



Experimental effects of elevated temperature and nitrogen deposition on high-elevation aquatic communities

Daniel L. Preston¹ · Eric R. Sokol^{2,3} · Katherine Hell³ · Diane M. McKnight^{3,4} · Pieter T. J. Johnson⁵

Received: 30 March 2019 / Accepted: 14 October 2019 / Published online: 16 November 2019
© Springer Nature Switzerland AG 2019

Abstract

Two widespread drivers of change in high-elevation lakes are climate warming and atmospheric nitrogen deposition, which may have interactive effects on aquatic ecosystems. Using an outdoor mesocosm experiment at 2900 m above-sea level along the Colorado Front Range, we investigated the individual and combined effects of realistic increases in temperature (ambient versus 2.4 °C increase) and nitrogen concentrations (three levels) on lake plankton and hydrochemistry. Relative to the low temperature treatment, enhanced temperatures decreased the overall density of *Daphnia pulicaria* by ~40% and of gravid females specifically by ~20%. Increased nitrogen also reduced *Daphnia* density, especially in the low-temperature treatments, leading to a significant nitrogen-by-temperature interaction. The calanoid copepod *Hesperodiaptomus shoshone*, in contrast, was unaffected by experimental manipulations of temperature and nitrogen, and declined in abundance over time regardless of treatment. Chlorophyll-a increased to a maximum in week 4 and was unaffected by the temperature manipulation, suggesting that observed effects on *Daphnia* were likely direct physiological responses to warming rather than bottom-up effects. Nitrate additions caused transient increases in chlorophyll-a, which converged across treatments by the end of the study as nutrients were assimilated. Nitrogen additions also led to progressive increases in dissolved organic carbon concentrations throughout the experiment. Our results suggest that warming has the potential to reduce zooplankton production, consistent with observed decreases in large-bodied *Daphnia* density with decreasing elevation in the Colorado Rockies. Future work should evaluate how the observed effects on plankton communities scale-up to natural lakes, particularly the relative importance of species-specific stress responses versus indirect food web effects.

Keywords Climate change · Alpine lake · Warming · Nitrogen deposition · Plankton · Hydrochemistry

Electronic supplementary material The online version of this article (<https://doi.org/10.1007/s00027-019-0678-4>) contains supplementary material, which is available to authorized users.

✉ Daniel L. Preston
daniel.preston@wisc.edu

¹ Department of Forest and Wildlife Ecology, University of Wisconsin-Madison, Madison, WI 53706, USA

² National Ecological Observatory Network, Boulder, CO 80301, USA

³ Institute of Arctic and Alpine Research, University of Colorado, Boulder, CO 80309, USA

⁴ Department of Civil, Environmental and Architectural Engineering, University of Colorado, Boulder, CO 80309, USA

⁵ Department of Ecology and Evolutionary Biology, University of Colorado, Boulder, CO 80309, USA

Introduction

Lakes are often sensitive to terrestrial and atmospheric change because they integrate broad scale processes through the movement of water across the landscape (Williamson et al. 2008). As a result, lakes can be useful indicators of environmental change occurring on scales ranging from a single basin (e.g., shifts in land use) to entire regions (e.g., climate change) (Carpenter et al. 1992; Schindler 2009; Moser et al. 2019). Lakes also provide vital ecosystem services including the provisioning of water for drinking and irrigation, highlighting the need to understand how ongoing changes alter lake functioning (Wilson and Carpenter 1999).

Warming temperatures represent a ubiquitous driver of change in lake ecosystems across the globe (Williamson et al. 2009). Climate can influence multiple aspects of lake functioning, including hydrology, lake mixing, ice phenology, and water chemistry (Adrian et al. 2009). Lake food

webs are also sensitive to climate change, with documented shifts in productivity and the phenology of species interactions in response to warming and/or shifts in precipitation (Scheffer et al. 2001). For instance, warming can alter the timing of phytoplankton blooms relative to the abundance of zooplankton grazers, leading to a mismatch in species interactions that control lake ecosystem processes (Winder and Schindler 2004).

Alongside shifts in climate, atmospheric nitrogen deposition represents an important potential driver of aquatic ecosystem change, particularly at high elevations (Fenn et al. 2003; Saros et al. 2011; Moser et al. 2019). Atmospheric transport of anthropogenic reactive nitrogen has been increasing globally due to intensifying use of fossil fuels and agricultural production (Galloway et al. 2008). Nitrogen deposition now represents a widespread nutrient source in ecosystems in remote areas, such as high-mountain lakes (Wolfe et al. 2003; Holtgrieve et al. 2011). While phosphorus is often a critically limiting nutrient in lake ecosystems (Schindler 1977; Carpenter 2005), some high-elevation lakes may instead be nitrogen limited, or co-limited by both nutrients (e.g., Elser et al. 2009). Indeed, nitrogen deposition into oligotrophic high-elevation lakes in Europe and North America has been linked to increases in phytoplankton biomass (Bergstrom and Jansson 2006) and shifts from nitrogen to phosphorus limitation (Elser et al. 2009).

Lakes in the Colorado Rocky Mountains in particular have experienced concurrent increases in temperatures and atmospheric nitrogen deposition. The subalpine Colorado Front Range (3048 m) has experienced warming of ~ 0.2 °C per decade over 56 years (McGuire et al. 2012) and the timing of snowmelt and resulting seasonal peaks in streamflow have shifted earlier by 2–3 weeks over 29 years throughout the Colorado Rockies (Clow 2010). Warming has decreased the duration of lake ice cover in the Colorado Rockies and is associated with stronger lake stratification, longer water residence times, greater ion and nutrient concentrations, and more chlorophyll-a (Preston et al. 2016). Among mountainous regions of the western US, the eastern Colorado Rockies have also experienced among the highest levels of nitrogen deposition recorded (Baron et al. 2000), which peaked in the mid-2000's (Mast et al. 2014). Elevated nitrogen deposition has variable effects on primary production in high-elevation Colorado lakes, in part due to differences in hydrology, concentrations of limiting nutrients other than nitrogen (e.g., phosphorus), and top-down control by zooplankton (Nydick et al. 2003). Benthic primary production may also take up a considerable amount of nitrate relative to phytoplankton (Nydick et al. 2004), driving lake-specific responses to nitrogen deposition.

