

**Ecological Impacts of Extreme Climatic Events on Mountain Lakes and Ponds**

by

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## **Abstract**

Global warming is contributing to extreme climatic events, especially at higher elevations and latitudes. Phytoplankton assemblages are highly sensitive to these climate-related environmental changes, which include heatwaves and drought events. Knowledge gaps exist concerning the cumulative impact of environmental warming and drought on aquatic alpine communities, and whether it depends on the order of exposure to each stressor. In this thesis, I investigated climate-related predictors of alpine phytoplankton assemblages across 72 lakes in national mountain parks, and the effects of an experimental heatwave and drought event on periphyton, phytoplankton, macroinvertebrate, and rotifer assemblages in fishless alpine pond mesocosms. Mean annual precipitation, lake depth, and lake surface area were all identified as significant predictors of phytoplankton biomass. Mean annual precipitation and air temperature explained among-site variance in taxonomically diagnostic algal pigment concentration, but not genera biomass. In the mesocosm experiment, simultaneous exposure to a heatwave and drought resulted in antagonistic impacts as drought overrode the effect of heating on most taxonomic groups, especially in the case of the phytoplankton and benthic invertebrate assemblages. However, sequential exposure to the two stressors resulted in a multiplicative effect on the phytoplankton assemblage. Altering the order of exposure to drought and warming had a significant effect on periphyton biomass and assemblage. My findings highlight the dominant role that increasingly frequent drought events will likely play in structuring shallow mountain lake and pond communities under a rapidly warming climate.

## **Dedication**

I dedicate this thesis to the algae, without whom the world we know and love would not exist.

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# Chapter 1 Introduction

## Background and Rationale

### *Mountain lakes as sentinels of global change*

Mountain lakes are considered sensitive indicators of the effects of climate change for several reasons. In general, these waterbodies are relatively remote and their responses to climatic events are often not confounded by the impacts of local human activities. Further, climatic warming is occurring more quickly at higher elevations in mountains than at lower elevations elsewhere (Wang et al. 2014; Calanca 2007). Mountain lakes and ponds function as integrators of the broader landscape effects of climate change as these funnel down into waterbodies as a result of their lower position within catchments (Williamson, et al. 2009). Thus, investigations into the cumulative impacts of climate change on aquatic environments and ecological communities in mountainous regions are needed for our understanding of how these remote and protected ecosystems will respond to future global warming.

My graduate research spanned lentic communities across five national mountain parks in Canada. A typical community structure in these mountain lakes includes phytoplankton, zooplankton, periphyton and macroinvertebrates, and fish in certain cases (Loewen et al. 2019; Parker et al. 2008). Schindler (1987) described the phytoplankton assemblage as especially important and useful to study as they are small rapidly reproducing organisms that disperse far and quickly. Significant shifts in species composition can happen rapidly after environmental changes and are readily detected if monitoring occurs at least on a biweekly basis (Schindler 1987). Many organisms in these lakes form resting structures in the sediments, which then

function as “seed banks” enabling species to later emerge and become re-established (Barbiero and Welch 1992; Brock et al. 2003). Barbiero and Welch (1992) found that recruitment of cyanobacteria from the sediment made up, on average, 40% of the population in the water column. Hansson (1996) saw that algal recruitment from sediment increased phytoplankton abundance by up to 50% a day, and that this was highest in shallow (<10m deep) lakes. Zooplankton egg banks can act as a reserve during unfavourable conditions, and diapausing eggs can persist for decades and still be viable (Hairston 1996). Resting structures in sediments convey information about communities’ responses to climate stress in the past, present, or future. This is an approach to limnology and paleolimnology called ‘resurrection ecology’ (Kerfoot et al. 1999). Resurrection ecology is an important aspect to consider when using freshwater communities as sentinel of global change, as resistant structures in the sediments can survive unfavourable conditions and repopulate the water column when favourable conditions return (Brock et al. 2003).

The communities in these lakes and ponds can be regulated through bottom-up and top-down dynamics affected by climatic factors, such as temperature (Kratina et al. 2012). Bottom-up effects can involve chemical and physical factors that control primary producers, which in turn provides the template for the structure of higher trophic levels. Top-down effects can involve top predators like fish or invertebrates reducing the population of secondary consumers, thereby reducing consumption of primary producers. These effects are exemplified in several mountain lake and pond experiments. Manipulation of nutrient addition and predation by calanoid copepods in alpine lakes of the Canadian Rockies demonstrated bottom-up and top-down effects respectively (Paul et al. 1994). Here, predation suppressed rotifer abundance, particularly that of soft bodied rotifers, overcoming the bottom-up stimulating effects of nutrient

addition. In Canadian alpine ponds, additions of ammonium nitrate had indirect effects on the whole ecosystem (Vinebrooke et al. 2014). Nitrogen-limited phytoplankton assemblages were stimulated by the increased nitrogen deposition while being suppressed by periphytic competition and increased grazing pressure. Stress can change the stability of a community by altering the top-down or bottom-up effects, and these effects can, in-turn, hide or illuminate the impact of stress on the community (Kratina et al. 2012).

