Variations in Zooplankton Communities as Indicators of Biological Responses to Climate Change and Recovery From Acidification in Northeastern and Maine Mountain Lakes

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VARIATIONS IN ZOOPLANKTON COMMUNITIES AS INDICATORS OF BIOLOGICAL RESPONSES TO CLIMATE CHANGE AND RECOVERY FROM ACIDIFICATION IN NORTHEASTERN AND MAINE MOUNTAIN LAKES

By

Stephanie Dykema

B.S. Fort Lewis College, 2013

A THESIS

Submitted in Partial Fulfillment of the Requirements for the Degree of Master of Science (in Ecology and Environmental Sciences)

The Graduate School The University of Maine

May 2021

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Thesis Advisors: Dr. Sarah J. Nelson, Dr. Ivan J. Fernandez

An Abstract of the Thesis Presented
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May 2021

Since the Clean Air Act Amendments of 1990, lakes in Maine and much of the Northeastern US have seen significant shifts in response to reduced atmospheric deposition as well as climate change. The organisms that inhabit lakes are susceptible to environmental change, but our understanding about how biological communities react to simultaneous changes in geochemistry and climate is incomplete. This research investigates how zooplankton communities respond to geochemical changes and warming, on both long-term and seasonal scales. These small organisms are sensitive to changes in physical lake conditions, and variation within zooplankton communities could indicate larger ecosystem shifts.

We evaluated historical water chemistry and zooplankton composition data from 143 lakes throughout the northeastern US from the EPA’s Eastern Lake Survey (ELS) to understand how recovery from acidification and subsequent chemical shifts influenced zooplankton. Surface water sulfate concentrations decreased by 22% between the two sample years of 1986 and 2004. Sites with closer proximity to development saw significant increases in chloride concentration likely due to increased use of road deicers, which caused a subsequent increase in calcium and magnesium base cations.
Zooplankton body size increased significantly between 1986 and 2004 and \textit{Daphnia} species, which have high calcium requirements, increased in correlation with increases in calcium. Zooplankton community structure, however, was most strongly influenced by variation in ANC, sulfate, and dissolved organic carbon (DOC). Our findings indicate that, though surface water acidity influences zooplankton community structure, recovery of zooplankton populations to a pre-acidification composition is unlikely because other chemical shifts along with changes in climate and land-use, are also eliciting responses in zooplankton communities.

A significant result of climate change is shifts in the timing of seasonally reoccurring events which have already been observed in Northeastern lakes including earlier ice break-up and longer periods of thermal stratification. Biotic responses to shifting seasons in lakes depends on species specific life-history traits and the seasonal events that drive them. We examined zooplankton phenology in lakes across a range of elevation and climate zones in Maine to understand the importance of spring surface water warm-up in high elevation versus low elevation sites. We collected zooplankton, chlorophyll-a, and water temperature in eight remote Maine lakes from the extremes of elevations (94m – 955m above sea level), representing the endmembers of climate variability in the state. High elevation lakes were distinguished by a short and rapid period of spring warming compared to more gradual warm-up in low elevation lakes. Zooplankton abundances increased in correlation with spring warm-up, most significantly in high elevation lakes. The link between surface water warming and zooplankton phenology varied among taxa, with rotifers and calanoid copepods more dependent on spring temperature than cladocerans and cyclopoid copepods. The variable response among zooplankton taxonomic groups to spring water warmup indicates that responses to climate change will be taxa specific. Disentangling the drivers of zooplankton populations will help provide a mechanistic understanding of longer-term climate and geochemical shifts observed in northeastern lake ecosystems and potential consequences of climate change.
DEDICATION

This is dedicated to my mother, Elizabeth Berbette, as well as Anne and John Whitman. I am grateful for your constant encouragement and generosity. Your love and support were paramount for my success and fulfilment throughout my time in Maine and continue to encourage my growth.
ACKNOWLEDGEMENTS

The US EPA–USGS LTM Network project, which includes HELM, were funded by EPA ORD to J.S. Kahl, W. McDowell, S.J. Nelson, K.E. Webster; and EPA CAMD to W.H. McDowell, J.S. Kahl, S.J. Nelson (IAG 06HQGR0143), processed through Grant/Cooperative Agreement G11AP20128 from the United States Geological Survey. The authors are solely accountable for the contents which do not necessarily represent the official opinions of USGS. HELM also received funding from the Maine DEP. Lake geochemical data are available to researchers upon request and are archived by the US EPA annually as part of the LTM (Long Term Monitoring) program (https://www.epa.gov/airmarkets/clean-air-markets-monitoring-surface-water-chemistry). Funding was also provided from the University of Maine, MAFES and the Graduate Student Government Grants Program. I would like to thank my advisor, Sarah Nelson, for unparalleled mentorship and inspiration. Sarah provided opportunities and support that encouraged my growth as a scientist and a female leader and helped make my graduate school experience rewarding and successful. I would also like to thank my advisor, Ivan Fernandez for jumping into a mentorship role last minute and whole heartedly supporting my development as a researcher with kindness and seasoned experience. My committee member, Rachel Hovel, not only provided research expertise and guidance but also joined me for fun and often chilly field work, inspired me as a passionate female ecologist, and advised me in my career endeavors. I also thank my committee member, Jasmine Saros for her expert research input and inspiration. I would like to thank the Niwot Ridge LTER PIs and staff for supporting me as I worked to complete my masters while managing my job duties. Julia Daily’s admirable dedication to the HELM project has allowed the program to continue and I thank her for implementing and managing in situ temperature loggers in the HELM lakes which provided valuable data for this project. Katherine Webster, the PI of the Eastern Lake Survey (ELS) zooplankton project at University of Maine in the early 2000s, provided valuable knowledge and guidance as I studied the historical ELS dataset. The National Park Service and Biologist Bill Gauley with Acadia National Park
permitted me to install a temperature probe in Sargent Mountain Pond and to sample for zooplankton. Thank you to my technician, Isaiah McFarlane for identifying and counting many zooplankton samples. Thank you to Ken Johnson, Amanda Gavin, Paige Strasko, Isaiah McFarlane, Leah Beck, and Elyse DeFranco for field assistance and making every field day fun no matter the conditions. Lastly, I want to thank my family, my boyfriend, Mike, and my dog, Rio for their unfaltering support.
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CHAPTER 1

ZOOPLANKTON COMMUNITY RESPONSE TO RECOVERY FROM ACIDIFICATION AND BIOGEOCHEMICAL SHIFTS IN NORTHEASTERN US LAKES

Abstract

Lakes in the northeastern US and around the globe saw significant declines in water quality and ecosystem health in the mid to late 20th century as atmospheric deposition from industrial emissions caused waters to acidify. Significant chemical shifts have been detected in northeastern lakes since the 1980’s when surface water acidification was widely reported and, subsequently, programs were established by the U.S. Environmental Protection Agency (EPA) to monitor environmental responses to limit emissions regulations. Since the Clean Air Act Amendments of 1990 intensified regulations to limit emissions of sulfur and nitrogen, lakes in much of the Northeast have recovered from acidification, just as other chemical and physical changes have been detected in response to acid reduction and climate change. The organisms that inhabit lakes are susceptible to these shifts, but our understanding about how different biological communities react to co-occurring changes in geochemistry is incomplete. We evaluated a large historical dataset from the EPA’s Eastern Lake Survey (ELS) of 143 lakes throughout the northeastern U.S. to understand how recovery from acidification and subsequent chemical changes influenced zooplankton body size and community composition. Surface water sulfate concentrations decreased by 22% in the ELS lakes between the two sample years of 1986 and 2004. Increasing DOC is a common result from the combined influence of deacidification and climate change and the ELS lakes also saw a 26% increase in DOC. Notably, in the more developed regions of our study area, chloride concentration in lakes tripled, likely due to increased use of road deicers, which caused a subsequent increase in calcium and magnesium base cations. Zooplankton body size increased significantly between 1986 and 2004 and *Daphnia* species, which require higher amounts of calcium than other zooplankton
species, increased in correlation with increases in calcium and magnesium. Shifts in zooplankton community structure, however, were most strongly influenced by variation in ANC, sulfate, and DOC. Our findings indicate that, though surface water acidity has a strong influence on zooplankton community structure, recovery of zooplankton populations to a pre-acidification composition is unlikely because other chemical changes are occurring in lakes in response to acid recovery and these, along with changes in climate and land-use, also elicit responses in zooplankton communities.

**Introduction**

Acidic surface waters were first recognized as a threat to freshwater ecosystems in the U.S. in the early 1970’s, and reports revealed a strong connection to emissions of sulfur and nitrogen from industrial pollution. National and international policies successfully reduced sulfur emissions in the U.S. by almost 70% by regulating stationary sources, especially coal burning power plants, and also reduced nitrogen by 50% between 1980 and 2010 (Strock et al., 2014). The Clean Air Act, first instated in 1963, has been revised by Congress several times and since the Amendments of 1990 intensified regulations to limit emissions of sulfur and nitrogen, assessment programs were established by the U.S. Environmental Protection Agency (EPA) to monitor environmental responses to emissions regulations (Kahl et al., 2004). These EPA monitoring programs began sampling lakes and streams in acid-sensitive regions of the U.S. as early as 1982 (Adirondack Long Term Monitoring) and have changed in number and rigor over the decades but continue to sample 94 lakes and 76 streams for surface water chemistry in Maine, Vermont, New York, and Virginia (US EPA LTM Network, https://www.epa.gov/airmarkets/clean-air-markets-monitoring-surface-water-chemistry). Results from these monitoring networks and other regional research indicate that lakes and streams in much of the Northeast have recovered from acidification. Here we define “recovery” as significant reductions in surface water SO4 that results in improved water quality for lake ecosystems
(Stoddard et al., 1999). Strock et al. (2014) evaluated 74 sites in New England and New York’s Adirondack mountains from the EPA’s Temporally Integrated Monitoring of Ecosystems (TIME) project and found rapid reductions in lake SO$_4^-$ between 1980 and 2010, accelerating after 2000, and simultaneous increases in acid neutralizing capacity (ANC); however, nitrate (NO$_3^-$) in surface water showed no directional trend. Analyses from other monitoring programs in the region have found similar patterns of decreases in sulfate (Kahl et al., 2004; Stoddard et al., 1999; Strock et al., 2014; Sullivan et al., 2018; Driscoll et al., 2003; Fuss et al., 2015). However, additional chemical and physical changes have been detected in response to shifts in water chemistry, and other anthropogenic factors like climate change have confounded the success of acid recovery (Finstad et al., 2016; Gavin et al., 2018; W. Keller et al., 2007; Catherine H. Rosfjord et al., 2007; Strock et al., 2016; Yan et al., 2008).

Evidence for the success of recovery from acidification in freshwaters of the Northeast have almost entirely focused on chemical change over time with a few projects collecting physical data like water temperature and Secchi transparency, but the dramatic shifts in chemistry that have been observed could also affect lake biota. Evidence suggests that full biological recovery from acidification, or a return to pre-acidification lake community structure, is unlikely due to confounding influences like changing land use and climate, and only a few studies have assessed biological responses to changing geochemistry in northeastern lakes. For example, analysis of a 20 year data set from 28 lakes in the Adirondack Park indicated that shifts in phytoplankton were driven by increasing DOC and not by acid recovery, and that zooplankton composition was most sensitive to declines in calcium (Leach et al., 2019). Similarly, Stager et al. (2019) found that acid-sensitive diatom species increased in an Adirondack lake as pH increased but other changes such as increases in DOC and water temperature likely contributed to a general increase in phytoplankton productivity that could not be attributed solely to declining acidity. Additionally, variation in predation pressures and inconsistent fish stocking practices made it difficult to assess biological recovery alongside acid recovery (Stager et al., 2019).
Reestablishment of fish species, however, has been detected in some recovering lakes. For instance, a study in Norway found that stocked brown trout (Salmo trutta) began reproducing naturally after 2008 when a pH threshold of 5.1 was reached (Lund et al., 2018). Similarly, research on experimentally manipulated lakes and naturally deacidified lakes with remnant fish populations in Ontario detected increases in lake trout (Salvelinus namaycush) populations; however, other acid sensitive fish species remained suppressed (Gunn & Mills, 1998). A long-term study of Adirondack Mountain lakes found that significant increases in ANC did not correlate with increased richness of fish species or abundance of brook trout (Salvelinus fontinalus) (Baldigo et al., 2016).