In addition to effects on primary production, both warming and nitrogen deposition have potential to directly or indirectly affect zooplankton in high-elevation Colorado

lakes. Increased nitrogen deposition may drive bottom-up increases in zooplankton productivity in nitrogen limited lakes, whereas warming has potential to either increase or decrease zooplankton populations (Thompson et al. 2008). Warming may be associated with direct increases in physiological rates driving zooplankton growth and reproduction (Yurista 1999), leading to increased population abundances (Fischer et al. 2011). Conversely, warming may also cause declines in zooplankton populations through direct thermal stress responses at high temperatures (Moore 1996). Most high-elevation lakes in Colorado support cladocerans (e.g., *Daphnia* spp.) and diaptomid copepods (e.g., *Hesperodiaptomus* spp.), both of which are locally adapted to cold water, and may cease reproduction or experience direct mortality if temperatures exceed their thermal optima (Gillooly and Dodson 2000; Strecker et al. 2004; Holzapfel and Vinebrooke 2005). Previous research indicates that high-elevation lake cladocerans tend to have higher thermal tolerances than co-occurring copepods (Weidman et al. 2014), suggesting that responses to warming are likely to be specific.

Although climate change and nitrogen deposition represent two widespread stressors to aquatic ecosystems (Baron et al. 2013), relatively few studies have evaluated their joint effects on alpine aquatic ecosystems (Thompson et al. 2008; Bergström et al. 2013). Because many aquatic ecosystems experience multiple, concurrent drivers of change, empirically isolating the effects of specific factors and their potential synergies remains a persistent challenge (Williamson et al. 2008). Experimental approaches offer an important complement to long-term and comparative studies because they allow isolation of individual drivers of change, including warming temperatures and increased inorganic nitrogen concentrations. In the present study, we used outdoor aquatic mesocosms at 2900 m elevation near the continental divide in Colorado, USA, to evaluate the individual and combined effects of warming and increases in nitrate on lake water chemistry and plankton communities. Our outdoor mesocosms allowed the concurrent manipulation of nitrogen and temperature, thereby complementing prior studies from Colorado Lakes that used large volume in-lake mesocosms, which are challenging to warm experimentally (e.g., Nydick et al. 2004). We predicted that increases in temperature and nitrogen would enhance primary production, leading to additive or synergistic effects when both factors were elevated together. Increased temperature could either decrease zooplankton production (due to direct thermal stress) or increase production (due to increased resource availability) (Moore 1996). We also predicted that responses of zooplankton would be species specific and depend on organismal traits, including body size and thermal tolerance (Holzapfel and Vinebrooke 2005; Daufresne et al. 2009). While aquatic mesocosms may be limited in their capacity to replicate ecosystem-scale processes, they can be especially

useful in developing mechanistic hypotheses to be further evaluated in the field. Given the relatively simplified pelagic community of many alpine lakes (Anderson 1972), they are also informative for testing drivers of high-elevation lake plankton dynamics.

Methods

Mesocosm experiment

We conducted a 2-by-3 factorial mesocosm experiment manipulating temperature (ambient or elevated) and nitrogen concentrations (low, medium, high) in 378 L polyethylene tanks (Rubbermaid tanks measuring 1.34 m long by 0.78 m wide by 0.64 m high). Treatments were replicated five times each ($n=30$ tanks total). The experiment was conducted at the University of Colorado Mountain Research Station north of Nederland, Colorado (40.030855°, -105.533786°) at an elevation of 2900 m above sea level. We filled mesocosms with 200 L of water from a local spring and 50 L of water from Como Creek, which flows through the research station property. Creek water was added because it was similar in water chemistry to nearby alpine lakes and provided an initial source of dissolved organic carbon. Each mesocosm was seeded with sediment (65 mL) and concentrated zooplankton (250 mL) collected with an Ekman grab and a Wisconsin zooplankton net (80 μm mesh), respectively, from Green Lake 4 (3550 m) within the Niwot Ridge Long-Term Ecological Research site (Bowman and Seastedt 2001). The sediment addition was used to introduce phytoplankton and microbial communities from the lake. Zooplankton additions consisted of two primary taxa, cladocerans (*Daphnia pulicaria*) and calanoid copepods (*Hesperodiaptomus shosone*) which were collected using 30 vertical tows of the zooplankton net in the deepest location of Green Lake 4 (~13 m depth). Each mesocosm was outfitted with a mesh lid (DeWitt UV PE 60% shade cloth) and a drainage hole ~50 cm above the tank bottom. Nitrogen concentrations were manipulated by adding sodium nitrate (NaNO_3) at initial concentrations of 3, 15, and 30 $\mu\text{mol NO}_3 \text{ L}^{-1}$, which reflects variation observed in local lakes (Gardner et al. 2008; Barnes et al. 2014). Water temperatures were increased in half of the mesocosms using greenhouse structures consisting of plexiglass and wooden A-frames that rested above the mesh lids (measuring 1.5 m long \times 0.8 m high \times 0.8 m wide; see Paull and Johnson 2014 for details). The lids were designed to create a 2–3 °C difference in water temperature across treatments, which is within the range of temperature increases predicted for mountain lakes in the next century in the Rockies (Roberts et al. 2017). While the plexiglass structures were highly effective at generating a realistic temperature gradient, it is possible that they may

have also affected UV radiation levels. In ten mesocosms (five ambient, five elevated), we suspended Hobo loggers (Onset Computer Corp., Bourne, MA USA) ~10 cm from the tank bottom to record water temperatures. The study began on 30 July 2014 and lasted for 6 weeks.