### *Current climate related risks to mountain lake ecosystems*

There are many climatic stressors that mountain lakes will face before 2100 (Sala et al. 2000). Here, I address two that will likely cause substantial changes in biodiversity. Warming and drought are climate related stressors that are likely to unequally affect mountain freshwater ecosystems (Bradley et al. 2004; Easterling et al. 2000; O'Reilly et al. 2015). Global summer surface water temperatures are predicted to increase by 0.34°C per decade, primarily due to increased air temperature and increased solar radiation (O'Reilly et al. 2015). Warming is even more pronounced in mountain lakes due to elevation-dependent amplification (O'Reilly et al. 2015), although local conditions will affect the overall temperature increase. Elevation-dependent amplification is due to less heat loss in air parcels as they travel upwards because the high elevation areas function closer to the saturated adiabatic lapse rate than the unsaturated adiabatic lapse rate (O'Reilly, et al. 2015). Typically, the altitude temperature gradient predicts a loss of 9.8°C per kilometre according to the unsaturated adiabatic lapse rate, but only 4.0°C per kilometre by the saturated adiabatic lapse rate (O'Reilly, et al. 2015).

Small lakes and ponds are shrinking under warmer and drier conditions from the compounding effects of several causes (Smol and Douglas 2007; Riordan et al. 2015). These

include unprecedented heat waves, loss of snowpack and glaciers, and inconsistent precipitation (ICPP 2022; Magnuson 1997). Sudden and persistent high temperatures can lead to complete desiccation of small ponds, as seen in a 50-year study from Alaska that found that pond size decreased as mean annual temperature and length of the growing season increased across the study area (Riordan et al. 2015). Smol and Douglas (2007) found that ponds that had persisted for thousands of years in the Arctic were now completely drying due to climate change, this included larger and deeper ponds. Drought events in alpine regions are rare historically but droughts globally are increasing in severity and frequency in the 21st century (Calanca 2007, IPCC 2022).

#### *Expected effects of warming*

Physical and chemical properties of lakes can change as air temperatures increase (Oleksy et al. 2020; Parker 2008). Thermally stratified lakes can become mixed or develop deeper epilimnions that track air temperature closely. This can have consequences on the light penetration, pH, and chemical processes in the water column. Temperature-sensitive algal groups may be extirpated if the hypolimnion refugium disappears, while taxa that prefer warming may increase in abundance. A common response to warming is a shift towards smaller body size and faster reproduction across ectothermic aquatic organisms (Daufresne et al. 2009). Strecker et al. (2004), found that an increase of 3.6°C in water temperature in fishless alpine ponds from Banff National Park, Canada, reduced zooplankton biomass by causing a shift from large cladocerans to rotifers. Phytoplankton and periphyton responded to this warming with significant assemblage compositional shifts. In a 16-month pond mesocosm experiment in Vancouver Canada, Kratina et al. (2012) found that warming of approximately 3°C above ambient temperature decreased

primary producer biomass. Moore et al. (1996) observed zooplankton in temperate lakes of the U.S.A. exhibit changes in behaviour, growth, and development in response to warming. They saw a shift towards smaller body size, earlier reproduction, and the loss of temperature-sensitive species. Petchey et al. (1999) found that aquatic food webs in aquatic microcosms were altered by warming as communities lost top predators and herbivores, and autotroph biomass increased. Increases in decomposition rate and nutrient cycling were also observed.

### *Expected effects of drought*

Physical and chemical properties of lakes can also change due to drought (Schindler et al. 1996; Oleksy et al. 2020; Parker et al. 2008). Increased evaporation can concentrate nutrients in the water column; alternatively, reduced precipitation can reduce nutrients and dissolved organic carbon (DOC) that enter the lake (Schindler et al. 1996; Oleksy et al. 2020; Parker et al. 2008). Changes to DOC type and concentration can affect the light penetration in these lakes, affecting the impact of damaging ultraviolet radiation on algal species. In clear oligotrophic lakes, found commonly in the alpine zone of my study area, a reduction in DOC caused by warming and drought would likely cause a significant increase in UV radiation penetrating the water (Schindler et al. 1996).