Zooplankton are useful organisms for studying lake ecosystem responses because these small and short-lived organisms are sensitive to changes in lake conditions due to their short life cycles, and variation within zooplankton communities could indicate larger ecosystem shifts. Pertinent zooplankton research provides evidence to suggest that the chemical shifts observed in northeastern lakes as surface water acidity declines likely elicited a reorganization of zooplankton communities. For instance, several studies on lakes in the Canadian shield found that lakes that recovered to a pH > 6.0 experienced shifts in zooplankton community composition progressing towards a pre-acidification structure with abundances of already-present species increasing first, followed by recolonization of acid sensitive species (W. Keller & Yan, 1998; Locke et al., 1994). A similar study, however, observed that variable responses among different zooplankton groups slowed recovery. Cladoceran species recovered more slowly due to increases in fish with acid recovery while copepods’ lack of parthenogenic reproduction inhibited dispersal, slowing their response to increasing pH (Gray et al., 2012). Other studies that have focused on fish recovery indicate that the decline in predators that resulted from acidification restructured aquatic food webs and that recovery is unlikely to follow a linear path or to result in a community similar to the pre-acidification system (Gunn & Mills, 1998). These inconsistent findings
emphasize the need for continued monitoring and further research to disentangle the mechanisms and trends of biological responses to chemical shifts (Baldigo et al., 2016; Driscoll et al., 2001).

One of the EPA programs that contributed to the monitoring of northeastern freshwaters during acidification was the Eastern Lake Survey (ELS), which was also one of the few regional-scale projects that sought to understand biological reactions to shifting water chemistry. ELS was initiated in 1984 as part of the National Surface Water Survey (NSWS) and phase I sampled 768 lakes for surface water chemistry with the purpose of linking atmospheric deposition with ANC. ELS-II resampled a subset of 145 of the most acid sensitive ELS-I lakes in 1986 and lakes were sampled again in 2004 via the University of Maine and added the collection of zooplankton in both years. These lakes extend through five different subregions of the Northeast defined by the EPA in 1984 and include Maine, Central New England, Southern New England, the Adirondack Mountains of New York, and the Pocono and Catskill Mountains of Pennsylvania and New York (Figure 1) (Rosfjord, 2005).

In accordance with trends observed throughout the Northeast, the chemistry of lakes in the ELS-II program changed significantly over the study period from 1984 to 2004 with most surface waters recovering from acidity. Over 90% of the ELS-II lake population experienced significant decreases in surface water SO$_4$, shifting from an average of 111 µeq/L in 1984 to 86 µeq/L in 2004 with ANC increasing from 86 µeq/L to 102 µeq/L. Also, in concurrence with other long-term studies, dissolved organic carbon (DOC) increased in 86% of the ELS-II lakes and aluminum (Al) concentration decreased, although differing filtration methods for the Al analyses may have influenced the variation (Rosfjord, 2005). The most noteworthy and unexpected chemical change that Rosfjord detected in the ELS-II lakes was an increase in chloride with a correlated increase in base cations (Ca+Mg). A thorough investigation of these parameters illustrated that about half of the ELS-II lakes were affected by anthropogenic sources of salt, generally lakes in closer proximity to roads, and these lakes saw steep increases in Cl.
between 1984 and 2004. Rosfjord et al. (2007) concluded that NaCl road deicers were inducing cation exchange of Na for Ca, resulting in an increase of Ca in surface waters. While other studies have indicated decreasing trends in base cation concentrations in surface waters which slow recovery from acidification, the ELS-II lakes in more developed regions appear to be an anomaly due to the influence of road salts (Evans et al., 2008; Kirchner & Lydersen, 1995). The ELS lake set is large and diverse and, although region and site specific variability complicate disentangling the drivers of change, significant trends of chemical change were observed between the 1984 and 2004 data and likely resulted from decreases in atmospheric deposition combined with shifts in climate and changes in land use (Gavin et al. 2018; Rosfjord et al. 2007; Stoddard et al. 1999; Strock et al. 2016).

ELS-II lakes were sampled for zooplankton in addition to surface water chemistry, providing the opportunity to investigate the linkage between chemical change and biological response. This dataset includes zooplankton abundance counts as well as body size estimates and although thorough analyses of chemical data showed significant shifts in these lake systems, analysis of zooplankton responses have yet to be explored. This research aims to 1) document the ELS-II legacy zooplankton dataset for publication, 2) investigate relationships among shifting water chemistry parameters and zooplankton communities, and 3) summarize the implications of findings and potential areas for further data exploration. The literature suggests that zooplankton community composition was radically restructured with the acidification of freshwaters and that community shifts have continued to occur in lakes throughout the Northeast as climate and water quality endure continued change (Fernandez et al., 2020; Locke et al., 1994; Walseg et al., 2003). We predict that zooplankton in the ELS-II lakes experienced significant shifts in community composition relative to the extent of acid recovery experienced between 1986 and 2004, with increases in acid sensitive species and overall species richness. Shifts in ANC and calcium are likely to be the strongest drivers of zooplankton community reorganization. With the success of emissions regulations and reduction in acidic deposition in
freshwater bodies (Sullivan et al., 2018), scientists and regulators are particularly interested in biological recovery associated with acid recovery and the ELS-II dataset provides an opportunity to explore the biological implications of recovery in a statistically-representative population of lakes in the northeastern U.S.

**Materials and Methods**

**Site description**

This research evaluates data from phase II of the U.S. EPA’s Eastern Lake Survey (ELS-II) which sampled 145 lakes in the northeastern U.S. in 1986 and 2004. The U.S. EPA established the National Surface Water Survey (NSWS) in 1983 with the intention of evaluating the effects of atmospheric deposition on freshwater bodies in regions of the northeastern U.S. where lakes and streams were most acid sensitive. Phase I of the program was called the Eastern Lake Survey I (ELS-I) and sites for the program were selected from all lakes with a surface area > 4 ha within acid-sensitive regions of the U.S. using a stratified random design method; the resulting 768 lakes were sampled in 1984 for surface water chemistry. ELS-II, the focus of this study, sampled a subset of the ELS-I lakes reducing site number to minimize variability by eliminating lakes with confounding anthropogenic influences or landscape characteristics and selecting lakes with the lowest ANC. The resulting 145 lakes were sampled in 1986, before major legislation restricted industrial emissions, and again in 2004, almost two decades later and 14 years after the Clean Air Act Amendments of 1990 limited atmospheric deposition of S and N. The ELS-II lakes are located in the northeastern U.S. in five different subregions designated by the EPA (see Figure 1): 1) The Adirondack mountains of New York, 2) The Pocono/Catskills mountains in New York and Pennsylvania, 3) Southern New England, 4) Central New England, 5) Maine. A thorough outline of the ELS-I and ELS-II site selection methods and detailed description of subregions can be found in Rosfjord (2005).
Data collection and sampling

Water samples and zooplankton tows were collected at each site between July 23-August 11, 1986 and between July 14- August 31, 2004. Samples were collected from the deepest area of each lake, and accessed by boat, helicopter, or float plane. Water samples were collected from the epilimnion of each pond and sampling methods, laboratory analyses and quality assurance protocols followed ELS-II protocols described by Merritt and Sheppe (1988) and standard EPA methods from the Long Term Monitoring (LTM) and Temporally Integrated Monitoring of Ecosystems (TIME) programs (Rosfjord, 2005; Strock et al., 2014).

Zooplankton were sampled with a Wisconsin style net with 80 µm mesh and vertical tows were collected from 0.5 to 1 meter off the bottom of the lake to the surface. Samples were fixed with 4%
sugar buffer formalin solution (1986) or ethanol (2004) and stored in a dark space. Zooplankton samples were identified to the species level by Alan Tessier in 1986. Katherine Webster’s laboratory in the Biological Sciences Department of the University of Maine analyzed the 2004 zooplankton samples; and species were grouped by higher-order taxa with cladocerans identified to the genus level and copepods distinguished as Calanoid or Cyclopoid. Abundance counts were recorded, and an ocular micrometer (1986) or FlowCam and ImageJ software (2004) was used to measure the body length of each taxa.

The ELS lakes have experienced significant shifts in chemistry over the course of the two decades between the two initial samples in 1984 and 1986 and the most recent sample in 2004. Over this time period, the northeastern U.S. also faced significant changes in climate including increases in air temperature and extreme precipitation events (Thibeault et al., 2014). Lakes, in turn, were experiencing shorter ice-over periods, warmer surface water temperatures, and other more complex shifts due to their close connection with the terrestrial environment (Hodgkins et al., 2002; O’Reilly et al., 2015). Average summer rainfall and air temperatures were determined using Parameter-elevation regression on independent slopes model (PRISM). PRISM uses an interpolation method to calculate climate variables at 4km resolution based on data from existing data stations (Daly et al., 2008).

**Data exploration and analysis**

The ELS dataset has passed hands and has been added to, managed, and amended by various investigators over the decades. Our initial work began with evaluating, cleaning, and organizing the data and identifying subsets of data with statistical potential. We approached this long-term dataset to address ecological questions beginning with an evaluation of changes in water chemistry over two decades of the study. Rosfjord (2005) thoroughly examined chemical changes in the ELS II lakes between 1984, the first year the lakes were sampled for water chemistry by ELS I, and 2004 when she oversaw the resampling of the ELS II lake population. The results illustrated significant shifts in water chemistry
over the 20-year period particularly in relation to acidity, chloride, and base cations (Rosfjord, 2005; Rosfjord et al. 2007). This research similarly explored trends in water chemistry, but used the years 1986-2004, to evaluate changes in water chemistry for the years in which zooplankton sampling took place. Chemistry data were first assessed for normality and transformed when necessary, to allow for use of parametric tests. A paired sample t-test was run on chemical variables including SO$_4$, NO$_3$, pH, ANC/alkalinity, Cl, and Ca + Mg to assess whether significant differences existed between 1986 and 2004.

Rosfjord (2005) used chemical and regional groupings in her analyses of chemistry, dividing the lake population among three ANC classes based on 1984 values: class 1. ANC ≤ 25µeq/L, class 2. 25 µeq/L ≤ ANC ≤ 100µeq/L, class 3. 100 µeq/L ≤ ANC ≤ 400 µeq/L. Lakes were also grouped based on high and low chloride to distinguish lakes affected by road salt. Cl groupings were calculated based on 2004 concentrations and marine inputs of Cl were estimated using the regression equation,

$$\ln(\text{Cl}_{\text{sea}}) = 5.35 - 0.0365 \times \text{distance from coast} + 0.00012 \times (\text{distance from coast})^2,$$

which allows for the following groupings: low – Cl < 20 µeq/L or, if within 100km of the coast Cl < 1.5 x Cl$_{\text{sea}}$; high – remaining lakes (see Rosfjord, 2005 for detailed grouping methods). These same groupings were used as categorical variables in our analysis, along with the five subregions, and analysis of variance tests (ANOVA) and PCA were performed to explore differences among subregions and chemical groupings between the two years of data.

Under the context of chemical change in the ELS-II lakes, we next sought to explore how different shifting chemical parameters influenced zooplankton communities in these lakes. Two zooplankton datasets of interest were: 1) relative abundance counts (individuals per L lake water) of major genera for cladocerans and order for copepods from 2004 and, 2) average body length of zooplankton genera or orders from 143 lakes in 1986 and 2004. Relative abundances provide an
opportunity to explore shifts in community composition while body size is a useful gauge of grazing pressure and growth potential (Arnott et al., 2017; Moore et al., 1996; Stemberger & Miller, 2003). Although zooplankton abundance counts were recorded in 1986, the database of legacy data contains zooplankton densities as number of individuals per meter of water sampled. We were unable to locate raw count data for 1986 or tow depth for 2004 and therefore could not compute relative abundances that would be comparable between the two sample years.

A paired t-test of average body length between 1986 and 2004 indicated that body size significantly increased from one decade to the next. Given this finding, data across both years were explored using simple and multiple linear regression analysis in R. Since we only have two sample years, year did not function as a viable explanatory variable. Regressions evaluated trends in body size corresponding to gradients in chemical data. Chemical variables included all analytes listed in Table 1 and were log transformed excluding pH and Akaike information criterion (AIC) was used to determine the best fitting model and number of model parameters.

Ordination methods allow representation of multiple dimensions in low dimensional space and can provide information on relative importance of variables. Nonparametric multidimensional scaling (NMDS) is effective for exploring abundance data where many unique species function as individual variables (Clarke & Ainsworth, 1993). We used NMDS to explore the relationship between zooplankton community composition and chemical and physical variables for the 2004 samples using the ‘vegan’ package in R and ‘metaMDS’ (Oksanen et al., 2019). In 2004, zooplankton were identified to the genus level for cladocerans, and order level for copepods so the following taxonomic groups were used for this analysis: Calanoid copepods, Cyclopoid copepods, Diaphanosoma, Daphnia, Holopedium, Polyphemus, Bosmina, Ceriodaphnia, Chydomus, and Leptodora. A Wisconsin double standardization was used to transform abundance values relative to each other and lessen the influence of zeros, and a Bray Curtis
distance matrix created values for each taxon based on dissimilarity. Stress, a measure of goodness of fit, was used to select the model that best described the data and obtain a p-value to quantify significance (Clarke, 1993). Chemical parameters and species abundances were log transformed and significant variables (p>0.05) were plotted as loadings. Community differences among Rosfjord’s (2005) groupings of ANC class, region, and Cl were assessed using Analysis of Similarity test (ANOSIM), a hypothesis test similar to ANOVA that is useful for evaluating ranked dissimilarities (Clarke 1993). Additionally, the survival threshold of 1.5mg/L Ca reported by several studies for Daphnia species was used to group lakes by high and low calcium to test community differences between these groups (Ashforth & Yan, 2008; Jeziorski et al., 2012a). Indicator species analysis was used to evaluate if certain species were significantly associated with chemical groupings or regions (De Cáceres et al., 2010). Mantel tests with Spearman’s correlations were used to explore which continuous chemical variables influenced community structure (Legendre, 1993). All significance tests used the Bray Curtis dissimilarity matrix computed from relative zooplankton abundances.

