Fall 2021

An elevational gradient in thermal tolerance among Daphnia from Western Maine lakes

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Submitted in partial fulfillment of the requirements for the
University Honors Scholar Designation

January 28, 2021

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WESTERN MAINE LAKES

ABSTRACT

With climate change threatening biodiversity worldwide, it is important to understand
species' physiological responses to changing thermal environments. This study examined
whether thermal tolerance (measured as time to immobilization, T_{imm}) in the zooplankton
Daphnia catawba and *D. schødleri* varied along an elevational gradient in Western Maine.
Specimens collected from five lakes were subjected to heat stress trials to look for
inter-population variation. Thermal tolerance was strongly correlated with several
elevation-driven lake temperature variables, with the percent of variation explained ranging from
13-37%. *Daphnia* from cooler, high-elevation lakes were more sensitive to elevated
temperatures. While latitudinal gradients have been examined extensively, this study represents
one of the first records of an elevational gradient in thermal response in *Daphnia*, and marks an
essential first step in establishing whether local adaptation in response to changing
temperatures will be possible in this system.

INTRODUCTION

Climate change is impacting species and ecosystems worldwide (Root et al. 2003,
Parmesan and Yohe 2003, Niinemets et al. 2017, Sheldon 2019, Weiskopf et al. 2020). It is
therefore important to understand species' physiological responses to changing thermal
environments. Due to thermal inertia, freshwater organisms experience less dramatic short-term
changes in temperature, and thus may be less adapted to thermal fluctuations, making them
vulnerable to the increasing number of extreme temperature events stemming from climate
change (Heino et al. 2009, Geerts et al. 2015b). This is acutely the case for poikilotherms
(organisms incapable of regulating their internal temperature): because dissolved oxygen (DO)
levels decline with rising temperatures, poikilotherms experience increased metabolic rates

under high temperatures while simultaneously suffering the effects of reduced DO (Zeis et al. 2004, Bruijning et al. 2018).

Poikilotherms of the Cladoceran genus *Daphnia* are keystone grazers of phytoplankton and important elements of freshwater food webs, where they are consumed by planktivorous fish (Lampert 2006, Miner et al. 2012). As cyclic parthenogens, *Daphnia* maintain multiple discrete clonal lineages, reproducing asexually throughout most of the year and only switching to sexual reproduction under stressful conditions or at the end of the active season, when they produce resting eggs (ephippia), which can overwinter in lakebed sediments (Henning-Lucass et al. 2016).

Interclonal variation in temperature tolerance has been documented in *Daphnia* (MacIsaac et al. 1985), even within the same lake (Werner et al. 2019). Where different clonal lineages are adapted to different temperatures, clonal frequency can vary over the course of the season (Carvalho and Crisp 1987). The presence of differently-adapted clones provides the resident variation necessary for *Daphnia* populations to undergo local adaptation, one mechanism which can underlie responses to climate change (Bruijning et al. 2018, Werner et al. 2019). Henning-Lucass et al. (2016) and Geerts et al. (2015a) compared 40-year-old post-ephippial clones with present-day specimens (a method known as resurrection ecology) and found modern individuals to be more heat-tolerant, suggesting microevolution to climate. Microevolution in response to temperature has been observed on even shorter timeframes (one to two seasons) by Van Doorslaer et al. (2009a,b) and Vanvelk et al. (2021). The rapidity of this response gives us reason to believe that individuals from populations in different thermal environments will show local adaptation to those conditions.

Another source of variation responsive to temperature is phenotypic plasticity which, unlike local adaptation, is a non-genetic developmental response to the conditions in which an

organism matured. Multiple studies in *Daphnia* have demonstrated phenotypic plasticity through common garden experiments and acclimation to non-native temperatures (Carvalho 1987, Zeis et al. 2004, Yampolsky et al. 2014, and Brans et al. 2017 examining thermal tolerance in *D. magna*; Williams et al. 2012 examining thermal tolerance in *D. pulex;* and Vanvelk et al. 2021 examining both thermal tolerance and life-history fitness components in co-occurring *D. magna*, *D. pulicaria*, and *D. galeata*).

Local adaptation and phenotypic plasticity are not mutually exclusive; indeed, they often co-occur (Yampolsky et al. 2014), and both contribute to how organisms respond physiologically to the thermal environment. However, before the effects of local adaptation and phenotypic plasticity can be disentangled, as they were by Williams et al. (2012) and Yampolsky et al. (2014), it must first be established that interpopulation variation in thermal tolerance is actually present within the scale of the study system.

Prior studies in *Daphnia* have primarily focused on variations in thermal tolerance across broad latitudinal gradients, from continental (Williams et al. 2012, Geerts et al. 2015b) to global (Yampolsky et al. 2014) scales. In all cases, thermal tolerance was higher in warmer environments. These cross-latitudinal studies have been useful for establishing broad trends of thermal tolerance, but even latitudinally adjacent populations may experience different temperature regimes (Yampolsky et al. 2014), and local adaptation to these conditions has been shown to arise on very fine scales. Brans et al. (2017) found both local adaptation and phenotypic plasticity in *D. magna* sampled from 13 ponds along a 150 km urban-rural gradient in Flanders, Belgium, and Declerck et al. (2002) detected local adaptation in water bodies separated by less than five meters.

Elevational gradients can provide similar extent in thermal variation as observed in latitudinal gradients, over much shorter geographic distances. Yet elevational gradients of

thermal tolerance are understudied in aquatic systems. This is surprising, since habitats in close proximity have the potential to act as climate refugia or as source populations in the context of climate change. Haileselasie et al. (2016) have shown *Daphnia* to be highly capable of dispersing between glacial lakes (ca. 11 km maximal inter-lake distance). However, even with the potential for gene flow among geographically proximate lakes, genetic differentiation among *Daphnia* populations is common (Allen et al. 2010), highlighting the importance of studying the extent of local adaptation among populations of *Daphnia* in neighboring lakes.

The foothills and mountains of Western Maine provide a suitable context in which to study local variation in thermal response, due to the geographic heterogeneity caused by the history of glaciation and the large number of lakes in close proximity. In this study, I examined the extent of variation in physiological response and thermal tolerance among *Daphnia* populations from high- and low-elevation lakes in Western Maine, and related it to local temperature regimes. Thermal tolerance was assessed using time to immobilization (T_{imm}), the time until cessation of swimming activity in individuals exposed to lethal high temperatures. Acute measures like T_{imm} and lethal temperature (critical thermal maximum, or CT_{Max}), have been noted to more closely track local temperature regimes than do life-history fitness components (growth, maturation, and fecundity), common metrics also used in studies of thermal response (Yampolsky et al. 2014).

I expected that *Daphnia* from colder, high-elevation lakes would exhibit lower tolerance for acute temperature stress than their low-elevation congeners, paralleling observations from latitudinal studies. Differences in thermal tolerance, if subsequently shown to be at least in part due to local adaptation, could provide insight into the emerging question of whether freshwater aquatic species such as *Daphnia* will respond to climate change through latitudinal and elevational range shifts, or through rapid local microevolution *in situ* (Van Doorslaer et al. 2009b,

Geerts et al. 2015a).

METHODS

Two high-elevation and three low-elevation lakes in Western Maine (Eddy, Midway, Little Wilson, Wilson, and Webb) were chosen for study based on the availability of temperature data from at least three years between 2014 and 2021 (Table 1, Figure 1). *Daphnia* were collected between 11 and 18 September 2021 (Table 1), via multiple vertical tows with a Wisconsin-style 80 micron mesh plankton net with a 30 cm opening (Wildco) deployed from a canoe or kayak in water depths at least 75% of the maximum depth of each lake (Figure 2). Specimens were transported in plastic sample jars to the laboratory where they were transferred to open glass beakers within four hours of collection, provided with food (*Nannochloropsis* grown in Alga-Gro® Freshwater Medium, Carolina), and maintained at room temperature (ca. 22.5°C).