Brock et al. (2003) observed the survival and ability to break dormancy in egg and seed banks in wetlands after prolonged drought. They concluded that, while the resting structures are resistant to drought, the bank may be exhausted under continued stress with limited opportunity to replenish. After a two-year drought in one Canadian lake, Arnott et al. (2002) saw an increase in crustacean species richness, which they posit may be due to the reduced DOC and increase in temperature and oxygen in the hypolimnion, post drought. They also observed interactive effects

of drought and temperature and drought and light on the hatching rates of some zooplankton taxa.

### *Expected effects of multiple stressors*

Single stressors are less common than multiple stressors in real systems (Sala et al. 2000; Orr et al. 2020), and mountain lakes will likely face both warmer and drier summer conditions simultaneously (Oleksy et al. 2020; Parker et al. 2008) as. While there are many recent studies that look at the interaction of multiple stressors, which can produce unexpected effects known as ‘ecological surprises’ (Paine et al. 1998), few of them focus on the alpine systems that are likely to be most affected (Jackson et al. 2016). In a meta-analysis by Jackson et al. (2016), the authors found that among multiple stressors studies in freshwater systems, the majority found antagonistic interactions between the stressors, where the combined effect was less than the sum of the individual effects. Previous studies have explored the effects of warming alone or in tandem with other stressors on lake and pond communities. Kratina et al. (2012), found that warming in pond mesocosms interacted with other stressors in significant ways. Warming increased the indirect effects of predation on primary producers but decreased the effects of nutrient addition. Thompson et al. (2008), performed an in-vitro experiment on alpine lake communities and found that the increase of phytoplankton abundance expected when treated with increased nitrogen only occurred when there was simultaneous warming. They also found an interaction between warming and sediment presence. Sediment did not act as a stress buffer as expected, but rather, they propose, as a stressor itself on filter feeding organisms, and a source of phosphorus for previously phosphorus-limited phytoplankton. These effects were also more pronounced in the sediment-warming treatments indicating an interaction between warming and sediment as well.

The effect of multiple stressors is complex and depends on the direction of response to each stressor by the community, as well as tolerances or sensitivities of individual taxa, and the cumulative impacts from one trophic level to the next (Shurin et al. 2012; Thompson et al. 2018). Therefore, understanding which null model to apply to various stressor interactions is important. Using an additive model to predict the effects of two stressors, it is easy to distinguish antagonistic interaction and synergistic interaction. However, when stressors elicit opposing effects on a community, it can be difficult to identify the type of interaction occurring. Comparing multiple null models allows us to account for stressor combinations where one may have a positive or neutral effect and the other has a detrimental effect. (Piggot et al. 2015).

#### *Exposure order effects*

Having a better understanding of the temporal effects of these common climate stressors will give us a better mechanistic understanding of the combined effects. As many ecosystems under climate stress will be hit by simultaneous and sequential stressors, this knowledge is key to predicting potential loss of biodiversity and ecosystem function. The importance of order of exposure often depends on the relative direction of the species responses to the stressor (MacLennan and Vinebrooke 2021). Using the co-tolerance theory, the order of stressor exposure would have a more prominent effect on the community if two stressors elicit different responses from the community independently. If two stressors have similar effects on the community and affect the same portion of the community, then the order should not matter when they are applied sequentially as they are functionally interchangeable. For example, if warming increased phytoplankton biomass and drought decreased it, then the order in which they are applied would likely affect the total community response. MacLennan and Vinebrooke (2021)

provided evidence for the co-tolerance theory of order of exposure effects. Increased water temperature and invasive predators were positively correlated stressors, which did not demonstrate order of exposure effects on mountain lake communities. In a marine setting, Brooks and Crowe (2019) observed that altering the order and timing of exposure of marine epifaunal communities to copper and biocide did influence the effects of these stressors. These stressors affected different portions of the community and elicited different responses in ecosystem function. Meng et al. (2020) found that the synergism seen between heat and toxicant stress on mosquito larvae was much more pronounced when heat was applied before pesticides.