At weekly intervals we collected samples for water chemistry (two 125 mL bottles), chlorophyll-a (500 mL amber bottles), dissolved organic carbon (125 mL amber bottles), and zooplankton. Water chemistry responses included total dissolved nitrogen (TDN), nitrate, dissolved inorganic nitrogen (DIN), dissolved organic carbon (DOC), and dissolved phosphorus (TDP). Conductivity and pH were measured with a handheld YSI meter just below the water's surface at each sampling date. Samples for nitrogen, phosphorus, DOC, and chlorophyll were filtered (1.0 or 1.2 μm glass fiber filters), stored at 4 °C and then processed at the Arikaree environmental chemistry laboratory using standardized methods (Williams et al. 1996; see <https://instaar.colorado.edu/research/labs-groups/arikaree-environmental-lab/> for additional details on water sample processing). Five replicate zooplankton samples were collected from different positions within each mesocosm on each sampling date using a PVC pipe placed vertically into the water column and capped on removal (~4.5 L water was collected per mesocosm by combining the five individual samples). Zooplankton samples were filtered (80 μm mesh) and preserved in 80% ethanol. Zooplankton were later identified, counted, and a subsample of 50 randomly selected adults of each species were measured for body length (mm) under a dissecting microscope. The number of gravid females of all species and *Daphnia ephippia* were also quantified.

Analyses

Most response variables were analyzed using generalized linear mixed models (GLMMs) with a random intercept term for mesocosm identity to account for repeated measurements on the same tanks (Zuur et al. 2009). We used a zero-inflated negative binomial GLMM for counts of zooplankton and a Gaussian distribution for all other responses (which were log-transformed when needed). We initially used models with all possible interactions of nitrogen (categorical variable with three levels), temperature (categorical variable with two levels), and time (numeric variable corresponding to sampling week). For all responses other than *Daphnia* density, the three-way interactions were non-significant ($p>0.05$ for all comparisons). To simplify interpretation, we therefore dropped the three-way interactions from all models other than the one predicting *Daphnia* density. Resulting models incorporated all possible two-way interactions (temperature-by-nitrogen, nitrogen-by-time, and temperature-by-time). For zooplankton adult size, we grouped both zooplankton taxa as the response variable and included a

species identity predictor term to quantify whether *Daphnia* and *Hesperodiaptomus* differed in body size. We used likelihood-ratio tests to evaluate the significance of main effects and interactions. We report the output from the full models (i.e., with all relevant interactions) in the Appendix (Table S1) and focus the results in the main text on the likelihood ratio tests. Analyses were conducted in the R computing environment using version 3.5.2 (R Core Team 2018). Most analyses were conducted using the glmmADMB package (Fournier et al. 2012).

Results

The temperature and nitrogen manipulations resulted in realistic variation in both variables across treatments. Mean water temperatures increased 2.4 °C from the ambient to the elevated temperature mesocosms (low temperature mean = 12.1 °C, elevated temperature mean = 14.5 °C; Fig. 1). Water temperatures differed by ~3 to ~9 °C from daytime highs to nighttime lows over the period when temperature loggers were deployed. The high temperature treatments showed slightly higher temperature variation over the study (mean CV within each mesocosm = 20.1) than the low temperature treatments (mean CV = 16.2). The nitrogen additions led to the expected differences in nitrate across treatments at the onset of the study, with mean values of 2.0 (high), 1.2 (medium) and 0.3 mg L⁻¹ (low) on the first sampling date (Fig. S1). Nitrate concentrations declined over time and reached approximately zero by week three in all treatments.

In general, nitrogen additions influenced water chemistry more so than the temperature manipulation. As with

nitrate, TDN declined over time, converging by week six to around 0.1 mg L⁻¹ in all treatments (nitrogen*time, df = 2, $\chi^2 = 39.5$, $p < 0.001$; Fig. 2). This pattern contributed to a decline in DIN:TDP over time as well (nitrogen*time, df = 2, $\chi^2 = 137.3$, $p < 0.001$; Fig. S1). DOC increased over the course of the experiment, especially in the high-nitrogen treatment (nitrogen*time, df = 2, $\chi^2 = 10.7$, $p = 0.005$; Fig. 3) and in the low-temperature treatment during weeks one through four (temp*time, df = 1, $\chi^2 = 4.1$, $p = 0.04$; Fig. 3).

Interactions between nitrogen and temperature influenced *D. pulicaria* density (nitrogen*temp*time, df = 3, $\chi^2 = 8.6$, $p = 0.035$) and conductivity (nitrogen*temp, df = 2, $\chi^2 = 6.5$, $p < 0.038$). *Daphnia* density remained low for the first 4 weeks in all treatments, and then increased in weeks five and six (Fig. 2). At the conclusion of the study, *Daphnia* were 2 × more abundant in the low-temperature treatment than the high-temperature treatment. Increases in *Daphnia* were greatest in the low- and medium-nitrogen treatments, especially at low temperature; *Daphnia* were 3 × more abundant in the low- and medium-nitrogen treatments compared to the high-nitrogen treatment (Fig. 2). The mean density of gravid *Daphnia* was also 20% higher in the low temperature treatment compared to the high temperature treatment (Fig. S2). Average *Daphnia* body size and the number of eggs per gravid female were not influenced by any of the treatments (Figs. S2, S4). We observed an increasing number of *Daphnia* ephippia in the low temperature treatment over time, whereas no ephippia were observed in the elevated temperature treatment (Fig. S3). Lastly, conductivity was ~11% lower at low temperature relative to high temperature by the end of the study. Within the high temperature treatment, however, conductivity was highest at medium nitrogen relative to low and high.

Fig. 1 Mean water temperatures from mesocosms under ambient (blue) and elevated (red) temperature treatments. Greenhouse enclosures were used to increase temperatures by an average of 2.4 °C in the elevated temperature treatment (Color figure online)