Individuals were selected for temperature trials based on size, owing to the need for specimens whose swimming motions could be seen and in an attempt to reduce the number of potentially confounding variables. Subjects were pipetted into individual glass vials (ca. 10 cm length x 2 cm diameter) and submerged in a room temperature (ca. 22.5°C) Microyn Labnique Digital Thermostatic water bath whose temperature was gradually increased (as described below) to prevent thermal shock. Several pilot trials were run with different temperature ramps and final temperatures to find a regimen that resulted in eventual (within 120 minutes) but not immediate immobilization of all specimens. Based on these pilot trials, a challenge temperature of 35°C, arrived at through 1.5°C increases over 35 minutes, was selected. Eight experimental trials were run with 10-11 subjects in individual vials per trial. Each trial included specimens from at least two lakes, but full randomization was not attempted due to the constraints of travel time between the collection sites and lab.

Subjects in their vials were removed briefly from the water bath at five minute intervals,

examined under magnification, and scored as either active or immobile. Because individuals
occasionally became active again after a single interval of immobility, those scored immobile
were observed for at least three more intervals. When an individual was determined to be
permanently immobilized, T_{imm} was recorded as the first interval where no swimming activity was
observed, even if it had displayed a level of undirected twitching. At the trial's conclusion,
individuals were preserved in 70% ethanol for measurement and identification.

Body length (measured from the dome of the head to the tip of the tail spine) was
determined using a dissecting microscope and ocular micrometer. Individuals were then keyed
to species using a compound microscope and an image-based key (Haney et al. 2013).

Lake temperature data was compiled from two sources. Long-term records maintained
by the Maine Department of Environmental Protection and the Lake Stewards of Maine (Bacon
2019) were used to supply temperatures for Wilson between 2015 and 2018, and for Little
Wilson and Webb. These consisted of temperature profiles (1 m increments) collected manually
on a semi-monthly basis during ice-free months. Temperatures for Eddy, Midway, and post-2018
Wilson came from automated HOBO loggers that collected year-round data at 20-30 minute
intervals from three depths: surface (0.5-1 m), 2 m, and the bottom (Rachel Hovel and Julia
Daly, pers. comm.).

These data sets were edited to remove the winter months (when *Daphnia* are inactive)
and, since manual collection occurred during daylight hours, HOBO data was cropped to include
only observations between 1000 and 1600 hours. Because high temperature stress was the
focus of this study, surface observations (1 m for manual data and 0.5-1 m for HOBO data) were
selected, excepting the HOBO data from Wilson, where 2 m data was the shallowest available.
Monthly daylight temperature averages for May through September, as well as the maximum
yearly temperature, were calculated from this data (Table 2).

In addition to these direct measures of temperature, two seasonal markers of thermal regime were also used in analyses: the date of maximum temperature (T_{max} date) and ice-out date. Average ice-out dates for Midway and Eddy (2010-2019) were obtained from Rachel Hovel and Julia Daly (pers. comm.), while members of lake residents' associations provided annual data for Wilson (Rob Lively, Friends of Wilson Lake, pers. comm.) and Little Wilson (Denise McCourt, Little Wilson Pond Improvement Association, pers. comm.). Ice-out dates from Webb came from the Maine Department of Agriculture, Conservation & Forestry (DACF 2021). For these three lakes, average ice-out date was computed as the mean of observations between 2014 and 2021.

Linear regressions were used to determine the relationship between T_{imm} and various temperature-related variables, geomorphological characteristics, and body length, and to relate temperature variables to geomorphological characteristics of each lake. ANOVA followed by Tukey's HSD was used to look for differences in T_{imm} and body length among lakes. Student's t-tests were used to determine whether T_{imm} and body size differed between species. These analyses were carried out using R (v4.1.2; R Core Team 2021) in the open-source program RStudio (v2021.09.1+372; RStudio, 2021).

RESULTS

Seven species were identified among the 70 individuals tested (Table 3). Of these, 57 were either *Daphnia catawba* or *Daphnia schødleri*, phenotypically similar species classified within the same species group (Kořinek and Hebert 1996). Only *D. catawba* was found in all five lakes, while *D. schødleri* was found in all barring Webb. *Daphnia pulex* was represented by single specimens from three different lakes, and the remaining four species (*D. dubia*, *D. laevis*, *D. mendotae*, and *Ceriodaphnia reticulata*) were unique to Webb. Because of their abundance and relative ubiquity, analyses were restricted to *D. catawba* and *D. schødleri*. This decision was

further informed by the fact that T_{imm} did not differ between the two species (t = 0.606, df = 54, p = 0.547, Figure 3).

Since the *Daphnia* assemblage from Webb appeared to differ from that in the other lakes (Table 3) and the number of *D. catawba* / *D. schødleri* tested from that lake was small (n = 5), analyses were performed both with and without the inclusion of Webb. In all cases, the two analyses produced similar results unless otherwise noted, and when results were comparable, only the statistics from the 5-lake analysis are reported.

Thermal tolerance (T_{imm}) was highly correlated with most, but not all, of the temperature-related variables (Table 4). The strongest predictor of thermal tolerance by far was the date at which the lake experienced its highest daylight temperature (T_{max} date, Figure 4), with *Daphnia* from lakes where temperatures peaked late in the season showing the highest T_{imm}. However, the actual maximum temperature (T_{max}), while significant in the 5-lakes analysis, explained less than a third as much of the variability in T_{imm} as did T_{max} date (Table 4), and lost significance altogether in the 4-lake analysis. In contrast to all other temperature variables, this relationship was negative, with higher maximum temperatures associated with lower T_{imm}.

Mean monthly daylight temperatures (i.e. the average of a month's daylight temperatures across all data years) generally increased in explanatory strength (r^2) as the season progressed and approached the date of specimen collection (Figure 4), with the exception of June, which did not correlate with T_{imm}. Ice-out was a notable non-predictor of T_{imm} (Table 4).

While T_{imm} did not differ between species, as noted above, it did differ by lake (F = 8.008, df = 4,51, p < 0.001, Figure 5a). Body length differed by species (t = -3.541, df = 54, p < 0.001, Figure 3b) and lake (F = 4.162, df = 4,51, p =0.005, Figure 5b), but was not a predictor of T_{imm} (Table 4).

Both elevation and area were good predictors of thermal response (elevation: $r^2 = 0.210$,

$p < 0.001$, Figure 6; area: $r^2 = 0.233$, $p < 0.001$). Latitude showed weak significance in the

4-lake analysis and was not significant in the 5-lake analysis (4-lake: $r^2 = 0.083$, $p = 0.04$,

5-lake: $r^2 = 0.057$, $p = 0.078$). However, these geomorphological characteristics are most likely

to affect T_{imm} indirectly, through their impacts on temperature regimes (Figure 6). I therefore

regressed each geomorphological variable on the array of temperature variables; their relative

importance can be assessed through the combined strength of the two-step pathways through

which they act on T_{imm} (Table 5). In agreement with the direct correlation results, this analysis

showed elevation to be a better predictor of the temperature variables that were better

predictors of T_{imm}, followed by latitude, and area.

DISCUSSION

Across five lakes in Western Maine that exist along an elevational gradient, I found that

Daphnia exhibited variability in their physiological responses to thermal stress, with individuals

from cooler, higher-elevation lakes being more sensitive to acute temperature stress than those

from warmer, lowland ones. Measures of lake temperature were excellent predictors of

physiological response to thermal stress: the three best predictors (mean daylight temperatures

in July, August, and September), while highly intercorrelated, each explained between 13 and

25% of the variation in thermal tolerance (T_{imm}) (Table 4). These results are congruent with those

of Yampolsky et al. (2014) showing higher thermal tolerance in *Daphnia* native to regions with

warmer temperatures. In the lakes I studied, mean daylight temperatures became more reliable

predictors of thermal tolerance as the season progressed and approached the date when

Daphnia were collected. Springtime temperatures and events show more inter-annual variation

as lakes warm and experience turnover, before stabilizing as the summer progresses. Thus,

when springtime temperatures are averaged over multiple years, their ability to predict thermal

tolerance, especially in late-summer *Daphnia* populations, may be limited.