**Results**

**Shifts in water chemistry**

Similar to Rosfjord’s (2005) findings assessing changes between 1984 and 2004, surface water chemistry shifted between 1986 and 2004 with significant increases in ANC, DOC, Ca, and Cl, and decreases in SO\(_4\) (p < 0.001) (Figure 2; Table 1). SO\(_4\) decreased at all sites from a median of 105.4 µeq/L to 81.9 µeq/L. Median alkalinity in 1986 was 57.7 µeq/L which improved to a median ANC of 72.1 µeq/L in 2004. Cl increased significantly in all lakes from a median of 38.1 µeq/L to 78.3 µeq/L but in the high Cl lake class concentrations tripled from 79.4 µeq/L to 238.7 µeq/L. In contrast, the low Cl lakes saw a decrease in Cl concentration from a median of 15.8 µeq/L to 9.30 µeq/L. Similarly, base cations (Ca+Mg) increased most significantly in the high Cl lakes with a median increase of 22.1 µeq/L while low Cl lakes
decreased by 5.94 µeq/L. DOC also significantly increased in the majority of the ELS-II lakes from a median of 3.7 to 4.7 mg/L (Table 1).

Figure 1.2. Violin plot of the kernel probability density of geochemical variables compared between 1986 (blue) and 2004 (red) in ELS-II lakes. Internal boxplots depict the median (bold horizontal line) and 25th and 75th quartiles (box). The whiskers indicate a 1.5x interquartile range and dots represent outliers. a) SO₄ concentration from all sites. b) DOC from all sites. c) Cl from sites in the high chloride group d) Ca from sites in the high chloride group. All variables were log transformed for analysis, but untransformed values are plotted for ease of interpretation.
Table 1.1 Summary statistics of surface water chemistry parameters in 1986 and 2004. Calcium and chloride values for the high chloride lake group (lake water Cl content > 20 µeq/L or, if within 100km of the coast >1.5 x Cl marine inputs) are included.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Min</th>
<th>Max</th>
<th>Median</th>
<th>Mean</th>
<th>SD</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkalinity 1986 (µeq/L)</td>
<td>-48.6</td>
<td>454.8</td>
<td>57.7</td>
<td>89.1</td>
<td>107.8</td>
<td>143</td>
</tr>
<tr>
<td>ANC 2004 (µeq/L)</td>
<td>-26.3</td>
<td>538.0</td>
<td>72.1</td>
<td>102.5</td>
<td>114.7</td>
<td>143</td>
</tr>
<tr>
<td>Air-equilibrated pH 1986</td>
<td>4.50</td>
<td>8.12</td>
<td>6.86</td>
<td>6.53</td>
<td>0.93</td>
<td>143</td>
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<tr>
<td>Air-equilibrated pH 2004</td>
<td>4.55</td>
<td>7.72</td>
<td>6.70</td>
<td>6.54</td>
<td>0.71</td>
<td>143</td>
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<tr>
<td>SO₄ 1986 (µeq/L)</td>
<td>30.8</td>
<td>331.7</td>
<td>105.4</td>
<td>106.4</td>
<td>42.1</td>
<td>142</td>
</tr>
<tr>
<td>SO₄ 2004 (µeq/L)</td>
<td>23.7</td>
<td>318.8</td>
<td>81.9</td>
<td>85.9</td>
<td>38.4</td>
<td>143</td>
</tr>
<tr>
<td>NO₃ 1986 (µeq/L)</td>
<td>0.03</td>
<td>22.7</td>
<td>0.5</td>
<td>1.7</td>
<td>3.5</td>
<td>143</td>
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<tr>
<td>NO₃ 2004 (µeq/L)</td>
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<td>19.5</td>
<td>0.05</td>
<td>1.1</td>
<td>3.3</td>
<td>143</td>
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<tr>
<td>DOC 1986 (mg/l)</td>
<td>0.3</td>
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<td>3.7</td>
<td>4.3</td>
<td>2.4</td>
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<tr>
<td>DOC 2004 (mg/l)</td>
<td>0.8</td>
<td>24.1</td>
<td>4.7</td>
<td>5.6</td>
<td>3.5</td>
<td>143</td>
</tr>
<tr>
<td>Cl 1986 (µeq/l)</td>
<td>4.5</td>
<td>612.7</td>
<td>38.1</td>
<td>78.5</td>
<td>119.6</td>
<td>85</td>
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<tr>
<td>Cl 2004 (µeq/l)</td>
<td>3.4</td>
<td>1303.2</td>
<td>78.3</td>
<td>207.8</td>
<td>291.4</td>
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<tr>
<td>Cl in high Cl class 1986 (µeq/l)</td>
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<td>612.7</td>
<td>79.4</td>
<td>132.5</td>
<td>149.0</td>
<td>43</td>
</tr>
<tr>
<td>Cl in high Cl class 2004 (µeq/l)</td>
<td>22.2</td>
<td>1303.2</td>
<td>238.7</td>
<td>356.3</td>
<td>318.9</td>
<td>80</td>
</tr>
<tr>
<td>Ca + Mg 1986 (µeq/l)</td>
<td>36.9</td>
<td>619.6</td>
<td>149.2</td>
<td>193.3</td>
<td>121.3</td>
<td>143</td>
</tr>
<tr>
<td>Ca + Mg 2004 (µeq/l)</td>
<td>35.9</td>
<td>838.5</td>
<td>176.6</td>
<td>222.3</td>
<td>158.4</td>
<td>143</td>
</tr>
<tr>
<td>Ca + Mg in high Cl class 1986 (µeq/l)</td>
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<td>619.6</td>
<td>188.4</td>
<td>222.5</td>
<td>132.4</td>
<td>80</td>
</tr>
<tr>
<td>Ca + Mg in high Cl class 2004 (µeq/l)</td>
<td>37.3</td>
<td>838.5</td>
<td>210.5</td>
<td>274.2</td>
<td>175.7</td>
<td>80</td>
</tr>
</tbody>
</table>
Zooplankton response

Zooplankton body lengths across all taxa were significantly greater in 2004 than 1986 based on a paired t-test ($t (731) = 5.27, p < 0.0001$) increasing from a median of 0.66mm to 0.74mm (Figure 3). We evaluated whether body size was related to increases in Ca as has been reported in previous studies (Jeziorski et al., 2012; Tessier & Horwitz, 1990), but linear and multiple regressions did not reveal a chemical driver of zooplankton body length when all lakes and all taxa were included. This analysis was repeated using only select cladoceran species and lakes from each region and ANC class to test for relationships between zooplankton body length and chemical parameters among more similar lakes, and this also yielded no significant results. However, when *Daphnia* species in the high Cl lake group were isolated and analyzed with linear regression, we saw that increases in Ca+Mg had a weak correlation with increased body length ($p = 0.10$, $r^2 = 0.013$) (Figure 4). In the high Cl lake group, *Daphnia* average body length increased significantly ($t (66) = 5.07, p< 0.0001$) from a median of 0.75mm to 0.88mm (Figure 3) and Ca+Mg concentrations increased from a median of 188.36 µeq/L to 210.46 µeq/L (Figure 2; Figure 1; Table 1).
Figure 1.3. Violin plots showing the kernel probability density of average body lengths of (a) all zooplankton taxon at all sites in 1986 (blue) and 2004 (red) and (b) average body lengths of *Daphnia* species from the high Cl lake group in 1896 (blue) and 2004 (red). Internal boxplots show the median (bold horizontal line) while the box represents the 25th and 75th quartiles. The whiskers indicate a 1.5 x interquartile range with dots representing outliers.
Figure 1.4. Average body length of *Daphnia spp.* from high Cl ELS-II lakes regressed against base cation concentrations (Ca + Mg). Ca + Mg was log transformed for analysis, but untransformed values are plotted for ease of interpretation. Linear regression indicates a marginal positive trend (\( p = 0.1, r^2 = 0.021 \)).

A three-dimensional NMDS of zooplankton abundances best explained community variability with a stress of 0.17 after 54 iterations (Figure 5). Loadings indicated that community dissimilarity among sites was influenced most prominently by *Holopedium, Daphnia*, closed cell pH, and Calcium. Color coding of sites based on the Ca threshold of 1.5mg/L illustrated a differentiation in community structure between high and low Ca lakes, and an indicator species analysis revealed that *Daphnia* species were associated with lakes in the high calcium group. However, ANOSIM analyses illustrated that community structure varied most significantly among regions (ANOSIM, \( R = 0.064, \text{significance} = 0.0009 \)) and ANC class (ANOSIM, \( R = 0.086, \text{significance} = 0.0001 \)) and not significantly between Ca groups.
Mantel tests evaluating correlation between individual chemical variables and community dissimilarity from the Bray Curtis dissimilarity matrix used in the NMDS indicated that variability in SO$_4^{2-}$ \( (p = 0.001) \) corresponded most strongly with variability in community structure among sites. Lakes in the 1$^{st}$ ANC class (ANC ≤ 25 µeq/L) were strongly associated with calanoid copepod species (indicator species analysis, \( p = 0.0021, \text{stat} = 0.29 \)) while lakes in the 3rd ANC class (100 µeq/L ≤ ANC ≤ 400 µeq/L) were associated with \textit{Daphnia} (indicator species analysis, \( p = 0.027, \text{stat} = 0.22 \)) and \textit{Ceriodaphnia} species (indicator species analysis, \( p = 0.0068, \text{stat} = 0.24 \)).

Table 1.2. Significance values and correlation coefficients from ANOSIM, Mantel, and indicator species tests. Significant relationships are in bold.

<table>
<thead>
<tr>
<th>Grouping variable</th>
<th>Significance</th>
<th>( R )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Region</td>
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</tr>
<tr>
<td>Calcium group</td>
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<td>-0.000072</td>
</tr>
<tr>
<td>ANC class</td>
<td>0.0001</td>
<td>0.086</td>
</tr>
</tbody>
</table>

<p>|Mantel|</p>
<table>
<thead>
<tr>
<th>Variable</th>
<th>Significance</th>
<th>( r )</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
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<td>0.036</td>
</tr>
<tr>
<td>Calcium</td>
<td>0.10</td>
<td>0.054</td>
</tr>
<tr>
<td>DOC</td>
<td>0.051</td>
<td>0.072</td>
</tr>
<tr>
<td>ANC</td>
<td>0.060</td>
<td>0.065</td>
</tr>
<tr>
<td>SO$_4$</td>
<td>0.001</td>
<td>0.12</td>
</tr>
<tr>
<td>NO$_3$</td>
<td>0.35</td>
<td>0.021</td>
</tr>
<tr>
<td>Conductivity</td>
<td>0.55</td>
<td>-0.0074</td>
</tr>
<tr>
<td>Cumulative chemical variables</td>
<td>0.026</td>
<td>0.088</td>
</tr>
</tbody>
</table>

|Indicator Species|
|------------------|---------|---------|
|Group| Taxa | \( p\)-value | \text{stat} |
|ANC class 1| \textit{Calanoida} | 0.002 | 0.23 |
|ANC class 3| \textit{Daphnia} | 0.027 | 0.22 |
|ANC class 3| \textit{Ceriodaphnia} | 0.006 | 0.24 |
|High Ca| \textit{Daphnia} | 0.031 | 0.20 |
Figure 1.5. Non-metric multidimensional scaling (NMDS) ordination plots showing zooplankton community assemblage by genus (cladocerans) or order (copepods) for ELS-II lakes sampled in 2004. Loadings of chemical variables (solid grey) and species (dashed black) are plotted over sites with vector lengths representing the relative influence of each variable on the NMDS axes. Only chemical variables and species with significant influence ($p < 0.05$) are included. Points are color coded to visually depict variability based on (a) ANC class and (b) Ca groupings based on the 1.5 mg/L threshold (74.85 µeq/L). Species loadings are coded as follows: Ho = *Holopedium*, Cal = calanoid copepod, Po = *Polyphemus*, Da = *Daphnia*. 
Discussion

Freshwaters of the Northeastern U.S. experienced significant geochemical shifts throughout the last two decades of the 20th century due to reduced atmospheric deposition from stricter regulation of industrial emissions as well as changes in climate and land use. Lakes integrate these multiple sources of change due to their close connection to the terrestrial and atmospheric environments, and chemical shifts in these systems have been well documented by research programs in the Northeast, with particular focus on increased acidity in the 1970’s and 1980’s followed by recovery from acidification over the course of the 1990’s and early 2000’s (Kahl et al., 2004; Strock et al., 2014; Sullivan et al., 2018).