## **Conclusion**

Mountain lakes and ponds are important indicators of the effects of extreme climatic events, or stressors (*sensu* Orr et al. 2020). Phytoplankton assemblages especially respond rapidly to changes in the environment (Schindler 1987). Climatic stressors will unequally affect alpine freshwater ecosystems (Bradley et al. 2004), and small lakes and ponds are shrinking under warmer and drier conditions (Magnuson 1997; Riordan et al. 2015), even ones that have persisted for thousands of years (Smol and Douglas 2007). Warming and drought are two of the biggest climate related threats to biodiversity in mountain lakes (Sala et al. 2000) but evidence of thermal and desiccation tolerance or resistance in cold-adapted aquatic alpine communities is a significant knowledge gap. These stressors may interact in unexpected ways (Jackson et al. 2016, Thompson et al. 2018). Temporal effects, including order of exposure effects, may change the community response to these stressors and remain an important area to explore (Brooks and Crowe 2019; MacLennan and Vinebrooke 2021).



## **Main Research Objectives**

1. Identify relationships between climate-related environmental variables and phytoplankton biomass across mountain lakes and ponds (Chapter 2).
2. Compare use of taxonomically diagnostic algal pigments versus their affiliated genera in identifying the suite of environmental variables that best explain variation in phytoplankton assemblages among mountain lakes and ponds (Chapter 2).
3. Experimentally test direct and interactive effects of a simulated drought event and heatwave in fishless alpine pond mesocosms (Chapter 3).
4. Test for exposure-order effects of warming and drought on alpine pond communities (Chapter 3).

The first two objectives were met using environmental and phytoplankton assemblage survey data from 72 mountain lakes in the Canadian Rocky Mountains. Phytoplankton assemblages were characterized based on taxonomically diagnostic pigment concentrations and as biovolumes of genera. Using a multiple linear model and multivariate analysis of these data I determined which climate-related variables were the best predictors of phytoplankton biomass and diversity.

The last two objectives were met using an in-vitro mesocosm experiment. I manipulated warming, drought, and stressor sequence to determine how an entire aquatic community emerging from the sediment across many taxonomic levels responded to single and combined climate stressors. Mesocosms contained biological inocula from alpine lake sediments. I measured total community compositional responses, zooplankton and benthic macroinvertebrate

abundances, and phytoplankton and periphyton total chlorophyll *a*, from the water column and sediments respectively. This holistic approach to resurrection ecology allowed me to determine how these important ecosystems may be susceptible or resistant to broad compositional changes under multiple climate stressors, and to assess the importance of stressor interactions and temporal effects.

## **Chapter 2 Climatic and lake size variables predict phytoplankton assemblages in the Canadian Rocky Mountains**

### **Introduction**

Phytoplankton assemblages are considered important predictors of extreme climate change events, or climate related stressors (*sensu* Orr et al. 2020), due to their responsiveness to changes in the surrounding landscape (Williamson et al. 2009). Mountain lake communities are especially important to study as they experience relatively more extreme climatic warming than occurs at lower elevation sites (Wang et al. 2014; Calanca 2007). Two stressors that will be increasing in frequency in these mountain lakes are drought and warming (Easterling et al. 2000; O'Reilly et al. 2015). Previous research has explored which environmental and chemical variables best predict phytoplankton assemblages. Loewen et al. (2020) found that local variables such as chemistry, light penetration, and air temperature were more important predicting variables than catchment features in north-temperate lakes in Alberta, Canada. Cook et al. (2023) identified total phosphorus, glacial coverage, underwater light penetration, and dissolved organic carbon as key predictors of phytoplankton assemblages in the Canadian Rockies. These findings suggest that current phytoplankton assemblages can be predicted by specific environmental variables that relate to higher risk of warming and drought. This is an important area of research as it could predict the impact of climate change on phytoplankton assemblages in the future.

Warming is the most frequently discussed aspect of climate change. The Intergovernmental Panel on Climate Change (IPCC) has modelled a minimum global increase of 1.5 °C by 2040, but this is expected to be much greater in mountain ecosystems (IPCC 2022). Mechanisms for this increased risk of warming in mountain lakes include increased solar

radiation and elevation-dependent amplification (Wang et al. 2014). Maximum lake depth and mean annual air temperature are related to increased risks of warming. Shallow lakes and ponds are less likely to be thermally stratified and more closely reflect changes in air temperature through the entire water column. Shallow sites lack a cool water refuge in the hypolimnion where temperature-sensitive algae could persist (Woolway and Merchant 2019; O'Reilly 2015; Magnuson et al. 1997; Parker et al. 2008).