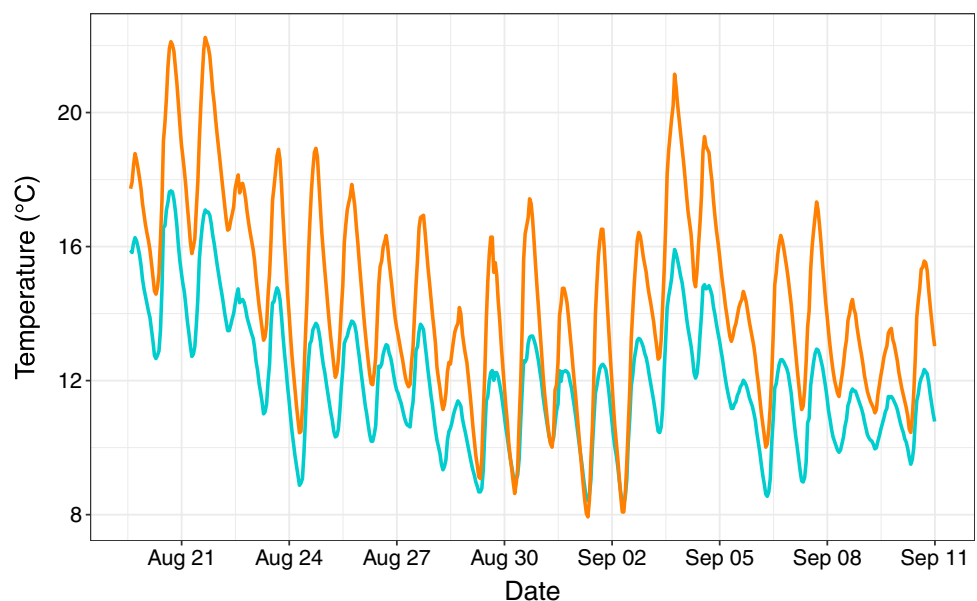


Fig. 2 Total dissolved nitrogen (TDN), total dissolved phosphorus (TDP), chlorophyll-a, and density of *Daphnia* and *Hesperodiaptomus* from mesocosms under the two temperature treatments (columns) and the three nitrate addition treatments (colored points and lines). The lines show mean values for each treatment combination and the points are individual replicates. Note the different units on the y-axis labels. The lines on the plot correspond to the smoothed averages across the different treatments (Color figure online)

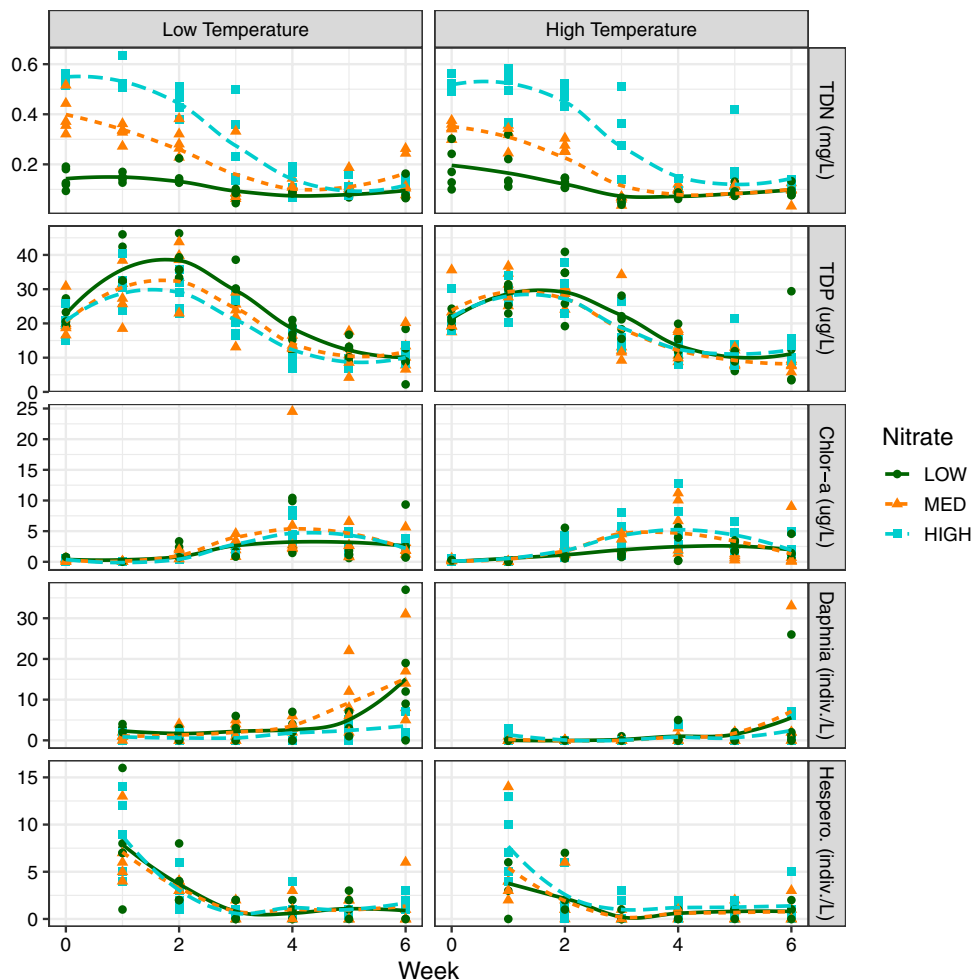
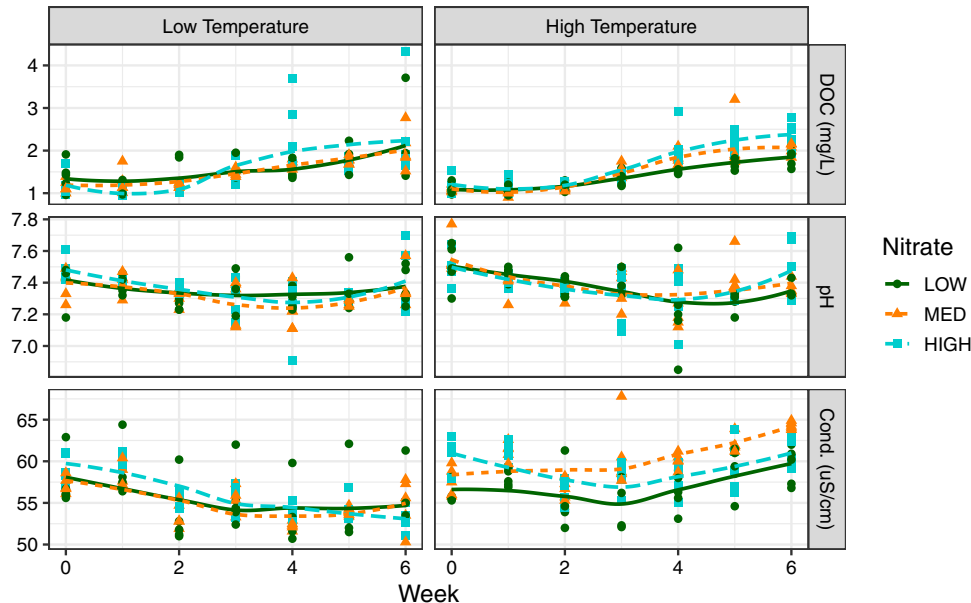


