendotae* survivorship and reproduction. We found short-term exposure (24 or 48h) to 22 °C decreased adult *D. mendotae* longevity and reduced egg laying and larval hatch success, which may reduce future *D. mendotae* population sizes. Disruptions in *D. mendotae* and other cold-adapted insect populations may have broad ramifications for groundwater-fed stream ecosystems. Our study highlights the need for further research on cold-adapted insect survivorship after short-term winter temperature spikes to understand impacts of climate change beyond mean annual temperature increases.

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Climate change; winter; Chironomidae; stream ecology; *Diamesa mendotae*

Introduction

Global mean surface air temperatures warmed 1.09 °C from 1850–1900 to 2011–2020 because of post-industrial greenhouse gas emissions (Gulev et al. 2021). Global mean surface air temperature is predicted to reach +1.5 °C by the early 2030s without extreme emission mitigation (Gulev et al. 2021). However, the intensity of winter warming is greater than other seasons (Liess et al. 2022; Notaro, Lorenz, Hoving, and Schummer 2014; Vose, Easterling, Kunkel, LeGrande, and Wehner 2017), and arctic and high-latitude regions are warming faster than the rest of the planet (Gulev et al. 2021; Liess et al. 2022; Post et al. 2019; Taylor, Maslowski, Perlwitz, and Wuebbles 2017). Mean December-March temperatures in the Upper Midwest of the United

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States are projected to increase 5.97 °C by 2100 in the most severe climate predictions (Notaro et al. 2014).

The frequency and intensity of warm periods are becoming more common in winter. Research in England concluded 5-day winter warm periods above 12–13 °C occurred every 2–7 years before 1910, but now occur every 0.5–3 years (Chapman, Murphy, Stainforth, and Watkins 2020). In the United States in northern Wisconsin, the number of days surface lake temperatures exceeded 20 °C in spring and fall increased by 0.72 days per year, or 25 additional days between 1981 and 2015 (Winslow, Read, Hansen, Rose, and Robertson 2017). Taken together, these predictions and temperature trends for northern-latitude winters indicate climate change may have significant impacts on winter-active organisms in the Holarctic Region.

The cold-adapted, winter-active chironomid *Diamesa mendotae* Muttkowski, 1915 is a common member of Minnesota groundwater-fed streams (e.g., Anderson, Kranzfelder, Bouchard, and Ferrington 2013; Hansen and Cook 1976; Mazack et al. 2014). Adult *D. mendotae* emergence is temperature dependent and begins in fall when mean water temperatures drop below 10 °C and ceases in spring when mean water temperatures rise above 10 °C (Bouchard and Ferrington 2009). Adult *D. mendotae* are freeze-intolerant, but are capable of supercooling below −20 °C before freezing, enabling them to walk, fly, and reproduce on the snow throughout winter (Bouchard, Carrillo, and Ferrington 2006; Carrillo, Cannon, and Ferrington 2004).

Mean *D. mendotae* longevity is 18.6 days at 6 °C (Ferrington, Bouchard, and Karns 2010) but increases with incubation at colder temperatures (Anderson et al. 2013, 2023; Mazack et al. 2014). Anderson et al. (2023) also reported longer longevities for *D. mendotae* held at 2 °C and 6 °C than those exposed to ambient outdoor air temperatures that ranged from −10 °C lows and 15 °C highs most days. Additionally, Icelandic species of *Diamesa* Meigen, 1835 had significantly lower longevities when incubated at 20 °C compared to 6 °C, with a mean longevity of 19.9 days at 6 °C compared to 3.8 days at 20 °C (Nyquist, Gíslason, Vondracek, and Ferrington 2021). Although *D. mendotae* is not found in Iceland, measured longevities for other *Diamesa* are likely applicable to *D. mendotae*. Therefore, we designed an experiment to examine the effects of short-term winter temperature spikes on *D. mendotae* to reflect the temperature variation cold-adapted insects may experience with climate change.

Climate change may directly affect *D. mendotae* reproductive success by changing oviposition, embryogenesis, or larval hatch rates, or may indirectly affect reproduction by reducing longevity and therefore shortening the total reproductive lifespan. Bouchard and Ferrington (2009) reported *D. mendotae* egg masses hatched 7.3 days on average after oviposition at ≈5 °C. Anderson et al. (2023) reported *Diamesa* females held at 6 °C had higher oviposition rates than those at 2 °C and those held outside in ambient temperatures. Females that oviposited also had significantly higher longevities than those that did not oviposit (Anderson et al. 2023). Schütz and Füreder (2019) also found European *Diamesa* eggs developed faster as temperature treatments increased from ≈2 °C to ≈12 °C, but hatching success rates were similar at all temperatures. *Diamesa mendotae* egg development is temperature dependent; therefore, more research is needed to determine how climate change and short-term temperature spikes will affect the timing of *D. mendotae* reproduction in response to winter warming.