Several studies have identified alarming decreases in the sizes of Arctic and alpine lakes and ponds. Calanca (2006) combined drought history and current European climate models to predict an increase in probability of drought occurrence to 50% from 15% currently, by 2100. In subarctic Alaska, Riordan et al. (2006) observed the surface area of closed-basin ponds decrease by up to 31% and the number per survey region decrease by up to 54% over 50 years. Drought events can be brought on by increases in evaporation, changes to precipitation, and increased air temperature (Riordan 2006; IPCC 2022). Environmental variables that relate directly to increased risk of drought in mountain lakes and ponds include mean annual air temperature, maximum lake depth, lake surface area, and mean annual precipitation. As air temperatures increase, evaporation from lake surfaces increases, which can lead to drought. By this same mechanism, lake surface area and depth also influence drought risk. Here, large shallow lakes and very small lakes are at highest risk of drought (Smol and Douglas 2007). Inconsistent precipitation is related to climate change and can also lead to increased risk of drought and alter sediment hydraulic properties which can further reduce stability (IPCC 2022; Caplan 2019). These four variables may predict increased risk of climate related warming and drought stress.

A classic method for measuring phytoplankton assemblages is taxonomic analysis of algal cells using light microscopy and the morphological species concept (Prescott 1982; Wehr

2015). A more modern approach is high performance liquid chromatography (HPLC). Here, phytoplankton assemblage is identified through concentrations of taxonomically diagnostic pigments (Jeffrey et al. 2005; Vinebrooke and Leavitt 1999). There is not a current consensus for the best methods and the one used is often determined by the requirements of the study. HPLC is faster and requires the least knowledge of algal morphology and taxonomy but does depend on access to HPLC equipment. Identifying algae by morphology under light microscope will provide the finest taxonomic level identity but is time consuming and required additional calculations to obtain biovolume (Hillebrand et al.1999). There is a lack of studies in which these two methods are compared in an environmental and community composition analysis.

In this study we aimed to use a dataset of temperature- and drought-related environmental variables and phytoplankton pigment and genera composition from 72 mountain lakes to meet three objectives: 1) determine the magnitude and direction of the relationship between total phytoplankton biomass and climatic and lake variables relating to higher risk of warming and drought using a multiple linear model, 2) compare two methods of measuring phytoplankton assemblages by determining if pigment concentration and/or genus composition shows a significant relationship with this set of environmental variables using multivariate analyses, and 3) establish which environmental variables related to warming and drought were significant predictors of phytoplankton assemblages using forward selection of the multivariate analyses. We used these three objectives to answer the question: do key variables related to increased risk drought and warming stress predict current phytoplankton assemblages?

## Methods

### *Limnological survey and environmental data*

The phytoplankton survey was conducted over the mid-summer growing season in 2017 and 2018. A total of 72 lakes spanning five national parks across the Canadian Rocky Mountains were surveyed. These parks were Banff, Jasper, Kootenay, Waterton, and Yoho. All lake sites ranged from 1024 m to 2423 m in elevation and were categorized as montane (1000-1499 m), subalpine (1500 – 1999 m) or alpine (>2000 m). We collected a 10-L depth-integrated water sample from the euphotic zone of each lake. From this sample, 50 mL was preserved with Lugol's solution for morphological taxonomic identification and biovolume calculation of phytoplankton, and one litre was filtered through Whatman GF/F filter paper, which was frozen for HPLC analysis. Maximum lake depth was measured for each site using bathymetric maps and a depth sounder. The catchment and environmental data were collected using satellite imagery and a geographic information system (ArcGIS 10.5.0.6491; Esri, Redlands, California, USA). Lake surface areas were calculated by measuring polygons drawn directly on the satellite imagery (Loewen et al. 2019). Mean annual air temperature and mean annual precipitation were calculated from 50 years of previous environmental data (Loewen et al. 2019).

### *Phytoplankton assemblage data*

We used two methods of calculating phytoplankton assemblages. The filter paper collected from each lake was frozen and the pigment was extracted using a standard 80:20 solution of acetone and methanol over 24 hours. The samples were filtered again through a 0.7- $\mu$ m Whatman GF/F filter and dried completely using N<sub>2</sub> gas (Jeffrey et al. 2005; Vinebrooke and

Leavitt 1999). HPLC was performed by standard protocol from Vinebrooke and Leavitt (1999) in an Agilent 1100 Series HPLC. Taxonomically diagnostic pigments were identified from each sample using Agilent ChemStation software. Algal identity was determined by light microscope analysis of morphology and biovolumes were calculated for each genus (Hillebrand et al. 1999). Genera that were not present in more than six lake (= 28 genera) were removed to downweigh rare taxa.