An initially surprising result was the negative relationship between maximum lake temperature (T_{max}) and T_{imm}, especially as several authors postulate that thermal tolerance is driven by extreme, rather than average, temperatures (Geerts et al. 2015a, Brans et al. 2017, Vanvelk et al. 2021). In this study, cooler high-elevation lakes recorded greater T_{max}. Smaller lakes are poorer heat sinks, making them prone to more rapid warming, and while the small lakes in this study are cooler due to their elevation, it is possible that their size makes them vulnerable to sudden temperature fluctuations, and thus greater extremes (Woolway et al. 2016). However, the most likely explanation for the negative relationship seen here between T_{max} and T_{imm} is that it is an artifact of the data collection methods. Eddy, Midway, and in later years Wilson, were all monitored by HOBO loggers providing continuous data, while the temperatures from Little Wilson and Webb were measured manually once or twice each month. Thus the likelihood of sampling the actual maximum temperatures at these lakes was drastically reduced, skewing the data.

One of the two seasonal indicators of thermal regime, the date of maximum temperature (T_{max} date), proved to be the strongest predictor of thermal tolerance, explaining 37% of the possible variation in T_{imm} within the 5-lakes analysis (Table 4). T_{max} date occurred later in low- than in high-elevation lakes, likely in part because lowland lakes were also larger (Table 1). As noted above, bigger basins take longer to warm, since their increased water volume magnifies their properties as a heat sink. In contrast, the other seasonal indicator, ice-out date, was a notably poor predictor of thermal tolerance, failing to achieve significance and explaining only 0.2% of the variation in T_{imm}. The tendency for temperatures and events to show more inter-annual variation early in the season may explain why ice-out date, similar to other spring temperature variables, is not an important predictor of thermal tolerance of *Daphnia* populations.

Two of the three geomorphological characteristics tested (elevation and area) were significant as predictors of thermal tolerance. However, because these variables likely act indirectly through their roles in driving lake thermal regimes, their impacts are best understood through their correlations with temperature. Intuitively, one sees that the importance of each geomorphological characteristic derives from the combined strength of the two-step pathways through which it acts on T_{imm}; thus, while latitude is the strongest predictor of ice-out, this is of little importance since ice-out is a non-predictor of T_{imm}. In contrast, elevation strongly predicts September temperature and this is enhanced by September temperature's strong correlation with T_{imm}. Overall, elevation was a better predictor of the temperature variables that were better predictors of T_{imm}. Several studies have shown relationships between thermal tolerance and latitude, which is often used as a proxy for temperature (Williams et al. 2012, Yampolsky et al. 2014, Geerts et al. 2015b), but I was unable to find literature reporting similar relationships with elevational gradients in *Daphnia*. It is not surprising that the impact of elevation was greater than that of latitude, since the study was designed to restrict the impact of latitude as a confounding variable. My results contribute one of the first examples of an elevational gradient in thermal tolerance among *Daphnia* populations.

The effect of lake area on temperature is more complex, acting through its relationships with both thermal mass and fetch (the distance over which wind can travel unobstructed). As noted earlier, larger lakes are more effective heat sinks, maintaining thermal inertia for longer. Conversely, larger lakes have greater fetch, allowing for greater mixing and circulation of warmer surface water. The balance between these opposing effects is governed by other lake geomorphological characteristics, such as depth. However, in the lakes I studied, there was a lake size gradient running parallel to elevation, with higher-elevation lakes being smaller, and lower-elevation lakes being larger. Thus the effect of area in this study is virtually inseparable from that of elevation; a more extensive sample of lakes chosen to decouple size and elevation

would be needed to address effects of lake area on temperature and, by extension, on thermal

tolerance.

Multiple studies in *Daphnia* have shown that at higher temperatures adult body size

decreases, since reduced size increases the ease of transporting oxygen to tissues in low

dissolved oxygen (DO) environments (Daufresne et al. 2009, Geerts et al. 2015a,b, Brans et al.

2017). This study found no correlation between body size and mean temperature of any month,

which was not surprising as I controlled for body size when selecting individuals, in an attempt

to limit the number of variables contributing to variation in T_{imm}. Despite this, body size did differ

significantly between lakes, but interestingly was not a predictor of T_{imm}, suggesting *Daphnia* in

this study may be using means other than body size to respond to thermal stress. *Daphnia*

populations respond to increased temperatures in a variety of other ways: by upregulating

hemoglobin (Yampolsky et al. 2014), increasing levels of heat shock proteins (Pauwels et al.

2007), maintaining optimal membrane fluidity through homeoviscous adaptation (Werner et al.

2019), altering resource allocation among life-history fitness components such as growth,

maturation, and fecundity (Carvalho 1987, Allen et al. 2010, Yampolsky et al. 2014, Bruijning et

al. 2018). Reductions in body size in response to temperature can be costly in terms of lowered

competitive strength and grazing capacity, meaning that some *Daphnia* may be selected to

respond to thermal stress via other means (Brans et al. 2017).

I found no difference in thermal tolerances between *D. catawba* and *D. schødleri*. This

may reflect that local adaptation to temperature occurs similarly in both of these species. This

contrasts with MacIsaac et al. (1985), who showed varying thermal tolerances among

co-occurring *Daphnia* species. It is also possible that *D. catawba* and *D. schødleri* hybridize

where they co-occur. Both species are members of the *D. pulex* species group (Kořinek and

Hebert 1996), and hybridization between *Daphnia* species is common (Wolf and Mort 1986,

Colbourne and Hebert 1996), including within the *D. pulex* complex (Hebert and Finston 1997). Several specimens in this study exhibited intermediate features, further supporting the possibility of hybridization.

Collections for this study were carried out in mid-September, a time that corresponds with the second of two major blooms reported for *D. catawba* in the northeastern United States, the first being in May (Tessier 1986). Future studies should ideally span the entire active season, with an eye on those specimens collected near the date of maximum temperature in hopes of identifying the clonal lineages with the highest thermal tolerance. Such a season-long study would also address whether the trend in explanatory power across monthly temperatures that I observed is robust and reflects the greater stability of late summer temperatures, or is merely a function of proximity to the date of collection. More importantly, a study carried out across a broader selection of lakes, chosen to decouple size and elevation, would help to clarify the trends observed here.

A unique feature of this study was that, unlike similar studies by Williams et al. (2012), Yampolsky et al. (2014), and Geerts et al. (2015b), who collected atmospheric temperature data from local weather stations, I correlated my thermal tolerance observations with temperature data collected over the last decade from the epilimnion of subject lakes. With the ready availability of inexpensive temperature loggers, this methodology should become routine.

Prior studies of thermal tolerance in *Daphnia* have used either acute measures such as T_{imm} or CT_{Max} (Geerts et al. 2015a,b, Henning-Lucass et al. 2016, Brans et al. 2017), or life-history fitness components such as growth, reproduction, and survival (Carvalho 1987, Allen et al. 2010, Bruijning et al. 2018). Yampolsky et al. (2014) and others have successfully used T_{imm} to study local adaptation in *Daphnia* and other aquatic organisms, arguing that acute measures reflect the ability to maintain crucial body functions in the face of thermal extremes. In

contrast, while fitness components reflect adaptive or plastic response to temperature, they also frequently covary with factors such as resource availability and predation, obscuring the effect of temperature (De Meester et al. 1999, Yampolsky et al. 2014). Tellingly, Geerts et al. (2015a) found no differences in fitness components across latitudes, but showed a positive correlation between CT_{Max} and average temperature of the warmest month. The need for an acute, sensitive, and easily-measured indicator of thermal tolerance was ultimately what drove my decision to use T_{imm} in this study (Yampolsky et al. 2014, Werner et al. 2019).

While the popular narrative around climate change is one of local extinctions, invasions, and the flight from rapidly warming environments, evidence suggests that local processes (adaptative and plastic) may in fact take center stage (Geerts et al. 2015a). For instance, Van Doorslaer et al. (2009b) showed that local microevolutionary adaptations to warming climates may enable resident populations to resist invasion by migrants from warmer climates, preventing the establishment of non-native genotypes. The assumed benefit of phenotypic plasticity is the speed with which it enables organisms to respond to changing regimes, however recent studies have shown *Daphnia* to be capable of rapid evolution in response to warming temperatures (Van Doorslaer et al. 2009a,b, Geerts et al. 2015a, Henning-Lucass et al. 2016, Vanvelk et al. 2021). Furthermore, phenotypic plasticity can itself be a target of selection (Williams et al. 2012, Yampolsky et al. 2014, Vanvelk et al. 2021). Thus both adaptation and phenotypic plasticity will be important in understanding how organisms respond physiologically to rapidly rising temperatures.