Fig. 3 Trends in dissolved organic carbon (DOC), pH, and conductivity from mesocosms under the two temperature treatments (columns) and the three nitrate addition treatments (colored points and lines). The lines show mean values for each treatment combination and the points are individual replicates. Note the different units on the y-axis labels. The lines on the plot correspond to the smoothed averages across the different treatments (Color figure online)



Several responses—including phosphorus, chlorophyll-a, *Hesperodiaptomus* density, and pH—were dynamic

over time, but unaffected by the nitrogen or temperature manipulations. Total dissolved phosphorus increased early

in the experiment, plateaued around week three, and then decreased through the rest of the experiment (time, $df=4$, $\chi^2=153.6$, $p<0.001$, Fig. 2). Chlorophyll-a, in contrast, increased until week four and then decreased slightly at the end of the study (time, $df=4$, $\chi^2=101.9$, $p<0.001$, Fig. 2). *Hesperodiptomus* decreased in density over the experiment (time, $df=4$, $\chi^2=71.1$, $p<0.001$, Fig. 2), but unlike *Daphnia*, their density was unaffected by either nitrogen or temperature treatments. *Hesperodiptomus* body size and the numbers of gravid females were also not significantly affected by the experimental treatments (Figs. S2, S4), although *Hesperodiptomus* were significantly larger than *Daphnia* (Table S1). Lastly, pH declined until week four and then increased for the final two weeks (time, $df=4$, $\chi^2=18.7$, $p<0.001$, Fig. 3).

Discussion

Warming and nitrogen deposition represent co-occurring drivers of ecosystem change in many lakes across the globe (Fenn et al. 2003; Williamson et al. 2009). Here, we evaluated their individual and combined effects on high-elevation (2900 m) plankton communities using semi-realistic mesocosms along the Front Range in Colorado, USA. Our results indicated that temperature reduced zooplankton density, especially for the large-bodied *D. pulicaria*. Nitrogen additions also negatively affected *Daphnia* density, resulting in interactive effects between nitrogen and temperature, but had relatively small effects on chlorophyll-a concentrations. Overall, we observed few interactions and the effects of nitrogen and temperature together were additive for most response variables. These results suggest that warming temperatures may lead to species-specific thermal stress responses in zooplankton communities, potentially driving either adaptation or shifts in community structure over time (Domis et al. 2007; De Meester et al. 2011).

Chlorophyll-a in the mesocosms was only weakly influenced by nitrogen additions, potentially due to nutrient limitation towards the end of the experiment. The high nitrogen treatment showed slightly higher chlorophyll-a levels in weeks three through five. Based on the observed chlorophyll concentrations, phytoplankton growth did not occur until weeks two to three of the study, leaving a relatively short period of growth prior to declines in nitrate. These patterns suggest that phytoplankton growth became increasingly nitrogen-limited over time, or co-limited by nitrogen and phosphorus, likely minimizing the effects of the nitrogen additions. Ratios of DIN:TDP generally declined over time in all treatments. Previous studies have indicated that phytoplankton in some mountain lakes in Colorado have shifted from nitrogen limitation (Morris and Lewis 1988) to phosphorus limitation with increases in nitrogen deposition in

recent decades (Gardner et al. 2008; Elser et al. 2009). For instance, phytoplankton in lakes along the Colorado Front Range with summer nitrate concentrations around $750 \mu\text{g L}^{-1}$ and total phosphorus concentrations around $5 \mu\text{g L}^{-1}$ were generally phosphorus limited (Elser et al. 2009). Although the mesocosms had higher phosphorus concentrations early on ($\sim 20 \mu\text{g L}^{-1}$; Fig. 1), we cannot rule out the possibility that phytoplankton were phosphorus limited at the onset of the study, thereby minimizing the effects of nitrogen addition on primary production. Further experimental work on the ecological stoichiometry of plankton communities would be useful to clarify how nitrogen deposition affects nutrient limitation of phytoplankton in mountain lakes. Additionally, the decline in chlorophyll-a towards the end of the study may also have been a result of increased grazing pressure as *Daphnia* populations grew. Nutrient limitation and grazing may therefore have jointly affected phytoplankton dynamics in the mesocosms.

The phytoplankton species that became established in the mesocosms were consistent with the phytoplankton community found in Green Lake 4, indicating that the mesocosms were effective at creating conditions suitable for local high-elevation phytoplankton from natural lakes. Across all treatments, the total phytoplankton biovolume followed a similar temporal pattern as chlorophyll-a; and the abundance of diatoms gradually decreased relative to several chlorophytes, including *Chlamydomonas* sp., and a cryptophyte *Plagioselmis* sp. (Olivier 2017). At the last time step, the chlorophyll-a and total phytoplankton biovolume decreased without an accompanying shift in the phytoplankton community composition. Further, the gradual decrease in diatoms relative to chlorophytes and cryptophytes that occurred in the mesocosms is also commonly observed in Green Lake 4 as the summer progresses (Olivier 2017; Gardner et al. 2008).

Both nitrogen additions and temperature influenced DOC concentrations, and DOC increased over time in all treatments. The primary source of DOC in the mesocosms was likely phytoplankton production, which increased over time until week four (based on chlorophyll-a). The increased DOC under high nitrogen conditions was consistent with the slightly higher chlorophyll-a values under high nitrogen. It is less clear what mechanisms led to the shift in DOC concentrations over time in response to elevated temperature, but it may have been related to differences in zooplankton survival, rates of waste excretion, and/or zooplankton decomposition. Furthermore, the DOC differences could also be related to differences in extracellular organic matter release by phytoplankton across treatments and/or rates of DOM degradation related to water chemistry or microbial community structure (Olivier 2017). Phytoplankton community composition was similar across treatments, suggesting that DOC differences were probably not the result of changes in

phytoplankton species composition (Olivier 2017). DOC in alpine lakes in the Colorado Front Range typically decreases over the summertime due to export and assimilation (Baron et al. 1991; Miller et al. 2009), which contrasts with the increases observed in the mesocosms.

