University of Alberta

Climate Change Impacts on Stoichiometry, Phytoplankton, and Zooplankton in Alpine Lake Food Webs

by

Raymond Paul Weidman

A thesis submitted to the Faculty of Graduate Studies and Research
in partial fulfillment of the requirements for the degree of

Doctor of Philosophy
in
Ecology

Department of Biological Sciences

Fall 2013
Edmonton, Alberta

ABSTRACT

The main hypothesis of this study was that warmer and drier conditions affect fishless alpine lakes by increasing (1) phosphorus (P)-availability, (2) P-limited autotrophs versus mixotrophic phytoflagellates, and (3) fast-growing P-limited cladocerans versus slower-growing nitrogen (N)-rich calanoid copepods. To test this hypothesis, I conducted (1) a spatial survey of 16 mountain lakes along 1016 m of elevation (summer water temperatures: 5–17°C) in Alberta; (2) a temporal survey of an alpine lake over 16 y (summer water temperatures: 3–14°C); and (3) a laboratory experiment using two alpine and montane plankton communities subjected to warming (10°C; 17°C) and increased dissolved organic carbon (DOC; +80%).

In the lake survey, higher temperatures and decreased lake depth were inferred using structural equation modeling (SEM) to decrease dissolved N:P, which increased chlorophytes and cyanophytes relative to phytoflagellates. Warming suppressed large-bodied cladocerans (*Daphnia middendorffiana*) and calanoid copepods (*Hesperodiaptomus arcticus*) and increased small-bodied zooplankton taxa. Small copepods increased relative to small cladocerans; this decreased particulate C:P via increased P-recycling by copepods.

In the temporal investigation, SEM revealed that decreased rain reduced flushing of dissolved P, which increased diatoms relative to phytoflagellates in Pipit Lake. Unlike the lake survey, more moderate warming increased *D. middendorffiana*, while *H. arcticus* increased with advanced ice-off. Thus, cladocerans increased relative to copepods, which increased particulate C:P via increased P-retention by zooplankton.

In the experiment, warming and DOC additions together increased P-availability, which increased autotrophs and offset the negative direct effects of warming and DOC. Warming increased *D. middendorffiana* but suppressed *H. arcticus*. Particulate C:P increased with DOC additions and increased cladocerans relative to copepods.

These findings partially supported my main hypothesis. Climate effects increased dissolved P-availability, thereby increasing autotrophs relative to phytoflagellates. Shorter-term warming (temporal survey and experiment) increased cladocerans relative to copepods, whereas longer-term warming (spatial survey) suppressed large-bodied zooplankton and increased small taxa. Particulate C:P varied with changes in cladocerans relative to copepods (via P-recycling) and increases in C-rich terrestrial inputs. Findings suggest climate change will increase primary productivity of alpine lakes over several decades by increasing P-availability, and decreasing grazing efficiency as copepods and smaller-bodied zooplankton supercede cladocerans.

ACKNOWLEDGMENTS

I would like to thank my co-supervisors Dr. David Schindler and Dr. Rolf Vinebrooke for providing me with an excellent educational experience and scientific opportunity. Their guidance with developing the research program and with writing the dissertation was invaluable. It was a privilege to study with them both.

I would like to thank Dr. John Spence for participating in my PhD committee and for his suggestions and enthusiasm for the research. I thank Dr. Heather Proctor and Dr. Beatrix Beisner (Université du Québec à Montréal) for participating in the final examination committee and for their engaging discussion and helpful comments on the dissertation.

Many people helped with field work, including Roseanna Radmanovich, Patrick Thompson, Quinn McCashin, Marie-Claire St. Jacques, Alyson Winkelaar, Heather Currie, Nathan Ballard, Iain Phillips, Dr. Chantel Nixon, Dr. Igor Lehnherr, and Dr. Will Hobbs. Working with them in the mountains of Alberta was very enjoyable and memorable.

Several people in the Department of Biological Sciences at the University of Alberta deserve special thanks. Dr. Brian Parker helped with many discussions about the research. Margaret Foxcroft was a great help with administration of the research program. Dr. Mingsheng Ma helped with discussing chemistry methods, particularly about isolating and analyzing dissolved organic C from lake water. Holly Bigelow, June Ng, and many summer students conducted the chemistry analysis at the University of Alberta Biogeochemistry Laboratory. Dr. David Hik and Dr. Lee Foote participated in the candidacy exam committee and provided helpful input on the research proposal. I also thank the many students in David Schindler and Rolf Vinebrooke's research groups for many interesting conversations and presentations.

Several other people helped with editing manuscripts and the dissertation, including Dr. Mohamed N. Mohamed (Ontario Ministry of Environment, Etobicoke), Dr. Will Hobbs (St. Croix Watershed Research Station, Minnesota), Dr. Jonathan Shurin (University of California, San Diego), Dr. Iain Phillips (Saskatchewan Watershed Authority, Saskatoon), Dr. Hamish Greig and Amanda Klemmer (University of Canterbury, Christchurch, New Zealand).

Dr. Jonathan Shurin and Dr. John Richardson hosted me at the University of British Columbia during the later stages of my research program. They provided me with an excellent opportunity to interact and collaborate with their group of students.

Dr. James Grace (US Geological Survey, Lafayette, Louisiana) and Dr. George Arhonditsis (University of Toronto, Scarborough) provided valuable comments and advice on structural equation modeling. Dr. Jeff Curtis (University of British Columbia, Okanagan) was generous in loaning a reverse osmosis unit and in discussing methods for concentrating dissolved organic C from lake water.

Parks Canada staff helped with coordinating field research, and facilitating access to backcountry sites. In particular, Cyndi Smith and Rob Watt at Waterton Lakes National Park (NP), Charlie Pacas at Banff NP, and Ward Hughson at Jasper NP were all very helpful.

Research funding was provided through grants to P.W. from the Alberta Conservation Association, Alberta Ingenuity Fund, and Canadian Circumpolar Institute, and through National Sciences and Engineering Research Council (NSERC) Discovery grants to D.S. and R.V. Scholarships were also provided to P.W. from NSERC, Alberta Ingenuity Fund, and the Walter John's Fund.

On a personal note, I thank my family for all their support during my entire journey through university. I have missed them greatly, especially during the later stages of finishing my dissertation.

Finally, I would like to thank my wife, Sarah Primeau, for her help with editing the dissertation, and all her love and support.

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CHAPTER 1: GENERAL INTRODUCTION

Climate change is having numerous impacts on lake food webs mainly via warming and changes in seasonal precipitation patterns (Parker et al. 2008, Garcia-Jurado et al. 2012). In western North America, seasonal and permanent snow packs are declining, winter precipitation is predicted to fall more as rain than snow, and summer precipitation is expected to decline (Plummer et al. 2006, IPCC 2007, Shepherd et al. 2010). Global average temperatures are predicted to increase by up to 6 ^{o}C in the next century and warming is predicted to be greatest in the planet's coldest regions, particularly at high elevations and high latitudes in the northern hemisphere (Bradley et al. 2004, IPCC 2007). These climatic impacts are expected to affect lake food webs via changes to external nutrient inputs and internal nutrient cycling, and via direct and interactive effects among biota (Hauer et al. 1997, Magnuson et al. 1997).

Lakes are considered sentinels of global change because they integrate the impacts of climate and landscape changes (Williamson et al. 2009, Schindler 2009). Increases in water temperature and variation in nutrient inputs due to altered precipitation patterns should be disproportionately greater in cold-water, oligotrophic lakes relative to warmer, more nutrient-rich lakes (Battarbee 2009). Thus, cold oligotrophic, species-poor lakes should be among the most sensitive sentinels of climate change. Furthermore, relatively pristine alpine lakes in undisturbed landscapes in the Rocky Mountain national Parks in Canada should reveal the impacts of climate change independently of the confounding effects of other human disturbances, such as landscape disturbance and nutrient pollution from sewage and livestock (but also see Schindler 2000, Holtgrieve et al. 2011).

Thus, a series of alpine lakes from Jasper, Banff, and Waterton Lakes National Parks in Alberta, Canada, were selected for this study as model ecosystems for determining the effects of multiple climate change stressors on lake food webs. The main objective of this dissertation was to determine how warming and decreased precipitation due to climate change will affect the productivity and composition of fishless alpine lake food webs via changes in dissolved and particulate P-content and composition of both phytoplankton and zooplankton assemblages.

Dissolved P-content

Climate change may increase dissolved P-content (i.e., decrease C:P and N:P) in alpine lakes in several ways. Reduced runoff in spring and lower flushing rates of alpine lakes in summer may reduce dissolved C relative to dissolved P because terrestrial inputs are relatively C-rich (Kopacek et al. 2011). For example, the opposite has occurred (i.e., high DOC and low dissolved P) in alpine lakes in Alberta in years with high snowpack and increased flushing rates in summer (Parker et al. 2008). Evaporation driven by warming and reduced summer precipitation are expected to further decrease flushing rates in alpine lakes, possibly concentrating dissolved P (Garcia-Jurado et al. 2012). Indeed, climate effects on runoff and water flushing rates may be particularly important in alpine lakes where soil and vegetation cover of catchments is low and flushing rates are high (weeks to months; Parker et al. 2001). In shallow more productive lakes, warming and decreased lake depth can also increase P recycled from sediments (Søndergaard et al. 2001), so the same may occur in shallow alpine lakes to a limited extent. In addition, warming and increased uptake of dissolved inorganic N (DIN) by bacteria in soil and vegetation may decrease inputs of DIN into alpine lakes, thereby increasing P-availability via decreased dissolved N:P (Brooks and Williams 1999). This has been shown in mountain lake-chains in California where DIN was negatively correlated with decreased elevation and increased soil and vegetation cover, while there was no change in TDP among lakes (Sadro et al. 2012).

In contrast, lake surveys and sediment records indicate that increased soil and vegetation cover of previously barren alpine catchments may increase inputs of C- and N-rich terrestrial matter into alpine lakes (Bunting et al. 2010, Kopacek et al. 2011). Warming can increase mineralization of terrestrial organic mater and further increase inputs of dissolved organic C (DOC) into lakes (Keller et al. 2008). Thus, climate change may have complex effects on concentrations and stoichiometric composition of dissolved C, N, and P in alpine lakes via warming, precipitation, water flushing times, lake depth, and terrestrial runoff.

Particulate P-content

Changes in dissolved P-availability due to climate change may be reflected in the particulate pool via uptake of nutrients by phytoplankton. For example, enriched dissolved C:P *in vitro* has increased cellular C:P of phytoplankton (Urabe et al. 2003). Thus, increases in dissolved P concentrations may increase particulate P-content in lakes, whereas increased inputs of C-rich terrestrial matter into lakes may have the opposite effect (i.e., decrease P-content; van de Waal et al. 2010).

Climate change may also affect particulate stoichiometry by altering taxonomic composition of phytoplankton. Eutrophic lakes provide an extreme example of this: low dissolved N:P supply ratios select for N-fixing cyanophytes, which have high cellular C- and N-content, and thereby increase particulate C:P and N:P (Hendzel et al. 1994). Thus, climate warming could potentially increase particulate C:P and N:P in alpine lakes by selecting for C- and N-rich chlorophytes or cyanophytes that outcompete phytoflagellates and diatoms at higher temperatures and higher P-availability (Kilham 1986, Tilman et al. 1986).

Particulate stoichiometry can also be affected by climate impacts on consumer-driven nutrient recycling. For example, warming may select for fast-growing P-rich cladocerans (Allan 1976) that preferentially retain P in their biomass and excrete nutrients at high N:P, thereby depleting P-content of the particulate pool (MacKay and Elser 1998). In contrast, climate warming may select for less edible phytoplankton, which promote omnivorous N-rich copepods over cladocerans (Moss et al. 1991), and thereby increase P-recycling in the water column (Elser and Urabe 1999).

Climate effects on particulate stoichiometry may also change the food quality of particulate matter for grazers. Growth of P-limited cladocerans can be limited by low P-content of particulate matter (Sterner and Hessen 1994, Urabe et al. 1997). Consequently, decreased particulate P-content due to increased terrestrial inputs could potentially suppress P-limited cladocerans by reducing their particulate food quality relative to N-limited copepods (van de Waal et al. 2010). In contrast, climate-driven enrichment of particulate P-content could have a positive effect on cladocerans.

Overall, climate change may alter the stoichiometric composition of particulate matter in lakes through complex effects among nutrient supply ratios, composition of phytoplankton, and nutrient recycling by zooplankton. In turn, changes in particulate stoichiometry may affect the food quality of particulate matter for grazers. Alpine lakes are ideal model systems for disentangling potentially complex climate effects on particulate stoichiometry because these food webs are relatively simple with few species.

Phytoplankton

Even in the absence of increased P-availability, climate warming could favour several different phytoplankton groups. Moderate warming by several degrees above average summer water temperatures combined with decreased P concentrations has increased abundance of phytoflagellates in both alpine lakes (Parker et al. 2008) and boreal lakes (Findlay et al. 2001). As many phytoflagellates are facultative phagotrophs, they may be more competitive than autotrophs under warmer P-limited conditions because they can rely on particulate nutrient sources and grazing rates increase with warming (Rothhaupt 1996, Kamjunke et al. 2007). In contrast, certain diatom and chlorophyte taxa (*Synedra* sp. *Ankyra* sp., respectively) have become more dominant in alpine lakes in Colorado under warming and drought conditions where concentrations of N and P did not change (Flanagan et al. 2009).

Any increases in dissolved P could increase total phytoplankton abundance in alpine lakes in Alberta because these lakes are predominately P-limited (Murphy et al. 2010). In alpine lakes in Spain, warming combined with decreased lake depth has concentrated dissolved P and stimulated total phytoplankton abundance (Garcia-Jurado et al. 2012). In addition, increases in dissolved P relative to DOC may also stimulate autotrophic production relative to heterotrophic production, as the opposite has occurred with whole-lake additions of DOC in an oligotrophic boreal lake in Sweden (Blomqvist et al. 2001). However, more extreme warming (e.g., up to 20 ^{o}C in water temperature) combined with increased P-availability (i.e., N:P < 20 molar) is expected to promote chlorophytes and cyanophytes over diatoms and phytoflagellates (Kilham 1986, Tilman et al. 1986).

Zooplankton

The herbivorous cladoceran *Daphnia middendorffiana* and omnivorous calanoid copepod *Hesperodiaptomus arcticus* are large-bodied (3–4 mm length) taxa that are key zooplankton species in fishless alpine lakes in the Rocky Mountains (McNaught et al. 1999). Smaller taxa (1–2 mm length) such as the cladocerans *Daphnia pulex* and *Chydorus sphaericus*, the calanoid copepod *Diaptomus tyrellii*, and the cyclopoid copepod *Diacyclops bicuspidatus thomasi* also occur in alpine lakes in the absence of competition and predation by larger zooplankton taxa and fish (Parker et al. 2001, Schindler and Parker 2002). Climate impacts may alter the relative abundance of these zooplankton taxa in alpine lakes through direct effects of warming or indirect effects on the quality of their food.

Laboratory studies and long-term monitoring indicate that moderate warming (from 10 ^{o}C up to 17 ^{o}C *in vitro* and from 10 ^{o}C up to 14 ^{o}C *in situ*) may increase abundance of *D. middendorffiana* in alpine lakes in Alberta (Thompson et al. 2008, Fischer et al. 2011), whereas more severe warming (from 10 ^{o}C up to 20 ^{o}C *in vitro*) has suppressed both *D. middenorffiana* and *H. arcticus* (Holzapfel and Vinebrooke 2005). Popluation growth rates of cladocerans and rotifers may increase more than copepods with warming from 10 ^{o}C up to 20 ^{o}C because copepods have longer, more complicated life histories (Allan 1976). However, longer-term warming may select for smaller sized taxa of both cladocerans and copepods because smaller taxa have shorter generation times (Gillooly 2000, Daufresne et al. 2009). Loss of large-bodied alpine specialist zooplankton taxa can drastically decrease grazing pressure on phytoplankton and decrease water clarity of alpine lakes (Parker and Schindler 2006).

Zooplankton populations in cold, thermally stable alpine lakes are likely more sensitive to warming than populations in shallower or lower elevation montane lakes (i.e., below treeline) that may be warmer and more thermally variable. Indeed, *in vitro* warming from 10 ^{o}C up to 20 ^{o}C suppressed biomass of alpine lake populations dominated by *D. middendorffiana* and *H. arcticus*, but warming did not affect populations from montane lakes that were dominated by smaller-bodied taxa (Holzapfel and Vinebrooke 2005). Similarly, upper lethal temperatures were much lower for northern populations of intertidal copepods in the western USA compared to southern populations from warmer regions in Mexico even after 10 generations of selection in the laboratory (Kelly et al. 2012). Thus, relatively cold alpine lakes are an ideal location to test for the effects of climate change on zooplankton populations.

Main hypotheses

In this study, four main hypotheses were tested to assess the potential impacts of climate change on dissolved and particulate P-content and associated composition of phytoplankton and zooplankton assemblages in fishless alpine lake food webs in Alberta. These hypotheses were derived based on the research discussed above. The main hypotheses 1A–3A pertained to the effects of warmer and drier conditions and increased P-availability due to climate change. The first three main hypothesis had one or two alternative hypotheses. Alternate hypotheses 1B-3B pertained to increasing inputs of C-rich terrestrial nutrients having a negative effect on P-availability. Alternate hypothesis 1C involved the top-down effects of nutrient recycling by zooplankton on particulate P-content. Alternate hypothesis 3C was related to the direct effects of warming on selection of smaller zooplankton taxa. Hypothesis 4 compared effects of warming on alpine versus montane zooplankton populations.

1A. Warmer and drier conditions and decreased lake depth will increase dissolved and particulate P-content (i.e., decrease C:P and N:P) in alpine lakes by increasing P concentrations via decreased water flushing (Søndergaard et al. 2001, Garcia-Jurado et al. 2012) and increased N-uptake from catchments (Brooks and Williams 1999, Sadro et al. 2012).

> 1B. Increased C-rich terrestrial inputs via advanced ice-off and increased soil and vegetation cover of catchments will decrease P-content (van de Waal et al. 2010, Bunting et al. 2010, Kopacek et al. 2011).
>
> 1C. Climate effects on cladocerans and copepods will affect particulate P-content via zooplankton nutrient recycling; higher abundance of P-rich cladocerans will increase C:P via increased P-retention, whereas higher copepod abundance will decrease via increased P-recycling C:P (Elser and Urabe 1999).

2A. Warming and increased dissolved P-content will promote autotrophs over potentially mixotrophic phytoflagellates in alpine lakes (Kilham 1986, Tilman et al. 1986).

2B. Increased C-rich terrestrial inputs and decreased P-content will promote phytoflagellates over autotrophs (Rothhaupt 1996, Findlay et al. 2001, Parker et al. 2008).

3A. Warming and increased particulate P-content will promote faster growing, P-limited cladocerans over slower-growing, N-limited copepods in alpine lakes (Allan 1976, Sterner and Hessen 1994).

3B. Increased C-rich terrestrial inputs and decreased particulate P-content will promote copepods over cladocerans.

3C. Direct effects of warming will promote smaller-bodied cladoceran and copepod taxa over larger-bodied taxa (Gillooly and Dodson 2000, Daufresne et al. 2009).

4. Direct effects of warming on zooplankton will be greater on alpine populations compared to montane populations that are adapted to warmer temperatures at lower elevations (Holzapfel and Vinebrooke 2005, Kelly et al. 2012).

Study approach

Three separate studies were conducted to investigate the impacts of climate change on alpine lake food webs and test the main hypotheses in this dissertation. These included a spatial survey (Chapter 2) of 16 lakes sampled over three years, a temporal survey (Chapter 3) of one alpine lake sampled over 16 years, and a 30-d *in vitro* experiment (Chapter 4) involving plankton from two alpine and two montane lakes.

The spatial and temporal scales in the three studies resulted in a range of environmental and biological conditions that were representative of future climate change conditions that are expected to occur over years to multiple decades (Table 1-1). For example, the range in elevation among lakes resulted in a wide range in average summer water temperatures (5–17 ^{o}C) and dissolved nutrient concentrations (e.g., DOC = 0.4–5.0 mg·L-1; TDP = 1–6 μg·L-1; DIN:TDP = 3–101 molar). The spatial survey represented the widest range of environmental and biological conditions, followed by the temporal survey and experiment. Variation in conditions in the spatial survey generally bracketed the conditions in the temporal survey and experiment. Thus, conditions in the spatial survey appear to be a reasonable surrogate for long-term climate change, particularly in shallow alpine lakes where warming and changes in dissolved nutrients may be most intense.

Spatial survey

The spatial survey was used to infer the effects of several climate factors on alpine lake food webs. Data were collected from 16 naturally fishless lakes positioned along an elevation and climatic gradient in three national parks in Alberta. Lakes all contained *D. middendorffiana* and *H. articus* or *Hesperodiaptomus shoshone*. Structural equation modeling (SEM) was used to determine the direct and indirect effects of warming, decreased precipitation, increased soil area:lake area, and advanced ice-off on food webs of these alpine lakes.

Temporal survey

In the temporal survey, I analyzed data collected from an alpine lake in Banff National Park over 16 years (1991–2006). Similar to the spatial survey, SEM was used to determine the effects of long-term variation in temperature and precipitation on the composition of dissolved and particulate nutrients and the phytoplankton and zooplankton communities.

Experiment

In the experiment, plankton from two fishless alpine lakes and two montane lakes were incubated *in vitro* for 30 days at 10 °C or 17 °C and with ambient or + 80 % DOC that was concentrated from each lake using reverse osmosis. These lakes were also included in the spatial and temporal surveys. DOC additions simulated long-term increases in terrestrial inputs due to increased soil and vegetation cover. The factorial design of this experiment was used to test the interactive effects of warming and DOC additions on the composition of dissolved and particulate nutrients and the phytoplankton and zooplankton communities.

Additional thesis sections

In Chapter 5, conclusions from each of the three main chapters are compared and contrasted in the context of the four main hypotheses of the thesis. In Appendix 1, SEM is compared with stepwise regression in order to explain the basic concepts and calculations underlying SEM. Appendix 2 reports findings on pelagic interactions among chironomid larvae, the amphipod *Gammarus lacustris*, and trout in alpine lakes in Alberta (Weidman et al. 2011); this research was conducted during the course of the present study but falls outside the main objective of this thesis. Appendix 3 contains summary-abstracts for Chapters 2–4.

References

Allan, J. D. 1976. Life history patterns in zooplankton. American Naturalist 110:165–180.

Battarbee, R. W. 2009. Threatened and stressed mountain lakes of Europe: Assessment and progress. Aquatic Ecosystem Health & Management 12:118–128.

Blomqvist, P., M. Jansson, S. Drakare, A. K. Bergstrom, and L. Brydsten. 2001. Effects of additions of DOC on pelagic biota in a clearwater system: Results from a whole lake experiment in northern Sweden. Microbial Ecology 42:383–394.

Bradley, R. S., F. T. Keimig, and H. F. Diaz. 2004. Projected temperature changes along the American cordillera and the planned GCOS network. Geophysical Research Letters 31:1–4.

Brooks, P. D., and M. W. Williams. 1999. Snowpack controls on nitrogen cycling and export in seasonally snow-covered catchments. Hydrological Processes 13:2177–2190.

Bunting, L., P. R. Leavitt, R. P. Weidman, and R. D. Vinebrooke. 2010. Regulation of the nitrogen biogeochemistry of mountain lakes by subsidies of terrestrial dissolved organic matter and the implications for climate studies. Limnology and Oceanography 55:333–345.

Daufresne, M., K. Lengfellner, and U. Sommer. 2009. Global warming benefits the small in aquatic ecosystems. Proceedings of the National Academy of Sciences of the United States of America 106:12788–12793.

Elser, J. J., and J. Urabe. 1999. The stoichiometry of consumer-driven nutrient recycling: Theory, observations, and consequences. Ecology 80:735–751.

Findlay, D. L., S. E. M. Kasian, M. P. Stainton, K. G. Beaty, and M. Lyng. 2001. Climatic influences on algal populations of boreal forest lakes in the Experimental Lakes Area. Limnology and Oceanography 46:1784–1793.

Fischer, J. M., M. H. Olson, C. E. Williamson, J. C. Everhart, P. J. Hogan, J. A. Mack, K. C. Rose, J. E. Saros, J. R. Stone, and R. D. Vinebrooke. 2011. Implications of climate change for *Daphnia* in alpine lakes: predictions from long-term dynamics, spatial distribution, and a short-term experiment. Hydrobiologia 676:263–277.

Flanagan, C. M., D. M. McKnight, D. Liptzin, M. W. Williams, and M. P. Miller. 2009. Response of the phytoplankton community in an alpine lake to drought conditions:

Colorado Rocky Mountain front range, USA. Arctic, Antarctic, and Alpine Research 41:191–203.

Garcia-Jurado, F., I. de Vicente, A. Galotti, A. Reul, F. Jimenez-Gomez, and F. Guerrero. 2012. Effect of drought conditions on plankton community and on nutrient availability in an oligotrophic high mountain lake. Arctic, Antarctic, and Alpine Research 44:50–61.

Gillooly, J. F. 2000. Effect of body size and temperature on generation time in zooplankton. Journal of Plankton Research 22:241–251.

Gillooly, J. F., and S. I. Dodson. 2000. Latitudinal patterns in the size distribution and seasonal dynamics of new world, freshwater cladocerans. Limnology and Oceanography 45:22–30.

Hauer, F. R., J. S. Baron, D. H. Campbell, K. D. Fausch, S. W. Hostetler, G. H. Leavesley, P. R. Leavitt, D. M. McKnight, and J. A. Stanford. 1997. Assessment of climate change and freshwater ecosystems of the Rocky Mountains, USA and Canada. Hydrological Processes 11:903–924.

Hendzel, L. L., R. E. Hecky, and D. L. Findlay. 1994. Recent changes of N2-fixation in Lake-227 in response to reduction of the N/P loading ratio. Canadian Journal of Fisheries and Aquatic Sciences 51:2247–2253.

Holtgrieve, G. W., D. E. Schindler, W. O. Hobbs, P. R. Leavitt, E. J. Ward, L. Bunting, G. Chen, B. P. Finney, I. Gregory-Eaves, S. Holmgren, M. J. Lisac, P. J. Lisi, K. Nydick, L. A. Rogers, J. E. Saros, D. T. Selbie, M. D. Shapley, P. B. Walsh, and A. P. Wolfe. 2011. A coherent signature of anthropogenic nitrogen deposition to remote watersheds of the Northern Hemisphere. Science 334:1545–1548.

Holzapfel, A. M., and R. D. Vinebrooke. 2005. Environmental warming increases invasion potential of alpine lake communities by imported species. Global Change Biology 11:2009–2015.

IPCC. 2007. International Panel on Climate Change - Fourth assessment report - The physical science basis: Regional climate projections. World Meteorological Organization, United Nations.

Kamjunke, N., T. Henrichs, and U. Gaedke. 2007. Phosphorus gain by bacterivory promotes the mixotrophic flagellate *Dinobryon* spp. during re-oligotrophication. Journal of Plankton Research 29:39–46.

Keller, W., A. M. Paterson, K. M. Somers, P. J. Dillon, J. Heneberry, and A. Ford. 2008. Relationships between dissolved organic carbon concentrations, weather, and acidification in small Boreal Shield lakes. Canadian Journal of Fisheries and Aquatic Sciences 65:786–795.

Kelly, M. W., E. Sanford, and R. K. Grosberg. 2012. Limited potential for adaptation to climate change in a broadly distributed marine crustacean. Proceedings of the Royal Society B-Biological Sciences 279:349–356.

Kilham, S. S. 1986. Dynamics of Lake Michigan natural phytoplankton communities in continuous cultures along a Si-P loading gradient. Canadian Journal of Fisheries and Aquatic Sciences 43:351–360.

Kopacek, J., J. Hejzlar, J. Vrba, and E. Stuchlik. 2011. Phosphorus loading of mountain lakes: Terrestrial export and atmospheric deposition. Limnology and Oceanography 56:1343–1354.

MacKay, N. A., and J. J. Elser. 1998. Nutrient recycling by *Daphnia* reduces N-2 fixation by cyanobacteria. Limnology and Oceanography 43:347–354.

Magnuson, J. J., K. E. Webster, R. A. Assel, C. J. Bowser, P. J. Dillon, J. G. Eaton, H. E. Evans, E. J. Fee, R. I. Hall, L. R. Mortsch, D. W. Schindler, and F. H. Quinn. 1997. Potential effects of climate changes on aquatic systems: Laurentian Great Lakes and Precambrian Shield Region. Hydrological Processes 11:825–871.

McNaught, A. S., D. W. Schindler, B. R. Parker, A. J. Paul, R. S. Anderson, D. B. Donald, and M. Agbeti. 1999. Restoration of the food web of an alpine lake following fish stocking. Limnology and Oceanography 44:127–136.

Moss, B., J. Stansfield, and K. Irvine. 1991. Development of daphnid communities in diatom-dominated and cyanophyte-dominated lakes and their relevance to lake restoration by biomanipulation. Journal of Applied Ecology 28:586–602.

Murphy, C. A., P. L. Thompson, and R. D. Vinebrooke. 2010. Assessing the sensitivity of alpine lakes and ponds to nitrogen deposition in the Canadian Rocky Mountains. Hydrobiologia 648:83–90.

Parker, B. R., and D. W. Schindler. 2006. Cascading trophic interactions in an oligotrophic species-poor alpine lake. Ecosystems 9:157–166.

Parker, B. R., D. W. Schindler, D. B. Donald, and R. S. Anderson. 2001. The effects of stocking and removal of a nonnative salmonid on the plankton of an alpine lake. Ecosystems 4:334–345.

Parker, B. R., R. D. Vinebrooke, and D. W. Schindler. 2008. Recent climate extremes alter alpine lake ecosystems. Proceedings of the National Academy of Sciences of the United States of America 105:12927–12931.

Plummer, D. A., D. Caya, A. Frigon, H. Cote, M. Giguere, D. Paquin, S. Biner, R. Harvey, and R. de Elia. 2006. Climate and climate change over North America as simulated by the Canadian RCM. Journal of Climate 19:3112–3132.

Rothhaupt, K. O. 1996. Laboratory experiments with a mixotrophic chrysophyte and obligately phagotrophic and phototrophic competitors. Ecology 77:716–724.

Sadro, S., C. E. Nelson, and J. M. Melack. 2012. The influence of landscape position and catchment characteristics on aquatic biogeochemistry in high-elevation lake-chains. Ecosystems 15:363–386.

Schindler, D. W. 2000. Aquatic problems caused by human activities in Banff National Park, Alberta, Canada. Ambio 29:401–407.

Schindler, D. W. 2009. Lakes as sentinels and integrators for the effects of climate change on watersheds, airsheds, and landscapes. Limnology and Oceanography 54:2349–2358.

Schindler, D. W., and B. R. Parker. 2002. Biological pollutants: alien fishes in mountain lakes. Water, Air, and Soil Pollution 2:379–397.

Shepherd, A., K. M. Gill, and S. B. Rood. 2010. Climate change and future flows of Rocky Mountain rivers: converging forecasts from empirical trend projection and down-scaled global circulation modelling. Hydrological Processes 24:3864–3877.

Sterner, R. W., and D. O. Hessen. 1994. Algal nutrient limitation and the nutrition of aquatic herbivores. Annual Review of Ecology and Systematics 25:1–29.

Søndergaard, M., J. P. Jensen, and E. Jeppesen. 2001. Retention and internal loading of phosphorus in shallow, eutrophic lakes. The Scientific World 1:427–442.

Thompson, P. L., M. C. St-Jacques, and R. D. Vinebrooke. 2008. Impacts of climate warming and nitrogen deposition on alpine plankton in lake and pond habitats: an *in vitro* experiment. Arctic, Antarctic, and Alpine Research 40:192–198.

Tilman, D., R. Kiesling, R. Sterner, S. S. Kilham, and F. A. Johnson. 1986. Green, bluegreen and diatom algae: Taxonomic differences in competitive ability for phosphorus, silicon and nitrogen. Archiv Fur Hydrobiologie 106:473–485.

Urabe, J., J. Clasen, and R. W. Sterner. 1997. Phosphorus limitation of *Daphnia* growth: Is it real? Limnology and Oceanography 42:1436–1443.

Urabe, J., J. Togari, and J. J. Elser. 2003. Stoichiometric impacts of increased carbon dioxide on a planktonic herbivore. Global Change Biology 9:818–825.

van de Waal, D. B., A. M. Verschoor, J. M. H. Verspagen, E. van Donk, and J. Huisman. 2010. Climate-driven changes in the ecological stoichiometry of aquatic ecosystems. Frontiers in Ecology and the Environment 8:145–152.

Weidman, R. P., D. W. Schindler, and R. D. Vinebrooke. 2011. Pelagic food web interactions among benthic invertebrates and trout in mountain lakes. Freshwater Biology 56:1081–1094.

Williamson, C. E., J. E. Saros, and D. W. Schindler. 2009. Climate change - Sentinels of change. Science 323:887–888.

Table 1-1. Time scale, spatial scale, and range in environmental and biological conditions among the three main studies. Representative time-scales for future climate change are approximate.

Variable	Units	Chapter 2 - Spatial survey	Chapter 3 - Temporal survey	Chapter 4 - Experiment
Sampling time-scale		monthly x 3 years	monthly x 16 years	1 month
Sampling spatial scale		16 lakes, 1016 m range in elevation	1 lake (long-term monitoring)	4 lakes, 677 m range in elevation
Representative time-scale for future climate change	y	decades – century	years – decades	months – years
Air temperature (cumulative degree days > 0 ^{o}C)	$^{o}C \cdot y^{-1}$	613 – 2341	780 – 1158	780 – 1114
Water temperature	^{o}C	5 – 17	3 – 14	10 or 17
DOC	$mg \cdot L^{-1}$	0.4 – 5.0	0.1 – 2.0	1.3 – 2.9
TDP	$\mu g \cdot L^{-1}$	1 – 6	1 – 6	1 – 8
DIN	$\mu g \cdot L^{-1}$	7 – 128	17 – 145	10 – 280
DOC:TDP	molar	785 – 2225	368 – 819	900 – 2500
DIN:TDP	molar	3 – 101	22 – 300	4 – 400
PC	$\mu g \cdot L^{-1}$	119 – 926	86 – 452	125 – 800
PN	$\mu g \cdot L^{-1}$	10 – 92	8 – 49	1 – 80
PP	$\mu g \cdot L^{-1}$	0.3 – 9.0	0.2 – 7.0	1.5 – 9.0
PC:PP	molar	117 – 410	116 – 1620	180 – 700
PN:PP	molar	10 – 36	4 – 141	15 – 40
chlorophyll *a*	$\mu g \cdot L^{-1}$	< 0.01 – 1.2	< 0.01 – 1.5	0.01 – 8.0
Non-flagellates	$\mu g \cdot L^{-1}$	< 0.01 – 1.2	< 0.01 – 1.2	0.02 – 1.0
Flagellates	$\mu g \cdot L^{-1}$	< 0.01 – 0.8	< 0.01 – 2.8	0.01 – 0.7
Non-flag:Flag	mass	< 0.01 – 1.5	< 0.01 – 0.4	1.4 – 2.0
Daphnia	$\mu g \cdot L^{-1}$	< 0.01 – 53.0	< 0.01 – 14.7	3 – 125
Hesperodiaptomus	$\mu g \cdot L^{-1}$	< 0.01 – 17.0	1.8 – 29.0	8 – 60
Cladoceran:Copepod	mass	< 0.01 – 20.0	< 0.01 – 0.5	0.4 – 2.1

CHAPTER 2: SPATIAL SURVEY – POTENTIAL CLIMATIC IMPACTS ON STOICHIOMETRY, PHYTOPLANKTON, AND ZOOPLANKTON IN ALPINE LAKES[1]

Introduction

Future global warming is predicted to be most pronounced at high elevations and high-latitudes in the northern hemisphere (Bradley et al. 2004; [IPCC] 2007). Higher temperatures and lower precipitation are expected to reduce the snowpack, advance ice-free periods (Magnuson et al. 2000, Morris et al. 2005), and amplify variation in chemistry and productivity of lakes, especially those in alpine environments (Hauer et al. 1997, Battarbee 2009). Thus, the goal of this study was to determine how co-occurring climatic variables affect the concentrations and composition of dissolved and particulate nutrients and plankton communities in naturally fishless alpine lakes.

Climate influences the physical and chemical characteristics of cold and dilute lakes via changes to terrestrial inputs and internal nutrient cycling. Drier summer conditions have resulted in certain alpine lakes becoming clearer and warmer (Parker et al. 2008). Decreased lake depth and slower water flushing rates due to warmer and drier conditions may concentrate dissolved P in oligotrophic lakes (Schindler et al. 1978, Garcia-Jurado et al. 2012). Lake surveys and sediment records also show increased soil and vegetation cover of alpine catchments will increase inputs of C-rich dissolved organic matter into alpine lakes over longer time periods (Bunting et al. 2010, Kopacek et al. 2011). Collectively, these climate effects may alter the stoichiometry of alpine lakes via bottom-up effects on dissolved nutrient supply (Vähätalo et al. 2003, van de Waal et al. 2010), and top-down effects on consumer-driven nutrient recycling by P-rich cladocerans (e.g., 14 N:P molar ratio of body tissue) and N-rich copepods (e.g., 48 N:P; Elser and Urabe 1999).

In turn, physical and chemical effects of climate can also affect the composition of phytoplankton and zooplankton communities of cold-water lakes. Moderate warming on the order of 1–4 ^{o}C above average ice-free season temperatures has increased biomass

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[1] This chapter is in preparation for Global Change Biology

of phytoflagellates in alpine lakes (in warm summers) and in experimental alpine ponds where average water temperatures are around 10–15 °C (Strecker et al. 2004, Parker et al. 2008). *In vitro* experiments show more extreme warming up to an average ice-free season temperature of 20 °C combined with greater P-availability (i.e., molar N:P < 20) increase biomass of chlorophytes and cyanophytes relative to diatoms and phytoflagellates in warmer lakes (Kilham 1986; Tilman et al. 1986). Further, warming up to 13 °C in alpine lakes and up to 17 °C *in vitro* have increased abundances of the cladoceran *Daphnia middendorffiana* and suppressed abundances of the calanoid copepod *Hesperodiaptomus arcticus* (Thompson et al. 2008, Fischer et al. 2011), which are key zooplankton taxa in many fishless alpine lakes in western North America. However, more extreme summer warming events, raising temperatures up to 20 °C, may suppress both of these alpine specialists (Holzapfel and Vinebrooke 2005) and select for smaller-bodied cladoceran and copepod species (Gillooly and Dodson 2000, Daufresne et al. 2009).

In this study, I inferred the direct and interactive effects of multiple climatic factors on alpine lake food webs using structural equation modeling (SEM) of data collected from 16 fishless lakes positioned along a thermal gradient in Alberta, Canada. My main hypothesis was that the cumulative effects of warming (i.e., increased air and water temperatures), advanced ice-off, decreased precipitation, decreased lake depth, and increased soil cover alter the nutritional status (i.e. stoichiometry) and taxonomic composition of phytoplankton and zooplankton communities in oligotrophic alpine lakes. In particular, I expected that warming combined with drought-induced increased dissolved P levels (e.g., Garcia-Jurado et al. 2012) would result in greater abundance of chlorophytes and cyanophytes relative to diatoms and phytoflagellates (Tilman et al. 1986; Magnuson et al. 1997). I also anticipated warming to directly suppress large-bodied alpine zooplankton (e.g., Holzapfel and Vinebrooke 2005) and promote smaller bodied taxa (e.g., Gillooly and Dodson 2000, Daufresne et al. 2009). Further, increased particulate P-content was expected to benefit P-limited cladocerans, whereas increased terrestrial inputs (i.e., decreased particulate P-content) were expected to promote N-limited copepods relative to cladcoerans (Sterner and Hessen 1994).

Methods

Study lakes

I selected 16 lakes from a database of 379 mountain lakes located in Jasper, Banff, and Waterton Lakes National Parks, Alberta (Anderson 1968, 1970, 1971, 1972, 1974, Donald et al. 2001). Lakes were chosen to be fishless, containing *Hesperodiaptomus* (*H. arcticus* or *H. shoshone*) and *Daphnia* (*D. middendorffiana* or *D. pulex*), which are often abundant alpine zooplankton taxa in the absence of fish (McNaught et al. 1999). I also selected lakes to span a wide range of elevations (1310−2350 m above sea level "asl"), which resulted in a broad range in cumulative degree days above 0 ^{o}C (613−2314 ^{o}C·y^{-1}), ice-off dates (13-April to 30-June), mean lake temperatures during the ice-free season (5.4−16.7 ^{o}C), total annual precipitation (354−899 mm), and water transparency (attenuation coefficient $K_d = 0.17−1.40$ m^{-1}). Lakes and catchments ranged by 1−2 orders of magnitude in lake area (0.4–28.4 ha), catchment area (24–1050 ha), maximum depth (1−29 m), and catchment area covered by soil relative to lake area (i.e., "soil area:lake area"; 0−250). Soil area to lake area ratio was calculated because the amount of terrestrial inputs into mountain lakes is influenced by soil coverage of catchments, as well as catchment area to lake area ratio (Kopacek et al. 2011). Catchment area, soil and vegetation cover, and lake area were calculated from satellite photos. Further description of the study lakes and catchments is given in Appendix 2-1. Average conditions for each lake including climate, dissolved and particulate nutrients, phytoplankton, and zooplankton are summarized in Table 2-1 and Appendices 2-2 to 2-9.

Climate and lake temperature and light data

Meteorological data for the study period were obtained from Environment Canada weather stations in Jasper National Park (52.93 N, 118.03 W; 1020 m asl), Banff National Park (51.43 N, 116.22 W; 1524 m asl), and Waterton Lakes National Park (49.13 N, 114.03 W; 1524 m asl). Weather stations were within 8–39 km of the study lakes in each park, but at much lower elevations. Air temperatures were corrected for elevation using an atmospheric lapse rate of -0.65 ^{o}C per 100 m increase in elevation (Huang et al. 2008).

Cumulative degree days above freezing were calculated for air temperature at each lake for each year. I determined if total daily precipitation fell as snow or rain based on whether lapse-corrected average daily temperature was above or below 0 ^{o}C at each lake. I then calculated annual snow and rain for each lake, although only total precipitation was used in our analyses.

Lake temperatures were measured for the upper 5 m of the water column of each lake (or less for shallow lakes) using data loggers deployed at 1-m intervals. Ice-off and ice-on dates were determined using data from water temperature loggers at the lake surface. Attenuation of photosynthetically active radiation ($Kd \cdot m^{-1}$) was measured at 1-m intervals on each sampling occasion.

Sample collection and analysis

The study lakes were sampled from 2005-2007, and additional long-term monitoring data (1991-2006) were used for Pipit, Snowflake, and Bighorn lakes. All lakes were sampled 5 times per year on average (3 to 17 times per year) for a total of 421 lake-days. Structural equation modeling (SEM) was conducted on the calculated average variables for each lake (described below).

Sampling was conducted by boat at the deepest point of each lake. Surface water was collected and filtered in the field through glass fiber filters (0.45 μm pore-size Whatman glass fiber filters GF/F) for analysis of particulate nutrients and phytoplankton pigments. Filters and unfiltered and filtered water were kept in coolers with ice during transport to the laboratory.

Dissolved and particulate nutrients

Dissolved and particulate nutrients were analyzed at the University of Alberta Biogeochemistry Laboratory (Parker and Schindler 2006). Filtered lake water was analyzed for dissolved organic C (DOC), dissolved inorganic N (DIN; i.e., nitrate + nitrite + ammonium), soluble reactive silica (SRSi), and total dissolved P (TDP). Unfiltered lake water was analyzed for total P (TP). Particulate P (PP) was calculated by subtracting TDP from TP. Particulate C (PC) and N (PN) were analyzed from a single filter. Molar ratios were calculated for DOC:TDP, DIN:TDP, SRSi:TDP, PC:PP, PN:PP, and PC:PN.

Phytoplankton pigments

Chlorophylls and carotenoids concentrated on glass-fiber filters were analyzed using reverse-phase high-pressure liquid chromatography (HPLC; Vinebrooke et al. 2002). Pigment data were then analyzed with CHEMTAX software to estimate biomass of the major phytoplankton classes (version 1.95, Wright, S.W. and Mackey, M.D., Hobart, Tasmania, Australia; Mackey et al. 1996). Biomass data were pooled into three main groups of phytoplankton: chlorophytes + cyanophytes, diatoms, and phytoflagellates (including chrysophytes, cryptophytes, and dinoflagellates). Additional details about HPLC and CHEMTAX methods are given in Appendix 2-10.

Zooplankton

Zooplankton were collected using 30-cm diameter Wisconsin-style plankton nets having 64-μm pore-sized mesh netting. Nets were towed vertically through the water column five times from 1 m above the lake bottom. Zooplankton were concentrated and preserved in 70% ethanol. Adult cladocerans, copepods, and rotifers were identified to species at 100−400 times magnification with an inverted microscope (Ward and Whipple 1966; Pennak 1978). All zooplankton and eggs were enumerated at 25−50 times magnification with a dissecting microscope. Zooplankton biomass in dry weight was calculated by applying standard length-weight regressions for each taxon (McCauley 1984). Cyclopoid copepods only appeared in a few of the survey lakes (Appendix 2-8), so climate effects on their abundance could not be tested. Similarly, individual rotifer species only occrued in some of the survey lakes (Appendix 2-8), so their biomass was pooled.

Structural equation modeling (SEM)

SEM is a multivariate statistical method used to test for direct and indirect effects within a network of related variables. SEM is a direct extension of the multiple regression approach. In fact, SEM can be described as “multiple, multiple regression” because it involves solving two or more multiple regression equations simultaneously, where there is one equation for each response variable in the model (Grace and Bollen 2005). One of the main differences between SEM and multiple regression is that SEM allows for the testing of effects among endogenous response variables, in addition to testing the effects of exogenous explanatory variables on response variables. Consequently, SEM allows for the calculation of direct, indirect, and total effects on response variables. Furthermore, SEM allows the user to diagrammatically represent the underlying series of inter-related multiple regression equations. SEM is explained in detail by Grace (2006) and more briefly by Grace and Bollen (2005) (see Appendix 1).

SEM was used to calculate the direct, indirect, and total effects of each climate variable on key response variables in our study lakes. Two food web models were constructed that tested for the effects of climate variables on 1) nutrient concentrations, phytoplankton biomass, and zooplankton biomass; and 2) nutrient stoichiometry and relative composition of phytoplankton and zooplankton taxa.

Response variables for the food web models were chosen from a series of multivariate stepwise regression models, which tested only the direct effects of climate variables on a larger set of response variables (methodology explained in Appendix 2-10; direct effects models shown in Appendix 2-11). Moreover, the stepwise regression models were used to validate the more complicated SE food web models (compared in Appendix 2-12).

Model building procedure

SEM was used in exploratory mode rather than to test specific model structures (Arhonditsis et al. 2006). Each structural equation (SE) model was built using an iterative model selection procedure using the software package AMOS (version 18, SPSS Statistics, Chicago, IL, USA). This modeling procedure used a Bayesian information criterion (BIC) index to select the most parsimonious combination of significant partial regression coefficients (referred to as “paths” or “path coefficients”; described below) that best explained the covariance among variables in each model. Model selection was conducted on all theoretically possible combinations of paths among variables. Furthermore, some of the study lakes are relatively well studied (e.g., McNaught et al. 1999; Wilhelm and Schindler 1999; Schindler and Parker 2002; Strecker et al. 2004; Holzapfel and Vinebrooke 2005; Parker et al. 2008), so each step in our model selection procedure was informed by *a priori* knowledge of the food web structure.

I used a relatively simple form of SEM with only observed variables and not theoretically constructed latent variables. However, I did use coding variables to control whether each model tested for an increase or decrease in each climate variable (Grace 2006). In particular, precipitation, lake depth, and ice-off date are expected to decrease with climate change, so these variables were negatively coded. Cumulative degree days, and soil area:lake area are expected to increase with climate change and were consequently positively coded. Average summer surface water temperatures were closely related to cumulative degree days for air temperature (water temperature ^{o}C = 0.006 x degree days ^{o}C + 3.88, $R^2 = 0.75$), so only degree days were used in our analysis. It is also worth noting that alternate climate change scenarios (e.g., warm and wet versus warm and dry conditions) could be re-calculated by reversing the coding variables of precipitation variables, although this was outside the scope of the present study.

The model building approach was analogous to conducting multivariate stepwise regression analysis in a sequential manner between each trophic level of the food web (e.g., Wells et al. 2008). Each food web model was constructed in six steps using the model selection procedure. Firstly, I tested for significant non-causal (i.e., unexplained) correlations among the four main climate variables, which were the same for both food web models (and the stepwise regression models). Secondly, the effects of climate variables were tested on ice-off date and water transparency (i.e., lake physical variables). Soil area:lake area was not expected to affect ice-off, although its effect on transparency was tested. Thirdly, I tested the effects of climate variables and ice-off on dissolved nutrients (DOC and TDP or DOC:TDP and DIN:TDP). Transparency was not expected to directly affect dissolved nutrients, but the effect of DOC was tested on transparency. Fourthly, I also tested for the effects of climate and lake physical variables and dissolved nutrients on particulate nutrients (PC or PN:PP) and phytoplankton (chl *a* or percent chlorophytes + cyanophytes). The reciprocal effects of PC and phytoplankton chl *a* on transparency were also tested in this step. I also tested the effects of climate and lake physical variables, particulate nutrients, and phytoplankton on zooplankton (*Daphnia* and *Diaptomus* or the cladoceran:copepod ratio). Dissolved nutrients were not expected to directly affect zooplankton. Finally, the top-down effects of zooplankton on particulate nutrients and phytoplankton (i.e., via grazing) and dissolved nutrients (i.e., via excretion) were examined. I summarized the standardized partial regression coefficients from both food web models (and stepwise regression models) in a way that was similar to comparing effect sizes in a meta-analysis.

Treatment of data

Lake-day data (i.e., samples collected on individual days) were screened for outliers that were greater or less than 3 SD from the mean for each lake. I then calculated 16 lake-means (i.e., averages based on lake-day data) for each measured variable for each lake from the 421 lake-day dataset. Lake-means were ln+1 transformed to improve homogeneity of variance and normality of all variables. Linearity of relationships between variables in each model was assessed using bivariate scatter plots. Then, a covariance matrix was constructed with all independent and dependent variables (Appendix 2-13), which was analyzed using the AMOS software package.

Path coefficients

There are two path types in our SE models, non-causal and causal paths, which are represented by several different types of path coefficients (Grace and Bollen 2005, Grace 2006). Non-causal paths are shown in the SE model diagrams as double-headed, curved arrows that describe correlation between two variables where causality is unexplained. Non-causal path coefficients “Pr” are in the form of Pearson’s *r* correlation coefficients.

Causal paths are shown in the SE diagrams as single-headed, straight arrows where causality is inferred to flow from the causal variable to the response variable. Standardized causal path coefficients “Pz” are in the form of partial regression coefficients where the underlying data are standardized as z-scores. Units of Pz indicate change in SD’s of the response variable Y per 1 SD increase of the independent variable X. Thus, standardized path coefficients allowed us to compare the magnitude of climatic effects across response variables that were measured on different scales, assuming each ln+1 transformed variable is normally distributed. For comparison, non-standardized causal path coefficients “Pc” with non-standardized units are also reported in Appendices 2-14 to 2-17.

Indirect effects “Iz” were calculated as the product of all path coefficients (Pz) along a compound path (Grace and Bollen 2005). Indirect effects coefficients (Iz) only appear occasionally in the text of the results section, but can be derived from the food web models. Total standardized effects "Tz" were calculated by summing the direct effects (Pz) and indirect effects (Iz) of each climate variable on each response variable. Cumulative standardized effects "Cz" were calculated by summing the total effects (Tz) of the four main climate variables on each response variable. The units of Cz indicate change in SD's of the response variable Y per 1 SD increase in all independent climate variables. Units of Pz, Iz, Tz, and Cz are only reported in the results section as "SD" for sake of brevity. Non-standardized units for all response variables appear in Table 2-1.

Model and path statistics

Maximum likelihood chi-square tests (X^2) are used in SEM to evaluate how well each model explains the observed covariance among variables. Unlike standard hypothesis testing, the predicted model is accepted and judged to adequately explain the observed covariance when X^2 has a P-value > 0.05 (Grace 2006). Each SE model was judged to adequately predict the observed covariance data when the P-value of the X^2 statistic is > 0.05. Both food web models were within the range of acceptable model fit (nutrient and plankton concentrations model: $X^2 = 53.1$, $P = 0.162$; nutrient and plankton composition model: $X^2 = 31.8$, $P = 0.623$; Appendices 2-14 and 2-15). All stepwise regression models were accepted with stronger model fit statistics ($X^2 = 7.2–16.2$, $P = 0.228–0.969$; Appendices 2-16 and 2-17).

Within each SE model, each path coefficient Pz is also tested with an individual t-test to determine if the coefficient is significantly different from zero at the $P < 0.05$ level of significance. Furthermore, I manually calculated the P-values for all Cz coefficients based on individual t-tests for Pz coefficients; the absolute value of Cz that corresponded to $P < 0.05$ was Cz ≥ 0.37 SD. Incidentally, some response variables had two or more significant but opposing effects (Pz) that resulted in a cumulative effect that was not significant (i.e., absolute value Cz < 0.37 SD). All cumulative effects (Cz) and individual effects (Pz) in the results section are significant ($P < 0.05$) unless otherwise noted. All other model statistics (X^2 and P-values) and path coefficients statistics (P-values and corresponding bootstrap values) are reported in Appendices 2-14 to 2-17.

Sample size considerations

Statistical power of the maximum likelihood X^2 statistic increases asymptotically with sample size (Grace 2006). Thus, SEM is generally thought to require large datasets, with roughly 5−10 samples per response variable. Other researchers argue that considerations of statistical power, sample size, and the ability to generalize findings from sample to population, are similar for SEM and other multivariate statistics such as multiple analysis of covariance (Arhonditsis et al. 2006). My comparison of stepwise regression models and food web models indicated that my small sample size (n = 16 lake means) did not appear to introduce unexpected effects in the SE models (Appendix 2-12). For example, there was a strong linear correlation in Cz between the stepwise regression and food web models ($Cz_{\text{food web model}} = 0.98 \times (Cz_{\text{stepwise model}}) + 0.09$, $R^2 = 0.88$, $P < 0.001$; data from Appendix 2-12). In addition, power to detect statistically significant effects in our dataset appeared to be relatively high because there were 1–2 orders of magnitude in variation in most response variables (Appendices 2-3 to 2-9).

I also used a bootstrapping procedure in AMOS to estimate the sensitivity of the regression coefficients to sample size in each model based on a randomly re-sampled dataset of n = 1000. Among all stepwise regression and food web models, Pz only varied by 1.7% ± 2.0 SD from the original model results (Appendices 2-14 to 2-17). Thus, bootstrapping results suggested that regression coefficients were not strongly affected by outliers in the dataset.

Results

Lake physical variables and dissolved nutrients

Warmer air temperatures were primarily associated with changes in ice-off, water transparency, dissolved nutrient concentrations, and stoichiometry (Figures 2-1 to 2-3). Higher temperatures together with the secondary influences of decreased precipitation and decreased lake depth were coupled with advanced ice-off (Cz = 1.56 SD), which in turn decreased water transparency (Cz = 1.44 SD) and increased concentrations of DOC (Cz = 1.15 SD). Further, warming and decreased lake depth were directly associated with increased concentrations of TDP (Cz = 0.95 SD). Stepwise regression models revealed warming was also significantly correlated with decreased concentrations of DIN (Cz = -0.67 SD; Appendix 2-11).

Dissolved nutrient stoichiometry results paralleled the model results with dissolved nutrient concentrations (Figures 2-1 and 2-3). Warming was mainly associated with a net increase in DOC:TDP (Cz = 0.78 SD) via advanced ice-off. Warming was also significantly correlated with decreased DIN:TDP (Cz = 0.82 SD).

Particulate nutrients

Decreased lake depth and increased soil area:lake area were the main variables that explained a net increase in concentrations of PC (Cz = 0.95 SD; Figure 2-1 and 2-2). Decreased lake depth was indirectly associated with an increase in PC due to advanced ice-off and higher TDP levels (Iz = 0.24 SD and 0.29 SD, respectively), whereas increased soil area:lake area was directly related with increased PC (Pz = 0.37 SD). On the other hand, warming and decreased precipitation were both negatively linked with concentrations of PC (Pz = -0.85 and -0.45 SD, respectively).

Warming was directly correlated with reduced PC:PP (Pz = -1.38 SD) and indirectly correlated via a reduction in the cladoceran:copepod ratio (Iz = -1.00 SD; Figure 2-1 and 2-3). This reduction in the relative abundance of cladcoerans was subsequently associated with a net decrease in PC:PP (Cz = -0.48 SD), although increased soil area:lake area partially increased PC:PP (Pz = 1.38 SD).

Phytoplankton

There was no net change in total phytoplankton chl *a* (Cz = 0.22 SD; Figure 2-1 and 2-2). Warming and decreased lake depth were indirectly linked with increased chl *a* via increased TDP (Iz = 0.37 and 0.29 SD, respectively), but this was offset by a direct negative association between chl *a* and decreased precipitation (Pz = -0.44 SD).

Warming alone was indirectly coupled with increased percent abundance of chlorophytes + cyanophytes (Cz = 0.58 SD) via decreased DIN:TDP (Iz = 0.58 SD; Figure 2-1 and 2-3). Also, warming was significantly correlated with decreased percent composition of phytoflagellates (Cz = -0.69 SD; Appendix 2-11). Decreased precipitation was related with reduced biomass of diatoms (Pz = -0.46 SD), although percent composition of this group of phytoplankton remained relatively low in abundance among lakes (around 11 %; Appendix 2-5).

Zooplankton

Similar to the phytoplankton results, there was no net change in total zooplankton biomass, but climate variables were associated with a change in zooplankton taxonomic composition (Figure 2-1 and 2-2). Warming and decreased lake depth were indirectly linked with increased biomass of *Daphnia* and *Diaptomus* via increased concentrations of TDP and phytoplankton chl *a*. However, increases in these taxa were indirectly offset by reduced phytoplankton chl *a* via decreased precipitation (multiple indirect paths in Figure 2-2). Warming was also coupled with a direct increase in *Diaptomus*, so there was a net increase in *Diaptomus* relative to *Daphnia* (Cz = 0.56 and 0.16 SD, respectively). Warming alone was significantly correlated with increased biomass of *Chydorus* + *Bosmina* (Cz = 0.74 SD; Appendix 2-11), but this did not increase total zooplankton biomass because these taxa comprised < 5 % of total biomass in most lakes (Appendix 2-6). There was no net change in total zooplankton biomass (Cz = 0.09 SD; Appendix 2-11) because a positive correlation with warming was offset by decreased precipitation.

Climatic effects were associated with in a net decline in the cladoceran:copepod ratio (Cz = -0.94 SD; Figures 2-1 and 2-3). Increased soil area:lake area was indirectly linked with reduced of abundance of cladocerans relative to copepods via increased PC:PP (Iz = -0.72 SD, Figure 2-3), even though there was a net decrease in PC:PP.

Although there was a large negative association with warming and the cladoceran:copepod ratio (Pz = -1.38 SD, Figure 2-3), this was largely offset by a positive, indirect link with warming via advanced ice-off (Iz = 0.88 SD). Decreased precipitation was negatively associated with phytoplankton chl *a*, which in turn reduced cladocerans relative to copepods (Figure 2-2).

The strongest potential climatic effect on *Hesperodiaptomus* was a weak positive linkage with decreased precipitation, but this path was not significant (Pz = 0.33 SD, P = 0.134; Figure 2-1). When examining the raw data, *Hesperodiaptomus* comprised the largest proportion of total zooplankton biomass in three of the five coldest lakes (Appendix 2-6). *Hesperodiaptomus* also comprised more than 40% of copepod biomass in eight of the nine coldest lakes (5.4–8.9 ^{o}C mean ice-free temperature) but comprised < 20% of copepod biomass in the seven warmest lakes (11.5–16.7 ^{o}C mean ice-free temperature; Appendix 2-8). *Daphnia pulex* were also more common in the three warmest lakes (16.0–16.7 ^{o}C mean ice-free temperature) compared to their larger congener *D. middendorffiana* (Appendix 2-8). In contrast, *D. middendorffiana* comprised more than 80% of daphnid biomass in 11 of the 13 coldest lakes in our study (5.4–14.8 ^{o}C mean ice-free temperature; Appendix 2-8). Small cladocerans *Chydorus* and *Bosmina* were most abundant in the warmest lakes (Appendix 2-6).

Discussion

My findings demonstrate that warming together with decreased water depth due to climate change can alter the stoichiometry and taxonomic composition of phytoplankton and zooplankton communities in alpine lakes. Warming was mainly associated with decreased water transparency via advanced ice-off. Warming was also linked with increased concentrations of TDP, decreased concentrations of DIN, and thus decreases in the DIN:TDP ratio. Warming and decreased lake depth appeared to stimulate phytoplankton via increased TDP, however decreased precipitation offset the increases in phytoplankton. Subsequently, decreased precipitation also inferred to offset increases in zooplankton biomass that had occurred due to warming and decreased depth. The abundance of herbivorous cladocerans declined relative to omnivorous copepods, mainly due to an increase in small calanoid copepod taxa and decreases in food quantity and quality. Below, I discuss the mechanisms behind these findings and consider the implications of continued climate change for alpine lake food webs.

Warmer and drier conditions increase dissolved P and decreased dissolved N

Climatic effects on dissolved P played a central role in determining the abundance of phytoplankton and zooplankton in the study lakes. Increased concentrations of TDP led to higher concentrations of phytoplankton, which positively affected herbivorous *Daphnia* and to a lesser extent omnivorous *Diaptomus*, which may rely on smaller zooplankton prey. These effects were moderated by decreased precipitation, which likely reduced external inputs of dissolved P, leading to a decrease in abundance of phytoplankton and zooplankton (total zooplankton, *Daphnia* and *Diaptomus*). Thus, my findings indicate that total phytoplankton abundance was strongly affected by the indirect effects of climate variables acting on dissolved P concentrations, rather than by direct effects of warming (*sensu* Magnuson et al. 1997).

Warming and decreased lake depth were associated with increased concentrations of TDP, although absolute P concentrations remain low (0.04–0.30 μmol TDP$\cdot$L^{-1}; Appendix 3). In alpine lakes in Spain, concentrations of dissolved P also increased during drought conditions with large reductions in lake depth (14 m to 4 m maximum depth) and increased sediment resuspension (Garcia-Jurado et al. 2012). Similarly, alpine ponds in our study area also have relatively higher concentrations of P and lower DIN:TDP ratios compared to deeper lakes (Murphy et al. 2010). Warmer drier summers with greater evaporation and reduced lake levels may thus concentrate dissolved P by increasing internal loading particularly in shallow lakes where there is greater diffusion and resuspension of P from sediments, which occurs in warmer more productive lakes (Søndergaard et al. 2001). In deep lakes, however, warming and increased thermal stratification may have the opposite effect by reducing P upwelling from the hypolimnion during the growing season (Posch et al. 2012).

Warming was most strongly associated with decreased concentrations of DIN and the decline in DIN:TDP in our survey lakes. Increased denitrification due to the direct effects of warming might have explained the loss of DIN in warmer lakes, but denitrification was most likely limited by low nitrate concentrations in our study lakes (i.e., $< 1–10$ μmol $DIN \cdot L^{-1}$; Veraart et al. 2011). Instead, lower concentrations of DIN in warmer lakes was likely due to catchments having lower snow cover, higher soil microbial activity, and consequently, lower nitrate exports (Brooks and Williams 1999). For example, in mountain lakes in California concentrations of DIN decreased from 10 to 0.1 μmol $NO_3 \cdot L^{-1}$ with decreasing elevation and increasing vegetation cover of catchments (Sadro et al. 2012). Thus, my findings suggest that warming will increase uptake of DIN in alpine catchments over several decades and reduce inorganic N inputs into alpine lakes. However, atmospheric deposition of N from anthropogenic sources (Holtgrieve et al. 2011) could offset increases in terrestrial N-uptake.

Bottom-up and top-down effects on particulate stoichiometry

Many ecological stoichiometry studies emphasize either the bottom-up effects of particulate food quality on consumers or top-down effects of consumer-driven nutrient recycling. The findings illustrate that particulate stoichiometry is affected by a complicated combination of climate influences via bottom-up effects on the dissolved and particulate nutrient supply and top-down effects via zooplankton nutrient recycling.

Bottom-up effects included increased supply of dissolved P relative to C that was associated with warming and decreased lake depth, which resulted in a net decrease in particulate C:P. Thus, climate effects on dissolved nutrient supply ratios were reflected in the particulate nutrient pool (van de Waal et al. 2010). This has also been shown *in vitro* where PC:PP increased when phytoplankton were supplied with higher levels of dissolved inorganic C relative to dissolved P (Urabe et al. 2003). In addition, increased soil cover among our study lakes partially increased PC:PP. This indicated that increased soil cover of catchments increased inputs of C-rich terrestrial particulate matter. Furthermore, the positive link between soil cover and PC:PP had a negative effect on the abundance of cladocerans relative to copepods. Decreased particulate P-content was expected to negatively effect the growth of P-limited cladocerans relative to copepods (Sterner and Hessen 1994).

Top-down effects on particulate stoichiometry involved an increase in PC:PP that was associated with a net decrease in cladocerans relative to copepods. An increase in copepods relative to cladocerans was expected to increase P-recycling in the water column because cladocerans preferentially retain P in their biomass and excrete less P than copepods (Sommer and Sommer 2006). This is an example of consumer-driven nutrient recycling (Elser and Urabe 1999).

Increased dominance of chlorophytes and cyanophytes

One of the main climatic effects on phytoplankton was that warming was indirectly linked with increased chlorophytes and cyanophytes via lower DIN:TDP. Chlorophytes and cyanophytes were expected to become more abundant as the DIN:TDP ratio decreased below 20 and average temperatures increased to 15–20 ^{o}C (Tilman et al. 1986). Indeed, average temperatures were near 15 ^{o}C and DIN:TDP ratios were < 20 in several of our lower elevation study lakes where chlorophytes and cyanophytes were most abundant. On the other hand, phytoflagellates have increased with warming by 1–3 ^{o}C above long-term averages in experimental alpine ponds in our study area (Strecker et al. 2004) and in several of our alpine study lakes (Parker et al. 2008); however, in these cases concentrations of TDP decreased or remained unchanged, whereas temperatures and concentrations of TDP increased with decreasing elevation among the study lakes. Diatoms would have been less competitive under warmer, more nutrient rich conditions (Tilman et al. 1986). In deeper lakes, warming may intensify thermal stratification and reduce upwelling of dissolved P, thereby promoting smaller phytoflagellate and diatom taxa that are more competitive under lower P and lower mixing conditions (Winder and Sommer 2012).