The primary result of this study was the demonstration of differences in thermal tolerance between *Daphnia* populations from geographically proximate lakes along an elevation and temperature gradient, an essential first step in establishing whether local adaptation in response to changing temperatures will be possible in this system. Having shown these

differences, further research should undertake common-garden and acclimatization experiments to tease apart the underlying causes, and ascertain how *Daphnia* in Western Maine lakes will respond to a changing climate.

ACKNOWLEDGMENTS

LITERATURE CITED

Allen, M.R., Thum, R.A. and Caceres, C.E., 2010. Does local adaptation to resources
explain genetic differentiation among *Daphnia* populations?. *Molecular ecology*,
19(15), pp.3076-3087.

Bacon, L., 2019, *Maine lakes water quality - temperature & dissolved oxygen profiles*,
electronic dataset, Maine Department of Environmental Protection & Volunteer Lake
Monitoring Program, viewed 28 November 2021,
<http://www.gulfofmaine.org/kb/2.0/record.html?recordid=9214>.

Brans, K.I., Jansen, M., Vanoverbeke, J., Tüzün, N., Stoks, R. and De Meester, L., 2017. The
heat is on: Genetic adaptation to urbanization mediated by thermal tolerance and
body size. Global change biology, 23(12), pp.5218-5227.

Bruijning, M., ten Berge, A.C. and Jongejans, E., 2018. Population‑level responses to
temperature, density and clonal differences in *Daphnia magna* as revealed by integral
projection modelling. *Functional Ecology*, *32*(10), pp.2407-2422.

Carvalho, G.R. and Crisp, D.J., 1987. The clonal ecology of *Daphnia magna* (Crustacea:
Cladocera): I. Temporal changes in the clonal structure of a natural population. *The
Journal of Animal Ecology*, pp.453-468.

Carvalho, G.R., 1987. The clonal ecology of *Daphnia magna* (Crustacea: Cladocera): II.
Thermal differentiation among seasonal clones. *The Journal of Animal Ecology*,
pp.469-478.

Colbourne, J.K. and Hebert, P.D., 1996. The systematics of North American *Daphnia*
(Crustacea: Anomopoda): a molecular phylogenetic approach. *Philosophical
Transactions of the Royal Society of London. Series B: Biological Sciences*,
351(1337), pp.349-360.

DACF, 2021, *Ice Out Dates*, electronic dataset, Maine Department of Agriculture,
Conservation & Forestry, viewed 30 November 2021,
<https://www.maine.gov/dacf/iceout>.

Daufresne, M., Lengfellner, K. and Sommer, U., 2009. Global warming benefits the small
in aquatic ecosystems. Proceedings of the National Academy of Sciences,
106(31), pp.12788-12793.

Declerck, S., Cousyn, C. and De Meester, L., 2001. Evidence for local adaptation in
neighbouring *Daphnia* populations: a laboratory transplant experiment.
Freshwater Biology, 46(2), pp.187-198.

De Meester, L., Boersma, M. and Spaak, P., 1999. Environmental stress and local adaptation
in *Daphnia magna*. Limnology and Oceanography, 44(2), pp.393-402.

Geerts, A.N., Vanoverbeke, J., Vanschoenwinkel, B., Van Doorslaer, W., Feuchtmayr, H.,

Atkinson, D., Moss, B., Davidson, T.A., Sayer, C.D. and De Meester, L., 2015a. Rapid evolution of thermal tolerance in the water flea *Daphnia*. *Nature Climate Change*, *5*(7), pp.665-668.

Geerts, A.N., De Meester, L. and Stoks, R., 2015b. Heat tolerance and its evolutionary potential along a latitudinal gradient in *Daphnia magna*. Evolutionary Ecology Research, 16(6), pp.517-528.

Google, 2021, *Google Maps*, viewed 29 November 2021, <https://www.google.com/maps>.

Haileselasie, T.H., Mergeay, J., Weider, L.J., Sommaruga, R., Davidson, T.A., Meerhoff, M., Arndt, H., Jürgens, K., Jeppesen, E. and De Meester, L., 2016. Environment not dispersal limitation drives clonal composition of Arctic *Daphnia* in a recently deglaciated area. *Molecular ecology*, *25*(23), pp.5830-5842.

Haney, J.F. *et al*. 2013. "An-Image-based Key to the Zooplankton of North America" version 5.0. University of New Hampshire Center for Freshwater Biology viewed 28 Nov 2021 <cfb.unh.edu>

Hebert, P.D. and Finston, T.L., 1997. A taxonomic reevaluation of North American *Daphnia* (Crustacea: Cladocera). III. The *D. catawba* complex. *Canadian journal of zoology*, *75*(8), pp.1254-1261.

Heino, J., Virkkala, R. and Toivonen, H., 2009. Climate change and freshwater biodiversity: detected patterns, future trends and adaptations in northern regions. Biological Reviews, 84(1), pp.39-54.

Henning‑Lucass, N., Cordellier, M., Streit, B. and Schwenk, K., 2016. Phenotypic plasticity in life‑history traits of *Daphnia galeata* in response to temperature–a comparison across clonal lineages separated in time. *Ecology and Evolution*, *6*(4), pp.881-891.

Kořinek, V. and Hebert, P.D.N., 1996. A new species complex of *Daphnia* (Crustacea, Cladocera) from the Pacific Northwest of the United States. *Canadian Journal of Zoology*, *74*(8), pp.1379-1393.

Lake Stewards of Maine 2021, *Lakes of Maine*, viewed 29 November 2021, <https://www.lakesofmaine.org/>.

Lampert, W., 2006. *Daphnia*: model herbivore, predator and prey. *Polish journal of ecology*, *54*(4), pp.607-620.

MacIsaac, H.J., Hebert, P.D. and Schwartz, S.S., 1985. Inter-and intraspecific variation in acute thermal tolerance of *Daphnia. Physiological zoology*, *58*(3), pp.350-355.

Miner, B.E., De Meester, L., Pfrender, M.E., Lampert, W. and Hairston Jr, N.G., 2012. Linking genes to communities and ecosystems: *Daphnia* as an ecogenomic model. *Proceedings of the Royal Society B: Biological Sciences*, *279*(1735), pp.1873-1882.

Niinemets, Ü., Kahru, A., Noges, P., Tuvikene, A., Vasemägi, A., Mander, Ü. and Nõges, T., 2017. Environmental feedbacks in temperate aquatic ecosystems under global change: why do we need to consider chemical stressors?. *Regional Environmental Change*, *17*(7), pp.2079-2096.

Parmesan, C. and Yohe, G., 2003. A globally coherent fingerprint of climate change impacts across natural systems. Nature, 421(6918), pp.37-42.

Pauwels, K., Stoks, R., Decaestecker, E. and De Meester, L., 2007. Evolution of heat shock protein expression in a natural population of *Daphnia magna*. *The American Naturalist*, *170*(5), pp.800-805.

R Core Team, 2021, *R: A Language and Environment for Statistical Computing*, Vienna, Austria: R Foundation for Statistical Computing, viewed 28 November 2021, <https://www.R-project.org>.

Root, T.L., Price, J.T., Hall, K.R., Schneider, S.H., Rosenzweig, C. and Pounds, J.A., 2003. Fingerprints of global warming on wild animals and plants. Nature, 421(6918), pp.57-60.

Sheldon, K.S., 2019. Climate change in the tropics: ecological and evolutionary responses at low latitudes. *Annual Review of Ecology, Evolution, and Systematics*, *50*, pp.303-333.

Tessier, A.J., 1986. Comparative population regulation of two planktonic cladocera (*Holopedium gibberum* and *Daphnia catawba*). *Ecology*, *67*(2), pp.285-302.

Vanvelk, H., Govaert, L., Van den Berg, E.M., Brans, K.I. and De Meester, L., 2021. Interspecific differences, plastic, and evolutionary responses to a heat wave in three co‐occurring *Daphnia* species. *Limnology and Oceanography*, *66*(4), pp.1201-1220.