The objectives of this study were to examine longevity and reproduction in *D. mendotae* subjected to short-term temperature spikes to expand our understanding of the effects of climate change on winter-active insects. We collected *D. mendotae* from two Minnesota groundwater-fed streams and reared them at either constant 6 °C or 6 °C with 24- or 48-h exposures to 22 °C. Our short-term high temperature exposure of 22 °C demonstrates the effect of climate change in the case of severe winter warming likely to occur at the beginning and end of the *D. mendotae* emergence period. Although we were not able to test a range of temperatures or exposure periods, our study serves as a range-finding test to determine the effects of extreme exposure to short-term temperature spikes. We hypothesised that 22 °C exposure would reduce mean longevity, and reduce oviposition and larval hatch rates of *D. mendotae*. As climate change increases mean winter temperatures and frequencies of winter warm periods, *D. mendotae* population dynamics could be altered with potential widespread repercussions in groundwater stream ecosystems.

Material and methods

Field sites and Chironomidae collections

Chironomidae were collected from Ike's Creek in Hennepin County and Pickwick Creek in Winona County, Minnesota (Figure 1, Table 1). Collections were made from Ike's Creek on 5 January 2020, and 23 January 2020, from two contiguous sites along the stream: Site 1 from 44.849°N, 93.236°W and Site 2 from 44.847°N,

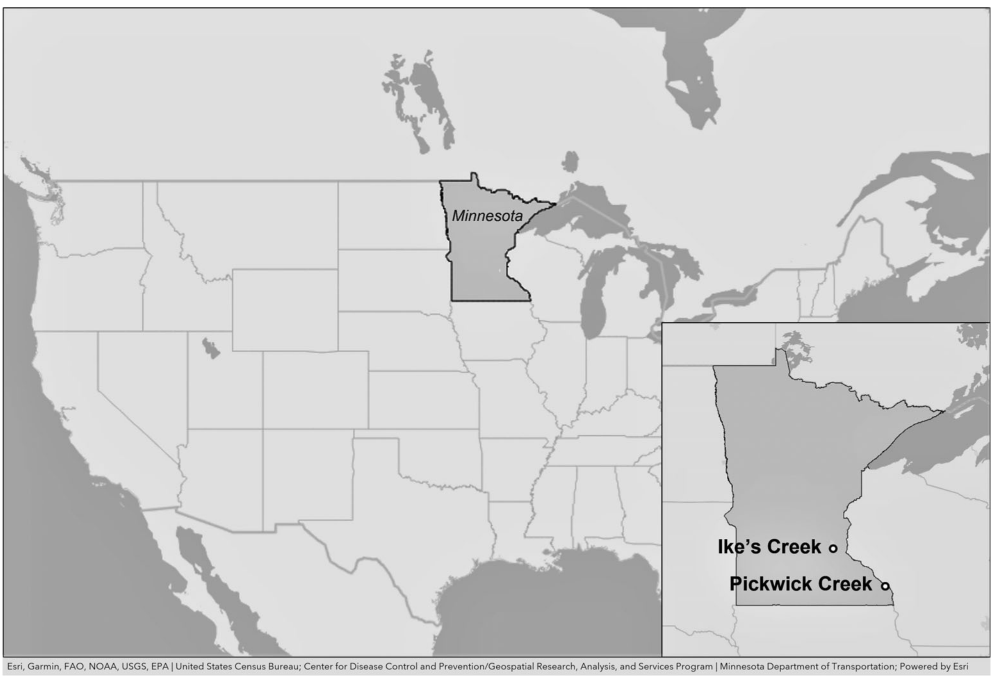

Figure 1. Stream locations for *Diamesa mendotae* Muttkowski, 1915 collections. Map created with ArcGIS® software by Esri with map layer data from the Center for Disease Control and Prevention (2020) and the Minnesota Department of Transportation (2022).

Table 1. Summary of *Diamesa mendotae* Muttkowski, 1915 collections in winter 2019–2020 and 2020–2021.

Stream	Date	Site	Males	Females
Ike's Creek	1/5/2020	2	36	6
	1/23/2020	1	47	4
Pickwick Creek	1/4/2021	1	113	34
		2	105	40

Note. Female *Diamesa* were assumed to be *D. mendotae* due to high numbers of male *D. mendotae*. Ike's Creek sites 1 and 2 were combined for all analyses. Pickwick Creek sites 1 and 2 collections were combined for reproduction analyses to increase sample size of females that oviposited.

93.233°W to the stream terminus at Long Meadow Lake. One collection was made from Pickwick Creek on 4 January 2021, from two discontiguous sites: Site 1 at 43.951°N, 91.511°W and Site 2 at 43.960°N, 91.504°W.

Adult *Diamesa* walking on snow were scooped with a small amount of snow into 1-dram snap-top vials following protocol from Ferrington et al. (2010). Our collection method is similar to previous studies of *D. mendotae* (Anderson et al. 2023; Ferrington et al. 2010; Mazack et al. 2014). However, *D. mendotae* could be several days old when collected off the snow, so longevities reported in this study represent minimum adult lifespans.

After collection, *D. mendotae* were transported to the University of Minnesota in coolers packed with snow to prevent warming. Vials were removed from the coolers and placed in an incubator (Percival E-54 U) at 6 °C, a temperature consistent with previous *D. mendotae* longevity studies (Anderson and Ferrington 2013; Ferrington et al. 2010). Melted snow in the vials hydrated the midges, but no food was provided, because adult *D. mendotae* are not known to feed (Bouchard and Ferrington 2009).