Neither increased concentrations of DOC nor increased DOC:TDP were significantly correlated with concentrations or composition of phytoplankton. The strongest potential association with increased DOC was a slightly positive but non significant correlation with concentrations of phytoplankton in the food web models (data not shown), likely due to an increase in dissolved P via mineralization of dissolved organic matter (Vähätalo et al. 2003). Increased DOC has been shown to increase production of bacteria over phytoplankton in an experimental whole-lake addition of DOC to an oligotrophic alpine lake in Sweden (Blomqvist et al. 2001). It was possible that increased DOC concentrations may have stimulated bacterial growth and zooplankton grazing on bacteria, but not total particulate nutrient concentrations.

Decreased abundance of cladocerans relative to copepods

Warming, decreased precipitation, and increased soil cover were inferred to reduce food quantity and quality for herbivorous grazers, thereby decreasing the abundance of cladocerans relative to copepods. Decreased precipitation was associated with reduced phytoplankton and consequently led to a decrease in *Daphnia* relative to copepod taxa, suggesting there was a decline in food *quantity* for herbivorous grazers. Reduced phytoplankton abundance likely had a larger negative effect on cladocerans because they are strictly herbivorous, whereas calanoid copepods in our study lakes can rely on phytoplankton or smaller zooplankton prey (e.g., Schindler and Parker 2002). In particular, *Hesperodiaptomus* prey on rotifers (Paul and Schindler 1994), which may be supported by autotrophic but also bacterial production. Particulate food *quality* partially declined for P-limited grazers due to the positive association between increased soil cover and PC:PP, even though there was a net decrease in PC:PP. This contributed to a decrease in abundance of cladocerans relative to copepods, probably because growth of cladocerans was P-limited (Urabe et al. 2003). Food quality for grazers was also affected by an increased percent abundance of chlorophytes and cyanophytes, which may have corresponded to an increase in less edible forms of phytoplankton for herbivorous cladocerans. This was found in several lakes in England where copepods replaced cladocerans (in the absence of fish predation) when the phytoplankton community became dominated by cyanophytes relative to diatoms (Moss et al. 1991).

Warming was inferred to increase the abundance of *Diaptomus* relative to *Daphnia*, which also contributed to the reduction in the cladoceran:copepod ratio. This was somewhat unexpected because *in vitro* experiments have shown that warming increases the growth rate and abundance of cladocerans more than copepods (Allan 1976, Thompson et al. 2008). On the other hand, an increase in *Diaptomus* may have occurred because warming is expected to select for smaller-bodied taxa. For example, warming *in situ* from 1 ^{o}C to 6 ^{o}C has decreased body size of species of calanoid copepods (*Pseudocalanus* sp.) in marine mesocosms in western Europe (Daufresne et al. 2009). Increased lake temperatures at lower latitudes in North America have also been found to decrease body-sizes of cladocerans (Gillooly and Dodson 2000). Indeed, *D. pulex* were more abundant than their larger congener *D. middendorffiana* in our warmest study lakes. I also found that warming increased biomass of *Chydorus* + *Bosmina* relative to larger-bodied *Daphnia*. Consequently, increased abundance of smaller-bodied cladoceran and copepod taxa due to continued climate warming may decrease filtration efficiency of the entire zooplankton community (Peters and Downing 1984).

The decline in cladocerans relative to copepods has several implications for mountain lake food webs. Greater abundance of raptorial feeding copepods that feed on large algal cells should increase the proportion of smaller phytoplankton taxa (Sommer et al. 2003, Parker and Schindler 2006). A shift towards dominance by copepods should also increase N-limitation of phytoplankton because copepod nutrient recycling can reduce dissolved N:P and potentially promote the abundance of N-fixing cyanophytes at warmer temperatures (i.e., ~ 20 ^{o}C); this is because copepods preferentially retain N in their biomass relative to cladocerans (Sommer and Sommer 2006). Additionally, a decrease in the cladoceran:copepod ratio may decrease the transfer of energy to higher trophic levels, such as predatory amphipods, anostracans, and chaoborids. These taxa typically prey on cladocerans to a much greater extent than copepods in mountain lakes in our study area (Wilhelm and Schindler 1999).

Considerations: Ultraviolet radiation and species-specific effects

Ultraviolet radiation (UVR) exposure can limit the growth of some plankton taxa, particularly in highly transparent lakes (Williamson et al. 2001). Indeed, *in situ* UVR exposure has suppressed rotifers, juvenile *D. middendorffiana*, and benthic algae in alpine lakes in our study area, but did not affect biomass of highly pigmented adult *D. middendorffiana* and *H. arcticus*, nor did it affect UVR-adapted phytoplankton (Vinebrooke and Leavitt 1999, Fischer et al. 2011). Although I did not directly assess UVR exposure, the measurements of water transparency via attenuation of photosynthetically active radiation provided an estimate of UVR penetration into the study lakes. Warming was mainly responsible for decreasing water transparency in the lakes via advanced ice-off. Advanced ice-free season length likely coincided with increased allochthonous inputs because advanced ice-off was also the main predictor of increased concentrations of DOC. Thus, climate-related decreases in water transparency may benefit UVR-sensitive plankton taxa in mountain lakes. In contrast, melting of permanent ice and reduced inputs of glacial flour can dramatically increase water transparency and UVR exposure in alpine lakes (Hylander et al. 2011). None of the study lakes had appreciable amounts of glacial flour, however, so future increases in water transparency are not anticipated.

Another consideration for my study is that some climatic effects may be species-specific. In Lake Washington, for example, long-term warming by about 1.5 ^{o}C from 1962−2002 delayed the peak abundances of the rotifer *Conochilus hippocrepis* in the fall, and advanced the peak abundance of *Conochilus unicornis* in the spring (Hampton 2005). In addition, *Daphnia pulicaria* decreased in abundance whereas *D. thorata* increased in abundance in spring (Hampton et al. 2006). Pooling groups of species by genera or class may thus obscure the effects of climate factors on individual species. In contrast to examining species-specific effects of climate, our approach emphasized climatic effects on functional groups of species such as large herbivorous daphnids and large omnivorous diaptomids. Similarly, I determined climatic effects on total biomass of zooplankton and phytoplankton despite potential species-specific effects.

Decline of alpine zooplankton species?

Several observations suggest that *Hesperodiaptomus arcticus*, *H. shoshone* and *D. middendorffiana* are adapted to relatively cold temperatures and oligotrophic conditions. These taxa are typically found in lakes or ponds in the Rocky Mountains of western North America or in the Arctic (Ward and Whipple 1966). Further, in my study there was a weak positive association between *Hesperodiaptomus* spp. and reduced precipitation, which also coincided with lower phytoplankton chl *a*. *Hesperodiaptomus* were also least abundant in the warmest survey lakes where mean ice-free temperatures were > 10 ^{o}C and where smaller species of *Diaptomus* copepods were dominant. *D. middendorffiana* were similarly least abundant in the warmest survey lakes. Thus, these observations suggest that continued climate warming may suppress these alpine specialists and promote smaller-bodied zooplankton taxa that were abundant in the warmer, more productive study lakes.

Suppression of predatory *Hesperodiaptomus* by warming will potentially reduce predation pressure on smaller-bodied zooplankton species. For example, *D. pulex* was found to be a superior competitor over its larger congener *D. middendorffiana* in the absence of invertebrate predation by the calanoid copepod *Heterocope septentrionalis* in Alaskan lakes (Dzialowski and O'Brien 2004). Similarly, *D. pulex* was dominant in Snowflake Lake in the absence of *Hesperodiaptomus* after the lake was stocked with non-native trout species in the 1960s (McNaught et al. 1999); populations of *D. middendorffiana* later recovered after fish populations collapsed and *Hesperodiaptomus* were experimentally reintroduced. Lower predation by *Hesperodiaptomus* may partially explain why *D. pulex* were more abundant than *D. middendorffiana* in study lakes where *Hesperodiaptomus* were least abundant (in addition to it being potentially favoured by warming due to its small body size). Lake surveys and mesocosm experiments have also shown that rotifer abundance is suppressed by *Hesperodiaptomus* predation (Paul and Schindler 1994). Thus, suppression of *Hesperodiaptomus* due to warming would likely increase abundance of rotifers in alpine lakes.

Conclusions: Cumulative climatic impacts on alpine lake food webs

My findings demonstrate that climate change may alter dissolved and particulate nutrient stoichiometry and composition of phytoplankton and zooplankton communities in alpine lakes. Warmer and drier conditions modeled in the study were mainly associated with increased concentrations of TDP relative to DIN, increased percent abundance of chlorophytes and cyanophytes relative to phytoflagellates, decreased abundance of herbivorous cladocerans relative to omnivorous copepods, and increased abundance of smaller-bodied copepods and cladocerans. These potential changes to alpine lakes due to climate change are a particular concern for the preservation of ecosystems in the Rocky Mountain National Parks of Canada, which is a main priority of the National Parks Act of Canada (Dearden and Dempsey 2004). Futhermore, a major implication of our findings is that these impacts of climate change may exacerbate eutrophication in other relatively shallow cold-water lakes where P-pollution is also an issue (Moss 2012).

References

Allan, J. D. 1976. Life history patterns in zooplankton. American Naturalist 110:165–180.

Anderson, R. S. 1968. The zooplankton of five small mountain lakes in southwestern Alberta. National Museum of Canada Natural History Papers 39:1–19.

Anderson, R. S. 1970. Predator-prey relationships and predation rates for crustacean zooplankters from some lakes in western Canada. Canadian Journal of Zoology 48:1229–1240.

Anderson, R. S. 1971. Crustacean plankton of 146 alpine and subalpine lakes and ponds in western Canada. Journal of the Fisheries Research Board of Canada 28:311–321.

Anderson, R. S. 1972. Zooplankton composition and change in an alpine lake. Verhandlungen der Internationalen Limnologie 18:264–268.

Anderson, R. S. 1974. Crustacean plankton communities of 340 lakes and ponds in and near the national parks of the Canadian Rocky Mountains. Journal of the Fisheries Research Board of Canada 31:855–869.

Anderson, R. S., and L. G. Raasveldt. 1974. *Gammarus* and *Chaoborus* predation and the possible effects of *Gammarus* and *Chaoborus* feeding on the zooplankton composition in some small lakes and ponds in western Canada. Canadian Wildlife Service Occasional Paper No. 18:1–24.

Arhonditsis, G. B., C. A. Stow, L. J. Steinberg, M. A. Kenney, R. C. Lathrop, S. J. McBride, and K. H. Reckhow. 2006. Exploring ecological patterns with structural equation modeling and Bayesian analysis. Ecological Modelling 192:385–409.

Battarbee, R. W. 2009. Threatened and stressed mountain lakes of Europe: Assessment and progress. Aquatic Ecosystem Health & Management 12:118–128.

Blomqvist, P., M. Jansson, S. Drakare, A. K. Bergstrom, and L. Brydsten. 2001. Effects of additions of DOC on pelagic biota in a clearwater system: Results from a whole lake experiment in northern Sweden. Microbial Ecology 42:383–394.

Bradley, R. S., F. T. Keimig, and H. F. Diaz. 2004. Projected temperature changes along the American cordillera and the planned GCOS network. Geophysical Research Letters 31:1–4.

Brooks, P. D., and M. W. Williams. 1999. Snowpack controls on nitrogen cycling and export in seasonally snow-covered catchments. Hydrological Processes 13:2177–2190.

Bunting, L., P. R. Leavitt, R. P. Weidman, and R. D. Vinebrooke. 2010. Regulation of the nitrogen biogeochemistry of mountain lakes by subsidies of terrestrial dissolved organic matter and the implications for climate studies. Limnology and Oceanography 55:333–345.

Daufresne, M., K. Lengfellner, and U. Sommer. 2009. Global warming benefits the small in aquatic ecosystems. Proceedings of the National Academy of Sciences of the United States of America 106:12788–12793.

Dearden, P. and J. Dempsey. 2004. Protected areas in Canada: decade of change. Canadian Geographer 48: 225–239.

Donald, D. B., R. S. Anderson, and D. W. Mayhood. 1994. Coexistence of fish and large *Hesperodiaptomus* species (Crustacea, Calanoida) in sub-alpine and alpine lakes. Canadian Journal of Zoology 72:259–261.

Donald, D. B., R. D. Vinebrooke, R. S. Anderson, J. Syrgiannis, and M. D. Graham. 2001. Recovery of zooplankton assemblages in mountain lakes from the effects of introduced sport fish. Canadian Journal of Fisheries and Aquatic Sciences 58:1822–1830.

Dzialowski, A. R., and W. J. O'Brien. 2004. Is competition important to arctic zooplankton community structure? Freshwater Biology 49:1103–1111.

Elser, J. J., and J. Urabe. 1999. The stoichiometry of consumer-driven nutrient recycling: Theory, observations, and consequences. Ecology 80:735–751.

Fischer, J. M., M. H. Olson, C. E. Williamson, J. C. Everhart, P. J. Hogan, J. A. Mack, K. C. Rose, J. E. Saros, J. R. Stone, and R. D. Vinebrooke. 2011. Implications of climate change for *Daphnia* in alpine lakes: predictions from long-term dynamics, spatial distribution, and a short-term experiment. Hydrobiologia 676:263–277.

Garcia-Jurado, F., I. de Vicente, A. Galotti, A. Reul, F. Jimenez-Gomez, and F. Guerrero. 2012. Effect of drought conditions on plankton community and on nutrient availability in an oligotrophic high mountain lake. Arctic, Antarctic, and Alpine Research 44:50–61.

Gillooly, J. F., J. H. Brown, G. B. West, V. M. Savage, and E. L. Charnov. 2001. Effects of size and temperature on metabolic rate. Science 293:2248–2251.

Gillooly, J. F., and S. I. Dodson. 2000. Latitudinal patterns in the size distribution and seasonal dynamics of new world, freshwater cladocerans. Limnology and Oceanography 45:22–30.

Grace, J. B. 2006. Structural equation modeling and natural systems, 1st edition. Cambridge University Press, Cambridge, UK.

Grace, J. B., and K. A. Bollen. 2005. Interpreting the results from multiple regression and structural equation models. Bulletin of the Ecological Society of America 86:283 – 295.

Hampton, S. E. 2005. Increased niche differentiation between two *Conochilus* species over 33 years of climate change and food web alteration. Limnology and Oceanography 50:421–426.

Hampton, S. E., P. Romare, and D. E. Seiler. 2006. Environmentally controlled *Daphnia* spring increase with implications for sockeye salmon fry in Lake Washington, USA. Journal of Plankton Research 28:399–406.

Hauer, F. R., J. S. Baron, D. H. Campbell, K. D. Fausch, S. W. Hostetler, G. H. Leavesley, P. R. Leavitt, D. M. McKnight, and J. A. Stanford. 1997. Assessment of climate change and freshwater ecosystems of the Rocky Mountains, USA and Canada. Hydrological Processes 11:903–924.

Holzapfel, A. M., and R. D. Vinebrooke. 2005. Environmental warming increases invasion potential of alpine lake communities by imported species. Global Change Biology 11:2009–2015.

Huang, S., P. M. Rich, R. L. Crabtree, C. S. Potter, and P. Fu. 2008. Modeling monthly near-surface air temperature from solar radiation and lapse rate: application over complex terrain in Yellowstone National Park. Physical Geography 29:158–178.

Hylander, S., T. Jephson, K. Lebret, J. von Einem, T. Fagerberg, E. Balseiro, B. Modenutti, M. Sol Souza, C. Laspoumaderes, M. Joensson, P. Ljungberg, A. Nicolle, P. A. Nilsson, L. Ranaker, and L. A. Hansson. 2011. Climate-induced input of turbid glacial meltwater affects vertical distribution and community composition of phyto- and zooplankton. Journal of Plankton Research 33:1239–1248.

IPCC. 2007. International Panel on Climate Change - Fourth assessment report - The physical science basis: Regional climate projections. World Meteorological Organization, United Nations.

Kilham, S. S. 1986. Dynamics of Lake Michigan natural phytoplankton communities in continuous cultures along a Si-P loading gradient. Canadian Journal of Fisheries and Aquatic Sciences 43:351–360.

Kopacek, J., J. Hejzlar, J. Vrba, and E. Stuchlik. 2011. Phosphorus loading of mountain lakes: Terrestrial export and atmospheric deposition. Limnology and Oceanography 56:1343–1354.

Kosten, S., V. L. M. Huszar, E. Bécares, L. S. Costa, E. van Donk, L. A. Hansson, E. Jeppesen, C. Kruk, G. Lacerot, N. Mazzeo, L. De Meester, B. Moss, M. Lürling, T. Nõges, S. Romo, and M. Scheffer. 2011. Warmer climates boost cyanobacterial dominance in shallow lakes. Global Change Biology 18:118–126.

Mackey, M. D., D. J. Mackey, H. W. Higgins, and S. W. Wright. 1996. CHEMTAX - A program for estimating class abundances from chemical markers: Application to HPLC measurements of phytoplankton. Marine Ecology-Progress Series 144:265–283.

Magnuson, J. J., D. M. Robertson, B. J. Benson, R. H. Wynne, D. M. Livingstone, T. Arai, R. A. Assel, R. G. Barry, V. Card, E. Kuusisto, N. G. Granin, T. D. Prowse, K. M. Stewart, and V. S. Vuglinski. 2000. Historical trends in lake and river ice cover in the Northern Hemisphere. Science 289:1743–1746.

Magnuson, J. J., K. E. Webster, R. A. Assel, C. J. Bowser, P. J. Dillon, J. G. Eaton, H. E. Evans, E. J. Fee, R. I. Hall, L. R. Mortsch, D. W. Schindler, and F. H. Quinn. 1997. Potential effects of climate changes on aquatic systems: Laurentian Great Lakes and Precambrian Shield Region. Hydrological Processes 11:825–871.

McCauley, E. 1984. The estimation of the abundance and biomass of zooplankton in samples. Pages 228–265 *in* J. A. Downing and F. H. Rigler, editors. A manual on methods for the assessment of secondary productivity in fresh waters. Blackwell Scientific, Oxford, UK.

McNaught, A. S., D. W. Schindler, B. R. Parker, A. J. Paul, R. S. Anderson, D. B. Donald, and M. Agbeti. 1999. Restoration of the food web of an alpine lake following fish stocking. Limnology and Oceanography 44:127–136.

Morris, K., M. Jeffries, and C. Duguay. 2005. Model simulation of the effects of climate variability and change on lake ice in central Alaska, USA. Annals of Glaciology 40:113–118.

Moss, B. 2012. Cogs in the endless machine: Lakes, climate change and nutrient cycles: A review. Science of the Total Environment 434:130–142.

Moss, B., J. Stansfield, and K. Irvine. 1991. Development of daphnid communities in diatom-dominated and cyanophyte-dominated lakes and their relevance to lake restoration by biomanipulation. Journal of Applied Ecology 28:586–602.

Parker, B. R., and D. W. Schindler. 2006. Cascading trophic interactions in an oligotrophic species-poor alpine lake. Ecosystems 9:157–166.

Parker, B. R., R. D. Vinebrooke, and D. W. Schindler. 2008. Recent climate extremes alter alpine lake ecosystems. Proceedings of the National Academy of Sciences of the United States of America 105:12927–12931.

Paul, A. J., and D. W. Schindler. 1994. Regulation of rotifers by predatory calanoid copepods (subgenus *Hesperodiaptomus*) in lakes of the Canadian Rocky Mountains. Canadian Journal of Fisheries and Aquatic Sciences 51:2520–2528.

Pennak, R. W. 1978. Fresh-water invertebrates of the United States, 2nd edition. Wiley, University of Michigan.

Peters, R. H., and J. A. Downing. 1984. Empirical-analysis of zooplankton filtering and feeding rates. Limnology and Oceanography 29:763–784.

Posch, T., O. Köster, M. M. Salcher, and J. Pernthaler. 2012. Harmful filamentous cyanobacteria favoured by reduced water turnover with lake warming. Nature Climate Change 2:809–813.

Sadro, S., C. E. Nelson, and J. M. Melack. 2012. The influence of landscape position and catchment characteristics on aquatic biogeochemistry in high-elevation lake-chains. Ecosystems 15:363–386.

Schindler, D. W., E. J. Fee, and T. Ruszczynski. 1978. Phosphorus input and its consequences for phytoplankton standing crop and production in the Experimental

Lakes Area and in similar lakes. Journal of the Fisheries Research Board of Canada 35:190–196.

Schindler, D. W., and B. R. Parker. 2002. Biological pollutants: alien fishes in mountain lakes. Water, Air, and Soil Pollution 2:379–397.

Sommer, U., and F. Sommer. 2006. Cladocerans versus copepods: the cause of contrasting top-down controls on freshwater and marine phytoplankton. Oecologia 147:183–194.

Sommer, U., F. Sommer, B. Santer, E. Zollner, K. Jurgens, C. Jamieson, M. Boersma, and K. Gocke. 2003. *Daphnia* versus copepod impact on summer phytoplankton: functional compensation at both trophic levels. Oecologia 135:639–647.

Sterner, R. W., and D. O. Hessen. 1994. Algal nutrient limitation and the nutrition of aquatic herbivores. Annual Review of Ecology and Systematics 25:1–29.

Strecker, A. L., T. P. Cobb, and R. D. Vinebrooke. 2004. Effects of experimental greenhouse warming on phytoplankton and zooplankton communities in fishless alpine ponds. Limnology and Oceanography 49:1182–1190.

Søndergaard, M., J. P. Jensen, and E. Jeppesen. 2001. Retention and internal loading of phosphorus in shallow, eutrophic lakes. The Scientific World 1:427–442.

Thompson, P. L., M. C. St-Jacques, and R. D. Vinebrooke. 2008. Impacts of climate warming and nitrogen deposition on alpine plankton in lake and pond habitats: an *in vitro* experiment. Arctic, Antarctic, and Alpine Research 40:192–198.

Tilman, D., R. Kiesling, R. Sterner, S. S. Kilham, and F. A. Johnson. 1986. Green, bluegreen and diatom algae: Taxonomic differences in competitive ability for phosphorus, silicon and nitrogen. Archiv Fur Hydrobiologie 106:473–485.

Urabe, J., J. Togari, and J. J. Elser. 2003. Stoichiometric impacts of increased carbon dioxide on a planktonic herbivore. Global Change Biology 9:818–825.

Veraart, A. J., J. J. M. de Klein, and M. Scheffer. 2011. Warming can boost denitrification disproportionately due to altered oxygen dynamics. Public Library of Science 6:1–6.

Vinebrooke, R. D., S. S. Dixit, M. D. Graham, J. M. Gunn, Y. W. Chen, and N. Belzile. 2002. Whole-lake algal responses to a century of acidic industrial deposition on the Canadian Shield. Canadian Journal of Fisheries and Aquatic Sciences 59:483–493.

Vinebrooke, R. D., and P. R. Leavitt. 1999. Differential responses of littoral communities to ultraviolet radiation in an alpine lake. Ecology 80:223–237.

Vähätalo, A. V., K. Salonen, U. Münster, M. Järvinen, and R. G. Wetzel. 2003. Photochemical transformation of allochthonous organic matter provides bioavailable nutrients in a humic lake. Archiv Fur Hydrobiologie 156:287–314.

van de Waal, D. B., A. M. Verschoor, J. M. H. Verspagen, E. van Donk, and J. Huisman. 2010. Climate-driven changes in the ecological stoichiometry of aquatic ecosystems. Frontiers in Ecology and the Environment 8:145–152.

Ward, B., and G. C. Whipple. 1966. Freshwater biology, 2nd edition. John Wiley and Sons, Inc., New York.

Wells, B. K., J. C. Field, J. A. Thayer, C. B. Grimes, S. J. Bograd, W. J. Sydeman, F. B. Schwing, and R. Hewitt. 2008. Untangling the relationships among climate, prey and top predators in an ocean ecosystem. Marine Ecology-Progress Series 364:15–29.

Wilhelm, F. M., and D. W. Schindler. 1999. Effects of *Gammarus lacustris* (Crustacea : Amphipoda) on plankton community structure in an alpine lake. Canadian Journal of Fisheries and Aquatic Sciences 56:1401–1408.

Williamson, C. E., O. G. Olson, S. E. Lott, N. D. Walker, D. R. Engstrom, and B. R. Hargreaves. 2001. Ultraviolet radiation and zooplankton community structure following deglaciation in Glacier Bay. Ecology 82:1748–1760.

Winder, M., and U. Sommer. 2012. Phytoplankton response to a changing climate. Hydrobiologia 698:5–16.

Table 2-1. Average physical, climate, chemical, and plankton conditions among 16 mountain lakes in Alberta, Canada.

Model	Variable	Units	Mean	SD	Min	Max
Physical and climate	Summer water temperature	^{o}C	10.7	3.9	5.4	16.7
	Cumulative degree days (air)	^{o}C·y^{-1}	1129.3	562.1	613.4	2341.2
	Total precipitation	mm	583.1	223.4	354.3	899.2
	Maximum depth	m	9.5	8.3	1.0	29.0
	Soil area:Lake area	$^{-1}$	37.1	70.6	0.0	260.4
	Ice-off	d	151.2	27.9	103.6	185.5
	Water transparency	(Kd, m^{-1})	0.5	0.4	0.2	1.4
Dissolved nutrients	Dissolved organic C (DOC)	µmol·L^{-1}	135.5	111.4	29.9	384.9
	Total dissolved P (TDP)	µmol·L^{-1}	0.1	0.1	0.04	0.2
	Soluble reactive silica (SRSi)	µmol·L^{-1}	48.3	36.8	17.6	153.8
	Dissolved inorganic N (DIN)	µmol·L^{-1}	3.5	2.7	0.5	9.1
	DOC:TDP	molar	1225.9	404.8	784.9	2225.2
	Si:TDP	molar	548.9	307.5	119.4	1179.9
	DIN:TDP	molar	44.6	35.8	2.6	100.8
Particulate nutrients	Particulate C (PC)	µmol·L^{-1}	25.1	18.9	9.9	77.1
	Particulate N (PN)	µmol·L^{-1}	2.3	1.8	0.7	6.6
	Particulate P (PP)	µmol·L^{-1}	0.1	0.1	0.0	0.3
	PC:PP	molar	200.4	78.4	116.9	409.7
	PN:PP	molar	18.5	7.2	10.0	35.8
	PC:PN	molar	11.9	2.6	8.6	18.8
Phytoplankton biomass	Chlorophyll *a* (chl a)	µg·L^{-1}	0.6	0.4	0.1	1.2
	Chlorophytes + Cyanophytes	µg·L^{-1}	0.2	0.3	0.0	0.9
	Diatoms	µg·L^{-1}	0.1	0.1	0.0	0.3
	Phytoflagellates	µg·L^{-1}	0.3	0.2	0.1	0.8
Phytoplankton % composition	Chlorophytes + Cyanophytes	%	0.3	0.3	0.0	0.9
	Diatoms	%	0.1	0.1	0.0	0.3
	Phytoflagellates	%	0.5	0.3	0.1	0.9
Zooplankton biomass (dry weight)	Total zooplankton	µg·L^{-1}	34.0	45.1	0.1	138.6
	Daphnia	µg·L^{-1}	13.9	17.3	0.0	53.4
	Chydorids	µg·L^{-1}	1.5	4.2	0.0	15.4
	Rotifers	µg·L^{-1}	0.1	0.2	0.0	0.6
	Hesperodiaptomus	µg·L^{-1}	3.8	4.6	0.0	17.0
	Diaptomus	µg·L^{-1}	14.5	23.8	0.0	72.1
	Cyclopoids	µg·L^{-1}	0.7	1.5	0.0	4.9
Plankton ratios	Cladocerans:Copepods	mass	3.3	6.4	0.0	20.4
	Zooplankton:Phytoplankton	mass	62.5	53.7	0.1	195.2
	chl a:TP	mass	2.6	1.4	0.8	6.7

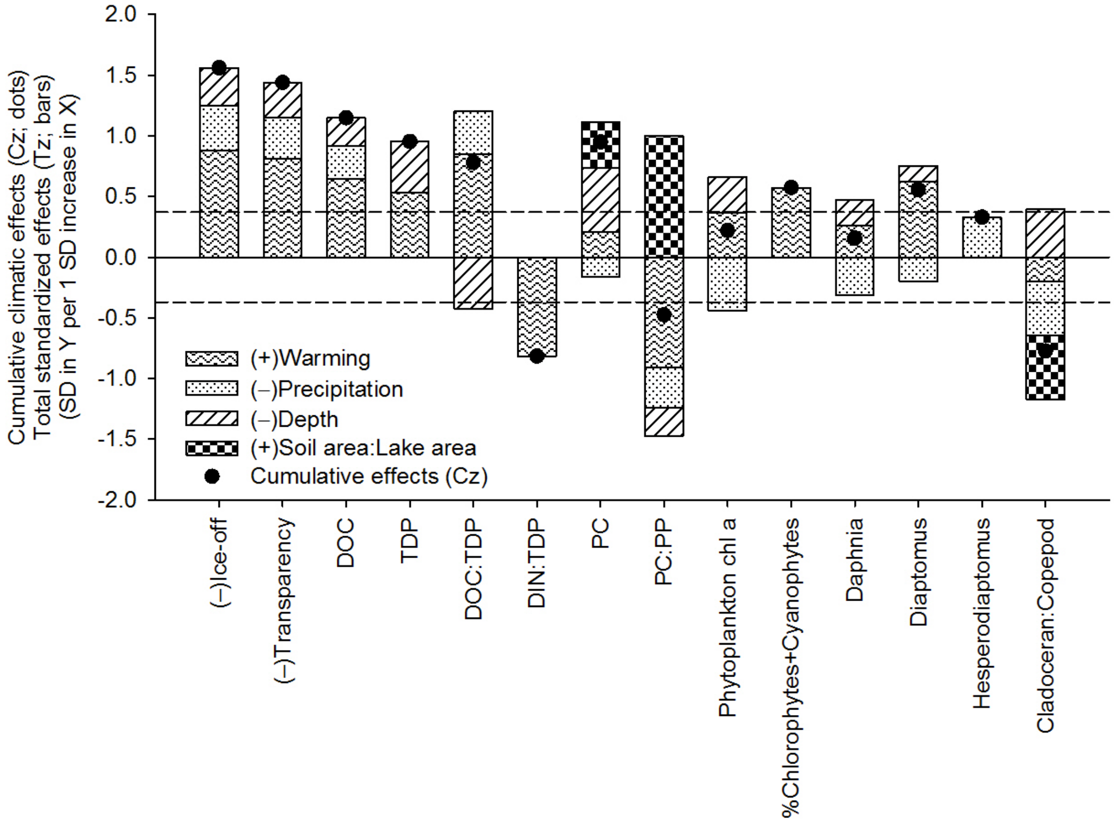


Figure 2-1. Cumulative and total effects of warming, decreased precipitation, decreased lake depth, and increased soil area:lake area on food web variables in 16 mountain lakes in Alberta, Canada. Results are from two separate structural equation models (Figure 2-2 and 2-3; see Appendices 2-14 and 2-15 for SEM statistics). Bars indicate standardized path coefficients (Pz). Dots indicate cumulative effects of four climate variables (Cz). Horizontal dashed lines indicate *P* < 0.05 level of significance.

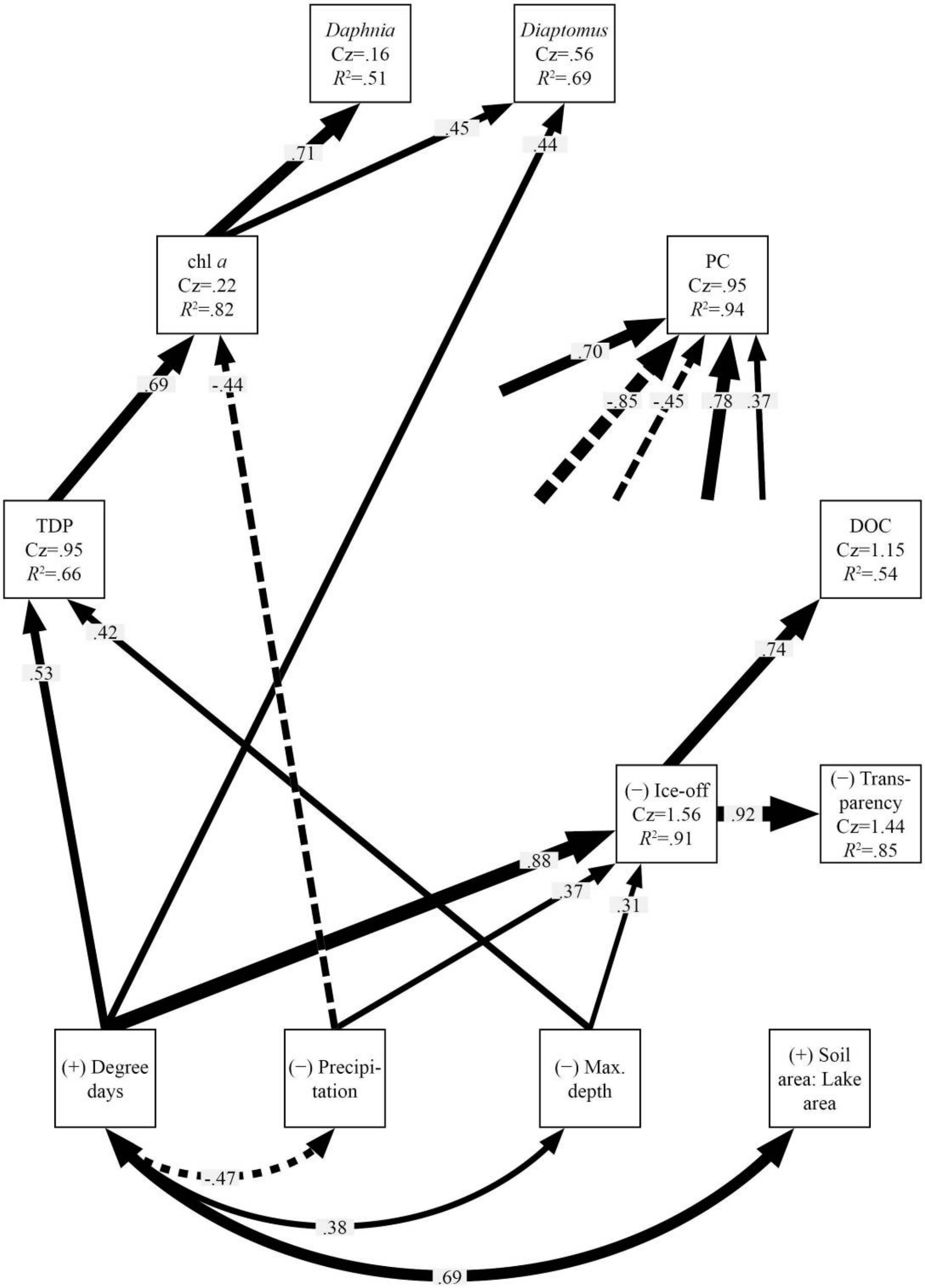


Figure 2-2. Structural equation model of nutrient concentrations and plankton biomass. Caption on next page.

Figure 2-2. Structural equation model of climatic effects of warming, decreased precipitation, decreased lake depth, and increased soil area:lake area on advanced ice-off, decreased water transparency, and concentrations of dissolved organic C (DOC), total dissolved P (TDP), particulate C (PC), total phytoplankton chl *a*, and biomass of *Daphnia*, *Diaptomus*, and *Hesperodiaptomus* in 16 fishless mountain lakes in Alberta, Canada. Overall model fit: $X^2 = 53.1$, $P = 0.162$. Curved double-headed arrows indicate non-causal Pearson's r correlation coefficients (Pr). Straight single-headed arrows indicate standardized partial regression coefficients (Pz) with units that equal changes in SDs of response variable Y per 1 SD increase of independent variable X. Solid and dashed lines indicate positive and negative coefficients, respectively. Path thickness is proportional to Pz. Paths for PC are truncated for clarity. Cumulative effects of all four climate variables on each response variable = "Cz" with units that equal change in SDs of response variable Y per 1 SD increase in all four climate variables. Symbols (+) and (−) indicate these variables are coded for positive or negative changes, respectively. See Appendix 2-14 for model statistics.

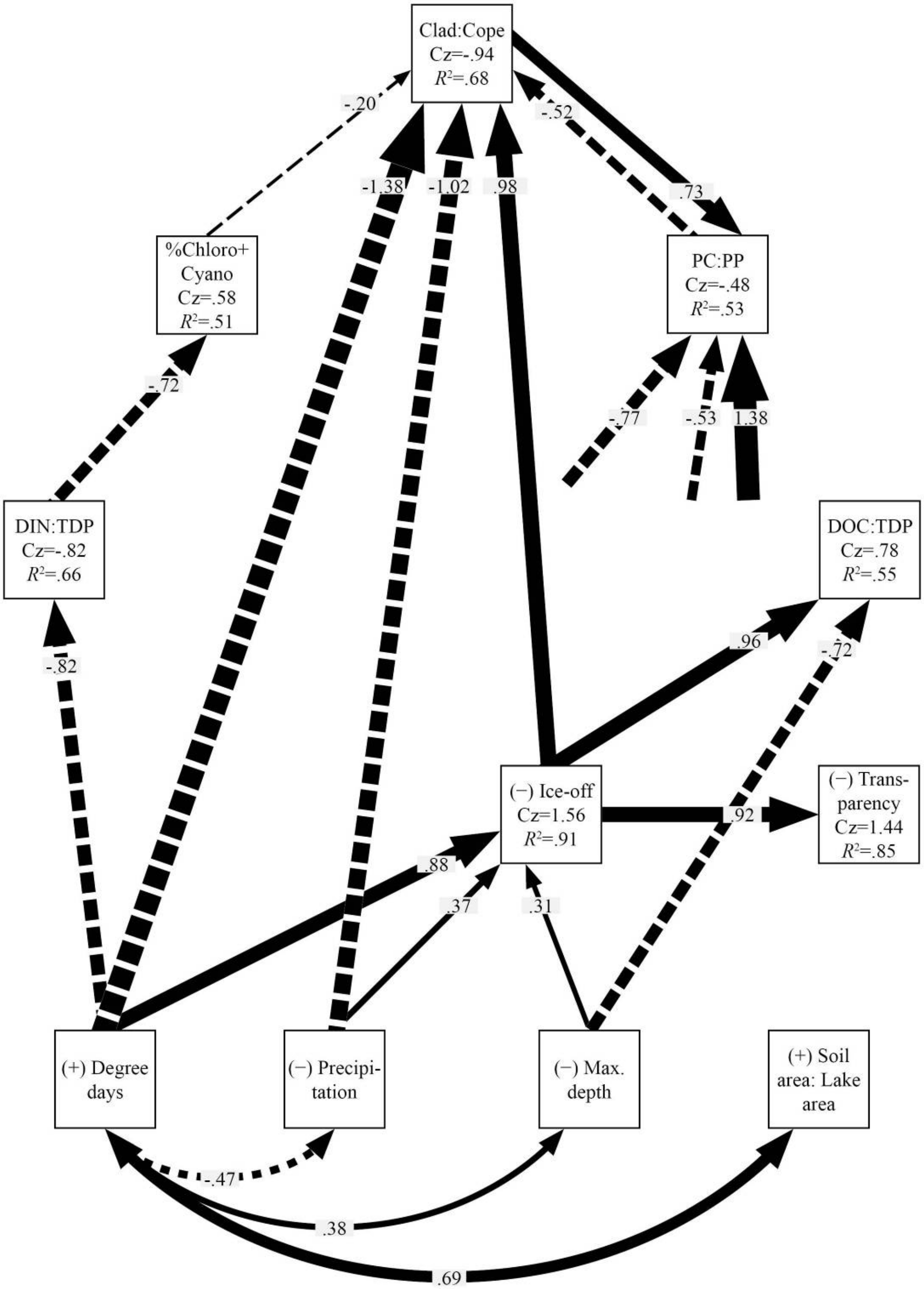


Figure 2-3. Structural equation model of nutrient ratios and plankton relative abundance. Caption on next page.

Figure 2-3. Structural equation model of climatic effects of warming, decreased precipitation, decreased lake depth, and increased soil area:lake area on advanced ice-off, decreased water transparency, and molar ratios of dissolved organic C to total dissolved P (DOC:TDP), dissolved inorganic N to TDP (DIN:TDP), particulate C to P (PC:PP), percent composition of chlorophytes + cyanophytes (%Chloro+Cyano), and mass ratio of cladocerans to copepods (Clad:Cope) in 16 fishless mountain lakes in Alberta, Canada. Overall model fit: $X^2 = 31.8$, $P = 0.623$. Paths for PC:PP are truncated for clarity. See caption of Figure 2 for model description. See Appendix 2-15 for model statistics.

Appendix 2-1. Physical characteristics of lakes and description of catchments for 16 survey lakes in Jasper, Banff, and Waterton Lakes National Parks (JNP, BNP, WLNP, respectively). (a.s.l.) above sea level; (*) unofficial names. Lakes are ordered by mean water temperature during the ice-free season.

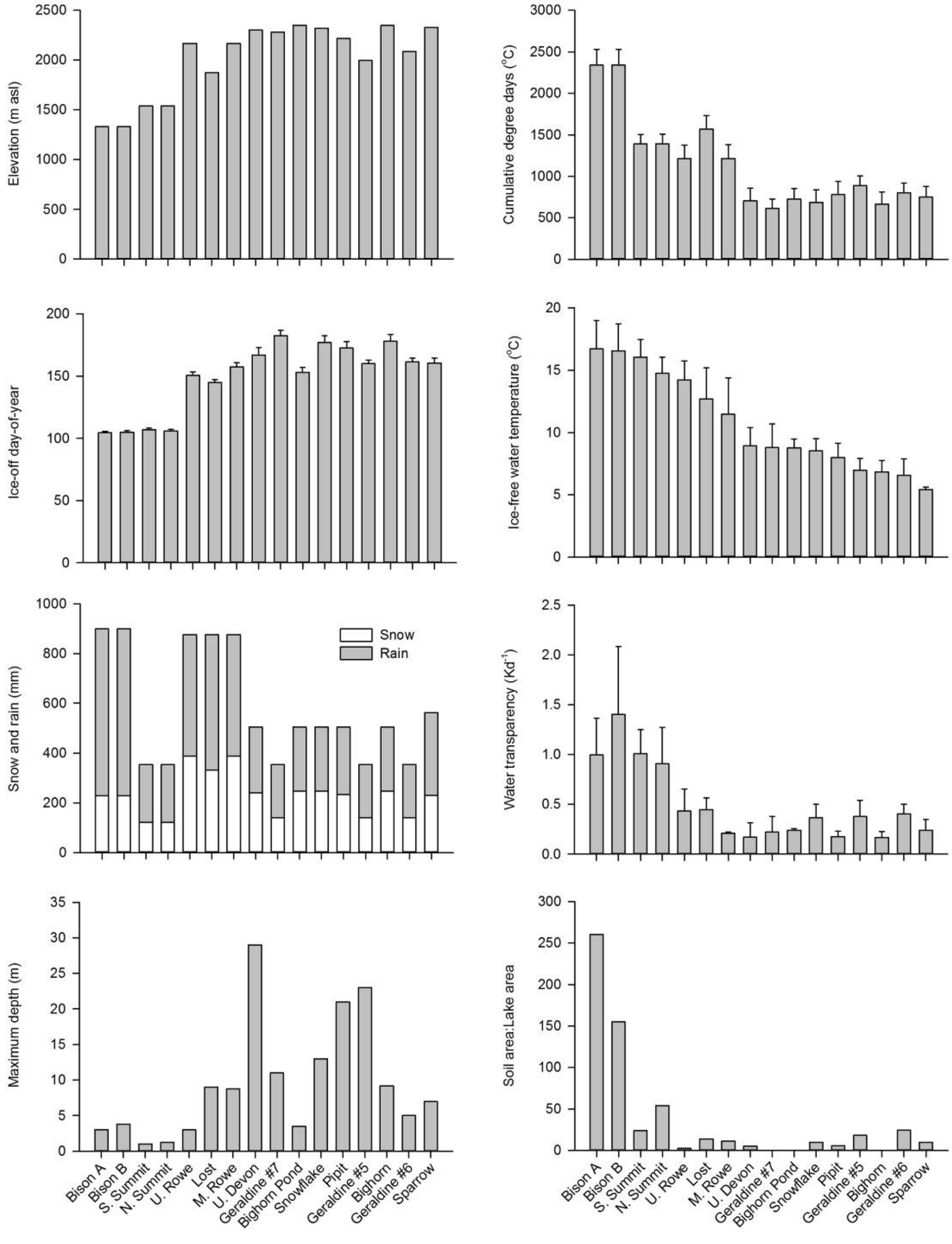
Lake	Park	Elevation (m a.s.l.)	Ice-free temp. (°C)	Max depth (m)	Lake area (ha)	Catchment area (ha)	Latitude (°N)	Longitude (°W)
Bison A*	WLNP	1331	16.7	3.0	3.1	818.6	49.127261	113.861268
Bison B*	WLNP	1331	16.6	3.8	5.4	845.5	49.128830	113.858348
South Summit	JNP	1540	16.0	1.4	6.4	428.7	52.886494	117.755769
North Summit	JNP	1540	14.8	1.3	1.8	357.1	52.891696	117.764021
Upper Rowe	WLNP	2168	14.2	3.0	6.5	58.2	49.050624	114.059549
Lost	WLNP	1875	12.7	9.0	1.7	24.1	49.147019	114.144879
Middle Rowe	WLNP	2165	11.5	8.8	2.1	68.9	49.052817	114.055843
Upper Devon	BNP	2300	8.9	29.0	28.4	952.7	51.728614	116.254520
Geraldine #7*	JNP	2280	8.8	11.0	3.7	308.4	52.558573	117.932857
Bighorn Pond*	BNP	2340	8.8	3.5	1.5	101.2	51.464244	115.650555
Snowflake	BNP	2320	8.5	13.0	5.8	221.1	51.598438	115.833168
Pipit	BNP	2217	8.0	21.0	8.0	234.2	51.616985	115.864196
Geraldine #5*	JNP	1995	7.0	23.0	17.3	1049.7	52.569933	117.949014
Bighorn	BNP	2347	6.8	9.2	0.4	103.9	51.464529	115.647938
Geraldine #6*	JNP	2085	6.6	5.0	3.2	325.0	52.576724	117.959433
Sparrow	KPP	2328	5.4	7.0	0.6	314.7	50.918744	115.270869
Range		1016	11.3	27.8	28.0	1025.5	3.841072	4.101085

Description of the study lakes and their catchments

Bighorn, Sparrow, and Geraldine 7 lakes are located in cirque basins that are well above the tree line with trace amounts of soil or vegetation in their catchments. Snowflake, Upper Devon, and Pipit are located near the tree line in alpine catchments that are covered by mosses and peaty soils or exposed rock. Upper and Middle Rowe lakes are located in an alpine/subalpine catchment that is partly covered by stunted coniferous forest and thin soils or exposed rock. Geraldine 5 and 6 are located in subalpine catchments that are partly covered by mature coniferous forest and soils or alpine meadow with alder, willow, grasses and mosses. Lost and North and South Summit lakes are located in montane catchments surrounded by mature mixed deciduous and coniferous forest with well developed soils. Bison lakes A and B are located within a grassland catchment with bluffs of alder and willow, but are just 6 km from the nearest alpine region.

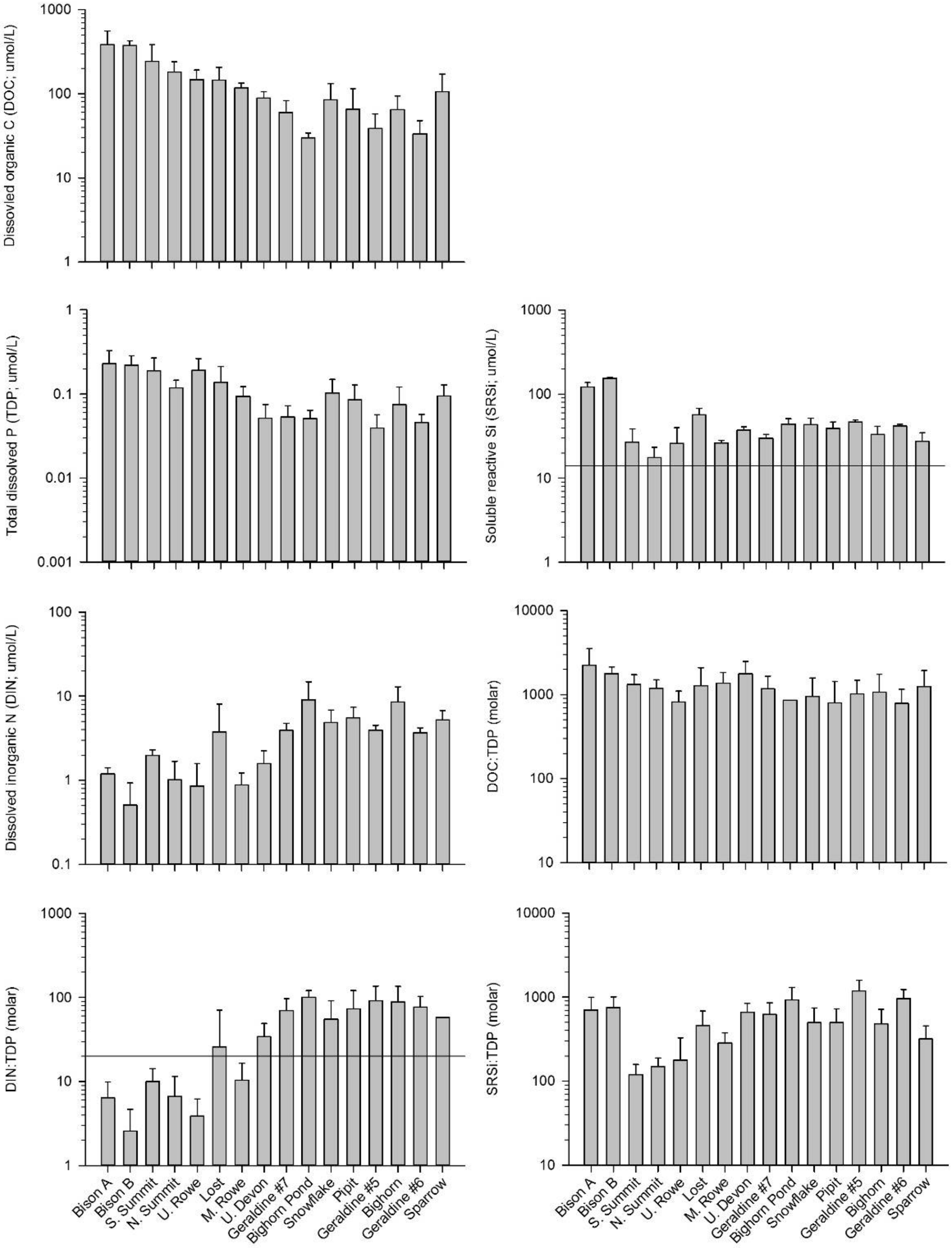
The Bison lakes are located within an enclosed pasture that is managed by Parks Canada and is 125 ha in size and contains approximately 12 bison (*Bison bison*) in the summer months. These lakes did not appear to be impacted by the presence of these animals. Canonical discriminant analysis (not shown) was used to classify lakes based on concentrations of dissolved and particulate nutrients and phytoplankton. Concentrations of TDP, DOC, particulate C, N, and P, and phytoplankton chl *a* were similar among the Bison lakes and the other five warmest, most productive lakes in our survey, including North and South Summit, Upper and Middle Rowe, and Lost. The largest chemical difference among lakes was that the Bison lakes had 3–8 times more SRSi (around 150 μmol Si$\cdot$L^{-1}) than all other study lakes (around 20–50 μmol Si$\cdot$L^{-1}).

Absence of fish in our study lakes was documented in technical reports prepared for Parks Canada by the Canadian Wildlife Service (R.S. Anderson, D.B. Donald, and others, various technical reports). The stocking and natural collapse or experimental removal of introduced trout populations has also been well-documented for our study lakes in Banff National Park (McNaught *et al*. 2002 Limnol. Oceanogr. 44:127-136; Schindler and Parker 2002 Water Air Soil Poll. 2:379-397; Parker and Schindler 2006 Ecosystems 9:157-166). We confirmed the fishless status of Geraldine Lakes 5–7 in Jasper National Park by deploying mixed-mesh gill nets (25–75 mm stretched mesh, 35 m long) near the inflows of each lake continuously for 48 h in September 2007. No fish were caught during that time.



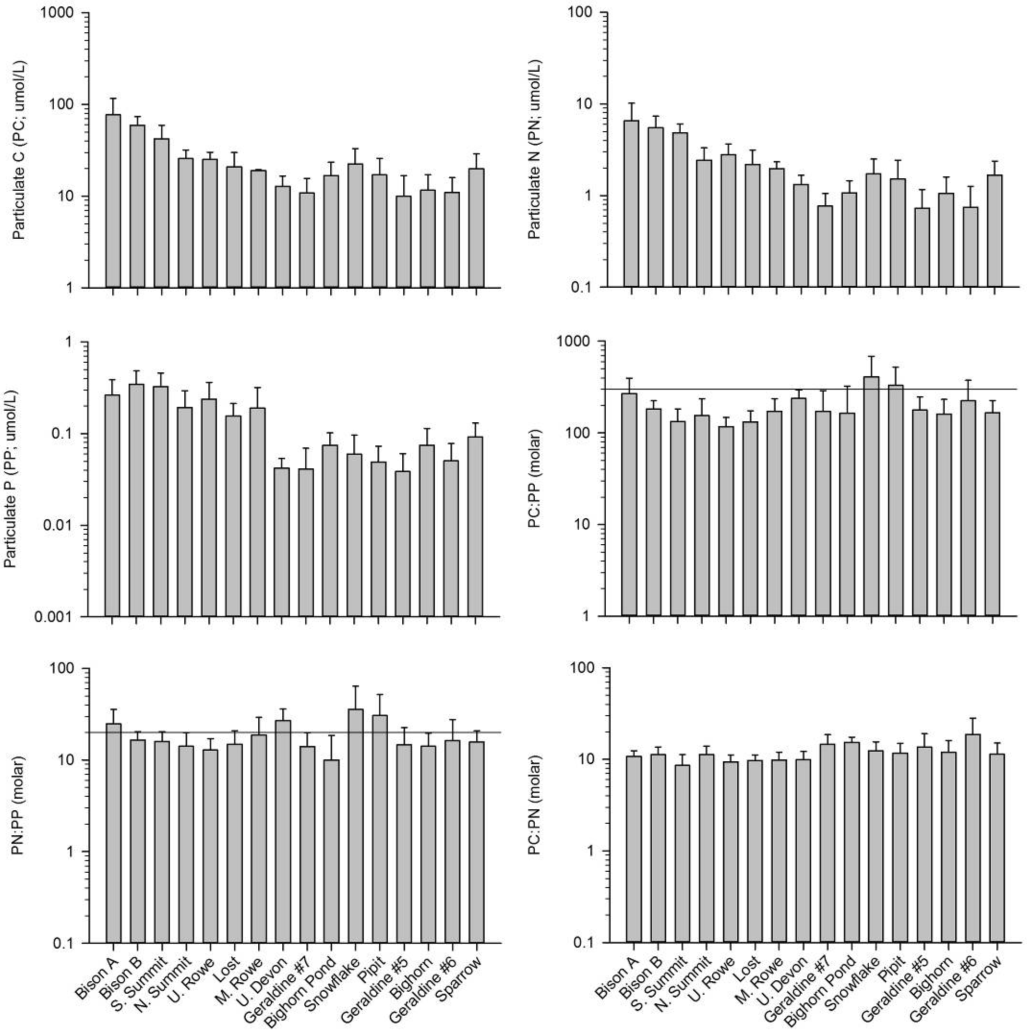
Appendix 2-2. Physical and climatic characteristics of survey lakes. Caption on next page.

Appendix 2-2. Physical and climatic characteristics of 16 fishless mountain lakes in Jasper, Banff, and Waterton Lakes National Parks, Alberta. Lakes were sampled 5 times on average during the ice-free season from 2005−2007, although Pipit, Snowflake, and Bighorn were sampled from 1991–2007. Lakes are ordered by mean water temperature during the ice-free season.

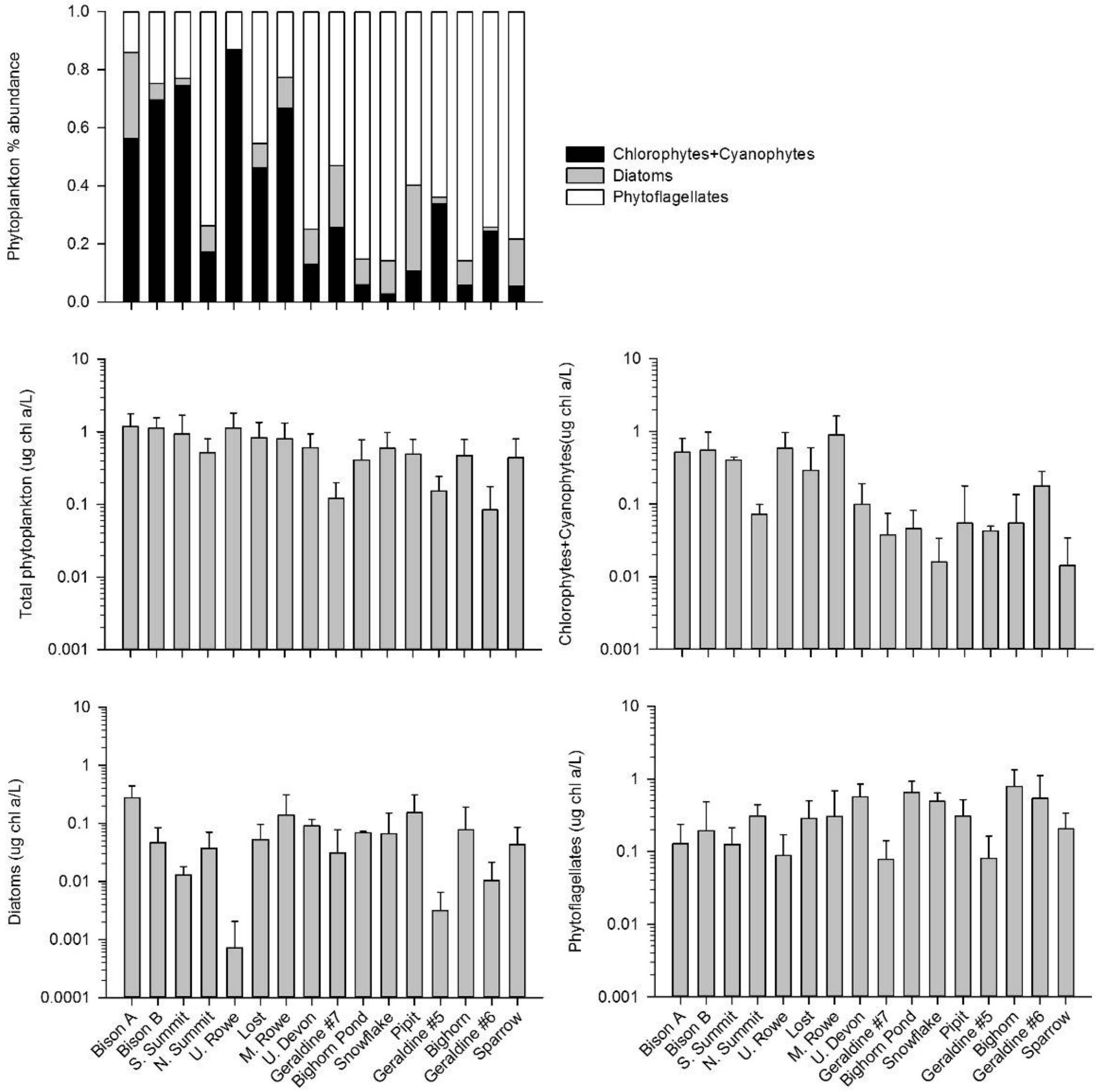


Appendix 2-3. Dissolved nutrient concentrations and stoichiometry ratios. Caption on next page.

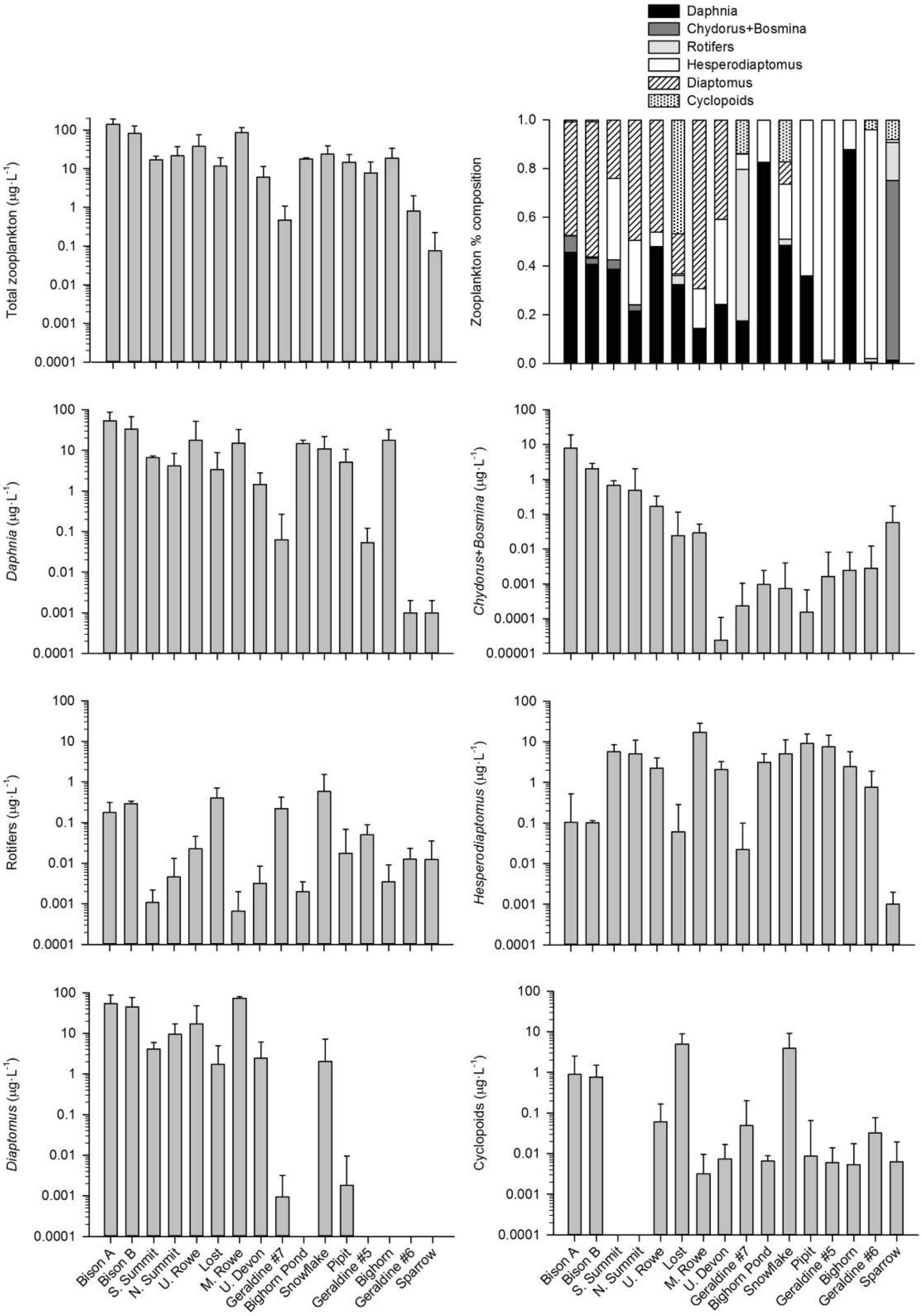
Appendix 2-3. Dissolved nutrient concentrations and stoichiometry ratios. Horizontal lines: SRSi = 0.5 mg or 14 μmol Si·L^{-1} indicates limiting concentration for diatom growth; DIN:TDP < 20 indicates N-limitation of phytoplankton growth. See Appendix 2-2 captions for details.



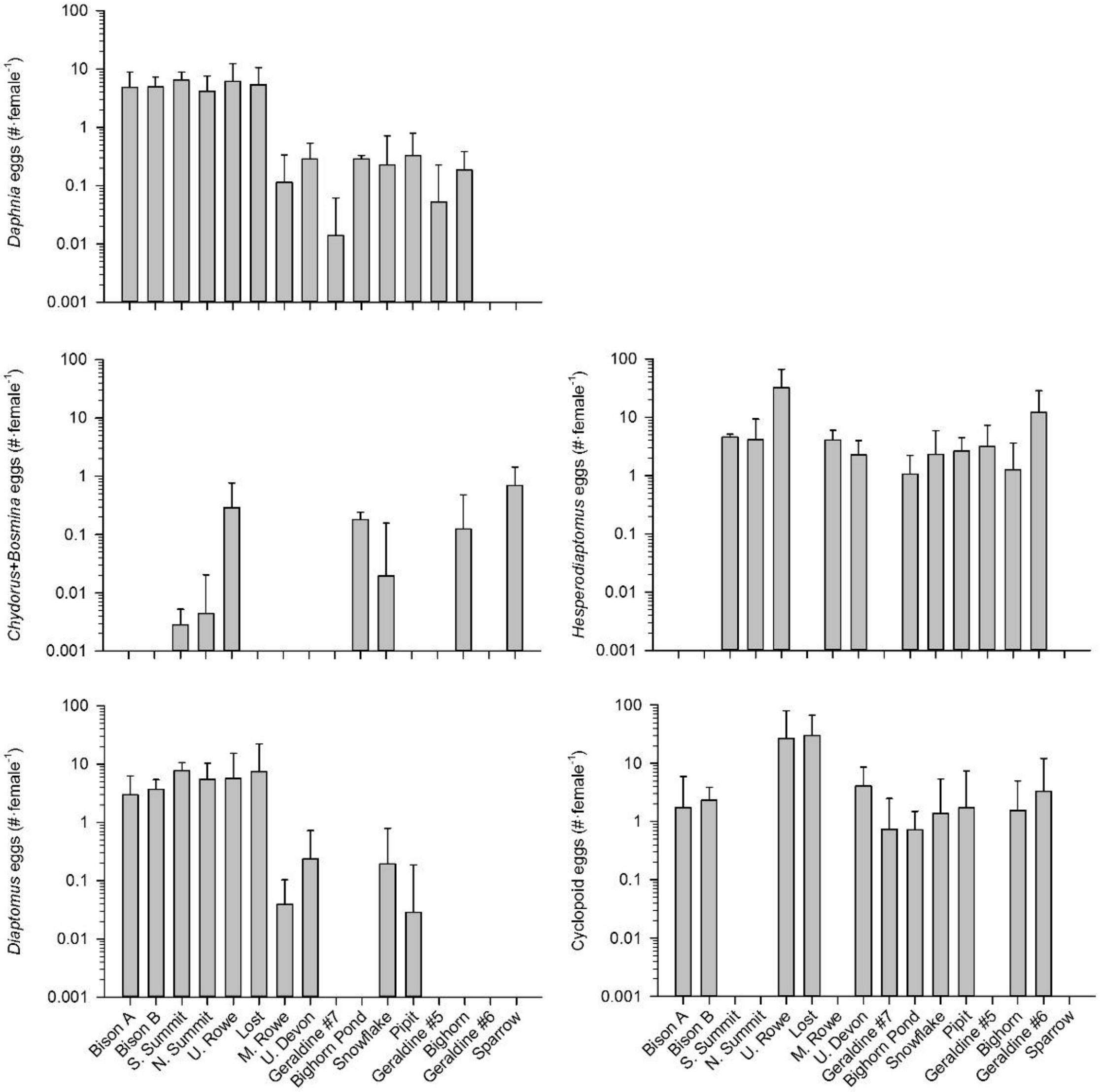
Appendix 2-4. Particulate nutrient concentrations and stoichiometry ratios. Horizontal lines: PC:PP > 300 indicates P-limitation for cladocerans; PN:PP < 20 indicates N-limitation of phytoplankton growth. See Appendix 2-2 captions for details.