Van Doorslaer, W., Stoks, R., Duvivier, C., Bednarska, A. and De Meester, L., 2009a. Population dynamics determine genetic adaptation to temperature in *Daphnia*. *Evolution: International Journal of Organic Evolution*, *63*(7), pp.1867-1878.

Van Doorslaer, W., Vanoverbeke, J., Duvivier, C., Rousseaux, S., Jansen, M., Jansen, B., Feuchtmayr, H., Atkinson, D., Moss, B., Stoks, R. and De Meester, L.U.C., 2009b. Local adaptation to higher temperatures reduces immigration success of genotypes from a warmer region in the water flea *Daphnia*. Global Change Biology, 15(12), pp.3046-3055.

Weiskopf, S.R., Rubenstein, M.A., Crozier, L.G., Gaichas, S., Griffis, R., Halofsky, J.E., Hyde, K.J., Morelli, T.L., Morisette, J.T., Muñoz, R.C. and Pershing, A.J., 2020. Climate change effects on biodiversity, ecosystems, ecosystem services, and natural resource management in the United States. *Science of the Total Environment*, *733*, p.137782.

Werner, C., Ilic, M. and von Elert, E., 2019. Differences in heat tolerance within a *Daphnia magna* population: the significance of body PUFA content. *Hydrobiologia*, *846*(1),

pp.17-26.

Williams, P.J., Dick, K.B. and Yampolsky, L.Y., 2012. Heat tolerance, temperature acclimation, acute oxidative damage and canalization of haemoglobin expression in *Daphnia*. *Evolutionary Ecology*, *26*(3), pp.591-609.

Wolf, H.G. and Mort, M.A., 1986. Inter-specific hybridization underlies phenotypic variability in *Daphnia* populations. *Oecologia*, *68*(4), pp.507-511.

Woolway, R.I., Jones, I.D., Maberly, S.C., French, J.R., Livingstone, D.M., Monteith, D.T., Simpson, G.L., Thackeray, S.J., Andersen, M.R., Battarbee, R.W. and DeGasperi, C.L., 2016. Diel surface temperature range scales with lake size. *Plos one*, *11*(3), p.e0152466.

Yampolsky, L.Y., Schaer, T.M. and Ebert, D., 2014. Adaptive phenotypic plasticity and local adaptation for temperature tolerance in freshwater zooplankton. Proceedings of the Royal Society B: Biological Sciences, 281(1776), p.20132744.

Zeis, B., Maurer, J., Pinkhaus, O., Bongartz, E. and Paul, R.J., 2004. A swimming activity assay shows that the thermal tolerance of *Daphnia magna* is influenced by temperature acclimation. *Canadian journal of zoology*, *82*(10), pp.1605-1613.

TABLES AND FIGURES

Table 1. Characteristics of the five Western Maine lakes included in this study. MIDAS is a Maine statewide lake identification code. Dates are for collection of live *Daphnia* used in thermal tolerance testing. MIDAS, elevation, area, and depths are from the Lakes of Maine website (Lake Stewards of Maine 2021); latitude and longitude are from Google Maps (Google 2021).

Lake name	MIDAS	Latitude	Longitude	Elevation (m)	Area (ha)	Mean depth (m)	Max depth (m)	Date collected
Midway	3544	44.931221	-70.541840	827	2.83	3.35	8.84	9.11.2021
Eddy	3546	44.920000	-70.526667	803	5.67	1.83	6.71	9.11.2021
Webb	3572	44.697490	-70.448440	207	887.90	7.31	12.80	9.18.2021
Wilson	3682	44.594900	-70.246770	174	229.87	12.19	26.82	9.12.2021
Little Wilson	3784	44.204160	-70.287820	90	45.33	6.71	17.07	9.12.2021

Table 2. Means of temperature variables and seasonal indicators of thermal regime used in analyses for five Western Maine lakes. The range of data years used to calculate means for each lake is indicated. Daylight refers to the period between 1000 and 1600 hours, while summer refers to the months May to September.

Lake	MIDAS	Data years	Mean monthly daylight temperature (°C)					Maximum summer temperature (°C)		Ice-out date (Julian)
			May	June	July	August	September	Value	Date (Julian)	
Midway	3544	2014-2021	9.34	18.16	22.35	21.42	17.66	26.10	195.75	118.00
Eddy	3546	2014-2021	9.15	17.97	22.03	20.77	16.87	26.90	203.13	122.00
Webb	3672	2014-2018	-	18.31	23.42	22.94	22.25	24.08	219.40	118.86
Wilson	3682	2015-2021	11.83	19.71	24.24	24.61	21.58	25.88	211.57	115.86
Little Wilson	3784	2017-2018	13.90	21.80	24.67	24.32	21.10	25.53	207.00	104.63

Table 3. Species identifications of the animals used in thermal tolerance trials. Identifications were made after trials were run using an image-based key (Haney et al. 2013).

Lake	Daphnia catawba	Daphnia schødleri	Daphnia pulex	Daphnia dubia	Daphnia laevis	Daphnia mendotae	Ceriodaphnia reticulata	Totals
Eddy	3	10	1	-	-	-	-	14
Midway	5	8	1	-	-	-	-	14
Little Wilson	3	9	1	-	-	-	-	13
Wilson	8	6	-	-	-	-	-	14
Webb	5	-	-	3	3	2	2	15
Total	24	33	3	3	3	2	2	70

Table 4. Regressions of time to immobilization (T_{imm}) on temperature-related variables from study lakes in Western Maine. Since the *Daphnia* assemblage from Webb appeared to differ from that in the other lakes (Table 3) and the number of *D. catawba* / *D. schødleri* tested from that lake was small (n = 5), analyses were performed both with and without the inclusion of Webb.

Independent variable		5 lakes (Webb included) r^2	5 lakes (Webb included) p	4 lakes (Webb excluded) r^2	4 lakes (Webb excluded) p
Mean monthly daylight temperature	May	0.105	0.021	0.105	0.02
	June	0.023	0.26	0.07	0.06
	July	0.129	0.007	0.165	0.003
	August	0.159	0.002	0.205	0.0008
	September	0.245	0.0001	0.21	0.0007
Maximum summer temperature	Value	0.123	0.008	0.028	0.24
	Date	0.374	$5.55\,e^{-7}$	0.353	$4.34\,e^{-6}$
Ice-out date		0.002	0.723	0.014	0.393
Body length		0.015	0.368	0.021	0.31

Table 5. Correlations of T_{imm} with temperature-related variables, and of temperature variables with geomorphological characteristics for the study lakes in Western Maine, allowing an assessment of the two-step impacts of geomorphological variables on T_{imm} through their effects on temperature. Since the *Daphnia* assemblage from Webb appeared to differ from that in the other lakes (Table 3) and the number of *D. catawba* / *D. schødleri* tested from that lake was small (n = 5), analyses were performed both with and without the inclusion of Webb.