In congruence with these studies, our results indicate that the ELS-II lakes also saw significant decreases in SO$_4$ and increases in pH and ANC between 1986 and 2004. Historical studies illustrated that biological communities were restructured and even eliminated due to surface water acidification in the 1970’s and 1980’s; however, the biological response to acid recovery and the other geochemical changes that have followed recovery is not well understood, and consensus indicates a need for further research (Baldigo et al., 2016). Our findings indicate that recovery from acidification in Northeastern lakes has likely resulted in changes in zooplankton communities (Figure 4; Figure 5), but that biological communities are unlikely to recover to post-acidification composition because changes in other chemical and physical parameters continue to impact these systems.

Changes in water chemistry

Acidity

We found overall recovery from acidification in the 143 ELS-II lakes in terms of SO$_4$, pH, and ANC with a 22% decrease in SO$_4$ and a 25% increase in ANC (Table 1, Figure 2). Other studies in the region support these trends and the U.S. EPA declared the Clean Air Act Amendments (CAAA) of 1990 an example of regulatory success (Kahl et al., 2004; Sullivan et al., 2018). Federally enforced restrictions on
industrial emissions resulted in decreased emissions of sulfur dioxide (SO$_2$) and oxidized nitrogen (NO$_x$) which caused a subsequent decline in wet deposition of sulfur (S) and nitrogen (N) into surface waters. Sullivan et al. (2018) reported a 60% decrease since the 1960’s in acid content of precipitation measured at Hubbard Brook Experimental Forest in NH. Freshwaters in the Northeast benefited greatly from these reductions and acidity of lakes and streams declined significantly and rapidly between 1980 and 2010 (Driscoll et al., 2003; Fuss et al., 2015; Kahl et al., 2004). The consistent trends of decreases in acidity between 1986 and 2004 in the large and varied ELS-II lake set further illustrates the improvements in water quality that resulted from the CAAA and the value of long-term monitoring programs.

**Cl and base cations**

We observed significant increases in Cl and base cations (Ca +Mg) in the ELS-II lakes between 1986 and 2004 (Figure 2), a result that matches Rosfjord’s (2007) finding. However, this pattern is not consistent with acid recovery as indicated in other studies. On the contrary, Ca depletion has often been associated with historically acidified lakes, presumably due to the lasting legacy of acidification as well as timber harvesting in some watersheds (Stoddard et al. 1999; Watmough and Aherne 2008). Studies in these regions indicate that acid deposition caused an accelerated leaching of base cations (primarily Ca and Mg) from soils, causing an initial increase of base cations in surface waters, followed by declining surface water concentrations as soil base cations were depleted (Likens et al. 1996; Fernandez et al. 2003). Rosfjord et al. (2007) concluded that increases in Ca correlating with increases in Cl in the ELS-II lakes indicated that a similar base cation exchange was occurring in soils due to increased use of NaCl road deicers. Increases in Cl and Ca+Mg were significantly higher in the lakes with closer proximity to roads, and other studies in the Northeast present evidence that road salt use has increased since the 1950’s and resulted in salinization of urban freshwaters (Dugan et al., 2017; Godwin et al., 2003; Kaushal et al., 2005). NaCl deicers exchange Na for Ca, when encountering soils, washing Ca and Cl into the environment or catchment, the likely cause of the uncharacteristic increase in base cations in the ELS-II.
Lakes in the high Cl lake group, most of which are close to human development, saw a 200% increase in Cl concentration from 1986 to 2004 and a 12% increase in Ca + Mg (Table 1). Though these lakes exhibited patterns of deacidification congruent with other studies, the increases in Cl and Ca+Mg base cations were more significant and likely confounded the influence of acid recovery on biota.

**Zooplankton response**

**Body size**

Our results indicate that zooplankton body size increased significantly from 1986 to 2004 in all species (Figure 3), but the overall increase was not driven significantly by chemical changes. An increase in *Daphnia* body size in the high Cl lake group was, however, weakly correlated with increases in Ca + Mg in these lakes (Figure 4). Daphniid species are often larger bodied with highly calcified carapaces and our findings support evidence from previous studies indicating that daphniids are particularly sensitive to changes in Ca (Arnott, Azan, and Ross 2017; Jeziorski, Paterson, and Smol 2012b). Repeated molting throughout the daphniid lifecycle is dependent on a continuous source of calcium that is primarily obtained through the aqueous environment, not through feeding (Alstad et al., 1999). Rapid declines in survival of *Daphnia ssp.* have been observed below a Ca threshold of 0.1-0.5 mg/L Ca and below 1.5 mg/L Ca, reproduction and maturation have been seen to decline significantly in daphnid species (Ashforth and Yan 2008; Hessen et al. 2000; Jeziorski and Yan 2006). The ELS lakes ranged from 0.42 to 12.16 mg/L Ca (20.96 to 606.78 µeq/L), with one lake below the 0.5 mg/L (24.95 µeq/L) Ca threshold in 1986, and 37 lakes below the reproduction and maturation threshold of 1.5 mg/L (74.85 µeq/L). Calcium requirements vary among different daphniid species and larger species like *Daphnia pulex/pulicaria* and *Daphnia magna* are intolerant of low calcium concentrations while other smaller species including *Daphnia catawba*, and *Daphnia ambiguа* can thrive in soft waters when competitive pressure from larger species is suppressed (Jeziorski et al., 2015; Ross, 2015). Increased zooplankton body size in the ELS-II lakes between 1986 and 2004 likely resulted from shifts in size distribution, particularly among
Daphniid species, with large, Ca-rich species becoming more dominant as aqueous Ca increased. Thus far, research has focused on the consequences of declining Ca and no study has explored how significant and rapid increases in Ca influence zooplankton communities in lakes. Our results suggest that increases in Ca caused by increased use of anthropogenic salts in Northeastern lakes may enhance reproduction and maturation of large-bodied \textit{Daphnia} that are more efficient feeders and outcompete smaller species, causing a shift in community structure (Arnott, Azan, and Ross 2016).

Salinization of freshwaters is a mounting concern in the Northeast as pollution from road deicers increases (Arnott et al. 2020; Kaushal et al. 2005). The pronounced increase in Cl in the high Cl ELS-II lakes indicates that increased use of road salt significantly altered water chemistry in these systems. Consensus among researchers is that freshwater salinization is a threat to aquatic organisms as well as water potability (Godwin et al., 2003; Hintz & Relyea, 2019). However, research illustrates that \textit{Daphnia} species can rapidly develop a tolerance to road salts within as few as 2.5 months or 5-10 generations of exposure to high levels of chloride in lake waters (Coldsnow et al., 2017; Hintz et al., 2019). In the high Cl lakes group, \textit{Daphnia} body length increased by 12% from 1986 to 2004 despite a 200% increase in Cl (Figure 3b). We can speculate that the increased body size of \textit{Daphnia} species in the salt-affected ELS-II lakes over time was likely due to a combination of interacting mechanisms, but that increases in Ca as well as evolved tolerance to salinization likely contributed to their success.

Although the effect of anthropogenic salt may have benefited the growth of \textit{Daphnia} zooplankton species, the shift from smaller bodied to larger bodied individuals likely elicited a response among other individuals of the food web. Zooplankton body size is often correlated with grazing rate and phytoplankton consumption and Wu and Culver (1991) concluded that \textit{Daphnia} species were the primary determinants of grazing pressure in Lake Erie. Increased zooplankton body size in the ELS-II lakes, particularly in \textit{Daphnia}, likely led to a decrease in phytoplankton abundance and larger bodied
Daphnia likely increased competitive pressure on other zooplankton species. Additionally, planktivorous fish may selectively feed on larger bodied zooplankton when they are available (Kim and Joo 2004), a result that provides evidence that a shift to larger bodied Daphnia likely influenced predators in the ELS-II lakes as well. The increase in body length that corresponded with increasing base cations and Cl in the ELS-II lakes likely had implications for zooplankton community composition as well as for the greater trophic structure of the lake ecosystems.

**Community composition**

Our analyses of the 2004 data highlight chemical drivers of community variation. ANC class and SO$_4$ concentration were the most significant drivers of variation in community structure among the ELS-II lakes (Figure 5). Many studies have evaluated biological reactions in freshwater systems recovering from acidification, primarily in Canada and Europe, and changes in zooplankton community structure are well documented (Driscoll et al. 2003; Keller, Gunn, and Yan 1992). Acidification of alpine lakes in Switzerland resulted in the extirpation of almost all crustacean zooplankton species. Species quickly recolonized in some lakes as soon as recovery began in the 1990’s; however, other lakes did not see the return of zooplankton communities until 2008 (Stuchlik et al., 2017). In Killarney Park, Ontario, lakes recovering from severe acidification did not experience increases in zooplankton species richness, but zooplankton shifted from a diminished community composition to a community more characteristic of non-acidified lakes (Holt & Yan, 2003). The response of fish to acid recovery has also been inconsistent among species and regions. Studies in Norway and the Canadian Shield saw increases in reproduction and abundance of native trout species in previously acidified lakes as waters neutralized (Gunn & Mills, 1998; Lund et al., 2018). On the contrary, fish populations in several Adirondack lakes did not increase in richness or abundance over a 28 year period of steadily increasing pH, and it might be that higher trophic level species require more time to recover (Baldigo, Roy, and Driscoll 2016).
Studies recognize that shifts in other chemical and physical conditions in lakes, either co-occurring with acid recovery or resulting from acid recovery, contribute to shifts in biological communities, rendering a true “biological recovery” unlikely (Baldigo, Roy, and Driscoll 2016). Results of significance tests associated with our NMDS analysis of the 2004 zooplankton communities implicated DOC as another significant driver of community variability among lakes (Figure 5), but research indicates that the influence of DOC on primary consumers is complex. Leach et al. (2019) concluded that increases in DOC provided an energy subsidy for phytoplankton but not zooplankton and other studies support this finding, suggesting that the supplemental nutrients provided by allochthonous DOC are only consumable by primary producers (Brett et al., 2017; Kelly et al., 2014). However, increased phytoplankton production often stimulates increased zooplankton production (Kissman et al., 2017) but the increase in food availability stimulated by increasing DOC is outweighed by the degradation of habitat caused by increased light attenuation in browning waters (Kelly et al., 2014). These mechanisms likely contributed to variability in community structure that correlated with varying concentrations of DOC. With a 26% increase in DOC from 1986 to 2004 (Table 1) in the ELS-II lakes, zooplankton communities likely shifted due to direct and indirect effects of these increases.

Though calcium concentration was not a significant driver of community variability when grouped in categories according to ANOSIM, Ca concentration as a continuous variable was significant according to a Mantel test (p < 0.06), and an NMDS plot grouping lakes at a calcium threshold of 1.5 mg/L illustrates that zooplankton community composition was differentiated between low and high Ca lakes. This finding supports evidence from previous studies that cladoceran zooplankton species, *Daphnia* in particular, rely on Ca concentrations in water to promote survival and reproduction (Arnott, Azan, and Ross 2017; Jeziorski, Paterson, and Smol 2012). The NMDS loadings vector for *Daphnia* species further illustrates the interaction between Ca and daphniids with the high Ca lakes clustering in the direction of the *Daphnia* vector and an indicator species analysis highlights that *Daphnia* species are
associated with high Ca lakes. Given the sharp decline in acidity observed in the ELS-II lakes and the simultaneous increases in DOC and Ca, we can infer that zooplankton communities shifted between 1986 and 2004 as these lakes recovered from acidification and reacted to changes in land use and climate and that higher trophic levels likely subsequently responded.

Community analyses of the 2004 ELS-II data illustrate that zooplankton communities in northeastern lakes are influenced by changes in water biogeochemistry. Zooplankton are responsive to change due to their shorter lifecycles and their position as primary consumers in the food web and serve as valuable indicators of trophic shifts. Biota with shorter life cycles such as zooplankton may be quicker to respond to acid recovery; however, as food webs shift and other environmental changes occur simultaneously, biological responses are complex, and drivers are difficult to tease apart. Continued monitoring of geochemical, physical, and biological parameters is imperative to inform our understanding of the trajectory of freshwater ecosystems after acid recovery as climate and land use continue to shift.

Implications and further research

The ELS-II lake set encompasses 143 lakes from Pennsylvania to Maine and includes a statistically representative population of northeastern lakes that were heavily affected by acid deposition and have subsequently recovered. This dataset is unique, not only due to the large sample size and variability among sites but also because zooplankton counts and body lengths were collected, which provide a window into the biological systems influenced by the chemical shifts that have been heavily documented in this region. The ELS-II dataset provides evidence that changes in surface water chemistry in northeastern lakes, including recovery from acidification, associated increased in DOC, and shifts due to increased influence of anthropogenic salt, elicited changes in zooplankton production and community structure. A full understanding of the drivers of zooplankton communities in the ELS-II lakes
would benefit from a more complete dataset, including viable abundances from 1986 to allow for comparative study. The 1986 data was lacking sufficient information to confirm or convert units of zooplankton density to match the 2004 dataset. This serves as a testament to the value of keeping thorough records and metadata to ensure that data can be replicated or utilized in future research.