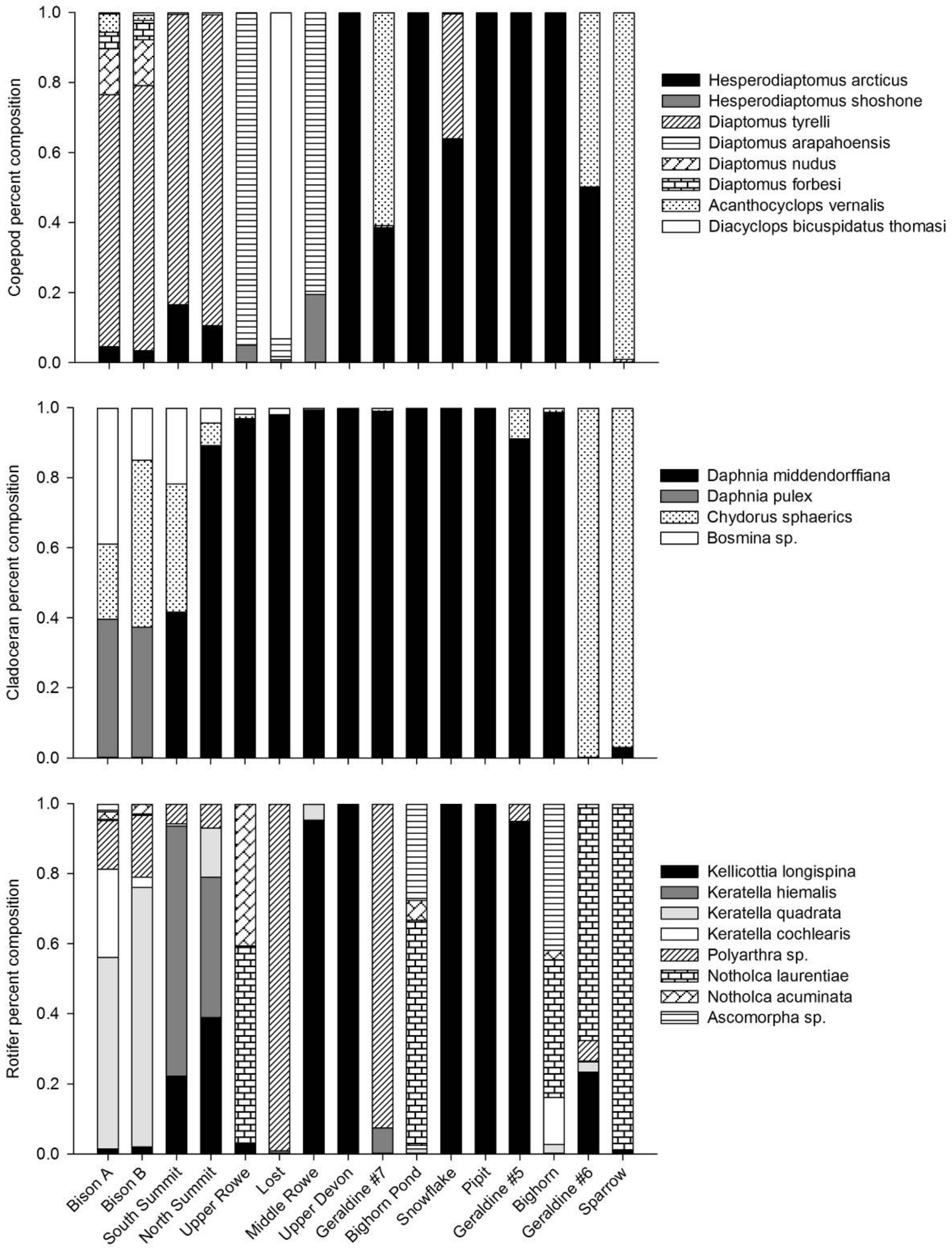
Appendix 2-5. Phytoplankton percent composition and biomass. See Appendix 2-2 captions for details.



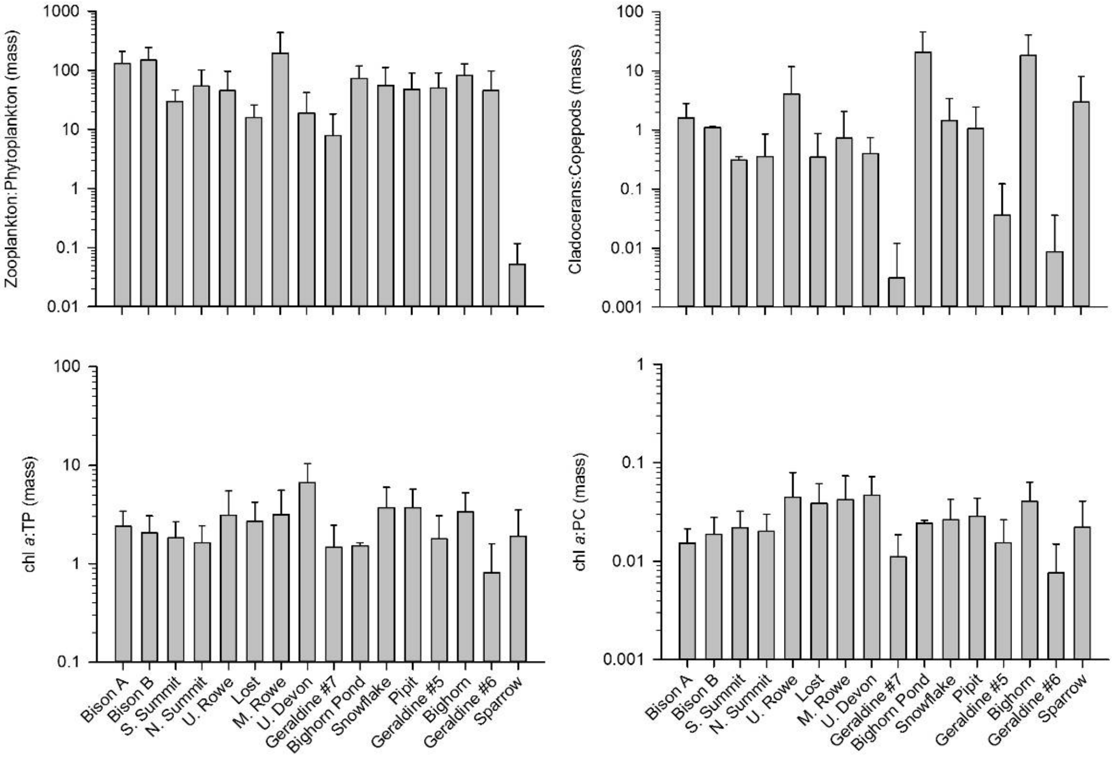
Appendix 2-6. Zooplankton biomass and percent composition. See Appendix 2-2 captions for details.



Appendix 2-7. Zooplankton eggs per female. See Appendix 2-2 captions for details.



Appendix 2-8. Zooplankton species percent composition. See Appendix 2-2 captions for details.



Appendix 2-9. Plankton composition ratios. See Appendix 2-2 captions for details.

Appendix 2-10. Additional methodological details for phytoplankton pigments and structural equation modeling

Phytoplankton pigments

Phytoplankton pigments were analyzed on filters using reverse-phase high-pressure liquid chromatography (HPLC; Vinebrooke *et al*. 2002 Can. J. Fish. Aquat. Sci. 59:483-493). We extracted pigments from frozen filters using a solution of 70 % acetone, 25 % ion-pairing reagent, and 5 % methanol. Pigments were injected into an Agilent 1100 Series HPLC analyzer, which had a Varian Microsorb 100Å C18 column, in-line diode array, and fluorescence detectors. Chlorophylls and carotenoids were quantified using calibration equations derived from standardized samples purchased from DHI Water and Environment, Denmark (Jeffrey *et al*. 2005 UNESCO Publishing).

We used carotenoid to chl *a* ratios to calculate biomass of the major algal classes (Mackey *et al*. 1996 Mar. Ecol-Prog. Ser. 144:1-3). Although pigment concentrations vary with the physiological state of phytoplankton and environmental conditions, carotenoid to chl *a* ratios are diagnostic of algal class under a range of temperature and light conditions (Schluter *et al*. 2006 Freshwater Biol. 8:1474-1485; Greisberger and Teubner 2007 J. Phycol. 43:1108-1119). Carotenoid to chl *a* ratios of some phytoplankton taxa can vary seasonally (Lauridsen *et al*. 2011 Freshwater Biol. 56:1638-1651). Thus, we calculated average carotenoid to chl *a* ratios for each lake from 2005−2007 in order to minimize any effects of temporal variance in these ratios. We also calculated the mass ratios chl *a*:TP and chl *a*:PC to determine the amount of phytoplankton chl *a* per unit of particulate matter (Jeppesen *et al*. 2005 Freshwater Biol. 50:1747-1771).

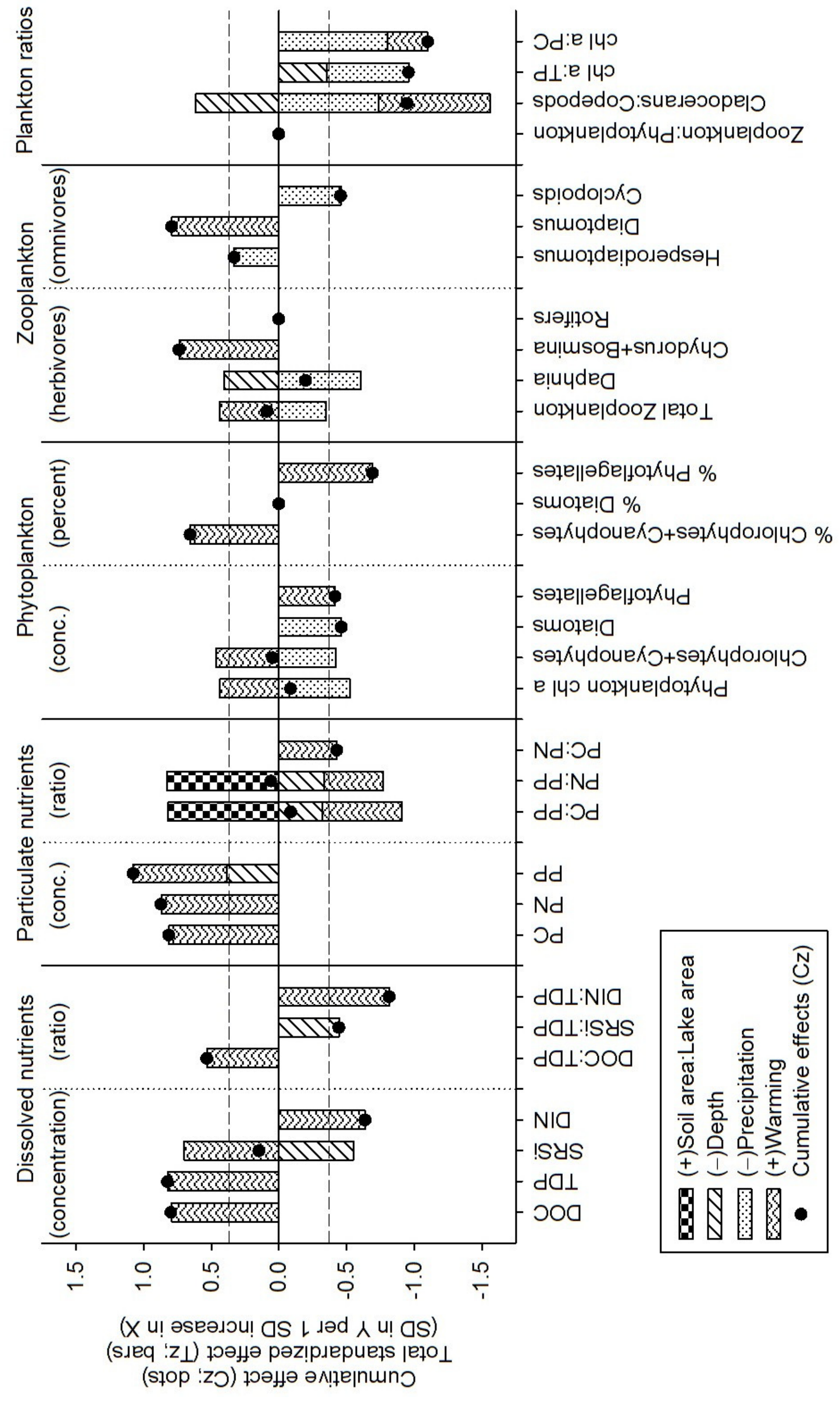
We analyzed carotenoid to chl *a* ratios using CHEMTAX software (version 1.95, Wright, S.W. and Mackey, M.D., Hobart, Tasmania, Australia; Mackey *et al*. 1996 Mar. Ecol-Prog. Ser. 144:265-283). CHEMTAX uses multiple carotenoid to chl *a* ratios for each class of phytoplankton to estimate phytoplankton biomass as a proportion of total chl *a*. We used average carotenoid to chl *a* ratios for the major freshwater phytoplankton classes from several recent studies (Schluter *et al*. 2006; Greisberger and Teubner 2007; Lauridsen *et al*. 2011). Alloxanthin, peridinin, and chlorophyll *b* (chl *b*) indicated the abundance of cryptophytes, dinoflagellates, and chlorophytes, respectively, as these pigments are specific to each algal class. Canthaxanthin was used to distinguish between cyanophytes and diatoms. Fucoxanthin was used to distinguish between diatoms and chrysophytes. Violaxanthin was used to distinguish between chrysophytes and chlorophytes, although it can be produced by dinoflagellates in trace amounts.

We were not able to distinguish between lutein (chlorophytes) and zeaxanthin (cyanophytes) in our HPLC procedure. Thus, we calculated biomass of chlorophytes and cyanophytes together. Chlorophytes were interpreted to be more abundant than cyanophytes due to the presence of chl *b* (chlorophytes) and the absence of most other carotenoids produced by cyanophytes, such as oscillaxanthin, apahanizophyll, astaxanthin, and myxoxanthin (Schluter *et al*. 2006). To further reduce the chance of taxonomic misidentification, we pooled all phytoflagellate groups including chrysophytes, cryptophytes, and dinoflagellates. Chrysophytes were judged to be the most abundant of the three phytoflagellate groups because of the lack of alloxanthin (cryptophytes) and peridinin (dinoflagellates) in most samples.

Comparison of stepwise regression modeling and structural equation modeling

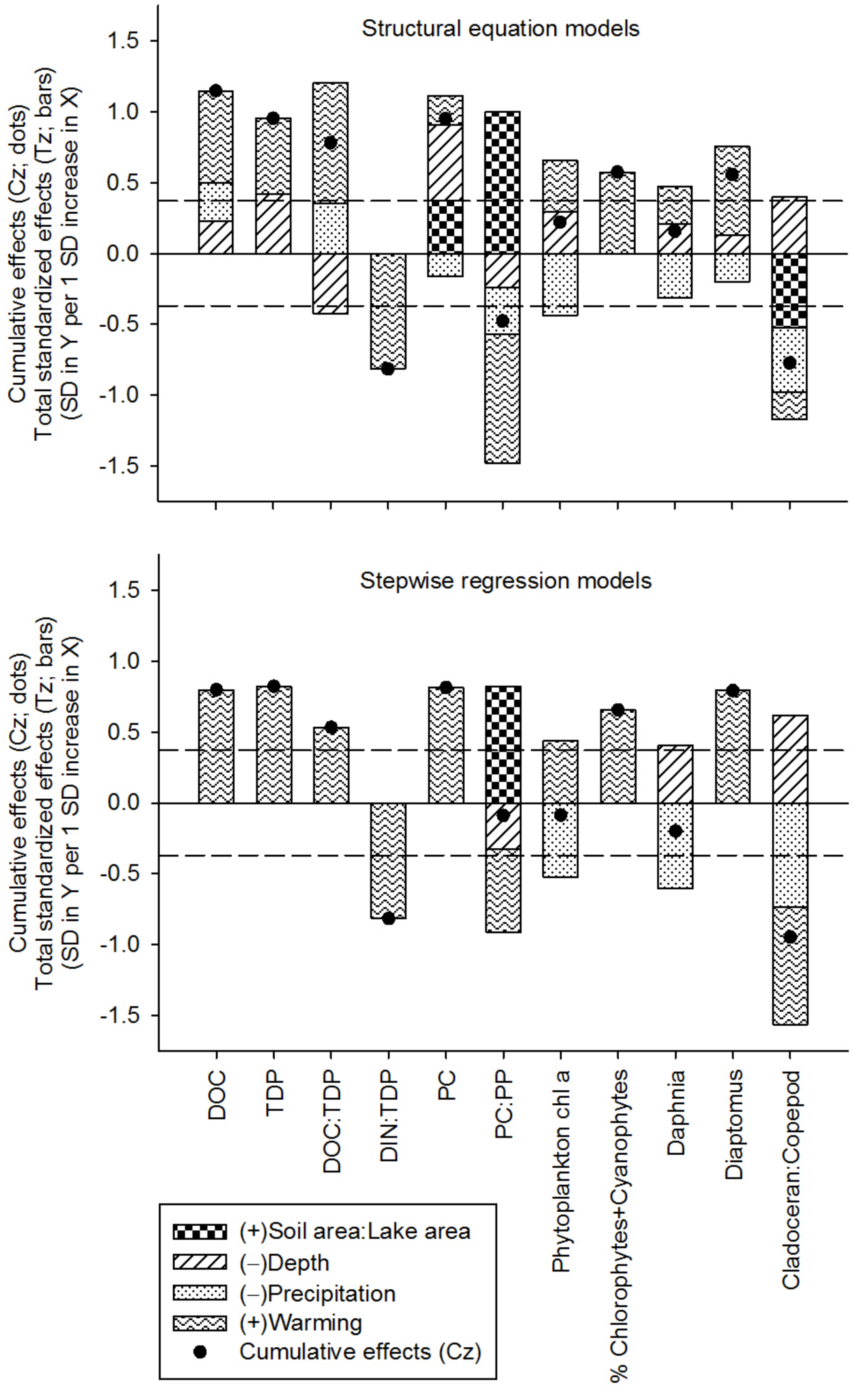
We used the SEM approach to construct several multivariate stepwise regression models that analyzed only the direct effects of the four main climate variables (warming, decreased precipitation, decreased lake depth, and increased soil area:lake area) on several groups of related response variables (dissolved and particulate nutrients, phytoplankton, and zooplankton). These multivariate stepwise regression models allowed us to test groups of related response variables together in several models instead of in numerous individual stepwise regressions. We used the stepwise regression models to identify the main response variables that were analyzed using SEM. Moreover, the stepwise regression models were used to validate the more complicated SE food web models.

Each stepwise regression model was built in three steps. Firstly, the model selection procedure in the AMOS software package (version 18, SPSS Statistics, Chicago, IL, USA) was used to test for significant non-causal (i.e., unexplained) correlations among the four main climate variables. These correlations among climate variables were the same for all of the stepwise regression models. Secondly, the model selection procedure was used to determine significant causal effects of climate variables on each group of response variables in each model. Finally, the selection procedure was used to test for significant residual covariance (i.e., non-casual correlations) among response variables. We then compared the standardized partial regression coefficients from all stepwise regression models and the SE food web models (Appendix 2-12).



Appendix 2-11. Stepwise regression models. Caption on next page.

Appendix 2-11. Stepwise regression models of the climatic effects of warming, decreased precipitation, decreased lake depth, and increased soil area:lake area on food web variables among 16 mountain lakes in Alberta, Canada. Results are from nine separate multivariate stepwise regression models (separated by vertical lines; see Appendices 2-16 and 2-17 for SEM statistics) for concentrations and stoichiometry ratios of dissolved and particulate nutrients, concentrations and percent abundance of phytoplankton, biomass of herbivorous and omnivorous zooplankton, and plankton composition ratios. Average overall model fit: $X^2 = 10.6 \pm 3.5$ SD, $P = 0.739 \pm 0.238$ SD. Bars indicate standardized path coefficients (Pz). Dots indicate cumulative effects of all four climate variables (Cz). Horizontal dashed lines indicate $P < 0.05$ level of significance.



Appendix 2-12. Comparison of climatic effects on response variables occurring in both the food web structural equation models (Figures 2-1 to 2-3) and the stepwise regression models (Appendix 2-11).

	Units	**CDD**	**Precipitation**	**Depth**	**Soil:lake area**	**Ice-off**	**Transparency**	**DOC**	**TDP**	SRSi	DIN	**DIN:TDP**	**PC**	PN	PP	**PC:PP**	PN:PP	**chl a**	Chloro+Cyano	Diatoms	Flagellates	**% Chloro+Cyano**	% Diatoms	% Flagellates	Total zoop.	**Daphnia**	Chydorus	Rotifers	**Hesperodiaptomus**	**Diaptomus**	Cyclopoids	**Clad:Cope**	Zoop:Phytop	chl a:TP
CDD	°C																																	
Precipitation	mm	**0.57**																																
Depth	mm	**-0.56**	-0.08																															
Soil:lake area	$^{-1}$	**0.80**	0.23	-0.35																														
Ice-off	d	**-0.90**	-0.23	**0.77**	**-0.78**																													
Transparency	Kd·m^{-1}	**0.85**	0.17	**-0.69**	**0.80**	**-0.96**																												
DOC	µmol·L^{-1}	**0.85**	**0.52**	**-0.50**	**0.68**	**-0.79**	**0.77**																											
TDP	µmol·L^{-1}	**0.86**	**0.59**	**-0.61**	**0.61**	**-0.80**	**0.79**	**0.92**																										
SRSi	µmol·L^{-1}	**0.53**	0.50	0.04	**0.52**	-0.41	0.47	0.35	0.44																									
DIN	µmol·L^{-1}	**-0.73**	-0.38	0.36	**-0.63**	**0.63**	**-0.62**	**-0.74**	**-0.63**	-0.16																								
DIN:TDP	molar	**-0.85**	**-0.53**	**0.60**	**-0.61**	**0.78**	**-0.75**	**-0.88**	**-0.86**	-0.22	**0.91**																							
PC	µmol·L^{-1}	**0.85**	**0.52**	**-0.62**	**0.71**	**-0.85**	**0.83**	**0.89**	**0.94**	**0.52**	**-0.59**	**-0.79**																						
PN	µmol·L^{-1}	**0.88**	**0.53**	**-0.60**	**0.70**	**-0.85**	**0.83**	**0.94**	**0.96**	0.46	**-0.66**	**-0.85**	**0.98**																					
PP	µmol·L^{-1}	**0.89**	0.49	**-0.73**	**0.60**	**-0.87**	**0.83**	**0.88**	**0.92**	0.30	**-0.72**	**-0.91**	**0.87**	**0.92**																				
PC:PP	molar	-0.23	-0.09	0.47	0.18	0.24	-0.14	-0.14	-0.17	0.31	0.18	0.30	0.01	-0.08	-0.39	-0.28																		
PN:PP	molar	-0.04	0.08	0.46	0.30	0.15	-0.06	0.18	0.07	0.22	-0.10	0.02	0.17	0.16	-0.15	**0.87**																		
chl *a*	µg·L^{-1}	**0.75**	**0.76**	**-0.41**	0.44	**-0.58**	**0.55**	**0.85**	**0.89**	0.35	**-0.62**	**-0.82**	**0.83**	**0.88**	**0.83**	-0.14	0.17																	
Chloro+Cyano	µg·L^{-1}	**0.73**	**0.68**	-0.39	0.43	**-0.52**	0.44	**0.62**	**0.67**	0.27	**-0.75**	**-0.78**	**0.58**	**0.66**	**0.77**	-0.29	-0.04	**0.73**																
Diatoms	µg·L^{-1}	0.27	0.46	0.12	0.28	-0.14	0.01	0.30	0.24	0.40	-0.10	-0.13	0.39	0.35	0.10	**0.51**	**0.54**	0.37	0.25															
Flagellates	µg·L^{-1}	-0.47	-0.11	0.20	-0.39	0.42	-0.43	-0.47	-0.48	-0.09	0.49	0.47	-0.39	-0.43	-0.45	0.27	0.09	-0.25	-0.37	0.09														
% Chloro+Cyano	%	**0.71**	0.38	-0.44	0.44	**-0.60**	**0.58**	**0.57**	**0.65**	0.23	**-0.73**	**-0.74**	**0.50**	**0.59**	**0.76**	**-0.50**	-0.26	**0.55**	**0.84**	-0.13	**-0.64**													
% Diatoms	%	0.02	0.12	0.21	0.11	0.00	-0.08	0.14	0.04	0.27	0.07	0.09	0.20	0.13	-0.14	0.49	0.43	0.00	-0.14	**0.73**	-0.18	-0.33												
% Flagellates	%	**-0.74**	-0.50	0.39	-0.46	**0.61**	**-0.56**	**-0.67**	**-0.76**	-0.33	**0.71**	**0.76**	**-0.64**	**-0.72**	**-0.78**	0.31	0.06	**-0.66**	**-0.85**	-0.18	**0.71**	**-0.91**	-0.03											
Total zooplankton	µg·L^{-1}	**0.70**	**0.62**	-0.33	0.43	**-0.56**	**0.53**	**0.61**	**0.67**	0.43	**-0.52**	**-0.68**	**0.69**	**0.70**	**0.69**	0.04	0.16	**0.79**	**0.67**	0.47	-0.07	0.46	-0.06	**-0.54**										
Daphnia	µg·L^{-1}	**0.56**	**0.62**	**-0.45**	0.21	-0.50	0.46	**0.54**	**0.67**	0.43	-0.30	**-0.56**	**0.70**	**0.68**	**0.65**	0.01	0.05	**0.77**	**0.56**	0.45	0.09	0.31	-0.01	-0.43	**0.91**									
Chydorus	µg·L^{-1}	**0.79**	0.47	-0.39	**0.72**	**-0.77**	**0.80**	**0.73**	**0.74**	**0.79**	**-0.56**	**-0.66**	**0.82**	**0.78**	**0.70**	0.09	0.10	**0.58**	0.48	0.37	-0.32	0.44	0.26	**-0.56**	**0.60**	**0.58**								
Rotifers	µg·L^{-1}	0.22	0.29	0.13	0.23	-0.12	0.26	0.27	0.29	**0.52**	-0.02	-0.10	0.29	0.23	0.09	0.35	0.35	0.21	0.01	0.05	-0.12	0.00	0.10	-0.01	0.18	0.17	0.31							
Hesperodiaptomus	µg·L^{-1}	-0.18	-0.24	0.15	-0.11	0.20	-0.21	-0.20	-0.22	-0.47	-0.04	0.04	-0.20	-0.15	-0.08	0.14	0.23	-0.03	0.08	0.03	0.11	-0.02	-0.29	0.05	0.29	0.09	-0.40	-0.35						
Diaptomus	µg·L^{-1}	**0.82**	**0.58**	**-0.50**	**0.62**	**-0.71**	**0.67**	**0.83**	**0.79**	0.28	**-0.87**	**-0.93**	**0.79**	**0.84**	**0.87**	-0.14	0.12	**0.82**	**0.88**	0.33	-0.38	**0.73**	-0.03	**-0.76**	**0.79**	**0.66**	**0.65**	0.11	0.11					
Cyclopoids	µg·L^{-1}	0.38	0.46	0.05	0.34	-0.21	0.31	0.36	0.39	**0.55**	-0.06	-0.19	0.38	0.34	0.21	0.25	0.32	0.38	0.13	0.10	-0.01	0.06	-0.01	-0.04	0.30	0.27	0.33	**0.93**	-0.33	0.19				
Clad:Cope	mass	-0.22	0.27	-0.15	-0.49	0.20	-0.26	-0.19	-0.02	0.00	0.46	0.19	-0.01	-0.07	-0.06	-0.14	-0.32	0.13	-0.13	0.13	**0.51**	-0.31	-0.09	0.25	0.20	**0.50**	-0.05	-0.20	-0.05	-0.17	-0.13	-0.28		
Zoop:Phytop	mass	0.41	0.28	-0.17	0.27	-0.35	0.32	0.16	0.26	0.36	-0.33	-0.35	0.29	0.29	0.34	0.18	0.11	0.34	0.49	0.35	0.19	0.31	-0.18	-0.32	**0.81**	**0.69**	0.39	0.05	0.43	**0.52**	0.10	0.13		
chl *a* :TP	mass	-0.11	0.36	**0.53**	-0.16	0.36	-0.32	0.16	0.04	-0.01	-0.11	-0.07	-0.01	0.07	-0.10	0.32	**0.60**	0.41	0.07	0.39	0.25	-0.16	0.12	0.04	0.29	0.23	-0.10	0.09	0.28	0.14	0.16	0.12	0.10	
Mean ln+1	ln	6.93	6.30	2.06	2.43	5.01	0.37	4.63	0.10	3.73	1.34	3.33	3.08	1.08	0.13	5.25	2.92	0.46	0.20	0.06	0.27	0.27	0.10	0.41	2.74	1.94	0.38	0.10	1.15	1.49	0.33	0.88	3.68	1.22
SD ln+1	ln	0.44	0.38	0.81	1.65	0.20	0.23	0.78	0.06	0.55	0.61	1.18	0.58	0.46	0.09	0.34	0.33	0.22	0.20	0.06	0.16	0.21	0.08	0.17	1.47	1.41	0.84	0.15	0.94	1.66	0.61	0.95	1.26	0.34
SD non-log	original	1.55	1.46	2.24	5.19	1.22	1.26	2.17	1.06	1.73	1.83	3.25	1.78	1.59	1.10	1.40	1.39	1.25	1.22	1.06	1.18	1.23	1.08	1.19	4.34	4.10	2.32	1.16	2.57	5.26	1.84	2.59	3.52	1.41

Appendix 2-13. Pearson's correlation matrix. Caption on next page.

Appendix 2-13. Pearson's correlation matrix of physical, climatic, chemical, and plankton variables measured in 16 fishless mountain lakes in Jasper, Banff, and Waterton Lakes National Parks, Alberta. Pearson's correlation coefficients in bold are significant ($P < 0.05$). Correlations were based on ln+1 transformed data. Highlighted variables were used in structural equation food web models. Highlighted coefficients indicate significant total effects in models. Non-transformed mean and SD values are also shown to indicate the range in the raw data. Note that precipitation, depth, and ice-off are not negatively coded as they are in structural equation models.

Appendix 2-14. Structural equation model statistics for concentrations of DOC, TDP, PC, and phytoplankton chl a and biomass of Daphnia, Diaptomus, and Hesperodiaptomus in 16 lakes in Jasper, Banff, and Waterton Lakes National Parks, Alberta (2005−2007). Overall model fit: X^2 = 53.1, P = 0.162. Pz = standardized partial regression coefficient, BsPz = bootstrapped standardized path coefficient, % Diff. = BsPz - Pz / Pz , Pc = unstandardized path coefficient and related standard error (SE) and P-value. Symbols (+) and (−) indicate these variables are coded for positive or negative changes, respectively.

Path description				**Individual path statistics**					
Sub-model	**Dependent**		**Independent**	**Pz**	**BsPz**	**% Diff.**	**Pc**	**SE**	***P*-value**
Climate	(+) Warming (°C)	<-->	(–) Precip. (mm)	-0.47	-0.46	-2.3	-0.06	0.03	0.020
		<-->	(–) Max. depth (m)	0.38	0.38	0.8	0.11	0.05	0.033
		<-->	(+) Soil area:lake area	0.69	0.68	-1.6	0.40	0.16	0.012
Physical	Ice-off (date)	<---	(+) Warming	0.88	0.89	0.8	0.42	0.05	<0.001
		<---	(–) Precip.	0.37	0.37	1.1	0.18	0.05	<0.001
		<---	(–) Max. depth	0.31	0.30	-2.9	0.07	0.02	0.002
	Transparency (Kd)	<---	Ice-off	0.92	0.92	0.1	1.06	0.10	<0.001
Dissolved nutrients	TDP (μmol/L)	<---	(+) Warming	0.53	0.53	-0.4	0.07	0.02	<0.001
		<---	(–) Max. depth	0.42	0.41	-1.9	0.03	0.01	<0.001
	DOC (μmol/L)	<---	Ice-off	0.74	0.73	-0.4	2.89	0.55	<0.001
Phytoplankton and particulate nutrients	chl *a* (μg/L)	<---	(–) Precip.	-0.44	-0.43	-2.1	-0.22	0.07	0.001
		<---	TDP	0.69	0.69	0.1	2.64	0.44	<0.001
	PC (μmol/L)	<---	(+) Warming	-0.85	-0.87	2.2	-1.08	0.28	<0.001
		<---	(–) Precip.	-0.45	-0.46	2.0	-0.57	0.16	<0.001
		<---	(+) Soil are:lake area	0.37	0.38	1.6	0.11	0.03	0.001
		<---	Ice-off	0.78	0.79	1.4	2.09	0.46	<0.001
		<---	TDP	0.70	0.69	-1.4	6.86	1.13	<0.001
Zooplankton	*Daphnia* (μg/L)	<---	chl *a*	0.71	0.70	-1.4	4.90	1.03	<0.001
	Diaptomus (μg/L)	<---	(+) Warming	0.46	0.46	-0.7	1.75	0.71	0.014
		<---	chl *a*	0.45	0.46	0.9	3.51	1.41	0.013

Appendix 2-15. Structural equation model statistics for stoichiometric ratios of DOC:TDP, DIN:TDP, PC:PP, percent abundance of chlorophytes and cyanophytes, and the cladoceran:copepod ratio. Overall model fit: $X^2 = 31.8$, $P = 0.623$. See Appendix 2-14 caption for details.

Path description				Individual path statistics					
Sub-model	**Dependent**		**Independent**	**Pz**	**BsPz**	**% Diff.**	**Pc**	**SE**	***P*-value**
Climate	(+) Warming (°C)	<-->	(–) Precip. (mm)	-0.47	-0.46	-1.5	-0.06	0.03	0.020
		<-->	(–) Max. depth (m)	0.38	0.37	-1.6	0.11	0.05	0.033
		<-->	(+) Soil area:lake area	0.69	0.68	-1.9	0.40	0.16	0.012
Physical	Ice-off (date)	<---	(+) Warming	0.88	0.89	0.9	0.42	0.05	<0.001
		<---	(–) Precip.	0.37	0.37	1.1	0.18	0.05	<0.001
		<---	(–) Max. depth	0.31	0.30	-2.3	0.07	0.02	0.002
	Transparency (Kd)	<---	Ice-off	0.92	0.92	-0.1	1.06	0.10	<0.001
Dissolved nutrients	DIN:TDP (mol)	<---	(+) Warming	-0.82	-0.82	0.1	-2.28	0.36	<0.001
	DOC:TDP (mol)	<---	(–) Max. depth	-0.72	-0.71	-1.8	-0.28	0.10	0.006
		<---	Ice-off	0.96	0.96	-0.2	1.68	0.40	<0.001
Phytoplankton and particulate nutrients	Chlor.+Cyan. %	<---	DIN:TDP	-0.70	-0.70	-0.9	-0.13	0.03	<0.001
	PC:PP (mol)	<---	(+) Warming	-0.77	-0.80	4.6	-0.71	0.27	0.010
		<---	(–) Max. depth	-0.53	-0.55	4.7	-0.23	0.10	0.022
		<---	(+) Soil area:lake area	1.38	1.43	3.6	0.29	0.08	<0.001
		<---	Clad.:Cope	0.73	0.83	13.6	0.23	0.10	0.019
Zooplankton	Clad.:Cope (mass)	<---	(+) Warming	-1.22	-1.17	-4.3	-3.54	1.66	0.033
		<---	(–) Precip.	-0.95	-0.93	-1.5	-2.77	0.79	<0.001
		<---	Ice-off	0.88	0.85	-3.3	5.41	2.66	0.042
		<---	Chlor.+Cyan.	-0.40	-0.44	10.1	-2.23	1.51	0.138
		<---	PC:PP	-0.52	-0.56	6.9	-1.65	0.71	0.020

Appendix 2-16. Stepwise regression model statistics for dissolved and particulate nutrient concentrations and stoichiometry. Average overall model fit: X^2 = 11.7 ± 4.6 SD, *P* = 0.570 ± 0.266 SD. See Appendix 2-14 caption for details.

Path description				Individual path statistics					
Model	**Dependent**		**Independent**	**Pz**	**BsPz**	**% Diff.**	**Pc**	**SE**	***P*-value**
Climate	(+) Warming (^oC)	<-->	(–) Precip. (mm)	-0.47	-0.47	0.85	-0.06	0.03	0.020
		<-->	(–) Max. depth (m)	0.38	0.37	-0.80	0.11	0.05	0.033
		<-->	(+) Soil area:lake area	0.69	0.67	-2.46	0.40	0.16	0.012
Dissolved	DOC	<-->	(+) Warming	0.80	0.80	0.00	1.49	0.25	<0.001
nutrients	TDP	<-->	(+) Warming	0.82	0.82	0.00	0.11	0.02	<0.001
(μmol·L-1)	SRSi	<-->	(+) Warming	0.70	0.69	-0.57	1.07	0.28	<0.001
		<-->	(–) Max. depth	-0.55	-0.54	-1.27	-0.40	0.15	0.007
	DIN	<-->	(+) Warming	-0.67	-0.67	-0.15	-1.00	0.25	<0.001
Dissolved	DOC:TDP	<-->	(+) Warming	0.53	0.53	-0.56	0.41	0.15	0.005
stoichiometry	SRSi:TDP	<-->	(–) Max. depth	-0.45	-0.44	-0.90	-0.35	0.16	0.026
(molar)	DIN:TDP	<-->	(+) Warming	-0.82	-0.81	-0.25	-2.29	0.30	<0.001
Particulate	PC	<-->	(+) Warming	0.81	0.81	-0.12	1.12	0.18	<0.001
nutrients	PN	<-->	(+) Warming	0.87	0.87	-0.46	0.93	0.12	<0.001
(μmol·L^{-1})	PP	<---	(+) Warming	0.69	0.69	-0.29	0.14	0.02	<0.001
		<---	(–) Max. depth	0.39	0.39	-0.77	0.04	0.01	<0.001
Particulate	PC:PP	<---	(+) Warming	-0.59	-0.59	1.02	-0.58	0.27	0.029
stoichiometry		<---	(–) Max. depth	-0.32	-0.31	-4.94	-0.15	0.09	0.106
(molar)		<---	(+) Soil area:lake area	0.82	0.80	-2.79	0.19	0.06	0.003
	PN:PP	<---	(+) Warming	-0.44	-0.44	0.46	-0.46	0.25	0.064
		<---	(–) Max. depth	-0.34	-0.33	-2.98	-0.17	0.08	0.043
		<---	(+) Soil area:lake area	0.83	0.81	-2.41	0.20	0.06	<0.001
	PC:PN	<---	(+) Warming	-0.43	-0.43	-0.93	-0.21	0.10	0.032

Appendix 2-17. Stepwise regression model statistics for phytoplankton concentrations and percent composition, zooplankton biomass, and plankton composition ratios. Average overall model fit: $X^2 = 9.7 \pm 2.4$ SD, $P = 0.874 \pm 0.092$ SD. See Appendix 2-14 caption for details.

	Path description			Individual path statistics					
Model	**Dependent**		**Independent**	**Pz**	**BsPz**	**% Diff.**	**Pc**	**SE**	***P*-value**
Climate	(+) Warming (^{o}C)	<-->	(–) Precip. (mm)	-0.47	-0.47	0.85	-0.06	0.03	0.020
		<-->	(–) Max. depth (m)	0.38	0.37	-0.80	0.11	0.05	0.033
		<-->	(+) Soil area:lake area	0.69	0.67	-2.46	0.40	0.16	0.012
Phytoplankton biomass (µg/L)	Total chl *a*	<---	(+) Warming	0.44	0.45	1.59	0.24	0.08	0.003
		<---	(–) Precip.	-0.53	-0.52	-1.52	-0.29	0.10	0.003
	Chloro.+Cyano.	<---	(+) Warming	0.47	0.47	-0.21	0.23	0.09	0.007
		<---	(–) Precip.	-0.42	-0.42	-1.89	-0.21	0.10	0.037
	Diatoms	<---	(–) Precip.	-0.46	-0.45	-3.25	-0.08	0.04	0.043
	Phytoflagellates	<---	(+) Warming	-0.42	-0.42	0.72	-0.17	0.08	0.039
Phytoplankton % composition	Chloro.+Cyano.	<---	(+) Warming	0.66	0.65	-0.31	0.34	0.08	<0.001
	Diatoms	<---	not detected						
	Phytoflagellates	<---	(+) Warming	-0.69	-0.69	-0.14	-0.30	0.07	<0.001
Zooplankton biomass (µg/L)	Total zooplankton	<---	(+) Warming	0.44	0.44	0.68	1.67	0.32	<0.001
		<---	(–) Precip.	-0.35	-0.34	-3.43	-1.34	0.73	0.067
	Daphnia	<---	(–) Precip.	-0.60	-0.59	-1.99	-2.21	0.65	<0.001
		<---	(–) Max. depth	0.41	0.40	-0.49	0.69	0.12	<0.001
	Chydorus+*Bosmina*	<---	(+) Warming	0.74	0.74	0.14	1.50	0.31	<0.001
	Rotifers	<---	not detected						
	Hesperodiaptomus	<---	(–) Precip.	0.33	0.33	-2.40	0.87	0.58	0.134
	Diaptomus	<---	(+) Warming	0.77	0.77	-0.52	3.07	0.50	<0.001
	Cyclopoids	<---	(–) Precip.	-0.46	-0.45	-2.19	-0.74	0.37	0.047
Plankton ratios (mass)	Zoop.:Phytop.	<---	not detected						
	Clad.:Cope.	<---	(+) Warming	-0.82	-0.82	-0.36	-2.31	0.57	<0.001
		<---	(–) Precip	-0.74	-0.73	-0.54	-2.08	0.56	<0.001
		<---	(–) Max. depth	0.62	0.59	-3.58	0.81	0.26	0.002
	chl *a*:TP	<---	(–) Precip.	-0.40	-0.39	-2.49	-0.38	0.18	0.033
		<---	(–) Max. depth	-0.56	-0.56	-0.36	-0.24	0.07	<0.001
	chl *a*:PC	<---	(+) Warming	0.30	0.30	1.69	0.34	0.18	0.064
		<---	(–) Precip.	0.80	0.78	-2.37	0.92	0.24	<0.001

CHAPTER 3: TEMPORAL SURVEY – WARMING AND DROUGHT INCREASE ABUNDANCE OF DIATOMS, CLADOCERANS, AND COPEPODS IN A FISHLESS ALPINE LAKE[2]

Introduction

Alpine lakes are particularly sensitive to climate change because warming and changes in precipitation may result in proportionally large differences in water temperature and nutrient inputs relative to warmer, more nutrient-rich lakes at lower elevations (Battarbee 2009). Surface temperatures have been increasing in lakes around the world over the past few decades (Schneider and Hook 2010). Global climate models predict that warming over the next 50 years will be greatest at high elevations along the American Cordillera and at northern latitudes (Bradley et al. 2004, IPCC 2007). Regional climate models indicate snow pack will decline and summer precipitation will be reduced in western North America (IPCC 2007, Shepherd et al. 2010). Decreased snowpack will reduce the thickness and duration of snow and ice cover on lakes (Morris et al. 2005). Reduced winter snowpack and summer precipitation should decrease inputs of terrestrial nutrients to lakes, because the opposite conditions have occurred in years with high winter snowpack in alpine lakes (Parker et al. 2008). Thus, the purpose of this study was to determine the climatic effects of long-term variation in temperature and precipitation on fishless alpine lake food webs.

Warming and decreased precipitation affect dissolved and particulate nutrient pools, primary producers, and consumers in alpine lake food webs. Reduced precipitation should decrease inputs of C-rich terrestrial matter into alpine lakes, thereby decreasing C:P ratios, as has been found in mountain lakes in central Europe (Kopacek et al. 2011). Decreases in dissolved C:P can be reflected in the particulate nutrient pool (Urabe et al. 2003), which would consequently increase P availability for P-limited grazers (Sterner and Hessen 1994). Reductions in dissolved C relative to P can also reduce mixotrophic and heterotrophic production relative to P-limited autotrophic production, as was observed in a whole-lake addition of DOC to an oligotrophic boreal

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[2] This chapter is in preparation for Limnology & Oceanography

lake in Sweden (Blomqvist et al. 2001). Population growth rates of cladocerans and rotifers may increase more than copepods with warming because copepods have longer generation times and more complicated life histories (Allan 1976). However, warming may also select for smaller-bodied zooplankton irrespective of broad taxonomic categories because declines in generation time with warming may largely be explained by body size (Gillooly 2000).

A major challenge in ecology is to disentangle the effects of multiple ecological stressors caused by global warming (Ormerod et al. 2010), such as higher temperatures and decreased precipitation. To date, many studies on the effects of climate change on lake food webs have compared groups of years with above- versus below-average climatic conditions (Findlay et al. 2001, Pettersson et al. 2003, Parker et al. 2008). Other studies have used time-series analysis to correlate food web and climatic variables over time (Winder and Schindler 2004, Hampton 2005, Blenckner et al. 2007, Wagner and Adrian 2009). However, several recent studies have demonstrated the use of structural equation modeling (SEM) to determine a mechanistic understanding of the effects of multiple stressors on aquatic food webs (Arhonditsis et al. 2006, Wells et al. 2008, Shimoda et al. 2011, Gudimov et al. 2012). SEM modeling has the advantage of allowing the calculation of direct and indirect effects on a network of related variables.

I used SEM to simultaneously test multiple hypotheses on the effects of long-term (1991–2006) variation in temperature and precipitation on the planktonic food web of a fishless mountain lake in Alberta. I hypothesized that reduced precipitation would decrease dissolved and particulate C relative to P via decreased inputs of C-rich allochthonous matter (Kopacek et al. 2011), thereby increasing production of autotrophs relative to mixotrophs, since the latter are more competitive under P-limitation because they can rely on phagotrophy to aquire nutrients (Rothhaupt 1996, Blomqvist et al. 2001). I also hypothesized that warming and decreased C:P would increase asexually reproducing cladocerans and rotifers because they are faster-growing (Allan 1976) and P-limited relative to sexually reproducing copepods (Sterner and Hessen 1994).

Methods

Study lake

Pipit Lake is located in a cirque basin above the tree line at 2217 m asl in Banff National Park, Alberta, Canada (51.616°N, 115.862°W). The lake catchment (245 ha) is composed mainly of argillaceous limestone and dolomite in the form of bedrock, shale, and talus (Hobbs et al. 2010). The catchment has several perennial snow-packs. Less than 10 % of the catchment is covered with peaty soils, mosses, grasses, and subalpine fir trees. The lake has a maximum and mean depth of 20.6 m and 12.6 m, respectively, is weakly stratified for about one month in summer, and has a water renewal time of several months (Parker and Schindler 2006).

Pipit Lake was chosen for this study from several alpine lakes in the vicinity that were monitored from 1991 to the present. Pipit had the longest continuous data record, with some early data available from the 1960s and 1970s. Although non-native trout species were stocked in Pipit in the 1960s, these populations were extinct by the 1970s and the pelagic food web was fully recovered to a naturally fishless state by the early 1990s (Schindler and Parker 2002).

Pipit was sampled three to twelve times per year during the ice-free season from 1991–2006 (n = 112 lake-days). Although data from each sampling event are not independent, our analysis is comparable to other single-lake, long-term pelagic food web studies (e.g., Winder and Schindler 2004) and paleolimnological studies (e.g., Hobbs et al. 2010). This dataset also has the properties of between-year and within-year autocorrelation, which was controlled by transforming data into residuals of variable x time regressions (described below in "Treatment of data").

Climate and lake temperature data

Climate data for the study period (1991–2006) were obtained from the Environment Canada weather station at Lake Louise (51.43 N, 116.22 W; 1524 m asl), which is 32 km southwest of Pipit. I used a lapse rate of 0.65 ^{o}C per 100 m elevation to correct air temperatures at Pipit Lake for the increase in elevation from Lake Louise (Huang et al. 2008). Mean monthly air temperatures at Pipit were above freezing from mid-May through late-September (Figure 3-1). From 2005–2007, I measured lake temperatures hourly for the upper 5 m of the water column using data loggers that were deployed at 1-m intervals. Lake temperatures lagged behind air temperatures by about one month, so the ice-free season occurred from mid-June through mid- to late-October. Cumulative degree days above freezing for air temperature increased most rapidly for air and water from May through September. Mean monthly precipitation ranged from 25–76 mm and was highest in June. I calculated cumulative summer precipitation from May through October in order to include precipitation inputs from the early melting period before the lake became ice-free. I calculated cumulative winter precipitation from November, just after ice-on, through April of each year.

Lake sampling

Sampling was done by boat from the deepest point in the lake. Water temperature profiles were measured at 1 m intervals and were used to calculate mean monthly temperatures for the upper 5 m of the water column. Lake water was collected at 0.5 m depth from the surface of the lake and filtered through Whatman™ GF/F filters in the field. Unfiltered and filtered lake water along with filters were kept at around 10 ^{o}C during transport to the laboratory.

Lake water and filters were analyzed for dissolved and particulate nutrients at the University of Alberta Biogeochemistry Laboratory using standard methods (Parker and Schindler 2006). Filters were analyzed for particulate C (PC) and particulate N (PN) together, and for chlorophyll-a (chl-a). Lake water filtrate was analyzed for DOC and dissolved inorganic N (DIN; i.e., nitrate + nitrite + ammonium). Unfiltered and filtered lake water were retained for analysis of total P (TP) and total dissolved P (TDP), respectively, which were used to calculate particulate P (PP; i.e., TP minus TDP). Molar ratios were calculated for DIN:TDP, PC:PP, and PN:PP.

Phytoplankton dip samples were collected from 0.25 m below the lake surface, preserved with acid Lugol's solution, and later settled, identified to species, and enumerated using the Ütermohl technique (modified by Nauwerck 1963) . The geometric shape of each taxon was measured and used to calculate biomass assuming a specific gravity of one. The mass ratio of PC to total phytoplankton biomass (PC:phyto) was then calculated.

Zooplankton were collected using 30-cm diameter Wisconsin-style plankton nets having 64-µm pore-sized mesh. Five vertical tows were taken from 1 m above the lake bottom at the point of maximum depth. Zooplankton were preserved in sugared-formalin. Cladocerans, copepods, and rotifers were identified to species and enumerated (WJ Findlay, Winnipeg, Manitoba) using a dissecting microscope (Ward and Whipple 1966, Pennak 1978). Adult copepods were distinguished by the development of their 5th swimming leg. Eggs per adult female were calculated for each zooplankton taxon. Daphnid neonates and eggs were pooled to calculate total daphnid reproductive output (eggs per female).

Structural equation modeling (SEM)

SEM is a multivariate statistical method based on analysis of covariance that is used to analyze simultaneous influences and responses within a network of related variables (Grace 2006). In contrast to univariate statistics based solely on significance testing, SEM modeling combines 1) *a priori* knowledge of the multivariate model structure (i.e., positive/negative effects among variables), 2) tests of significance of individual relationships in the model, and 3) overall tests for how well the predicted model fits the observed covariance data. I used a simple form of SEM with only observed variables, which is referred to elsewhere as “modern path analysis” or “SEM with observed variables.” We conducted SEM using the software package AMOS (version 18). The analysis provides a case study for using SEM to separate the effects of long-term variation in multiple climatic variables on a food web. This approach should be expanded to compare multiple lakes simultaneously in future research.

A priori model structure

Average conditions of the food web in Pipit Lake were known from previous studies of fishless alpine lakes in our study area (McNaught et al. 1999, Schindler and Parker 2002, Parker and Schindler 2006; Table 3-1; Figure 3-2). The most abundant zooplankton predator is the calanoid copepod *Hesperodiaptomus arcticus*, which is up to 4 mm in length. The smaller diaptomid, *Diaptomus tyrelli* (about 2 mm in length), is rare. Also occasionally present are the cyclopoid copepods *Acanthocyclops vernalis* or *Diacyclops bicuspidatus*, which are about 1 mm in length. Naupliar life stages (approximately 0.2 mm in length) of each copepod taxon graze smaller phytoplankton cells. Later stage copepodids and adult copepods prey on nauplii and rotifers and graze larger phytoplankton cells (Schindler and Parker 2002). The most abundant cladoceran grazer is *Daphnia middendorffiana*, which is up to 4 mm in length. The cladocerans *Chydorus* sp. and *Alona* sp., which are about 0.5 mm in length, were only present on a few occasions throughout the study period. The five most abundant rotifer taxa (approximately 86 % of total rotifer densities; 1991–2007), which are about 0.1 mm in length, are *Kellicottia longispina* (29 %), an unidentified globular-shaped taxon (24 %), *Notholca* sp. (22 %), *Keratalla quadrata* (9 %), and *Rotaria* sp. (2 %).

The four most abundant phytoplankton groups in Pipit (1991–2007) include diatoms, dinoflagellates, chrysophytes, and cryptophytes, with taxa ranging in size from about 3–30 μm in greatest axial length (Table 3-1; Figure 3-2). Increases in concentrations of TDP were expected to benefit autotrophic diatoms, with the five most abundant species (about 32 % of total phytoplankton biomass) being *Cyclotella glomerata* (21 %), *C. bodanica* (3 %), *C. atomus* (3 %), *C. ocellata* (3 %), and *Eucocconeis flexella* (2 %). Warming and increased concentrations of DOC were expected to benefit heterotrophic phytoflagellates (Strecker et al. 2004, Parker et al. 2008), which were composed of dinoflagellates, chrysophytes, and cryptophytes. The five most abundant species of dinoflagellates (about 36 % of total phytoplankton biomass) were *Gymnodinium* sp. (21 %), *G. ordinatum* (6 %), *G. fungiforme* (4 %), *G. lacustre* (3 %), and *G. inversum* (2 %). The most abundant chrysophyte was an unidentified coccoid-shaped taxon that made up about 8 % of total phytoplankton biomass. The most abundant cryptophyte was *Rhodomonas minuta* that made up 11 % of total phytoplankton biomass. Biomass of chrysophytes and cryptophytes was combined for the analysis (i.e., “chrysophytes + cryptophytes”) because these groups were found to be highly collinear in a preliminary SEM.

The top macroinvertebrate predator in Pipit Lake is the amphipod *Gammarus lacustris* (about 2 cm in length), which preys on zooplankton in the water column in the absence of fish in alpine lakes in our study area (Wilhelm and Schindler 1999). I did not include pelagic macroinvertebrates in the present study in order to reduce model complexity. I have examined pelagic trophic interactions among gammarids, chironomid larvae, and trout in alpine lakes in our study area in a previous study (Appendix 2; Weidman et al. 2011).

PC:PP and PC:phyto were analyzed to determine whether decreased concentrations of DOC reduced the C-content of particulate matter and phytoplankton (Healey and Hendzel 1980, Vähätalo et al. 2003, van de Waal et al. 2010). Decreased PC:PP and PC:phyto were expected to increase particulate food quality for P-limited grazers (Sterner and Hessen 1994). A preliminary SEM indicated that PN:PP was highly collinear with PC:PP, so PN:PP was not included in the model.

Cumulative degree days above freezing (i.e., air temperature) were analyzed to determine the effects of climatic conditions on the food web over the entire season. Daily water temperature was also used to determine short-term thermal effects (i.e., daily versus seasonal) on food web parameters. There were no significant correlations with DIN and any particulate nutrients or phytoplankton groups (Appendix 3-1), so DIN was not included in the SEM. Indeed, primary production in Pipit and most alpine lakes in Alberta is P-limited (Murphy et al. 2010).

Treatment of data

Prior to analysis each variable was screened for outliers that were greater or less than 3 SD from the annual mean within each year the lake was sampled. All variables were ln+1 transformed to improve homogeneity of variance and normality. Relationships among variables in the models were confirmed to be linear by examining bivariate scatter plots.

To remove between-year and within-year autocorrelation, data were transformed into unstandardized residuals by regressing each variable against the covariates date (serial numer) and day-of-year (0–365) , which effectively eliminated temporal autocorrelation from the dataset. For example, before transformation, water temperatures increased slightly with date (regression slope = 0.0002 $^{o}C \cdot day^{-1}$, $R^2 = 0.01$, $P = 0.134$) and increased to a greater extent with day-of-year (regression slope = 0.0461 $^{o}C \cdot$day-of-year^{-1}, $R^2 = 0.16$, $P < 0.001$). After transformation into residuals, water temperatures were no longer correlated with either date or day-of-year (regression slope =

0.0000 °C·day^{-1} or 0.0000°C·day-of-year^{-1}, $R^2 = 0.00$, $P = 1.000$). Transformation of data into residuals also reduced the skew of the distribution of variables, which helped improve the fit of SEM.

A covariance matrix for all variables in the food web formed the basis for analysis using AMOS software (derived from correlation matrix in Appendix 3-1). A correlation matrix of Pearson's *r* coefficients was also calculated to evaluate bivariate relationships among variables.

Model specification and analysis

I calculated the total standardized effects ("Tz") of increased air temperature (i.e., cumulative degree days above freezing), increased water temperature, decreased summer precipitation, and decreased winter precipitation in each SE model by summation of the direct and indirect standardized effects of each climate variable on each independent variable (Grace 2006). Units of Tz indicate change in standard deviations of the response variable for every 1 SD increase in the predictor variable, although we refer to units as "SD" in the text for simplicity. I then calculated the cumulative climatic effects ("Cz") of all three climate variables plus water temperature on each response variable. The effects of decreased winter precipitation in the models largely reflected the effects of decreased snowpack (discussed below). Total effects (Tz) that were > 0.10 SD in absolute magnitude corresponded with significance values of $P < 0.05$ based on the t-statistics of individual paths in each model (described below).

I used coding variables to control whether each model tested for an increase or decrease in each climate variable (Grace 2006). Summer and winter precipitation are expected to decrease with climate change, so these variables were negatively coded (indicated as "(–)" in figures). Air and water temperatures are expected to increase with climate change and were consequently positively coded (indicated as "(+)" in figures).

I combined variables into four groups that included A) climate variables, B) lake temperature and dissolved nutrients, C) phytoplankton or particulate stoichiometry (tested in separate models), and D) zooplankton, which are later referred to as "submodels A–D" (i.e., rows A–D in Figure 3-2). Climate variables were considered as exogenous (i.e., independent), as we did not seek to explain the cause of variation in these variables. All other variables were considered endogenous (i.e., dependent) with estimates of residual error, although these error terms are not illustrated in order to simplify the presentation of the model figures. To further reduce model complexity, two separate models were constructed with either the group of phytoplankton variables (i.e., "phytoplankton-zooplankton model") or the group of particulate stoichiometry variables (i.e., "stoichiometry-zooplankton model").

I constructed each SEM by testing a series of submodels. Initially, I tested for significant non-causal correlations within each of the four groups of variables (i.e., in submodels A−D; Figure 3-2). In submodel A, air temperature was positively correlated with decreased winter precipitation, but no causal relationship was inferred from this association. Non-causal correlations subsequently controlled for collinearity among variables in each submodel. Next, I tested for causal relationships between variables among submodels A−D using a bottom-up approach. Wells et al. (2008) used a similar albeit top-down approach to modeling the effects of climate variation on the marine food web off the central California coast. Pathways were chosen based on my hypotheses and *a priori* knowledge of the food web structure. First, I tested for significant effects of climate variables on lake temperature and dissolved nutrients (i.e., "submodel A→B"). Second, I tested for significant effects of climate variables and lake temperature and dissolved nutrients on phytoplankton or particulate stoichiometry (i.e., "submodel A+B→C"). Third, I tested for significant effects of climate variables, lake temperature, and phytoplankton or particulate stoichiometry on zooplankton (i.e., "submodel A+B+C→D"). Any effects of dissolved nutrients were excluded from this submodel because these variables would not directly affect zooplankton. Finally, I tested for significant top-down effects of zooplankton on phytoplankton (i.e., "submodel D→C+B+A"). For each submodel, the model selection procedure "specification search" in AMOS was used with a Bayesian information criteria index to select the most parsimonious set of significant pathways that optimized the overall model fit (Schumacker 2006).

Non-causal correlation path coefficients ("Pr"), which are numerically equivalent to Pearson's *r* correlation coefficients, are indicated as double-headed dotted arrows in SE model figures. Causal path coefficients ("Pz") are in the form of multivariate partial regression coefficients that are standardized as z-scores. Causal paths are indicated as single-headed solid arrows in SEM figures. Path coefficients are controlled for partial correlations among independent variables (i.e., non-causal correlations) and joint effects of multiple independent variables on each dependent variable (Grace and Bollen 2005).

The overall fit of each submodel was tested with chi-square statistics using maximum likelihood estimation (MLX^2). Unlike more standard hypothesis testing, here we fail to reject the predicted model when the MLX^2 statistic has a P-value > 0.05 and judge that the predicted model adequately explains the observed covariance data (Grace 2006). Individual non-causal and causal path coefficients were tested with t-statistics ($P < 0.05$). A residual covariance matrix was also inspected for each submodel to confirm that all significant pathways were included.

The sample size of 112 lake-day samples was about 5 times greater than the number of variables in each model (20), which was roughly the minimum sample size required by SE modeling. To evaluate the adequacy of our sample size, I also used the bootstrapping random re-sampling procedure in AMOS to generate a dataset of 1000 samples and re-calculate path coefficients in each model (Arbuckle 2009).

Results

Cumulative climatic effects

Drier conditions (decreased summer and winter precipitation) and warming (increased air and water temperature) were associated with additive and antagonistic changes in the abiotic environment and lake food web (Figure 3-3). Decreased winter precipitation and increasing air temperature were coupled with an additive increase in lake temperature (Cz = 0.63 SD; see Figure 3-3 for levels of significance). Decreased summer precipitation alone was directly related with lower DOC (Cz = -0.40 SD). Reduced summer precipitation was also directly associated with increased PC:PP (Cz=0.51), PC:phyto (Cz=0.14 SD), and biomass of diatoms (Cz=0.25 SD). Increased air temperature and lake temperature were mainly linked with additive increases in biomass of *D. middendorffiana* (Cz = 0.58 SD) while reduced winter precipitation alone was associated with increased abundance of *H. arcticus* (Cz = 0.28 SD).

There were several significant antagonistic interactions. Warmer air temperatures suppressed the positive association between reduced summer precipitation and TDP (Cz = 0.04 SD). Reduced winter precipitation dampened the positive linkage between warming and dinophytes (Cz = 0.03 SD) and chrysophytes + cryptophytes (Cz = 0.01 SD).

Phytoplankton-zooplankton model

Precipitation, lake temperature, and dissolved nutrients (Submodel $A \rightarrow B$)

Inferred effects of decreased precipitation were greater than or equal to effects of warming on the abiotic environment (Figure 3-4; Appendix 3-2). Increased lake temperature was associated with decreased winter precipitation (Pz = 0.43 SD, $P < 0.001$) and warming (Pz = 0.19 SD, $P = 0.028$). Decreased summer precipitation was linked to increased concentrations of TDP (Pz = 0.18, $P = 0.049$), but warming offset this increase in TDP (Pz = -0.23, $P = 0.013$, respectively). Decreased summer precipitation was coupled with reduced concentrations of DOC (Pz = -0.40 SD, $P < 0.001$), which thereby dimished dissolved C relative to P. Air temperatures were correlated with low winter precipitation (Pr = 0.20 SD, $P = 0.003$) and low summer precipitation (Pr = -0.32 SD, $P =$

0.001). These non-causal correlations control for collinearity among climate variables, making them statistically independent of each other (Grace and Bollen 2005).

Phytoplankton (Submodel A+B→C)

Similar to the dissolved nutrient results, inferred effects of decreased precipitation were equal to or stronger than effects of warming on phytoplankton community structure (Figure 3-4; Appendix 3-2). Biomass of diatoms was positively correlated with biomass of dinoflagellates (Pr = 0.47, $P < 0.001$), but was negatively correlated with biomass of chrysophytes + cryptophytes (Pr = -0.29, $P < 0.001$). Once correlations among phytoplankton groups were controlled, SE modeling revealed that decreased summer precipitation was associated with increased biomass of diatoms (Pz = 0.37, $P < 0.001$). Reduced winter precipitation was linked to decreased the biomass of dinoflagellates (Pz = -0.29 $P < 0.001$) and chrysophytes + cryptophytes (Pz = -0.20, $P = 0.018$). Increased air and water temperatures offset the negative effects of winter precipitation on chrysophytes + crytophytes (Pz = 0.33, $P < 0.001$) and dinophytes (Pz = 0.32, P < 0.001), respectively.