### *Statistical analysis*

All statistics and ordinations were performed in R studio version 4.2.0 (2022-04-22 ucrt). We performed Pearson correlation analyses to determine the correlation between our four environmental and lake variables (mean annual precipitation, mean annual air temperature, maximum lake depth, and lake surface area); variables were considered highly correlated if their Pearson  $r$  value was greater than 0.6. Phytoplankton genus data were Hellinger-transformed, and pigment data were  $\log_{10}$ -transformed to make the data more normal and to adjust scaling between groups. Multiple linear regression was performed on the four environmental and lake variables and chlorophyll  $a$ -inferred phytoplankton biomass from the lakes to determine if these variables were significant predictors of phytoplankton growth. Mean annual air temperature and mean annual precipitation were strongly negatively correlated (Pearson  $r < -0.6$ : Figure 2.1) so mean annual air temperature was removed from this analysis as the precipitation data best met the assumptions of homoscedasticity and normality. Multiple linear model plots were produced to visualize the relationship between each environmental variable and biomass while other variables were held constant.

Ordinations were performed on the multivariate assemblage datasets. Principal component analysis (PCA) was performed using the abiotic climate and lake variables to characterize the environmental conditions of the lakes. A detrended correspondence analysis (DCA) of the pigment data for all 72 lakes showed an axis length of 1.8 standard deviations, indicating that a linear response model was best for further ordinations. Redundancy analyses (RDAs) of the pigment and environmental data and the genera and environmental data were performed. When the ordinations were deemed significant, ( $P < 0.05$ ) we used forward selection to determine which environmental variables were significant predictors of phytoplankton assemblages using Monte Carlo permutation tests ( $n = 999$ ). Centroids were calculated for the lake plots using the ggplot2 (version 3.4.1) package by grouping lakes by park of origin or elevation category, and polygons were drawn onto the corresponding ordination plots.

## Results

### *Environmental variables*

Phytoplankton biomass was predicted using a multiple linear model combining maximum lake depth, lake surface area, and mean annual precipitation across 72 lakes in the Canadian Rocky Mountains ( $R^2 = 15.8$ ,  $P = 0.008$ ). Chlorophyll *a*, a proxy for algal biomass, had a significant linear relationship with each variable when the other variables were held constant (Figure 2.2). According to the model, phytoplankton biomass had a negative relationship with precipitation ( $r^2 = -5.28$ ,  $P = 0.018$ ) and lake area ( $r^2 = -0.44$ ,  $P = 0.049$ ), and a positive relationship with lake depth ( $r^2 = 0.78$ ,  $P = 0.044$ ). PCA of environmental variables indicative of increased risk of warming and drought captured 98.5% of the total variance between the first and



second axes (Figure 2.3). The primary axis was described by mean annual air temperature and precipitation vectors while the secondary axis was described by lake area and depth (Figure 2.3). The elevational polygons showed a clear trend of the first axis as an elevational gradient, where montane sites had lower precipitation and higher air temperature than alpine sites. Lake depth and area were unrelated to the elevational gradient, a result supported by the Pearson correlation heat map (Figure 2.1). Lake area and lake depth were positively correlated ( $r = 0.5$ ), mean annual air temperature and mean annual precipitation were negatively correlated ( $r = -0.78$ ).

### *Phytoplankton Assemblages*

Phytoplankton assemblages as measured by taxonomically diagnostic pigments but not by genera, was predicted by the four warming and drought related environmental variables (Figure 2.4; Figure 2.5). RDA was significant ( $F = 0.01$ ) for the pigment analysis but was not significant for the analysis of genera ( $F > 0.05$ ). The RDA of pigment and environmental variables captured 9.63% of the total variance with the first two axes.

Mean annual air temperature and mean annual precipitation were the only significant ( $P < 0.05$ ) predictors of phytoplankton assemblages measured by taxonomically diagnostic pigments as determined by forward selection of variables using Monte Carlo permutation tests ( $n = 999$ ). Cryptophytes and chlorophytes, phytoplankton groups associated with the pigments alloxanthin and lutein respectively, were negatively associated with precipitation according to the RDA (Figure 2.5). Polygons of the calculated centroids for lakes grouped by park of origin and elevation showed no distinct patterns (Figure 2.6).

## Discussion

The study provided evidence of climate and lake-related factors associated with ecological risks of increased temperature and drought affecting phytoplankton assemblages across mountainous regions in western Canada. Mean annual precipitation, lake depth, and lake surface area were all significant predictors of phytoplankton biomass according to the multiple linear model. Mean annual precipitation and air temperature were significant explanatory variables of among-lake variance in phytoplankton assemblage based on taxonomically diagnostic algal pigments but not genera biovolumes. These findings indicate that climate warming and increased drought events are likely to alter mountain phytoplankton assemblages by affecting their productivity and taxonomic composition. Below, potential explanations for these key findings are considered.