Temperature treatment groups

Individuals were separated by sex and split into groups at random to ensure similar numbers of males and females among experimental groups and to account for different ages at collection. A control group was returned to constant 6 °C and treatment groups were placed in an incubator at 22 °C for 24 or 48h before being returned to 6 °C for the remainder of the experiment. Ike's Creek treatment groups were moved to the 22 °C incubator two days after collection, whereas Pickwick Creek treatment groups were moved three days after collection, to ensure the temperature was stabilised at 22 °C. The sizes and number of treatment groups depended on the number of midges collected at each site, with the goal to have approximately 20 individuals of at least one sex per experimental group.

Experimental temperature selection

Diamesa mendotae pupal exuviae were collected from Minnesota groundwater-dominated streams between October and May (Bouchard and Ferrington 2009). The National Weather Service reported daily maximum temperatures for Minneapolis, MN during the emergence periods of *D. mendotae* (available at www.weather.gov/wrh/climate?wfo=mpx). There were 38 days with maxima above 22 °C (8% of days) and 78 days with maxima above 18 °C (16% of days) in October 2019–May 2020 and October 2020–May 2021.

The majority of these high temperature days occurred in May, at the end of *D. mendotae*'s emergence period. Anderson et al. (2023) also reported daily high temperatures of 7–15 °C in March, with 18 °C on one date in western Minnesota.

If climate change models accurately predict future mean Minnesota winter temperatures, then a 5.97 °C winter temperature increase as predicted in Notaro et al. (2014) could increase the number of days temperature maxima exceed 22 °C during the emergence period of *D. mendotae* by the end of the century. Therefore, 22 °C was chosen as the exposure temperature to model the most extreme effects of climate change on *D. mendotae* possible by the end of the century. This temperature is also similar to the 20 °C treatment group investigated by Nyquist et al. (2021) to evaluate high temperature exposure on *Diamesa* in Iceland.

Longevity assessments and analyses

Vials containing *D. mendotae* were kept on ice and only handled when necessary to avoid excessive warming during observation. Individuals were inspected daily to record the date of death following Ferrington (2019). Motionless individuals were examined under 12X magnification to ensure they did not exhibit any minute movements. Dead individuals were preserved in >70% ethanol and identified to species (male) or genus (female) using Hansen and Cook (1976). No guide exists to identify females to species; therefore, female *Diamesa* were assumed to be *D. mendotae* due to the abundance of male *D. mendotae*. Male *D. nivoriunda* were collected in low numbers, thus some females identified as *D. mendotae* may have been *D. nivoriunda*. The data from both collections from Ike's Creek were combined because there were no statistically significant differences between *D. mendotae* longevities at both sites.

Differences in survivorship among experimental groups were assessed with boxplots and Kaplan–Meier survivorship curves for each treatment group, as boxplots display data distribution, and Kaplan–Meier curves illustrate survivorship over time. All figures were produced using RStudio 1.4.1717 (R Core Team 2021) with *ggplot2* package (v3.3.5; Wickham 2016). Packages *survival* (v3.2-13; Therneau 2020) and *survminer* (v0.4.9; Kassambara, Kosinski, Biecek, and Fabian 2021) were used in addition to produce Kaplan–Meier curves. JMP® Pro 15.2.0 (JMP® 2019) was used to perform statistical analyses. A Shapiro-Wilk test was used to determine if each treatment group was normally distributed. The constant 6 °C and 22 °C/48 hr groups from Pickwick were not normally distributed; therefore, non-parametric tests were performed for Pickwick analyses (Wilcoxon test for two treatments, Kruskal–Wallis with post hoc Steel–Dwass All Pairs test for more than two treatments). Ike's Creek *D. mendotae* longevities were normally distributed, so parametric tests were used. Log-rank tests were used to identify differences between Kaplan–Meier curves for both streams.

Reproductive assessments and analyses

Female *Diamesa* were inspected daily to determine whether oviposition occurred. Embryogenesis occurred when the egg interior condensed and darkened into a visible larval body. Larval hatch was recorded when the first larva was observed emerging from an egg mass. Only *Diamesa* from Pickwick Creek were used in reproduction analyses because Ike's Creek female sample sizes were small (Table 1). Data for reproduction were combined between both Pickwick Creek sites to increase statistical power.

RStudio with *ggplot2* was used to produce figures and JMP® was used for statistical analyses of reproductive data. Shapiro-Wilks tests were used to determine normality in all treatment groups. Non-parametric tests were used to evaluate differences in reproductive data because several treatments were not normally distributed and sample sizes were small. A Fisher's Exact Test was used to compare the proportion of females that did not oviposit or did not produce viable eggs to females whose eggs hatched into larvae.

Results

Diamesa mendotae *longevity decreased after 22 °C exposure*

An ANOVA indicated collection date had no effect on Ike's Creek *D. mendotae* longevity ($F = 0.005$, $p = 0.944$). Sex and oviposition status both significantly affected longevity across treatments, as non-ovipositing females had significantly lower longevities than males ($F = 4.576$, $p = 0.013$). However, low sample sizes for female *D. mendotae* in Ike's Creek limit conclusions relative to oviposition.