Zooplankton (Submodel D→C+B+A)

Climate variables were associated with changes in the most dominant crustacean species but not with rotifers (Figure 3-4; Appendix 3-2). Air and water temperatures increased along with densities of herbivorous *D. middendorffiana* (Pz = 0.25, $P = 0.009$ and Pz = 0.26, $P = 0.010$, respectively), which suppressed the biomass of dinoflagellates (Pz = -0.25 $P = 0.005$) and chrysophytes + cryptophytes (Pz = -0.32, $P < 0.001$). There was a weak coupling of *D. middendorffiana* with the biomass of diatoms (Pz = -0.15, $P = 0.062$), but this path was retained because it was marginally significant. Decreased winter precipitation was associated with increased omnivorous *H. arcticus* (Pz = 0.28, $P = 0.002$), which reduced diatoms (Pz = -0.30, $P < 0.001$). *H. arcticus* were expected to reduce rotifer biomass, but this correlation was not significant ($r = -0.17$, $P = 0.274$; Appendix 3-1), so this path was not included in the model.

Stoichiometry-zooplankton model

All pathways among physical, dissolved nutrient, and zooplankton variables were the same as in the phytoplankton-zooplankton model (Figure 3-5; Appendix 3-3). Only new pathways involving PC:PP and PC:phyto are reported for the stoichiometry-zooplankton model below. SE modeling revealed that PC:PP was most strongly associated with *D. middendorffiana* and rotifers (Pz = 0.44, $P < 0.001$ and Pz = 0.69, $P < 0.001$, respectively). PC:PP also increased along with lake temperatures (Pz = 0.17, $P < 0.001$), reduced summer precipitation (Pz = 0.12, $P < 0.001$), and increased DOC (Pz = 0.12, $P < 0.001$). Unexpectedly, higher TDP was also linked with increased PC:PP (Pz = 0.48, $P < 0.001$).

Associations with PC:phyto and other variables in the food web were similar to those with PC:PP. Warming and increased concentrations of TDP were correlated with increased PC:phyto (Pz = 0.17, $P = 0.008$ and Pz = 0.45, $P < 0.001$, respectively; Figure 3-5; Appendix 3-3). *D. middendorffiana* and rotifers also increased along with PC:phyto (Pz = 0.25, $P = 0.002$ and Pz = 0.23, $P = 0.003$, respectively). In contrast, *H. arcticus* was negatively linked with PC:phyto (Pz = -0.19, $P = 0.002$).

Although zooplankton reproduction was not considered in the SEM, bivariate correlations suggested that eggs per female *D. middendorffiana* decreased slightly with increased PC:PP ($r = -0.27$, $P = 0.273$; Appendix 3-1), although this correlation was not significant. In contrast, eggs per rotifer decreased significantly with increased PN:PP ($r = -0.66$, $P = 0.003$).

Discussion

SEM enabled me to elucidate the direct and indirect effects of long-term variation in temperature and precipitation on the chemical, physical, and biological components of a remote alpine lake. Changes in precipitation were associated with variation in lake temperatures and concentrations of dissolved nutrients, which favored diatoms relative to mixotrophic phytoflagellates and higher particulate C:P ratios. Warmer air and water temperatures were correlated with higer abundance of cladocerans, while advanced ice-off due to decreased winter precipitation was associated with an increase in calanoid copepods. Below, I discuss potential mechanisms underlying these changes and forecast possible changes to oligotrophic cold-water lakes under climate change.

Increased lake temperatures via warming and decreased snow pack

My results suggest that alpine lakes will become warmer as air temperatures increase and the snowpack in western North America decreases as predicted (Plummer et al. 2006, Shepherd et al. 2010). SEM revealed that winter precipitation, which fell mostly as snow in our dataset, was associated with a substantial decline in summer lake temperature. Global climate models predict warmer winter temperatures, resulting in more winter precipitation falling as rain instead of snow in western Canada over the next 100 years (Shepherd et al. 2010), which could reduce cold meltwater inputs into alpine lakes later in summer and result in warmer lake temperatures. Under the opposite conditions, high winter precipitation led to colder summer lake temperatures in Austrian mountain lakes; this was attributed to increased inputs of cold meltwater (Thompson et al. 2005). Likewise, in some Norwegian mountain lakes high winter precipitation reduced summer lake temperatures by delaying ice-off (Borgstrøm 2001).

Increased dissolved P relative to C via reduced precipitation

The findings also suggest that expected drier summers may result in alpine lakes having less allochthonous inputs of DOC, and higher concentrations of TDP owing to slower flushing rates. A recent study also found that, under somewhat different conditions, high winter snowpack increased mean annual concentrations of DOC while diluting TDP concentrations in Pipit Lake and three other alpine lakes nearby (Parker et al. 2008). While I found that summer precipitation was most strongly associated with concentrations of DOC, the previous study indicated that high winter snowpack increased DOC. This apparent difference was explained by the fact that I defined summer as May through October (6 months) under the assumption that precipitation and catchment runoff in early spring would enter the lake before ice-off, whereas the earlier study defined the summer season as June through September (4 months). Thus, reduced precipitation in the early ice-free period appeared to be important for decreasing inputs of DOC relative to TDP in alpine lakes in our study area.

Increased autotrophs relative to mixotrophs under drier conditions

The SEM indicated that the expected decreases in summer precipitation will favour autotrophic diatoms relative to mixotrophic phytoflagellates in alpine lakes in our study area. Drier summer conditions appear to favor production of diatoms over phytoflagellates in alpine lakes by reducing flushing rates and thereby reducing dilution of dissolved P. Although diatoms were not directly correlated with concentrations of TDP, diatoms and TDP both increased with reduced summer precipitation. Under opposing circumstances, mixotrophic phytoflagellates may outcompete diatoms when concentrations of P are reduced, since phytoflagellates are able to acquire nutrients via phagotrophy (Rothhaupt 1996). Indeed, mixotrophs increased in Lake Constance, Germany, following reductions of external P-loading (Kamjunke et al. 2007).

Advanced ice-off following reduced winter snow pack was also associated with an increase in diatoms relative to phytoflagellates in our study. Similarly, a one-month advance in ice-off suppressed phytoflagellates and advanced the spring diatom bloom in the Müggelsee, Germany, from 1979–2003 (Adrian et al. 2006). Mixotrophic phytoflagellates may persist in low-light under ice because they use phagotrophy to supplement their nutrient uptake (Rothhaupt 1996). Indeed, phytoflagellates were found to be more abundant than diatoms under ice in a mountain lake in Switzerland (Winder et al. 2003). Mixotrophic phytoflagellates were more abundant in boreal lakes at the Experimental Lakes Area (northwestern Ontario) during the 1990s when lakes were warmer, more transparent, and had lower P concentrations due to low stream flows (Findlay et al. 2001). These findings suggest that diatoms are more competitive than phytoflagellates under higher light and higher P conditions, such as occurs in most lakes after ice-off in spring.

There was no net change in biomass of either phytoflagellate group (dinophytes and chrysophytes + cryptophytes) because positive correlations with warmer temperatures were offset by negative correlations with decreased winter precipitation. Phytoflagellate production is expected to increase with warming because phagotrophic feeding rates increase with temperature (Jones and Rees 1994). In the absence of changes to dissolved nutrients, warming has also increased biomass of phytoflagellates from alpine lakes and ponds in the study area in experimental mesocosms and microcosms (Strecker et al. 2004, Thompson et al. 2008). In my study, however, reduced winter precipitation counteracted the positive association between warming and phytoflagellates, likely by advancing the ice-free season. Warming was also associated with increased densities of *Daphnia*, which in turn had stronger grazing effects on phytoflagellates than on diatoms. Thus, the cumulative effects of warming and drought conditions are predicted to increase biomass of autotrophic diatoms relative to potentially mixotrophic phytoflagellates in alpine lakes in the study area. In deeper lakes, however, warming may intensify thermal stratification, reducing upwelling of dissolved P, and promote small phytoflagellates and diatoms that are more competitive under lower P conditions and less turbulent mixing (Winder and Sommer 2012).

Increased herbivores and omnivores by warming and advanced ice-off

Warming alone was associated with significantly greater abundance of daphnids, whereas abundance of calanoid copepods was not correlated with temeprature in our study lake. Summer lake temperatures had a long-term average of 7.7 °C ± 2.5 SD and ranged up to 14 °C during the study period. I expected that warming would stimulate asexually reproducing cladocerans relative to sexually reproducing copepods because the latter have slower growth rates and longer generation times (Allan 1976). In previous experiments, warming *in vitro* from 8 °C to 15 °C and from 10 °C to 17 °C increased production of *D. middendorffiana* but decreased production of *H. arcticus* from Pipit Lake (Thompson et al. 2008; R.P.W. unpublished data). More extreme warming *in vitro* from 10 °C to 20 °C suppressed both *D. middendorffiana* and *H. arcitcus* from Pipit (Holzapfel and Vinebrooke 2005). Thus, long-term SEM results combined with previous *in vitro* experiments suggest warming up to 14 ^{o}C should increase production of *D. middendorffiana*; however, more extreme warming up to 20 ^{o}C could potentially decrease both *D. middendorffiana* and *H. arcticus* in alpine lakes.

My findings also indicate that advanced ice-off due to reductions in mountain snowpack should stimulate *H. arcticus*. Delayed ice-off can increase mortality of over-wintering adults that survive on lipids stored from the previous summer (Vanderploeg et al. 1998). Late ice-off can also delay hatching of copepod resting eggs and reduce the number of individuals that reach maturity. For example, a second generation of *Leptodiaptomus sicilis* failed to reach maturity by the end of the ice-free season in subalpine Pyramid Lake, Jasper National Park, Alberta (Herzig et al. 1980). Advanced ice-off due to climate warming may thus increase survivorship and production of calanoid copepods because they have long and complex life histories relative to faster-growing asexually reproducing cladocerans. In contrast, effects of warming appeared to be more important than winter snow pack and season length for explaining variation in biomass of *Daphnia*.

I did not detect any significant climatic effects on rotifer abundance in Pipit Lake. This finding runs counter to evidence that warming by +4 ^{o}C (i.e., from 15 ^{o}C to 19 ^{o}C average summer water temperature) stimulated *Keratella cochlearis* and *Conochilus unicornis* in nearby alpine ponds (Strecker et al. 2004). In the present study, warming may have increased predation rates of *H. arcticus* on rotifers (e.g., Uye and Kayano 1994) and subsequently offset any increases in rotifer production due to warming. Indeed, *H. arcticus* suppressed non-armored rotifers in mesocosms and in a survey of 69 mountain lakes in Alberta (Paul et al. 1995). Experimental reintroduction of *H. arcticus* also suppressed rotifer biomass in nearby Snowflake Lake (McNaught et al. 1999). In Pipit Lake, rotifers appeared to decline slightly with increased densities of *H. arcticus*, but this correlation was not significant. It is possible that pooling of rotifer species into one group obscured the negative effect of *H. arcticus* predation on non-armored taxa. That is, increased abundance of *H. arcticus* may have increased predation on non-armored rotifers and favoured armoured taxa.

Decreased particulate P-content via consumer nutrient recycling and producer composition

The findings suggest that an increase in abundance of P-rich *Daphnia* relative to *Hesperodiaptomus* will reduce P recycling to the water column and suppress particulate P content relative to particulate C. In fact, the strongest pathways in my models were those that linked higher densities of *D. middendorffiana* and rotifers to increased PC:PP. Compared to copepods, cladocerans generally have higher P-requirements for growth, retain more P in their biomass, and thus excrete less P (Sterner and Hessen 1994, Sommer and Sommer 2006). Rotifers also appeared to decrease particulate P-content in a manner similar to daphnids.

Increased TDP unexpectedly increased PC:PP, which may be explained by changes in plankton composition. For example, increased TDP coincided with increased diatoms, which in turn may have been higher in PC:PP than phytoflagellates. Chrysophytes and cryptophytes appeared to be low in PC:PP ($R^2 = -0.26$, $P = 0.191$; Appendix 3-1), but this association was not significant. Increased TDP was also significantly associated with increased PC:phyto, suggesting there may have been an increase in non-algal biomass such as bacteria (excluding cyanophytes), which may outcompete phytoplankton for limited P (Danger et al. 2007). Increases in PC:PP above 500 may ultimately limit production of P-limited grazers in alpine lakes (Sterner and Hessen 1994, Urabe et al. 2003). Average PC:PP ratios in our study were 440 ± 317 SD (Table 3-1), which may explain why increased PC:PP did not reduce densities of grazers.

Increased lake temperatures due to continued climate warming can also intensify the mineralization of C-rich dissolved organic matter and potentially increase particulate C relative to P in lakes (van de Waal et al. 2010). Increases in both water temperature and DOC were significantly associated with increased particulate C relative to P in our study. Similarly, DOC in a Finnish lake was mineralized to CO_2 by solar radiation and bacteria, which increased phytoplankton uptake of dissolved C relative to P, and thus increased PC:PP (Vähätalo et al. 2003). However, correlations with particulate P-content and nutrient recycling by grazers were much stronger than correlations with DOC and particulate P content.

Considerations: Predicting future changes from past variation

Future changes in precipitation and temperature may differ from the trends observed in my dataset, thus resulting in different effects on alpine lake food webs. Although I modeled the effects of summer and winter drought over a 16-y period, longer term (e.g., 50–100 y) changes in seasonal precipitation are expected to be characterized by decreased summer precipitation and increased winter precipitation falling as rain versus snow, which may increase catchment runoff early in the ice-free season (Shepherd et al. 2010). Very little winter precipitation fell as rain in our dataset, so we could not model this scenario. Further, long-term warming over many decades may be accompanied by increased soil and vegetation cover of alpine catchments and increased terrestrial nutrient inputs into alpine lakes (Bunting et al. 2010), which are trends that were not observed over the 16-y dataset. In addition, more extreme warming events (e.g., with temperatures up to 15–20 ^{o}C) outside of the range of daily water temperatures observed in our dataset (3–14 ^{o}C) may suppress large-bodied zooplankton species that are alpine specialists (*D. middendorffiana* and *H. arcticus*) and promote smaller-bodied taxa (Holzapfel and Vinebrooke 2005). In addition, it is also important to limit generalizations from my findings to similar lakes in our study area because our model results were from a single lake. However, these data were collected over a fairly long time period (16 years) from a remote lake in an undisturbed landscape; such conditions are considered ideal for detecting the effect of climate change on aquatic ecosystems (Battarbee 2009).

In this study, variation in climate and lake food web variables were analyzed independently of inter- and intra-annual variation, which had certain benefits relative to a temporal anlysis. A previous study by Parker et al. (2008) analyzed variation in climate conditions, lake chemistry, and phytoplankton composition in four alpine lakes in our study area (including Pipit Lake). They identified years with significantly high winter snowpack, low summer precipitation, and more dilute lake conditions (i.e., cold and dry conditions; 2001, 2002, 2003) relative to years with low winter snowpack and high summer precipitation (i.e., cold and wet conditions; 1993, 1998, 1999). In contrast, SEM was used in the present study to model the effects of expected future climate change with both warmer and drier conditions. This approach had the advantage of providing a mechanistic understanding of the direct and indirect effects of *individual* climate factors using SEM. This approach also utilized data across all available years from 1991-2006.

Conclusions: Cumulative effects of warmer and drier conditions

My findings suggest that decreasing snow pack and advanced ice-off will amplify the effects of warming on alpine lake temperatures. Decreased summer precipitation appears to reduce catchment inputs of DOC, decrease flushing rates, and thereby increase the retention of dissolved P, which coincided with increased production of autotrophic diatoms. Warming was positively associated with mixotrophic phytoflagellates but this was counterbalanced by a negative association with advanced ice-off due to decreased snow pack. Increased autotrophic production may increase transfer efficiency of energy to zooplankton by eliminating a trophic step between dissolved nutrients, bacteria, and mixotrophic phytoflagellates (Jansson et al. 2007). Further, warming by itself should increase herbivorous *D. middendorffiana*, which will heighten grazing pressure on phytoflagellates and reduce the P-content of the particulate nutrient pool via increased P-retention by daphnids. Earlier ice-off will promote *H. arcticus*, which will increase grazing pressure on diatoms. Overall, warming, reduced winter snowpack, and decreased summer precipitation should stimulate production of autotrophs relative to mixotrophs, and increase biomass of zooplankton herbivores and omnivores in alpine lakes where conditions are similar to those in our study lake. It is also important to consider that more extreme climate change over longer time periods (outside the range in our dataset) may have different effects on alpine lake food webs.

References

Adrian, R., S. Wilhelm, and D. Gerten. 2006. Life-history traits of lake plankton species may govern their phenological response to climate warming. Global Change Biology 12:652–661.

Allan, J. D. 1976. Life history patterns in zooplankton. American Naturalist 110:165–180.

Arbuckle, J. 2009. Amos 18 user's guide. . SPSS Inc., Chicago, USA.

Arhonditsis, G. B., C. A. Stow, L. J. Steinberg, M. A. Kenney, R. C. Lathrop, S. J. McBride, and K. H. Reckhow. 2006. Exploring ecological patterns with structural equation modeling and Bayesian analysis. Ecological Modelling 192:385–409.

Battarbee, R. W. 2009. Threatened and stressed mountain lakes of Europe: Assessment and progress. Aquatic Ecosystem Health & Management 12:118–128.

Blenckner, T., R. Adrian, D. M. Livingstone, E. Jennings, G. A. Weyhenmeyer, D. G. George, T. Jankowski, M. Jarvinen, C. N. Aonghusa, T. Noges, D. Straile, and K. Teubner. 2007. Large-scale climatic signatures in lakes across Europe: a meta-analysis. Global Change Biology 13:1314–1326.

Blomqvist, P., M. Jansson, S. Drakare, A. K. Bergstrom, and L. Brydsten. 2001. Effects of additions of DOC on pelagic biota in a clearwater system: Results from a whole lake experiment in northern Sweden. Microbial Ecology 42:383–394.

Borgstrøm, R. 2001. Relationship between spring snow depth and growth of brown trout, *Salmo trutta*, in an alpine lake: Predicting consequences of climate change. Arctic, Antarctic, and Alpine Research 33:476–480.

Bradley, R. S., F. T. Keimig, and H. F. Diaz. 2004. Projected temperature changes along the American cordillera and the planned GCOS network. Geophysical Research Letters 31:1–4.

Bunting, L., P. R. Leavitt, R. P. Weidman, and R. D. Vinebrooke. 2010. Regulation of the nitrogen biogeochemistry of mountain lakes by subsidies of terrestrial dissolved organic matter and the implications for climate studies. Limnology and Oceanography 55:333–345.

Danger, M., J. Leflaive, C. Oumarou, L. Ten-Hage, and G. Lacroix. 2007. Control of phytoplankton-bacteria interactions by stoichiometric constraints. Oikos 116:1079–1086.

Findlay, D. L., S. E. M. Kasian, M. P. Stainton, K. G. Beaty, and M. Lyng. 2001. Climatic influences on algal populations of boreal forest lakes in the Experimental Lakes Area. Limnology and Oceanography 46:1784–1793.

Gillooly, J. F. 2000. Effect of body size and temperature on generation time in zooplankton. Journal of Plankton Research 22:241–251.

Grace, J. B. 2006. Structural equation modeling and natural systems, 1st edition. Cambridge University Press, Cambridge, UK.

Grace, J. B., and K. A. Bollen. 2005. Interpreting the results from multiple regression and structural equation models. Bulletin of the Ecological Society of America 86:283 – 295.

Gudimov, A., E. O'Connor, M. Dittrich, H. Jarjanazi, M. E. Palmer, E. Stainsby, J. G. Winter, J. D. Young, and G. B. Arhonditsis. 2012. Continuous bayesian network for studying the causal links between phosphorus loading and plankton patterns in Lake Simcoe, Ontario, Canada. Environmental Science and Technology 46:7283–7292.

Hampton, S. E. 2005. Increased niche differentiation between two *Conochilus* species over 33 years of climate change and food web alteration. Limnology and Oceanography 50:421–426.

Healey, F. P., and L. L. Hendzel. 1980. Physiological indicators of nutrient deficiency in lake phytoplankton. Canadian Journal of Fisheries and Aquatic Sciences 37:442–453.

Herzig, A., R. S. Anderson, and D. W. Mayhood. 1980. Production and population-dynamics of *Leptodiaptomus-sicilis* in a mountain lake in Alberta, Canada. Holarctic Ecology 3:50–63.

Hobbs, W. O., S. V. Lalonde, R. D. Vinebrooke, K. O. Konhauser, R. P. Weidman, M. D. Graham, and A. P. Wolfe. 2010. Algal-silica cycling and pigment diagenesis in recent alpine lake sediments: mechanisms and paleoecological implications. Journal of Paleolimnology 44:613–628.

Holzapfel, A. M., and R. D. Vinebrooke. 2005. Environmental warming increases invasion potential of alpine lake communities by imported species. Global Change Biology 11:2009–2015.

Huang, S., P. M. Rich, R. L. Crabtree, C. S. Potter, and P. Fu. 2008. Modeling monthly near-surface air temperature from solar radiation and lapse rate: application over complex terrain in Yellowstone National Park. Physical Geography 29:158–178.

IPCC. 2007. International Panel on Climate Change - Fourth assessment report - The physical science basis: Regional climate projections. World Meteorological Organization, United Nations.

Jansson, M., L. Persson, A. M. De Roos, R. I. Jones, and L. J. Tranvik. 2007. Terrestrial carbon and intraspecific size-variation shape lake ecosystems. Trends in Ecology and Evolution 22:316–322.

Jones, R. I., and S. Rees. 1994. Influence of temperature and light on particle ingestion by the fresh-water phytoflagellate *Dinobryon*. Archiv Fur Hydrobiologie 132:203–211.

Kamjunke, N., T. Henrichs, and U. Gaedke. 2007. Phosphorus gain by bacterivory promotes the mixotrophic flagellate *Dinobryon* spp. during re-oligotrophication. Journal of Plankton Research 29:39–46.

Kopacek, J., J. Hejzlar, J. Vrba, and E. Stuchlik. 2011. Phosphorus loading of mountain lakes: Terrestrial export and atmospheric deposition. Limnology and Oceanography 56:1343–1354.

McNaught, A. S., D. W. Schindler, B. R. Parker, A. J. Paul, R. S. Anderson, D. B. Donald, and M. Agbeti. 1999. Restoration of the food web of an alpine lake following fish stocking. Limnology and Oceanography 44:127–136.

Morris, K., M. Jeffries, and C. Duguay. 2005. Model simulation of the effects of climate variability and change on lake ice in central Alaska, USA. Annals of Glaciology 40:113–118.

Murphy, C. A., P. L. Thompson, and R. D. Vinebrooke. 2010. Assessing the sensitivity of alpine lakes and ponds to nitrogen deposition in the Canadian Rocky Mountains. Hydrobiologia 648:83–90.

Nauwerck, A. 1963. Die beziehungen zwischen zooplankton und phytoplankton im See Erken. Symbolae Botanicae Upsalienses 17:1–163.

Ormerod, S. J., M. Dobson, A. G. Hildrew, and C. R. Townsend. 2010. Multiple stressors in freshwater ecosystems. Freshwater Biology 55:1–4.

Parker, B. R., and D. W. Schindler. 2006. Cascading trophic interactions in an oligotrophic species-poor alpine lake. Ecosystems 9:157–166.

Parker, B. R., R. D. Vinebrooke, and D. W. Schindler. 2008. Recent climate extremes alter alpine lake ecosystems. Proceedings of the National Academy of Sciences of the United States of America 105:12927–12931.

Paul, A. J., P. R. Leavitt, D. W. Schindler, and A. K. Hardie. 1995. Direct and indirect effects of predation by a calanoid copepod (subgenus: *Hesperodiaptomus*) and of nutrients in a fishless alpine lake. Canadian Journal of Fisheries and Aquatic Sciences 52:2628–2638.

Pennak, R. W. 1978. Fresh-water invertebrates of the United States, 2nd edition. Wiley, University of Michigan.

Pettersson, K., K. Grust, G. Weyhenmeyer, and T. Blenckner. 2003. Seasonality of chlorophyll and nutrients in Lake Erken - effects of weather conditions. Hydrobiologia 506:75–81.

Plummer, D. A., D. Caya, A. Frigon, H. Cote, M. Giguere, D. Paquin, S. Biner, R. Harvey, and R. de Elia. 2006. Climate and climate change over North America as simulated by the Canadian RCM. Journal of Climate 19:3112–3132.

Rothhaupt, K. O. 1996. Laboratory experiments with a mixotrophic chrysophyte and obligately phagotrophic and phototrophic competitors. Ecology 77:716–724.

Schindler, D. W., and B. R. Parker. 2002. Biological pollutants: alien fishes in mountain lakes. Water, Air, and Soil Pollution 2:379–397.

Schneider, P., and S. J. Hook. 2010. Space observations of inland water bodies show rapid surface warming since 1985. Geophysical Research Letters 37.

Schumacker, R. E. 2006. Conducting specification searches with AMOS. Structural Equation Modeling 13:118–129.

Shepherd, A., K. M. Gill, and S. B. Rood. 2010. Climate change and future flows of Rocky Mountain rivers: converging forecasts from empirical trend projection and down-scaled global circulation modelling. Hydrological Processes 24:3864–3877.

Shimoda, Y., M. E. Azim, G. Perhar, M. Ramin, M. A. Kenney, S. Sadraddini, A. Gudimov, and G. B. Arhonditsis. 2011. Our current understanding of lake ecosystem response to climate change: What have we really learned from the north temperate deep lakes? Journal of Great Lakes Research 37:173–193.

Sommer, U., and F. Sommer. 2006. Cladocerans versus copepods: the cause of contrasting top-down controls on freshwater and marine phytoplankton. Oecologia 147:183–194.

Sterner, R. W., and D. O. Hessen. 1994. Algal nutrient limitation and the nutrition of aquatic herbivores. Annual Review of Ecology and Systematics 25:1–29.

Strecker, A. L., T. P. Cobb, and R. D. Vinebrooke. 2004. Effects of experimental greenhouse warming on phytoplankton and zooplankton communities in fishless alpine ponds. Limnology and Oceanography 49:1182–1190.

Thompson, P. L., M. C. St-Jacques, and R. D. Vinebrooke. 2008. Impacts of climate warming and nitrogen deposition on alpine plankton in lake and pond habitats: an *in vitro* experiment. Arctic, Antarctic, and Alpine Research 40:192–198.

Thompson, R., C. Kamenik, and R. Schmidt. 2005. Ultra-sensitive alpine lakes and climate change. Journal of Limnology 64:139–152.

Urabe, J., J. Togari, and J. J. Elser. 2003. Stoichiometric impacts of increased carbon dioxide on a planktonic herbivore. Global Change Biology 9:818–825.

Uye, S., and Y. Kayano. 1994. Predatory feeding-behavior of *Tortanus* (Copepoda, Calanoida) - life-stage differences and the predation impact on small planktonic crustaceans. Journal of Crustacean Biology 14:473–483.

Vanderploeg, H. A., J. F. Cavaletto, J. R. Liebig, and W. S. Gardner. 1998. *Limnocalanus macrurus* (Copepoda: Calanoida) retains a marine arctic lipid and life cycle strategy in Lake Michigan. Journal of Plankton Research 20:1581–1597.

Vähätalo, A. V., K. Salonen, U. Münster, M. Järvinen, and R. G. Wetzel. 2003. Photochemical transformation of allochthonous organic matter provides bioavailable nutrients in a humic lake. Archiv Fur Hydrobiologie 156:287–314.

van de Waal, D. B., A. M. Verschoor, J. M. H. Verspagen, E. van Donk, and J. Huisman. 2010. Climate-driven changes in the ecological stoichiometry of aquatic ecosystems. Frontiers in Ecology and the Environment 8:145–152.

Wagner, C., and R. Adrian. 2009. Exploring lake ecosystems: hierarchy responses to long-term change? Global Change Biology 15:1104–1115.

Ward, B., and G. C. Whipple. 1966. Freshwater biology, 2nd edition. John Wiley and Sons, Inc., New York.

Weidman, R. P., D. W. Schindler, and R. D. Vinebrooke. 2011. Pelagic food web interactions among benthic invertebrates and trout in mountain lakes. Freshwater Biology 56:1081–1094.

Wells, B. K., J. C. Field, J. A. Thayer, C. B. Grimes, S. J. Bograd, W. J. Sydeman, F. B. Schwing, and R. Hewitt. 2008. Untangling the relationships among climate, prey and top predators in an ocean ecosystem. Marine Ecology-Progress Series 364:15–29.

Wilhelm, F. M., and D. W. Schindler. 1999. Effects of *Gammarus lacustris* (Crustacea : Amphipoda) on plankton community structure in an alpine lake. Canadian Journal of Fisheries and Aquatic Sciences 56:1401–1408.

Winder, M., H. R. Burgi, and P. Spaak. 2003. Mechanisms regulating zooplankton populations in a high-mountain lake. Freshwater Biology 48:795–809.

Winder, M., and D. E. Schindler. 2004. Climate change uncouples trophic interactions in an aquatic ecosystem. Ecology 85:2100–2106.

Winder, M., and U. Sommer. 2012. Phytoplankton response to a changing climate. Hydrobiologia 698:5–16.

Table 3-1. Long-term average conditions for Pipit Lake (1991–2006) including climatic conditions, lake temperature, dissolved and particulate nutrient concentrations and stoichiometry, phytoplankton biomass, and zooplankton densities.

Category	Within models	Variable	Mean	SD	Min	Max
Climate	yes	Degree days (DegDay; ^{o}C)	701.3	202.4	780.1	1158.2
	yes	Winter precipitation (WinPpt; mm)	230.1	58.4	115.0	354.1
	yes	Summer precip. (SumPpt; mm)	210.2	80.1	84.3	451.0
Temperature & chemistry	yes	Summer water temperature (^{o}C)	7.7	2.5	2.8	13.9
	yes	Dissolved organic C (mg·L^{-1})	0.7	0.4	0.1	2.0
	yes	Total dissolved P (TDP; µg·L^{-1})	2.7	1.1	0.7	6.3
		Dissolved inorg. N (DIN; µg·L^{-1})	72.2	24.7	16.7	145.1
		DIN:TDP (molar)	73.4	50.7	22.0	300.0
Particulate nutrients		Particulate C (PC; µg·L^{-1})	226.5	103.9	86.2	451.6
		Particulate N (PN; µg·L^{-1})	24.9	11.9	8.1	49.2
		Particulate P (PP; µg·L^{-1})	1.7	1.1	0.2	6.7
	yes	PC:PP (molar)	440.6	316.7	116.0	1620.0
		PN:PP (molar)	41.0	32.2	4.0	141.0
		PC:PN (molar)	11.0	2.4	8.2	20.9
	yes	PC:phyto. (PC:phyto; mass)	5.9	9.1	0.2	36.1
Phytoplankton		Total phytop (Phyto; µg·L^{-1})	284.1	685.7	0.1	3996.6
	yes	Dinoflagellates (µg·L^{-1})	162.6	505.7	0.0	2794.0
	yes	Diatoms (µg·L^{-1})	84.1	220.9	0.0	1194.0
	yes	Chryso.+Crypto. (µg·L^{-1})	9.4	17.4	0.0	85.2
		Cyanophytes (µg·L^{-1})	1.1	3.4	0.0	15.0
		Chlorophytes (µg·L^{-1})	0.9	3.8	0.0	24.0
		Chlorophyll-*a* (µg·L^{-1})	0.5	0.3	0.0	1.5
		Phyto size ratio (Ratio < 30 µm^{3})	1.4	2.4	0.0	13.3
Zooplankton	yes	*Daphnia middendorffiana* (#·L^{-1})	0.10	0.10	0.00	0.37
		D. middendorffiana eggs (#·fem^{-1})	0.22	0.30	0.00	1.08
	yes	Total rotifers (#·L^{-1})	0.26	0.62	0.00	3.26
		Rotifer eggs (#·individual^{-1})	0.13	0.24	0.00	1.00
	yes	*Hesperodiaptomus arcticus* (#·L^{-1})	0.34	0.21	0.05	0.79
		H. arcticus eggs (#·fem^{-1})	2.09	1.06	0.20	5.06

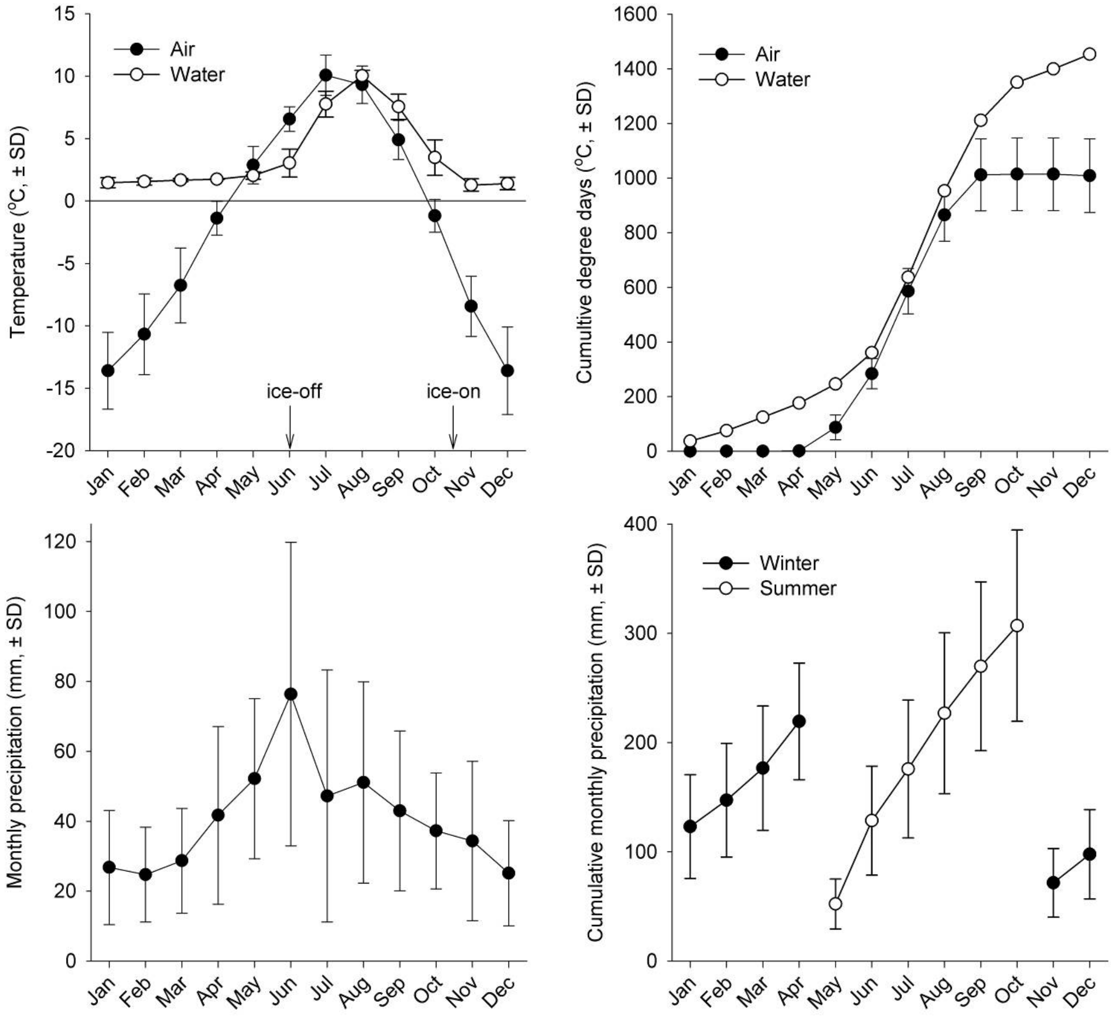

Figure 3-1. Average air temperature and monthly precipitation (1991–2006) and mean lake temperatures (2006 only) for Pipit Lake

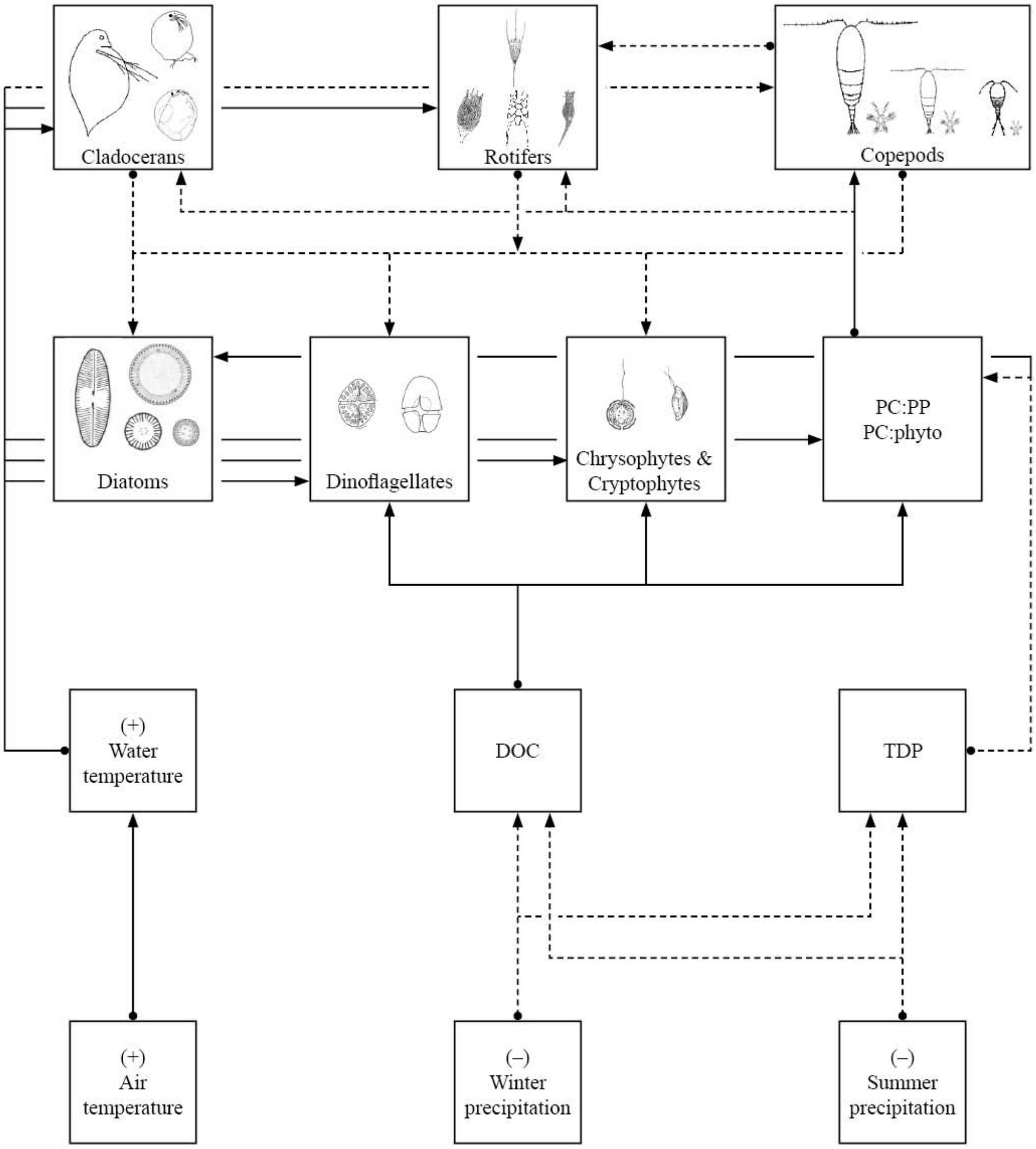

Figure 3-2. Food web model for Pipit Lake. Caption on next page.

Figure 3-2. Food web model for Pipit Lake illustrating the expected effects of air temperature and precipitation on dissolved and particulate nutrients, phytoplankton, and zooplankton. Solid lines indicate positive effects and dashed lines indicate negative effects. A dot indicates the origin of each causal path. Letters A–D indicated the four groups of variables referred to as submodels in the text. Cladocerans illustrated include *Daphnia middendorffiana* (left), *Alona* sp. (upper right), and *Chydorus* sp. (lower right). Rotifers include *Kellicottia longispina* (top), *Notholca* sp. (lower left), *Keratella quadrata* (lower centre), and *Rotaria* sp. (lower right). Copepods include *Hesperodiaptomus arcticus* (left), *Diaptomus tyrelli* (centre), and *Acanthocyclops vernalis* or *Diacyclops bicuspidatus* (right), and larval nauplii are shown to the lower right of each taxa. Diatoms include *Eucocconeis flexella* (left) and several species of *Cyclotella* spp. (right). Dinoflagellates include several species of *Gymnodinium* spp. Chrysophytes and cryptophytes include an unidentified coccoid taxa (left) and *Rhodomonas minuta* (right).

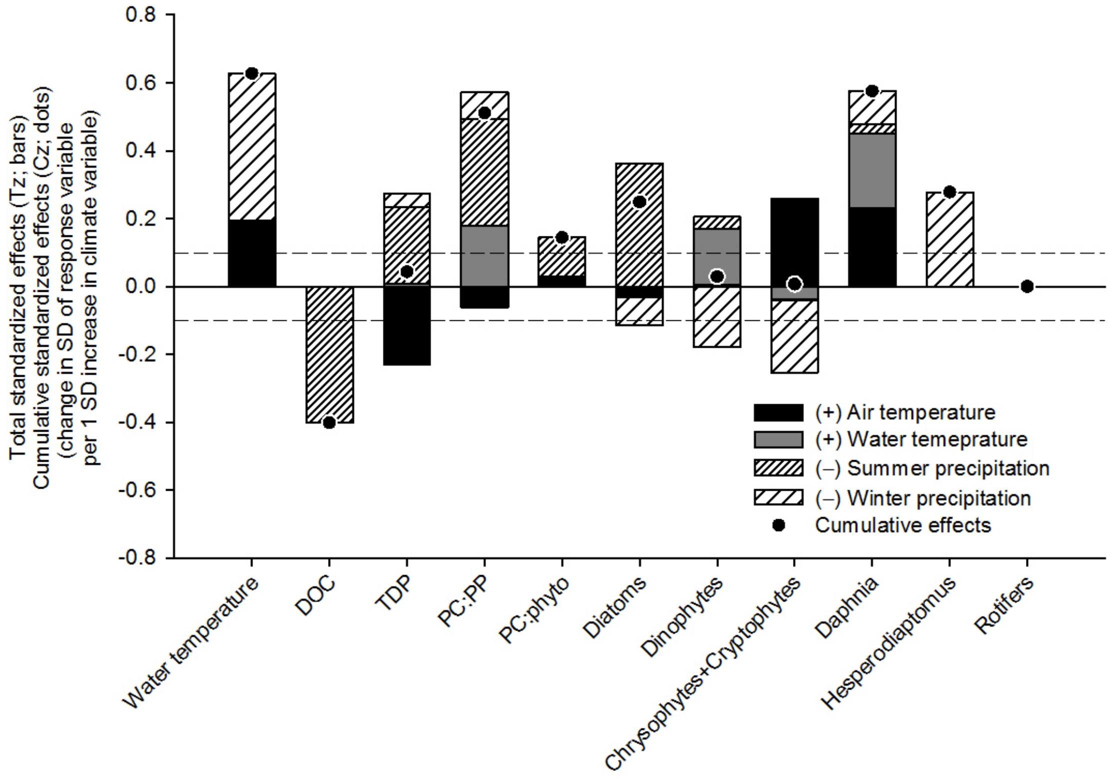

Figure 3-3 Total standardized effects (Tz, including direct and indirect effects) and cumulative climatic effects (Cz) of increased air temperature, increased water temperature, decreased winter precipitation, and decreased summer precipitation on lake temperature, dissolved nutrients, phytoplankton, particulate stoichiometry, and zooplankton in Pipit Lake (1991–2006).

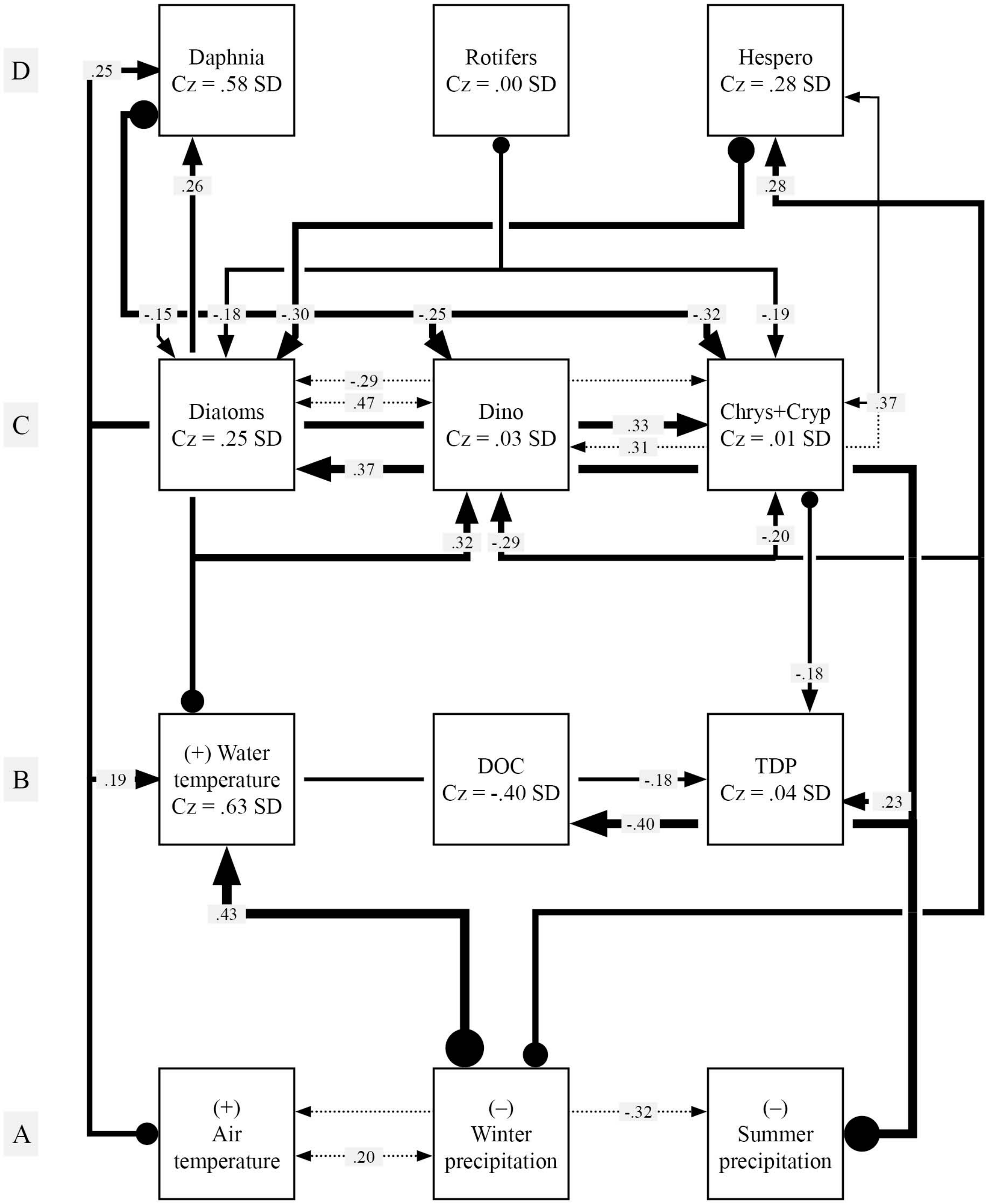

Figure 3-4. Phytoplankton-zooplankton structural equation model. Caption on next page.

Figure 3-4. Phytoplankton-zooplankton structural equation model: Effects of increased air and water temperature, and decreased winter and summer precipitation on lake temperature, dissolved nutrients, phytoplankton, and zooplankton in Pipit Lake (1991–2006). Overall model fit: $X^2 = 49.2$, $P = 0.105$. "Cz" = cumulative climatic effect of all three climate variables plus water temperature. Dotted lines with two-headed arrows indicate non-causal path coefficients equivalent to Pearson's ¬r correlations (i.e., "Pr" in text). Solid lines indicate standardized causal path coefficients (i.e., "Pz" in text). A dot indicates the origin of each causal path. Line weights are relative to the absolute magnitude of Pz. Letters A–D indicated the four groups of variables referred to as submodels in the text.

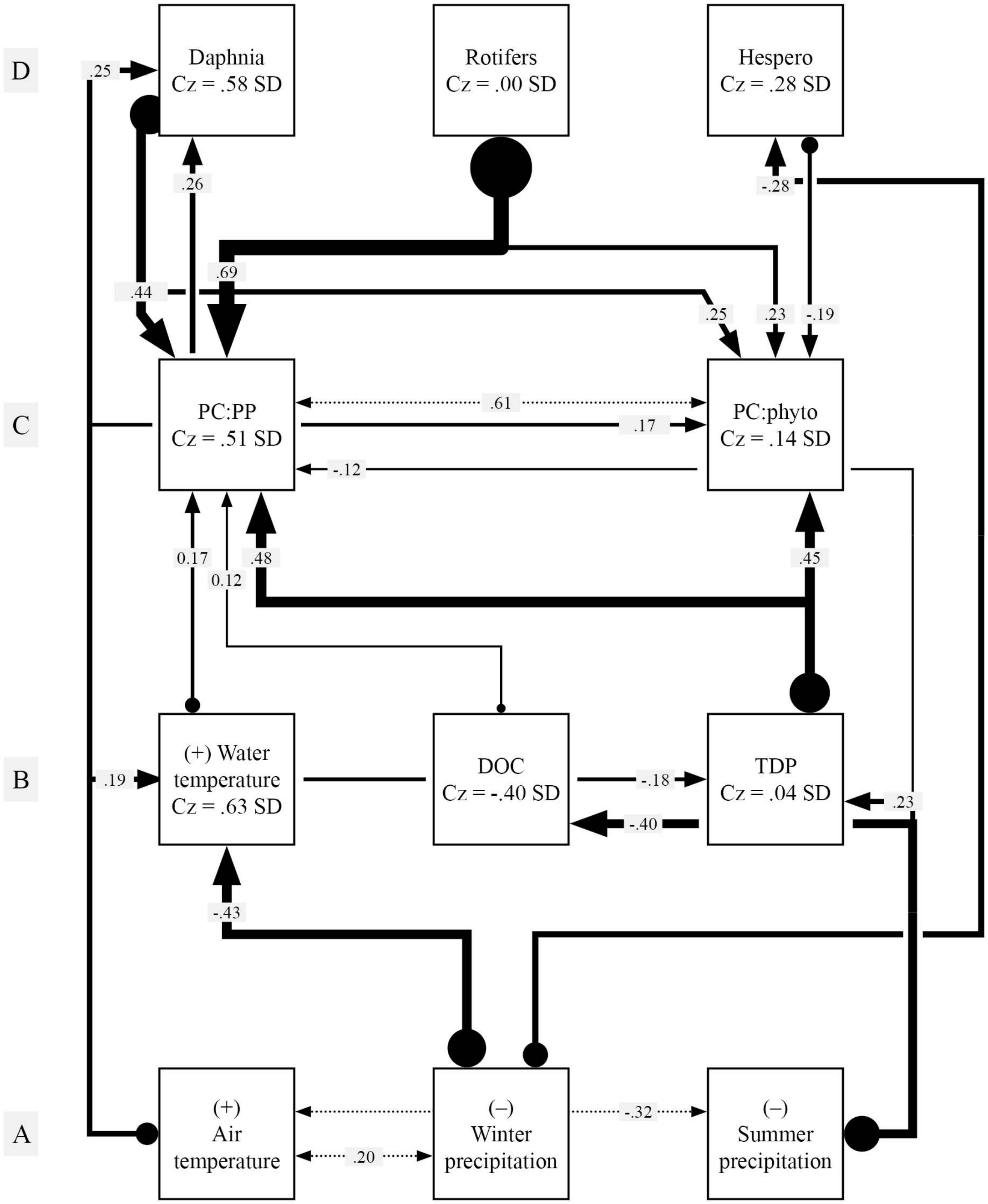

Figure 3-5. Stoichiometry-zooplankton structural equation model: Effects of increased air and water temperature and decreased winter precipitation and summer precipitation on lake temperature, dissolved nutrients, particulate stoichiometry, and zooplankton in Pipit Lake (1991–2006). Overall model fit: X^2 = 41.2, *P* = 0.104. See caption in Figure 4 for explanation.

	Units	**DegDay (air)**	**Snow**	**Rain**	**Temp (water)**	**DOC**	**TDP**	DIN	DIN:TDP	**PC:PP**	PN:PP	PC:PN	**PC:Phyto**	Phyto	**Diat**	**Dino**	**Chrys+Cryp**	Ratio<30um^3	**Daphnia**	Daphnia eggs	**Rotifer**	Rotifer eggs	**Hespero**	Hespero eggs
DegDay (air)	°C																							
Snow	mm	-0.17																						
Rain	mm	**0.20**	0.02																					
Temp (water)	°C	**0.24**	**-0.44**	-0.08																				
DOC	mg·L^{-1}	0.14	0.21	**0.37**	-0.08																			
TDP	µg·L^{-1}	**-0.22**	-0.09	**-0.32**	**0.10**	-0.14																		
DIN	µg·L^{-1}	-0.01	0.11	0.11	0.19	-0.10	-0.04																	
DIN:TDP	molar	0.19	0.21	**0.33**	-0.04	0.20	**-0.76**	**0.55**																
PC:PP	molar	-0.03	-0.09	**-0.30**	**0.17**	**-0.17**	**0.57**	-0.13	**-0.50**															
PN:PP	molar	-0.05	-0.09	-0.29	0.34	-0.18	**0.61**	0.02	**-0.45**	**0.73**														
PC:PN	molar	0.06	0.14	0.15	-0.28	0.26	-0.10	0.01	0.21	0.06	-0.32													
PC:Phyto	mass	**0.09**	-0.08	-0.14	-0.07	-0.14	**0.36**	-0.01	-0.39	**0.57**	**0.49**	-0.33												
Phytop	µg·L^{-1}	-0.09	0.19	-0.27	0.02	-0.19	0.18	0.03	0.05	-0.08	0.32	0.04	-0.38											
Diat	µg·L^{-1}	-0.02	0.04	**-0.39**	-0.04	-0.23	0.14	0.11	0.20	0.07	0.19	-0.20	-0.19	**0.64**										
Dino	µg·L^{-1}	0.02	**0.15**	-0.12	**0.12**	-0.15	0.15	0.04	0.03	-0.10	0.40	-0.13	-0.21	**0.90**	0.40									
Chrys+Cryp	µg·L^{-1}	**0.10**	**0.14**	0.22	-0.14	0.25	**-0.26**	-0.28	0.16	-0.26	-0.21	0.15	-0.14	0.07	-0.23	0.13								
Ratio<30um^3	%	-0.07	0.17	0.05	**-0.33**	-0.14	-0.14	0.18	0.17	-0.05	0.15	-0.18	0.15	0.02	0.31	-0.09	-0.13							
Daphnia	µg·L^{-1}	**0.24**	-0.14	-0.10	**0.33**	-0.14	0.09	0.18	0.08	**0.46**	0.20	0.17	**0.27**	-0.29	**-0.16**	**-0.12**	**-0.24**	-0.01						
Daphnia eggs	egg·fem^{-1}	-0.26	0.09	-0.13	-0.06	0.26	-0.18	-0.19	-0.07	-0.27	0.06	-0.20	0.04	-0.07	-0.20	-0.05	-0.01	-0.17	-0.19					
Rotifer	µg·L^{-1}	0.06	0.08	-0.10	-0.17	-0.12	0.05	-0.15	-0.10	**0.51**	0.24	0.22	**0.23**	-0.24	**-0.14**	-0.17	**-0.25**	0.09	0.04	-0.18				
Rotifer eggs	egg·fem^{-1}	0.11	-0.10	0.13	-0.18	-0.22	-0.33	0.01	0.23	-0.07	**-0.66**	0.27	0.08	-0.21	0.06	-0.22	-0.07	0.10	0.08	-0.28	0.04			
Hespero	µg·L^{-1}	-0.02	**-0.17**	-0.11	0.17	0.05	0.21	-0.18	**-0.38**	0.10	0.47	**-0.46**	**-0.11**	-0.06	**-0.29**	0.25	0.26	-0.18	0.13	0.01	-0.17	-0.19		
Hespero eggs	egg·fem^{-1}	0.18	-0.18	0.10	**0.34**	0.28	0.19	0.06	-0.07	0.20	0.14	-0.06	-0.02	-0.29	-0.13	-0.25	-0.33	0.04	0.18	0.12	0.07	-0.04	-0.10	
Mean resid ln+1	ln	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
SD resid ln+1	ln	0.11	0.24	0.33	0.28	0.23	0.29	0.33	0.47	0.61	0.70	0.18	0.45	1.93	1.87	1.99	0.94	1.50	0.07	0.19	0.29	0.18	0.15	0.37
SD exp(ln)	original	1.12	1.27	1.39	1.32	1.26	1.34	1.39	1.60	1.84	2.01	1.20	1.57	6.89	6.49	7.32	2.56	4.48	1.07	1.21	1.34	1.20	1.16	1.45

Appendix 3-1. Pearson's correlation matrix. Caption on next page.

Appendix 3-1. Pearson's correlation matrix of climate and limnological variables for Pipit Lake (1991–2006) after transformation into unstandardized residuals to removal of temporal autocorrelation (see methods for details). Abbreviations are explained in Table 1. Highlighted variables are used in structural equation models. Highlighted coefficients indicate significant total effects in models. Bold values indicate significant standardized path coefficients (Pz; *P* < 0.05) and underlined values indicate significant bivariate correlations (Pr; *P* < 0.05). Mean values of each variable are equal to zero because data were transformed into unstandardized residuals (ln-transformed). SD values are shown for ln-transformed residuals and in original units for each variable.

Appendix 3-2. Phytoplankton-zooplankton model: Structural equation model path statistics and overall model fit. Double-headed arrows indicate non-causal path coefficients "Pr". Single-headed arrows indicate standardized causal path coefficients "Pz".

Category	Dependent variable	Path	Independent variable	Pr or Pz	*P*-value
Physical	(+) Air temperature	<-->	(−) Winter precipitation	0.20	0.033
	(+) Air temperature	<-->	(−) Summer precipitation	-0.32	0.001
	(+) Water temperature	<---	(+) Air temperature	0.19	0.028
	(+) Water temperature	<---	(−) Winter precipitation	0.43	<0.001
Nutrients	DOC	<---	(−) Summer precipitation	-0.40	<0.001
	TDP	<---	(+) Air temperature	-0.18	0.049
	TDP	<---	(−) Summer precipitation	0.23	0.013
	TDP	<---	Chrysophytes+Cryptophytes	-0.18	0.045
Phytoplankton	Diatoms	<-->	Chrysophytes+Cryptophytes	-0.29	<0.001
	Diatoms	<-->	Dinophytes	0.47	<0.001
	Diatoms	<---	(−) Summer precipitation	0.37	<0.001
	Diatoms	<---	*Hesperodiaptomus*	-0.30	<0.001
	Diatoms	<---	Rotifers	-0.18	0.014
	Diatoms	<---	*Daphnia*	-0.15	0.062
	Dinophytes	<---	(+) Water temperature	0.32	<0.001
	Dinophytes	<---	(−) Winter precipitation	-0.29	<0.001
	Dinophytes	<---	*Daphnia*	-0.25	0.005
	Chrysophytes+Cryptophytes	<---	(+) Air temperature	0.33	<0.001
	Chrysophytes+Cryptophytes	<---	(−) Winter precipitation	-0.20	0.018
	Chrysophytes+Cryptophytes	<---	*Daphnia*	-0.32	<0.001
	Chrysophytes+Cryptophytes	<---	Rotifers	-0.19	0.015
Zooplankton	*Daphnia*	<---	(+) Air temperature	0.25	0.009
	Daphnia	<---	(+) Water temperature	0.26	0.010
	Hesperodiaptomus	<-->	Dinophytes	0.31	<0.001
	Hesperodiaptomus	<-->	Chrysophytes+Cryptophytes	0.37	<0.001
	Hesperodiaptomus	<---	(−) Winter precipitation	0.28	0.002

The overall fit of each submodel of the phytoplankton-zooplankton SE model was accepted using the MLX^2 statistic ($P > 0.05$) except for the last two submodels A+B+C→D and D→C+B+A (Figure 4). Submodel A+B+C→D was not accepted ($MLX^2 = 127.0$, $P < 0.001$) because none of the phytoplankton groups significantly affected zooplankton abundances. In submodel D→C+B+A, each of the zooplankton groups significantly grazed one or more phytoplankton groups, but there was still a high amount of unexplained covariance in this submodel ($MLX^2 = 59.7$, $P = 0.010$).

Inspection of the residual covariance matrix for the submodel D$\rightarrow$C+B+A suggested *H. arcticus* were positively associated with biomass of dinoflagellates and chrysophytes + cryptophytes. Once these pathways were included (Pr = 0.31, $P < 0.001$ and Pr = 0.37, $P < 0.001$, respectively) the overall fit of the final phytoplankton-zooplankton model was accepted (MLX^2 = 49.2, $P = 0.105$, RMSEA = 0.044), which indicated there were no major discrepancies between the model and the observed covariance matrix. Path coefficients re-calculated using the bootstrapping procedure only ranged by -2.7 to +2.2 % from the original coefficients.

Appendix 3-3. Stoichiometry-zooplankton structural equation model path statistics and overall model fit. Double-headed arrows indicate non-causal path coefficients “Pr”. Single-headed arrows indicate standardized causal path coefficients “Pz”.

Category	Dependent Variable	Path	Independent Variable	Pr or Pz	*P*-value
Physical	(+) Air temperature	<-->	(−) Winter precipitation	0.20	0.033
	(+) Air temperature	<-->	(−) Summer precipitation	-0.32	0.001
	(+) Water temperature	<---	(+) Air temperature	0.19	0.028
	(+) Water temperature	<---	(−) Winter precipitation	0.43	<0.001
Nutrients	DOC	<---	(−) Summer precipitation	-0.40	<0.001
	TDP	<---	(+) Air temperature	-0.20	0.028
	TDP	<---	(−) Summer precipitation	0.26	0.006
Stoichiometry	PC:PP	<-->	PC:phytoplankton	0.61	<0.001
	PC:PP	<---	(+) Water temperature	0.17	<0.001
	PC:PP	<---	(−) Summer precipitation	0.12	<0.001
	PC:PP	<---	DOC	0.12	<0.001
	PC:PP	<---	TDP	0.48	<0.001
	PC:PP	<---	*Daphnia*	0.44	<0.001
	PC:PP	<---	Rotifers	0.69	<0.001
	PC:phytoplankton	<---	(+) Air temperature	0.17	0.008
	PC:phytoplankton	<---	TDP	0.45	<0.001
	PC:phytoplankton	<---	*Daphnia*	0.25	0.002
	PC:phytoplankton	<---	Rotifers	0.23	0.003
	PC:phytoplankton	<---	*Hesperodiaptomus*	-0.19	0.002
Zooplankton	*Daphnia*	<---	(+) Air temperature	0.25	0.009
	Daphnia	<---	(+) Water temperature	0.26	0.010
	Hesperodiaptomus	<---	(−) Winter precipitation	0.28	0.003

The overall fit of each submodel of the stoichiometry-zooplankton SE model was accepted using the MLX^2 statistic ($P > 0.05$) with one exception (Figure 5). The second last submodel A+B+C→D was not accepted because there were no significant effects of particulate stoichiometry on any of the zooplankton groups (MLX^2 = 193.5, $P < 0.001$). However, the final submodel D→C+B+A was accepted (MLX^2 = 41.2, $P = 0.104$, RMSEA = 0.046) because there were significant effects of each zooplankton group on one or both particulate stoichiometry variables. Thus, the overall fit of the final stoichiometry-zooplankton SE model indicated there were no major discrepancies between the model and the observed covariance matrix.

Similar to the phytoplankton-zooplankton SE model, path coefficients only varied by -2.5 to 4.0 % from the original coefficients after bootstrapping was conducted. The bootstrapped path coefficients with the greatest variation (4 %) were not significant and were not included in the final SE model.

CHAPTER 4: EXPERIMENT – WARMING AND DISSOLVED ORGANIC CARBON HAVE COUNTERBALANCING EFFECTS ON PHYTOPLANKTON AND PROMOTE CLADOCERANS OVER COPEPODS IN ALPINE LAKES[3]

Introduction

Impacts of higher temperatures and changes in terrestrial nutrient inputs due to global warming should be proportionally larger in alpine lakes compared to warmer, more nutrient-rich lakes (Battarbee 2009). Global circulation models predict continued warming will be greatest at high latitudes and high elevations in the northern hemisphere (Bradley et al. 2004, [IPCC] 2007). In alpine catchments, melting of permanent snow and ice and changing precipitation patterns will alter the thermal and chemical properties of alpine lakes (Hauer et al. 1997). Periodic droughts reduce terrestrial runoff and cause lakes to become warmer and more transparent in boreal and alpine regions in North America (Schindler et al. 1996a, Parker et al. 2008). In contrast, melting of permanent snow and ice and increased soil and vegetation cover in alpine catchments can increase terrestrial inputs into alpine lakes. For example, spatial and paleoecological lake surveys show greater concentrations of dissolved organic matter in mountain lakes with vegetated versus non-vegetated catchments in Alberta and California (Bunting et al. 2010, Sadro et al. 2012). Thus, the purpose of our study was to determine the climatic effects of warming and inputs of dissolved organic C (DOC) on planktonic food webs in alpine lakes.

Climatic effects alter dissolved and particulate nutrient composition in alpine lakes though bottom-up effects on nutrient supply ratios (Vähätalo et al. 2003, van de Waal et al. 2010) and through top-down effects on nutrient recycling by zooplankton (Elser and Urabe 1999). Warming can stimulate the mineralization of DOC and increase availability of its constituent elements at high proportions of C relative to P (van de Waal et al. 2010). Subsequently, decreased particulate P-content could reduce growth rate of P-limited herbivorous cladoceran grazers, which have higher P-requirements than N-rich omnivorous copepods (Sterner and Hessen 1994). In contrast, warming may increase

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[3] This chapter has been submitted to Freshwater Biology

abundance of cladocerans relative to copepods, thereby decreasing recycling of P to the water column by copepods in alpine lakes (Sommer and Sommer 2006).

Climate change may also alter phytoplankton and zooplankton communities in alpine lakes through direct or indirect effects via nutrient composition. Increased levels of DOC may suppress phytoplankton abundance by interactively reducing P-availability and increasing competition with heterotrophs, as has occurred in a whole-lake addition of DOC in an oligotrophic boreal lake in Sweden (Blomqvist et al. 2001). Warming could also indirectly suppress phytoplankton in alpine lakes by increasing zooplankton grazing rates (Moore et al. 1996). Further, warming may directly stimulate faster-growing asexually reproducing cladocerans relative to longer-lived sexually reproducing copepods (Allan 1976). However, smaller sized taxa of both zooplankton groups may also increase in abundance with warming (Gillooly et al. 2002). Direct effects of warming on zooplankton are expected to be more extreme in alpine populations compared to populations in warmer montane lakes at lower elevations (Holzapfel and Vinebrooke 2005).