| | | | T_{imm} | 5 lakes (Webb included) | | | | | | 4 lakes (Webb excluded) | | | | | |
| | | | | Elevation | | Latitude | | Area | | Elevation | | Latitude | | Area | |
			r^2	r^2	p	r^2	p	r^2	p	r^2	p	r^2	p	r^2	p
Temperature variables	Mean monthly daylight temperature	May	0.105	0.911	0.046	0.988	0.006	0.160	0.600	0.911	0.046	0.988	0.006	0.160	0.600
		June	0.023	0.520	0.170	0.932	0.008	0.052	0.713	0.833	0.087	0.996	0.001	0.083	0.711
		July	0.129	0.899	0.014	0.864	0.022	0.035	0.762	0.983	0.009	0.871	0.067	0.374	0.389
		August	0.159	0.875	0.020	0.708	0.074	0.051	0.715	0.954	0.024	0.715	0.154	0.559	0.253
		September	0.245	0.905	0.013	0.454	0.213	0.463	0.206	0.947	0.027	0.696	0.166	0.580	0.238
	Maximum summer temperature	Value	0.123	0.425	0.234	0.119	0.569	0.798	0.041	0.630	0.206	0.650	0.194	0.149	0.614
		Date	0.374	0.581	0.134	0.137	0.540	0.746	0.059	0.693	0.168	0.394	0.372	0.650	0.194
	Ice-out date		0.002	0.423	0.235	0.874	0.020	0.047	0.725	0.642	0.199	0.909	0.047	0.012	0.892
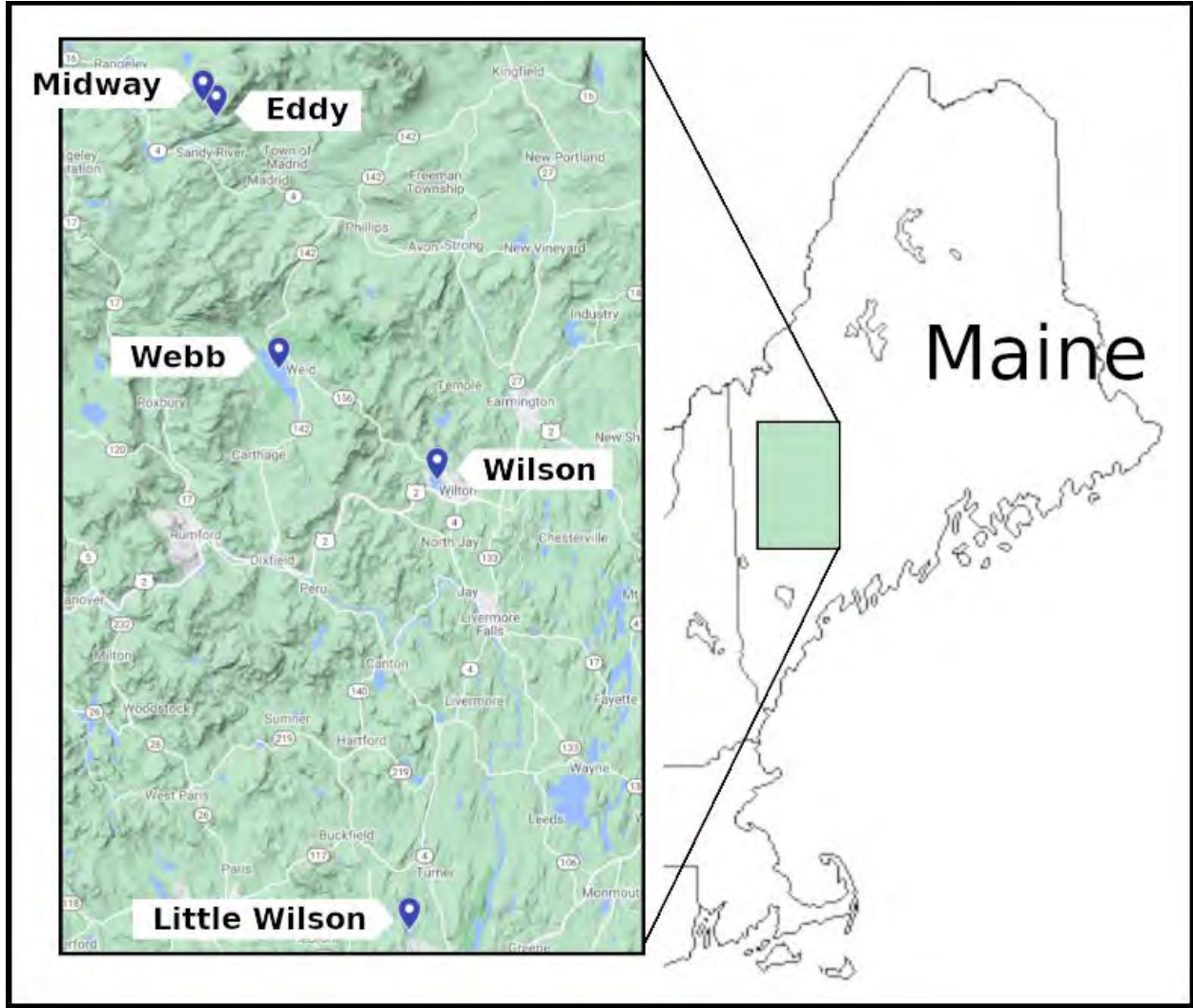
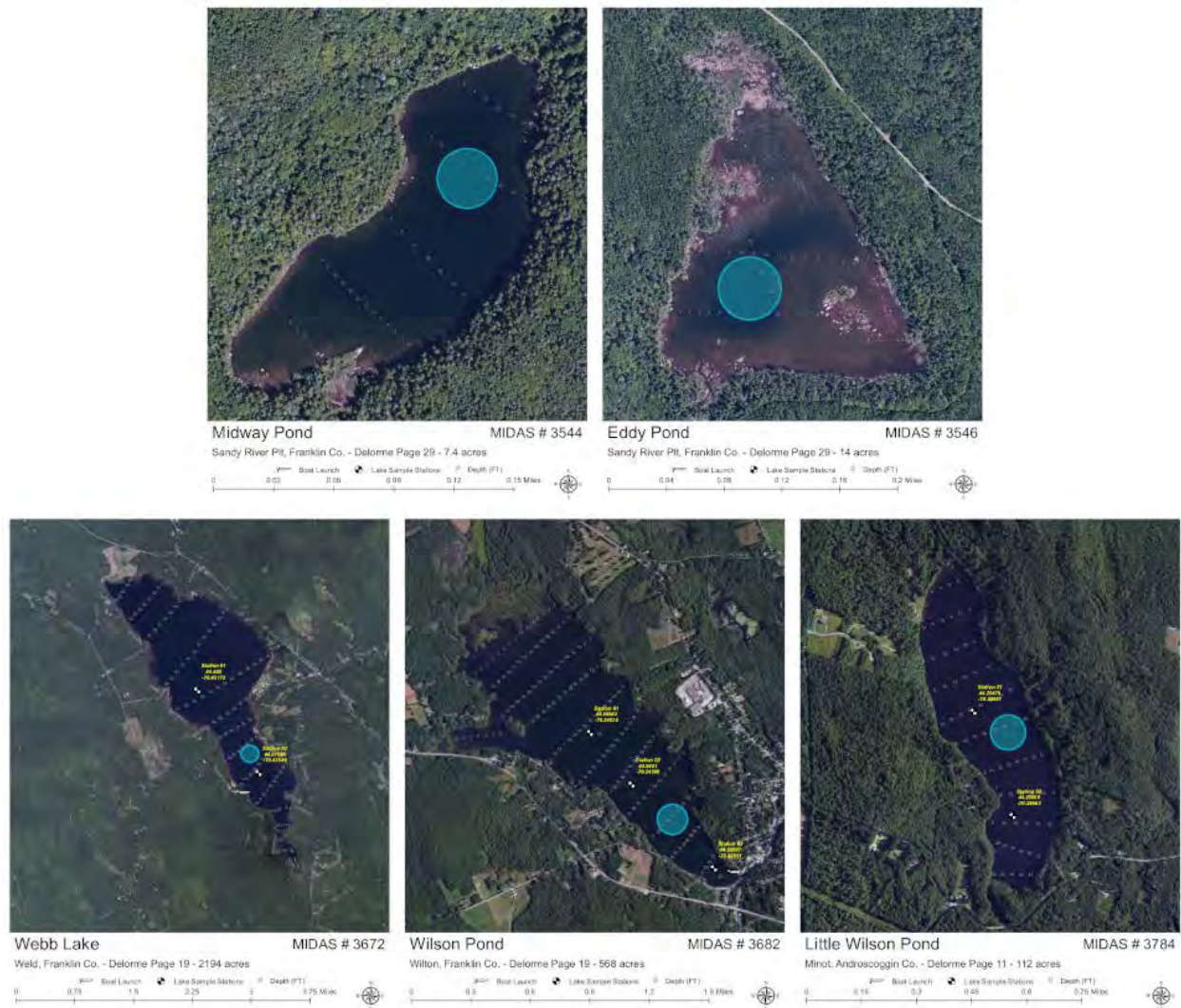Figure 1. Locations of the five Western Maine lakes used in the study. Distances between lakes ranged from 2 - 83 km.