Increased temperature resulted in lower population growth of *Daphnia* within the mesocosms most likely due to physiological stress limiting growth and reproduction (Yurista 1999). Previous work indicates that warming can either increase or decrease the survival, reproduction, and feeding rates of *Daphnia*, depending on the amount of warming, the temperature optimum for a given zooplankton population, and the response of phytoplankton resources (Moore 1996). Such effects can be variable even for specific zooplankton species. For instance, warming of 7 °C increased population growth of *D. pulex* in mesocosms located in Saskatchewan, Canada, leading to food limitation and subsequent population collapse (Beisner et al. 1997). In contrast, an increase of 3.6 °C strongly reduced populations of *D. pulex* in mesocosms at 2300 m in Alberta, Canada, likely due to direct physiological stress (Strecker et al. 2004). In general, warming is known to enhance thermal stress and food limitation in cold-adapted zooplankton, and both factors may act synergistically to limit zooplankton growth over time (Moore 1996; Wagner and Benndorf 2007). We also observed slightly greater temperature variation in the high temperature treatment, which is consistent with predicted warming trends and may have further stressed *Daphnia*. The negative effects of warming on *Daphnia* were particularly strong under the high nitrogen condition, which could reflect reductions in food quality (i.e., increased C:P of phytoplankton). If phytoplankton increased in nitrogen content, this would likely be associated with a relative decrease in their phosphorus content, which has been shown to negatively affect *Daphnia* growth (Elser et al. 2016).

We observed species-specific differences in zooplankton dynamics, suggesting that organismal traits, including thermal tolerance and/or diet, likely mediated their responses. The cladoceran *D. pulicaria* increased in density over time in most treatments, whereas the calanoid copepod *H. shoshone* decreased steadily over time. The *Daphnia* dynamics appear relatively consistent with observed temporal trends within a summer in the natural lake where the zooplankton were collected (Loria et al. unpublished). Even in the ambient condition, the mesocosms were slightly warmer than the summer temperature in the lake from which the zooplankton were collected, possibly explaining the population decline of *Hesperodiaptomus*. Based on preliminary sampling in Green Lake 4, *Hesperodiaptomus* appears to increase in density in early summer and then decline towards fall (Loria et al. unpublished). While the ambient temperature mesocosms averaged 12.1 °C over the study period, typical summer water temperatures at Green Lake 4 are ~9 °C, with a high

of ~14 °C recorded during an unusually warm summer (Gardner et al. 2008; Flanagan et al. 2009). Previous work indicates that *Hesperodiaptomus* is more sensitive to negative physiological effects of high temperature than *Daphnia* (Holzapfel and Vinebrooke 2005; Thompson et al. 2008; Weidman et al. 2014). It is further possible that declines in *Hesperodiaptomus* were driven by resource limitation; unlike *Daphnia*, *Hesperodiaptomus* are generalist omnivores (Anderson 1970), typically feeding on a mix of zooplankton and phytoplankton, and they may have been prey limited especially early in the experiment. *Daphnia* generally do not feed on other zooplankton.

Several of our findings can be evaluated within the context of predicted shifts in body size with temperature (Ohlberger 2013). The size-stress hypothesis, for instance, posits that larger-bodied organisms should be more sensitive to environmental stress than smaller-bodied organisms (Odum 1985). The copepods in our study had a larger adult body size than the cladocerans. If the declines in *Hesperodiaptomus* were driven by thermal stress, this pattern would be consistent with the prediction of the stress-size hypothesis that smaller zooplankton are better able to adapt to elevated temperatures (Moore and Folt 1993; Thompson et al. 2008). Recent comparative sampling across Colorado alpine lakes has also shown that average zooplankton body size correlates positively with lake elevation, consistent with the idea that warmer temperature favor smaller-bodied zooplankton. More broadly, there is support for individual, population, and community levels shifts towards smaller body sizes with elevated temperatures in bacteria, plankton, and fishes (Daufresne et al. 2009; Yvon-Durocher et al. 2011). These patterns may represent a general phenomenon due to a combination of mechanisms involving species interactions (e.g., size-selective predation at high or low temperatures; Dodson 1974), shifts in species composition, and intraspecific shifts in mean body size (Ohlberger 2013). Future work is needed to quantify long-term trends in plankton size distributions with climate change to evaluate the generality of these patterns and the consequences for food webs and ecosystems.

Our mesocosm study had several limitations that should be considered in interpreting and generalizing the results. Although the manipulations were effective at creating realistic temperature differences and capturing locally-relevant initial nitrogen concentrations, the small volume, short-time scale, and simplified biological communities limit their capacity to simulate lake ecosystems. It is also possible that collection of zooplankton and phytoplankton from a larger number of lakes, including lower elevation systems, would have generated more diverse zooplankton responses to the experimental treatments (i.e., shifts in composition as well as density). That said, several aspects of ecosystem functioning were relatively well-replicated in our mesocosms. For instance, nitrogen typically enters local lakes as a pulse

during snowmelt in spring and then declines over the summer due to phytoplankton uptake (Bowman et al. 2014), which was similar to the dynamics observed in our study. Additionally, the ecologically relevant phytoplankton community (Olivier 2017) and the magnitude and daily variation in warming between the low and high temperature treatments effectively represented warming lake conditions for a local alpine lake near the experimental site.

Taken together, results of this experiment emphasize the potential for additive effects of warming and nitrogen deposition on simplified aquatic communities. Complementing such manipulative studies with long-term field studies and comparative sampling of lakes across elevation gradients will be essential for clarifying the mechanisms through which climate and nutrient inputs are likely to alter aquatic communities at high elevations. The relative roles of direct (e.g., thermal stress) versus indirect (food web mediated) effects deserve greater attention in particular, as does the potential for community shifts versus adaptive responses of plankton.