I used an *in vitro* experiment to test for the interactive effects of warming and DOC additions on plankton communities from two alpine and two montane lakes. I hypothesized that warming and increased DOC would decrease dissolved and particulate P relative to C (van de Waal et al. 2010), thereby suppressing phytoplankton abundance (Blomqvist et al. 2001) and favouring omnivorous copepods over P-limited herbivorous cladocerans (Sterner and Hessen 1994). I also tested the alternative hypothesis that warming directly stimulates faster-growing asexually reproducing cladocerans relative to longer-lived, sexually reproducing copepods (Allan 1976). Further, effects of warming on zooplankton were expected to be greater in alpine lakes than in montane lakes, where populations would be adapted to warmer temperatures (Holzapfel and Vinebrooke 2005).

Methods

Study lakes

I selected four fishless lakes from a limnological database of 379 mountain lakes located in Alberta (Anderson 1968, 1970, 1971, 1972, 1974, Donald et al. 2001). The fishless status of these lakes was confirmed in previous studies (Schindler and Parker 2002, Weidman et al. 2011). Lakes were selected that contained both *Hesperodiaptomus arcticus* (or *Hesperodiaptomus shoshone*) and *Daphnia middendorffiana*, which are important taxa in fishless alpine lakes in Alberta (McNaught et al. 1999). Lakes were also selected to span the widest possible range in elevation (677 m). Mean annual air temperature decreases by about 0.65 ^{o}C per 100 m increase in elevation in mountain regions in western North America (Huang et al. 2008). Thus, this elevation gradient covered a range in local climatic conditions across montane and alpine regions.

Pipit Lake (Banff National Park) is a headwater lake in a cirque basin located above the tree line in an alpine catchment that is partly covered by mosses and peaty soils or exposed rock. Upper Geraldine Lake (Jasper National Park) is third in a glacial series of seven lakes and is located near the tree line of an alpine/subalpine catchment that is partly covered by mature coniferous forest and soils or alpine meadow with alder, willow, grasses and mosses. Middle Rowe Lake (Waterton Lakes National Park) is second in a glacial series of three lakes and is located below the tree line, perched on the ledge of a subalpine/montane catchment that is covered partly by stunted coniferous forest and thin soils or exposed rock. North Summit Lake (Jasper National Park) is located on the bottom of a montane valley and is surrounded by mature mixed deciduous and coniferous forest with well-developed soils.

Pipit and Upper Geraldine represented colder, larger, more oligotrophic alpine lakes, whereas Middle Rowe and North Summit represented warmer, smaller, more nutrient rich montane lakes (Table 4-1; Appendix 4-1 and 4-2). Pipit and Upper Geraldine were about 2−5 times larger by mean and maximum depth and volume, and about 16 times larger by area than Middle Rowe and North Summit. Concentrations of DOC and TDP were about 2−4 times lower in Pipit and Upper Geraldine compared to Middle Rowe and North Summit. Primary production was P-limited in Pipit and Upper

Geraldine (137 DIN:TDP ± 14 SD; by moles) but N-limited in Middle Rowe and North Summit (5 DIN:TDP ± < 1 SD; Healey and Hendzel 1980).

In addition to *H. arcticus* (or *H. shoshone* in Middle Rowe) and *D. middendorffiana* the alpine lakes contained the rotifer *Kellicottia longispina* and the montane lakes contained smaller diaptomid copepods (around 1 mm length); *Diaptomus tyrrelli* occurred in North Summit and *D. arapahoensis* occurred in Middle Rowe (Appendix 4-3). North Summit contained the cyclopoid copepod *Acanthocyclops vernalis*. In the historical dataset of 379 mountain lakes, *H. arcticus* or *H. shoshone* occurred in 44 % of lakes, *D. middendorffiana* (or *D. pulex*) occurred in 38 % of lakes, *D. tyrrelli* or *D. arapahoensis* occurred in 21 % of lakes, and *A. vernalis* occurred in 46 % of lakes (data for rotifers were not available). *Hesperodiaptomus arcticus*, *H. shoshone, D. middendorffiana*, and *D. arapahoensis* occurred in lakes at higher elevations (2108 m above sea level (a.s.l.) ± 201 SD) relative to the other species (1713 m a.s.l ± 423 SD).

Lake temperatures

From May 2005 to August 2007, I monitored water temperature profiles hourly in each lake using data loggers set at 1 m depth intervals and moored in the deepest part of each lake (Appendix 4-1). Average monthly temperatures were determined for the upper 10 m of the water column for Pipit, Upper Geraldine, and Middle Rowe and for the entire 1.3 m of the water column of North Summit. In May–July, temperatures in North Summit were 14.1 ^{o}C ± 3.4 SD, whereas those in Middle Rowe, Pipit and Upper Geraldine were 5.3 ^{o}C ± 3.4 SD. Maximum monthly temperatures ranged by 9.4 ^{o}C among lakes, with North Summit being warmest (18.1 ^{o}C) and Upper Geraldine being coldest (8.7 ^{o}C). Ice-free days ranged by 86 d among lakes, with North Summit having the most ice-free days (217 d) and Pipit having the fewest ice-free days (131 d). Average annual temperatures only ranged by 1.9 ^{o}C because North Summit (the warmest lake) froze to the bottom from November to March, whereas Upper Geraldine (the coldest lake) retained the most heat in winter because it has the greatest volume.

Lake sampling

I collected water and zooplankton from each lake during helicopter surveys in 2007. Lakes were sampled in order from warmest to coldest; North Summit was sampled on July 5, Middle Rowe and Pipit were sampled on August 14 and 22, respectively, and Upper Geraldine was sampled on September 26. Lakes were sampled and experiments were conducted in this order to sample the plankton community near the warmest time period in each lake. Live zooplankton were collected by boat from the center of each lake by vertical net hauls using a 64 μm mesh size, 30 cm diameter net. One thousand litres of water were collected in 20 L carboys from the outflow of each lake. Two hundred litres of lake water were later used to fill the experimental units and 800 L of lake water were later used to extract concentrated DOC. Lake water and the stock of live concentrated zooplankton were transported back to the University of Alberta the same day and stored at 10 ^{o}C in the environmentally-controlled chambers under a 12 hour light:dark cycle.

Concentration of dissolved organic carbon

I concentrated DOC from 800 L of water from each lake using reverse osmosis (Winter et al. 2007). The method generated concentrated DOC that better represented the chemistry of naturally occurring DOC in each lake compared to commercially available fulvic or humic acids. Lake water was sequentially screened though 64 μm and 10 μm pore size mesh by gravity, and filtered through 0.45 μm pore-sized, 144-mm diameter glass fiber filters using a peristaltic pump. Lake water was then concentrated from 800 L to about 1 L using a stainless steel reverse osmosis unit (Limnological Research Corporation, Kelowna, British Columbia; P.J. Curtis, University of British Columbia, Okanagan Campus, Kelowna, BC). The reverse osmosis membrane unit retained molecules > 400 Da (FilmTec FT30 US Filters thin composite RO membrane, Minneapolis, MN). Concentrated DOC was stored overnight at 10 ^{o}C to allow sedimentation of $CaCO_3$ precipitate. The DOC concentrate was decanted and filtered through 0.45 μm glass fiber filters. Concentrations of DOC were increased by 80% in the DOC(+) treatments in each lake. Bulk additions of DOC also contained N and P that were associated with the entire pool of dissolved organic matter (Vähätalo et al. 2003).

Experimental design

I conducted a randomized two factor (temperature x DOC) *in vitro* experiment by incubating plankton communities from two alpine and two montane lakes in their natural water. Temperature treatments were 10 ^{o}C and 17 ^{o}C. The 10 ^{o}C treatment level simulated mean summer temperatures of the study lakes during the ice-free period (9.8 ^{o}C ± 1.4 SD). The 17 ^{o}C treatment level simulated an increase of 7 ^{o}C, which represented the upper range of warming predictions for lakes in Canada with a doubling of atmospheric CO_2 concentrations (Magnuson et al. 1997). The DOC treatment had two levels, ambient concentration and 80 % increased concentration. The + 80 % DOC treatment level simulated recent increases in DOC of 40–120 % in alpine lakes in Banff National Park (Parker et al. 2008).

Lakes were treated as a blocking factor (i.e., “lake-block”) in the experimental design. The setup for each lake-block consisted of two racks each containing 12, 8 L transparent, cylindrical PVC Nalgene bottles that were 19 cm in diameter and 40 cm in height. The bottom 1 cm of each bottle was cut off, and then the bottles were capped, inverted, and held upside-down in each rack. The open aspect of the bottles allowed for gas exchange. One rack of 12 bottles was placed inside one of two environmentally-controlled growth chambers, set at either 10 ^{o}C or 17 ^{o}C to create the “Temp(∅)” and “Temp(+)” treatment levels, respectively. The two DOC treatment levels, ambient “DOC(∅)” and + 80 % “DOC(+)”, were randomly assigned within each temperature treatment. There were six replicates (i.e., six bottles) in each treatment level. Treatments within each lake-block were arranged randomly within each growth chamber. Growth chambers were identical in construction and were sterilized with 10 % bleach solution prior to each experiment.

Experimental procedure

I started each experiment 2 days after we sampled each lake by adding 7 L of lake water to each bottle after passing it through a 64-μm screen to remove zooplankton. The stock of live concentrated zooplankton was stirred to homogenize densities before 1 L was added to each bottle. After one day, dead zooplankton were removed from the bottom of each bottle by pipette. Densities of adult *Hesperodiaptomus* and *Daphnia* were made equal across bottles by visually counting and transferring individuals by pipette. Zooplankton were stocked at densities about 5 times greater than in each lake because ambient densities were very low (< 1 individual$\cdot$L^{-1}) to ensure that treatment effects could be detected.

The stock of concentrated zooplankton was also intended to increase dissolved nutrients and stimulate phytoplankton growth, thereby supporting zooplankton grazing in each experiment. Zooplankton were expected to supply dissolved nutrients via excretion and death during transport. Indeed, adding the stock of zooplankton increased concentrations of total dissolved P (TDP) and dissolved inorganic N (DIN) by about 2 times relative to background concentrations in each lake. In addition, bottles were stirred each day during the experiment to minimize sedimentation of phytoplankton and support zooplankton grazing.

One hundred mL of concentrated DOC was added to the DOC(+) treatments. An equivalent amount of water from each lake was added to the DOC(∅) treatments to keep equal volumes. The DOC(+) treatments were arranged randomly within each environmental chamber (i.e., 10 ^{o}C and 17 ^{o}C). I later tested for differences in DOC among treatments to determine the potential interactive effects of warming on DOC concentrations, despite the obvious circularity of using DOC as both a response and treatment variable.

After each experiment was incubated for 4 weeks, I analyzed water for dissolved and particulate nutrients at University of Alberta Biogeochemistry Laboratory using standardized methods (see Parker and Schindler 2006). I siphoned 2 L of water from the center of each bottle to avoid collecting detritus. Water was screened through a 64-μm sieve to remove zooplankton, which were preserved in 90 % ethanol. Water was then filtered through 0.45 μm pore size glass fiber filters (Whatman GF/F filters). Filters were analyzed for PC and PN together and phytoplankton pigments (described below). Filtrate was analyzed for DOC, total dissolved N (TDN), nitrate + nitrite, ammonium, and TDP. Concentrations of DIN were calculated as nitrate + nitrite + ammonia. Dissolved organic N was calculated as TDN - DIN. Total phosphorus (TP) was analyzed in unfiltered lake water that was passed through a 64-µm sieve to remove zooplankton. Particulate P was calculated as TP - TDP. Molar ratios were calculated for DOC:TDP, DON:TDP, DIN:TDP, PC:PP, PC:PN, and PN:PP.

I used reverse-phase high-pressure liquid chromatography (HPLC) to measure phytoplankton chlorophylls and carotenoids. Phytoplankton abundance was estimated using concentrations of chlorophyll *a* (chl *a*). Carotenoids were used to infer the composition of broad groups of phytoplankton taxa (i.e., chlorophytes and cyanophytes, diatoms, chrysophytes, cryptophytes, and dinophytes (Vinebrooke et al. 2002). Chlorophylls and carotenoids were extracted from frozen glass fiber filters using a solution of acetone and methanol. Pigments were analyzed using an Agilent 1100 Series HPLC equipped with a Varian Microsorb 100Å C18 column and in-line diode array and fluorescence detectors. Pigments were quantified using calibration equations that were calculated from purchased standards (DHI Water and Environment, Denmark) and by consulting Jeffrey et al. (2005). I pooled samples from 3 of the 6 bottles in each treatment combination within each lake-block to ensure pigments were above detection limits (~ 0.01 $\mu g \cdot L^{-1}$), resulting in two replicate samples per treatment.

All zooplankton from each bottle were collected using a 64um mesh and preserved in 90 % ethanol. Zooplankton were later identified at 200x magnification with a Zeiss inverted microscope and enumerated at 25−50x magnification with a Wild M5 dissecting microscope (Ward and Whipple 1966, Pennak 1978). Zooplankton included adult and juvenile cladocerans, juvenile copepod life stages (C1−3 and C4−5), adult (C6) male and female copepods, and rotifers. Mature copepods were distinguished by the morphology of their 5th swimming leg. Biomass of each zooplankton taxon was calculated using standard length-weight regression equations (McCauley 1984). Copepod nauplii were not found, probably because of predation by adult *Hesperodiaptomus*. Copepodid life stages C1−3 were only found for *D. tyrrelli* in North Summit and were summed with adults.

Statistical analysis

I used a randomized two-factor (temperature x DOC) multivariate analysis of variance (MANOVA) that was blocked by lake to test for interactive effects of warming and additions of DOC on dissolved and particulate nutrients, phytoplankton pigments, and zooplankton. Significant lake-block interaction terms (temperature x DOC x lake-block, temperature x lake-block, and DOC x lake-block) indicated that effects differed among lakes. Significant differences among lake-blocks were elucidated with univariate analysis of variance (temperature x DOC) within each lake-block. Dunnett's *post hoc* multiple comparisons were used within each lake-block to compare each treatment to the controls and not among all possible treatment combinations, which minimized the chance of type-I error. I conducted a single MANOVA for dissolved and particulate nutrients and zooplankton, although we presented these statistics in separate tables in the appendix. I conducted a separate MANOVA for phytoplankton pigments because there were only two replicates per treatment.

Significant temperature x DOC interactions were determined to be synergistic or antagonistic by subtracting the observed two-factor interactive effect,

Eq. 1: [T(+)D(+) minus control]

where "T" = temperature and "D" = DOC, from the additive effect that was predicted by the sum of the single factor effects,

Eq. 2: [i.e., [T(+)D(−) minus control] plus [T(−)D(+) minus control]]

Effects were synergistic when the observed interactive effect exceeded the predicted additive effect (i.e., Eq. 1 > Eq. 2). Effects were antagonistic when the observed interactive effect was less than the predicted additive effect (i.e., Eq. 1 < Eq. 2; Christensen et al. 2006). Only the calculated interactive effects (i.e., Eq. 2 minus Eq. 1) were presented in order to simplify the results.

Background levels of response variables varied among lake-blocks (Appendix 4-2 and 4-3), so data for each treatment were transformed into standard deviations relative to the controls (i.e., Z-scores) within each lake-block. Transformed data for the controls had a mean of zero with variance of ± 1 SD, so these data were not presented in the results for simplicity.

I also used a one-factor (zooplankton biomass) multivariate analysis of covariance (MANCOVA) that was blocked by lake to test the effects of biomass of *Daphnia* and *Hesperodiaptomus* on concentrations of phytoplankton chl *a*, PC, PP, and PC:PP and PN:PP ratios. Temperature and DOC treatments were pooled within each lake for a total sample size of $n = 24$.

All data were ln-transformed prior to analysis to improve homogeneity of variance and normality. Each response variable was increased by either 1 or 10 prior to ln-transformation to avoid negative Z-scores. The *a priori* level of significance was set at $P < 0.05$, except for phytoplankton pigments where the level of significance was relaxed to $P < 0.1$ because there were only two replicates. Only significant effects that occurred in more than one lake were considered in the results, which reduced the chance of type-I error. All statistical tests were conducted using the statistical package SPSS (version 18, 2009, Chicago, IL, USA).

Results

Dissolved nutrients

Warming interactively modified the effects of DOC additions on dissolved N and P in the experimental communities (Figure 4-1; Appendix 4-4). Warming reduced the positive effect that DOC additions had on concentrations of DIN in both alpine lakes ($F_{1,4\ \mathrm{Temp*DOC*Block}} = 18.2$, $P < 0.001$). Warming also reduced the slight positive effect that DOC additions had on TDP in Upper Geraldine and North Summit ($F_{1,4\ \mathrm{Temp*DOC*Block}} = 3.3$, $P = 0.015$). As expected, DOC additions directly increased concentrations of DOC by 86 % ± 6 % SD in the DOC(+) treatments relative to controls among the four experimental lake communities ($F_{1,4\ \mathrm{DOC*Block}} = 64.0$, $P < 0.001$).

Particulate nutrients

Warming and DOC additions together had interactive effects on particulate P-content; these effects differed between alpine and montane lakes (Figure 4-2; Appendix 4-5). The combination of warming and additions of DOC decreased PC:PP in Pipit and Upper Geraldine but increased PC:PP in Middle Rowe and North Summit ($F_{1,4\ \mathrm{Temp*DOC*Block}} = 4.4$, $P = 0.003$), likely due to the more terrestrial nature of DOC in Middle Rowe and North Summit (see discussion). Similarly, the combination of treatments decreased PN:PP in Upper Geraldine but increased PN:PP in Middle Rowe ($F_{1,4\ \mathrm{Temp*DOC*Block}} = 11.3$, $P < 0.001$). Also, warming alone directly increased concentrations of PC in Upper Geraldine, Middle Rowe, and North Summit ($F_{1,4\ \mathrm{Temp*Block}} = 35.2$, $P < 0.001$).

Phytoplankton pigments

Together, warming and DOC additions offset the direct negative effects that each factor alone had on concentrations of phytoplankton pigments (Figure 4-3; Appendix 4-6). In particular, the combination of treatments reduced the negative effect of each treatment on lutein + zeaxanthin ($F_{1,4\ \mathrm{Temp*DOC*Block}} = 14.8$, $P < 0.001$). These effects appeared the same in each lake where data were available for chl *a* and chl *b*. However, several of these cases were not significant because sample size of phytoplankton data was small (n = 2; see methods for explanation). One exception to this pattern was that concentrations of fucoxanthin were increased by warming alone in Upper Geraldine and North Summit ($F_{1,4\ \mathrm{Temp*Block}} = 3.1$, $P = 0.065$) and by DOC additions alone in Middle Rowe and North Summit ($F_{1,4\ \mathrm{DOC*Block}} = 22.3$, $P < 0.001$).

Zooplankton biomass

Warming had opposing direct effects on cladocerans and calanoid copepods in the three coldest lake communities (Figure 4-4; Appendix 4-7). Warming increased biomass of *Daphnia* in Pipit, Upper Geraldine, and Middle Rowe ($F_{1,4\ \mathrm{Temp*Block}} = 35.7$, $P < 0.001$). In contrast, warming decreased biomass of *Hesperodiaptomus* in these same three lakes ($F_{1,4\ \mathrm{Temp*Block}} = 18.4$, $P < 0.001$). Since *Hesperodiaptomus* were more abundant than *Daphnia* in these lakes, warming led to a decline in total zooplankton biomass in Pipit and Upper Geraldine ($F_{1,4\ \mathrm{Temp*Block}} = 10.6$, $P < 0.001$; Appendix 4-3). Warming did not reduce total zooplankton biomass in Middle Rowe because warming increased biomass of *Diaptomus*, which compensated for the decline in biomass of *Hesperodiaptomus* ($F_{1,4\ \mathrm{Temp*Block}} = 5.0$, $P = 0.001$). Warming increased the cladoceran:copepod ratio in all three lakes ($F_{1,4\ \mathrm{Temp*Block}} = 31.9$, $P < 0.001$).

Warming did not affect any zooplankton taxa in North Summit. *Keratella longispina* were only present in Pipit and Upper Geraldine, whereas *Acanthocyclops vernalis* were only present in North Summit, but neither of these taxa were significantly affected by the experimental treatments (data not shown).

Correlations between zooplankton and particulate nutrients

Zooplankton biomass was significantly correlated with particulate P-content in lakes where concentrations of particulate nutrients were highest (Appendices 4-8 to 4-10). Specifically, biomass of *Hesperodiaptomus* was positively correlated with concentrations of PP and negatively correlated with PC:PP and PN:PP in Upper Geraldine and Middle Rowe. In contrast, biomass of *Daphnia* was negatively correlated with concentrations of PP and positively correlated with PC:PP and PN:PP in Middle Rowe, although these trends were not significant in Upper Geraldine. Warming and not additions of DOC mainly caused the variation in zooplankton and particulate nutrients (see (+)Temp treatments in Appendices 4-9 and 4-10).

Discussion

The study highlights how warming and increased concentrations of DOC due to climate change may have interactive or direct effects on dissolved nutrient cycling, particulate stoichiometry, and phytoplankton and zooplankton community composition in alpine and montane lakes. Warming and DOC additions interactively increased particulate P-content in alpine lakes, but had the opposite effect in montane lakes. Warming and DOC alone each had direct negative effects on phytoplankton pigment concentrations, but interactions between these factors offset negative effects on phytoplankton. I infer that warming interacted with DOC to stimulate biological uptake of dissolved P by phytoplankton, leading to a net increase in phytoplankton and an overall decrease in TDP. Warming also stimulated cladocerans and had negative effects on copepods, particularly those from alpine lakes. The increase in cladocerans relative to copepods led to decreased P-recycling to the particulate nutrient pool. Below, I consider possible explanations for how warming and DOC affected these changes in planktonic communities.

Warming stimulated uptake of dissolved N and P supplied by DOC

Warming reduced the positive effect that DOC additions on concentrations of DIN and TDP in two of the four study lakes (Figure 4-1). Additions of DOC thus appeared to supply N and P, while warming likely stimulated uptake of N and P by phytoplankton, thereby leading to a reduction in DIN and TDP. Similarly, in 18 lakes across Europe, warming periods have reduced concentrations of dissolved P and Si by stimulating phytoplankton growth in winter and spring (Blenckner et al. 2007). These findings demonstrate the potential interactive effects between warming and increased inputs of DOC on the availability of dissolved N and P for producers in mountain lakes.

Additions of DOC increased concentrations of DIN in both alpine lakes, suggesting that dissolved organic matter was higher in N-content and possibly more labile in alpine lakes than in montane lakes. McKnight et al. (1997) also found that DOC from alpine lakes in Colorado was higher in N-content and less aromatic than DOC from lower elevation lakes and streams; this was attributed to it being more autochthonous compared to DOC from lower elevations. Greater inputs of N-rich melt water from permanent snow and ice packs may also explain higher N-content of the dissolved nutrient pool in alpine lakes (Slemmons and Saros 2012). Greater uptake of DIN in soil and vegetation could also explain lower DIN concentrations in lakes at lower elevations (Brooks and Williams 1999).

Contrasting effects on particulate P-content in alpine versus montane lakes

The combination of warming and additions of DOC altered particulate P-content in opposing ways in alpine and montane lakes (Figure 4-2). Warming and DOC additions synergistically increased PC:PP in the montane lakes, but antagonistically decreased PC:PP in the alpine lakes; there were similar effects on PN:PP in Upper Geraldine and Middle Rowe. The pool of DOC in montane lakes was expected to be lower in P-content (i.e., higher C-content) and more terrestrial in origin because the montane lakes are surrounded by forested catchments. For example, terrestrial inputs are high in C:P relative to atmospheric inputs in alpine and montane lakes in southern Poland (Kopacek et al. 2011). Dissolved organic matter is also less labile in lakes and streams in vegetated catchments compared to non-vegetated alpine catchments in Colorado (McKnight et al. 1997). Further, mineralization of DOC can increase dissolved and particulate C:P; this was observed in a boreal lake in Finland that is surrounded by coniferous forest (Vähätalo et al. 2003). Thus, our findings suggest that increased inputs of terrestrial DOC due to multi-decadal development of soil and vegetation in alpine catchments (Bunting et al. 2010) may lead to decreased P-content of the dissolved and particulate nutrient pool in alpine lakes. The opposite can occur, however, with shorter-term decreases precipitation, runoff, and inputs of DOC into mountain lakes (e.g., Schindler et al. 1996).

Warming combined with higher concentrations of dissolved C may decrease particulate P-content in several ways. First, increased C-content of the dissolved nutrient supply can be reflected in the particulate nutrient pool (Vähätalo et al. 2003, van de Waal et al. 2010), leading to increased particulate C relative to P. Second, warming may increase PC:PP of producers by promoting warm-acclimated algae and bacteria that have lower P-content than cold-acclimated conspecifics (Woods et al. 2003). Third, in warmer, eutrophic lakes it has been suggested that warming from 5 ^{o}C to 20 ^{o}C has increased rates of photosynthesis normalized per cell under P-limited conditions, thereby increasing PC:PP (Hessen et al. 2005); it possible that this same pattern may occur in colder, less productive lakes.

Interactive and direct climatic effects on phytoplankton

Warming and DOC additions combined to produce an antagonistic interaction that reduced the negative effect of each treatment alone on phytoplankton pigments (Figure 4-3). That is, DOC additions increased DIN and TDP, but alone these did not lead to increased phytoplankton. Warming combined with DOC additions appeared to stimulate the uptake of DIN and TDP, leading to increased phytoplankton abundance (Figure 4-1). Dissolved N and P are constituents of the pool of dissolved organic matter that can be made available for uptake by producers through bacterial and photochemical mineralization of DOC (Vähätalo et al. 2003). Thus, the individual effects of warming and DOC additions on phytoplankton pigments did not predict the positive counter-balancing interactive effect of combining both treatments.

In larger aquatic ecosystems, warming has decreased upwelling of dissolved P due to increased thermal stratification in large tropical meromictic lakes (Verburg et al. 2003), deep oligomictic lakes in North America (Coats et al. 2006), and warm regions of the ocean (Boyce et al. 2010). In contrast, warming can increase regeneration of P from the hypolimnion in eutrophic temperate lakes (Pettersson et al. 2003). In weakly stratified oligotrophic alpine lakes, however, effects of warming on lake stratification and internal nutrient regeneration should be relatively weak.

Although warming alone decreased phytoplankton in our study (Figure 4-3), it also increased concentrations of PC (Figure 4-2), which suggests that warming caused a shift in the composition of producers towards either bacteria or algal taxa with low pigment concentration. Further, warming and additions of DOC both increased fucoxanthin, which was the only pigment that did not decline in the experimental treatments. Fucoxanthin is produced by several groups of phytoplankton including diatoms, cryptophytes, and chrysophytes (Vinebrooke et al. 2002). However, the absence of other major carotenoids produced by diatoms (diatoaxanthin and diadinoxanthin), dinoflagellates (peridinin), and cryptophytes (alloxanthin) suggests that warming and additions of DOC increased biomass of non-chlorophyllous phytoflagellates such as chrysophytes. Several other studies have also found that warming and decreased dissolved P and/or increased DOC has stimulated phytoflagellates relative to other phytoplankton taxa in boreal lakes and alpine lakes and ponds (Findlay et al. 2001, Strecker et al. 2004, Parker et al. 2008). These findings warrant further investigation of the potential for warming and increases in DOC to promote the growth of phytoflagellates in cold-water lakes.

Warming increased cladocerans relative to copepods in alpine lakes

Warming increased the abundance of herbivorous *Daphnia* relative to omnivorous *Hesperodiaptomus* in three of four of the experimental communities (Figure 4-4). Intrinsic population growth rates predict that faster-growing asexually reproducing cladocerans may increase relative to slower-growing sexually reproducing copepods with warming from 10 ^{o}C to 25 ^{o}C (Allan 1976). Indeed, cladocerans tend to reach maximum seasonal abundances at around 15–20 ^{o}C in lakes throughout the western hemisphere (Gillooly and Dodson 2000). However, warming also decreases generation times of cladocerans, copepods, and rotifers at the same rate when scaled using body size (Gillooly et al. 2002). Nevertheless, a net increase in abundance of cladocerans relative to copepods with warming can have important impacts on alpine lake food webs. Increased cladocerans will increase grazing pressure on phytoplankton (Parker and Schindler 2006) and can increase P-retention by daphnids, thereby reducing P-recycling in the water column (Sommer and Sommer 2006).

Increased biomass of *Daphnia* relative to *Hesperodiaptomus* appeared to increase grazing pressure on phytoplankton in lakes where concentrations of chl *a* were highest. That is, there were no significant correlations between biomass of *Hesperodiaptomus* and concentrations of chl *a* in the three lakes where data were available (Appendix 4-9). Biomass of *Daphnia* was negatively correlated with concentrations of chl *a* only in Middle Rowe where chl *a* was $> 1\ \mu g \cdot L^{-1}$ (Appendix 4-10). *Daphnia* did not appear to indescriminatly filter the water column of particulate matter. Instead, biomass of *Daphnia* was positively correlated with concentrations of PC in one lake (Upper Geraldine). Thus, effects of grazing by *Daphnia* appeared to be relatively weak among our study lakes. In alpine pond mesocosms in our study area, warming by + 3.6 ^{o}C suppressed biomas of *Daphnia pulex*, increased biomass of rotifers, and shifted the phytoplankton community towards dominance by phytoflagellates without changing total phytoplankton abundance (Strecker et al. 2004). Grazing pressure was also weak in a mesocosm experiment in a boreal lake in northwestern Ontario (Christensen et al. 2006); here, warming (+3 ^{o}C) and decreased concentrations of DOC (- 40%) reduced phytoplankton abundance, likely due to reduced P-availability. However, the recovery of *D. middendorffiana* after experimental removal of a non-native trout population in a nearby alpine lake resulted in large increases in phytoplankton abundance over five years (Parker and Schindler 2006). Thus, increases in large cladoceran grazers due to climate warming can result in increased top-down effects on the phytoplankton community, although these impacts may be difficult to detect in alpine lakes where productivity is very low.

My findings also suggest that alpine zooplankton may be more sensitive to warming than zooplankton from warmer montane lakes. Compared to alpine populations in our study, none of the zooplankton taxa from North Summit lake were affected by warming (Figure 4-4); mean maximum seasonal temperatures in North Summit were 5–10 ^{o}C warmer than in the other study lakes (Appendix 4-1). In a previous study, warming *in vitro* from 13 ^{o}C to 20 ^{o}C also suppressed zooplankton from Pipit Lake but did not affect zooplankton from several lower elevation lakes in Alberta (Holzapfel and Vinebrooke 2005). Elsewhere, upper lethal temperatures of an intertidal copepod (*Tigriopus californicus*) from northern California and Oregon were about 4 ^{o}C lower than for the same species in more southerly populations from Baja, Mexico (Kelly et al. 2012); northern populations showed little ability to adapt to warmer temperatures even after 10 generations in the laboratory. Similarly, populations of *Hesperodiaptomus* may be adapted to local temperatures because members of this genus have low dispersal rates, even among nearby ponds in the Arctic (Boileau and Hebert 1988). Thus, climate warming may increase *Daphnia* relative to *Hesperodiaptomus* in alpine lakes, but effects may not be uniform on populations in warmer regions (lower elevations and/or latitudes).

Low species diversity may also cause alpine zooplankton assemblages to be particularly sensitive to warming. Despite the positive effects on cladoceran biomass, warming decreased total zooplankton biomass in the alpine lakes by suppressing biomass of *Hesperodiaptomus* (Figure 4-4). On the other hand, warming did not suppress total zooplankton biomass in Middle Rowe because an increase in biomass of *Diaptomus* compensated for the decline in *Hesperodiaptomus* in this lake. In warmer experimental mesocosms, experimental additions of zooplankton species (i.e., increased species richness) has also reduced the negative effect of warming on total zooplankton biomass (Thompson and Shurin 2012). In alpine lakes, low crustacean species richness will likely limit the compensation of the zooplankton community to future climate warming.

Consumer-driven nutrient recycling versus effects of food quality on consumers

Correlations between zooplankton and particulate nutrients indicate that the net effect of decreased biomass of *Hesperodiaptomus* and increased biomass of *Daphnia* was decreased particulate P content (Appendix 4-9 and 4-10). That is, decreased biomass of *Hesperodiaptomus* due to warming was correlated with decreased concentrations of PP and increased PC:PP and PN:PP. In contrast, increased biomass of *Daphnia* with warming was correlated with decreased PP and increased PC:PP and PN:PP. These correlations can not demonstrate causal effects, so it is possible that the changes in zooplankton biomass and particulate concentrations and stoichiometry were coincidental. However, cladocerans are expected to retain more P in their biomass and excrete less P than copepods (Sommer and Sommer 2006). Thus, our findings suggest that an increase in biomass of cladocerans relative to copepods due to warming should decrease consumer-driven nutrient recycling of P by copepods in our study lakes, and therefore decrease P availability (*sensu* Elser and Urabe 1999).

Low particulate P-content (i.e., PC:PP > 300) can inhibit growth of P-limited *Daphnia* (Sterner and Hessen 1994, Urabe et al. 1997). Particulate P-content did not appear to limit growth of *Daphnia* except Upper Geraldine (Appendix 4-2). Indeed, *Daphnia* were least abundant in Upper Geraldine where PC:PP was ~ 800.

Considerations: Time-scale and magnitude of warming and ultraviolet radiation

I must consider that the effects of warming on alpine zooplankton may be dependant on the duration and magnitude of experimental warming. Long-term (1991–2005) data from Pipit Lake has shown that mild warming from 6 ^{o}C to 12 ^{o}C in ice-free lake temperatures have increased densities of *D. middendorffiana* (Fischer et al. 2011), whereas advanced ice-free season length that will accompany climate warming has increased *Hesperodiaptomus* (R.P.W. unpublished data). Stronger warming *in vitro* from 8 °C to 14 °C has increased densities and reproduction of *D. middendorffiana* and decreased reproduction of *Hesperodiaptomus* from Pipit Lake (Thompson et al. 2008), which was similar to our findings with warming from 10 °C to 17 °C. More extreme *in vitro* warming from 13 °C to 20 °C has suppressed densities of both *D. middendorffiana* and *Hesperodiaptomus* (Holzapfel and Vinebrooke 2005). Similarly, in a survey of 16 lakes in Alberta, biomass of both taxa was lowest in the warmest lakes where mean summer water temperatures ranged from 6 °C to 18 °C (R.P.W. unpublished data); these lakes were dominated by smaller-bodied species (*Diaptomus* spp., *Daphnia pulex*, *Bosmina* spp., and *Chydorus sphaericus*). Collectively, these studies suggest that mild warming up to about 13 °C water temperature should increase both *D. middendorffiana* and *Hesperodiaptomus*, moderate warming to around 13–17 °C, may increase *D. middendorffiana* relative to *Hesperodiaptomus*, but more severe warming > 17 °C should suppress both taxa and promote smaller-bodied zooplankton species in alpine lakes.

Another consideration is that exposure to ultraviolet radiation (UVR) may limit the colonization success of some plankton taxa in highly transparent lakes (Williamson et al. 2001). In alpine lakes in my study area, *in situ* UVR exposure has suppressed juvenile *D. middendorffiana*, rotifers, and benthic algae, although it has not affected phytoplankton or highly pigmented adult *Hesperodiaptomus* and *D. middendorffiana* (Vinebrooke and Leavitt 1999, Fischer et al. 2011). Thus, it is possible that increased concentrations of DOC will reduce UVR exposure and benefit some plankton taxa in alpine lakes. Conversely, drought conditions may reduce terrestrial inputs and increase UVR exposure of plankton communities, which has occurred in boreal lakes in northwestern Ontario (Schindler et al. 1996b).

Conclusions: Impacts of climate warming and increased DOC on alpine lake food webs

The results suggest that warming and increased inputs of DOC due to climate change will have a number of interactive and direct effects on concentrations and composition of nutrients and plankton communities in alpine lake food webs. Firstly, warming alone or increased DOC alone may decrease production of autotrophic phytoplankton (Blomqvist et al. 2001). However, warming combined with DOC may stimulate the availability and biological uptake of dissolved N and P by producers (Vähätalo et al. 2003), thereby increasing autotroph production. Secondly, warming combined with increased DOC inputs can reduce the P-content of the particulate nutrient pool in lakes with forested catchments (Kopacek et al. 2011), but the reverse may be true in lakes where DOC is more autochthonous in origin. Finally, warming can directly increase the abundance of herbivorous *D. middendorffiana* while suppressing omnivorous *Hesperodiaptomus* spp. over the 10–17 ^{o}C temperature range, and alpine populations of these taxa appear to be more sensitive to warming than those from warmer lakes (Holzapfel and Vinebrooke 2005). Consequently, increased abundance of cladocerans relative to copepods appeared to reduce P-recycling and particulate P-content in the water column (Sommer and Sommer 2006).

References

Allan, J. D. 1976. Life history patterns in zooplankton. American Naturalist 110:165–180.

Anderson, R. S. 1968. The zooplankton of five small mountain lakes in southwestern Alberta. National Museum of Canada Natural History Papers 39:1–19.

Anderson, R. S. 1970. Predator-prey relationships and predation rates for crustacean zooplankters from some lakes in western Canada. Canadian Journal of Zoology 48:1229–1240.

Anderson, R. S. 1971. Crustacean plankton of 146 alpine and subalpine lakes and ponds in western Canada. Journal of the Fisheries Research Board of Canada 28:311–321.

Anderson, R. S. 1972. Zooplankton composition and change in an alpine lake. Verhandlungen der Internationalen Limnologie 18:264–268.

Anderson, R. S. 1974. Crustacean plankton communities of 340 lakes and ponds in and near the national parks of the Canadian Rocky Mountains. Journal of the Fisheries Research Board of Canada 31:855–869.

Battarbee, R. W. 2009. Threatened and stressed mountain lakes of Europe: Assessment and progress. Aquatic Ecosystem Health & Management 12:118–128.

Blenckner, T., R. Adrian, D. M. Livingstone, E. Jennings, G. A. Weyhenmeyer, D. G. George, T. Jankowski, M. Jarvinen, C. N. Aonghusa, T. Noges, D. Straile, and K. Teubner. 2007. Large-scale climatic signatures in lakes across Europe: a meta-analysis. Global Change Biology 13:1314–1326.

Blomqvist, P., M. Jansson, S. Drakare, A. K. Bergstrom, and L. Brydsten. 2001. Effects of additions of DOC on pelagic biota in a clearwater system: Results from a whole lake experiment in northern Sweden. Microbial Ecology 42:383–394.

Boileau, M. G., and P. D. N. Hebert. 1988. Genetic differentiation of freshwater pond copepods at arctic sites. Hydrobiologia 167/168:393–400.

Boyce, D. G., M. R. Lewis, and B. Worm. 2010. Global phytoplankton decline over the past century. Nature 466:591–596.

Bradley, R. S., F. T. Keimig, and H. F. Diaz. 2004. Projected temperature changes along the American cordillera and the planned GCOS network. Geophysical Research Letters 31:1–4.

Brooks, P. D., and M. W. Williams. 1999. Snowpack controls on nitrogen cycling and export in seasonally snow-covered catchments. Hydrological Processes 13:2177–2190.

Bunting, L., P. R. Leavitt, R. P. Weidman, and R. D. Vinebrooke. 2010. Regulation of the nitrogen biogeochemistry of mountain lakes by subsidies of terrestrial dissolved organic matter and the implications for climate studies. Limnology and Oceanography 55:333–345.

Christensen, M. R., M. D. Graham, R. D. Vinebrooke, D. L. Findlay, M. J. Paterson, and M. A. Turner. 2006. Multiple anthropogenic stressors cause ecological surprises in boreal lakes. Global Change Biology 12:2316–2322.

Coats, R., J. Perez-Losada, G. Schladow, R. Richards, and C. Goldman. 2006. The warming of Lake Tahoe. Climatic Change 76:121–148.

Donald, D. B., R. D. Vinebrooke, R. S. Anderson, J. Syrgiannis, and M. D. Graham. 2001. Recovery of zooplankton assemblages in mountain lakes from the effects of introduced sport fish. Canadian Journal of Fisheries and Aquatic Sciences 58:1822–1830.

Elser, J. J., and J. Urabe. 1999. The stoichiometry of consumer-driven nutrient recycling: Theory, observations, and consequences. Ecology 80:735–751.

Fischer, J. M., M. H. Olson, C. E. Williamson, J. C. Everhart, P. J. Hogan, J. A. Mack, K. C. Rose, J. E. Saros, J. R. Stone, and R. D. Vinebrooke. 2011. Implications of climate change for *Daphnia* in alpine lakes: predictions from long-term dynamics, spatial distribution, and a short-term experiment. Hydrobiologia 676:263–277.

Gillooly, J. F., E. L. Charnov, G. B. West, V. M. Savage, and J. H. Brown. 2002. Effects of size and temperature on developmental time. Nature 417:70–73.

Gillooly, J. F., and S. I. Dodson. 2000. Latitudinal patterns in the size distribution and seasonal dynamics of new world, freshwater cladocerans. Limnology and Oceanography 45:22–30.

Hauer, F. R., J. S. Baron, D. H. Campbell, K. D. Fausch, S. W. Hostetler, G. H. Leavesley, P. R. Leavitt, D. M. McKnight, and J. A. Stanford. 1997. Assessment of climate change and freshwater ecosystems of the Rocky Mountains, USA and Canada. Hydrological Processes 11:903–924.

Healey, F. P., and L. L. Hendzel. 1980. Physiological indicators of nutrient deficiency in lake phytoplankton. Canadian Journal of Fisheries and Aquatic Sciences 37:442–453.

Hessen, D. O., E. van Donk, and R. Gulati. 2005. Seasonal seston stoichiometry: effects on zooplankton in cyanobacteria-dominated lakes. Journal of Plankton Research 27:449–460.

Holzapfel, A. M., and R. D. Vinebrooke. 2005. Environmental warming increases invasion potential of alpine lake communities by imported species. Global Change Biology 11:2009–2015.

Huang, S., P. M. Rich, R. L. Crabtree, C. S. Potter, and P. Fu. 2008. Modeling monthly near-surface air temperature from solar radiation and lapse rate: application over complex terrain in Yellowstone National Park. Physical Geography 29:158–178.

IPCC. 2007. International Panel on Climate Change - Fourth assessment report - The physical science basis: Regional climate projections. World Meteorological Organization, United Nations.

Jeffrey, S. W., R. F. C. Mantoura, and S. W. Wright. 2005. Phytoplankton pigments in oceanography: guidelines to modern methods, 2nd edition. UNESCO Publishing, Paris.

Kelly, M. W., E. Sanford, and R. K. Grosberg. 2012. Limited potential for adaptation to climate change in a broadly distributed marine crustacean. Proceedings of the Royal Society B-Biological Sciences 279:349–356.

Kopacek, J., J. Hejzlar, J. Vrba, and E. Stuchlik. 2011. Phosphorus loading of mountain lakes: Terrestrial export and atmospheric deposition. Limnology and Oceanography 56:1343–1354.

Kratina, P., H. S. Greig, P. L. Thompson, T. S. A. Carvalho-Pereira, and J. B. Shurin. 2012. Warming modifies trophic cascades and eutrophication in experimental freshwater communities. Ecology 93:1421–1430.

Magnuson, J. J., K. E. Webster, R. A. Assel, C. J. Bowser, P. J. Dillon, J. G. Eaton, H. E. Evans, E. J. Fee, R. I. Hall, L. R. Mortsch, D. W. Schindler, and F. H. Quinn. 1997. Potential effects of climate changes on aquatic systems: Laurentian Great Lakes and Precambrian Shield Region. Hydrological Processes 11:825–871.

McCauley, E. 1984. The estimation of the abundance and biomass of zooplankton in samples. Pages 228–265 *in* J. A. Downing and F. H. Rigler, editors. A manual on methods for the assessment of secondary productivity in fresh waters. Blackwell Scientific, Oxford, UK.

McKnight, D. M., R. Harnish, R. L. Wershaw, J. S. Baron, and S. Schiff. 1997. Chemical characteristics of particulate, colloidal, and dissolved organic material in Loch Vale Watershed, Rocky Mountain National Park. Biogeochemistry 36:99–124.

McNaught, A. S., D. W. Schindler, B. R. Parker, A. J. Paul, R. S. Anderson, D. B. Donald, and M. Agbeti. 1999. Restoration of the food web of an alpine lake following fish stocking. Limnology and Oceanography 44:127–136.

Moore, M. V., C. L. Folt, and R. S. Stemberger. 1996. Consequences of elevated temperatures for zooplankton assemblages in temperate lakes. Archiv Fur Hydrobiologie 135:289–319.

Moss, B. 2010. Climate change, nutrient pollution and the bargain of Dr Faustus. Freshwater Biology 55:175–187.

Parker, B. R., and D. W. Schindler. 2006. Cascading trophic interactions in an oligotrophic species-poor alpine lake. Ecosystems 9:157–166.

Parker, B. R., R. D. Vinebrooke, and D. W. Schindler. 2008. Recent climate extremes alter alpine lake ecosystems. Proceedings of the National Academy of Sciences of the United States of America 105:12927–12931.

Pennak, R. W. 1978. Fresh-water invertebrates of the United States, 2nd edition. Wiley, University of Michigan.

Pettersson, K., K. Grust, G. Weyhenmeyer, and T. Blenckner. 2003. Seasonality of chlorophyll and nutrients in Lake Erken - effects of weather conditions. Hydrobiologia 506:75–81.

Sadro, S., C. E. Nelson, and J. M. Melack. 2012. The influence of landscape position and catchment characteristics on aquatic biogeochemistry in high-elevation lake-chains. Ecosystems 15:363–386.

Schindler, D. W., S. E. Bayley, B. R. Parker, K. G. Beaty, D. R. Cruikshank, E. J. Fee, E. U. Schindler, and M. P. Stainton. 1996a. The effects of climatic warming on the properties of boreal lakes and streams at the Experimental Lakes Area, northwestern Ontario. Limnology and Oceanography 41:1004–1017.

Schindler, D. W., P. J. Curtis, B. R. Parker, and M. P. Stainton. 1996b. Consequences of climate warming and lake acidification for UV-B penetration in North American boreal lakes. Nature 379:705–708.

Schindler, D. W., and B. R. Parker. 2002. Biological pollutants: alien fishes in mountain lakes. Water, Air, and Soil Pollution 2:379–397.

Slemmons, K. E. H., and J. E. Saros. 2012. Implications of nitrogen-rich glacial meltwater for phytoplankton diversity and productivity in alpine lakes. Limnology and Oceanography 57:1651–1663.

Sommer, U., and F. Sommer. 2006. Cladocerans versus copepods: the cause of contrasting top-down controls on freshwater and marine phytoplankton. Oecologia 147:183–194.

Sterner, R. W., and D. O. Hessen. 1994. Algal nutrient limitation and the nutrition of aquatic herbivores. Annual Review of Ecology and Systematics 25:1–29.

Thompson, P. L., and J. B. Shurin. 2012. Regional zooplankton biodiversity provides limited buffering of pond ecosystems against climate change. Journal of Animal Ecology 81:251–259.

Thompson, P. L., M. C. St-Jacques, and R. D. Vinebrooke. 2008. Impacts of climate warming and nitrogen deposition on alpine plankton in lake and pond habitats: an *in vitro* experiment. Arctic, Antarctic, and Alpine Research 40:192–198.

Urabe, J., J. Clasen, and R. W. Sterner. 1997. Phosphorus limitation of *Daphnia* growth: Is it real? Limnology and Oceanography 42:1436–1443.

Verburg, P., R. E. Hecky, and H. Kling. 2003. Ecological consequences of a century of warming in Lake Tanganyika. Science 301:505–507.

Vinebrooke, R. D., S. S. Dixit, M. D. Graham, J. M. Gunn, Y. W. Chen, and N. Belzile. 2002. Whole-lake algal responses to a century of acidic industrial deposition on the Canadian Shield. Canadian Journal of Fisheries and Aquatic Sciences 59:483–493.

Vinebrooke, R. D., and P. R. Leavitt. 1999. Differential responses of littoral communities to ultraviolet radiation in an alpine lake. Ecology 80:223–237.

Vähätalo, A. V., K. Salonen, U. Münster, M. Järvinen, and R. G. Wetzel. 2003. Photochemical transformation of allochthonous organic matter provides bioavailable nutrients in a humic lake. Archiv Fur Hydrobiologie 156:287–314.

van de Waal, D. B., A. M. Verschoor, J. M. H. Verspagen, E. van Donk, and J. Huisman. 2010. Climate-driven changes in the ecological stoichiometry of aquatic ecosystems. Frontiers in Ecology and the Environment 8:145–152.

Ward, B., and G. C. Whipple. 1966. Freshwater biology, 2nd edition. John Wiley and Sons, Inc., New York.

Weidman, R. P., D. W. Schindler, and R. D. Vinebrooke. 2011. Pelagic food web interactions among benthic invertebrates and trout in mountain lakes. Freshwater Biology 56:1081–1094.

Williamson, C. E., O. G. Olson, S. E. Lott, N. D. Walker, D. R. Engstrom, and B. R. Hargreaves. 2001. Ultraviolet radiation and zooplankton community structure following deglaciation in Glacier Bay. Ecology 82:1748–1760.

Winter, A. R., T. A. E. Fish, R. C. Playle, D. S. Smith, and P. J. Curtis. 2007. Photodegradation of natural organic matter from diverse freshwater sources. Aquatic Toxicology 84:215–222.

Woods, H. A., W. Makino, J. B. Cotner, S. E. Hobbie, J. F. Harrison, K. Acharya, and J. J. Elser. 2003. Temperature and the chemical composition of poikilothermic organisms. Functional Ecology 17:237–245.

Table 4-1. Physical characteristics of the four study lakes in Banff, Jasper, and Waterton Lakes National Parks, Alberta.

Variable	Units	Pipit	Upper Geraldine	Middle Rowe	North Summit
Coordinates	^{o} North	51.6169	52.5699	49.0528	52.8917
	^{o} West	-115.8642	-117.9490	-114.0558	-117.7640
Elevation	m a.s.l.	2217	1995	2162	1540
Mean Depth	m	13.0	13.4	5.0	0.6
Maximum Depth	m	21.0	30.0	12.0	1.3
Area	ha	8.0	17.3	2.1	1.8
Volume	m^{3} x 10^{4}	134	356	30	6

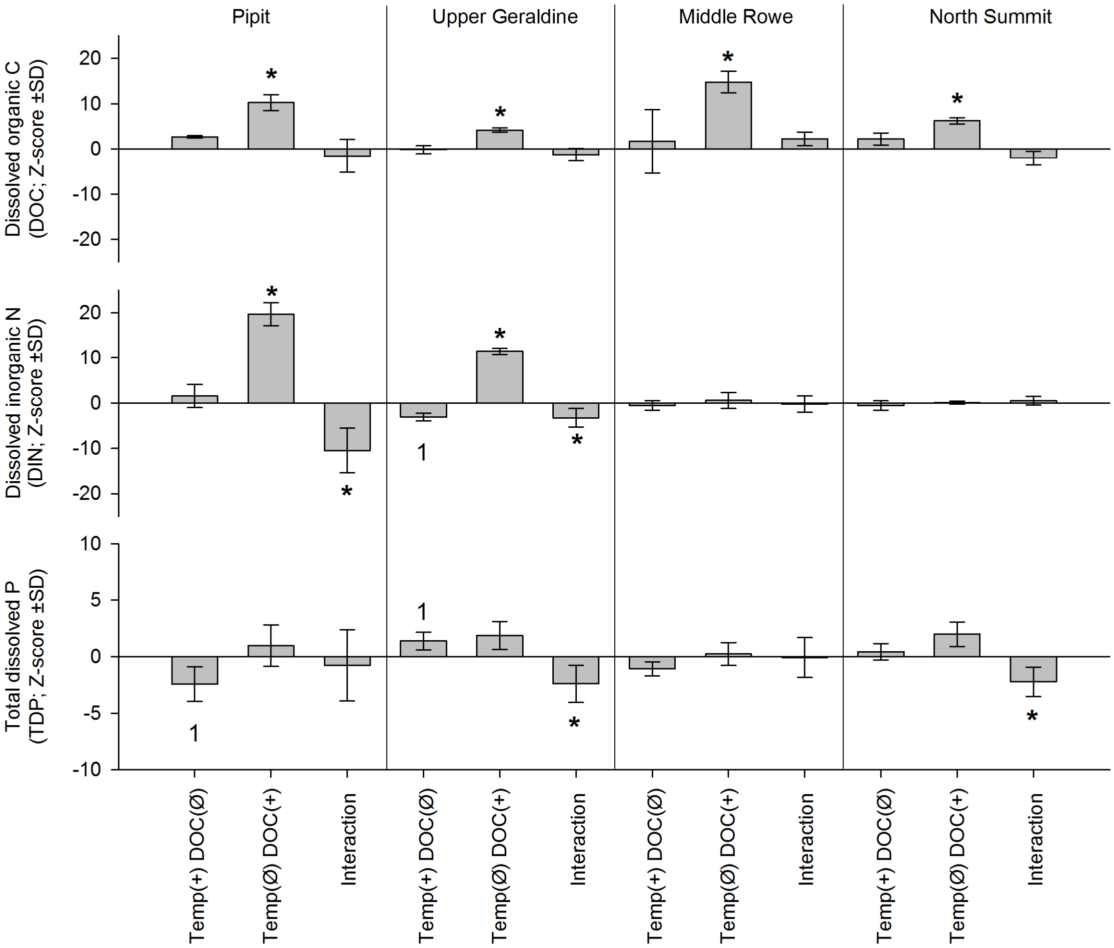


Figure 4-1. Dissolved nutrient concentrations: Effects of warming (Temp) and additions of dissolved organic C (DOC) in the four study lakes. Y-axis units = Z-scores, error bars = SD, and sample size = 6 replicates per treatment. Standardized controls (not shown) have mean = 0 and SD ± 1. Single-factor treatment effects are calculated relative to controls. Interaction terms are calculated as the two-factor treatment effect minus the sum of the single-factor treatment effects (see methods for equations). Dunnett's post hoc comparisons are used to test significant differences relative to controls, where * *P* < 0.05 and "1" = significant difference in only one lake-block.

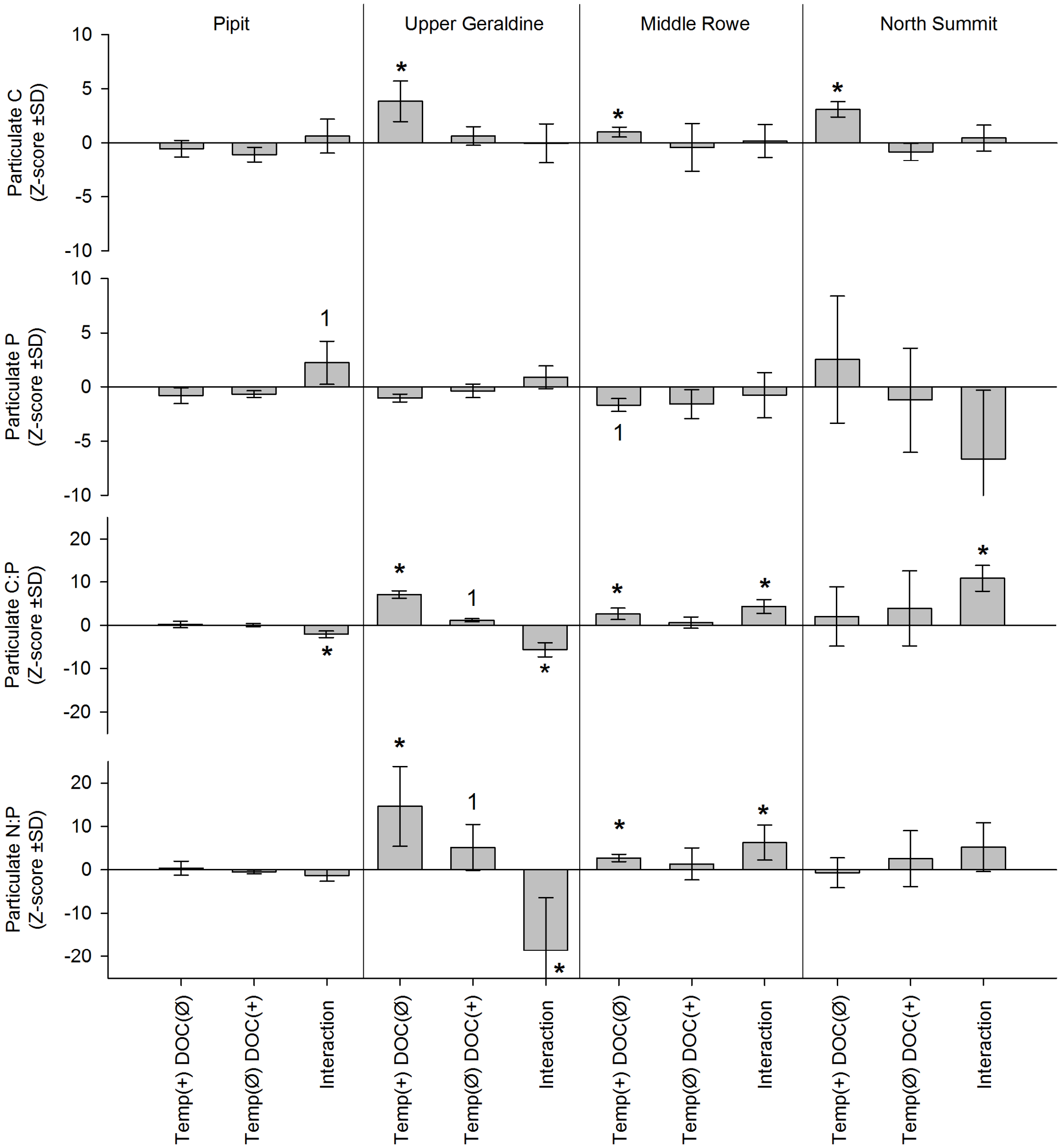


Figure 4-2. Particulate nutrient concentrations and stoichiometry: Effects of warming (Temp) and additions of dissolved organic C (DOC) in the four study lakes. See Figure 1 caption for details.

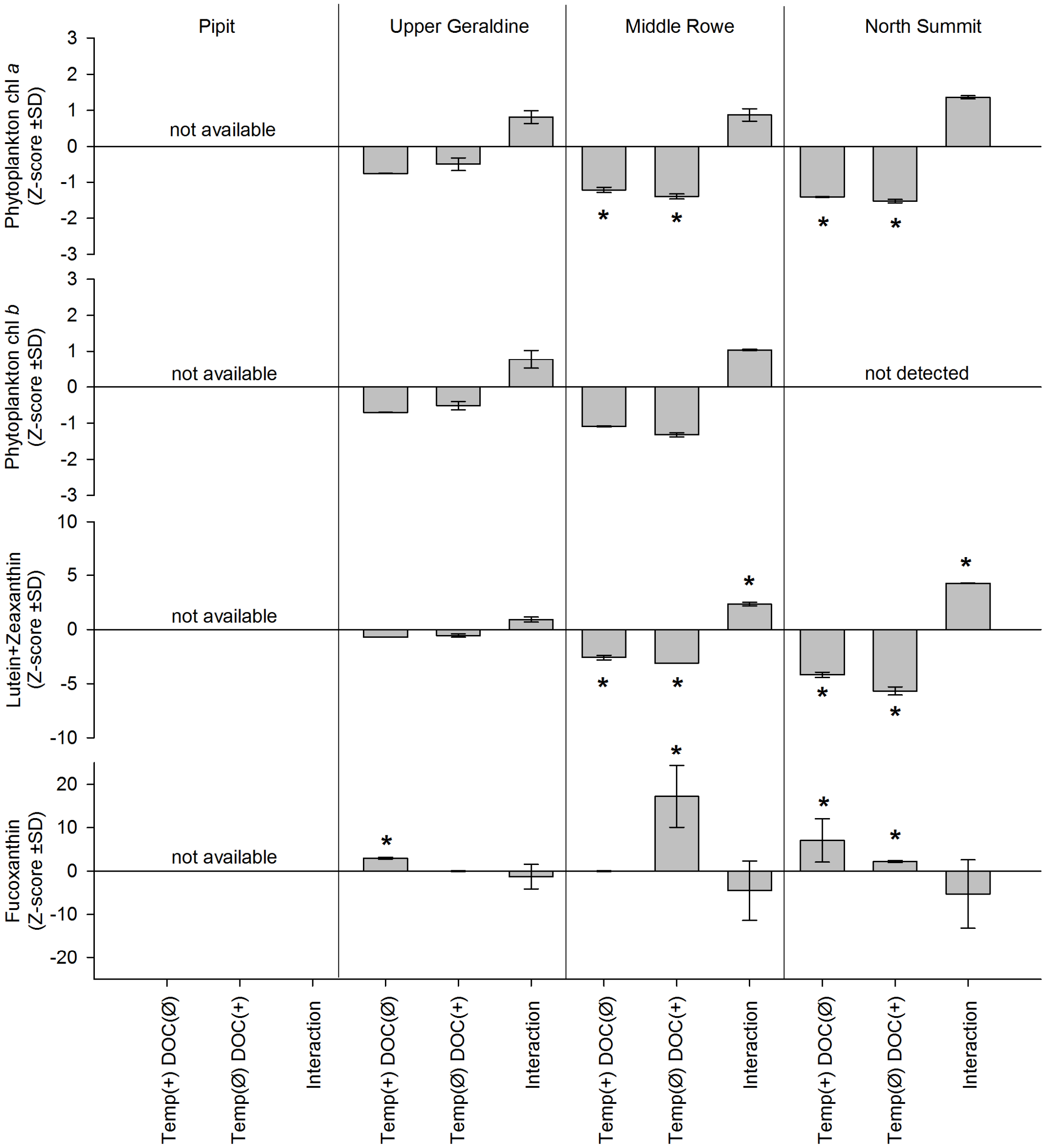


Figure 4-3. Phytoplankton pigment concentrations: Effects of warming (Temp) and additions of dissolved organic C (DOC) in the four study lakes. Sample size = 2 replicates per treatment; * $P < 0.1$. See Figure 1 caption for details.