Figure 2. Aerial photographs of the five Western Maine lakes studied. The circular marks represent the locations where plankton tows were made.
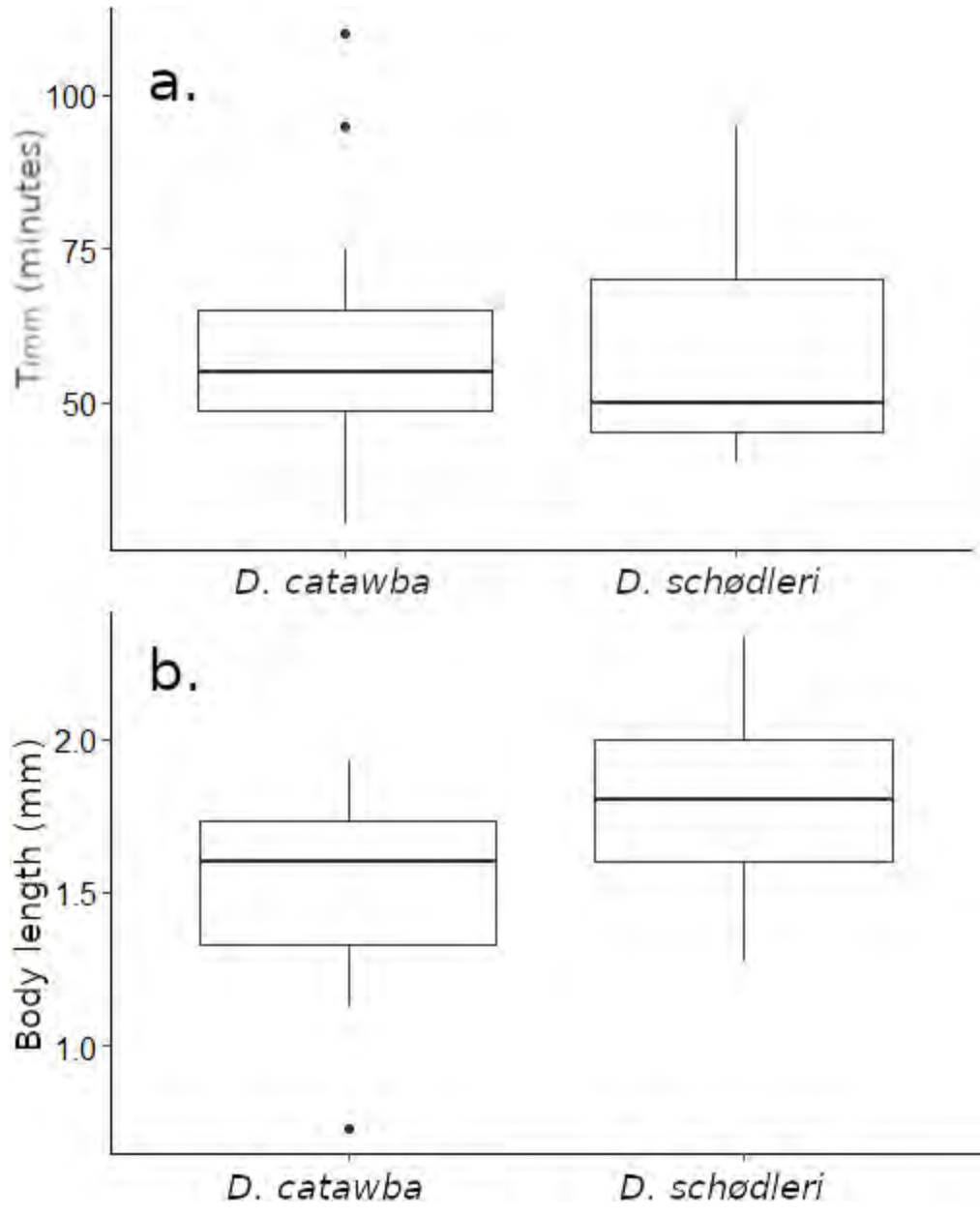
Figure 3. **a.** Time to immobilization (T_{imm}) did not differ between *Daphnia catawba* and *Daphnia schødleri* used in the study, but body length **b.** did. Box-and-whisker plots report median, first and third quartile, and range.
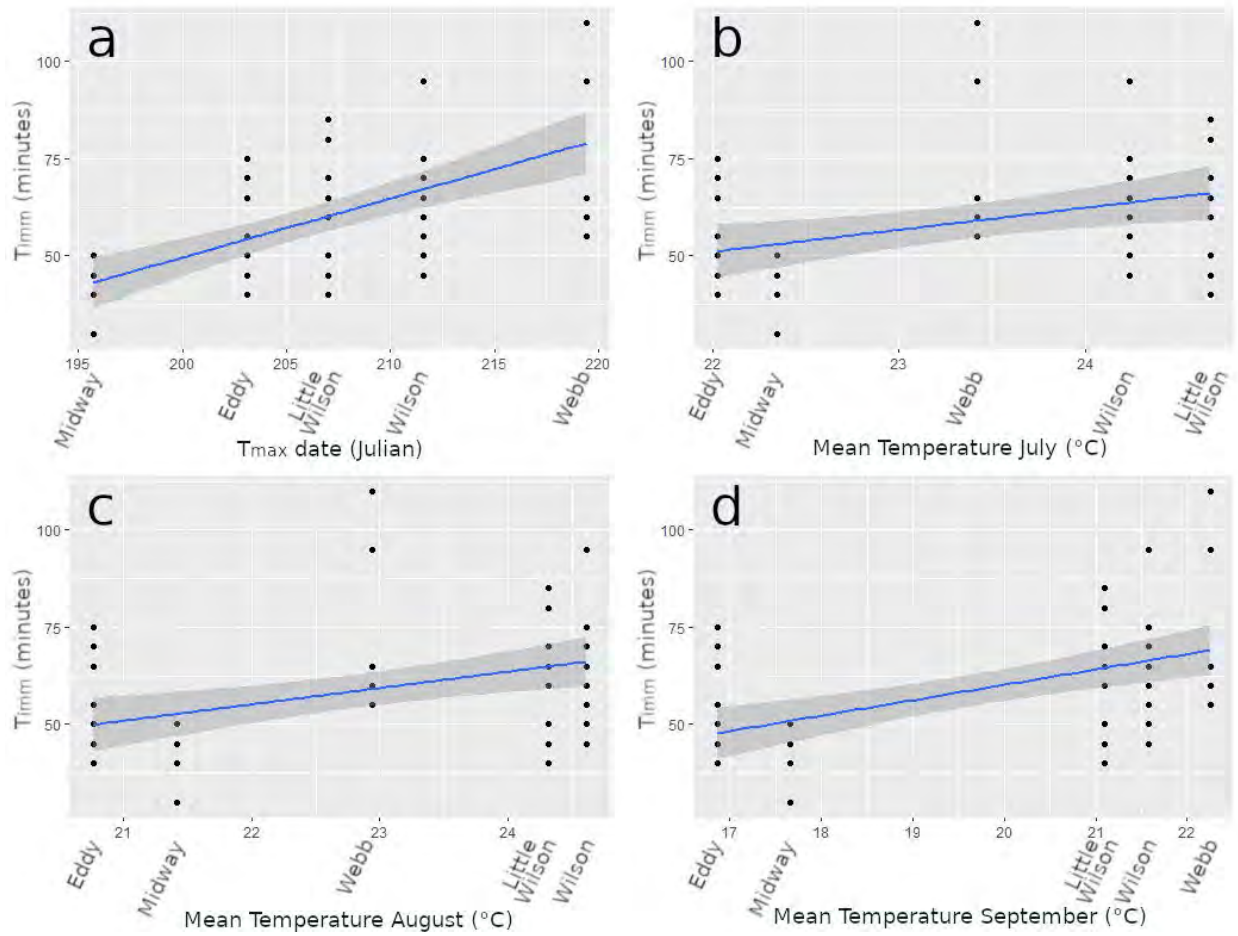
Figure 4. Dependence of thermal tolerance (time to immobilization T_{imm}) on four temperature-related variables (date of maximum temperature and mean monthly daylight temperatures for July, August, and September) in the 5 lakes studied in Western Maine. Correlation coefficients (r^2) and significance (p) of each relationship are reported in Table 4.