Acknowledgements We thank Matt Carson and Matt Olivier for assistance with the experiment, and Sara Paull for constructing the greenhouse warming structures. Funding for this research has come from the NSF Niwot Ridge LTER program (DEB 1027341), NSF grants to P. Johnson (DEB-0841758 and DEB-1149308) and D. Preston (DEB-1311467), the University of Colorado, and the University of Wisconsin-Madison Office of the Vice Chancellor for Research and Graduate Education.

References

- Adrian R, O'Reilly CM, Zagarese H et al (2009) Lakes as sentinels of climate change. *Limnol Oceanogr* 54:2283–2297
- Anderson RS (1970) Predator–prey relationships and predation rates for crustacean zooplankters from some lakes in western Canada. *Can J Zool* 48:1229–1240
- Anderson RS (1972) Zooplankton composition and change in an alpine lake. *Internationale Vereinigung für Theoretische und Angewandte Limnologie: Verhandlungen* 18:264–268
- Barnes RT, Williams MW, Parman JN et al (2014) Thawing glacial and permafrost features contribute to nitrogen export from Green Lakes Valley, Colorado Front Range, USA. *Biogeochemistry* 117:413–430
- Baron J, McKnight D, Denning AS (1991) Sources of dissolved and particulate organic material in Loch Vale watershed, Rocky Mountain National Park, Colorado, USA. *Biogeochemistry* 15:89–110
- Baron JS, Rueth HM, Wolfe AM et al (2000) Ecosystem responses to nitrogen deposition in the Colorado Front Range. *Ecosystems* 3:352–368
- Baron JS, Hall EK, Nolan BT et al (2013) The interactive effects of excess reactive nitrogen and climate change on aquatic ecosystems and water resources of the United States. *Biogeochemistry* 114:71–92
- Beisner BE, McCauley E, Wrona FJ (1997) The influence of temperature and food chain length on plankton predator prey dynamics. *Can J Fish Aquat Sci* 54:586–595
- Bergstrom A-K, Jansson M (2006) Atmospheric nitrogen deposition has caused nitrogen enrichment and eutrophication of lakes in the northern hemisphere. *Glob Change Biol* 12:635–643
- Bergström A-K, Faithfull C, Karlsson D, Karlsson J (2013) Nitrogen deposition and warming—effects on phytoplankton nutrient limitation in subarctic lakes. *Glob Change Biol* 19:2557–2568
- Bowman WD, Nemergut DR, McKnight DM, Miller MP, Williams MW (2014) A slide down a slippery slope - alpine ecosystem responses to nitrogen deposition. *Plant Ecol Divers*. <https://doi.org/10.1080/17550874.2014.984786>
- Bowman WD, Seastedt TR (2001) Structure and function of an alpine ecosystem: niwot ridge. Oxford University Press, Colorado
- Carpenter SR (2005) Eutrophication of aquatic ecosystems: bistability and soil phosphorus. *Proc Natl Acad Sci* 102:10002–10005
- Carpenter SR, Fisher SG, Grimm NB, Kitchell JF (1992) Global change and freshwater ecosystems. *Annu Rev Ecol Syst* 23:119–139
- Clow DW (2010) Changes in the timing of snowmelt and streamflow in Colorado: a response to recent warming. *J Clim* 23:2293–2306
- Daufresne M, Lengfellner K, Sommer U (2009) Global warming benefits the small in aquatic ecosystems. *Proc Natl Acad Sci* 106:12788–12793
- De Meester L, Van Doorslaer W, Geerts A et al (2011) Thermal genetic adaptation in the water flea *Daphnia* and its impact: an evolving metacommunity approach. Oxford University Press, Oxford
- Dodson SI (1974) Zooplankton competition and predation: an experimental test of the size-efficiency hypothesis. *Ecology* 55:605–613
- Domis LNDS, Mooij WM, Huisman J (2007) Climate-induced shifts in an experimental phytoplankton community: a mechanistic approach. *Hydrobiologia* 584:403–413
- Elser JJ, Kyle M, Steger L et al (2009) Nutrient availability and phytoplankton nutrient limitation across a gradient of atmospheric nitrogen deposition. *Ecology* 90:3062–3073
- Elser JJ, Kyle M, Learned J et al (2016) Life on the stoichiometric knife edge: effects of high and low C: P ratio on growth, feeding, and respiration in three *Daphnia* species. *Inland Waters* 6:136–146
- Fenn ME, Baron JS, Allen EB et al (2003) Ecological effects of nitrogen deposition in the western United States. *Bioscience* 53:404–420
- Fischer JM, Olson MH, Williamson CE et al (2011) Implications of climate change for *Daphnia* in alpine lakes: predictions from long-term dynamics, spatial distribution, and a short-term experiment. *Hydrobiologia* 676:263
- Flanagan CM, McKnight DM, Liptzin D et al (2009) Response of the phytoplankton community in an alpine lake to drought conditions: Colorado Rocky Mountain Front Range, USA. *Arct Antarct Alp Res* 41:191–203
- Fournier DA, Skaug HJ, Ancheta J, Ianelli J, Magnusson A, Maunder MN, Nielsen A, Sibert J (2012) AD Model Builder: using automatic differentiation for statistical inference of highly parameterized complex nonlinear models. *Optim Methods Softw* 27:233–249
- Galloway JN, Townsend AR, Erismann JW et al (2008) Transformation of the nitrogen cycle: recent trends, questions, and potential solutions. *Science* 320:889–892
- Gardner EM, McKnight DM, Lewis WM, Miller MP (2008) Effects of nutrient enrichment on phytoplankton in an alpine lake, Colorado, USA. *Arct Antarct Alp Res* 40:55–64
- Gillooly JF, Dodson SI (2000) Latitudinal patterns in the size distribution and seasonal dynamics of new world, freshwater cladocerans. *Limnol Oceanogr* 45:22–30
- Holtgrieve GW, Schindler DE, Hobbs WO et al (2011) A coherent signature of anthropogenic nitrogen deposition to remote watersheds of the northern hemisphere. *Science* 334:1545–1548
- Holzappel AM, Vinebrooke RD (2005) Environmental warming increases invasion potential of alpine lake communities by imported species. *Glob Change Biol* 11:2009–2015