Mean and median longevity in male and female *D. mendotae* from Ike's Creek were not statistically significant between temperature groups (Figure 2). Kaplan–Meier

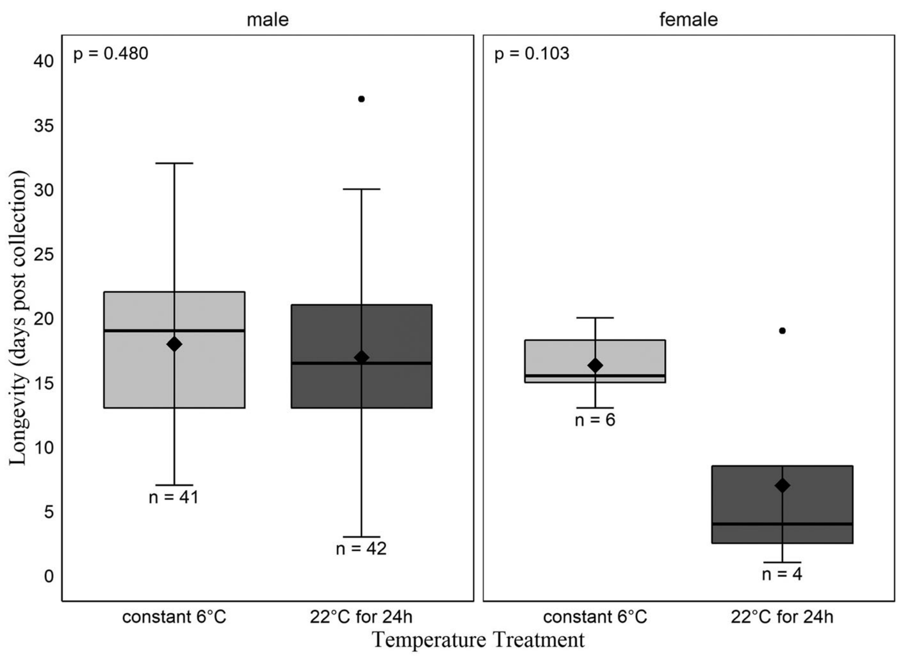

Figure 2. Male and female *Diamesa mendotae* Muttkowski, 1915 longevity from Ike's Creek combined collections from 5 January 2020 and 23 January 2020. Box and whisker boundaries signify the maximum, 75th percentile, median, 25th percentile, and minimum longevity values. Black diamonds (◆) indicate mean longevity and dots (•) indicate outliers. No significant differences were found between constant 6 °C and 22 °C for 24h groups (*T*-test).

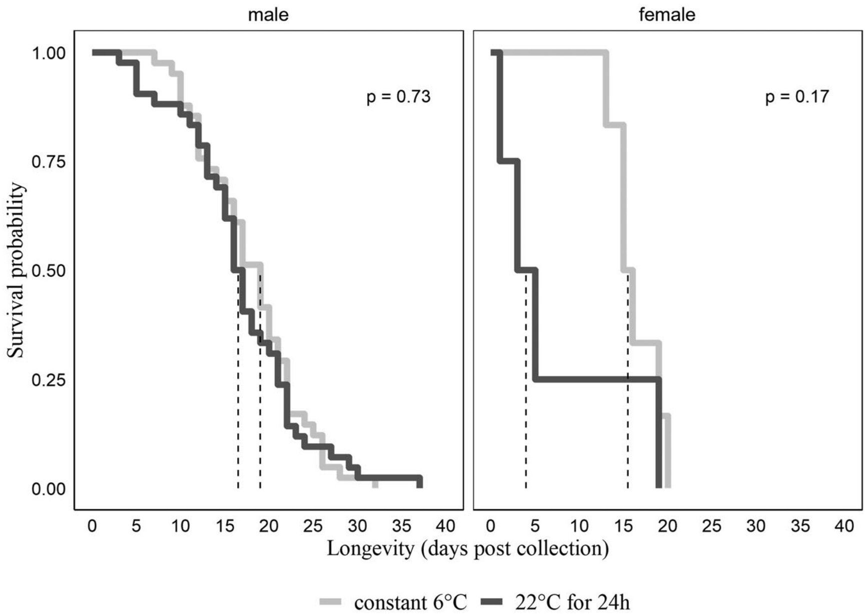

Figure 3. Male and female *Diamesa mendotae* Muttkowski, 1915 survivorship curves for combined collections from Ike's Creek from 5 January 2020 and 23 January 2020. Kaplan–Meier survivorship curves indicate the proportion of individuals alive on a given day. Vertical dashed lines indicate longevity values at 50% survivorship in each group. There were no significant differences between treatment groups (Log-Rank test).

curves for male survivorship followed similar patterns over time between treatment groups and sites, with relatively linear progressions from 100% to 0% survivorship (Figure 3).

A nonparametric Wilcoxon test indicated stream site had no effect on *D. mendotae* longevity in Pickwick Creek across treatments. Non-ovipositing females had significantly lower longevities than ovipositing females and males across treatments ($p < 0.001$), but the longevity of combined ovipositing and non-ovipositing females did not differ significantly from males ($p = 0.076$). Sex and oviposition status had no effect on *D. mendotae* longevity within each temperature treatment.