A full understanding of drivers of zooplankton community dynamics in the ELS-II lakes would also require integrating the influence of climate change and of top-down and bottom-up pressure from other trophic levels. The process of recovery of zooplankton communities is dependent on the dispersal, colonization, and expansion abilities of species and is often mediated by interspecific competition and predator/prey dynamics. The extirpation of fish species in response to lake acidification resulted in expansion of predatory invertebrate species in some cases, which altered plankton community structure. Patterns of zooplankton recovery, therefore, will be linked to reestablishment of fish species and other shifts in predation and in some cases, biological recovery might manifest as a novel community equilibrium, not as the original community (Keller & Yan, 1998). The presence of fish in the ELS-II lakes during the two sample years was difficult to ascertain but it is likely that many sites contained populations of stocked or natural fish populations that were predators of zooplankton. The large number of sites and use of multivariate statistics allowed us to explore relationships despite this missing information.

The Northeastern U.S. is warming more quickly than other regions of the U.S. with a significant increase in warming rate since 1960 and we are also experiencing marked increases in precipitation with more frequent extreme events (Fernandez et al. 2015). Over the eighteen-year study period, these changes in climate were certain to have contributed to change in the ELS-II lakes and were likely to have influenced the zooplankton communities. Similar to the effect of chemical changes, changes in climate elicit complex reactions in freshwater ecosystems and responses are often species-specific. The most
notable and frequently reported effect of climate change on zooplankton communities is shift in phenology. Phenological responses to shorter winters and longer summers depend on specific life-history strategies the variable response among different members of food webs have been seen to result in trophic mismatches (Adrian et al., 2006; O’Reilly et al., 2015). Community structure shifts between 1986 and 2004 were likely influenced by climate induced phenology shifts, however, since we only examined the 2004 community dataset, we can assume that climate variables were insignificant for our community analysis. Changes in body length could have been influenced by shifting climate; however, research indicates that warming surface waters result in decreases in zooplankton body size (Strecker et al., 2004), which contrasts with the significant increase in body size observed in the ELS-II lakes. Nonetheless, including climate variability in future analyses of the ELS-II dataset would add value to the implications of zooplankton shifts.

Our findings illustrate the complexity of interacting mechanisms that drive change in small, short lived aquatic organisms. The detectable shifts and drivers, despite the extreme variability among sites and limited data, also indicate that zooplankton are useful for understanding biological responses to change. Zooplankton are primary consumers and an integral part of the food chain and shifts in zooplankton community structure have been seen to influence the entire lake food web (Carter et al., 2017; Stemberger et al., 2001). Although water quality in northeastern lakes has significantly improved since acidification, changes continue to occur in response to climate change and human development and continued monitoring is warranted. Biological monitoring should be included in this effort in order to understand fully how anthropogenic influences determine water quality and ecosystem health.
CHAPTER 2

ELEVATION DETERMINES SPRING PHENOLOGY: IMPLICATIONS OF ZOOPLANKTON TEMPERATURE DEPENDENCE IN MAINE LAKES IN A CHANGING CLIMATE

Abstract

Climate change is influencing the timing of seasonally reoccurring events in ecosystems across the globe. Shifts have already been detected in northeastern lakes including earlier ice breakup and longer periods of thermal stratification. Environmental warming imposes varying levels of pressure on plankton taxonomic groups depending on their life-history traits, and the variability in response rate among different species has resulted in measurable shifts in community structure. This research aims to understand how spring thermal dynamics in lakes across the extremes of elevations and climate zones in Maine influence zooplankton phenology in order to understand how lake communities might react to a warming climate. Zooplankton, chlorophyll-a, and surface water temperature were measured throughout the spring season in eight remote lakes from the extremes of elevations (94m – 955m above sea level). Following ice-out, low elevation lakes warmed at a relatively gradual rate (0.2 °C/day) while rate of warming following ice-out in the high elevation lakes was significantly more rapid (0.3 °C/day) with surface waters reaching maximum temperature 45 days quicker on average than low elevation lakes. Results of linear mixed effects models indicate that zooplankton abundances increased in response to warming surface waters in the spring and temperature was a particularly strong driver of zooplankton spring phenology in high elevation lakes. However, the influence of surface water temperature on zooplankton phenology varied among taxa, with rotifers and calanoid copepods more dependent on spring warming than cladocerans and cyclopoid copepods. The variable response among zooplankton taxonomic groups to spring surface water warmup indicates that responses to climate change will be taxa specific. Interpreting the drivers of phenological shifts among lake ecosystem
members will be imperative for understanding and predicting the complex effects of climate change on freshwater systems.

**Introduction**

Climate shifts are influencing the timing of seasonally recurring events including physical events such as when snow melts in the spring or the ground first freezes in the fall, as well as phenological events such as when plants first leaf out, flower, and go to seed or when migrating birds arrive in breeding grounds, mate, and reproduce. Many species’ phenology is driven by temperature cues, while others respond to day length. Intra-seasonal temperatures can vary from year to year and are now trending warmer due to climate change; however, day length remains the same and even among organisms that synchronize with temperature cues, individual species respond at different rates depending on their life history traits (Salinger, 2005; Adrian et al., 2006). Variable response mechanisms of species-specific phenology can result in trophic mismatches and a reorganization of ecological communities (Adrian et al., 2006; Miller-Rushing et al., 2010). For example, evaluation of historical data from an Illinois temperate forest revealed that the disruption of plant-pollinator relationships and the local extinction of 50% of bee species was largely due to asynchronous phenological shifts (Burkle et al., 2013). Similarly, juvenile Coho salmon (*Oncorhynchus kisutch*) that depend on large populations of zooplankton in the subarctic Northeast Pacific for their development and migration expenditures have experienced population declines because of earlier peak abundance of copepod zooplankton in warm years (Mackas et al., 2007). Interpreting the drivers and consequences of phenological shifts among ecosystem members is imperative for understanding and predicting the complex effects of climate change.

Significant environmental shifts have already been detected in northeastern lakes where milder winters, earlier springs, and longer summers are causing seasonally reoccurring events to shift (O’Reilly
et al., 2015). Ice forms later in the season or does not form at all, warmer surface waters produce earlier onset of thermal stratification, and ice breaks up earlier in the spring (Austin & Colman, 2007; Magnuson et al., 2000; Hodgkins 2013). In turn, the community of organisms that inhabits a lake under these changing conditions is forced to shift as well. Environmental warming imposes varying levels of pressure on plankton taxonomic groups depending on their life-history traits, and the variability in response rate among different species has resulted in measurable shifts in community structure (Adrian et al., 2006; Winder & Schindler, 2004a). Response rate to warming can vary among different trophic levels as illustrated in Windermere, UK, where *Daphnia* phenology advanced more rapidly with warming than phytoplankton and perch phenology, subsequently shortening time between phenophases of the three trophic levels and interrupting historical interactions (Thackeray et al., 2013). Similarly, in a large temperate lake in Washington, US, diatom blooms increased in abundance and peaked earlier in response to increases in vernal temperature but *grazing Daphnia* populations declined because their phenology did not synchronize with the warming trend (Winder & Schindler, 2004a). Variation in response rate has also been seen within trophic levels, for instance, *Daphnia* in a large eutrophic lake in Germany advanced in synchrony with warmer spring water temperatures while slower-growing zooplankton species with more complex life-cycles including copepods and mussel larvae did not shift phenology (Adrian et al., 2006). Lake community phenology is shaped by species-specific thermal, physical, or biogeochemical requirements at crucial life-stages such as emergence from diapause or spawning. Community-scale phenological shifts are, therefore, determined by seasonal and sub-seasonal temperature patterns combined with subsequent shifts in physical and biogeochemical lake properties that influence lake biota development (Adrian et al., 2006; Donnelly et al., 2011; Kharouba et al., 2018). The variation in responses of different plankton groups is likely to have implications for the rest of the lake ecosystem like the population decline documented by Mackas et al. (2007). Such
behavioral variability and resulting phenological mismatches are likely occurring at all levels of trophic and ecosystem scales and have the potential to dramatically modify biological communities.

Zooplankton are useful indicators of ecosystem health in lakes, but they are most often sampled mid-summer. However, the winter-spring transition period in lakes is one of significant activity for both physical and biological lake components that shape the remainder of the growing season and this period is advancing earlier and lengthening with climate change (Haberman & Haldna, 2017; Hodgkins, 2013; Powers & Hampton, 2016). Ice-out timing and onset of stratification mark the beginning and end of the spring transition in lakes (Gronchi et al., 2021) and determine onset of peak algal blooms. Ice break-up is necessary for the depth-integrated intensity of photosynthetically active radiation (PAR) to increase enough for phytoplankton net growth to begin exceeding losses in seasonally ice-covered lakes, which varies depending on both regional climate and local lake characteristics (Gronchi et al., 2021). Additionally, spring turnover is a significant driver of lake biogeochemistry at the onset of the growing season and mixing redisperses nutrients and oxygen that settled in the benthos during winter ice-cover (Judd et al., 2005). In Upper Lake Constance in central Europe, interannual variation in early spring weather that led to differing intensities in mixing events resulted in significant year to year variability in the composition of herbivore zooplankton that initiated the clear water phase (Tirok & Gaedke, 2006). In years with shallower mixing events, ciliates and rotifers were the dominant drivers of the clear water phase while *Daphnia* were the dominant grazers contributing to the clear water phase when mixing was deeper (Tirok & Gaedke, 2006). To understand the dynamics and drivers of phenological change in lakes under a warming climate, an increased focus on the spring transition season is warranted.

Maine is a relatively large state with three designated climate divisions (1. northern, 2. southern interior, 3. coastal) and variability among divisions is pronounced due to a gradient of elevations, latitude, and proximity to the coast (Fernandez et al., 2015). Analysis of historical ice-out data between
1880 and 2000 indicates that lakes in Maine’s northern climate division lose ice 20 to 40 days later than lakes in the southern interior and coastal climate divisions, a result of significantly more snowfall in the north with a 50% greater median of maximum snow depth occurring in the northern mountains than the southern region (Hodgkins et al., 2002). Additionally, the average annual temperature in the northern climate division is 5.6°C cooler than that of the coastal climate division (Fernandez et al., 2020). Climate-ecosystem relationships compared among sites along a latitudinal or elevational gradient can effectively model temporal ecological change (Brown, 2014). The large gradient in lake seasonality within the state of Maine offers a valuable opportunity to study the implications of climate variability in a short-term study.

This research aims to understand the importance of spring surface water temperature for driving zooplankton phenology and how variation in spring temperature dynamics influences zooplankton communities in lakes across the range of elevation and climate zones in Maine. Specifically, we hypothesize that a significant difference in the onset and rate of spring surface water warming between high elevation and low elevation lakes will drive differences in the rate of zooplankton population growth; specifically, low elevation lakes will warm earlier and more gradually, driving earlier growth and greater abundances in zooplankton, while high elevation lakes will warm later and more quickly with corresponding later zooplankton growth and lower overall abundances limited by the shorter growing period. We also predict that zooplankton taxa will have different relationships with spring surface water warming depending on their life-history strategies; faster growing rotifers and cladocerans will tightly track spring temperature while slower growing copepod species might not be strongly linked with spring warming but more reliant on other seasonal drivers like day length. We tracked zooplankton phenology from under-ice through spring in eight small lakes at the endmembers of Maine’s climate divisions and evaluated the relative influence of surface water warming compared with day length, chlorophyll-a concentration, and fish pressure. Disentangling the drivers of zooplankton
phenology will provide a basis for understanding the consequences of climate change and potential for persistence of cold refugia for northeastern lentic ecosystems.

**Methods**

**Study sites**

A network of U.S. Environmental Protection Agency (EPA) programs has monitored lakes in the northeastern US since the 1980’s to understand effects of industrial emissions producing acid rain on eastern water bodies. Since the Clean Air Act Amendments (CAAA) of 1990, lakes in Maine have largely recovered from acidification in terms of sulfate concentration in water (Kahl et al., 2004; Strock et al., 2014). A subset of lakes in Maine continue to be monitored by the EPA’s Long Term Monitoring program (https://www.epa.gov/airmarkets/clean-air-markets-monitoring-surface-water-chemistry) and High Elevation Monitoring program (HELM) (Gavin et al., 2018; Strock et al., 2016).

In this study, a subset of lakes was selected from among the LTM and HELM projects at varying elevations and in different climate divisions in order for our sample lake population to encompass maximum variability in climate and length of the summer season in Maine (Figure 1, Table 1). Most of the HELM and LTM lakes are remote from human development and therefore relatively isolated from direct anthropogenic influence making them ideal sites to study ecosystem responses to changes in physical and chemical lake properties. Details of long-term monitoring sites and characteristics for broader HELM and LTM lake sets are provided in Gavin et al. (2018) and Strock et al. (2016).