### *Precipitation as a driver of phytoplankton biomass*

There are a few possible mechanisms that explain the negative relationship between mean annual precipitation and chlorophyll-inferred phytoplankton biomass. High precipitation in the winter and moderate precipitation in the summer both decrease total algal biomass (Oleksy et al. 2020; Parker et al. 2008). High winter snowpack reduces the length of the ice-free season, decreases spring water temperatures, and dilutes nutrient concentrations. High winter snowpack years are associated with high spring flushing which reduces nutrient concentrations and favours smaller fast-growing phytoplankton species (KcKnight et al. 1991). Even in lakes with high nutrient contributions from glacial run-off, increased melting snowpack can dilute the in-lake nutrient concentrations (Park et al. 2004; Parker et al. 2008; Sadro et al. 2018).

High phytoplankton biomass has been linked to low levels of summer precipitation (Oleksy et al. 2020). Low summer precipitation increases lake nutrient concentrations due to evaporation relative to inflow (Lewis et al. 2015), and shallow lakes are more likely to track increases in air temperatures, resulting in high phytoplankton biomass in the epilimnion as metabolic rates increase (Kelly et al. 2018; Kraemer 2016). Oleksy et al. (2020) observed an increase in DOC in years with warmer drier summers. Due to the decreased lake inflow, this is attributed mainly to autochthonous DOC, which can increase populations of small mixotrophic cryophytes and diatoms, that feed on DOC and DOC producing plankton (Parker et al. 2008; Bird and Kalff 1986; Findlay et al. 2001; Rothhaupt 1996). During an extended drought period in boreal lakes, Findlay et al. (2001), observed a similar shift towards an increase in mixotrophic dinoflagellates and cryophytes able to use bacteria as a food source.

High summer precipitation levels increase allochthonous inputs of dissolved organic nutrients and carbon from run-off and bedrock weathering, stimulating algal production (Schindler et al. 1996). Increased run-off can also decrease the clarity of the water column, protecting phytoplankton from damage by UV-B radiation, which can otherwise suppress mountain phytoplankton (Neale et al. 1998; Williamson et al. 2010). Thunderstorms associated with high summer precipitation also induce wind-driven mixing that further increase epilimnion nutrients and turbidity, stimulating phytoplankton growth (Sadro and Melack 2012; Perga et al. 2018).

#### *Air temperature as a driver of phytoplankton biomass*

Air temperature influences mountain phytoplankton through changes to water temperature, stratification, snowmelt timing, absorbance, and turbidity (Schindler, et al. 1996).

Warmer air causes deeper thermoclines, which can increase frequency of nutrient mixing. Warmer, shallow, and frequently mixed lakes are associated with high chlorophyll *a* in their epilimnion (Michelutti et al. 2016; Kraemer et al. 2016; Kelly et al. 2018) due to heat increasing the metabolic rates of algae (Kraemer 2016). Increased temperature and lower N:P ratios increase phytoplankton biomass by reducing energy and nutrient limitations (Cross et al. 2015), as many of these lakes are phosphorus-limited (Cook et al. 2020). Deeper thermoclines especially benefit species that are adapted for warmer water, which can cause a species composition and biomass shift (Strecker, et al 2003). Warmer spring air temperatures are associated with earlier snowmelt timing, reducing the negative impact of snowpack on phytoplankton biomass (Fassnacht et al. 2018).

Evidence of precipitation and air temperature as drivers of mountain phytoplankton can be found in other lake surveys that also investigated the effects of climate-driven environmental variables. Parker et al. (2008) used long-term limnological and meteorological data, to show that alpine lakes in the Canadian Rockies between 1991 and 2003 group into two distinct climate groups. Lakes in the 2000's were nutrient poor and had lower phytoplankton biomass than those in the 1990's. This is attributed to clear water and deeper mixing in the 2000's due to colder winters with more snow, later snowmelt timing and hotter drier summers. Average summer lake temperatures were 0.5 – 1.2 °C higher in the 2000's, lakes were also less turbid, allowing deeper light penetration of damaging UV-B radiation. Total phosphorus, silica, and other dissolved organic nutrient concentrations were lower in the 2000's due to less weathering of bedrocks. DOC was higher but was mainly non-chromatic and allochthonous and did not increase turbidity. This bottom-up control of lake communities by climate related variables interacted with the top-down effects of the reintroduction of native zooplankton to further suppress phytoplankton

biomass. Higher phytoplankton biomass seen in the 1990's was attributed to higher winter air temperatures and low winter snowfall, early melt, and mild wet summer.