Males and females collected from Pickwick Creek Sites 1 and 2 had lower mean and median longevity with 22 °C exposure compared to constant 6 °C individuals (Figure 4). However, no significant differences were found between treatment groups from Pickwick Creek Site 1 (Figure 4). Significant differences were found for Pickwick Creek Site 2 males between constant 6 °C and 22 °C/48h groups ($p = 0.003$), and females between constant 6 °C and 22 °C/24h groups ($p = 0.042$) and constant 6 °C and 22 °C/48h groups ($p = 0.007$) (Figure 4). Kaplan–Meier curve log-rank analyses confirmed these differences and also indicated a significant difference between Site 2 males at constant 6 °C and 22 °C/24h ($p = 0.028$) (Figure 5). There were no differences between 24 and 48h exposure groups based on Kruskal–Wallis and Kaplan–Meier log-rank analyses. Therefore, short-term exposure to 22 °C reduced *D. mendotae* longevity, but depended on the collection site on Pickwick Creek.

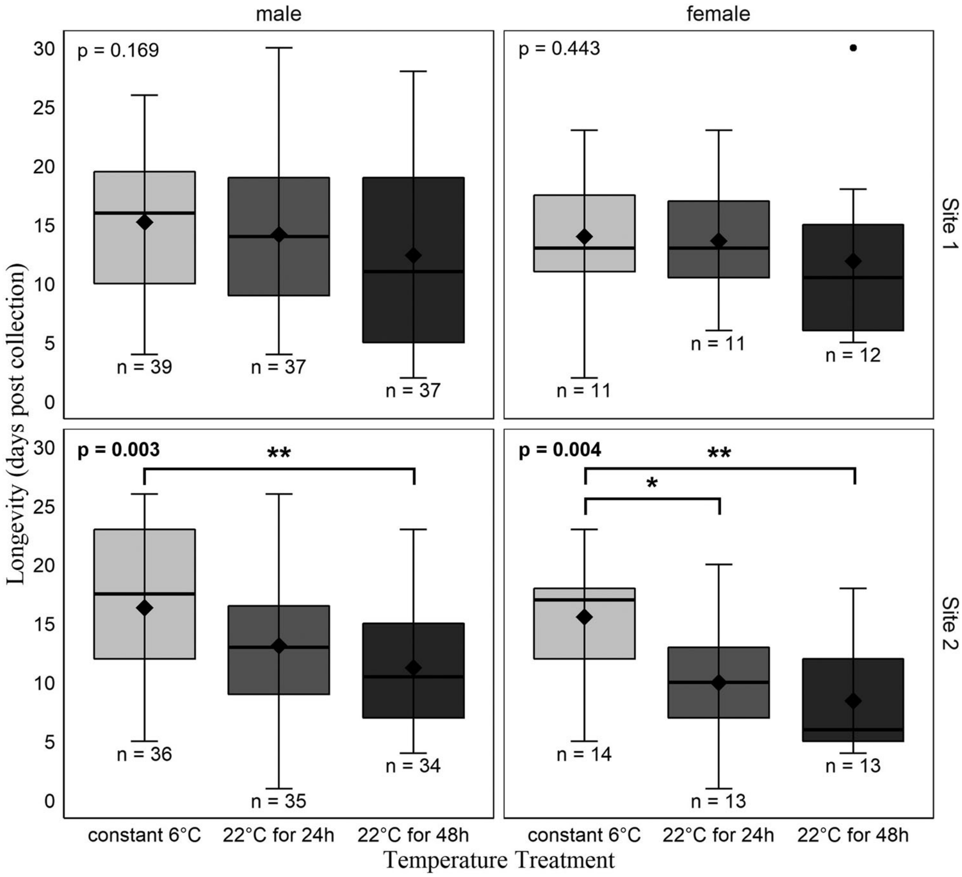

Figure 4. Male and female *Diamesa mendotae* Muttkowski, 1915 longevity from Pickwick Creek Sites 1 and 2 collected on 4 January 2021. Box and whisker boundaries signify the maximum, 75th percentile, median, 25th percentile, and minimum longevity values. Black diamonds (◆) indicate mean longevity and dots (•) indicate outliers. Asterisks denote significant differences (*$p < 0.05$; **$p < 0.01$) between control and treatment groups (Kruskal–Wallis with post-hoc Steel–Dwass All Pairs test).

Reproduction decreased with 22 °C exposure

Oviposition rate was 88% in constant 6 °C groups, 67% for 22 °C/24h, and 72% for 22 °C/48h groups; but were not significantly different (Fisher's test). The date of oviposition, embryogenesis, and larval hatch were also not significantly different among treatment groups (Table 2).

Oviposition dates for females in each treatment were grouped based on occurrence of embryogenesis to determine if the timing of oviposition affected probability of embryogenesis (Figure 6). Embryogenesis occurred more often in eggs that were oviposited earlier than those oviposited later (Figure 6). However, significant differences between oviposition dates with or without embryogenesis were only found in constant 6 °C and 22 °C/48h groups (Figure 6). Eggs that underwent embryogenesis were oviposited 2.6, 1.7, and 2.8 days after collection, whereas eggs that did not undergo