Sites were selected based on winter accessibility and landscape characteristics and were evaluated to minimize variability in size, depth, and relative influence of watershed and wetland characteristics among candidate lakes. Sites were grouped into two cohorts:
High elevation group – four high elevation lakes (675 m – 975 m) from the HELM project and located in Maine’s Northern climate division that historically experiences later ice-out, greater snowfall, and colder average temperatures (Hodgkins et al., 2002). These lakes range in size from 1.6 to 11.9 hectares and are headwater, drainage ponds surrounded by coniferous boreal forest dominated by the spruce-fir forest type (Kahl, 1998).

Low elevation group – four low elevation (94 m – 332 m) lakes in the Southern Interior and Coastal climate divisions of Maine that experience earlier spring warming and ice-out, and higher average temperatures (Hodgkins et al., 2002). Three lakes are from the LTM project. The fourth is Sargent Mountain Pond in Acadia National Park which was added to the study because it imitates a high elevation tarn with vegetative cover and geomorphology similar to that of the high elevation lakes. Lakes in this group range in size from 0.4 to 27 hectares and are drainage ponds except for Bracey which is a seepage pond, defined here as a lake with no inlet or outlet. The vegetation is mixed deciduous-coniferous forests.

Landscape characteristics considered in site selection are summarized in Table 1. Watershed and waterbody data were obtained from the National Hydrography Dataset Plus (NHDPlus HR) from the U.S. Geological Survey. Wetland data were acquired from the National Wetlands Inventory (NWI) managed by the U.S. Fish and Wildlife Service. Spatial analyses to determine watershed characteristics were performed using ArcGIS Pro and Python. Fish status was determined from the Maine Department of Inland Fisheries & Wildlife fish stocking reports and lake survey maps (https://www.maine.gov/ifw/fishing-boating/fishing/index.html, accessed 28 March 2021). All lakes except for Sargent Mountain Pond (low elevation) and Cranberry Pond (high elevation) were determined to contain populations of stocked or natural fish including brook trout (Salvelinus fontinalis).
Table 2.1. Landscape characteristics used to select sites. MIDAS is the State of Maine’s unique identification number for each lake.

<table>
<thead>
<tr>
<th>MIDAS</th>
<th>Lake name</th>
<th>Elevation (m)</th>
<th>Lake area (ha)</th>
<th>Ratio of watershed area to lake area</th>
<th>Ratio of watershed area to wetland area</th>
<th>Project</th>
<th>Max depth (m)</th>
<th>Temperature probe</th>
</tr>
</thead>
<tbody>
<tr>
<td>8601</td>
<td>Horns</td>
<td>955</td>
<td>1.6</td>
<td>4.6</td>
<td>4.6</td>
<td>HELM</td>
<td>7.0</td>
<td>Y</td>
</tr>
<tr>
<td>3544</td>
<td>Midway</td>
<td>824</td>
<td>2.8</td>
<td>36.9</td>
<td>9.3</td>
<td>HELM</td>
<td>8.8</td>
<td>Y</td>
</tr>
<tr>
<td>3540</td>
<td>Mountain-R</td>
<td>737</td>
<td>11.9</td>
<td>11.0</td>
<td>7.9</td>
<td>HELM</td>
<td>10.9</td>
<td>Y</td>
</tr>
<tr>
<td>8603</td>
<td>Cranberry</td>
<td>743</td>
<td>3.1</td>
<td>12.6</td>
<td>9.6</td>
<td>HELM</td>
<td>5</td>
<td>Y</td>
</tr>
<tr>
<td>4422</td>
<td>Salmon</td>
<td>94</td>
<td>4.0</td>
<td>3.7</td>
<td>7.4</td>
<td>LTM</td>
<td>10.9</td>
<td>Y</td>
</tr>
<tr>
<td>4508</td>
<td>Bracey</td>
<td>117</td>
<td>8.0</td>
<td>11.4</td>
<td>4.8</td>
<td>LTM</td>
<td>8.1</td>
<td>N*</td>
</tr>
<tr>
<td>441</td>
<td>Second</td>
<td>126</td>
<td>27.0</td>
<td>10.9</td>
<td>6.1</td>
<td>LTM</td>
<td>42.7</td>
<td>Y</td>
</tr>
<tr>
<td>8473</td>
<td>Sargent</td>
<td>332</td>
<td>0.4</td>
<td>110.4</td>
<td>25.2</td>
<td>none</td>
<td>13.0</td>
<td>Y**</td>
</tr>
</tbody>
</table>

*Bracey was instrumented with a temperature probe, but we were unable to locate the probe after the winter. ** Sargent was instrumented with a probe on 4/28/2019 which ceased logging due to malfunction on 7/11/2019.
Figure 2.1. Location of study sites and Maine’s climate divisions. Data from NOAA/NCEI U.S. Climate Division.
Sampling design

The eight selected lakes were sampled multiple times in 2019 to characterize zooplankton communities during each season and throughout the spring. Except for Bracey Pond (MIDAS 4508) which was inaccessible in the winter, all lakes were sampled under ice in the winter of 2019 between February and April to capture a baseline productivity estimate. Lakes were then sampled repeatedly over the course of spring warming beginning after lake ice-out, which we defined as when the majority of the lake surface was open water and could be navigated by a boat, a criterion used by a statewide volunteer monitoring program (lakestewardsofmaine.org). Each lake was sampled three to four times after ice-out throughout the spring with 8–14 days between each successive sample. A summer sample was collected in each lake between July and August during summer thermal stratification defined by when the lake epilimnion was greater than 10° C warmer than the hypolimnion. Finally, a sample in the fall was collected at each site between October and November during fall mixing, prior to ice formation but after cooling temperatures and wind interrupted thermal stratification causing the water column to be a consistent temperature throughout.

Sample collection

Samples were collected at the deepest point in each lake which was previously determined using bathymetric maps and a depth finder and marked with a GPS. Samples during the open water season were taken from an inflatable boat and winter samples were accessed by drilling through the ice with a 20 cm hand auger.

At each sampling event, two vertical zooplankton tows were collected with a Wisconsin plankton sampler with a 13 cm mouth diameter and 63 µm mesh. Additional tows were taken when populations were sparser to ensure the capture of at least 100 individual organisms. Samples were preserved with 70% ethanol in clean, 250 mL polypropylene jars. Zooplankton were identified under a
3.5 X-90X LED trinocular zoom stereo microscope with a dichotomous key (Haney et al. 2013) and counted using a Bogorov chamber. Calanoid copepods were identified to family while rotifers, cladocerans, and cyclopoid copepods were identified to genus or species. Zooplankton density (-L) was calculated by dividing the number of individuals of a species or taxa counted in a sample by the volume of lake water sampled.

To increase understanding of bottom-up pressure on zooplankton populations and forage availability, chlorophyll-a samples were also collected at each sampling visit based on the Maine Department of Environmental Protection’s methods (Pearsall, 1997). A weighted Tygon tube with an inner diameter of 1.27 cm was used to take a core from 0.25 meters above the lake bottom to the surface at a maximum depth of nine meters. Like the vertical tow method used for zooplankton sampling, this approach integrates the lake water from the entire water column. One or two cores were taken depending on lake depth to fill the sample container with a minimum of 0.5 L and a maximum of 1 L of water. Samples were stored in clean, opaque Nalgene bottles on ice and filtered within 24 hours of collection through Whatman 47 mm GF/F filters (0.7 μm pore size). Chlorophyll-a analysis was performed at the Sawyer Stable Isotope Laboratory where the filters were pulverized with 90% acetone and analyzed with a Varian 50 Bio UV-Visible Spectrophotometer.

Sites were instrumented with HOBO Pendant Temperature/Light Data Loggers (Onset; Bourne, MA) that recorded surface water temperature every six hours, continuously throughout the entire study period. Thermistors were suspended at ~0.5 m depth between an anchor and a buoy and also situated at the deepest point in each lake. In Bracey, Second, and Salmon Ponds, thermistors were deployed in spring 2018 and remained in the lakes over the winter of 2018–2019 to provide ice-out and spring warming data. We were unable to locate the thermistor chain in Bracey Pond in the spring of 2019. Thermistors were installed in Sargent Mountain Pond in April of 2019. In the high elevation ponds,
thermistors have been collecting data since as early as the spring of 2015 and are regularly serviced by University of Maine, Farmington faculty. During each sample collection site visit, additional temperature profiles and dissolved oxygen (DO) were also collected at 1 m increments throughout the water column at the deepest point using a YSI ProPlus or EXO multiparameter sonde.

**Data preparation and Statistical analysis**

We used temperature data to compute limnological degree day (LDD), a metric we defined that is indexed to a thermal threshold. Metabolism of lake biota is dependent on temperature (Kraemer et al., 2017), however, it is often the cumulative thermal energy over an unknown period of time that exerts influence. Additionally, among-site variation limits the explanatory power of temperature on a given sample day. Use of degree-days in limnology and fish science is increasing and this method is well suited to our study because it allows us to compare sites with different climates and assumes that biotic growth and warming are linearly correlated which we expect during spring in our study lakes (Chezik et al., 2014; M. Keller et al., 2020; Thompson et al., 2005). The degree-day scales temperature based on a biologically relevant starting temperature that represents a threshold value for the initiation of growth in a study system. A degree-day is commonly calculated by subtracting a determined base temperature ($T_0$) from the mean temperature on a given day:

$$ DD = \left[ \frac{T_{\text{max}} + T_{\text{min}}}{2} \right] - T_0 $$

Where $T_{\text{max}}$ is the maximum daily temperature and $T_{\text{min}}$ is the minimum daily temperature. For LDD, we used a base temperature of 4 °C, the temperature of maximum density of water, representing the start of spring surface water warming when surface waters of our study sites consistently ceased to refreeze. In seasonally ice-covered lakes, surface waters begin warming once the lake is free of ice, which is marked by the first spring mixing event when the water column shifts from winter stratification, where
the denser 4 °C water sits at the bottom, to a water column at a uniform temperature. This seasonally recurring event marks the point when surface water temperatures gradually increase, and summer stratification slowly establishes. We therefore calculated Limnological Degree Day as

$$LDD = \left[ \frac{T_{max} + T_{min}}{2} \right] - 4°C$$

To quantify the difference in rate of spring surface water warming between high elevation and low elevation lakes we used a 2-sample t-test on the slopes of the springtime lake water temperature series. The slope of temperature in the seven instrumented lakes over the course of spring 2019 were extracted with LDD > 0 as the starting point and the point where maximum LDD was reached as termination. Slopes were grouped by lake group and run through a 2-sample t-test and Wilcoxon test to evaluate if the difference between slopes of the high and low elevation lakes were significantly greater than zero.

We used linear mixed effects models to assess the influence of spring surface-water warming on zooplankton abundances relative to the role of Julian date, chlorophyll-a concentration, and fish presence and we examined variation in patterns between high and low elevation lakes and among taxonomic groups. Models were built in R version 4.0.2 (R Core Team 2020) with the nlme package (Pinheiro et al., 2011) and maximum likelihood estimation was used to test the significance of fixed effects while accounting for non-independence among samples from the same site by including site as a random intercept (Zuur et al., 2009). Total zooplankton density was included as the response variable for the primary model and additional models were run separately to evaluate rotifer, cladoceran, calanoid and cyclopoid copepod responses. All densities were log_{10} transformed (+1 when zeros were present) to fit normality and temporal variables were centered around zero. We tested the influence of temperature averaged over the 10-day period leading up to each sampling event in our models as well as LDD. Variance inflation indicated collinearity between LDD and 10-day means so we selected LDD as
our primary thermal variable after assessing model fit. We were concerned that temporally-proximate samples might be similar and introduce non-independence, but the autocorrelation function (ACF) from the stats package revealed that temporal autocorrelation among samples was negligible. Models were assessed for variance inflation using the VIF function from the car package and final model fit was determined through examination of residual distributions and AICc scores implemented in the MuMIn package (Bartoń, 2020). Significance of fixed effects were estimated using maximum likelihood and marginal and conditional R² values were reported using the function r.squaredGLMM from the MuMIn package.

Results

Spring warm up in high elevation vs. low elevation lakes

The timing and duration of surface water warming in the spring varied significantly between high and low elevation lakes (Figure 2). The low elevation group lost ice cover in late April 2019 while the high elevation group did not ice-out until mid- to late May 2019. Following ice-out, low elevation lakes warmed at a relatively gradual rate (0.2 °C/day), reaching maximum temperature over the course of approximately 105 to 120 days since ice-out (Figure 2a). In contrast, rate of warming following ice-out in the northern/mountain lakes was significantly more rapid (0.3 °C/day) with surface waters reaching maximum temperature around 60 - 75 days after ice-out (Figure 2a). Maximum surface water temperatures were reached in the high elevation lakes between July 5th and 6th (24.5 °C mean, n = 4) and in the low elevation lakes on July 31st (27.9 °C mean, n = 2). The slope of surface water temperature over time between ice-out and date of maximum temperature was significantly steeper in the high elevation lakes than the low elevation lakes (t (7) = 6.8176, p <0.01) (Figure 2b).
Figure 2.2. Difference in warming rate between northern/mountain lakes and southern/coastal lakes depicted in (a) a timeseries of surface water temperatures through spring 2019 and (b) comparison of the median slope of temperature over time between the two lake groups.