- Mast MA, Clow DW, Baron JS, Wetherbee GA (2014) Links between N deposition and nitrate export from a high-elevation watershed in the Colorado Front Range. *Environ Sci Technol* 48:14258–14265
- McGuire CR, Nufio CR, Bowers MD, Guralnick RP (2012) Elevation-dependent temperature trends in the Rocky Mountain Front Range: changes over a 56-and 20-year record. *PLoS One* 7:e44370
- Miller MP, McKnight DM, Chapra SC, Williams MW (2009) A model of degradation and production of three pools of dissolved organic matter in an alpine lake. *Limnol Oceanogr* 54:2213–2227
- Moore MV (1996) Consequences of elevated temperature for zooplankton assemblages in temperate lakes. *Arch Hydrobiol* 135:289–319
- Moore M, Folt C (1993) Zooplankton body size and community structure: effects of thermal and toxicant stress. *Trends Ecol Evol* 8:178–183
- Morris DP, Lewis WM Jr (1988) Phytoplankton nutrient limitation in Colorado mountain lakes. *Freshw Biol* 20:315–327
- Moser KA, Baron JS, Brahney J, Oleksy IA, Saros JE, Hundey EJ, Sadro SA, Kopacek J, Sommaruga R, Kainz MJ, Strecker AL, Chandra S, Walters DM, Preston DL, Michelutti N, Lepori F, Spaulding SA, Christianson KR, Melack JM, Smol JP (2019) Mountain lakes: eyes on global environmental change. *Glob Planet Change* 178:77–95
- Nydick KR, Lafrancois BM, Baron JS, Johnson BM (2003) Lake-specific responses to elevated atmospheric nitrogen deposition in the Colorado Rocky Mountains, USA. *Hydrobiologia* 510:103–114
- Nydick KR, Lafrancois BM, Baron JS (2004) NO₃ uptake in shallow, oligotrophic, mountain lakes: the influence of elevated NO₃ concentrations. *J N Am Benthol Soc* 23:397–415
- Odum EP (1985) Trends expected in stressed ecosystems. *Bioscience* 35:419–422
- Ohlberger J (2013) Climate warming and ectotherm body size—from individual physiology to community ecology. *Funct Ecol* 27:991–1001
- Olivier MR (2017) The effects of climate change on an alpine lake system: a mesocosm experiment. M.S. Thesis. Environmental Studies Program, University of Colorado-Boulder
- Paull SH, Johnson PT (2014) Experimental warming drives a seasonal shift in the timing of host-parasite dynamics with consequences for disease risk. *Ecol Lett* 17:445–453
- Preston DL, Caine N, McKnight DM et al (2016) Climate regulates alpine lake ice cover phenology and aquatic ecosystem structure. *Geophys Res Lett* 43:5353–5360
- R Core Team (2018) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. <https://www.Rproject.org/>
- Roberts JJ, Fausch KD, Schmidt TS, Walters DM (2017) Thermal regimes of Rocky Mountain lakes warm with climate change. *PLoS One* 12:e0179498
- Saros JE, Clow DW, Blett T, Wolfe AP (2011) Critical nitrogen deposition loads in high-elevation lakes of the western US inferred from paleolimnological records. *Water Air Soil Pollut* 216:193–202
- Scheffer M, Straile D, van Nes EH, Hosper H (2001) Climatic warming causes regime shifts in lake food webs. *Limnol Oceanogr* 46:1780–1783
- Schindler DW (1977) Evolution of phosphorus limitation in lakes. *Science* 195:260–262
- Schindler DW (2009) Lakes as sentinels and integrators for the effects of climate change on watersheds, airsheds, and landscapes. *Limnol Oceanogr* 54:2349–2358
- Strecker AL, Cobb TP, Vinebrooke RD (2004) Effects of experimental greenhouse warming on phytoplankton and zooplankton communities in fishless alpine ponds. *Limnol Oceanogr* 49:1182–1190
- Thompson PL, St. Jacques MC, Vinebrooke RD (2008) Impacts of climate warming and nitrogen deposition on alpine plankton in lake and pond habitats: an in vitro experiment. *Arct Antarct Alp Res* 40:192–198
- Wagner A, Benndorf J (2007) Climate-driven warming during spring destabilises a *Daphnia* population: a mechanistic food web approach. *Oecologia* 151:351–364
- Weidman PR, Schindler DW, Thompson PL, Vinebrooke RD (2014) Interactive effects of higher temperature and dissolved organic carbon on planktonic communities in fishless mountain lakes. *Freshw Biol* 59:889–904
- Williams MW, Losleben M, Caine N, Greenland D (1996) Changes in climate and hydrochemical responses in a high-elevation catchment in the Rocky Mountains, USA. *Limnol Oceanogr* 41:939–946
- Williamson CE, Dodds W, Kratz TK, Palmer MA (2008) Lakes and streams as sentinels of environmental change in terrestrial and atmospheric processes. *Front Ecol Environ* 6:247–254
- Williamson CE, Saros JE, Vincent WF, Smol JP (2009) Lakes and reservoirs as sentinels, integrators, and regulators of climate change. *Limnol Oceanogr* 54:2273–2282
- Wilson MA, Carpenter SR (1999) Economic valuation of freshwater ecosystem services in the United States: 1971–1997. *Ecol Appl* 9:772–783
- Winder M, Schindler DE (2004) Climatic effects on the phenology of lake processes. *Glob Change Biol* 10:1844–1856
- Wolfe AP, Van Gorp AC, Baron JS (2003) Recent ecological and biogeochemical changes in alpine lakes of Rocky Mountain National Park (Colorado, USA): a response to anthropogenic nitrogen deposition. *Geobiology* 1:153–168
- Yurista PM (1999) Temperature-dependent energy budget of an Arctic Cladoceran, *Daphnia middendorffiana*. *Freshw Biol* 42:21–34
- Yvon-Durocher G, Montoya JM, Trimmer M, Woodward G (2011) Warming alters the size spectrum and shifts the distribution of biomass in freshwater ecosystems. *Glob Change Biol* 17:1681–1694
- Zuur AF, Ieno EN, Walker N, Saveliev AA, Smith GM (2009) Mixed effects models and extensions in ecology with R. Springer, Berlin

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.