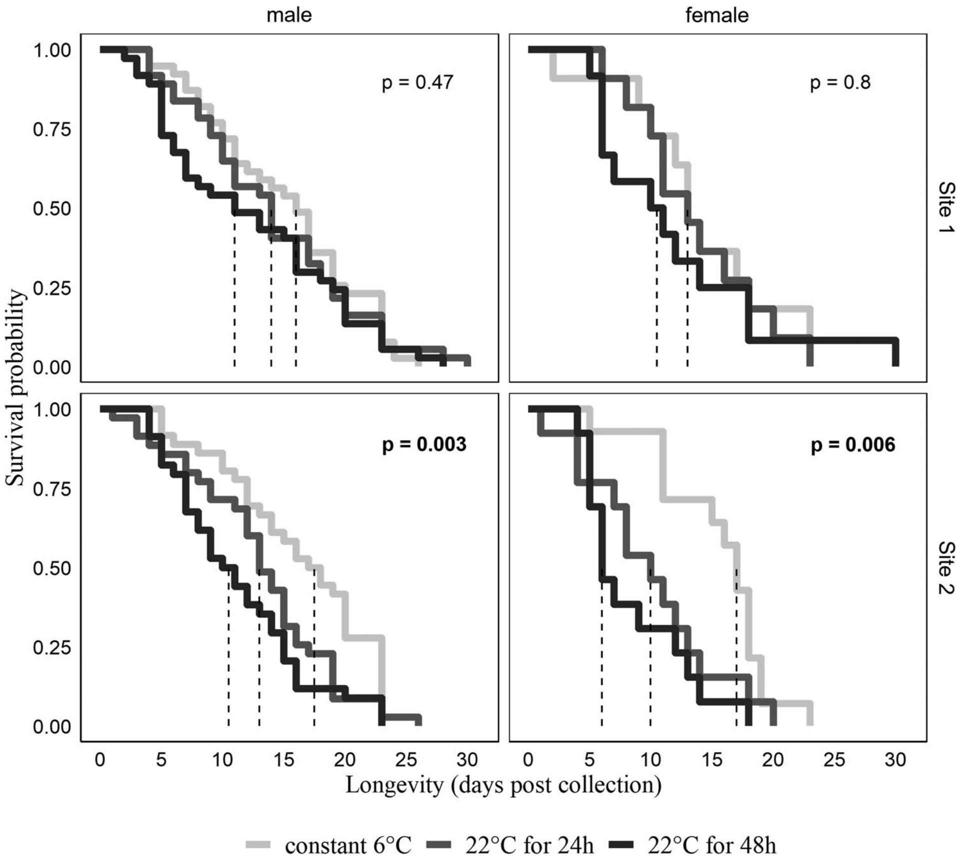

Figure 5. Male and female *Diamesa mendotae* Muttkowski, 1915 survivorship curves from Pickwick Creek Sites 1 and 2 on 4 January 2021. Kaplan–Meier survivorship curves indicate the proportion of individuals alive on a given day. Vertical dashed lines indicate longevity values at 50% survivorship in each group. Individual survivorship was significantly different among treatment groups in Pickwick Site 2 (Log-Rank test).

Table 2. *Diamesa mendotae* Muttkowski, 1915 oviposition, embryogenesis, and larval hatch timing collected from Pickwick Creek on 4 January 2021.

Treatment	Oviposition *days post collection*	Embryogenesis *days post oviposition*	Larval hatch *days post oviposition*
Constant 6 °C	4.4	2.1	8.6
22 °C for 24h	4.3	2.2	8.17
22 °C for 48h	3.8	1.7	7*
p-value	*0.764*	*0.448*	*0.090*

Note. One outlier from the 22 °C/48h oviposition group (oviposition = 26 days) was removed because it was greater than three standard deviations from the mean. Asterisk (*) denotes only one egg mass hatched into larvae in the 22 °C/48h group. No significant differences were found between treatment groups (Kruskal–Wallis test).

embryogenesis were oviposited 6.3, 5.2, and 5.2 days after collection in constant 6 °C, 22 °C/24h, and 22 °C/48h groups, respectively (Figure 6).

Females were categorised into groups of those whose egg masses did or did not develop into larvae to determine the effects of 22 °C exposure on larval hatch rate (Figure 7). The proportion of females in each category varied significantly between