Zooplankton response to spring warming

Across the eight study lakes, zooplankton abundance ranged from 3.1 L\(^{-1}\) to 223.5 L\(^{-1}\) with a mean of 47.5 individuals per liter. The largest densities occurred in Cranberry and Horns, the highest elevation ponds, in June and July and were dominated by > 70% rotifers, primarily Keratella spp. In contrast, under-ice densities were significantly higher in low ponds than in high ponds with the highest
abundance observed in Sargent Mountain Pond at 188.6 L\(^{-1}\) consisting of 59% *Eubosmina longispina*.

Total zooplankton abundance across all study lakes throughout the spring was strongly driven by surface water temperature, with a highly significant effect of LDD and high goodness of fit values for the best-fit model (\(\beta_{\text{LDD}} = 0.047\pm0.01, P < 0.001, R^2_c = 0.63\); Table 2, Figure 3). In line with our predictions this illustrates that, as surface waters warm in the spring, zooplankton abundances subsequently increase. Additionally, LDD was the only variable that significantly influenced total zooplankton abundances throughout the spring growing season when all lakes were included in the model (Table 2).

As we expected, the influence of spring warming on different zooplankton taxa among the seven study lakes with temperature data was highly variable. Increases in rotifer abundance were most strongly correlated with increasing LDD (\(\beta_{\text{LDD}} = 0.071\pm0.015, p < 0.0001, R^2_c = 0.70\); Table 2, Figure 3) and LDD was the single, significant effect. Calanoid copepods were also significantly driven by LDD (\(\beta_{\text{LDD}} = 0.043\pm0.012, p < 0.01, R^2_c = 0.71\); Table 2); however, model selection indicated fish presence as an important variable for model fit as well (Table 2, Figure 3). In contrast, cyclopoid copepod and cladoceran abundances were not driven by LDD. Model selection indicated that cyclopoid abundances were influenced positively by both fish presence and, significantly, chl-a (\(\beta_{\text{chl-a}} = 0.18\pm0.047, p < 0.01, R^2_c = 0.51\); Table 2). The best-fit model for cladoceran abundance indicated that day length may have played a more important role in increasing spring abundances, with Julian day as the single most important variable. Goodness of fit for the model was high; however, the effect of Julian day was non-significant (\(\beta_{\text{Julian}} = 0.01\pm0.002, p > 0.05, R^2_c = 0.59\); Table 2).

Surface water warming in the spring was a more powerful driver in the high elevation lakes than in the low elevation lakes. The best fit model for the high elevation lake group indicated LDD as a strong driver and the only influential variable for total zooplankton abundance (\(\beta_{\text{LDD}} = 0.053\pm0.015, p < 0.01, R^2_c = 0.68\); Table 2, Figure 3). In contrast, model selection for the low elevation lakes showed no significant
influence of LDD, with fish presence as the only variable in the best-fit model, with a positive but non-significant effect ($\beta_{\text{fish}} = 0.15\pm0.088$, $p > 0.05$, $R^2 = 0.18$; Table 2). It must be noted, however, that when the two lake groups are included in models separately, significant degrees of freedom are lost due to small sample size. We did not assess whether the time series of LDD affected zooplankton abundance because continuous temperature data were only available for three of the four lakes from the low elevation group and only two lakes had complete spring temperature data.
Table 2.2. Model results of fitted LMMs. Tested fixed effects are listed in columns with parameter estimates and p-values (<0.0001***, <0.001**, <0.01*, <0.05’) ± SE. Goodness of fit indicated with marginal and conditional R² and change in AICc based on the AICc score of the best fit model. LDD represents limnological degree day, fish presence refers to the presence or absence of fish in a lake.

<table>
<thead>
<tr>
<th>Response Variable</th>
<th>Formula</th>
<th>Intercept</th>
<th>LDD</th>
<th>Julian day</th>
<th>Fish presence</th>
<th>Chl-a</th>
<th>LDD*Chl-a</th>
<th>ΔAICc</th>
<th>Marginal &amp; conditional R²</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>total zooplankton abundance</td>
<td>LDD</td>
<td>1.54</td>
<td>0.047**±0.01</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.39, 0.63</td>
<td>25</td>
</tr>
<tr>
<td>total zooplankton abundance</td>
<td>LDD + fish + Julian day + LDD*chl-a</td>
<td>1.57</td>
<td>0.073'±0.03</td>
<td>-0.002±0.008</td>
<td>-0.04±0.2</td>
<td>0.004±0.08</td>
<td>-0.009±0.01</td>
<td>15.3</td>
<td>0.48, 0.66</td>
<td>23</td>
</tr>
<tr>
<td>total zooplankton abundance</td>
<td>GDD + fish + chl-a + Julian day</td>
<td>1.66</td>
<td>0.061'±0.03</td>
<td>-0.001±0.007</td>
<td>-0.04±0.2</td>
<td>-0.044±0.062</td>
<td></td>
<td>11.23</td>
<td>0.48, 0.64</td>
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<td>total zooplankton abundance</td>
<td>LDD + fish + Julian day</td>
<td>1.59</td>
<td>0.047'±0.021</td>
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<td></td>
<td>6.48</td>
<td>0.41, 0.64</td>
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<td>total zooplankton abundance</td>
<td>LDD + fish</td>
<td>1.59</td>
<td>0.046**±0.011</td>
<td>-0.076±0.19</td>
<td></td>
<td></td>
<td></td>
<td>2.98</td>
<td>0.41, 0.64</td>
<td>25</td>
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<tr>
<td>Rotifer abundance</td>
<td>LDD</td>
<td>1.13</td>
<td>0.071***±0.015</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.39, 0.70</td>
<td>25</td>
</tr>
<tr>
<td>Calanoid copepod abundance</td>
<td>LDD + fish</td>
<td>0.68</td>
<td>0.043*±0.012</td>
<td>-0.41±0.20</td>
<td></td>
<td></td>
<td></td>
<td>0</td>
<td>0.52, 0.71</td>
<td>25</td>
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<tr>
<td>Cyclopoid copepod abundance</td>
<td>chl-a + fish</td>
<td>-0.18</td>
<td>0.27±0.13</td>
<td>0.18*±0.047</td>
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<td></td>
<td></td>
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<tr>
<td>Cladoceran abundance</td>
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<td></td>
<td></td>
<td></td>
<td>0</td>
<td>0.41, 0.59</td>
<td>29</td>
</tr>
<tr>
<td>total zooplankton abundance in high elevation lakes</td>
<td>LDD</td>
<td>1.55</td>
<td>0.053*±0.015</td>
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<td></td>
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<td></td>
<td>0</td>
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<td>14</td>
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<tr>
<td>total zooplankton abundance in low elevation lakes</td>
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<td>0.15±0.088</td>
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<td></td>
<td></td>
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<td>0</td>
<td>0.18, 0.18</td>
<td>15</td>
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</table>
Figure 2.3. Linear regression plots illustrating zooplankton responses to LDD throughout spring sampling events of 2019. Plots show (a) total zooplankton abundance in all lakes, (b) total zooplankton abundance in southern/coastal lakes, (c) total zooplankton abundance in northern/mountain lakes, (d) rotifer abundance in all lakes, (e) cladoceran abundance in all lakes, (f) calanoid copepod abundance in all lakes, (g) cyclopoid copepod abundance in all lakes. Abundance variables were log10 transformed for analysis, but untransformed values are plotted for ease of interpretation.
**Discussion**

Elevational variation in patterns of spring surface water warming foreshadows shifts under a warming climate

Our findings illustrate that spring thermal processes in high elevation lakes are unique compared to low elevation lakes. High elevation lakes in our study began warming later than low elevation lakes but peak surface temperatures were reached at a 50% faster rate in high elevation lakes than in low elevation lakes (Figure 2). This distinguishes spring in high elevation lakes, marked by a short and rapid period of warming, from the significantly longer spring period in low elevation lakes. Guzzo & Blanchfield (2017) observed a similar pattern in Canadian Shield lakes when comparing interannual variability in ice-out and seasonal temperatures. In years when ice-out occurred earlier, lake surface waters warmed more slowly, and onset of summer occurred around the same time despite earlier ice-out. Lake trout (*Salvelinus namaycush*) in these lakes migrate to the pelagic zone once surface waters increase above 15 °C (onset of summer) but throughout the spring they feed on littoral prey which are more abundant than pelagic prey. Longer springs, therefore, may have contributed to increased growth for lake trout in years with earlier ice-out (Guzzo & Blanchfield, 2017). In our study the spring warming period in low elevation lakes was 45 days longer on average than in high elevation lakes. The difference in length of spring is related to the timing of ice-out; earlier ice-out in low elevation lakes exposes surface waters to persistent cold night-time temperatures, slowing the course of warming. In contrast, later ice-out in the high elevation lakes results in a shorter spring period because the angle of the sun and length of daylight generate more substantial heat (Shuter et al., 2013). Elevation is a characteristic relevant to among-lake thermal differences (Christianson et al., 2019; Moser et al., 2019) and it is well documented that elevation is an important determinant of interannual warming trends in lakes around the globe (O’Reilly et al., 2015). The relationship between elevation and season-specific lake thermal
processes is not well understood; however, our study presents novel insight into the role of elevation and, more broadly, local climate for determining spring lake thermal phenology.

Research on lake responses to global change and biotic-thermal relationships has widely focused on summertime or annual average water temperature (Winslow et al., 2017). Recognition is emerging, however, that seasonal and intra-seasonal temperature patterns are vital for understanding lake-climate relationships and for predicting aquatic ecosystem responses to climate change (Niedrist et al., 2018; Winslow et al., 2017). Global surface waters are warming (O’Reilly et al., 2015) but not all months or seasons are warming at the same rate (Winslow et al., 2017). Understanding seasonal temperature heterogeneity is important because many lake processes are dependent on temperature patterns during specific seasons (Winslow et al., 2017). For example, the strongest determinants of the timing of ice breakup in northeastern US lakes are March and April air temperatures (Beyene & Jain, 2015; Hodgkins et al., 2002); onset of the clear water phase in Europe’s Lake Constance is driven by early spring wind patterns (Tirok & Gaedke, 2006); and migration of lake trout in boreal Canadian Shield lakes to their summer, deep water habitat occurs when surface waters reach 15 °C, which depends on March through May air temperatures (Guzzo & Blanchfield, 2017). These examples also illustrate that the spring period in lakes is highly dynamic and important for both physical and biological lake processes.

The relative velocity of climate change and subsequent severity of ecosystem change in mountain lakes is also up for debate. Climate change refugia, ecosystems where climate induced change is suppressed or moderated due to local or regional landscape characteristics, hydrology, and/or biotic interactions (Morelli et al., 2016). In many mountain streams in the Northwestern US, warming has been inconsequential compared to surrounding systems and cold-water vertebrates, including several threatened fish and amphibian species, are thriving (Isaak et al., 2016). Connectivity to groundwater or permanent snowfields and forest canopy shading are potentially driving the cooler microclimates of
mountain streams (Isaak et al., 2016). Researchers speculate that high elevation lakes could also serve as refugia (Morelli et al., 2016) and evidence suggests that high elevation ecosystems are warming at a slower rate than low elevation systems. The ice-free period in mountainous regions has not lengthened as substantially as lower elevation lakes (Hodgkins et al., 2002; O’Reilly et al., 2015). In Maine, minimum temperatures between 1895 and 2018 in Northern climate divisions, where the mountain lakes we examined are situated, increased 8-16% less than the rest of the state (Fernandez et al., 2020). However, research relating warming trends in the Northeastern US to spruce-fir forests, the dominant vegetative cover at our high elevation lake sites, suggests that by 2100 lower spruce-fir ranges will no longer be present and mountains will not serve as refugia for species dependent on this forest type (Wason et al., 2017). Furthermore, climate change refugia in mountain ecosystems are often defined by high habitat connectivity (Isaak et al., 2016; Morelli et al., 2017) which is not a distinct characteristic of lakes where migration and dispersal of many biota are limited by lake or catchment boundaries (Havel & Shurin, 2004). Clarifying if mountain lakes will provide refugia for species vulnerable to warming will require a deeper understanding of the relationships among warming trends and lake morphological, hydrological, and biological characteristics (Morelli et al., 2016).

If high elevation lakes are not buffered from the effects of climate change, seasonal processes in mountain lakes could gradually become more like those of low elevation lakes. Latitudinal and elevational range shifts have been widely observed in response to climate change in studies of terrestrial and aquatic systems and many species have shifted or expanded their distributions northward or upward in elevation as warming renders lower habitats unsuitable (Lenoir & Svenning, 2015). Furthermore, high elevation phenology is expected to shift toward low elevation phenology and such convergence has been observed in terrestrial studies (Chen et al., 2018; Lenoir & Svenning, 2015). For instance, records of leaf unfolding from Europe indicate that spring forest phenology at high elevations is shifting towards a timing similar to low elevation forests (Chen et al., 2018). Maine lakes offer a
distinctly different thermal phenology pattern in high elevation lakes in the spring compared to low
elevation lakes. As projected warming trends continue to increase the length of the open water season
and surface water temperatures in lakes, high elevation lake phenology could shift towards mirroring
the timing and processes of low elevation lakes. Under such a scenario, mountain lakes would lose their
distinct, rapid spring warm-up, which, as our findings illustrate, is an important driver of zooplankton
phenology. A thorough understanding of the drivers and mechanisms of spring phenology in lakes is
paramount for predicting how lakes will react to climate change and our results contribute to this body
of knowledge.