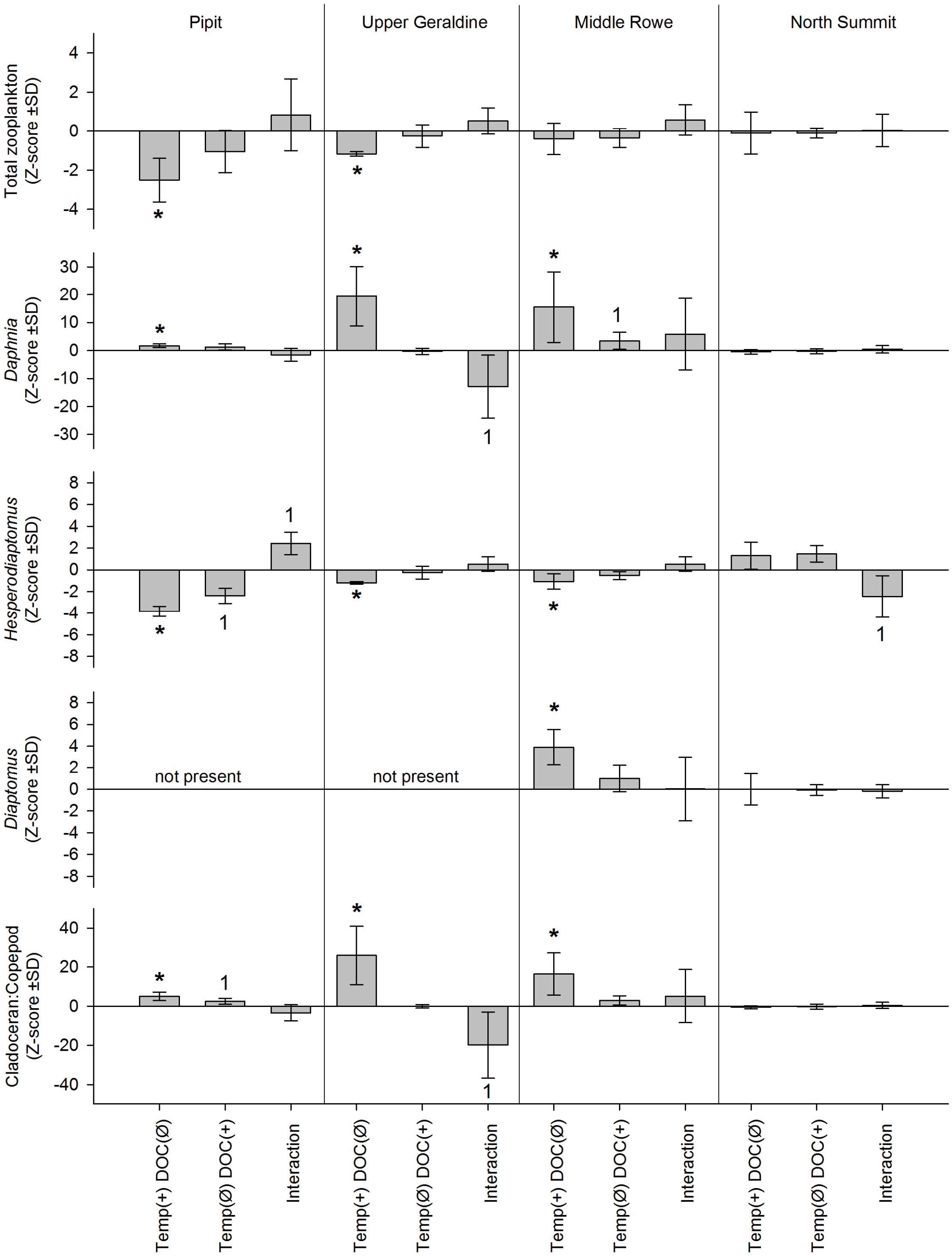
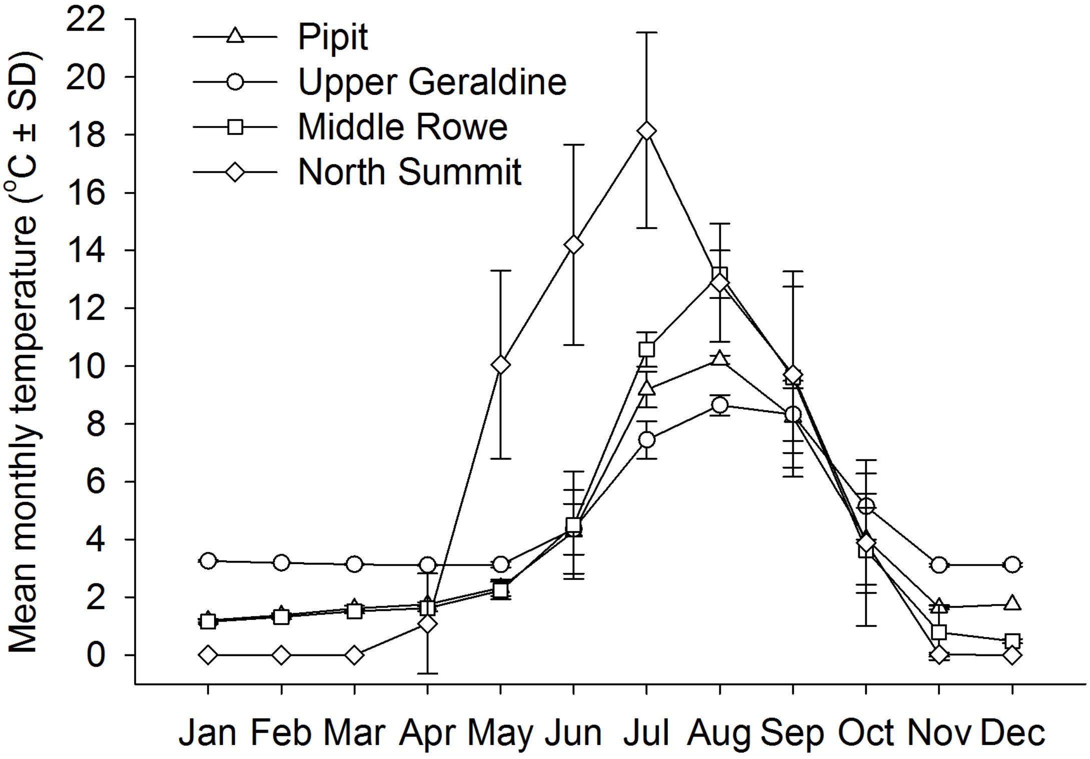
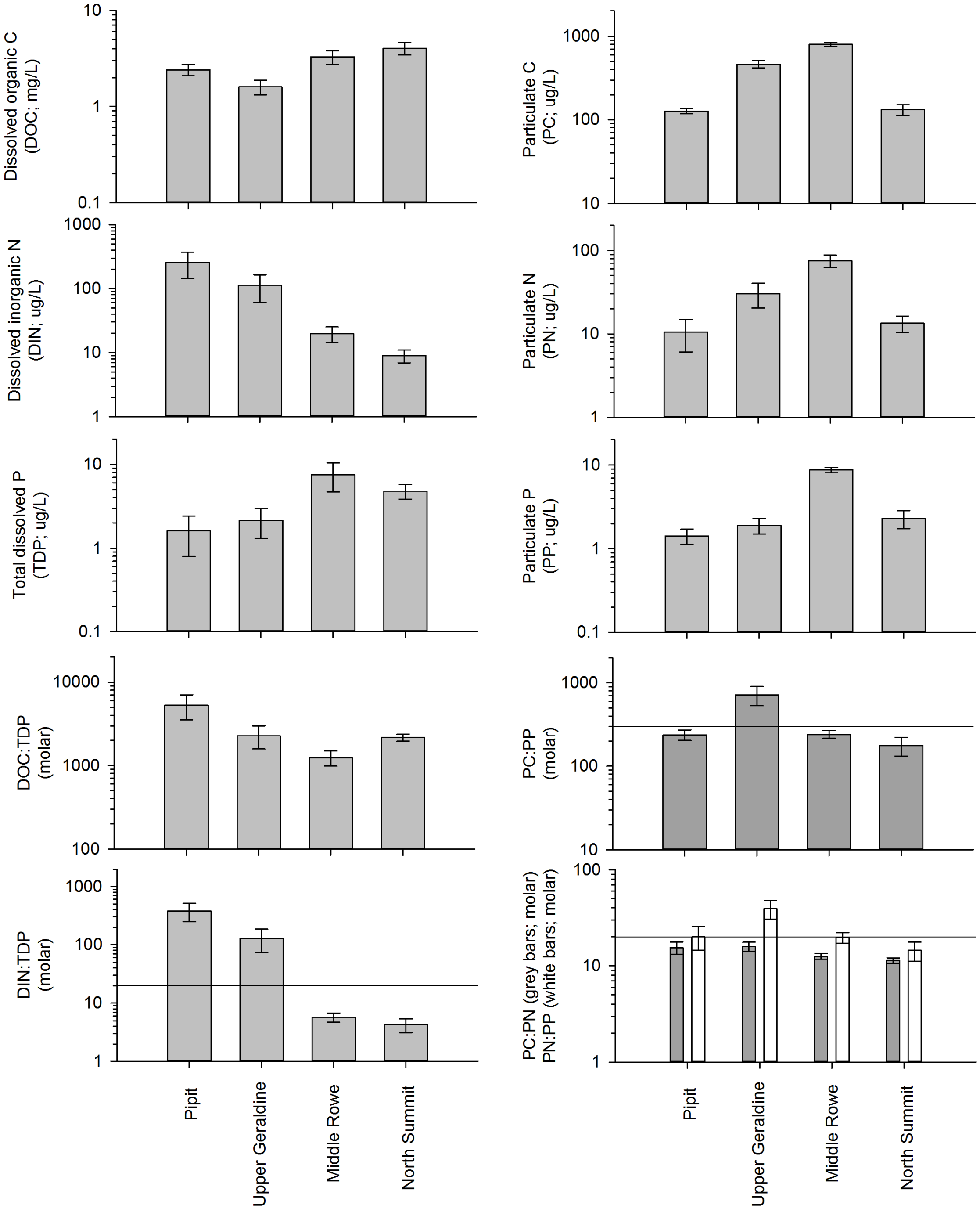


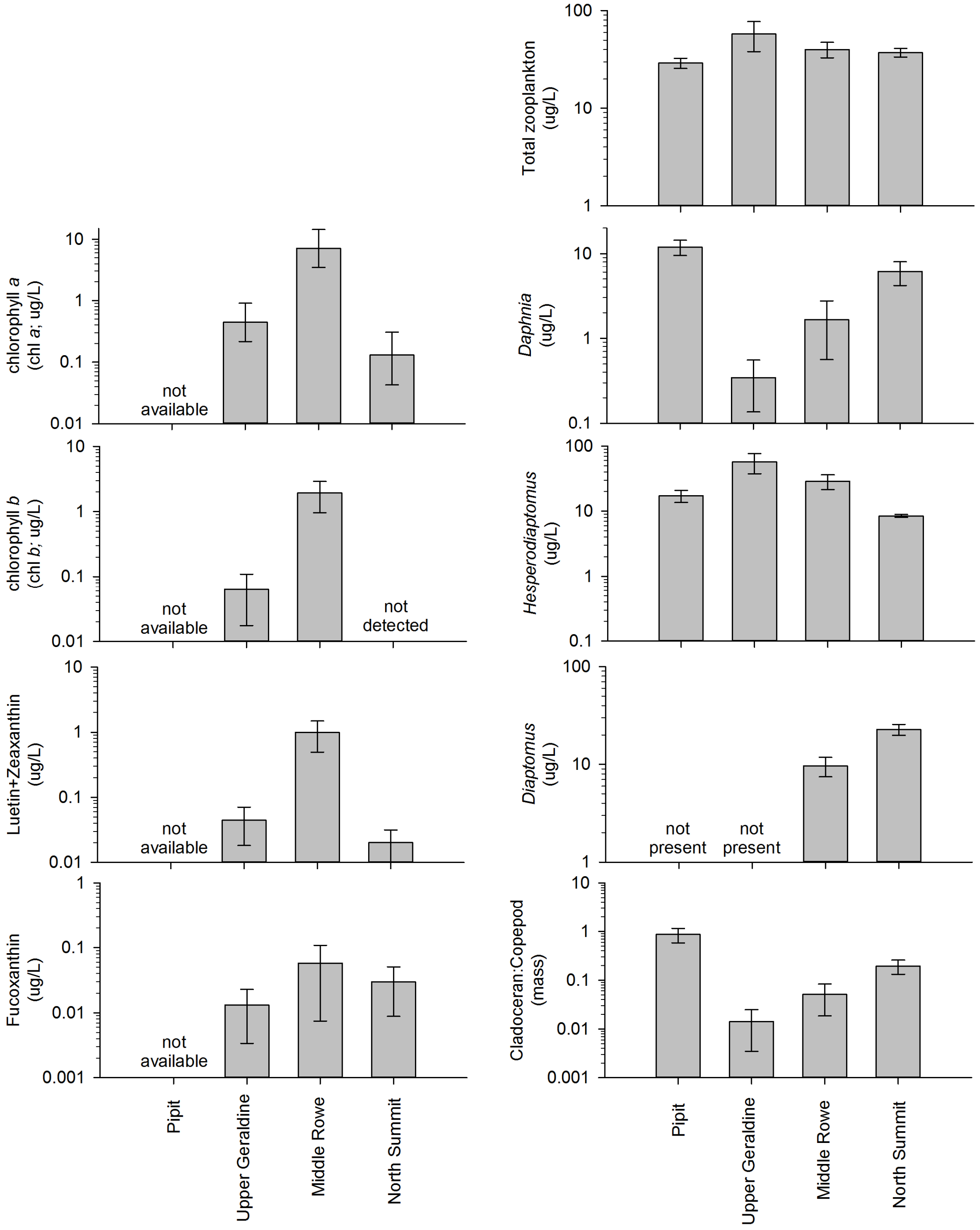
Figure 4-4. Zooplankton biomass: Effects of warming (Temp) and additions of dissolved organic C (DOC) in the four study lakes. See Figure 1 caption for details.



Appendix 4-1. Mean monthly water temperatures of the four study lakes in 2006 (n = 30 d, ± SD).



Appendix 4-2. Dissolved and particulate nutrient concentrations and stoichiometry among treatments in the four study lakes (n = 24 replicates, ± SD).



Appendix 4-3. Phytoplankton pigment concentrations and zooplankton biomass among treatments in the four study lakes (n = 24 replicates, ± SD).

Appendix 4-4. Dissolved nutrient concentrations and stoichiometry: Multivariate analysis of variance (MANOVA) of the effects of increased temperature (Temp) and additions of dissolved organic carbon (DOC) in four study lakes. The MANOVA was blocked by lake with n = 6 replicates per treatment. Dunnett’s post hoc comparisons were used to determine if differences were significantly different (*P* < 0.05) from controls, where “+” = positive effect, “–” = negative effect, and “1” = significant difference in only one lake-block. Statistics in Appendix 4-4, 4-5, and 4-7 are from a single MANOVA.

MANOVA (temperature x DOC) blocked by lake				**Dunnett's post hoc comparisons (treatment vs. control)**			
Response variable	**Source of variation**	***F*-ratio**	***P*-value**	**Pipit**	**Upper Geraldine**	**Middle Rowe**	**North Summit**
Dissolved organic C	Temp. * Block	1.3	0.283				
	DOC * Block	64.0	<0.001	+	+	+	+
	Temp. * DOC * Block	0.8	0.503				
Dissolved inorganic N	Temp. * Block	23.7	<0.001		1		
	DOC * Block	210.1	<0.001	+	+		
	Temp. * DOC * Block	18.2	<0.001	–	–		
Total dissolved P	Temp. * Block	11.7	<0.001	1	1		
	DOC * Block	2.0	0.108				
	Temp. * DOC * Block	3.3	0.015		–		–

Appendix 4-5. Particulate nutrient concentrations and stoichiometry: Multivariate analysis of variance of the effects of increased temperature (Temp) and additions of dissolved organic carbon (DOC) in the four study lakes. See Appendix 4-4 caption for details.

MANOVA (temperature x DOC) blocked by lake				Dunnett's post hoc comparisons (treatment vs. control)			
Response variable	**Source of variation**	***F*-ratio**	***P*-value**	**Pipit**	**Upper Geraldine**	**Middle Rowe**	**North Summit**
Particulate C (PC)	Temp. * Block	35.2	<0.001		+	+	+
	DOC * Block	2.1	0.087				
	Temp. * DOC * Block	0.4	0.838				
Particulate P (PP)	Temp. * Block	5.2	0.001			1	
	DOC * Block	1.7	0.165				
	Temp. * DOC * Block	2.5	0.048	1			
PC:PP	Temp. * Block	11.7	<0.001		+	+	
	DOC * Block	10.3	<0.001		1		
	Temp. * DOC * Block	4.4	0.003	–	–	+	+
PN:PP	Temp. * Block	7.4	<0.001		+	+	
	DOC * Block	7.3	0.000		1		
	Temp. * DOC * Block	11.3	<0.001		–	+	

Appendix 4-6. Phytoplankton pigment concentrations: Multivariate analysis of variance (MANOVA) of the effects of increased temperature (Temp) and additions of dissolved organic carbon (DOC) in the four study lakes. "n/a" = not available; "n/d" = not detected. A separate MANOVA was conducted for phytoplankton data where n = 2 and the level of significant was *P* < 0.1. See Appendix 4-4 caption for details.

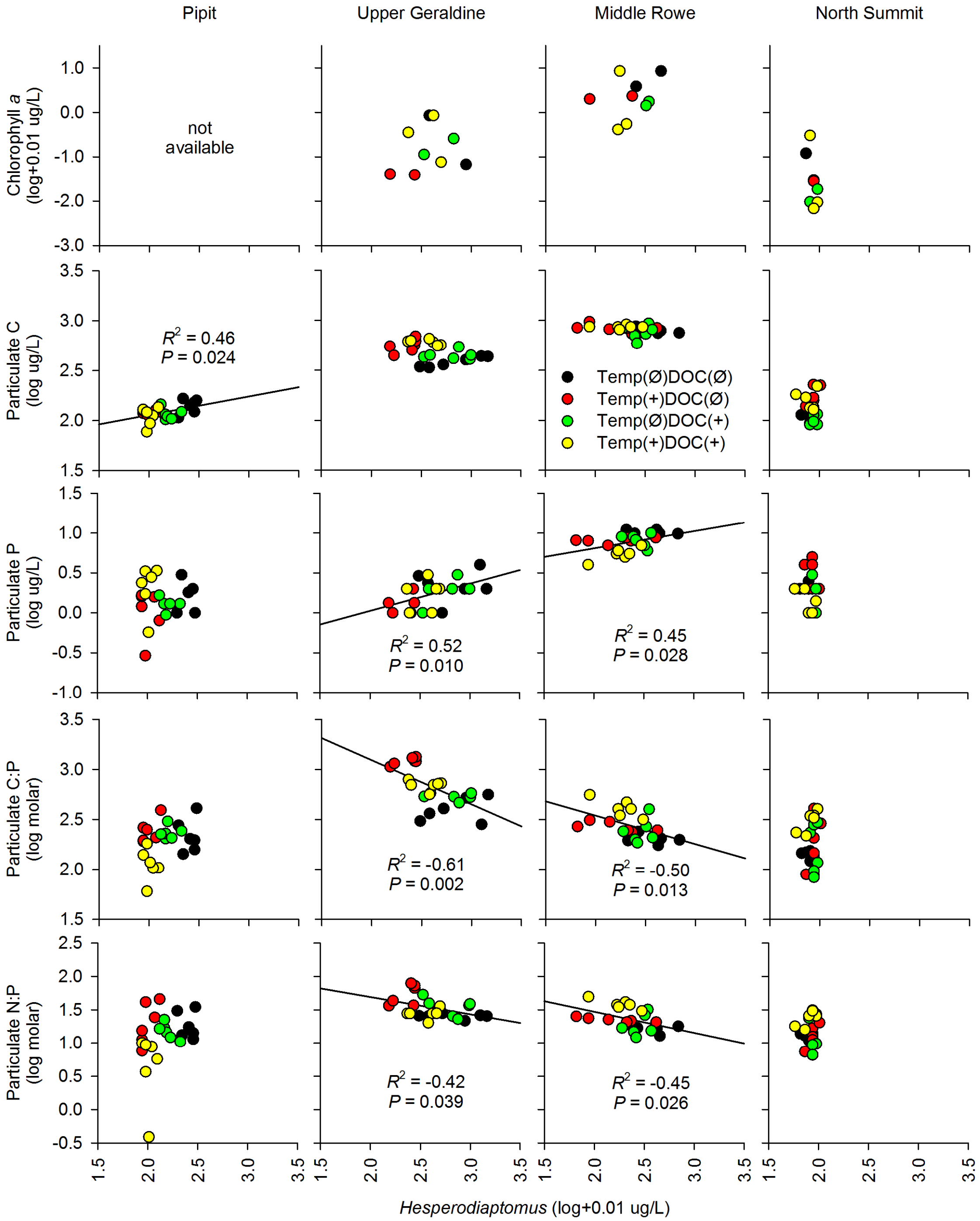
MANOVA (temperature x DOC) blocked by lake				**Dunnett's post hoc comparisons (treatment vs. control)**			
Response variable	**Source of variation**	***F*-ratio**	***P*-value**	**Pipit**	**Upper Geraldine**	**Middle Rowe**	**North Summit**
Chlorophyll *a*	Temp. * Block	3.2	0.063	n/a		–	–
	DOC * Block	4.2	0.031	n/a		–	–
	Temp. * DOC * Block	2.1	0.156	n/a			
Chlorophyll *b*	Temp. * Block	1.6	0.244	n/a			n/d
	DOC * Block	2.5	0.113	n/a			n/d
	Temp. * DOC * Block	1.6	0.249	n/a			n/d
Lutein + Zeaxanthin	Temp. * Block	14.8	<0.001	n/a		–	–
	DOC * Block	39.5	<0.001	n/a		–	–
	Temp. * DOC * Block	14.8	<0.001	n/a		+	+
Fucoxanthin	Temp. * Block	3.1	0.065	n/a	+		+
	DOC * Block	22.3	<0.001	n/a		+	+
	Temp. * DOC * Block	1.2	0.351	n/a			

Appendix 4-7. Zooplankton biomass: Multivariate analysis of variance of the effects of increased temperature (Temp) and additions of dissolved organic carbon (DOC) in the four study lakes. See Appendix 4-4 caption for details.

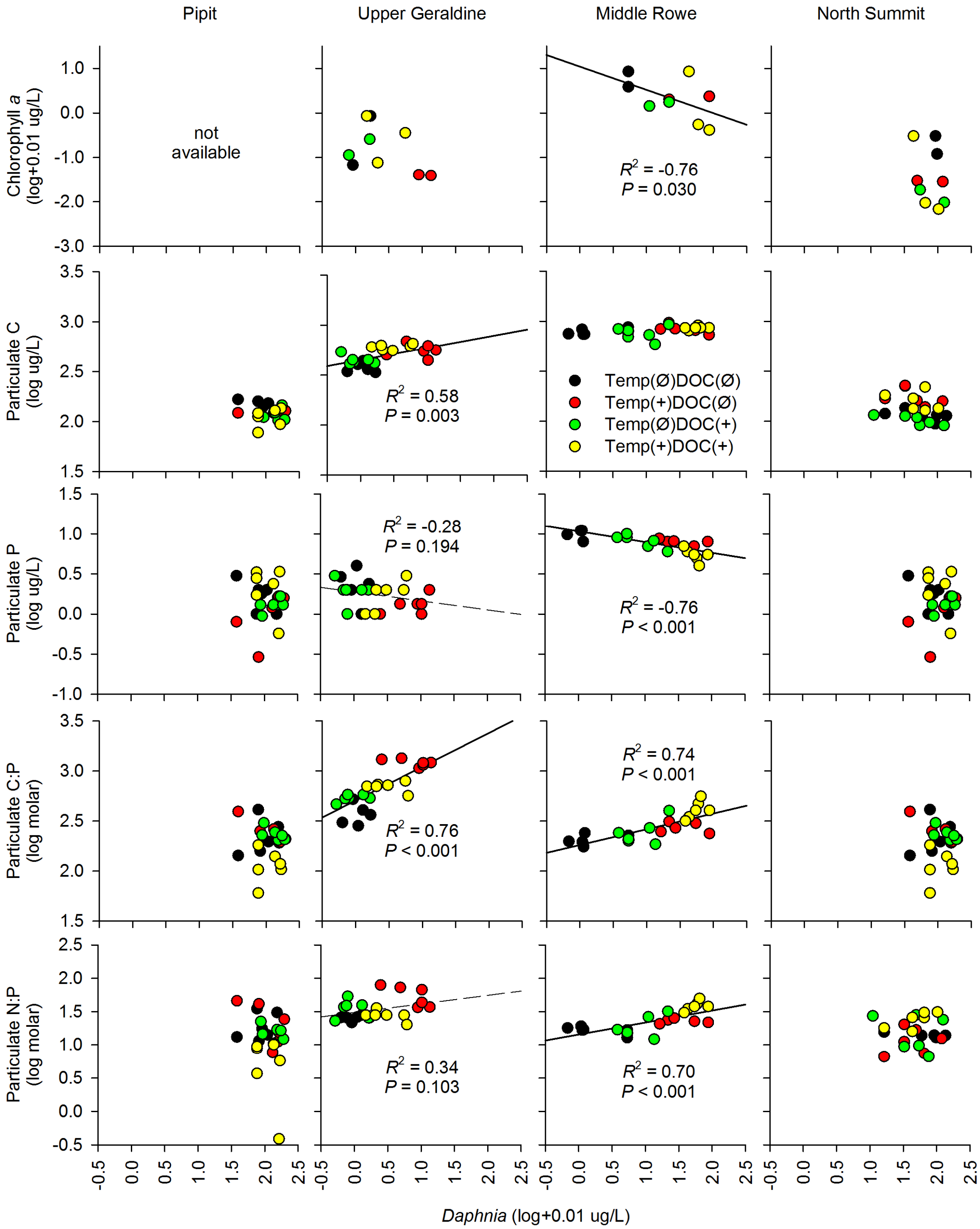
MANOVA (temperature x DOC) blocked by lake				Dunnett's post hoc comparisons (treatment vs. control)			
Response variable	**Source of variation**	***F*-ratio**	***P*-value**	**Pipit**	**Upper Geraldine**	**Middle Rowe**	**North Summit**
Total zooplankton	Temp. * Block	10.6	<0.001	–	–		
	DOC * Block	0.8	0.511				
	Temp. * DOC * Block	0.6	0.632				
Daphnia	Temp. * Block	35.7	<0.001	+	+	+	
	DOC * Block	5.9	<0.001			1	
	Temp. * DOC * Block	3.4	0.013		1		
Hesperodiaptomus	Temp. * Block	18.4	<0.001	–	–	–	
	DOC * Block	3.3	0.015	1			
	Temp. * DOC * Block	6.7	<0.001	1			1
Diaptomus	Temp. * Block	20.5	<0.001			+	
	DOC * Block	1.4	0.240				
	Temp. * DOC * Block	0.0	1.000				
Cladocerans:Copepods	Temp. * Block	31.9	<0.001	+	+	+	
	DOC * Block	6.1	<0.001	1			
	Temp. * DOC * Block	5.0	0.001		1		

Appendix 4-8. Multivariate analysis of covariance (MANCOVA) of the effects of biomass of *Daphnia* and *Hesperodiaptomus* on concentrations of phytoplankton chlorophyll a (chl a) and particulate C (PC) and P (PP) and the PC:PP and PN:PP molar ratios in four study lakes. The MANCOVA was blocked by lake. Temperature and dissolved organic C treatments were pooled for n = 24 replicates per lake (except for chl a where n = 8; tested in a separate MANCOVA). *Post hoc* comparisons were used to determine if regression coefficients were positive = “+” = positive, negative = “–” , or “1” = significant in only one lake-block.

MANCOVA (*Daphnia* or *Hesperodiaptomus*) blocked by lake				***Post hoc* comparisons (regression coefficient different from zero)**			
Response variable	**Source of variation**	***F*-ratio**	***P*-values**	**Pipit**	**Upper Geraldine**	**Middle Rowe**	**North Summit**
Phytoplankton chl *a*	*Daphnia* * Block	4.0	0.026			1	
	Hesperodiaptomus * Block	1.3	0.305				
Particulate C (PC)	*Daphnia* * Block	7.9	<0.001		1		
	Hesperodiaptomus * Block	11.1	<0.001	1			
Particulate P (PP)	*Daphnia* * Block	0.8	0.548		–	–	
	Hesperodiaptomus * Block	16.3	<0.001		+	+	
PC:PP	*Daphnia* * Block	7.3	<0.001		+	+	
	Hesperodiaptomus * Block	8.4	<0.001		–	–	
PN:PP	*Daphnia* * Block	4.2	<0.001		+	+	
	Hesperodiaptomus * Block	3.4	0.012		–	–	



Appendix 4-9. Correlations with *Hesperodiaptomus* and concentrations of phytoplankton chl *a*, particulate C (PC), particulate P (PP), and the PC:PP and particulate N:P molar ratios. Temperature and dissolved organic C treatments were pooled for n = 24 replicates per lake (except for chl *a* where n = 8; tested separately).



Appendix 4-10. Correlations with *Daphnia* and concentrations of phytoplankton chl *a*, particulate C (PC), particulate P (PP), and the PC:PP and particulate N:P molar ratios. See Appendix 4-9 caption for details.

CHAPTER 5: GENERAL CONCLUSIONS

Major findings among the three main studies suggest that warming, decreased precipitation, and decreased lake depth due to climate change will increase P-availability, promote the production of autotrophs relative to potentially mixotrophic phytoflagellates, and increase small-bodied zooplankton taxa over large-bodied taxa. These results support the general observation that climate change may intensify the symptoms of eutrophication in relatively shallow temperate lakes where P pollution is also a problem (Moss 2012).

Among the three studies there were relatively consistent effects on dissolved P-content and phytoplankton, whereas effects on particulate P-content and zooplankton were more complicated. All three studies supported the hypothesis (1A) that warmer and drier conditions due to climate change will increase dissolved P-content (i.e., decrease C:P and N:P) in alpine lakes. Effects on particulate P-content were mixed: warming increased particulate P-content (hypothesis 1A), greater terrestrial inputs partially offset this negative effect on P-content (hypothesis 1B), and variation in the relative abundance of cladocerans and copepods also affected P-content (hypothesis 1C). Effects on phytoplankton were more consistent, with autotrophs increasing relative to phytoflagellates in all three studies as a result of warming and increased dissolved P-availability (hypothesis 2A). Effects on zooplankton depended on the time-scale in each study: short-term warming increased cladocerans relative to copepods (hypothesis 3A), whereas longer-term warming suppressed large cladocerans and copepods and promoted small-bodied copepods relative to small cladocerans (hypothesis 3C). In addition, the experiment supported the hypothesis that alpine zooplankton populations are more sensitive to warming than those from warmer montane lakes (hypothesis 4). Mechanisms to explain these conclusions are summarized below along with the main implications of the findings.

Warmer and drier conditions increased dissolved P-content

The three main studies indicated that climate change will increase dissolved P relative to N and C in alpine lakes (hypothesis 1A; Table 5-1). In the spatial survey, warming and decreased depth both increased total dissolved P (TDP) relative to dissolved inorganic N (DIN). In the temporal survey, decreased rain increased TDP relative to dissolved organic C (DOC), although warming offset this effect as it stimulated TDP uptake by phytoplankton. In the experiment, DOC additions slightly increased TDP, whereas the combination of warming and DOC additions decreased TDP also due to increased uptake by phytoplankton.

Several mechanisms may explain these increases in dissolved P-content in alpine lakes. Firstly, long-term warming and decreased lake depth are expected to concentrate dissolved P in alpine lakes (Garcia-Jurado et al. 2012). Effects of warming and decreased depth may be particularly important in shallow alpine lakes and ponds where there is greater contact of water volume with sediment, which may also become more easily resuspended by wind-driven mixing. Indeed, concentrations of TDP are higher in alpine ponds (10 μg·L-1) than in deeper alpine lakes (1 $\mu g \cdot L^{-1}$) in Alberta (Murphy et al. 2010). Secondly, long-term warming over several decades may increase dissolved P-availability by decreasing DIN:TDP via increased microbial uptake of DIN in alpine catchments and decreased DIN inputs into alpine lakes (Brooks and Williams 1999, Sadro et al. 2012). However, increased atmospheric deposition of N from anthropogenic sources (Holtgrieve et al. 2011) may offset reductions in DIN:TDP related to warming. Thirdly, decreased snowpack and reduced summer rain may increase dissolved P relative to C by reducing C-rich terrestrial inputs and decreasing flushing of TDP, as the opposite situation has occurred in alpine lakes in Alberta in years with high snowpack (Parker et al. 2008). Finally, dissolved oarganic matter can be mineralized into its constituent molecules, which may be a source of TDP (Vähätalo et al. 2003).

The one exception to these findings was that in the spatial survey, advanced ice-off due to warming increased inputs of DOC relative to TDP (hypothesis 1B; Table 5-1). This result supported the alternative hypothesis 1B that increased melting and runoff will increase inputs of C-rich terrestrial matter into mountain lakes. Similarly, greater terrestrial inputs are correlated with higher dissolved C:P among mountain lakes in central Europe (Kopacek et al. 2011). Thus, although warming and decreased lake depth increased P-availability via decreased DIN:TDP, advanced ice-off also appeared to increase inputs of C-rich terrestrial matter relative to TDP.

Overall, findings suggested that warmer and drier conditions may increase dissolved P relative to N in alpine lakes on 1 y (experiment) to 20 y (temporal survey) time scales. However, over multiple decades (spatial survey) advanced ice-off and a longer ice-free period may be accompanied by increased inputs of DOC into these lakes. Increased concentrations of TDP and decreased DIN:TDP due to climate change would stimulate phytoplankton growth in alpine lakes, which are P-limited (Murphy et al. 2010). However, long-term increases in DOC relative to TDP could potentially stimulate heterotrophic production and reduce energy flow to higher trophic levels in alpine lake food webs (Jansson et al. 2007).

Bottom-up and top-down effects on particulate P-content

Effects on particulate P-content were more complicated than effects on the dissolved nutrient pool, as there was evidence to support each of the three alternative hypotheses. The spatial survey and results from the alpine lake-blocks in the experiment supported the hypothesis that climate change would increase particulate P-content in alpine lakes (hypothesis 1A; Table 5-2). In the spatial survey, there was a net decrease in PC:PP mainly due to the direct effects of warming. In the alpine lake-blocks in the experiment, warming and the combination of warming and DOC additions also decreased PC:PP.

The spatial survey and results from the montane lake-blocks in the experiment provided some evidence to support the alternative hypothesis that climate change factors might partially offset increases in particulate P-content in alpine lakes (hypothesis 1B; Table 5-2). Increased soil cover partially increased PC:PP in the spatial survey, although the net effect of all climate factors on PC:PP was negative. In the experiment, the combination of warming and DOC additions also increased PC:PP in montane lakes. DOC in the montane lakes was likely more terrestrial in origin than in the alpine lakes where DOC can be from algal sources (McKnight et al. 1997).

All three studies supported the hypothesis that climate effects on the relative abundances of cladocerans and copepods would affect particulate P-content (hypothesis 1C; Table 5-2). Increases in cladocerans due to warming were associated with increases in PC:PP in the temporal survey and the experiment. In the spatial survey, however, the decline in cladocerans relative to copepods had a negative effect on PC:PP. Thus, shorter term increases in cladocerans due to warming may decrease particulate P-content, but the opposite may occur with longer-term warming over multiple decades selecting for small diaptomid copepods relative to smaller cladocerans. Decreases in the abundance of cladocerans relative to copepods have also been found to decrease particulate P-content in freshwater and marine mesocosm experiments (Sommer and Sommer 2006).

There was some evidence to suggest that reduced particulate P-content had a negative effect on P-limited cladocerans relative to N-limited copepods (Sterner and Hessen 1994). In the spatial survey, the partial increase in PC:PP had a negative effect on abundance of cladocerans relative to copepods. However, increased PC:PP did not appear to have a negative effect on cladocerans in the temporal survey or experiment (except in one lake) where mean PC:PP ratios were generally below the level (300) thought to inhibit growth of cladocerans (Urabe et al. 1997).

Together, results indicated that particulate P-content in alpine lakes was affected by bottom-up effects on dissolved nutrient supply ratios (Urabe et al. 2003, Frost et al. 2007) and top-down effects of nutrient recycling by zooplankton (Elser and Urabe 1999). Particulate P-content is expected to increase with both short-term and long-term climate change. Short-term increases are expected to occur due to increased concentrations of dissolved P. Longer-term increases are expected due to warming and increases in small diaptomid copepods, which was observed in the spatial survey. Increases in C-rich terrestrial inputs, which are expected with increased soil and vegetation cover of alpine catchments (Bunting et al. 2010, Sadro et al. 2012), may partially offset increases in particulate P-content and have a negative effect on P-limited cladocerans (Sterner and Hessen 1994, Urabe et al. 1997).

Warming and increased P increase autotrophs relative to phytoflagellates

All three studies supported the hypothesis that warming combined with increased P-availability would increase autotrophs relative to potentially mixotrophic phytoflagellates (hypothesis 2A; Table 5-3). In the spatial survey, warming combined with decreased DIN:TDP to increase chlorophytes and cyanophytes relative to phytoflagellates. Indeed, phytoflagellates were the most abundant phytoplankton group in the coldest alpine lakes with lowest concentrations of TDP, whereas chlorophytes and cyanophytes were dominant in warmer, low elevation lakes with higher TDP. In the temporal survey, decreased rain combined with increased TDP to increase biomass of diatoms relative to phytoflagellates. In the experiment, warming combined with DOC additions to increase availability of P and offset the negative effects of the single-factor treatments on the most abundant phytoplankton pigments chlorophyll *a* and *b* and lutein + zeaxanthin.

These findings indicate that warmer and drier conditions should stimulate autotrophic production in alpine lakes via increased P-availability. *In vitro* studies also predict that chlorophytes and cyanophytes would become more abundant than diatoms and phytoflagellates as average temperatures increase from 10 ^{o}C to 20 ^{o}C and the molar DIN:TDP ratio decreases below 20 (Kilham 1986, Tilman et al. 1986). Under the opposite conditions of reduced P conditions, mixotrophic phytoflagellates may outcompete diatoms by acquiring nutrients via phagotrophy (Rothhaupt 1996). Indeed, phytoflagellates have increased with reduced P-availability in boreal lakes and alpine lakes (Findlay et al. 2001, Parker et al. 2008). In deeper lakes (e.g., > 50 m maximum depth), however, climate warming may play an important role in intensifying thermal stratification, reducing upwelling of dissolved P during the growing season, and promoting small diatoms and phytoflagellates (Winder and Sommer 2012).

The greatest impact of increased dissolved P-content in the three main studies was the observed change in phytoplankton community composition where autotrophs increased relative to potentially mixotrophic phytoflagellates. Increased autotrophic production may ultimately increase alpine lake productivity by increasing energy transfer efficiency to zooplankton. That is, under increased autotrophy a trophic step is eliminated between dissolved nutrients, bacteria, and mixotrophic phytoflagellates (Jansson et al. 2007). Thus, increased autotrophy can potential increase the productivity of alpine lake food webs.

Variation in concentrations of DOC were not significantly associated with phytoplankton abundance in the spatial and temporal surveys. This finding provided evidence against the idea that increased inputs of DOC would increase heterotrophic production at the expense of autotrophic production (Blomqvist et al. 2001). In the experiment, short-term additions of DOC by itself did appear to reduce autotrophic pigments. However, the combination of warming and DOC additions offset this negative effect on phytoplankton. It is possible that increased DOC inputs due to advanced ice-off in spring may affect the composition of early successional phytoplankton taxa in alpine lakes relative to the assembelage later in summer and fall; this could be explored in a future study.

Short-term versus long-term effects of warming on zooplankton

Effects of warming on zooplankton depended on the duration of warming (i.e., study time-scale) and the magnitude of warming. Warming increased the abundance of cladocerans relative to copepods in the short-term experiment, as predicted by hypothesis 3A (Table 5-4; Allan 1976). Over the intermediate time-scale (i.e. 16 y), the temporal survey partially supported hypothesis 3A as well, because warming increased *D. middendorffiana* (Table 5-4); at the same time, however, advanced ice-off due to decreased precipitation unexpectedly increased *Hesperodiaptomus*. Although all findings in the temporal and spatial surveys are correlative, decreased winter precipitation was the only variable that was significantly associated with changes in abundance of *Hesperodiaptomus*. Advanced ice-off due to decreased winter precipitation was statistically independent of temperature (air or water) or food sources (phytoplankton or rotifers). Thus, advanced ice-off was inferred to have benefited *Hesperodiaptomus* because it provided a longer amount of time for *Hesperodiaptomus* to complete its life cycle. Under the opposite conditions, delayed ice-off was observed to increase mortality of over-wintering calanoid copepods in Lake Michigan (Vanderploeg et al. 1998). Similarly, early ice-on increased mortality of a second generation of *Leptodiaptomus sicilis* in mountain lakes in Alberta (Herzig et al. 1980). Over the longest-term time scale, represented by the spatial survey, warming promoted the abundance of smaller-bodied copepods (*Diaptomus* spp.) and cladocerans (*Daphnia pulex* and *Chydrous spahericus*) over larger taxa, which supported hypothesis 3C (Gillooly and Dodson 2000, Daufresne et al. 2009). Warming also increased the abundance of small copepods relative to small cladocerans.

Together these findings suggest that short-term warming may promote herbivorous cladocerans in alpine lakes, while moderate warming (e.g., up to 14 ^{o}C mean summer water temperatures) and advanced ice-off (up to 30 d), as observed in the temporal survey, may increase production of both *D. middendorffiana* and

Hesperodiaptomus in alpine lakes. More extreme warming and advanced ice-off (up to 17 °C and up to 75 d, respectively), as represented in the spatial survey, could suppress both *D. middendorffiana* and *Hesperodiaptomus* and promote smaller-bodied taxa.

Ecological impacts from the loss of large-bodied alpine zooplankton species can be inferred by observing the effects of introductions of non-native trout species in alpine lakes. Loss of *D. middendorffiana* and *H. arcticus* following trout introductions resulted in dramatic reductions in water clarity and increases in phytoplankton abundance due to reduced grazing in alpine lakes (Parker and Schindler 2006). Maintenance of the ecological integrity of ecosystems is a main priority of the National Parks Act of Canada (Dearden and Dempsey 2004). Thus, loss of native alpine zooplankton species and alteration of plankton community composition due to climate change is a particular concern for the preservation of alpine lake ecosystems in Rocky Mountain National Parks of Canada.

Variation in concentratios of DOC did not affect abundances of zooplankton in the spatial or temporal surveys, nor did DOC additions affect zooplankton abundance in the experiment. These findings did not support the idea that terrestrial inputs of C may support zooplankton production (Cole et al. 2011). Instead, abundance of zooplankton appeared to be supported by autotrophic production (Mohamed and Taylor 2009).

Alpine zooplankton are more sensitive to warming than montane populations

The experiment was the only study that directly tested the relative sensitivities of alpine and montane zooplankton populations to warming. Results supported the hypothesis that populations from warmer, lower elevation lakes would be less sensitive to warming than alpine populations (hypothesis 4; Table 5-4). Similarly, Holzapfel and Vinebrooke (2005) found that zooplankton populations from alpine lakes in Alberta were suppressed by warming from 10 °C to 20 °C, whereas zooplankton from warmer montane lakes were not affected. Populations of intertidal copepods also exhibited adaptation to higher upper lethal temperatures in warmer regions of Mexico compared to the colder climate of northern California (Kelly et al. 2012). Thus, effects of warming may not be uniform on zooplankton populations in alpine lakes compared to populations in warmer lakes at lower elevations and latitudes.

Future research directions

Use of SEM to analyze food webs dynamics is relatively new approach in aquatic ecology (Arhonditsis et al. 2006, Shimoda et al. 2011, Gudimov et al. 2012). In this study, SEM was effective for constructing simple food web models by analyzing linkages between trophic levels in a step-wise manner (Wells et al. 2008). Use of SEM to analyze long-term monitoring data is also a fairly novel application of the statistical tool (Grace et al. 2012). Further, SEM was used in this study to test multiple hypotheses simultaneously, which is a major advantage of the statistical method (Grace 2006). In this way, numerous ecological concepts can be integrated, such as nutrient stoichiometry, grazing, predation, and competition. Thus, these approaches should be applied in the analysis of food web dynamics in other long-term ecological datasets and whole-lake experiments.

SEM was also useful for disentangling the effects of multiple climate stressors on lake food webs in this study. Elucidating the impacts of multiple anthropogenic stressors on food webs represents a major challenge in ecology (Ormerod et al. 2010). Thus, SEM should be used in future studies to tease apart and understand the relative impacts on aquatic food webs from multiple anthropogenic stressors, such as climate change, nutrient pollution, and species losses and invasions.

One of the main implications of the findings in this study was that climate change may intensify symptoms of eutrophication in other temperate lakes where P pollutions is a problem (Moss 2012). This general hypothesis could be tested further using lakes with and without anthropogenic P inputs across a range in climatic conditions in order to disentangle the impacts of climate change and P-loading on lake productivity. Responses of lake food webs to climate change could be compared using SEM with long-term monitoring data and data from whole-lake experiments in boreal lakes at the Experimental Lakes Area in northwestern Ontario. It would also be useful to include data from deeper lakes to test the effects of increased thermal stratification on water column mixing, internal nutrient P-loading, and primary productivity (Posch et al. 2012, Winder and Sommer 2012). This research would be valuable for setting nutrient management ojectives for controlling eutrophication of inland waters under future climate change conditions.

Increasing inputs of C-rich terrestrial matter can potentially increase bacterial production relative to phytoplankton production in oligotrophic lakes (Blomqvist et al. 2001). However, long-term and intermidate scale findings in the spatial and temporal surveys, respectively, suggested that inputs of DOC did not suppress autotrophic production. In the experiment, short-term DOC additions alone suppressed phytoplankton abundance. Thus, it would be useful to investigate whether increased inputs of C-rich terrestrial matter could potentially reduce energy transfer to higher trophic levels in alpines lakes (Jansson et al. 2007). Food web analysis using SEM could also be used to determine the effects of DOC on the balance of autotrophic and heterotrophic production in alpine lakes. In addition, a whole-lake experiment where DOC-rich waters may be diverted into an alpine lake with low DOC concentrations would be particularly effective way at testing this problem.

Impacts of climate change on interactions among zooplankton, pelagic macroinvetebrates, and trout in alpine lakes could also be investigated using SEM and long-term and spatial survey data. The omnivorous amphipod *Gammarus lacustris* is often found in the water column of many alpine lakes in Alberta (Wilhelm and Schindler 1999). Detritivorous chironomid larvae and non-feeding chironomid pupae are also found in the water column of alpine lakes, where they are preyed on by gammarids and trout (Weidman et al. 2011). Climate warming and increased ice-free season length may increase the abundance of pelagic macroinveterbates with flexible life histories (Wilhelm and Schindler 2000). Warming may also affect predation of trout on plankton (Messner et al. 2013). Thus, impacts of climate change on higher trophic level interactions may have important implications for the productivity and structure of alpine lake food webs.

Main implications

Overall, the main potential impacts of climate change on alpine lake food webs observed in this study include increased P-availability, increased abundance of autotrophs relative to potentially mixotrophic phytoflagellates, suppression of large-bodied alpine-specialist zooplankton, increased abundance of small zooplankton taxa, and increased abundance of small copepods relative to small cladocerans. These findings suggest that climate change will ultimately increase phytoplankton productivity and reduce grazing efficiency in alpine lakes. The main symptoms of eutrophication, such as dramatic increases in the phytoplankton standing crop and cyanobacteria blooms, were not observed in this study. Also, in deeper lakes warming can increased thermal stratification and reduce nutrient upwelling, which may decrease P-availability during the growing season (Winder and Sommer 2012). However, the findings from this study offer support for the idea that climate change will exacerbate symptoms of eutrophication in temperate, relatively shallow lakes where P-pollution is also a problem (Moss 2012).

References

Allan, J. D. 1976. Life history patterns in zooplankton. American Naturalist 110:165–180.

Arhonditsis, G. B., C. A. Stow, L. J. Steinberg, M. A. Kenney, R. C. Lathrop, S. J. McBride, and K. H. Reckhow. 2006. Exploring ecological patterns with structural equation modeling and Bayesian analysis. Ecological Modelling 192:385–409.

Blomqvist, P., M. Jansson, S. Drakare, A. K. Bergstrom, and L. Brydsten. 2001. Effects of additions of DOC on pelagic biota in a clearwater system: Results from a whole lake experiment in northern Sweden. Microbial Ecology 42:383–394.

Brooks, P. D., and M. W. Williams. 1999. Snowpack controls on nitrogen cycling and export in seasonally snow-covered catchments. Hydrological Processes 13:2177–2190.

Bunting, L., P. R. Leavitt, R. P. Weidman, and R. D. Vinebrooke. 2010. Regulation of the nitrogen biogeochemistry of mountain lakes by subsidies of terrestrial dissolved organic matter and the implications for climate studies. Limnology and Oceanography 55:333–345.

Cole, J. J., S. R. Carpenter, J. Kitchell, M. L. Pace, C. T. Solomon and B. Weidel. 2011. Strong evidence for terrestrial support of zooplankton in small lakes based on stable isotopes of carbon, nitrogen, and hydrogen. Proceedings of the National Academy of Sciences of the United States of America 108:1975–1980.

Daufresne, M., K. Lengfellner, and U. Sommer. 2009. Global warming benefits the small in aquatic ecosystems. Proceedings of the National Academy of Sciences of the United States of America 106:12788–12793.

Dearden, P. and J. Dempsey. 2004. Protected areas in Canada: decade of change. Canadian Geographer 48: 225–239.

Elser, J. J., and J. Urabe. 1999. The stoichiometry of consumer-driven nutrient recycling: Theory, observations, and consequences. Ecology 80:735–751.

Findlay, D. L., S. E. M. Kasian, M. P. Stainton, K. G. Beaty, and M. Lyng. 2001. Climatic influences on algal populations of boreal forest lakes in the Experimental Lakes Area. Limnology and Oceanography 46:1784–1793.

Frost, P. C., C. T. Cherrier, J. H. Larson, S. Bridgham, and G. A. Lamberti. 2007. Effects of dissolved organic matter and ultraviolet radiation on the accrual, stoichiometry and algal taxonomy of stream periphyton. Freshwater Biology 52:319–330.

Garcia-Jurado, F., I. de Vicente, A. Galotti, A. Reul, F. Jimenez-Gomez, and F. Guerrero. 2012. Effect of drought conditions on plankton community and on nutrient availability in an oligotrophic high mountain lake. Arctic, Antarctic, and Alpine Research 44:50–61.

Gillooly, J. F., and S. I. Dodson. 2000. Latitudinal patterns in the size distribution and seasonal dynamics of new world, freshwater cladocerans. Limnology and Oceanography 45:22–30.

Grace, J. B. 2006. Structural equation modeling and natural systems, 1st edition. Cambridge University Press, Cambridge, UK.

Grace, J. B., J. Keeley, D. Johnson, and K. A. Bollen. 2012. Structural equation modeling and the analysis of long-term monitoring data. *in* R. A. Gitzen, J. J. Millspaugh, A. B. Cooper, and D. S. Licht, editors. Design and Analysis of Long-Term Ecological Monitoring Studies. . Cambridge University Press, Cambridge, UK.

Gudimov, A., E. O'Connor, M. Dittrich, H. Jarjanazi, M. E. Palmer, E. Stainsby, J. G. Winter, J. D. Young, and G. B. Arhonditsis. 2012. Continuous bayesian network for studying the causal links between phosphorus loading and plankton patterns in Lake Simcoe, Ontario, Canada. Environmental Science and Technology 46:7283–7292.

Herzig, A., R. S. Anderson, and D. W. Mayhood. 1980. Production and population-dynamics of *Leptodiaptomus-sicilis* in a mountain lake in Alberta, Canada. Holarctic Ecology 3:50–63.

Holtgrieve, G. W., D. E. Schindler, W. O. Hobbs, P. R. Leavitt, E. J. Ward, L. Bunting, G. Chen, B. P. Finney, I. Gregory-Eaves, S. Holmgren, M. J. Lisac, P. J. Lisi, K. Nydick, L. A. Rogers, J. E. Saros, D. T. Selbie, M. D. Shapley, P. B. Walsh, and A. P. Wolfe. 2011. A coherent signature of anthropogenic nitrogen deposition to remote watersheds of the Northern Hemisphere. Science 334:1545–1548.

Holzapfel, A. M., and R. D. Vinebrooke. 2005. Environmental warming increases invasion potential of alpine lake communities by imported species. Global Change Biology 11:2009–2015.

Jansson, M., L. Persson, A. M. De Roos, R. I. Jones, and L. J. Tranvik. 2007. Terrestrial carbon and intraspecific size-variation shape lake ecosystems. Trends in Ecology and Evolution 22:316–322.

Kelly, M. W., E. Sanford, and R. K. Grosberg. 2012. Limited potential for adaptation to climate change in a broadly distributed marine crustacean. Proceedings of the Royal Society B-Biological Sciences 279:349–356.

Kilham, S. S. 1986. Dynamics of Lake Michigan natural phytoplankton communities in continuous cultures along a Si-P loading gradient. Canadian Journal of Fisheries and Aquatic Sciences 43:351–360.

Kopacek, J., J. Hejzlar, J. Vrba, and E. Stuchlik. 2011. Phosphorus loading of mountain lakes: Terrestrial export and atmospheric deposition. Limnology and Oceanography 56:1343–1354.

McKnight, D. M., R. Harnish, R. L. Wershaw, J. S. Baronand S. Schiff. 1997. Chemical characteristics of particulate, colloidal, and dissolved organic material in Loch Vale Watershed, Rocky Mountain National Park. Biogeochemistry 36:99–124.

Messner, J. S., M. M. Maclennan, and R. D. Vinebrooke. 2013. Higher temperatures enhance the effects of invasive sportfish on mountain zooplankton communities. Freshwater Biology 58:354–364.

Mohamed, M. N. and W. D. Taylor. 2009. Relative contribution of autochthonous and allochthonous carbon to limnetic zooplankton: A new cross-system approach. Fundamental and Applied Limnology 175:113–124.

Moss, B. 2012. Cogs in the endless machine: Lakes, climate change and nutrient cycles: A review. Science of the Total Environment 434:130–142.

Murphy, C. A., P. L. Thompson, and R. D. Vinebrooke. 2010. Assessing the sensitivity of alpine lakes and ponds to nitrogen deposition in the Canadian Rocky Mountains. Hydrobiologia 648:83–90.

Ormerod, S. J., M. Dobson, A. G. Hildrew, and C. R. Townsend. 2010. Multiple stressors in freshwater ecosystems. Freshwater Biology 55:1–4.

Parker, B. R., and D. W. Schindler. 2006. Cascading trophic interactions in an oligotrophic species-poor alpine lake. Ecosystems 9:157–166.

Parker, B. R., R. D. Vinebrooke, and D. W. Schindler. 2008. Recent climate extremes alter alpine lake ecosystems. Proceedings of the National Academy of Sciences of the United States of America 105:12927–12931.

Posch, T., O. Köster, M. M. Salcher, and J. Pernthaler. 2012. Harmful filamentous cyanobacteria favoured by reduced water turnover with lake warming. Nature Climate Change 2:809–813.

Rothhaupt, K. O. 1996. Laboratory experiments with a mixotrophic chrysophyte and obligately phagotrophic and phototrophic competitors. Ecology 77:716–724.

Sadro, S., C. E. Nelson, and J. M. Melack. 2012. The influence of landscape position and catchment characteristics on aquatic biogeochemistry in high-elevation lake-chains. Ecosystems 15:363–386.

Shimoda, Y., M. E. Azim, G. Perhar, M. Ramin, M. A. Kenney, S. Sadraddini, A. Gudimov, and G. B. Arhonditsis. 2011. Our current understanding of lake ecosystem response to climate change: What have we really learned from the north temperate deep lakes? Journal of Great Lakes Research 37:173–193.

Sommer, U., and F. Sommer. 2006. Cladocerans versus copepods: the cause of contrasting top-down controls on freshwater and marine phytoplankton. Oecologia 147:183–194.

Sterner, R. W., and D. O. Hessen. 1994. Algal nutrient limitation and the nutrition of aquatic herbivores. Annual Review of Ecology and Systematics 25:1–29.

Tilman, D., R. Kiesling, R. Sterner, S. S. Kilham, and F. A. Johnson. 1986. Green, bluegreen and diatom algae: Taxonomic differences in competitive ability for phosphorus, silicon and nitrogen. Archiv Fur Hydrobiologie 106:473–485.

Urabe, J., J. Clasen, and R. W. Sterner. 1997. Phosphorus limitation of *Daphnia* growth: Is it real? Limnology and Oceanography 42:1436–1443.

Urabe, J., J. Togari, and J. J. Elser. 2003. Stoichiometric impacts of increased carbon dioxide on a planktonic herbivore. Global Change Biology 9:818–825.

Vanderploeg, H. A., J. F. Cavaletto, J. R. Liebig, and W. S. Gardner. 1998. *Limnocalanus macrurus* (Copepoda: Calanoida) retains a marine arctic lipid and life cycle strategy in Lake Michigan. Journal of Plankton Research 20:1581–1597.

Vähätalo, A. V., K. Salonen, U. Münster, M. Järvinen, and R. G. Wetzel. 2003. Photochemical transformation of allochthonous organic matter provides bioavailable nutrients in a humic lake. Archiv Fur Hydrobiologie 156:287–314.

Weidman, R. P., D. W. Schindler, and R. D. Vinebrooke. 2011. Pelagic food web interactions among benthic invertebrates and trout in mountain lakes. Freshwater Biology 56:1081–1094.

Wells, B. K., J. C. Field, J. A. Thayer, C. B. Grimes, S. J. Bograd, W. J. Sydeman, F. B. Schwing, and R. Hewitt. 2008. Untangling the relationships among climate, prey and top predators in an ocean ecosystem. Marine Ecology-Progress Series 364:15–29.

Wilhelm, F. M., and D. W. Schindler. 1999. Effects of *Gammarus lacustris* (Crustacea : Amphipoda) on plankton community structure in an alpine lake. Canadian Journal of Fisheries and Aquatic Sciences 56:1401–1408.

Wilhelm, F. M., and D. W. Schindler. 2000. Reproductive strategies of *Gammarus lacustris* (Crustacea : Amphipoda) along an elevation gradient. Functional Ecology 14:413–422.

Winder, M., and U. Sommer. 2012. Phytoplankton response to a changing climate. Hydrobiologia 698:5–16.

Table 5-1. Dissolved nutrients: Support for hypotheses among the spatial and temporal surveys and experiment

Study[1]	Hypothesis	Response	Factor(s)	Notes
Spatial	1A	(–)DIN	(+)Temp	
	1A	(+)TDP	(+)Temp, (–)Depth	
	1A	(–)DIN:TDP	(+)Temp	
	1B	(+)DOC	(+)Temp x (–)Ice-off	
	1B	(+)DOC:TDP	(+)Temp x (–)Ice-off	
Temporal		(n/s)DIN	n/s	x
	1A	(n/c)TDP	(–)Rain x (+)Temp	a
	1A	(n/c)DIN:TDP	(–)Rain x (+)Temp	x, a
	1A	(–)DOC	(–)Rain	
	1A	(–)DOC:TDP	(–)Rain	x
Experiment	1A	(+)DIN	(+)DOC	
	1A	(+)TDP	(+)DOC	b
		(n/s)DIN:TDP	n/s	x
	1A	(–)DIN	(+)Temp x (+)DOC	c
	1A	(–)TDP	(+)Temp x (+)DOC	c
		(n/s)DIN:TDP	n/s	x
	1A	(+)DOC	(+)DOC	d
		(n/s)DOC:TDP	n/s	x

[1] Ordered by study, hypothesis, response

Hypotheses:
1A - Warming, decreased precipitation, and decreased lake depth will increase P-content (decrease C:P)
1B - Increased terrestrial inputs (advanced ice-off and increased soil cover) will decrease P-content
1C - Increased cladocerans will decrease P-content, whereas increased copepods will increase P-content

Notes:
n/s - not significant
n/c - no net change
a - (–)Rain increased TDP, but this was offset by (+)Temp
b - (+)DOC slightly increased TDP
c - (+)Temp x (+)DOC increased availability and uptake of DIN and TDP
d - (+)DOC did not increase DOC:TDP
x - Data not shown in results

Table 5-2. Particulate nutrients: Support for hypotheses among the spatial and temporal surveys and experiment

Study	Hypothesis[1]	Response	Factor(s)	Notes
Spatial	1A	(–)PC:PP	(+)Temp	a
Experiment	1A	(–)PC:PP alpine	(+)Temp x (+)DOC	
Spatial	1B	(+)PC	(–)Depth, (+)Soil	
Spatial	1B	(+)PC:PP	(+)Soil	a
Experiment	1B	(+)PC:PP montane	(+)Temp x (+)DOC	b
Spatial	1C	(–)PC:PP	(+)Temp x (–)Clad:Cope	
Temporal	1C	(+)PC:PP	(+)Temp x (+)Daphnia	
Experiment	1C	(+)PC:PP	(+)Temp x (+)Daphnia (–)Hespero	

[1] Ordered by hypothesis, study, response

Hypotheses:
1A - Warming, decreased precipitation, and decreased lake depth will increase P-content (decrease C:P)
1B - Increased terrestrial inputs (advanced ice-off and increased soil cover) will decrease P-content
1C - Increased cladocerans will decrease P-content, whereas increased copepods will increase P-content

Notes:
a - (+)Soil partially increased PC:PP, but net change was negative
b - (+)PC:PP in montane where DOC appeared to be more terrestrial in origin than alpine
c - (+)TDP unexpectedly increased PC and PC:PP
x - Data not shown in results

Table 5-3. Phytoplankton: Support for hypotheses among the spatial and temporal surveys and experiment

Study[1]	Hypothesis	Response(s)	Factor(s)	Notes
Spatial	2A	(+)Chlorophytes+Cyanophytes	(+)Temp x (–)DIN:TDP	
	2A	(–)Flagellates	(+)Temp x (–)DIN:TDP	
Temporal	2A	(+)Diatoms	(–)Rain x (+)TDP	
	2A	(–)Flagellates	(–)Rain x (+)TDP	
Experiment	2A	(+)chl a, chl b, Lutein+Zeaxanthin	(+)Temp x (+)DOC	
	2B	(–)chl a, chl b, Lutein+Zeaxanthin	(+)Temp, (+)DOC	
	2B	(+)Fucoxanthin	(+)Temp, (+)DOC	a

[1] Ordered by study, hypothesis, response

Hypotheses:
2A - Warming and increased P-content will increased autotrophs relative to mixotrophs
2B - Increased terrestrial inputs and decreased P-content will increase mixotrophs relative to autotrophs

Notes:
a - (+)Temp and (+)DOC each increased fucoxanthin, which in the absence of other pigments produced by diatoms, dinophytes or cryptophytes, suggested an increase in chrysophytes

Table 5-4. Zooplankton: Support for hypotheses among the spatial and temporal surveys and experiment

Study[1]	Hypothesis	Response(s)	Factor(s)	Notes
Spatial	3C	(–)D. middendorffiana	(+)Temp	a
Spatial	3C	(–)Hesperodiaptomus	(+)Temp	b
Spatial	3C	(+)D. pulex, Chydorus, Diaptomus	(+)Temp	
Spatial	3B	(–)Cladoceran:Copepod	(+)Soil x (+)PC:PP	
Temporal	3A	(+)D. middendorffiana	(+)Temp	
Temporal	see note "c"	(+)Hesperodiaptomus	(–)Precip x (–)Ice-off	c
Experiment	3A	(+)D. middendorffiana	(+)Temp	
Experiment	3A	(–)Hesperodiaptomus	(+)Temp	
Experiment	3A	(+)Cladoceran:Copepod	(+)Temp	
Experiment	3C	(+)Diaptomus	(+)Temp	
Experiment	4	(+)Clad:Cope in alpine only	(+)Temp	

[1] Ordered by study, hypothesis, response

Hypotheses:
3A - Warming and increased P-content will increase cladocerans relative to copepods
3B - Increased terrestrial inputs and decreased P-content will increase copepods relative to cladocerans
3C - Warming will decrease large zooplankton taxa and increase small taxa
4 - Effects of warming on zooplankton will be greater in alpine than montane lakes

Notes:
a - Warming appeared to promote *D. pulex* over *D. middendorffiana*
b - Warming and increased primary productivity appeared to promote *Diaptomus* over *Hesperodiaptomus*
c - Advanced ice-free season unexpectedly increased *Hesperodiaptomus*

APPENDIX 1: STRUCTURAL EQUATION MODELING – BASIC CONCEPTS AND CALCULATIONS[4]

Introduction

Structural equation modeling (SEM) is a multivariate statistical method used to test for direct and indirect effects within a network of related variables. SEM was used in the present study to test for the direct and indirect effects of climate variables on and among key food web variables including dissolved and particulate nutrients, phytoplankton, and zooplankton in the spatial survey (Chapter 2) and temporal survey (Chapter 3). In this appendix-chapter, SEM is explained in terms of its history in ecology and basic concepts and calculations. Examples and concepts from Grace and Bollen (2005) and Grace (2006) were adapted along with examples using data from the present study to equip the reader with the basic concepts of SEM.

A brief history of structural equation modeling

Geneticist Sewall Wright is credited for developing path analysis from the analysis of multiple regression coefficients, in order to understand multiple causes and multiple responses in observational survey data (Wright 1918, 1920, 1934, 1960). From 1950–1990, path analysis and structural equation modeling were developed mainly by quantitative economists, psychologists, and sociologists. In the early 1970s, modern SEM was synthesized by Jöreskog (1973) in the software program LISREL.

In recent decades, use of SEM increased steadily across scientific fields including ecology (Figure A1-1). Since 1990, there have been about twice as many papers in ecology using multiple regression (1,170) as SEM (589). By 2009-2011, however, there were roughly equal numbers of papers in ecology using SEM and multiple regression.

There are several helpful reviews of the use of path analysis and SEM in ecology. Mitchell (1992) published an early review and provided a simple explanation of the use

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[4] The two primary references for this appendix-chapter were 1) Grace, J. B. (2006). Structural Equation Modeling and Natural Systems, and 2) Grace, J. B., and K. A. Bollen (2005). Interpreting the results from multiple regression and structural equation models. Bulletin of the Ecological Society of America 86:283 – 295.

of path analysis in ecology. Another early, highly cited ecological study using SEM was conducted by Wootton (1994) who examined the direct and indirect effects of shorebirds on intertidal invertebrates. Several studies have highlighted problems associated with SEM in ecological studies. Petraitis et al. (1996) demonstrated how collinearity among predictor variables can inflate effect sizes (i.e. path coefficients) in SE models. They also critized authors in ecology using SEM that appeared to be violating assumptions of large sample size, multivariatenormality, linearity, and additivity. Pugesek and Grace (1998) addressed these criticisms by suggesting that SEM has similar requirments for representative sample size and linearity as other multivariate statistics, including MANOVA and multiple regression. They also outlined various techniques for dealing with expected non-linear and non-additive responses . Furthermore, more recent reviews of SEM in ecology include Graham (2003) who explained how SEM can be useful with dealing with multicollinearity in ecological datasets. O'Brien (2007) provided a clear explanation of the mathematical effects of collinearity on inflation of variance estimates in SEM. Dealing with effects of collinearity among predictor and response variables is explained further in this appendix chapter (below).

James B. Grace (US Geological Survey, Lafayette Louisiana) is currently among the most published authors using SEM in ecology. His text 'Structural Equation Modeling and Natural Systems' (Grace 2006) provides a thorough explanation of the calculations and assumptions underlying the method. Grace and Bollen (2005) provides a particularly simple explanation of the basic mathematics underlying SEM by comparing it with multiple regression. Several examples from Grace and Bollen (2005) are explained in further detail below.

Several recent studies in aquatic ecology have demonstrated the use of SEM to determine the direct and indirect effects of multiple ecological stressors on aquatic food webs (Arhonditsis et al. 2006, Shimoda et al. 2011, Gudimov et al. 2012). In particular, George B. Arhonditsis (University of Toronto, Scarborough, Ontario) is among the most published authors using SEM in aquatic ecology. In the present study, SEM was used to conduct basic food web analysis in an analysis of survey data from 16 mountain lakes and long-term monitoring data from an alpine lake. A similar approach was followed by

Wells et al. (2008) in their analysis of climate impacts on a California coastal marine food web.

A few of the SEM software programs include LISREL (Linear Structural Relations), R Statistics, and AMOS (Analysis of Moment Structures). User manuals for these programs often provide simpler explanations of SEM methods than text books and scientific publications, although they are not adequate for a complete understanding of the method. Nevertheless, manuals for AMOS, a windows based platform, are particularly useful (Arbuckle 2009, Byrne 2009). Other resources include the journal 'Structural Equation Modeling: A Multidisciplinary Journal' (Taylor and Francis Publishing), which is dedicated to theoretical and statistical issues involved in SEM. In addition, 'SEMNET: Structural Equation Modeling Discussion Group' (University of Alabama) is an excellent online resource.

What is structural equation modeling?

Definitions and diagrams

Structural equation modeling is a direct extension of multiple regression. That is, each SE model is made up of two or more multiple or simple regression equations that control for each other and are solved together in a single model. Thus, SEM can be described as "multiple, multiple regression" because it involves solving two or more multiple regression equations simultaneously, where there is one equation for each response variable in the model (Grace and Bollen 2005). One of the main differences with stepwise regression is that SEM allows for the testing of effects among response variables, in addition to testing the effects of explanatory variables on response variables.

In Figure A1-2A, a single multiple regression equation is used to calculate the effects of X_1 and X_2 on Y_1, using one partial regression coefficient for each X variable. Double-headed curved arrows represent bivariate correlation coefficients that are non-causal, i.e., correlation between these variables is unexplained. Single-headed arrows represent simple or partial regression coefficients where the predictor variable is inferred to have a casual effect on the response variable. However, if X_1 is expected to directly affect X_2, this collinearity can be calculated as a causal effect using SEM. In Figure A1-2B, two regression equations are used to calculate the direct and indirect effects of X_1 on

Y_1 and the direct effects of X_1 on Y_2. Thus, instead of just controlling $X_1 \rightarrow Y_1$ and $X_2 \rightarrow Y_1$ for the collinearity between $X_1 \leftrightarrow X_2$, SEM can be used to calculate the direct effect of $X_1 \rightarrow X_2$ and the indirect of $X_1 \rightarrow Y_2 \rightarrow Y_1$.