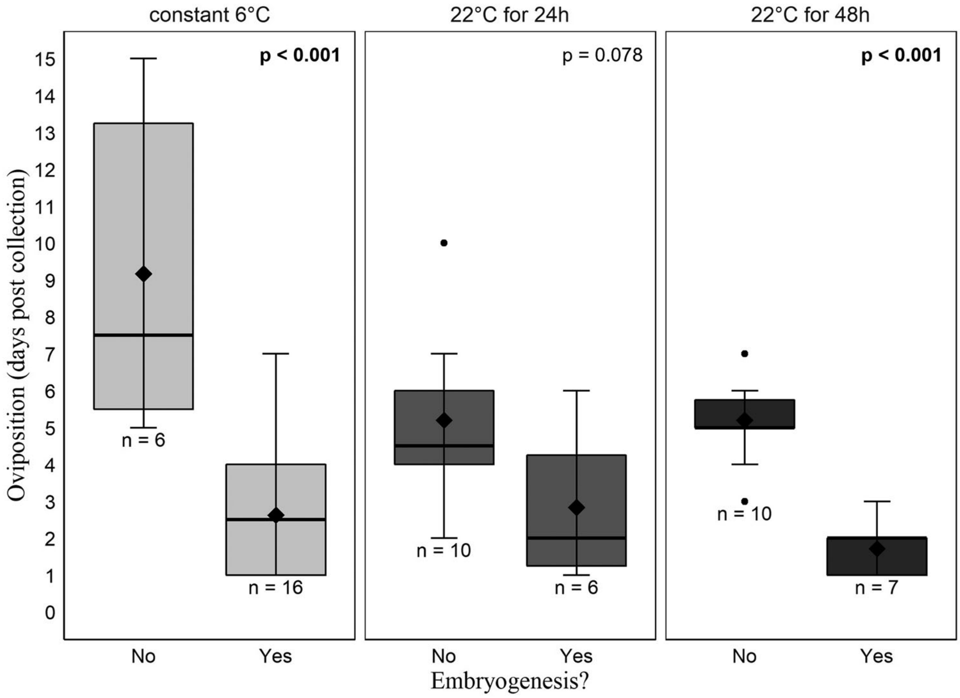

Figure 6. Female *Diamesa mendotae* Muttkowski, 1915 oviposition date with or without occurrence of embryogenesis from Pickwick Creek collected 4 January 2021. Box and whisker boundaries signify the maximum, 75th percentile, median, 25th percentile, and minimum longevity values. Black diamonds (◆) indicate mean longevity. One outlier from the 22 °C/48h (oviposition = 26 days) was removed. Significant differences were found in the constant 6 °C and 22 °C/48h groups (Wilcoxon test).

treatment groups from each site (Fisher's Test: $p < 0.001$) (Figure 7). Females in Pickwick Creek's constant 6 °C group had the highest rates of larval hatching, with 60% of egg masses hatching into larvae (Figure 6). Conversely, the 22 °C/48h group had the lowest rate of larval hatching success (4%), with only one of 25 egg mass hatching (Figure 6).

Discussion

Cold-adapted Chironomidae and other winter-active insects require an extended cold period to complete their life cycles (Bouchard and Ferrington 2009; Soszyńska-Maj, Paasivirta, and Giłka 2016). Climate change is increasing mean winter temperatures and the frequency of winter warm spikes, especially in northern climates (Chapman et al. 2020; Guirguis, Gershunov, Schwartz, and Bennett 2011; Liess et al. 2022; Notaro et al. 2014). Chironomidae development, as in other insects, is thermally dependent (Frouz, Ali, and Lobinske 2002; Reynolds and Benke 2005), and may be negatively affected by winter warming. Studies on the impacts of climate change on cold-adapted insects have evaluated how increasing air temperatures affect insect longevity or community assemblage (Fitzgerald et al. 2021; Nyquist et al. 2021).

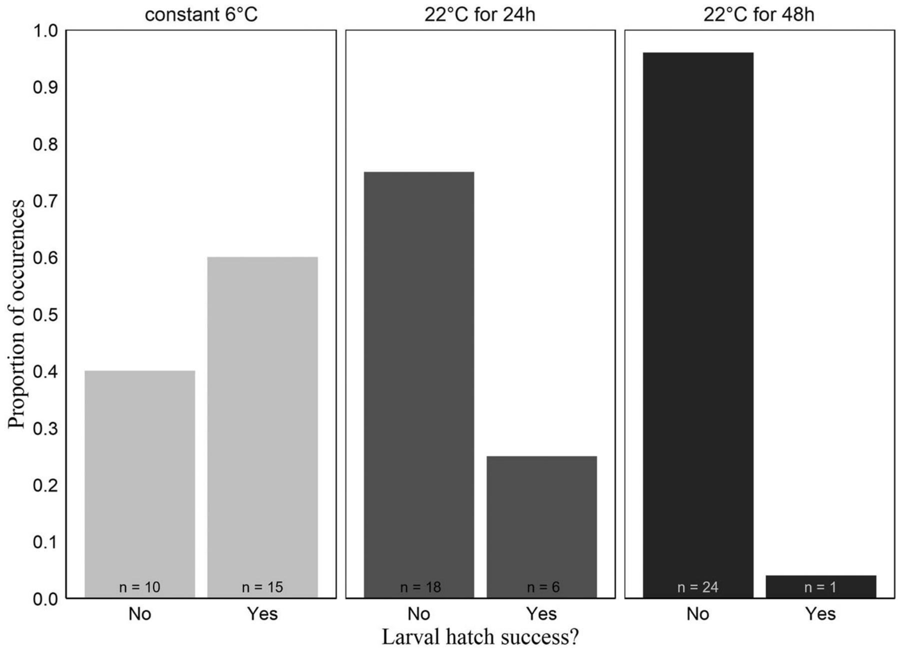

Figure 7. Female *Diamesa mendotae* Muttkowski, 1915 larval hatch success from Pickwick Creek collected 4 January 2021. Females were divided into groups of those whose egg masses hatched into larvae ('Yes') and those that did not oviposit or whose egg masses did not hatch into larvae ('No'). Proportions of females were significantly different among the three treatment groups ($p < 0.001$; Fisher's Test).