Zooplankton dependence on spring temperatures and variation among taxa implicates a changing
future for lake communities

Results of linear mixed effects models indicate that zooplankton abundances increase in
response to warming surface waters in the spring (Table 2, Figure 3) and temperature is a particularly
strong driver of zooplankton spring phenology in high elevation lakes (Table 2, Figure 3). Temperature is
known to be a key driver of biological processes and increases in temperature cause increases in
metabolic rate, growth, and egg maturation in many organisms as long as temperature is within a safe
threshold (Elser et al., 2020). Therefore, our findings support our hypotheses—following ice-out,
temperature drives zooplankton abundance development as spring surface waters warm. Haberman &
Haldna (2017) observed a similar tight link between zooplankton abundances and temperature in a
polymictic lake in Estonia. For every one-degree C of rise in spring water temperature zooplankton
abundance increased by 27% and autumn water temperatures also correlated with changes in
abundance. Other zooplankton life history events and behaviors also correlate with temperature.
Summer development of zooplankton may be linked to sub-seasonal water temperatures occurring
around the clear water phase (Huber et al., 2010) and vertical diel migration also appears to increase
with higher temperatures in some lakes (Simoncelli et al., 2019). Analyses from our study highlight the
importance of the spring warming period in high elevation lakes for phenology and understanding the link between temperature and zooplankton phenology will aid in our understanding of how climate warming will manifest in lake ecosystems.

Current warming trends suggest continued shifts in northeastern US lakes towards earlier ice-out, warmer surface waters, and longer open water seasons (Beyene & Jain, 2015; Fernandez et al., 2020; O’Reilly et al., 2015) and under future conditions the spring warm-up period in mountain lakes is likely to lengthen. Given the tight link revealed in this study between zooplankton development and spring surface water warm-up, zooplankton communities will respond to the lengthening of spring in high elevation lakes. Shifts in spring plankton phenology in response to climate change have been widely reported in temperate lakes around the globe. The onset of the spring algal bloom and the clear-water phase are occurring earlier in response to indirect effects of warming temperatures including earlier ice-out and onset of stratification (Gronchi et al., 2021; Straile & Adrian, 2000; Tirok & Gaedke, 2006; Winder & Schindler, 2004b). Zooplankton spring phenology has generally responded in synchrony with warming trends and earlier phytoplankton phenology (Adrian et al., 2006; Gerten & Adrian, 2002; Thackeray et al., 2012; Winder & Schindler, 2004a); however, phenological responses to change in other seasons are more variable and depend on species specific life-history traits (Adrian et al., 2006). Adrian et al. (2006) reported a synchronous, 2-4 week advancement in spring phytoplankton and zooplankton phenology in a small, polymictic lake in Germany in response to two decades of warming; however, summer copepod phenology responded variably. Extension of the summer pelagic phase occurred in several copepod species as the lake grew warmer, while for some species timing did not shift but peak abundances increased, and for others abundances increased in addition to an extension of the pelagic phase (Adrian et al., 2006). Focusing on spring for monitoring phenological changes in lake biotic communities could provide the advantage of more traceable responses and should be prioritized given the importance of spring for lake ecosystem processes.
*Daphnia* are the most commonly studied zooplankton in the context of phenological change in lakes and analyses from several studies in Europe, as well as experimental manipulations indicate that climate warming is causing earlier spring arrival and peak abundances of daphnids and the shift is primarily driven by warmer spring water temperatures and earlier phytoplankton blooms (Berger et al., 2007; Straile & Adrian, 2000; Thackeray et al., 2012; Winder et al., 2012). However, in our study lakes, cladoceran development did not correlate with water temperature in the spring but instead Julian day was the only predictor variable present in the best fit model though it was not significant. This indicates that day length and light availability might be more important for cladoceran spring growth than temperature in these lakes. *Daphnia* egg development and release from diapause is widely accepted to be dependent on light availability (Ślusarczyk & Flis, 2019; Stross, 1966). The lack of a relationship between spring cladoceran abundance and temperature could also be due to relatively low abundances of cladocerans which made up only 8% of total zooplankton on average in our study lakes. *Daphnia* were particularly uncommon, appearing in small numbers in 30% of samples, 60% of which were summer or autumn samples. Many cladoceran species are less tolerant to cold temperatures and often appear in the water column later than other taxa and reach peak abundances in the autumn (Allan, 1976; Kalff, 2002). In our study sites, cladoceran species appeared more frequently in summer and autumn samples compared to spring samples. Therefore, cladoceran phenology in our region might be more dynamic later in the growing season and examination of summer and autumn will be key for understanding drivers of cladoceran phenology in these lakes.

Research also indicates that phytoplankton phenology and spring mixing events play an important role for spring cladoceran development (Thackeray et al., 2012; Tirok & Gaedke, 2006; Winder et al., 2012). In a large lake in the UK, spring phenology of *Daphnia geleata* was determined by the timing of the phytoplankton peak (Thackeray et al., 2012); mesocosm manipulations indicated algal biomass regulated by light availability as the key driver of *Daphnia* spring phenology (Winder et al.,
and phytoplankton phenology paired with spring mixing intensity defined the onset of *Daphnia* growth in Europe’s Lake Constance (Tirok & Gaedke, 2006). Deeper and more intense mixing in Lake Constance resulted in higher *Daphnia* abundances, likely because intense mixing delayed the phytoplankton bloom, allowing *Daphnia* to outcompete faster growing zooplankton that dominate grazing under early blooms (Tirok & Gaedke, 2006). Experimental manipulation in a large lake in Quebec revealed a tight link between *Bosmina* growth, a group that was more common in our study lakes, and increased thermocline depth due to warming (Gauthier et al., 2014).

Cyclopoid copepod abundances were similarly unaffected by spring surface water warming in our study but were instead driven by fish presence and significantly by chlorophyll-a concentration (Table 2). Interestingly, the effect of fish presence was positive which could be a result of reduced competition provided by fish that often select for larger-bodied cladoceran species (Kalff, 2002). Copepods develop more slowly and cycle through multiple juvenile stages before reaching adulthood (Allan, 1976; Nicolle et al., 2012) so they could be slower to respond to the quick spring warmup observed in high elevation lakes or could remain in nauplii or copepodite stages through spring. Research also highlights other drivers of cyclopoid phenology besides temperature. A tight link with the timing of the clear-water phase in a shallow lake in Germany corroborates our finding that chl-a drives spring phenology of cyclopoids (Huber et al., 2010). Other evidence points to spring mixing and summer thermocline depth as key determinants of cyclopoid growth, indicating that deeper thermoclines caused by climate warming and increased mixing with intensified wind events will advance cyclopoid phenology (Gauthier et al., 2014). Monitoring continuous sub-surface temperatures in our study lakes to map mixing dynamics and analyzing phytoplankton growth could provide further insight into the drivers of cladoceran and cyclopoid copepod phenology in northeastern US temperate lakes.
Generally, small dimictic lakes, and mountain lakes, are largely missing from the body of research evaluating zooplankton phenological shifts. Our findings suggest that in small northeastern US ponds, phenology of rotifers and calanoid copepods might be more sensitive to climate change due to their dependence on spring temperature. Though phenological advances have been reported in these groups, only a handful of studies have examined copepod species and rarely rotifer species. In lake Washington, US, earlier peak abundances of *Keratella* rotifers were strongly correlated with warming surface waters and the calanoid copepod, *Leptodiaptomus ashlandi*, declined in abundance but increased in biomass due to a climate driven shift from a 12 month cycle to a 6 month cycle (Winder et al., 2009; Winder & Schindler, 2004b). Our results indicate that an increased focus on rotifer species in plankton phenological research will provide valuable insight into effects of warming in small temperate lakes.

Rotifers were abundant throughout the spring in our lakes and an average of 50% of total abundances were rotifer species. Additionally, the tight relationship between rotifer abundances and spring water temperature (Table 2, Figure 3) highlights their sensitivity to warming. Quick development, short lifecycles, and parthenogenetic reproduction allow rotifers to dominate grazing in the early spring and to respond quickly to changing environmental conditions (Allan, 1976). Our results imply that lengthening of spring in high elevation lakes could significantly alter rotifer phenology since they closely track warming in the spring. Understanding whether a longer spring in mountain lakes will mean an earlier onset of rotifer peak abundance or significantly greater growth will require further study of species interactions and change over time with either outcome having implications for the rest of the lake food web. In lakes with planktivorous fish populations, rotifers often serve as the first available food source for juvenile fish and advancement in rotifer phenology could cause reduced fish recruitment if rotifer phenology and fish hatches become decoupled (Nicolle et al., 2011). A comprehensive understanding of potential phenological shifts in northeastern US and high elevation lakes under climate
warming will require further monitoring of a variety of zooplankton taxa and species-specific life-cycle drivers.

The variable response among zooplankton taxonomic groups to spring surface water warmup in our study forecasts that responses to long-term climate warming in lakes will be taxa specific. As plankton species and other lake biota adjust distinctively to earlier ice-out, longer springs, and interacting geochemical, physical, and biological shifts, asynchronies will develop in lake communities. Such trophic mismatches have been reported in freshwater and marine ecosystems among plankton and between plankton and higher trophic levels (Mackas et al., 2007; Winder & Schindler, 2004a). A crash in Daphnia populations was observed in Lake Washington, US, when diatom blooms advanced over time with warming temperatures but Daphnia grazers did not shift with temperature and consequently matured after declines in key food sources (Winder & Schindler, 2004a). Increased stratification in marine systems advanced the onset of spring phytoplankton blooms by 16 days; however, temperature driven fish spawning occurred 32 days earlier resulting in a drastic mismatch between phytoplankton and fish phenology. Subsequent declines in fish recruitment are expected because zooplankton food sources tightly track phytoplankton phenology in these systems (Asch et al., 2019). The responsiveness of rotifers and calanoid copepods to spring temperature along with the absence of a link to spring temperatures for cladoceran and cyclopoid copepod species in this research suggests that zooplankton composition in northeastern US lakes will reorganize under climate change. Asynchronies among lake zooplankton will elicit responses in the algal food sources and vertebrate predators of these species and could drastically alter lake food webs. Our examination of drivers of zooplankton phenology at varying elevations in Maine lakes deepens our understanding of phenological mechanisms in remote and mountain systems and is critical to understand and anticipate the consequences of climate change.
REFERENCES


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Table A.1 Summary of landscape characteristics of ELS-II lakes by region.

<table>
<thead>
<tr>
<th>Region</th>
<th>Statistic</th>
<th>Elevation (m)</th>
<th>Lake area (ha)</th>
<th>Maximum depth (m)</th>
<th>Watershed : Lake area</th>
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</thead>
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<tr>
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<td>Max</td>
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Table A.2 Summary of zooplankton composition in ELS-II lakes in 2004 by region. Zooplankton taxonomic group abbreviations are as follows: Cal = calanoid copepod; Cyc = cyclopoid copepod; Da = Daphnia; Bo = Bosmina; Ho = Holopedium; Po = Polyphemus; Di = Diaphanasoma; Ce = Ceriodaphnia; Ch = Chydorus

<table>
<thead>
<tr>
<th>Region</th>
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Table A.3 Summary of zooplankton composition in high elevation and low elevation lakes during winter and spring samples. Grey rows indicate low elevation ponds and white rows indicate high elevation ponds.

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BIOGRAPHY OF THE AUTHOR

Stephanie Dykema was born in Boulder, Colorado on July 12, 1988. She was raised in South Boulder and graduated from Fairview Highschool in 2007. She attended Fort Lewis College in Durango, Colorado and graduated in 2013 with a Bachelor of Science degree in Ecology and Environmental Biology. Following college, Stephanie worked as a riparian Botanist for PacFish/InFish Biological Opinion Monitoring Program (PIBO), a US Forest Service project that monitors fish habitat through Montana, Idaho, Washington, and Oregon. She worked for this program for two and a half years surveying vegetation and managing field crews. In 2015 Stephanie moved to Jackson Wyoming where she worked on a variety of wildlife and vegetation monitoring projects and spent a season as the monitoring lead for Grand Teton National Park’s revegetation program. In June 2020, Stephanie accepted a Professional Research Assistant position with Niwot Ridge LTER and the University of Colorado’s Institute for Arctic and Alpine Research (INSTAAR). She moved back to her hometown of Boulder and currently manages the field work and data collection for a long-term alpine lake monitoring project in the Colorado Rockies. Stephanie is a candidate for the Master of Science degree in Ecology and Environmental Sciences from the University of Maine in May 2021.