Basic equations and coefficients

Figure A1-3 represents a more detailed description of path coefficients and equations used in multiple regression models and SE models. SEM uses the same basic equations as multiple regression. All regression coefficients in the examples in this appendix are Z-transformed, so the units are in standard deviations (SD). Path coefficients are interpreted the same as any slope coefficient. Standardized coefficients are interpreted to indicate the change in SD's of Y per 1 SD increase in X. Standardized coefficients > 2–3 SD are generally considered to be inflated often due to high collinearity among the predictor variables. In the extreme case, a coefficient of 6 SD would represent an increase in 99% of the total range in the response variable for every 1 SD increase in the predictor variable. This is the case because 6 SD represents 99% of the range of a normally distributed variable. In practice, standardized path coefficients are > 1 SD only when strong indirect effects occur combined with direct effects on the response variables. A more detailed explanation of the calculations of path coefficients is given below.

Why use structural equation modeling?

Direct, indirect, and total effects[5]

In Figure A1-4, Grace and Bollen (2005) compare multiple regression and SEM and highlight the similarities between the two methods plus the advantages of SEM. This example illustrates how a multiple regression model can be rearranged into a SE model to test the direct, indirect, and total effects among dependent and independent variables. In both models, elevation had a positive effect on plant cover after a forest fire. However, the SE model showed that elevation also had a positive, indirect effect on plant cover via decreased stand age and increased fire severity. The largest discrepancy between the two

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[5] Adapted from Grace and Bollen 2005. Interpreting the results from multiple regression and structural equation models ESA Bulletin 86:283-295

models was that the SE model showed that stand age had a negative, indirect effect on plant cover; this effect was not apparent in the multiple regression model. The direct effect of fire severity was the same in both models; fire severity had a direct, negative effect on plant cover in both models. Thus, this example shows how SEM can reveal more detailed information about the interactions among a network of related variables.

Dealing with collinearity

Regression coefficient inflation scenarios

In any multiple regression model, as collinearity among X variables increases, the strongest partial correlations between X and Y variables will increase in a non-linear manner. Thus, collinearity may result in over-estimation of the effects of the strongest X variables and obscure the effects of weaker partial correlations. Figure A1-5A is a simulated multiple regression model, where X_1 is moderatley correlated with Y_1 ($r^2 = 0.50$), X_2 is weakly correlated with Y_1 ($r^2 = 0.15$), and X_1 and X_2 are moderately collinear ($r^2 = 0.35$). In the numerator of the equation for the partial regression coefficient, the effect of X_1 on Y_1 ($\gamma_{1,1}$) is subtracted from the effect of X_2 on Y_1, while the denominator standardizes the coefficient to the unique variance (i.e., not shared) between X_1 and X_2.

To illustrate the effect of collinearity between X_1 and X_2 on partial regression coefficients, in Figure A1-5B, each term of the partial regression equation was increased from 0 to 0.95 SD in four separate scenarios. In scenario 1, as the correlation between X_1 and Y_1 (r_{x1y1}) increased, $\gamma_{1,1}$ increased from 0 to 1.02 SD. This result demonstrated that maximum potential effect of X_1 on Y_1 was around 1.02 SD when the other terms in the regression were held constant. This effect of r_{x1y1} on $\gamma_{1,1}$ was approximately linear. In scenario 2, as the correlation increased between X_2 and Y_1 (r_{x2y1}) the effect of X_1 on Y_1 ($\gamma_{1,1}$) decreased from around 0.57 to 0.19 SD in roughly a linear manner. In scenario 3, as collinearity increased between X_1 and X_2 (r_{x1x2}) the effect of X_1 on Y_1 ($\gamma_{1,1}$) increased exponentially from 0.50 to 3.67 SD. This result demonstrated how increasing collinearity can exponentially inflate partial regression coefficients especially when r_{x1x2} was > 0.8. In scenario 4, as both r_{x1x2} and r_{x1y1} were increased, $\gamma_{1,1}$ increased exponentially from 0 to 9.82 SD. This result showed how small increases in the partial regression coefficient can become inflated when collinearity of predictor variables is high.

Effects of temperature and ice-off on DOC from a survey of 16 mountain lakes in Alberta (2005–2007)

In Figure A1-6, the example illustrates how SEM can be used to rearrange a multiple regression model to avoid potential problems with collinear independent variables and to calculate the direct and indirect effects on response variables. In the multiple regression model in Figure A16-A, temperature and ice-off are significant predictors of DOC, although both independent variables are highly collinear ($r^2 = -0.88$). High collinearity can inflate partial regression coefficients because the correlation between X_1 and X_2 is included in the denominator of the partial regression coefficient equation. This term ($1 - r_{2x1x2}$) represents the unique variance between X_1 and X_2. The partial regression coefficient can increase non-linearly as unique variance between X_1 and X_2 decreases.

Inflation of regression coefficients can exclude weaker predictors from the model. For example, DOC may also be affected by precipitation and soil cover of catchment, but these variables may be excluded by the step-wise selection process if the coefficients for temperature and ice-off are inflated.

It is expected that increased temperature advances ice-off date of lakes. Thus, in Figure A1-6B, the model is rearranged into a SE model to predict the effect of temperature on ice-off and to predict the indirect effect of temperature on DOC via ice-off. The indirect effect of temperature on DOC via ice-off is calculated with the equation for a compound path coefficient, which is not affected by collinearity as is the partial regression equation.

Comparing multiple regression and SEM

Figure A1-7 represents the first in a series of three step-wise regression and SE models that were compared and modified to control for colinearity among climate variables. Standardized partial regression coefficients are shown for all dissolved nutrients, however, the comparison is focused on the results for dissolved Si, which illustrate the advantages of SEM. Dashed and solid lines indicate negative and positive coefficients, respectively. The symbols (+) and (−) indicate positive and negative changes, respectively, in climate variables. Thick lines indicate high collinearity among climate variables and inflated regression coefficients for response variables. Standardized partial regression coefficients are given for both SWR models (upper panel) and SE models (lower panel). Variables marked with "x" have inflated regression coefficients (i.e., > 2–3 SD) due to high collinearity among climate variables.

Step-wise regression and SEM provided almost equivalent results using this model structure. However, high collinearity among precipitation variables (snow, summer rain, and winter rain) inflated the partial regression coefficients (i.e., > 2–3 SD) between summer rain and winter rain and Si. Indeed, bivariate correlation coefficients (r^2) were > 0.8 among the three precipitation variables. Collinearity among precipitation variables could not be explained without adding variables to the model (i.e., elevation and latitude). Thus, precipitation variables were eliminated and total precipitation was used instead in the subsequent models.

In Figure A1-8, comparison with the previous models (those with 7 climate variables) illustrated how collinearity and inflation of coefficients can over-estimate the effects of some predictor variables and exclude the effect of other predictors. In the previous model, summer rain and winter rain were selected as predictors of Si, ice-off and temperature were excluded from the model. In this model, advanced ice-off, increased temperature, decreased precipitation, and decreased depth were all significant predictors of Si. However, partial regression coefficients for Si were still inflated by high collinearity between temperature and ice-off in this model. Temperature, precipitation, and lake depth were all expected to affect ice-off. Thus, instead of eliminating ice-off from the analysis to avoid the problem of collinearity and inflation of regression coefficients, the effects of temperature, precipitation, and depth on ice-off were modeled using SEM in the subsequent analysis.

In Figure A1-9, the SE model allowed for the calculation of direct effects of climate variables on dissolved nutrients and the indirect effects of climate variables via advanced ice-off. All partial regression coefficients were within the expected range of 0-2 SD because collinearity among precipitation variables and between temperature and ice-off was avoided. Precipitation had a much weaker effect on Si compared to the initial models; decreased precipitation directly reduced Si, but also indirectly increased Si to a lesser extend via advanced ice-off. Advanced ice-off was shown to increase Si, which was an effect that was not apparent in the initial step-wise regression model. Increased temperature was shown to indirectly increase Si via advanced ice-off; temperature had the opposite effect in the previous step-wise regression model. Decreased depth directly decreased Si, but it also indirectly increased Si by advancing ice-off. Thus, SEM revealed more detailed information than step-wise regression about the network of direct and indirect effects of climate variables on dissolved nutrients in the lake survey.

Direct, indirect, and reciprocal effects[6]

SE models can be setup in a number of ways depending on the expected relationships among variables. In Figure A1-10, models A and B represent the most basic forms of multiple regression and SE models. The advantage of SEM in model B is that collinearity between X_1 and X_2 (X_2 is now Y_2) is measured as a causal effect, and the direct and indirect effects on Y_1 can be measured.

In model C, effects on multiple dependent variables can be calculated along with collinearity among X and Y variables when there is no justification for causal effects between X variables and between Y variables. This model is still equivalent to two multiple regression equations, one for each Y variable, with the added feature that residual covariance is explained between Y_1 and Y_2.

In model D, the effect of Y_2 on Y_1 is calculated as a causal effect instead of as an unexplained correlation. In this case, $Y_2 \rightarrow Y_1$ is expected from *a priori* theory. Model D and B are a clear departure from multiple regression because of the capacity to calculate the effect of one response variable on another response variable.

In model E, all effects among X and Y variables are calculated as casual effects. In addition, reciprocal effects between Y variables were also be calculated. Thus, SEM allows for modeling direct, indirect, total, and reciprocal effects, which are all expected in food web models.

In model F, use of unmeasured "latent" variables distinguishes structural equation modeling from path analysis. In this simple case, a coding variable was used to reverse the sign of the regression coefficient for X_1. Latent variables can also include composite

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[6] Adapted from Grace, J.B. 2006. Structural Equation Modeling and Natural Systems.

variables with multiple indicators. This model structure is used in chapters 2 and 3 of this dissertation.

How to calculate path coefficients in SEM[7]

Main concepts in multiple regression and SEM

SEM is based on the same basic concepts involved in multiple regression which include: variance, covariance, z-transformation, correlation, regression coefficients (standardized and unstandardized), and partial regression coefficients (standardized and unstandardized).

Variance

[Eq.] $VAR_x = \Sigma(x_{i\text{-}j} - x_{mean})^2 / (n - 1)$

Covariance

[Eq.] $COV_{xy} = \Sigma(x_{i\text{-}j} - x_{mean}) \bullet (y_{i\text{-}j} - y_{mean}) / (n - 1)$

Because units and scales of x and y can be different, it is helpful to standardize covariance using the z-transformation:

Z-score transformation

[Eq.] $Z_i = x_i - x_{mean} / SD_x$

Pearson correlation coefficient

[Eq.] $r_{xy} = \Sigma(Zx_{i\text{-}j} \bullet Zy_{i\text{-}j}) / (n - 1)$

The correlation coefficient can also be calculated as:
[Eq.] $r_{xy} = COV_{xy} / (SD_x \bullet SD_y)$

Thus, correlation can be standardized by either using z-scores or by dividing covariance by the product of SD's of X and Y.

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[7] Adapted from Grace 2006 - Structural Equation Modeling and Natural Systems

Linear regression

[Eq.] $y = b_{yx} \bullet x + a$

The distinction between the regression coefficient (b_{yx}) and correlation coefficient (r_{xy}) is that with b_{yx}, x is considered a predictor and y is a response variable.

Standardized regression coefficient

[Eq.] $b_{yx} = r_{xy} \bullet (SD_y / SD_x)$

When X and Y are standardized as Z-scores, SD_y and $SD_x = 1$, so $b_{yx} = r_{xy}$. However, un-standardized b_{yx} is not equivalent to un-standardized r_{xy}.

Un-standardized regression coefficient

[Eq.] $b_{yx} = COV_{xy} / VAR_x$

The un-standardized regression coefficient is normalized to variance of the predictor, whereas the standardized coefficient is normalized to the cross-product of SD's of X and Y.

Eight rules for calculating path coefficients

Grace (2006) articulated eight basic rules for calculating path coefficients in SEM, which provide a good introductory framework for interpreting SE models and path coefficients.

Rule #1 Non-causal paths

"The path coefficient of a non-causal (i.e., unanalyzed) path is the bivariate correlation coefficient (standardized) or covariance coefficient (un-standardized) between the two variables involved."

Rule #2 Simple paths

"When two variables are connected by a single path, the coefficient is equal to the bivariate regression coefficient (standardized or un-standardized)." For example, in Figure A1-11, r_{x1y1} is a simple path coefficient.

Rule #3 Compound paths

"The strength of a compound path is equal to the product of coefficients along that path." In Figure A1-11, for example, the compound path $X_1 \rightarrow Y_1 \rightarrow Y_2$ is $r_{x1y2} = r_{x1y1} \bullet r_{y1y2}$

Rule #4 Partial regression coefficients

"When two variables are connected by more than one causal path, a partial regression coefficient is involved." For example, in Figure A1-12, the partial regression equation for path $\gamma_{2,1}$ is:

[Eq.] $\gamma_{2,1} = [r_{x1y2} - (r_{x1y1} \bullet r_{y1y2})] / (1 - r^2_{x1y1})$

where γ = effects of X variables on Y variables. The numerator is equal to the direct correlation between $X_1 \rightarrow Y_2$ minus the indirect correlation among $X_1 \rightarrow Y_1 \rightarrow Y_2$ (i.e., Rule #3). The denominator is a term that standardizes the effect of $X_1 \rightarrow Y_2$ to the degree which variance is not shared between X_1 and Y_1. If X_1 and Y_1 were not correlated, $X_1 \rightarrow Y_2$ would be independent of $Y_1 \rightarrow Y_2$. This equation has the property of allowing the sum of all path coefficients between two variables (X_1 and Y_2) to equal the bivariate correlation between those variables (see Rule #8).

In Figure A1-12, the partial regression equation for path $\beta_{2,1}$ is:

[Eq.] $\beta_{2,1} = [r_{y1y2} - (r_{x1y1} \bullet r_{x1y2})] / (1 - r^2_{x1y1})$

where β = effects of Y variables on other Y variables, and $Y_1 \rightarrow Y_2$ is controlled for $X_1 \rightarrow Y_2$ and $X_1 \rightarrow Y_1$.

Rule #5 Error terms

"Coefficients for error terms are correlation or covariance coefficients representing the error of prediction for the Y variable." Error terms represent unexplained effects on the response variable. In Figure A1-13, the equation for the error term for Y_1 is:

[Eq.] $e_1 = \sqrt{(1 - r^2_{y1})}$

Rule #6 Unanalyzed correlations

"The unanalyzed correlation between two endogenous variables is represented by a partial correlation or covariance coefficient." In Figure A1-13, the equation for partial correlation between e_1 and e_2 ($\psi_{1,2}$) is:

[Eq.] $r_{y1y2(x1)} = \psi_{1,2} = r_{y1y2} - (r_{x1y1} \bullet r_{x1y2}) / \sqrt{[(1 - r^2_{x1y1})(1 - r^2_{x1y2})]}$

Note that if e_1 and e_2 were not correlated, the bivariate correlation between Y_1 and Y_2 could still be calculated as: $r_{y1y2} = r_{x1y1} \bullet r_{x1y2}$. If Y_1 and Y_2 are truly independent, their bivariate correlation should equal $r_{y1y2}= r_{x1y1} \bullet r_{x1y2}$. However, if the observed correlation between Y_1 and Y_2 is much larger than expected, this indicates some factor is missing from the model that may explain the correlation between Y_1 and Y_2. Thus, the partial correlation between e_1 and e_2 should be included in the model.

Rule #7 Total effects

"The total effect of one variable on another is the sum of its direct and indirect effects through causal paths." In Figure A1-14A, the total effect of X_1 on Y_2 = 0.24 + (0.55 x 0.47) = 0.50, which happens to equal the bivariate correlation (not shown), so there is no evidence for a partial correlation between e_1 and e_2.

In Figure A1-14B, all causal paths involve partial regressions, and standardized partial regression coefficients are shown. The total effect of X_1 on Y_1 is 0.64 because there is only one casual effect between these variables. The total effect of X_1 on Y_2 is 0.15 + (0.64 x 0.27) = 0.32. The total effect of X_2 on Y_2 = -0.11 x 0.27 = -0.03.

Rule #8 Total correlations

"The sum of all causal and non-causal pathways connecting two variables equals the total correlation (i.e., bivariate correlation) between those two variables." Total correlations involve total effects and non-causal correlations. For example, in Figure A1-15A the total correlation for $X_1 \rightarrow Y_1 = 0.64 + (0.80 \times -0.11) = 0.55$. The total correlation $X_2 \rightarrow Y_2 = (-0.11 \times 0.27) + (0.80 \times 0.15) + (0.80 \times 0.64 \times 0.27) = 0.23$. Note that the partial path coefficient $\gamma_{1,2} = -0.11$ (i.e., $X_2 \rightarrow Y_1$) was much less than the bivariate correlation $X_2 \leftrightarrow Y_1 = 0.40$, which indicated that the effect of X_2 on Y_1 was much weaker than predicted by the bivariate correlation (Figure A1-15 B and C).

Advantages of SEM – Recapitulation

Direct, indirect, and total effects

SEM allows for the simultaneous calculation of effects of X variables on Y variables, plus the effects of Y variables on other Y variables, which facilitates the calculation of direct, indirect, and total effects on all response variables.

Collinearity

All regression models suffer from the same problems with collinearity. However, SEM allows models and underlying equations to be rearranged to avoid collineariy in some cases where there is a theoretical basis from *a priori* information to do so. Thus, SEM can be used to model collinearity instead of just subtracting it from effects in the model. In some cases, highly collinear independent variables may have to be excluded from the model if there is no theoretical basis for explaining collinearity among these variables without greatly increasing model complexity.

Reciprocal effects

Calculating the effects of Y variables on other Y variables allows for the calculation of reciprocal effects. This is useful where, for example, bottom-up effects of climate variables on nutrients, phytoplankton, and zooplankton can be calculated and compared simultaneously with top-down effects of nutrient excretion and grazing of phytoplankton by zooplankton (chapters 2 and 3). Thus, SEM facilitates the testing of food web models (e.g., Wells et al. 2008).

Visualizing equations

SEM diagrams allow the researcher and the reader to visualize the hypothesized relationships among a network of related variables, instead of only presenting a series of related equations.

Whole model fit

In addition to what was described above, SEM allows numerous equations to be tested together in a single model using a chi-square statistic. This model fit statistic indicates whether the hypothesized model adequately predicts the covariance among variables in the observed dataset. In the same way, MANOVA provides an overall test of multiple ANOVAs.

References

Arbuckle, J. 2009. Amos 18 user’s guide. . SPSS Inc., Chicago, USA.

Arhonditsis, G. B., C. A. Stow, L. J. Steinberg, M. A. Kenney, R. C. Lathrop, S. J. McBride, and K. H. Reckhow. 2006. Exploring ecological patterns with structural equation modeling and Bayesian analysis. Ecological Modelling 192:385–409.

Byrne, B. M. 2009. Structural equation modeling with AMOS: basic concepts, applications, and programming. . CRC Press, New York, USA.

Grace, J. B. 2006. Structural equation modeling and natural systems, 1st edition. Cambridge University Press, Cambridge, UK.

Grace, J. B., and K. A. Bollen. 2005. Interpreting the results from multiple regression and structural equation models. Bulletin of the Ecological Society of America 86:283 – 295.

Graham, M. H. 2003. Confronting multicollinearity in ecological multiple regression. Ecology 84:2809–2815.

Gudimov, A., E. O’Connor, M. Dittrich, H. Jarjanazi, M. E. Palmer, E. Stainsby, J. G. Winter, J. D. Young, and G. B. Arhonditsis. 2012. Continuous bayesian network for studying the causal links between phosphorus loading and plankton patterns in Lake Simcoe, Ontario, Canada. Environmental Science and Technology 46:7283–7292.

Jöreskog, K. G. 1973. A general method for estimating a linear structural equation system. Pages 85–112 *in* A. S. Goldberger and O. D. Duncan, editors. Structural Equation Models in the Social Sciences. Seminar Press, New York.

Mitchell, R. J. 1992. Testing evolutionary and ecological hypotheses using path-analysis and structural equation modeling. Functional Ecology 6:123–129.

Petraitis, P. S., A. E. Dunham, and P. H. Niewiarowski. 1996. Inferring multiple causality: The limitations of path analysis. Functional Ecology 10:421–431.

Pugesek, B. H., and J. B. Grace. 1998. On the utility of path modelling for ecological and evolutionary studies. Functional Ecology 12:853–856.

Shimoda, Y., M. E. Azim, G. Perhar, M. Ramin, M. A. Kenney, S. Sadraddini, A. Gudimov, and G. B. Arhonditsis. 2011. Our current understanding of lake ecosystem response to climate change: What have we really learned from the north temperate deep lakes? Journal of Great Lakes Research 37:173–193.

Wells, B. K., J. C. Field, J. A. Thayer, C. B. Grimes, S. J. Bograd, W. J. Sydeman, F. B. Schwing, and R. Hewitt. 2008. Untangling the relationships among climate, prey and top predators in an ocean ecosystem. Marine Ecology-Progress Series 364:15–29.

Wootton, J. T. 1994. Predicting direct and indirect effects: An integrated approach using experiments and path-analysis. Ecology 75:151–165.

Wright, S. 1918. On the nature of size factors. Genetics 3:367–374.

Wright, S. 1920. Correlation and causation Part I. Method of path coefficients. Journal of Agricultural Research 20:557–585.

Wright, S. 1934. The method of path coefficients. Annals of Mathematical Statistics 5:161–215.

Wright, S. W. 1960. Path coefficients and path regression: Alternative or complementary concepts. Biometrics 16:189–202.

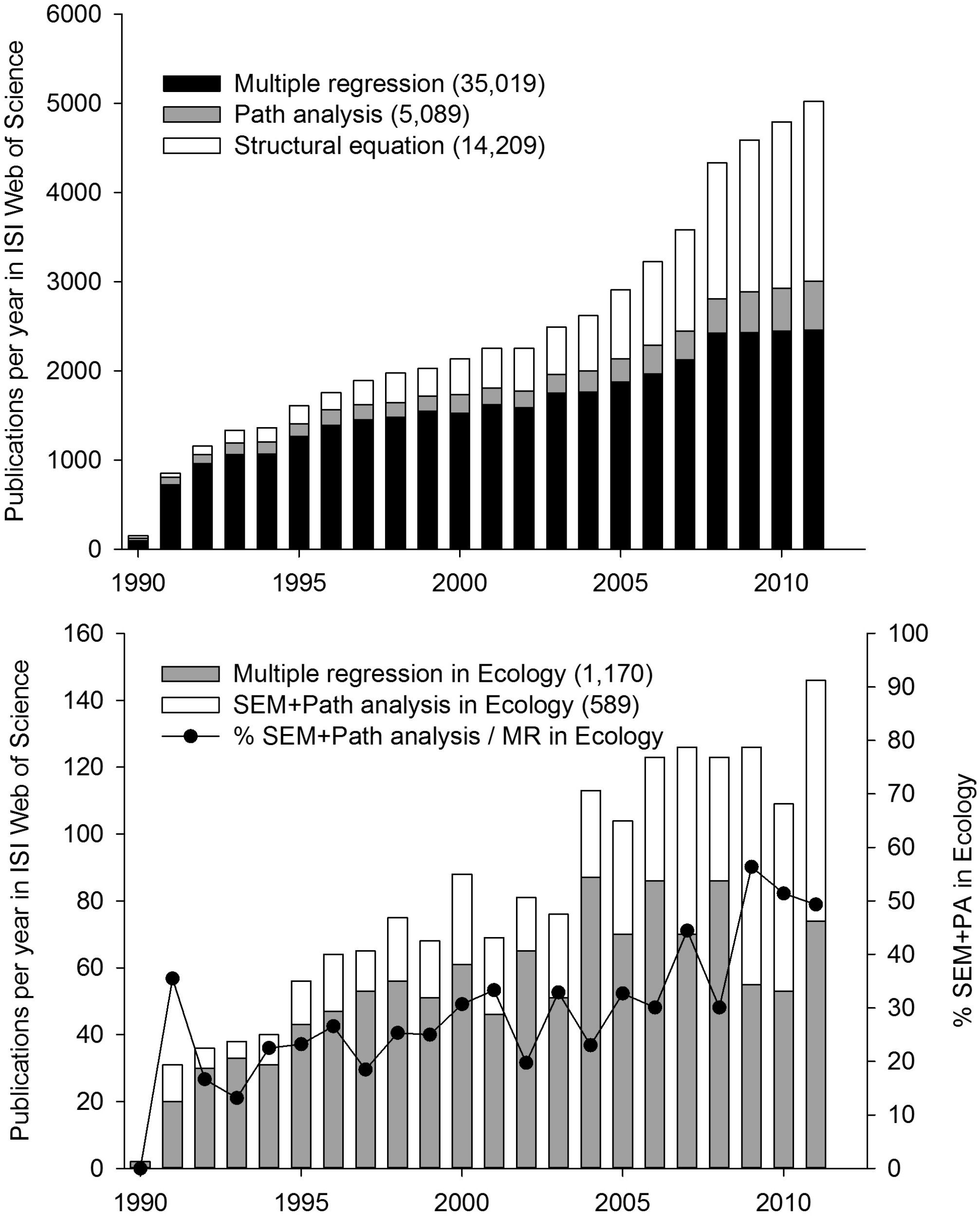


Figure A1-1. Publications that use the term "path analysis", "structural equation", or "multiple regression" in ISI Web of Science, within the whole database (upper panel) and within the field of ecology (lower panel).

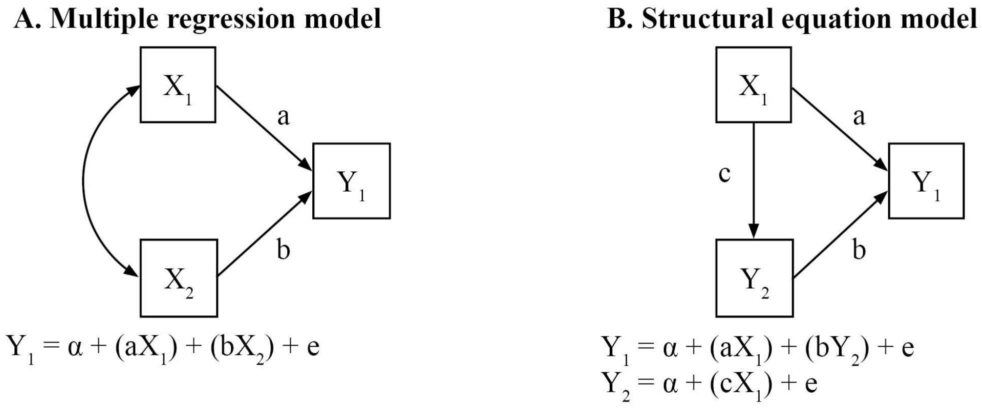
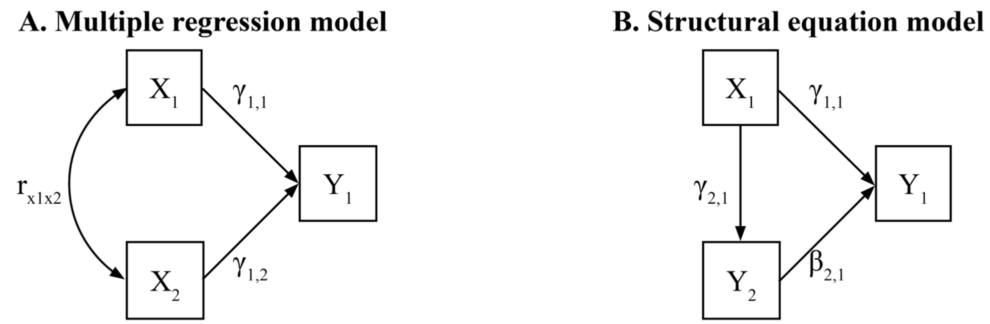


Figure A1-2. Simplest form of a multiple regression model (left) and rearrangement of the multiple regression model into the simplest form of structural equation model (right).



A. Multiple regression model

B. Structural equation model

$$Y_1 = \alpha + (\gamma_{1,1}X_1) + (\gamma_{1,2}X_2) + e$$

$$Y_1 = \alpha_1 + (\gamma_{1,1}X_1) + (\beta_{2,1}Y_2) + e_1$$
$$Y_2 = \alpha_2 + (\gamma_{2,1}X_1) + e_2$$

γ = effects on dependent variables by independent variables, e.g. $\gamma_{1,1}$ = effects on Y_1 by X_1
β = effects on dependent variables by other dependant variables, e.g. $\beta_{1,2}$ = effects on Y_1 by Y_2
r = bivariate correlation, e.g. r_{x1x2} = correlations between X_1 and X_2
α = intercept, and e = error

Panel A:
r_{x1x2} = non-causal (i.e. unexplained) bivariate correlation between X_1 and X_2
$\gamma_{1,1}$ and $\gamma_{1,2}$ = partial regression coefficients

Partial regression coefficient:
[Eq.] $\gamma_{1,1} = [(r_{x1y1}) – (r_{x1x2} \bullet r_{x2y1})] / (1 – r^2_{x1x2})$

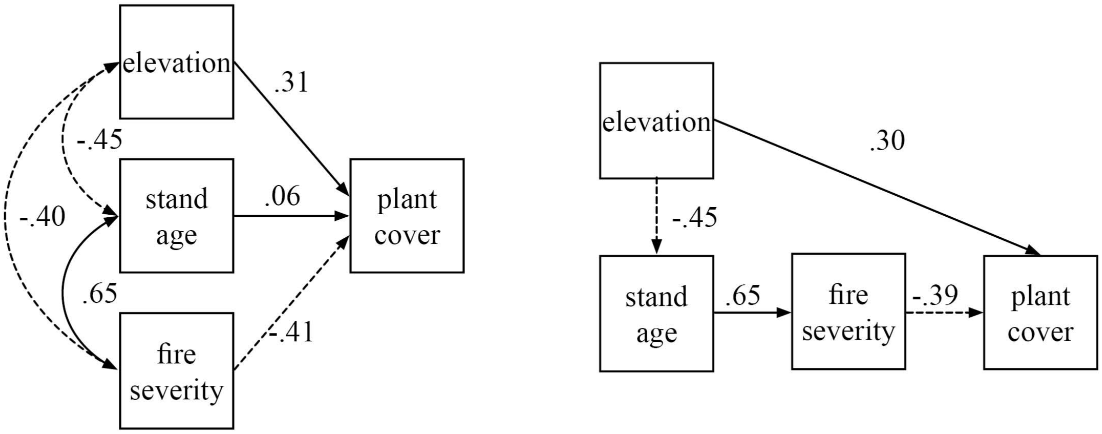
Panel B:
$\gamma_{1,1}$ and $\beta_{2,1}$ = partial regression coefficients
$\gamma_{2,1}$ = simple regression coefficient

Simple regression coefficient:
[Eq.] $\gamma_{2,1} = r_{x1y2} \bullet (SD_{Y2} / SD_{X1})$

Figure A1-3. Multiple regression model (left) and rearrangement of the multiple regression model into a structural equation model (right) with path coefficients and equations.

A. Multiple regression model: Effects on post-forest fire plant cover

B. Structural equation model: Effects on post-forest fire plant cover



C. Multiple regression model: Standardized regression coefficients

D. Structural equation model: Standardized regression coefficients

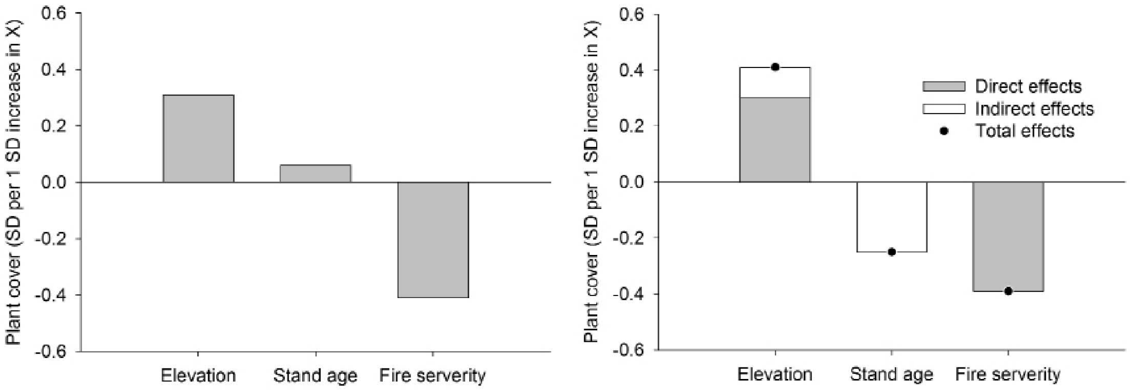
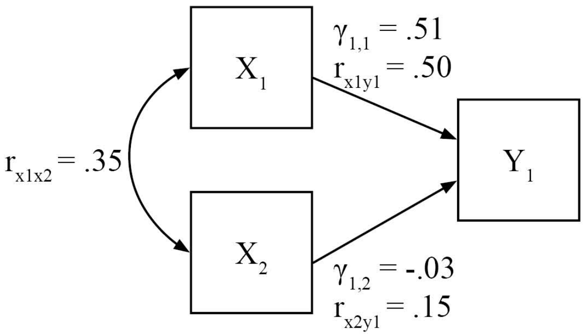


Figure A1-4. Direct, indirect, and total effects in multiple regression and structural equation modeling.

A. Simulated multiple regression model



Effect of $X_1 \rightarrow Y_1$: Partial regression coefficient:

[Eq.] $\gamma_{1,1} = [(r_{x1y1}) - (r_{x1x2} \bullet r_{x2y1})] / (1 - r^2_{x1x2})$

B. Simulated effects of collinearity on $X_1 \rightarrow Y_1$ (i.e. $\gamma_{1,1}$)

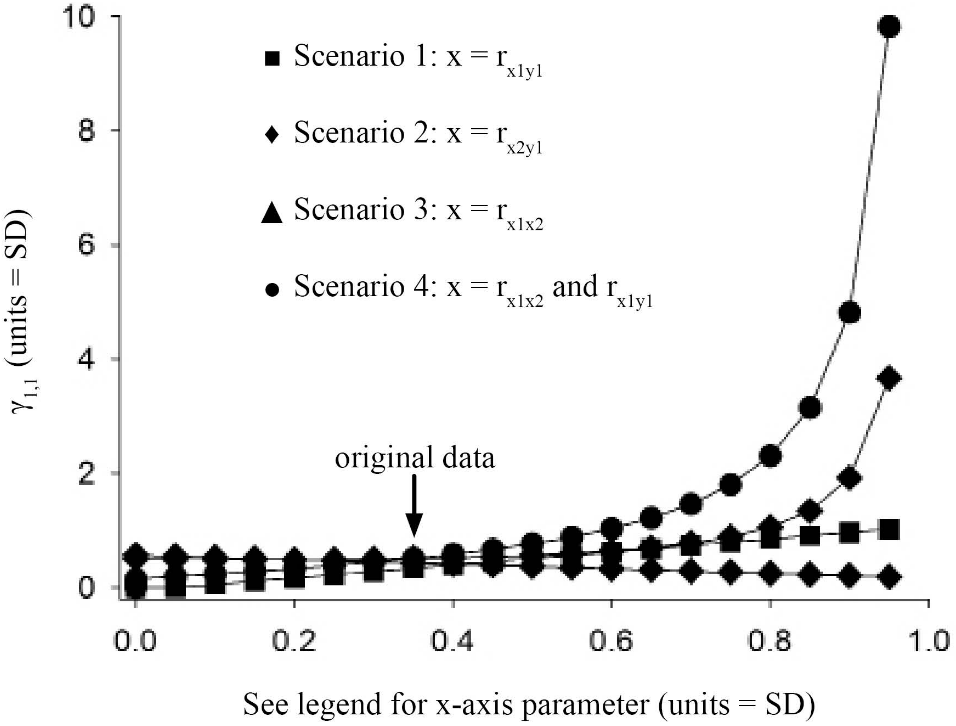
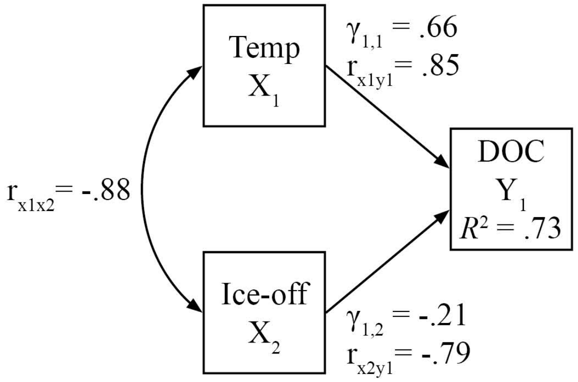


Figure A1-5. Regression coefficient inflation scenarios.

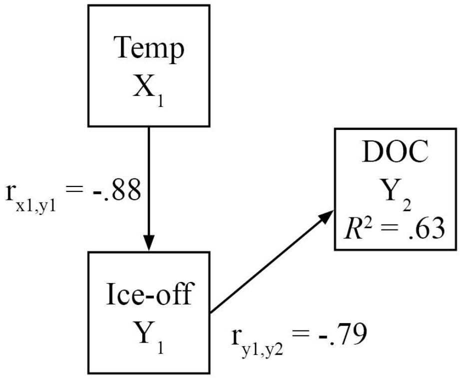
A. Multiple regression model



Effect of Temperature (X_1) on DOC (Y_1): Partial regression coefficient:

[Eq.] $\gamma_{1,1} = [(r_{x1y1}) - (r_{x1x2} \cdot r_{x2y1})] / (1 - r^2_{x1x2}) = 0.66$

B. Structural equation model



Effect of Temperature (X_1) on DOC (Y_2): Compound path coefficient:

[Eq.] $\gamma_{2,1} = (r_{x1y1} \cdot r_{y1y2}) = 0.70$

Figure A1-6. Comparing multiple regression and SEM by analyzing the effects of temperature and ice-off on DOC from a survey of 16 mountain lakes in Alberta (2005–2007).

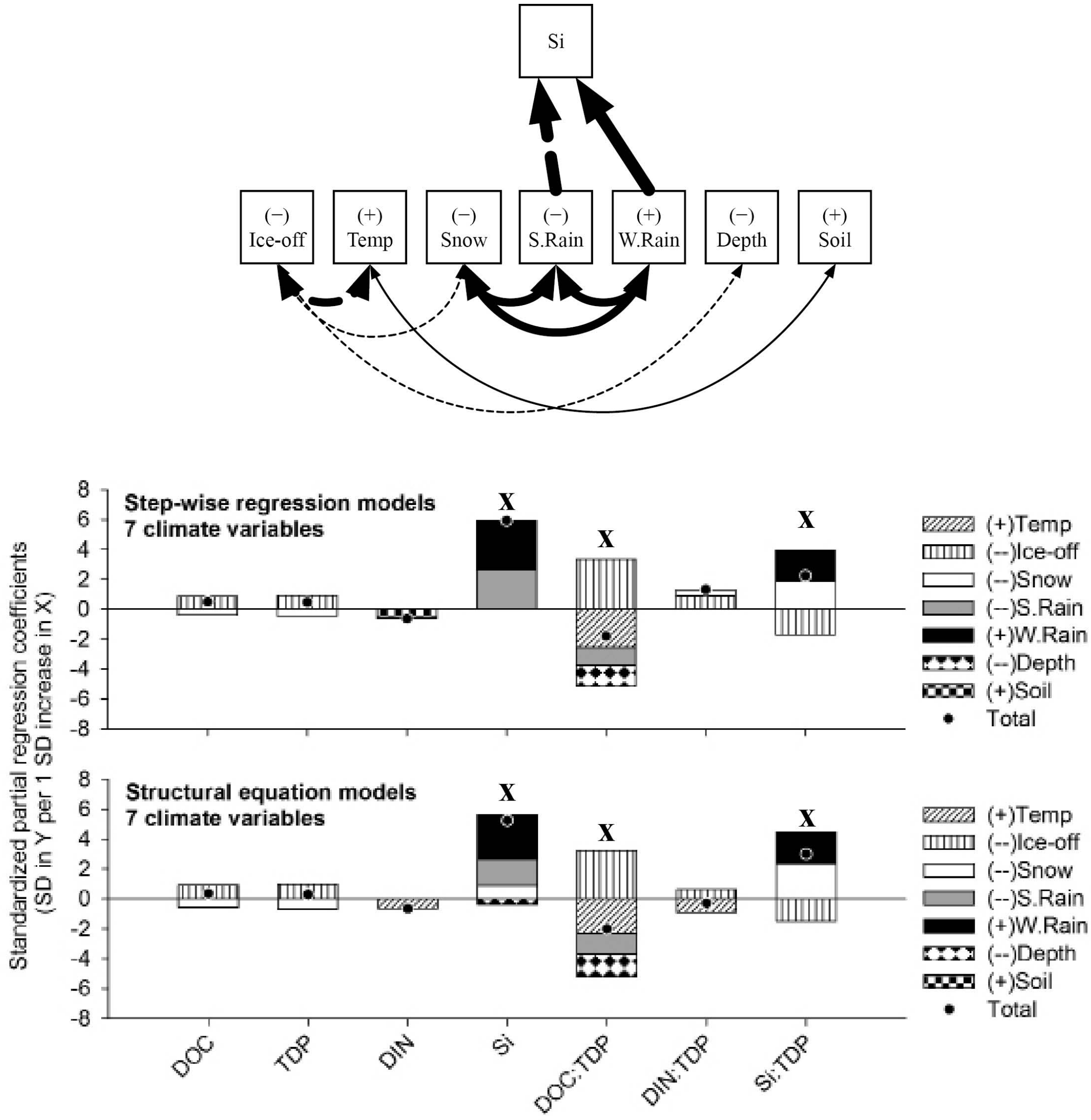


Figure A1-7. Model structure and partial regression coefficients of step-wise regression (SWR) and SE models used to analyze the effects of 7 climate variables on dissolved nutrients from a survey of 16 mountain lakes in Alberta (2005–2007). There was one model for each dissolved nutrient variable, but only Si is shown in the model diagram. Model structure was the same for SWR and SEM in this scenario with 7 climate variables. In the lower panel, variables marked with "x" have inflated partial regression coefficients (i.e., > 2–3 SD) due to high collinearity among climate variables. Figure explanation continued in text.

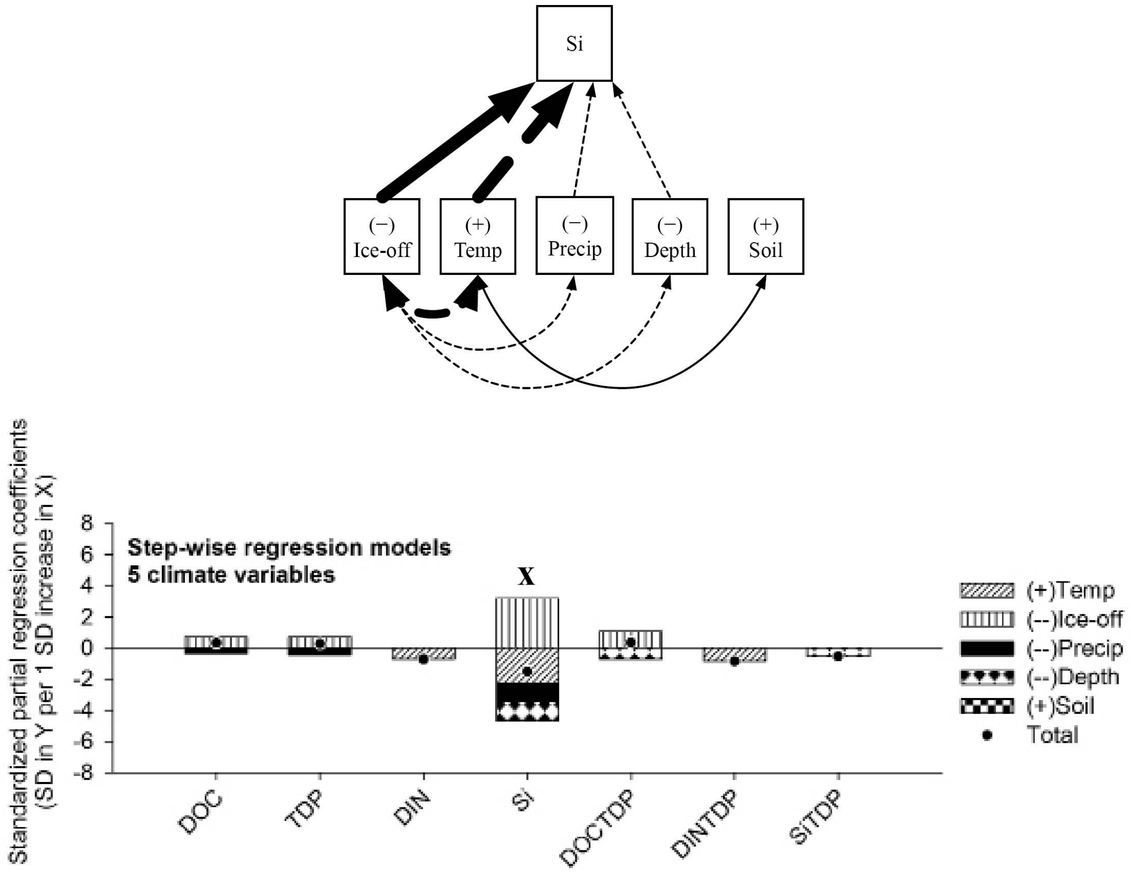


Figure A1-8. Model structure and partial regression coefficients of step-wise regression (SWR) models used to analyze the effects of 5 climate variables on dissolved nutrients (only Si is shown in the model diagram) in a survey of 16 mountain lakes in Alberta (2005–2007). SEM was not considered in this scenario. See previous figure caption for additional details.

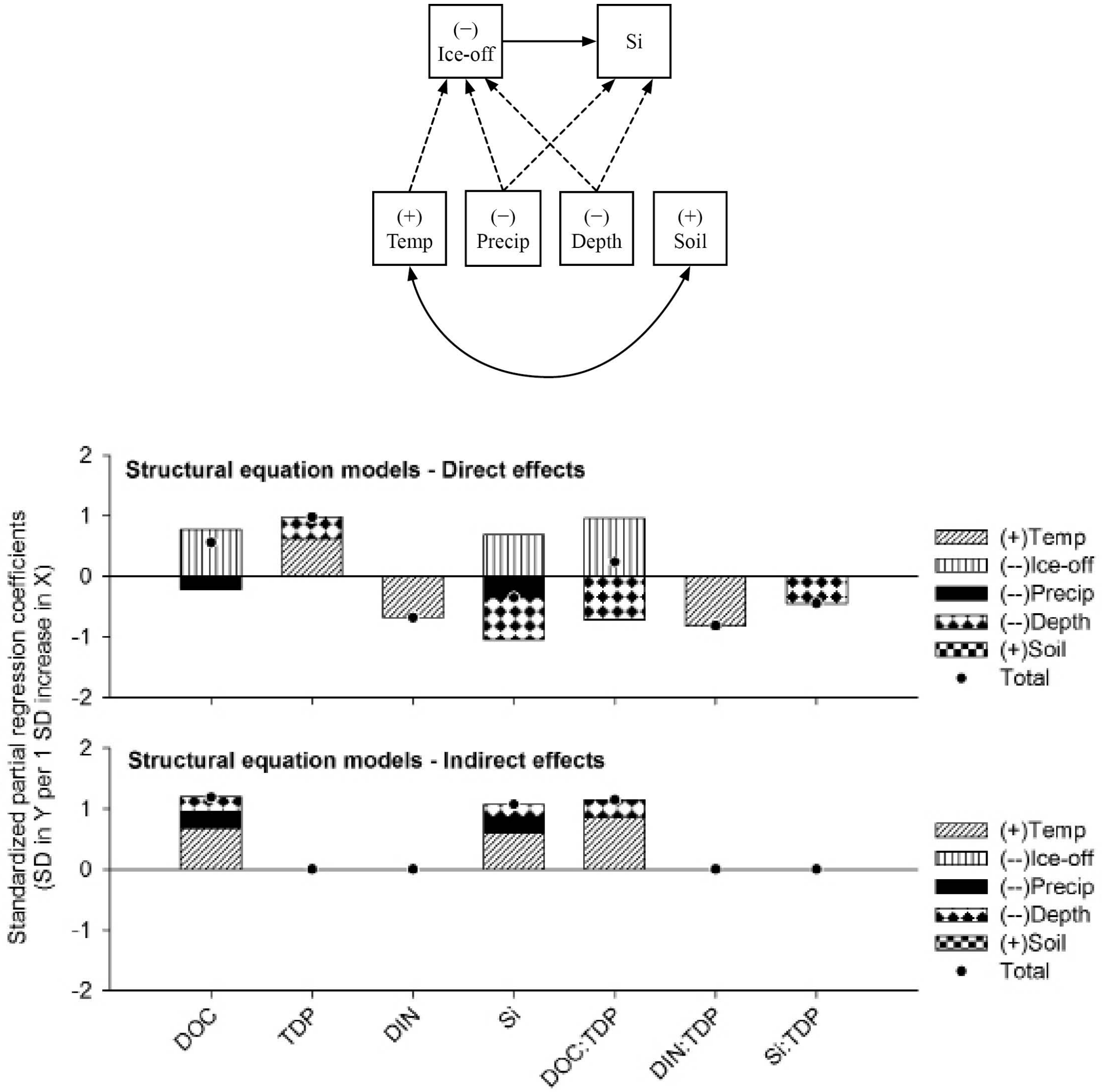
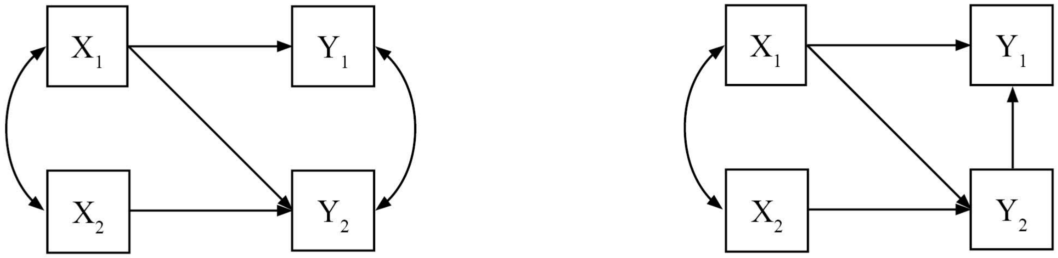


Figure A1-9. Model structure and partial regression coefficients of structural equation models analyzing the effects of 5 climate variables on dissolved nutrients in a survey of 16 mountain lakes in Alberta (2005–2007). Effects of climate variables on ice-off are included as causal effects in the models. Standardized partial regression coefficients are given for the direct effects (middle panel) and indirect effects (lower panel) of climate variables. See captions of previous figures for additional details.


A. Multiple regression model with collinear X variables ($X_1 \leftrightarrow X_2$)

B. Structural equation (SE) model with causal effects among X and Y variables


C. SE model with collinear X and Y variables ($X_1 \leftrightarrow X_2$ and $Y_1 \leftrightarrow Y_2$)

D. SE model with collinear X variables ($X_1 \leftrightarrow X_2$) and causal effects among Y variables


E. SE model with causal effects among X and Y variables and reciprocal effects among Y variables

F. SE model with a latent coding variable (circle) to reverse the effects of X_1

Figure A1-10. Calculation of direct, indirect, and reciprocal effects in structural equation modeling. adapted from Grace 2006 structural equation modeling and natural systems.

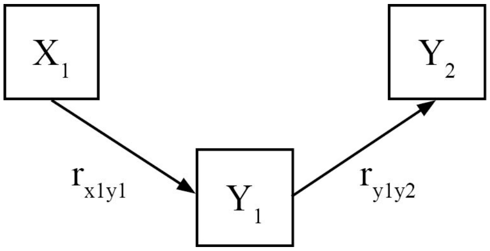


Figure A1-11. Structural equation model demonstrating rules #2 and #3 for calculating simple and compound path coefficients.

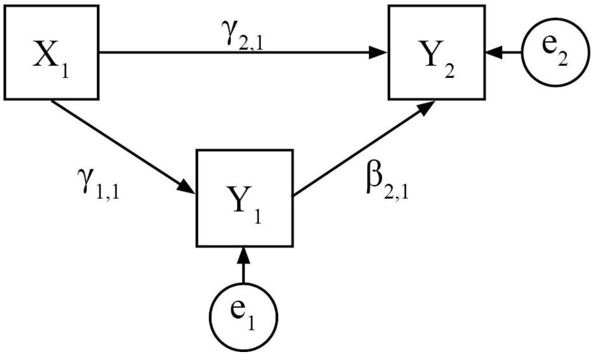


Figure A1-12. Structural equation model demonstrating rule #4 for calculating partial regression coefficients.

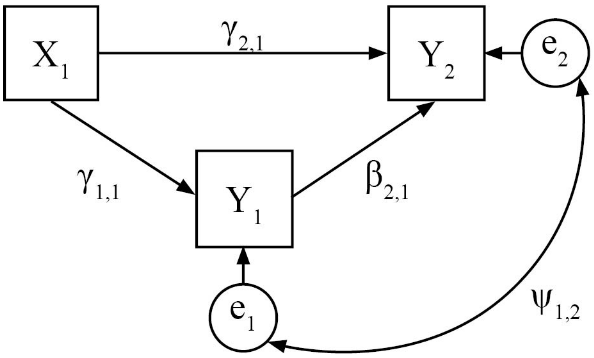


Figure A1-13. Structural equation model demonstrating rules # 5 and 6 for calculating error terms and unanalyzed correlations.

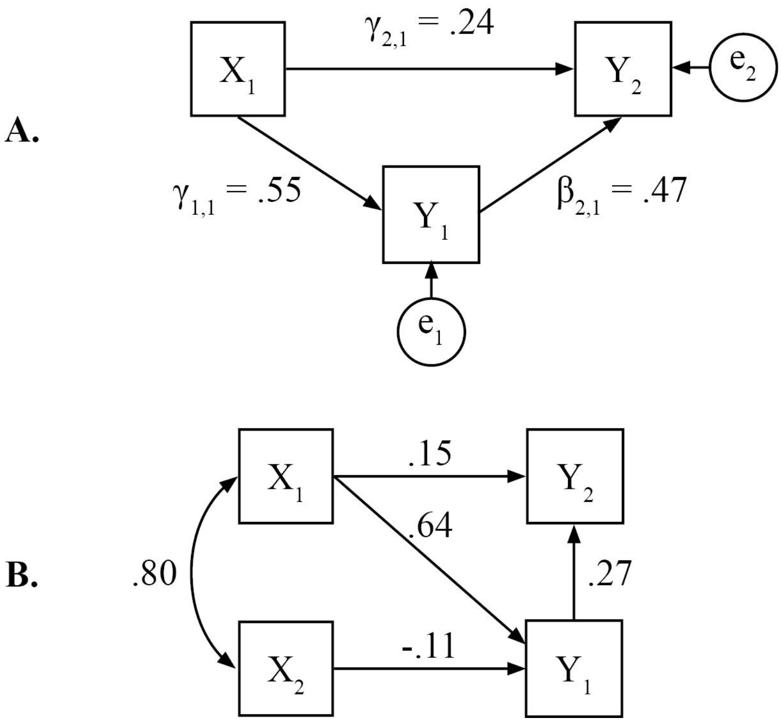
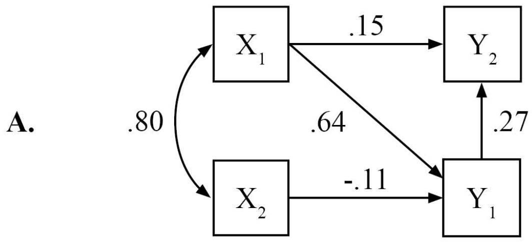


Figure A1-14. Structural equation models demonstrating rule # 7 for calculating total effects.



B. Bivariate correlation coefficients **C. Direct and indirect SEM path coefficients**

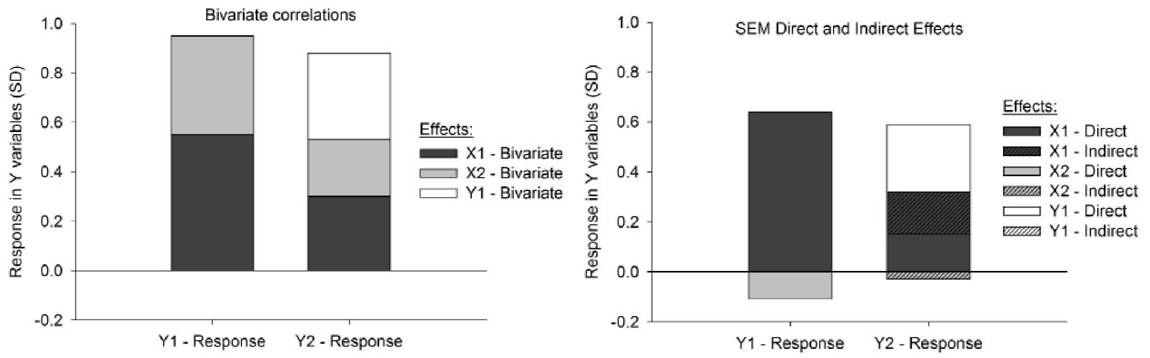


Figure A1-15. Structural equation model demonstrating rule # 8 for calculating total correlations.

APPENDIX 2: PELAGIC FOOD WEB INTERACTIONS AMONG BENTHIC INVERTEBRATES AND TROUT IN MOUNTAIN LAKES[8]

Abstract

Benthic chironomid larvae and the amphipod *Gammarus lacustris* have been observed in the pelagic habitats of many mountain lakes. The main goal of this study was to determine if chironomid larvae and gammarids potentially affect predator-prey and nutrient dynamics in pelagic food webs of mountain lakes. Eighty six mountain lakes were surveyed in Alberta and eastern British Columbia during the years 1965–1984, 1991–2004, and 2005–2007. Pelagic chironomid larvae were found in 86% of these lakes and pelagic gammarids were found in 29% of lakes. Densities of pelagic chironomid larvae were 92% lower in lakes with pelagic gammarids and 76% lower in lakes with trout ($P < 0.05$). Intraguild predation of trout on gammarids appeared to reduce predation pressure on chironomid larvae. Gammarids consumed *in vitro* about 1 chironomid$\cdot$gammarid$^{-1}\cdot$d^{-1}, or about 20% of their body mass in chironomid biomass per day. Concentrations of total dissolved P and N, particulate C, and chlorophyll *a* increased with increasing densities of pelagic gammarids and chironomid larvae *in situ* ($R^2 = 0.14 \pm 0.19$ SD, $P < 0.1$) and *in vitro* ($P < 0.001$). Our findings suggest that gammarids and chironomid larvae are linked as predators and prey in pelagic food webs, possibly stimulating phytoplankton abundance via nutrient release.

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[8] This chapter has been published: Weidman, Schindler, and Vinebrooke 2011. Freshwater Biology 56: 1081-1094.

Introduction

Benthic macroinvertebrates that migrate into the water column can be ecologically important linkages between benthic and pelagic habitats in freshwater ecosystems (Vander Zanden and Vadeboncoeur 2002). These migrants may prey on smaller pelagic invertebrates. For example, the amphipod *Gammarus lacustris* (Sars 1863) moves between benthic and pelagic habitats and fills the role of top pelagic predator in fishless mountain lakes in Alberta (Wilhelm and Schindler 1999). Benthic macroinvertebrates also become vulnerable to predation in the water column, such as gammarids and chironomid larvae and pupae, which are important food sources for native bull trout (*Salvelinus confluentus*, Suckley 1859) in mountain lakes (Wilhelm *et al*. 1999a). Similarly, late instar pelagic chironomid larvae that migrate into the water column in Loch Ness, Scotland, are important prey for Arctic charr (*Salvelinus alpinus*, Linnaeus 1758) (Winfield, Bean, and Hewitt. 2002).

Macroinvertebrates can link benthic and pelagic habitats by releasing nutrients into the water column. Gammarids release up to 30% of dissolved P that is recycled in the water column each day in mountain lakes in Alberta (Wilhelm, Hudson, and Schindler 1999b). Further, release of nutrients into the water column by macroinvertebrates can stimulate pelagic primary production (Vanni 2002). Concentrations of dissolved and particulate nutrients and chlorophyll-a (chl-a) have been shown to increase in the water column in the presence of chironomid larvae and gammarids in mesocosm experiments (Fukuhara and Sakamoto 1987; Wilhelm and Schindler 1999).

There are many reports of presumably obligate benthic chironomid larvae occurring in the water column in lakes and rivers in many regions of the world (Davies 1974; Armitage, Cranston, and Pinder 1995; Takagi et al. 2005). Larvae may be present in pelagic regions due to active vertical migration and swimming (Nachtigall 1961; Brackenbury 2000) and suspension by currents (Lewis 1957; Wülker 1963; Winfield *et al*. 2000). Pelagic chironomid larvae may thus be preyed on by pelagic predators and release dissolved nutrients into the water column. Gammarids have been observed to prey on chironomid larvae in the water column of lakes and ponds in Alberta and Saskatchewan (Anderson and Raasveldt 1974). Despite the reported occurrences of pelagic chironomid larvae and the widespread distribution of chironomids in freshwater ecosystems, their ecological roles in pelagic communities have not been determined.

We conducted lake surveys, synthesized a historical lake database, and conducted an *in vitro* experiment to examine predation and nutrient release among pelagic gammarids, chironomid larvae, and trout. Our two main testable hypotheses were: 1) gammarids and trout suppress chironomid larvae in pelagic habitats; and 2) pelagic chironomid larvae and gammarids enhance nutrient concentrations and phytoplankton abundance.

Methods

Lake Surveys

Pelagic invertebrates were sampled in eighty six mountain lakes in Alberta and eastern British Columbia during the ice-free season (June–October) in three lake surveys over 1965−1984 (R.S. Anderson, Canadian Wildlife Service, and D.B. Donald, Environment Canada; various Government of Canada data reports), 1991−2004 (D.W.S.), and 2005−2007 (R.P.W.; Table A2-1). Ten lakes were also sampled several times in the winter of 2005/2006. Sampling was done by boat from the centre of each lake. Pelagic invertebrates were collected using 30-cm diameter Wisconsin-style plankton nets having 64-µm pore-sized mesh. Five vertical tows were taken from 1 m above the lake bottom at the point of maximum depth. Invertebrates were preserved in sugared-formalin or ethanol and later enumerated at 40−60 times magnification.

First to forth instar chironomid larvae were pooled in a single count, which did not include pupae. In the 2005−2007 survey, Chironomidae subfamilies included Chironominae (46%), Orthocladiinae (41%), and Tanypodinae (14%). In lentic environments, Chironominae are usually associated with profundal sediments, Orthocladiinae are mainly found in littoral regions and feed on rock surfaces or macrophytes, whereas Tanypodinae tend to be free-swimming epibenthic predators (Armitage *et al*. 1995).

In the 1991−2004 and 2005−2007 surveys lake water was collected from the centre of each lake at 0.5 m depth from the surface. Lake water was screened through 500-µm pore-sized mesh and then filtered through GF/F filters for analysis of particulate C (PC) and chl-a. Filtered lake water was retained for analysis of total dissolved P and N (TDP and TDN, respectively). Lake water and GF/F filters were chilled at about 10 ^{o}C until analyzed at University of Alberta Biogeochemistry Laboratory (Parker and Schindler 2006).

Predation

Annual mean densities of pelagic chironomid larvae and gammarids were calculated for each lake in each survey in order to minimize any temporal autocorrelation between pelagic chironomid larvae and gammarids due to differences in their phenology. Average densities were then calculated among years for each lake.

In the 1965−1984 survey, densities of pelagic invertebrates had been rounded to the nearest higher order of magnitude to speed enumeration. The range of invertebrate densities were thus about one order of magnitude greater in the 1965−1984 survey (10−100 individuals$\cdot$m^{-3}) than in the 1991−2004 and 2005−2007 surveys (1−10 individuals$\cdot$m^{-3}). Consequently, invertebrate densities were divided by 10 in each lake in the 1965−1984 survey to better approximate the range in densities in the more recent surveys where enumerations were more precise.

The status of fish in lakes in the 1965−1984 survey was documented in various data reports prepared for Parks Canada by the Canadian Wildlife Service (R.S. Anderson, D.B. Donald, and others). For lakes in the 1991−2004 survey, the status of native bull trout populations and the stocking, natural collapse, or experimental removal of introduced rainbow trout (*Oncorhynchus mykiss*, Walbaum 1792) and brook trout (*Salvelinus fontinalis*, Mitchill 1814) populations has also been well-documented (Schindler and Parker 2002; McNaught *et al*. 1999; Parker and Schindler 2006). For lakes in the 2005−2007 survey, the status of fish was uncertain for three lakes in Jasper National Park (NP; Upper Geraldine Lake and two adjacent unnamed lakes). To confirm status of fish in these lakes, mixed-mesh gill nets (25–75 mm stretched mesh) that were 35 m long were deployed near the inflows in each lake continuously for 48 h in September 2007. No fish were caught, so the lakes were considered to be fishless.

The effects of pelagic gammarids and fish on densities of pelagic chironomid larvae were tested using a one-factor (± gammarids/fish) analysis of variance (ANOVA) using type IV sum-of-squares model to adjust for unequal sample sizes among treatment levels. The three treatment levels in the ANOVA were: lakes with no gammarids or fish "Gamm(−)Fish(−)", lakes with fish but no gammarids "Gamm(−)Fish(+)", and lakes with gammarids but no fish "Gamm(+)Fish(−)". A "Fish(+)Gamm(+)" treatment level was not possible because gammarids were not present in the water column in lakes with fish, although gammarids were present in benthic regions of some lakes with fish. The lake survey period was included as a random effect in the ANOVA to test whether the effects of fish and gammarids were different across the three datasets. Lake mean depth and elevation were covariates in the ANOVA to test whether the effects of fish and gammarids were different across lakes of varying depth and elevation. The effect of gammarids pelagic chironomid larvae was also tested across all lakes using an analysis of covariance (ANCOVA).

For all statistical analyses in this study, pelagic invertebrate densities were ln+1-transformed and all other variables were ln-transformed prior to analysis. *Post hoc* tests were conducted using Bonferonni-corrected multiple comparisons for significant effects in ANOVA models. Single factor ANOVA/ANCOVAs were used to compare significant effects in multiple ANOVA/ANCOVA (MANOVA/MANCOVA) models (see below). The level of significance was set at $P < 0.05$. Significance levels were Huynh-Feldt corrected for MANOVA/MANCOVA models. All statistical analyses were conducted using SPSS software (version 18).