Previous research on *D. mendotae* longevity incubated individuals at constant temperatures (Ferrington et al. 2010; Mazack et al. 2014; Nyquist et al. 2021); however, these studies did not account for daily temperature fluctuations. This study sought to understand how short-term high temperature spikes affected *D. mendotae* longevity and reproduction as a model for winter-active insects. We hypothesised 22 °C exposure for 24 or 48h would reduce survivorship and alter reproductive timing but found only partial support for these hypotheses. We found significant decreases in *D. mendotae* longevity from Pickwick Creek Site 2 after 22 °C exposure, but not from Pickwick Creek Site 1 or from Ike's Creek. In addition, we did not find significant differences in the date of oviposition after collection. However, there were significantly different proportions of egg masses that developed into larvae among treatment groups, with reduced larval hatching in 22 °C exposure groups. Together, these findings indicate short-term high temperature exposure may have negative effects on *D. mendotae* longevity and reproduction, but these effects may vary across locations and separated *D. mendotae* populations.

Ferrington et al. (2010) reported *D. mendotae* mean longevity ranged from 12.1–33.0 days at constant 6 °C across 11 collection dates from six stream sites. *Diamesa mendotae* longevity at constant 6 °C was 17.6–18 days from Ike's Creek and 15–16.1 days from Pickwick Creek in this study, and was within the range reported by Ferrington et al. (2010). Mean longevity in Pickwick Creek was shorter than mean Ike's Creek longevity in the constant 6 °C and 22 °C/24h groups. These differences in longevity between Ike's Creek and Pickwick Creek could be due to differences in water temperature during *D. mendotae* development in the two streams. Mean Ike's Creek temperatures in January 2020 were 6.8 °C at Site 1 and 5.7 °C at Site 2. Comparatively, mean Pickwick Creek temperatures in January 2021 were 4.3 °C at Site 1 and 4.8 °C at Site 2. Therefore, the higher water temperature in Ike's Creek than Pickwick Creek may have affected the differences in *D. mendotae* longevity, and differences in water temperatures between Site 1 and Site 2 on Pickwick Creek could have led to differences in *D. mendotae*'s response to short-term high temperature exposure. However, further work is necessary to determine the underlying mechanisms in *D. mendotae* that regulate tolerance levels of high temperatures.

Previous studies have assumed *D. mendotae* collected on the snow emerged that same day (e.g., Ferrington et al. 2010), and many individuals we collected were teneral and likely emerged within the same day. However, Anderson et al. (2013) found *D. mendotae* were capable of surviving extended periods buried beneath the snow, and our study and previous research found *D. mendotae* capable of living over 30 days after collection (Anderson et al. 2013; Mazack et al. 2014), which suggests chironomids may have been several days old at the time of collection. *Diamesa mendotae* in this study were randomly assigned to treatment groups to reduce the effects of individual age on group longevity. However, our longevity estimates do not reflect the lifespan of adult *D. mendotae* from pupal eclosion to death, but instead predict minimum longevity in wild populations.

Short-term high temperature exposure did not significantly alter female *Diamesa* oviposition date relative to constant 6 °C. However, eggs that underwent embryogenesis in Pickwick Creek had significantly earlier oviposition dates than those that did not develop in constant 6 °C and 22 °C/48h groups. Bouchard and Ferrington (2009) reported females in mated pairs collected from the snow or from emergence traps oviposited within 9 days when held at 5 °C. Most ovipositing females in this study oviposited 1–10 days after collection, except for three females that oviposited 15 or 26 days after collection. We did not collect mated pairs; therefore, ovipositing females whose eggs underwent embryogenesis must have mated prior to collection. Some females that did not oviposit may have also oviposited prior to collection. A possibility is that not all females that oviposited had mated prior to collection, and instead laid unfertilised eggs. Unmated female *Belgica antarctica* Jacobs, 1900, a cold-adapted Antarctic chironomid, oviposited 4–5 days after pupation when reared at 6–7 °C, but laid fewer eggs than mated females (Edwards and Baust 1981). If *D. mendotae* females also oviposit regardless of mating status, then a lack of egg development may not be due to 22 °C exposure, but to differences in proportions of mated and unmated females in treatment groups. Further research could evaluate mating and oviposition habits of *D. mendotae* after pupal emergence to determine whether unmated *D. mendotae* oviposit.

Chironomids face different levels of risk from air temperature fluctuations as they develop from aquatic juvenile stages to the terrestrial adult stage. Eggs are exposed to air temperature fluctuations within the female's body once a female emerges from the aquatic pupa, but would be buffered from air temperature fluctuations in the water after oviposition. Many egg masses in our treatment groups were oviposited after females were returned to constant 6 °C after exposure to 22 °C. Egg masses in the 22 °C treatment groups had lower rates of larval hatching. Therefore, the temperature increase to 22 °C may have directly inhibited larval development or may have had indirect effects on female physiology reducing egg viability.

Winter-active insects require extended cold temperatures to complete development and reproduction. Therefore, winter-active species may be threatened by shorter, warmer winters and short-term temperature spikes as climate change progresses. We found *D. mendotae* populations experience site-dependent reductions in longevity and larval hatch rates after 24 or 48h exposure to 22 °C. However, constant 6 °C with 22 °C temperature spikes do not encompass the entirety of natural winter temperature variation. *Diamesa mendotae* and other cold-adapted insects will likely endure longer or more frequent periods of fluctuating temperatures against a background of higher mean winter temperatures. Therefore, further research should seek to determine how short-term temperature spikes affect insect longevity, reproduction, and behaviour to fully understand seasonal and regional effects of climate change on winter-active species and the broader impacts on winter stream ecosystems.

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