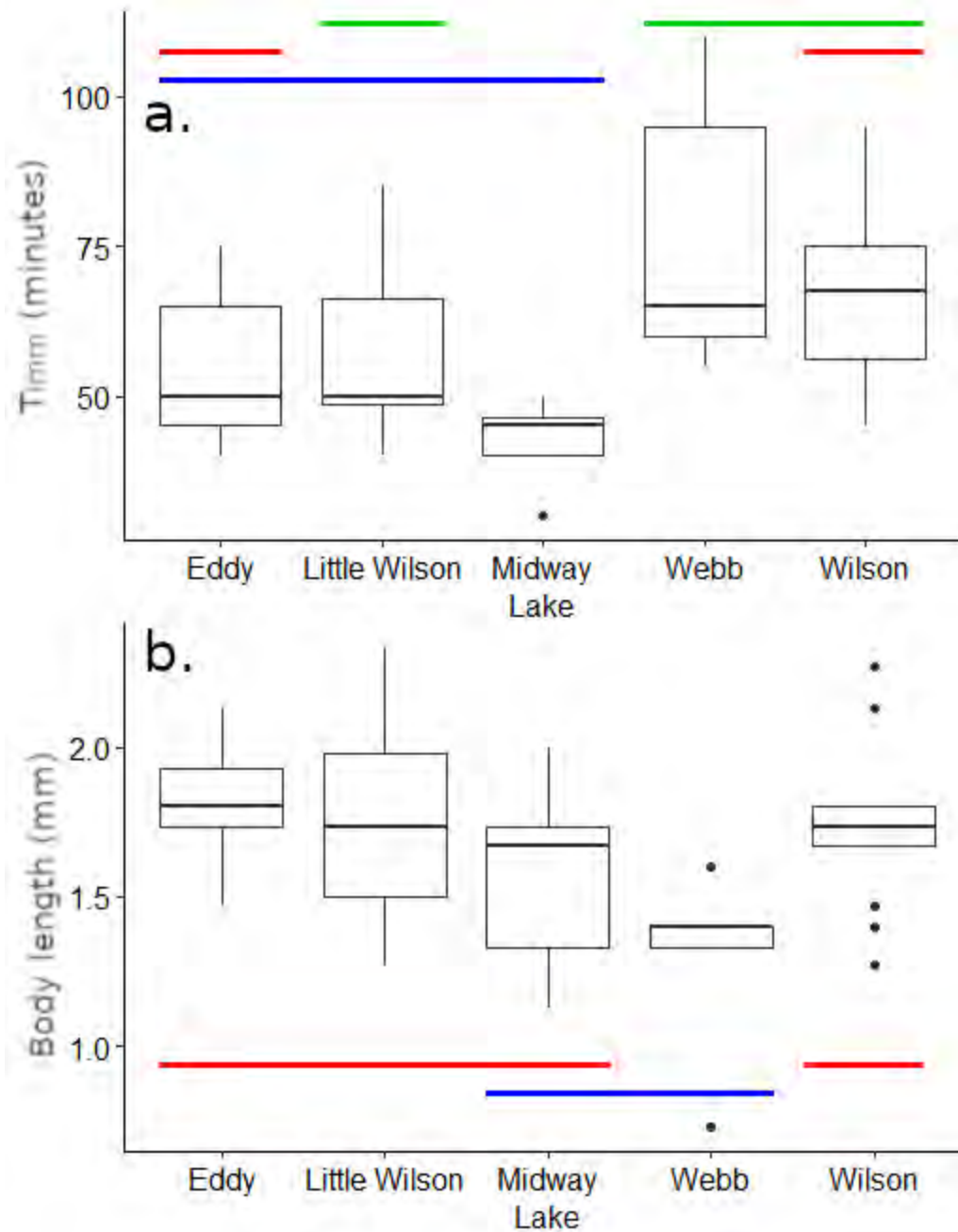
Figure 5. **a.** Time to immobilization (T_{imm}) and **b.** body length both differed among lakes used in the study. Box-and-whisker plots report median, first and third quartile, and range. Colored lines connect groups that did not differ significantly ($p > 0.05$) by *a posteriori* Tukey's HSD testing.
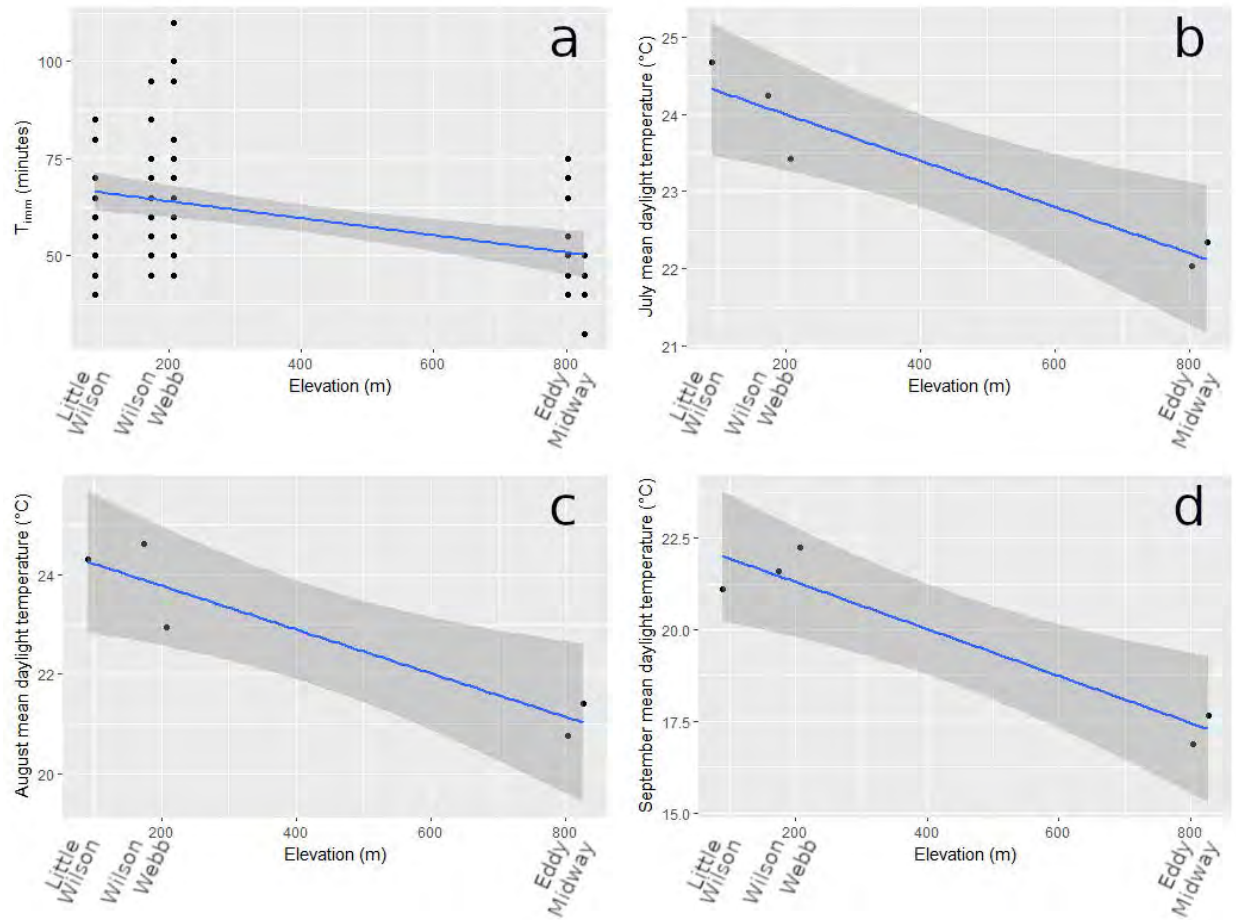
Figure 6. Dependence of thermal tolerance (time to immobilization, T_{imm}) and mean monthly daylight temperatures for July, August, and September on elevation in the five lakes studied in Western Maine. For T_{imm} v. elevation, $r^2 = 0.210$, $p < 0.001$; Correlation coefficients (r^2) and significance (p) of temperature-elevation relationships are reported in Table 5.