Nutrients

The effects of pelagic invertebrates on concentrations of nutrients and chl-a were analyzed two ways. Within-lake variation was analyzed for Bighorn and Snowflake lakes (Banff NP) in the 1991-2004 survey because they had the highest densities of gammarids and chironomid larvae, respectively. Among-lake variation was analyzed for all 23 lakes sampled in the 1991-2004 and 2005-2007 surveys. Analyses of within-lake variation in nutrients, chl-a, and invertebrates were conducted using lake-day data. Analyses of among-lake variation were conducted using lake-year data.

Data from Bighorn and Snowflake lakes in the 1991–2004 survey were corrected for temporal autocorrelation prior to analyses. Pelagic invertebrate densities and concentrations of nutrients and chl-a were regressed against date for each lake. The residuals (predicted minus observed values) from these regressions were then used for the analyses.

The effects of pelagic gammarids and chironomid larvae on concentrations of dissolved and particulate nutrients and chl-a were tested using MANCOVA models. One MANCOVA was used to test among-lake variation in invertebrates, nutrients, and chl-a. Separate MANCOVA models were used to test within-lake variation in Bighorn and Snowflake lakes because sample sizes were unequal between lakes. The dependent variables in the MANCOVAs were TDP, TDN, the molar ratio of TDN:TDP, PC, and chl-a.

The level of significance was set at $P < 0.10$ because statistical power was expected to be low in this component of our study. Low power was anticipated because gammarids release small concentrations of dissolved P into the water column, around 0.01–0.1 $\mu g\ P \cdot L^{-1} \cdot d^{-1}$, but these concentrations are biologically important for phytoplankton production in oligotrophic mountain lakes (Wilhelm *et al*. 1999b). We conducted *post hoc* power analysis for regressions between invertebrates and nutrients and chl-a (data not shown). Indeed, power to detect statistically significant regression slopes decreased exponentially from about 80% for slopes > 1 $mg \cdot invertebrate^{-1}$ (equivalent to $\mu g \cdot L^{-1}$; $P < 0.05$) to around 36% for slopes between 0.1–0.5 $mg \cdot invertebrate^{-1}$ ($P < 0.1$).

Experiment

In 2006, chironomid larvae, gammarids, and lake water were collected from Pipit Lake once in July, from Snowflake Lake once in July and August, and from Harrison Lake once in August. Invertebrates and lake water collected on these occasions were used to conducted four identical laboratory experiments to determine: 1) if pelagic gammarids preyed on chironomid larvae, and 2) if pelagic chironomid larvae and gammarids increased concentrations of dissolved and particulate nutrients and chl-a.

Chironomid larvae were collected using multiple Ekman dredge samples taken from the centre of each lake. Benthic sampling ensured that adequate numbers of chironomid larvae (about 500 per lake) could rapidly be collected for each experiment. Elsewhere, benthic substrates have contained chironomid taxa that were representative of those present in pelagic zones in Loch Leven, England (Davies 1976a). Only 4 gammarids were required for each experiment, so these individuals were collected using vertical plankton net hauls in Pipit and Snowflake lakes. Benthic grabs were used in Harrison Lake because predation by native bull trout restricts gammarids to benthic regions during the day (Schindler and Parker 2002).

Chironomids and gammarids were separated from sediment in the field using lake water and a 200-µm pore-sized mesh screen. Chironomids were stored in 1-L containers with lake water and a small amount of lake sediment. Gammarids were stored separately from chironomids in 1-L containers of lake water that contained concentrated amounts of zooplankton. Lake water and the invertebrates were transported to the University of Alberta in coolers at about 10 ^{o}C.

The day following field sampling, individual 4th instar chironomid larvae were separated from sediment in the laboratory. The chironomid genera *Einfeldia* (Kieffer 1924) and *Procladius* (Skuse 1889) were the only taxa observed in the three lakes. *Einfeldia* was selected for the experiment because it was about 10 times more abundant than *Procladius*.

The invertebrates from Pipit Lake were only used to conduct a preliminary experiment that showed gammarids consumed about 1 chironomid·day^{-1} (data not shown). For the experiments using invertebrates from Snowflake (in July and August) and Harrison lakes (August only), 7-L aquaria were stocked with chironomid larvae and with or without a single gammarid. There were four aquaria in each treatment (i.e., n = 4). There were also four aquaria with neither invertebrate to control for the effects on nutrients and chl-a (described below). There were enough chironomid larvae collected so that each aquarium could be stocked with 8 individuals in the experiment in July and 20 individuals in each experiment in August. Therefore, the experiment in July was run for six days whereas the two experiments in August were run for nine days, based on the preliminary predation rate of about 1 chironomid·gammarid^{-1}·d^{-1}. Aquaria were kept at 10 ^{o}C in an environmentally controlled chamber with a 12-h light-dark cycle. The number of chironomids remaining alive in each aquarium was counted at the same time each day.

Predation

At the end of each experiment, gammarids and the remaining chironomids were dried at 60 ^{o}C for 24 hr and then weighed (DW). Average chironomid body mass was determined for each aquaria in each experiment and these values were used to calculate total chironomid dry mass for each aquaria for each day of each experiment. The rate of predation of gammarids on chironomid larvae was expressed as chironomid mg DW consumed per gammarid mg DW per day for each day of each experiment. The % of chironomids remaining alive was also determined for each day of each experiment.

Predation rates and the % chironomids remaining alive were tested over time to account for any variation in feeding of gammarids due to changes in their diet and handling before the experiment. A three factor (± gammarids x experiment x day) repeated measures ANOVA (RM-ANOVA) was used to compare % chironomids remaining over days 0−6 in each experiment. Only days 0−6 were tested so that sample sizes were equal among the three experiments. A two factor (experiment x day) RM-ANOVA was used to compare predation rates over days 1−6 in each experiment. In addition, a singe factor (experiment) MANOVA was used to compare invertebrate biomass among the experiments where chironomids and gammarids were the independent variables.

Nutrients

At the end of each experiment, after invertebrates were removed by pipette, three L of water were siphoned out of each aquarium and filtered through GF/F filters and analyzed for PC and chl-a. Filtered water was analyzed for TDP and TDN. Sediment was not added to the aquaria so bioturbation would not have affected concentrations of particulate nutrients in the water column.

The effects of chironomid larvae and gammarids on dissolved and particulate nutrients and chl-a were tested using a two factor (± chironomids/gammarids x experiment) MANOVA. The first factor in the MANOVA had three treatment levels: aquaria with no chironomids or gammarids “Chiron(−)Gamm(−)”, aquaria with chironomids but no gammarids “Chiron(+)Gamm(−)”, and aquaria with chironomids and gammarids “Chiron(+)Gamm(+)”. Each of the three experiments comprised the three levels of the second factor in the MANOVA. The dependent variables in the MANOVA were TDP, TDN, TDN:TDP, PC, and chl-a. The level of significance was set at $P < 0.05$.

Results

Lake Surveys

Predation

In the 86 mountain lakes that were surveyed, pelagic chironomid larvae were found in 86% of lakes and pelagic gammarids were found in 29% of lakes. Maximum daily densities of pelagic chironomid larvae and gammarids were about 100 individuals·m^{-3} in several lakes. Averaged across all lakes, there were about 19.2 chironomids·m^{-3} in lakes with neither fish nor pelagic gammarids, about 4.4 chironomids·m^{-3} in lakes with only fish, and about 1.6 chironomids·m^{-3} in lakes with only pelagic gammarids ($F_{(\pm\text{Fish/Gammarids } 2,70)} = 8.8$, $P = 0.015$; Figure A2-1; Table A2-2). Gammarids only occurred in the water column of lakes where fish were absent (about 6.3 gammarids·m^{-3}). Thus, fish and pelagic gammarids appeared to reduce pelagic chironomid larvae by about 77% and about 92%, respectively. Fish also appeared to suppress predation pressure on pelagic chironomid larvae by about 14% by eliminating gammarids from the water column.

The estimated effects of fish and gammarids on densities of pelagic chironomid larvae were not significantly different across the three lake surveys ($F_{(\pm\text{Fish/Gammarids*Survey } 5,70)} = 1.3$, $P = 0.262$; Figure A2-1; Table A2-2). In the 1965–1984 survey, pelagic chironomid larvae were 29% lower in lakes with fish and 75% lower in lakes with gammarids compared to lakes with neither predator ($F_{(\pm\text{Fish/Gammarids } 2,58)} = 5.2$, $P = 0.008$). In the 1991–2004 survey, no lakes were sampled where both fish and gammarids were absent, but pelagic chironomid densities were 96% lower in lakes with gammarids compared to lakes with fish ($F_{(\pm\text{Fish/Gammarids } 1,6)} = 7.9$, $P = 0.031$). In the 2005–2007 survey, pelagic chironomid larvae were 89% lower in lakes with fish and 96% lower in lakes with gammarids compared to lakes with neither predator ($F_{(\pm\text{Fish/Gammarids } 2,12)} = 8.4$, $P = 0.007$). The inferred effects of fish and gammarids on densities of pelagic chironomid larvae also did not differ significantly across lakes of varying mean depth ($F_{(\pm\text{Fish/Gammarids*Depth } 3,70)} = 1.0$, $P = 0.389$) or elevation ($F_{(\pm\text{Fish/Gammarids*Elevation } 3,70)} = 2.0$, $P = 0.389$). Among all lakes, pelagic chironomids decreased by about 2 chironomids·gammaird^{-1} ($F_{(\text{Gammarids } 1,82)} = 7.2$, $P = 0.009$, $R^2 = 0.08$; Figure A2-2).

Nutrients

In the 1991–2004 survey, concentrations of TDP were significantly correlated with pelagic chironomid larvae in Bighorn Lake ($F_{(\text{Chironomids } 1,36)} = 3.5$, $P = 0.071$), where TDP increased by about 0.3 mg TDP·chironomid^{-1} (units are equivalent to mg·m^{-3} or µg·L^{-1}; Table A2-3). Concentrations of TDP were significantly correlated with pelagic gammarids in Snowflake Lake ($F_{(\text{Gammarids } 1,78)} = 2.4$, $P = 0.091$), where TDP increased by about 0.2 mg TDP·gammarid^{-1}. Concentrations of TDN were not significantly correlated with pelagic chironomids in Bighorn Lake ($P = 0.212$) or pelagic gammarids in Snowflake Lake ($P = 0.189$). Thus, the molar ratio of TDN:TDP was significantly correlated with pelagic chironomid larvae in Bighorn Lake ($F_{(\text{Chironomids } 1,36)} = 6.5$, $P = 0.015$) and pelagic gammarids in Snowflake Lake ($F_{(\text{Gammarids } 1,78)} = 14.7$, $P < 0.001$), where TDN:TDP decreased by about 9.0 per chironomid or gammarid in both lakes.

Concentrations of PC were significantly related to pelagic chironomid larvae in Bighorn Lake ($F_{(\text{Chironomids } 1,36)} = 3.5$, $P = 0.069$) and with pelagic gammarids in Snowflake Lake ($F_{(\text{Gammarids } 1,78)} = 3.2$, $P = 0.076$) where PC increased by about 0.2 mg PC·invertebrate^{-1} (Table A2-3). Concentrations of chl-a were not significantly correlated with pelagic chironomids in Bighorn Lake ($P = 0.349$), but were significantly related to pelagic gammarids in Snowflake Lake ($F_{(\text{Gammarids } 1,78)} = 7.4$, $P = 0.008$), where chl-a increased by about 0.3 mg chl-a·gammarid^{-1}.

Across all lakes in the 1991–2004 and 2005–2007 surveys, concentrations of TDP were significantly correlated with pelagic chironomid larvae ($F_{(\text{Chironomids } 1,20)} = 4.4$, $P = 0.071$) and pelagic gammarids ($F_{(\text{Gammarids } 1,20)} = 9.1$, $P = 0.019$), where TDP increased by about 1 mg TDP per gammarid or chironomid (Table A2-4). Concentrations of TDN were also significantly related to pelagic chironomid larvae ($F_{(\text{Chironomids } 1,20)} = 2.1$, $P = 0.089$) and pelagic gammarids ($F_{(\text{Gammarids } 1,20)} = 12.4$, $P = 0.010$), where TDN increased by about 1 mg TDN per chironomid or gammarid. The molar ratio of TDN:TDP was not significantly correlated with chironomids ($P = 0.843$) or gammarids ($P = 0.512$).

Concentrations of PC and chl-a were not significantly related to pelagic chironomid densities across all lakes in the 1991–2004 and 2005–2007 surveys ($P = 0.837$ and $P = 0.503$, respectively; Table A2-4). However, concentrations of PC and chl-a were significantly correlated with pelagic gammarids, where PC increased by about 2.7 mg PC·gammarid^{-1} ($F_{\text{(Gammarids 1,20)}} = 5.9$, $P = 0.046$) and chl-a increased by about 0.5 mg chl-a·gammarid^{-1} ($F_{\text{(Gammarids 1,20)}} = 3.4$, $P = 0.078$).

Experiment

Predation

Gammarids reduced the number of chironomid larvae surviving in all three experiments ($F_{\text{(Gammarids 1.7,30.3)}} = 69.2$, $P < 0.001$; Figure A2-3). The negative effect of predation by gammarids was significant on days 2–6 in all experiments ($F_{\text{(Gammarids*Day 1.7,30.3)}} = 56.3$, $P < 0.001$). However, the percent of chironomid larvae remaining alive on each day varied among the experiments ($F_{\text{(Experiment*Day 3.4,30.3)}} = 23.9$, $P < 0.001$). The decline in chironomid larvae was greatest in the experiment with invertebrates from Snowflake Lake in July ($F_{\text{(Gammarids*Day*Experiment 3.4,30.3)}} = 23.9$, $P < 0.001$).

Rates of predation by gammarids on chironomid larvae were normalized to mg DW of chironomids consumed per mg DW of gammarids per day and thus were relatively constant over time ($F_{\text{(Day 2.0,17.7)}} = 1.9$, $P = 0.174$; Figure A2-4). Predation rates were not significantly different among the three experiments ($F_{\text{(Day*Experiment 3.9,17.7)}} = 0.8$, $P = 0.552$). The mean predation rate across all days and experiments was 0.20 mg DW chironomid·mg DW gammarid^{-1}·d^{-1} ± 0.02 SE. Gammarids thus consumed about 20% of their biomass in chironomid biomass per day.

Mean gammarid biomass was 12 mg DW·individual^{-1} ± 4.0 SE, but was significantly higher in the experiment with invertebrates from Snowflake Lake in July ($F_{\text{(Experiment 2,9)}} = 20.9$, $P < 0.001$). Larger gammarids in the experiment in July likely explained why chironomid larvae densities declined more rapidly. Mean chironomid larvae biomass was 2.2 mg DW·individual^{-1} ± 0.4 SE, but was significantly lower in the experiment with invertebrates from Snowflake Lake in August ($F_{\text{(Experiment 2,9)}} = 9.0$, $P = 0.007$).

Nutrients

Concentrations of TDP and TDN were significantly greater (20–40%) in presence of chironomid larvae and gammarids in all three experiments ($F_{(\pm\text{Chironomids/Gammarids } 2,27)}$ = 91.5 and 87.8, respectively, $P < 0.001$; Table A2-5; Figure A2-5). The molar ratio of TDN:TDP was significantly lower (12%) in the presence of chironomid larvae and gammarids in all three experiments ($F_{(\pm\text{Chironomids/Gammarids } 2,27)}$ = 96.7, $P < 0.001$). Concentrations of PC and chl-a were also significantly greater (274–731%) in the presence of chironomid larvae and gammarids ($F_{(\pm\text{Chironomids/Gammarids } 2,27)}$ = 91.5 and 87.8, respectively, $P < 0.001$; Table A2-5; Figure A2-6). There were some differences in the effects of chironomid larvae and gammarids on concentrations of nutrients and chl-a among the three experiments (i.e., significant chironomid/gammarid x experiment interactions). These differences were due to the lack of significant effects of chironomids alone in the experiment with invertebrates from Snowflake in July.

Discussion

Predation on chironomids by gammarids and trout

Results from the lake surveys and *in vitro* predation experiments agreed with the first hypothesis that pelagic gammarids and trout suppressed pelagic chironomid larvae. Other lines of empirical evidence also support our hypothesized effects of predation by gammarids and trout on pelagic chironomid larvae. In Harrison Lake, Banff NP, chironomid larvae, pupae, and adults have been found in the stomach contents of bull trout (Wilhelm *et al*. 1999a; Parker *et al*. 2007). Anderson and Rassveldt (1974) observed gammarids preying on pelagic chironomid larvae and chaoborid larvae in several lakes in Alberta and Saskatchewan. Furthermore, in our 2005–2007 lake survey, half-eaten chironomid larvae were observed on several occasions in pelagic samples from Upper Geraldine Lake (Jasper NP) where pelagic gammarids were the only pelagic predator. The presence of larval carcasses in the water column of this deep lake (25 m maximum depth) suggested that gammarids preyed directly on chironomid larvae and released some of half-eaten prey in pelagic regions.

Although gammarids and trout suppressed pelagic chironomid larvae, trout also preyed on or displaced gammarids from pelagic habitats, thereby releasing chironomids from gammarid predation. Predation by trout on pelagic gammarids may be considered as intraguild predation, which occurs when two predators (gammarids and trout) share a common prey (chironomid larvae) but the middle predator (gammarids) is also a prey of the top predator (trout; Holt and Polis 1997; Polis, Myers, and Holt 1998). Intraguild predation may explain why densities of pelagic chironomid larvae were higher in lakes with trout than in lakes with pelagic gammarids. Furthermore, intraguild predation of trout on gammarids likely occurs even though gammarids did not appear to inhabit the water column of lakes with trout. In Harrison Lake, for example, gammarids are restricted to benthic regions during the day, but are preyed on by small trout in littoral regions and also appear in the stomach contents of large trout in offshore regions (Wilhelm *et al*. 1999a).

The well-known effect of size-selective predation by planktivorous fish (Brooks and Dodson 1965) may explain why pelagic chironomid larvae coexisted in the water column with trout while gammarids did not. Gammarids had much greater biomass than chironomid larvae in this study. Size-selective predation rates of trout should thus be much higher on gammarids than chironomid larvae.

In vitro predation rates agreed with those of Anderson and Raasveldt (1974) who found that gammarids, preferring live animal prey over plant material and detritus, consumed 0.5−1.0 chironomid larvae (*Clinotanypus* sp., Kieffer 1923)$\cdot$gammarid$^{-1}\cdot$d^{-1} and about 0.3 chaoborid larvae (*Chaoborus americanus*, Johannsen 1903)$\cdot$gammarid$^{-1}\cdot$d^{-1}. However, *in vitro* predation rates surely overestimated *in situ* predation rates. Wilhelm, Schindler, and McNaught (2000) found that gammarid predation on *Daphnia middendorffiana* (Fischer 1851) decreased from about 16 to 1 daphnids$\cdot$gammarid$^{-1}\cdot$d^{-1}, when compared across 20L microcosms, 1000L mesocosm, and whole-lake measurements in Snowflake Lake, Banff NP. *In situ* predation rates of gammarids are likely limited by predator-prey encounter rates, which are determined by predator and prey densities, spatial distributions, and migration behaviour, as well as physical refuges for pelagic prey. The 7 L aquaria in our experiments represented only the pelagic habitat where there was no refuge for chironomid larvae from predation. Furthermore, when gammarid predation is prey-limited then predation rates have been shown to increases linearly with increasing densities of *Daphnia* from about 0.1 to 4.0 *Daphnia*$\cdot$L^{-1} (Wilhelm *et al*. 2000). Chironomid larvae were stocked in excess so that *in vitro* predation by gammarids was not limited by prey abundance. This was confirmed by both the observation that chironomid larvae declined linearly over time and that predation rates of gammarids did not change over time. *In vitro* predation rates in the present study thus reflect the maximum potential rate at which gammarids can consume chironomid larvae.

Our *in vitro* experiments demonstrate that chironomid larvae are vulnerable to predation by gammarids. Gammarids began preying on chironomid larvae and consuming their internal body contents on the first day of each experiment. Gammarids often held onto chironomid larvae carcasses during the day when they were observed. Similarly, Dick, Platvoet, and Kelly (2002) found that chironomid larvae were the most vulnerable out of 11 prey taxa to *in vitro* gammarid predation. We divided the survival times they reported for prey by those for gammarids and found that chironomid larvae survived for only 0.06 days for every day that gammarids survived in their experiments. More mobile prey taxa such as Chydoridae, Isopoda, Corixidae, and chaoborid larvae survived up to 1–4 days per day survived by gammarids. Pelagic chironomid larvae in our study lakes are thus expected to be vulnerable to *in situ* gammarid predation given adequate encounter rates.

Nutrient release by gammarids and chironomids

Positive correlations among pelagic invertebrate densities and dissolved and particulate nutrients and chl-a *in situ* and *in vitro* agreed with previous studies. Concentrations of total P and chl-a have been shown to increase with increased densities of pelagic gammarids in 2800 L mesocosms in Snowflake Lake (Wilhelm and Schindler 1999). Fukuhara and Sakamoto (1987) also found that chironomid larvae (*Chironomus plumosus*, Linnaeus 1758) and annelids (*Limnodrilus* spp., Claparede 1862) increased *in vitro* concentrations of dissolved P and N. Our results combined with previous studies supported the second hypothesis that these invertebrates release dissolved nutrients into the water column, which in turn increases the algal standing crop in mountain lakes. The limitations of our lake survey correlations and small-scale experiments mentioned above also apply here. Consideration should be given to the possibility that positive correlations between nutrients and invertebrates across lakes in the survey may reflect more nutrient-rich lake conditions enhancing secondary production through increased food availability.

Gammarids and chironomids may also move into the water column of lakes at times of the season when particulate food concentrations and dissolved nutrient concentrations are higher. However, there were no significant correlations with day of year and gammarid or chironomid larvae densities or nutrient and chl-a concentrations across all lakes once data were corrected for temporal autocorrelation (data not shown). Ten lakes were sampled several times in the winter of 2005/2006. Pelagic gammarids and chironomid larvae were present in winter in lakes where they were also present in summer. In several lakes, the succession of chironomid larvae to pupae in the water column and then emergence of adults was observed over 6–8 weeks beginning after ice-out. Similarly, in Char Lake in the Canadian Arctic chironomid emergence occurs mainly in late winter and early spring through broken and melting ice around the lake perimeter (Welch 1973). These observations may indicate that chironomid larvae play a greater role in nutrient release and as prey in pelagic habitats in late winter and early spring in cold-water lakes. In Harrison Lake, chironomids are found in the diet of bull trout more frequently in spring, as trout switch to feeding on gammarids and *Daphnia* later in summer (Wilhelm *et al*. 1999a).

Sedimentation of gammarid and chironomid faecal pellets may increase transport of nutrients to the benthos. However, *in vitro* concentrations of dissolved and particulate nutrients and chl-a did not include faecal pellets that settled to the bottom of the aquaria. *In situ* concentrations of dissolved and particulate nutrients and chl-a were analyzed from the upper 0.5 m of the water column of lakes and would not have included appreciable amount of faecal pellets from gammarids or chironomids. *In situ* and *in vitro* particulate nutrient and chl-a concentrations thus would have reflected the net effect (minus sedimentation) of nutrient release by invertebrates in the water column.

Residence time by invertebrates in the benthos will affect the net transfer of nutrients between benthic and pelagic habitats. Gammarids are known to migrate from benthic to pelagic regions at night in Snowflake Lake, Banff NP (Wilhelm *et al*. 2000). Gammarids may thus consume pelagic prey and excrete nutrients in the benthos. However, Wilhelm *et al*. (1999b) determined that excretion by pelagic gammarids contributed up to 30% of P that was recycled in the water column each day in Snowflake Lake, which accounted for diurnal vertical migration and losses due to sedimentation of faecal pellets. Similarly, a review by Vanni (2002) showed that nutrient release by benthic invertebrates and zooplankton supplied about 23% of the dissolved P required to support pelagic primary production across 23 different lakes. Gammarids may transport some pelagic nutrients to the benthos, but they appear to cause a net increase in cycling of dissolved nutrients in the water column.

Larval dipterans that feed while swimming may simultaneously remove particulate nutrients from the water column while releasing dissolved nutrients, similar to gammarids. For example, predatory chironomid larval taxa in the subfamily Tanypodinae such as *Procladius* sp. migrate vertically to prey on smaller invertebrates above lake sediments (Baker and McLachlan 1979). *Chaoborus* spp. are well-known pelagic predators that undergo diurnal vertical migration (Fedorenko 1975). However, the majority of chironomid larvae found in the 2005–2007 survey were benthic taxa (Chironomidae and Orthocladiinae) and it is unlikely they undergo diurnal vertical migration. Vertical migration by typically benthic chironomid larvae is energetically costly and inefficient (Nachtigall 1961; Brackenbury 2000). Benthic larvae are likely starving while in pelagic regions because they are not capable of filter-feeding in the water column. Chironomid pupae are in a similar position because they do not have mouth parts and must metabolize stored energy as they migrate from sediments to the lake surface. Chironomid larvae may consume sediment particles off their posterior brushes and claws while swimming in the water column (Oliver 1971). However, chironomid larvae probably are not entering the water column in order to feed because pelagic regions are relatively devoid of particulate nutrients compared to benthic regions. Chironomid larvae are thus not expected to cause a net removal of nutrients from the water column.

Pelagic gammarids were positively correlated with concentrations of PC and chl-a in Snowflake Lake and across survey lakes, whereas pelagic chironomid larvae were only positively correlated with concentrations of PC and only in Bighorn Lake. Biomass of gammarids was about 6 times greater than biomass of chironomid larvae in our study. Therefore, the magnitude of nutrient release and our ability to detect nutrient release on a per capita basis was greater for gammarids than chironomid larvae.

The observed increases of around 0.2 mg TDP per chironomid or gammarid should be considered biologically important in oligotrophic mountain lakes where TDP concentrations are only about 2.9 mg TDP·m^{-3}. Furthermore, the TDN:TDP molar ratio was positively correlated with pelagic invertebrate densities across lakes, but remained well above (240 ± 161 SD) the threshold of 20 that indicates N-limitation for algal production (Healy and Hendzel 1980). Thus,_increases in concentrations of TDN with pelagic invertebrate densities were judged to be less important than increases in TDP for potentially increasing pelagic algal production.

Benthic invertebrates in pelagic habitats

Pelagic chironomid larvae and gammarids appeared to be a relatively important component of invertebrate communities in mountain lakes in our study. Mean densities of gammarids and pelagic chironomid larvae were potentially equivalent to about 10–30 mg DW invertebrate$\cdot$m^{-3} based on average biomass of gammarids and fourth instar chironomid larvae collected in the experiment. In comparison, zooplankton biomass (excluding pelagic gammarids and chironomid larvae) in alpine lakes in Banff NP is about 1–10 mg DW zooplankton$\cdot$m^{-3}, based on average densities of about 100 individuals$\cdot$m^{-3} (McNaught, Pavlik, and Schindler 2000) and length-weight regressions for zooplankton species 1–4 mm in length (Peters and Downing 1984).

Chironomid larvae may have occupied pelagic habitats in lakes in our study due to drift and active swimming. Invertebrate drift frequently occurs in rivers where chironomid larvae become prey for fish (e.g., Keeley and Grant 1997). Similarly, in the Blue Nile River, Sudan, chironomid larvae have reinvaded temporarily flooded reservoirs by swimming into the water column and drifting into these areas (Lewis 1957; Wülker 1963). Species of Orthocladiinae which typically inhabit the littoral zone in mountain lakes (Armitage *et al*. 1995) may be susceptible to being suspended by waves and wind-driven currents. In Loch Ness, Scotland, wind-induced currents increase the abundance of pelagic invertebrates including chironomid larvae, which are important prey for Arctic charr (Winfield *et al*. 2000). In Loch Leven, England, early instar larvae of several species of chironomid larvae use positive phototaxis and pelagic swimming ability to redistribute to more oxic and productive sediments (Davies 1974; 1976a). Active movement of larvae from profundal to littoral regions may thus increase their presence in the water column while optimizing their emergence as adults (Davies 1976b).

Chironomid larvae may migrate upward into the water column to avoid hypoxia. Oxygen depletion at the sediment-water interface (< 50% of surface O_2) was occasionally observed in the present study in several shallow (< 5 m) and more productive (about 10 mg TDP$\cdot$m^{-3}) mountain lakes in late fall and winter (data not shown). The highest densities of pelagic chironomid larvae (about 100$\cdot$m^{-3}) in the present study were first noticed in late winter in one alpine lake that had less than 5 m maximum depth and was subject to hypoxia. Anoxia and release of hydrogen sulfide trigger the pelagic swimming behaviour of chironomid larvae in Lake Katanuma, Japan (Takagi *et al*. 2005). Although some chironomid taxa are known to contain haemoglobin and be resistant to hypoxia, early instar larvae are sensitive to low oxygen conditions and mortality of late instar larvae increases after several weeks (Hamburger, Dall, and Lindegaard 1995).

Benthic-pelagic trophic linkages

Predation of trout and gammarids on pelagic chironomid larvae and stimulation of phytoplankton production by nutrients released from these invertebrates represent potentially important benthic-pelagic trophic linkages in mountain lake food webs. Conclusions about the importance of these food web linkages will depend on where predation and nutrient release occurs. If trout are consuming invertebrates in benthic regions, then they will transfer nutrients into the water column despite vertical migration of chironomid larvae and gammarids. However, vertical movement of benthic invertebrates must increase their vulnerability to predation and thus enhance the trophic linkage. In addition, the movement of gammarids and chironomids into the water column in fishless lakes creates a benthic-pelagic food web linkage that would not occur in the absence of trout.

There have been various reports of pelagic behaviour by chironomid larvae in lakes around the world, but integration of these observations into the plankton ecology literature has been lacking. Chironomid larvae may have been considered elsewhere as unintended by-catch in zooplankton net hauls. Our results show that chironomid larvae are involved in pelagic food web dynamics in mountain lakes as pelagic prey and suppliers of dissolved nutrients. Similarly, pelagic gammarids prey on smaller invertebrates, are preyed on by trout, and stimulate nutrient cycling in the water column. Our study highlights the potential for pelagic chironomid larvae and gammarids to link benthic and pelagic habitats in mountain lakes through predator-prey interactions and nutrient release.

References

Anderson, R. S., and L. G. Raasveldt. 1974. *Gammarus* and *Chaoborus* predation and the possible effects of *Gammarus* and *Chaoborus* feeding on the zooplankton composition in some small lakes and ponds in western Canada. Canadian Wildlife Service Occasional Paper No. 18:1–24.

Armitage, P. D., P. S. Cranston, and L. C. V. Pinder. 1995. The Chironomidae: biology and ecology of non-biting midges. . Chapman and Hall, London, UK.

Baker, A. S., and A. J. Mclachlan. 1979. Food preferences of Tanypodinae larvae (Diptera, Chironomidae). Hydrobiologia 62:283–288.

Brackenbury, J. 2000. Locomotory modes in the larva and pupa of *Chironomus plumosus* (Diptera, Chironomidae). Journal of Insect Physiology 46:1517–1527.

Brooks, J. L., and S. I. Dodson. 1965. Predation body size and composition of plankton. Science 150:28–35.

Davies, B. R. 1974. Planktonic activity of larval chironomidae in Loch Leven, Kinross. Proceedings of the Royal Society of Edinburgh Section B-Biological Sciences 74:275–283.

Davies, B. R. 1976a. A trap for capturing planktonic larval chironomids. Freshwater Biology 6:373–380.

Davies, B. R. 1976b. The dispersal of Chironomidae larvae: a review. Journal of the Entomological Society of South Africa 39:39–62.

Dick, J. T. A., D. Platvoet, and D. W. Kelly. 2002. Predatory impact of the freshwater invader *Dikerogammarus villosus* (Crustacea : Amphipoda). Canadian Journal of Fisheries and Aquatic Sciences 59:1078–1084.

Fedorenko, A. Y. 1975. Feeding characteristics and predation impact of *Chaoborus* (Diptera, Chaoboridae) larvae in a small lake. Limnology and Oceanography 20:250–258.

Fukuhara, H., and M. Sakamoto. 1987. Enhancement of inorganic nitrogen and phosphate release from lake sediment by tubificid worms and chironomid larvae. Oikos 48:312–320.

Hamburger, K. P., C. Dall, and C. Lindegaard. 1995. Effects of oxygen deficiency on survival and glycogen-content of *Chironomus-anthracinus* (Diptera, Chironomidae) under laboratory and field conditions. Hydrobiologia 297:187–200.

Healey, F. P., and L. L. Hendzel. 1980. Physiological indicators of nutrient deficiency in lake phytoplankton. Canadian Journal of Fisheries and Aquatic Sciences 37:442–453.

Holt, R. D., and G. A. Polis. 1997. A theoretical framework for intraguild predation. American Naturalist 149:745–764.

Keeley, E. R., and J. W. A. Grant. 1997. Allometry of diet selectivity in juvenile Atlantic salmon (*Salmo salar*). Canadian Journal of Fisheries and Aquatic Sciences 54:1894–1902.

Lewis, D. J. (1957). Observations on Chironomidae at Khartoum. Bulletin of Entomological Research 48:155–184.

McNaught, A. S., D. Pavlik, and D. W. Schindler. 2000. Patterns of zooplankton biodiversity in the mountain lakes of Banff National Park, Canada. Pages 494–499 *in* W. Williams, editor. International Association of Theoretical and Applied Limnology, Volume 27, Part 1, Proceedings.

McNaught, A. S., D. W. Schindler, B. R. Parker, A. J. Paul, R. S. Anderson, D. B. Donald, and M. Agbeti. 1999. Restoration of the food web of an alpine lake following fish stocking. Limnology and Oceanography 44:127–136.

Nachtigall, W. 1961. Zur locomotionsmechanik schwimmender dipteran larven. I. Mitteilung: Schwimmen ohne ruderorgane: Ceratopogoniden und Chironomiden. Zeitschrifte fur Vergleichende Physiologie 44:509–522.

Oliver, D. 1971. Life history of the Chironomidae. Annual Review of Entomology 16:211–230.

Parker, B. R., and D. W. Schindler. 2006. Cascading trophic interactions in an oligotrophic species-poor alpine lake. Ecosystems 9:157–166.

Parker, B. R., D. W. Schindler, F. M. Wilhelm, and D. B. Donald. 2007. Bull trout population responses to reductions in angler effort and retention limits. North American Journal of Fisheries Management 27:848–859.

Peters, R. H., and J. A. Downing. 1984. Empirical-analysis of zooplankton filtering and feeding rates. Limnology and Oceanography 29:763–784.

Polis, G. A., C. A. Myers, and R. D. Holt. 1989. The ecology and evolution of intraguild predation - potential competitors that eat each other. Annual Review of Ecology and Systematics 20:297–330.

Schindler, D. W., and B. R. Parker. 2002. Biological pollutants: alien fishes in mountain lakes. Water, Air, and Soil Pollution 2:379–397.

Takagi, S., E. Kikuchi, H. Doi, and S. Shikano. 2005. Swimming behaviour of *Chironomus acerbiphilus* larvae in Lake Katanuma. Hydrobiologia 548:153–165.

Vander Zanden, M. J., and Y. Vadeboncoeur. 2002. Fishes as integrators of benthic and pelagic food webs in lakes. Ecology 83:2152–2161.

Vanni, M. J. 2002. Nutrient cycling by animals in freshwater ecosystems. Annual Review of Ecology and Systematics 33:341–370.

Welch, H. E. 1973. Emergence of Chironomidae (Diptera) from Char-Lake, Resolute, Northwest-Territories. Canadian Journal of Zoology 51:1113–1123.

Wilhelm, F. M., J. J. Hudson, and D. W. Schindler. 1999a. Contribution of *Gammarus lacustris* to phosphorus recycling in a fishless alpine lake. Canadian Journal of Fisheries and Aquatic Sciences 56:1679–1686.

Wilhelm, F. M., B. R. Parker, D. W. Schindler, and D. B. Donald. 1999b. Seasonal food habits of bull trout from a small alpine lake in the Canadian Rocky Mountains. Transactions of the American Fisheries Society 128:1176–1192.

Wilhelm, F. M., and D. W. Schindler. 1999. Effects of *Gammarus lacustris* (Crustacea : Amphipoda) on plankton community structure in an alpine lake. Canadian Journal of Fisheries and Aquatic Sciences 56:1401–1408.

Wilhelm, F. M., D. W. Schindler, and A. S. McNaught. 2000. The influence of experimental scale on estimating the predation rate of *Gammarus lacustris* (Crustacea : Amphipoda) on Daphnia in an alpine lake. Journal of Plankton Research 22:1719–1734.

Winfield, I. J., C. W. Bean, and D. P. Hewitt. 2002. The relationship between spatial distribution and diet of Arctic charr, *Salvelinus alpinus*, in Loch Ness, UK. Environmental Biology of Fishes 64:63–73.

Wülker, W. 1963. Prospects for biological control of pest Chironomidae in the Sudan. World Health Organization Ethnobotanical Leaflets 11: 1–23.

Table A2-1. Eighty six mountain lakes surveyed in the Rocky Mountains of Alberta and eastern British Columbia (1965-1984, 1991-2004, and 2005-2007). National parks in Alberta included Banff NP (BNP), Jasper NP (JNP), and Waterton Lakes NP (WLNP) and provincial parks in British Columbia included Glacier PP (GPP) and Kootenay PP (KPP).

		Lakes	Samples	Samples·lake^{-1}·year^{-1}		Mean Depth (m)		Elevation (m asl)	
Dataset	Park			Mean	SD	Mean	SD	Mean	SD
1965–1984	BNP	19	65	3	2	4	3	2080	297
	GPP	7	12	2	1	4	5	1723	269
	JNP	8	11	1	1	3	1	1608	208
	KPP	15	21	1	1	4	6	1497	422
	WLNP	14	35	3	2	1	1	1373	231
1991–2004	BNP	8	171	3	1	7	4	2175	321
2005–2007	BNP	4	44	4	2	5	5	2303	58
	JNP	6	80	4	1	3	1	1861	296
	WLNP	5	46	4	2	2	2	1773	423
	Total:	86	485	3	1	4	2	1821	314

Table A2-2. Analysis of variance (ANOVA) of the effects of predation by gammarids and trout on chironomid larvae among mountain lakes surveyed with varying mean depth and elevation in 1965-1984, 1991-2004, and 2005-2007. A type IV sum-of-squares model was used in the ANOVA because sample sizes were unequal among treatment levels.

Model	Source	D.f. Source, Error	F-ratio	P-value	Sig.
Grand Mean	±Fish/Gammarids	2, 70	8.8	0.015	**
	±Fish/Gammarids*Survey	5, 70	1.3	0.262	-
	±Fish/Gammarids*Mean Depth	3, 70	1.0	0.389	-
	±Fish/Gammarids*Elevation	3, 70	2.0	0.123	-
1965–1974	±Fish/Gammarids	2, 58	5.2	0.008	**
1991–2004	±Fish/Gammarids	1, 6	7.9	0.031	**
2005–2007	±Fish/Gammarids	2, 12	8.4	0.007	**

Table A2-3. Multiple analysis of covariance of the effects of chironomids and gammarids on concentrations of total dissolved P and N (TDP and TDN, respectively), the molar ratio of TDN:TDP, and concentrations of particulate C (PC) and chlorophyll-a (chl-a) based on within-lake variation in Bighorn and Snowflake lakes (Banff NP) sampled in 1991-2004. Units for slope are mg·individual^{-1} for all dependent variables except for TDN:TDP where units are individual^{-1}.

Model	Source	Dependant	D.f. Source, Error	F-ratio	P-value	Sig.	Slope	R^2
Bighorn	Chironomids	TDP	1, 36	3.5	0.071	*	0.3	0.08
		TDN	1, 36	1.6	0.212	-	-0.2	0.04
		TDN:TDP	1, 36	6.5	0.015	**	-8.9	0.11
		PC	1, 36	3.5	0.069	*	0.3	0.08
		Chl-a	1, 36	0.9	0.349	-	-0.1	0.02
Snowflake	Gammarids	TDP	1, 78	2.4	0.091	*	0.2	0.03
		TDN	1, 78	3.2	0.189	-	-0.2	0.03
		TDN:TDP	1, 78	14.7	<0.001	***	-9.0	0.14
		PC	1, 78	3.2	0.076	*	0.2	0.03
		Chl-a	1, 78	7.4	0.008	**	0.3	0.07

Table A2-4. Multiple analysis of covariance of the effects of chironomids and gammarids on concentrations of total dissolved P and N (TDP and TDN, respectively), the molar ratio of TDN:TDP, and concentrations of particulate C (PC) and chlorophyll-a (chl-a) based on across-lake variation in 23 lakes sampled in 1991-2004 and 2005-2007. Units for slope are mg·individual^{-1} for all dependent variables except for TDN:TDP where units are individual^{-1}.

Source	Dependant	D.f. Source, Error	F-ratio	P-value	Sig.	Slope	R^2
Chironomids	TDP	1, 20	4.4	0.071	*	0.3	0.05
	TDN	1, 20	2.1	0.089	*	0.2	0.14
	TDN:TDP	1, 20	0.0	0.843	-	-2.1	0.00
	PC	1, 20	0.0	0.837	-	0.1	0.00
	Chl-a	1, 20	0.5	0.503	-	0.1	0.02
Gammarids	TDP	1, 20	9.1	0.019	**	1.6	0.57
	TDN	1, 20	12.4	0.010	**	1.8	0.64
	TDN:TDP	1, 20	0.4	0.512	-	-4.3	0.01
	PC	1, 20	5.9	0.046	**	2.7	0.46
	Chl-a	1, 20	3.4	0.078	*	0.5	0.23

Table A2-5. Multiple analysis of variance of the effects of pelagic gammarids and chironomids on concentrations of total dissolved P and N (TDP and TDN, respectively), the molar ratio of TDN:TDP, and concentrations of particulate C (PC) and chlorophyll-a (chl-a) in three laboratory experiments.

Source	Dependent	D.f. Source, Error	F-ratio	P-value	Sig.
±Chironomids/Gammarids	TDP	2, 27	91.5	<0.001	***
	TDN	2, 27	87.8	<0.001	***
	TDN:TDP	2, 27	96.7	<0.001	***
	PC	2, 27	97.0	<0.001	***
	chl-a	2, 27	22.7	<0.001	***
±Chironomids/Gammarids*Experiment	TDP	4, 27	11.7	<0.001	***
	TDN	4, 27	11.4	<0.001	***
	TDN:TDP	4, 27	12.2	<0.001	***
	PC	4, 27	12.1	<0.001	***
	chl-a	4, 27	9.1	<0.001	***

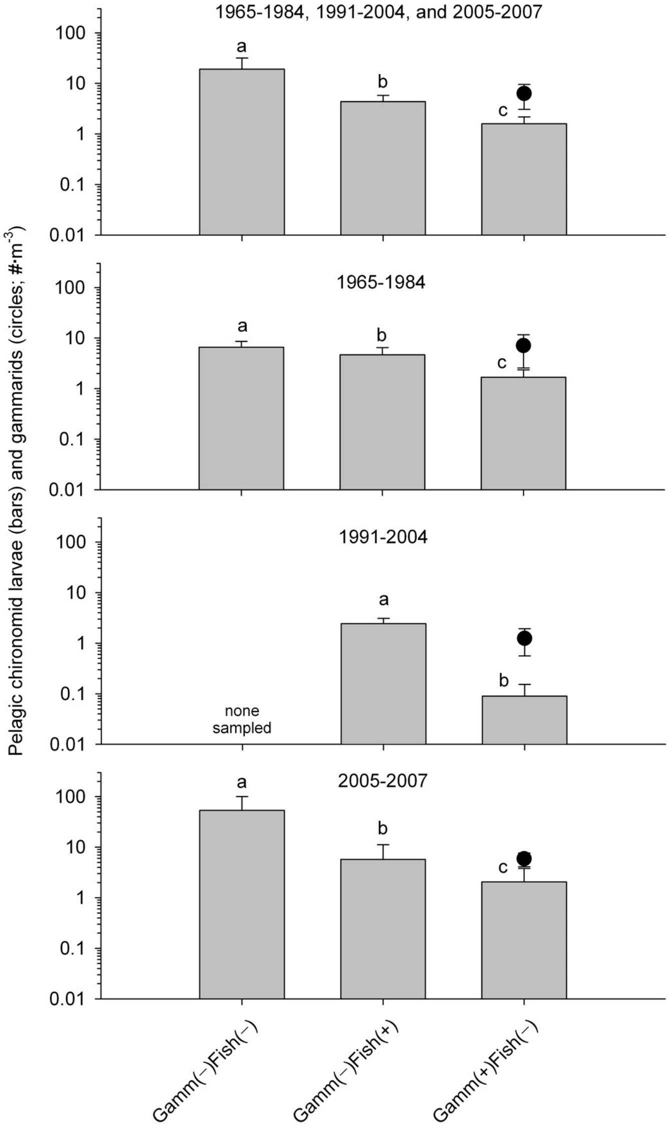

Figure A2-1. Densities of pelagic chironomid larvae (bars) and gammarids (circles) in mountain lakes with and without pelagic gammarids and fish sampled in three lake surveys in the Rocky Mountains of western Alberta and eastern British Columbia (1965-1984 n = 63; 1991-2004 n = 8 ; 2005-2007 n = 15; ± SE). Lakes with neither pelagic gammarids nor fish = "Gamm(–)Fish(–)"; lakes with fish but no pelagic gammarids = "Gamm(–)Fish(+)"; and lakes with pelagic gammarids but no fish = "Gamm(+)Fish(–)".

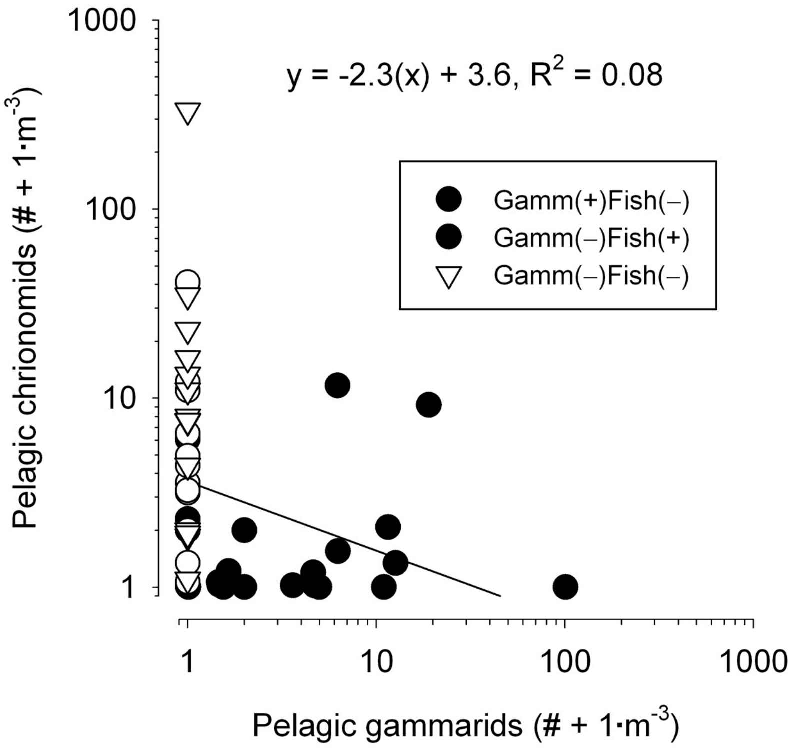

Figure A2-2. Densities of pelagic chironomid larvae regressed against densities of pelagic gammarids across 86 lakes with and without pelagic gammarids and fish in the Rocky Mountains of western Alberta and eastern British Columbia sampled in 1965-1984, 1991-2004 and 2005-2007. Lakes with neither pelagic gammarids nor fish = "Gamm(–)Fish(–)"; lakes with fish but no pelagic gammarids = "Gamm(–)Fish(+)"; and lakes with pelagic gammarids but no fish = "Gamm(+)Fish(–)".

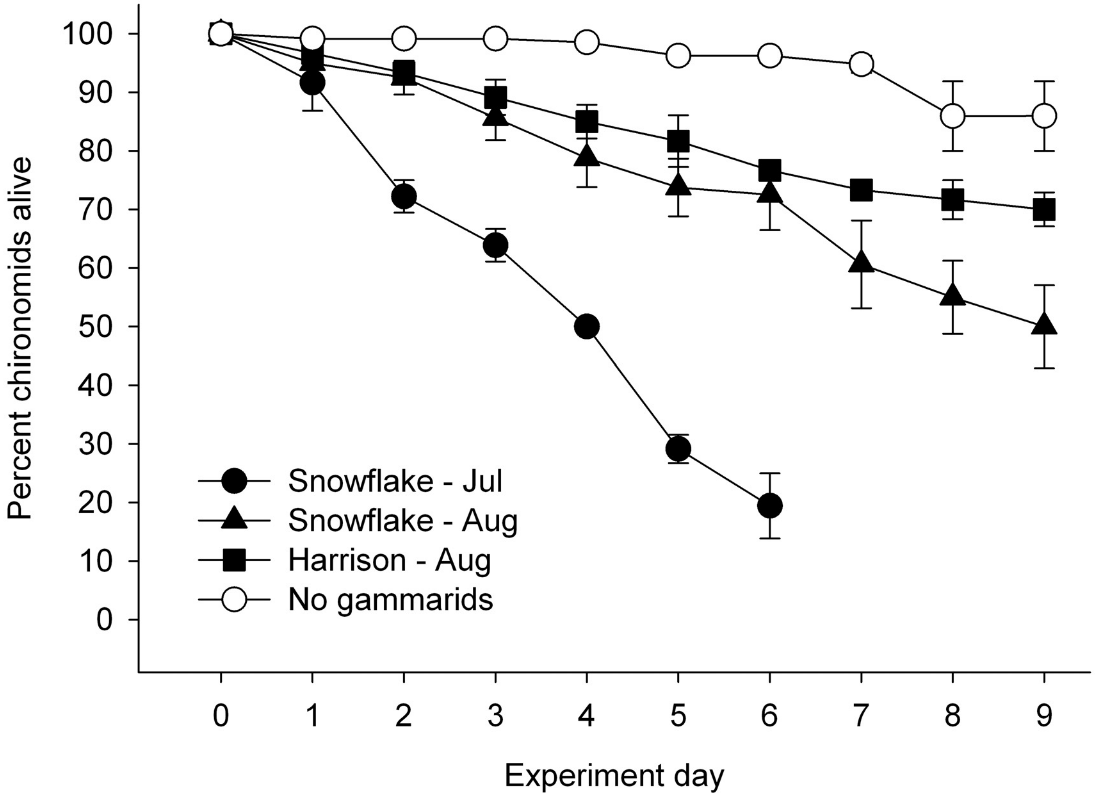

Figure A2-3. Percent chironomid larvae remaining alive in aquaria with and without pelagic gammarids collected from Snowflake Lake in July and August 2006 and from Harrison Lake in August 2006 (n = 4). Average values across experiments were presented for the control treatments where gammarids were absent. Statistical differences were tested only on days 0–6 so that sample sizes were equal across experiments. Bonferonni-corrected *post hoc* comparisons showed that treatments with and without gammarids were significantly different on days 2–6 for all experiments.

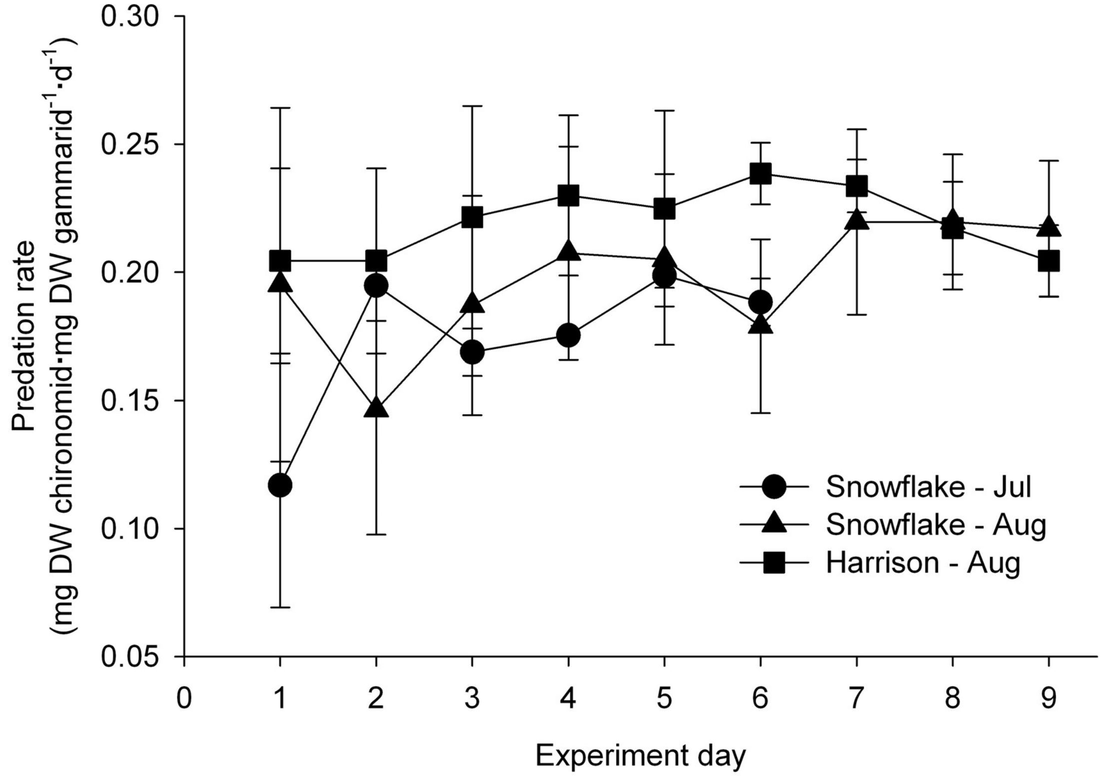

Figure A2-4. Predation rate of gammarids on chironomid larvae collected from Snowflake Lake in July and August 2006 and from Harrison Lake in August 2006 and held in aquaria for 6–9 days (n = 4). Statistical differences were tested only among days 0–6 so that sample sizes were equal. Predation rates were not significantly different across days.

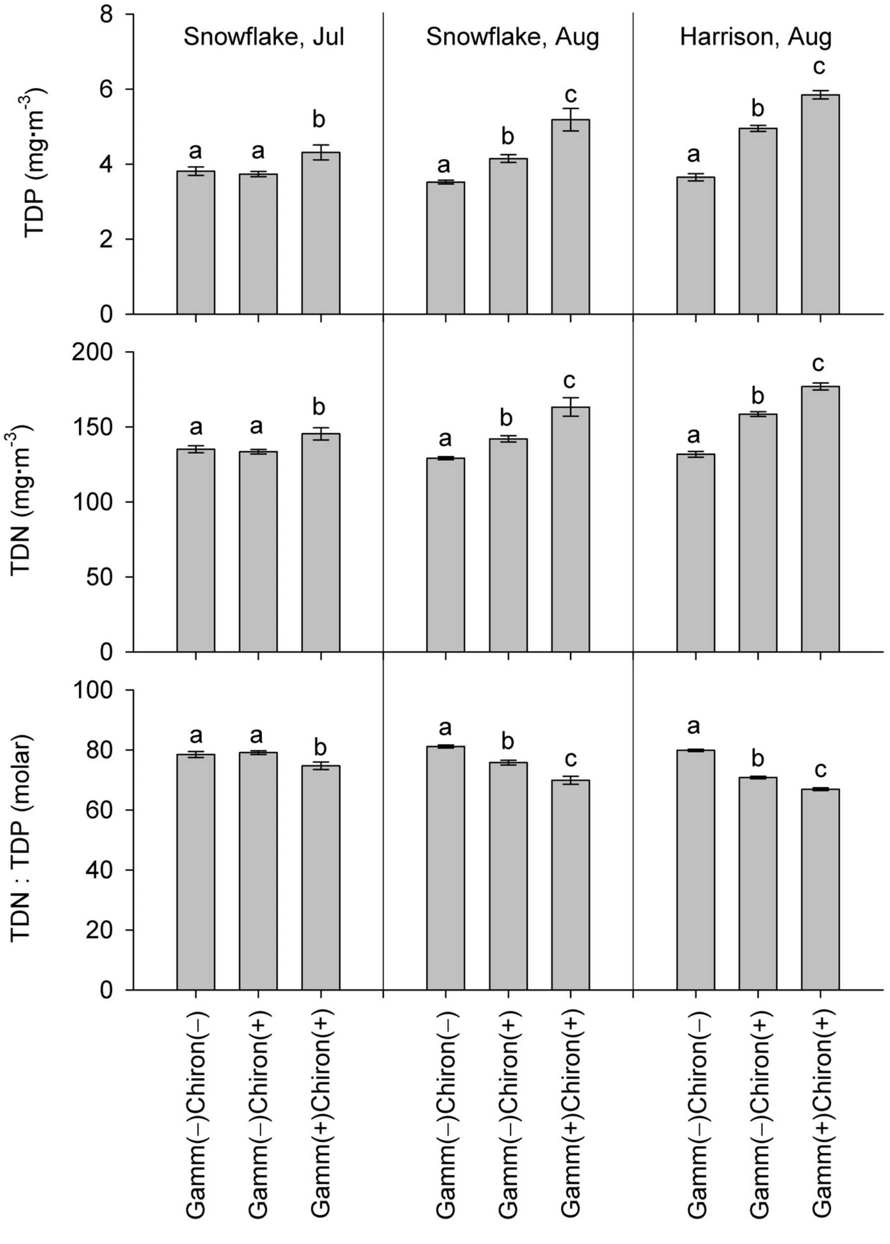

Figure A2-5. Total dissolved P and N (TDP and TDN, respectively) and the molar ratio of TDN:TDP in the presence and absence of chironomid larvae and gammarids collected from Snowflake Lake in July and August 2006 and from Harrison Lake in August 2006 and held in aquaria for 6–9 days (n = 4, ± SE). Treatments with neither invertebrate = "Gamm(–)Chiron(–)"; treatments with chironomids only = "Gamm(–)Chiron(+)"; and treatments with both invertebrates = "Gamm(+)Chiron(+)".

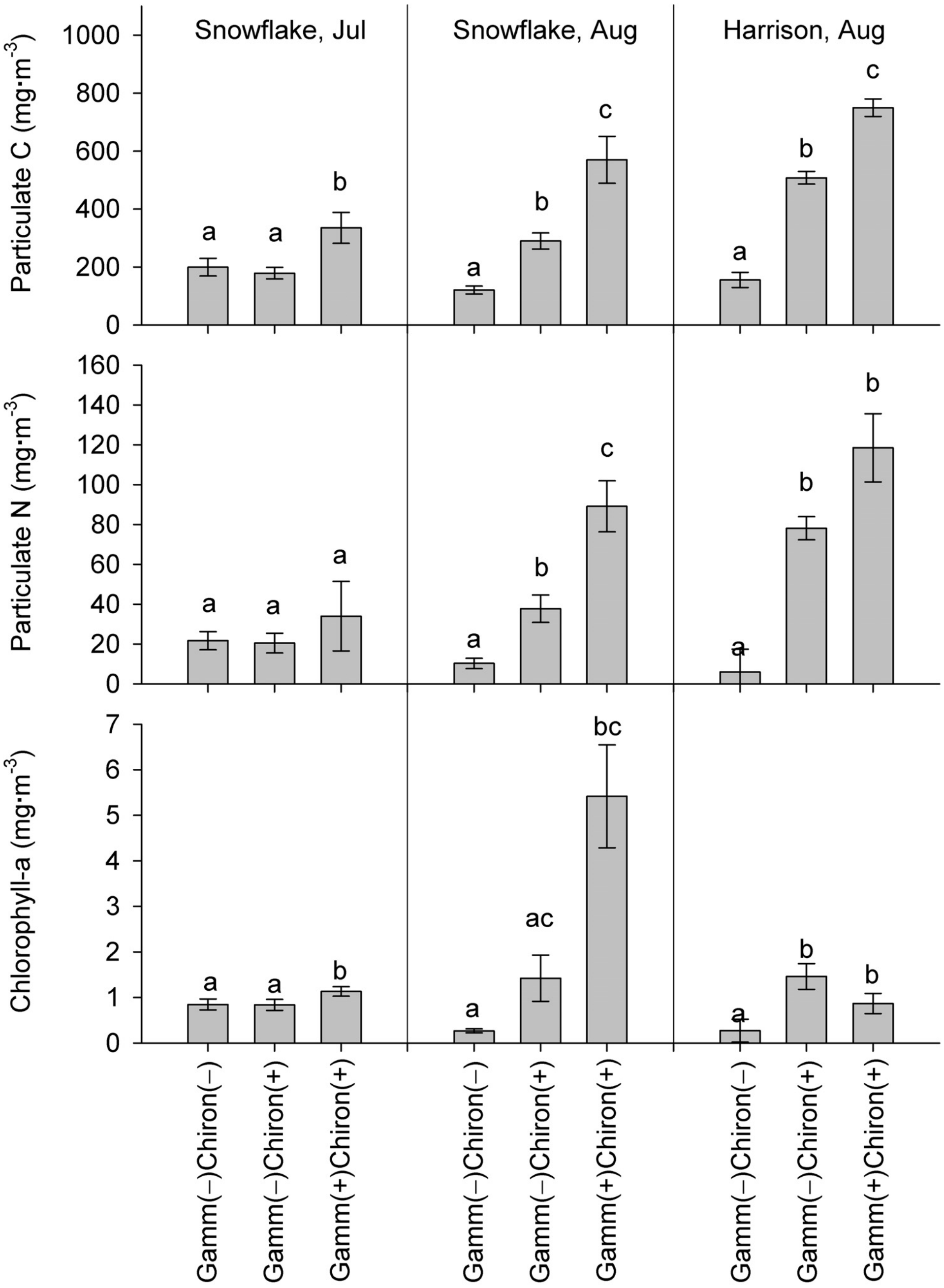

Figure A2-6. Particulate C (PC) and chlorophyll-a (chl-a) in the presence and absence of chironomid larvae and gammarids collected from Snowflake Lake in July and August 2006 and from Harrison Lake in August 2006 and held in aquaria for 6–9 days (n = 4, ± SE). Treatments with neither invertebrate = "Gamm(–)Chiron(–)"; treatments with chironomids only = "Gamm(–)Chiron(+)"; and treatments with both invertebrates = "Gamm(+)Chiron(+)".

APPENDIX 3: SUMMARY OF CHAPTERS 2–4

Chapter 2: Spatial Survey – Summary

We tested the hypothesis that higher temperatures and reduced precipitation due to climate change would increase dissolved phosphorus concentrations, thereby increasing autotrophic phytoplankton and P-limited cladoceran grazers in alpine lakes. We inferred the effects of climate-related factors on fishless alpine lake food webs using structural equation modeling and spatial survey data. We sampled 16 lakes positioned along an climatic gradient (5−17 ^{o}C mean ice-free water temperature) in three national parks in Alberta. Our primary hypothesis was partially confirmed as warming and reduced lake depths favoured 1) increased dissolved P relative to dissolved inorganic N, and 2) increased percent abundance of chlorophytes and cyanophytes relative to phytoflagellates. Counter to our predictions, reduced precipitation 1) decreased abundance of large-bodied herbivorous cladocerans relative to small-bodied omnivorous copepods, and 2) offset the positive effects of warming and shallower lake depth on total phytoplankton and zooplankton biomass. Under warmer, P-rich conditions key alpine cladocerans (*Daphnia middendorffiana*) and copepods (*Hesperodiaptomus arcticus* or *Hesperodiaptomus shoshone*) were replaced by smaller-bodied species (*Daphnia pulex*, *Chydorus* sp., *Bosmina* sp., and *Diaptomus* spp.). Our findings highlight how climate change can alter the composition of key limiting nutrients, primary producers, and herbivorous and omnivorous consumers in alpine lake food webs.

Chapter 3: Temporal Survey – Summary

Effects of climate change on lake food webs involve large-scale multiple stressors, such as long-term warming and decreased precipitation. We determined the effects of long-term (1991–2006) variation in temperature and precipitation on a fishless alpine lake food web in Banff National Park (Alberta, Canada) using structural equation modeling. We hypothesized that decreased precipitation would decrease inputs of C-rich allochthonous matter, thereby decreasing dissolved C relative to P and increasing production of P-limited autotrophs relative to mixotrophs. We also hypothesized that warming would promote faster-growing P-limited herbivorous cladocerans and rotifers relative to omnivorous copepods. We found that decreased summer precipitation significantly reduced concentrations of dissolved organic C and reduced dilution increased concentrations of total dissolved P, which coincided with an increase in diatoms relative to phytoflagellates. Despite increases in dissolved P, however, there was a net increase in particulate C:P primarily due to warming-related increases in *Daphnia*, which retained P and grazed on P-rich phytoflagellates. Calanoid copepods were not suppressed by warming, but instead increased with reduced winter snow pack and advanced ice-off, which subsequently increased copepod grazing on diatoms. Our findings suggest that warmer and drier conditions due to climate change will enrich alpine lakes in dissolved P relative to C, stimulating autotrophic production and secondary production by daphnids and copepods. However, comparison with other studies suggest that more extreme warming conditions outside the range in variation in our dataset may produce different effects on phytoplankton and zooplankton taxa.

Chapter 4: Experiment – Summary

We tested the hypothesis that warming and increased dissolved organic C (DOC) that are expected with climate change would decrease P-content, thereby reducing phytoplankton and favoring omnivorous copepods over P-limited herbivorous cladocerans in alpine lakes. Alternatively, warming was expected to stimulate faster-growing cladocerans relative to copepods in alpine lakes compared to montane lakes where populations were expected to be adapted to warmer temperatures. We incubated plankton from two alpine lakes and two montane lakes *in vitro* for 30 days at 10 ^{o}C or 17 ^{o}C and with ambient or + 80 % DOC. Warming and DOC additions alone significantly reduced phytoplankton abundance, but the interaction between treatments offset this effect by increasing availability of dissolved P. Montane lakes with forested catchments had significantly lower particulate P-content under the combination of warming and DOC additions, but had the opposite effect was observed in the alpine lakes. Warming directly increased biomass of *Daphnia* and reduced biomass of the calanoid copepod *Hesperodiaptomus* in the three coldest lakes, but had no effect on zooplankton in the warmest lake. In addition, decreased particulate P content was significantly correlated with increased abundance of *Daphnia* relative to *Hesperodiaptomus*. Our findings suggest that warming and increased DOC due to climate change will have counterbalancing effects on phytoplankton abundance. Increased soil and vegetation cover of alpine catchments due to climate warming should decrease particulate P-content in alpine lakes. Direct-effects of warming should also promote herbivorous *Daphnia* over omnivorous *Hesperodiaptomus* in temperature-sensitive alpine populations, which may further reduce P-recycling to the water column.