Oleksy et al. (2020) provided additional support for climatic factors of phytoplankton function, composition, and biochemical properties of mountain lakes in the Canadian Rockies. The summers of 2015 and 2016 were warmer and drier than the 1981-2010 average, while precipitation was lower in summer, and snowpack higher or the same in winter. Using boosted regression tree models for 28 mountain lakes, they found that phytoplankton biomass was driven primarily by snowpack, summer precipitation, and summer air temperature. High summer precipitation and air temperature increased phytoplankton biomass, and high winter snowpack decreased total phytoplankton biomass. Low snowpack led to higher spring water temperatures and nutrient concentrations; even after baseflow conditions were met, water temperatures continued to increase while the N:P ratio went down, increasing phytoplankton biomass. In years with average snowpack, high phytoplankton biomass in summer was attributed to warmer and drier summers, which reduce the snowpack quickly and reduce the negative stress on phytoplankton growth. The strength of the regulation of phytoplankton biomass by precipitation and temperature was also dependent on watershed variables. In glacier-fed lakes, inputs from glaciers increase nitrogen as  $\text{NO}_3$ , from microbial nitrification and atmospheric deposition. The nutrient dilution effect of high snowpack was not seen as strongly in glacier-fed lakes. Land cover and hydrologic connectivity also influenced the types and quality of nutrient delivery to lakes during high precipitation, as did lake morphology. For example, larger, deeper lakes below glaciers were less directly affected by variations in precipitation compared to subalpine shallow lakes.

### *Lake morphology as a driver of phytoplankton biomass*

The relationships we found between waterbody morphology (i.e. surface area and depth) and phytoplankton chlorophyll concentrations can potentially be attributed to chromatic acclimation and photoinhibition. Specifically, phytoplankton chlorophyll concentrations were higher in smaller deeper lakes than larger shallower lakes. Photosynthetically active radiation is rapidly attenuated with increasing depth, resulting in light limitation of phytoplankton at deeper depths (Sommaruga 2001). Phytoplankton can acclimate by producing higher cellular concentrations of chlorophyll to increase their capacity for light-harvesting (MacIntyre et al. 2002; Westberry et al. 2008), which could explain the positive relationship of chlorophyll concentrations and lake depth. In contrast, phytoplankton trapped in shallow surface waters at higher elevations can be exposed to very intense solar irradiance, resulting in photoinhibition and bleaching of chlorophyll (Krause 1988). The effects of air temperature and precipitation can also be modulated by lake bathymetry. Oleksy et al. 2020 found the highest chlorophyll concentrations in deeper lakes with smaller lake area-to-watershed ratios.

### *Drivers of phytoplankton assemblages*

Mean annual precipitation and air temperature were significant predictors of phytoplankton assemblage, as assessed by pigments, across the surveyed mountain lakes (Figure 2.5). Warming is typically associated with changes in total algal biomass as it causes a shift towards smaller cells or stimulates growth and leads to cyanobacteria dominance (Petchey et al. 1999; Daufresne et al. 2009; Kratina et al. 2012). Loewen et al. (2020) found that 2% of the total variation in phytoplankton assemblage in north temperate lakes was explained by spring air temperature. Mean annual precipitation is highly correlated with elevation; however in the RDA

of significant environmental variables, we did not see a trend in the elevation group centroids. This may indicate a relationship between the taxonomically diagnostic pigments and precipitation independent of elevation.

Cryptophytes and chlorophytes figured predominantly in the findings. These algae were negatively associated with precipitation (Figure 2.5). This may mean that if precipitation trends were to change in the way that climate scientist have predicted, we could see the strongest response in these two groups. These two groups were also found to be predictable by climate-related environmental variables in Vinebrooke and Leavitt's (1999) survey of mountain lakes and ponds. They found that cryptophytes were typically associated with high dissolved organic carbon (DOC) concentrations in lower montane lakes.

Phytoplankton assemblage could be predicted by this set of warming and drought related environmental variables but only as taxonomically diagnostic pigments. Genus-level assemblage and these variables did not produce a significant ordination. Phytoplankton genera can be redundant in function, as many genera can fill identical ecological niches. Some responses to stress may be more apparent when measured by broad taxonomic groups (e.g., cyanobacteria, chlorophytes, diatoms) diagnosed by pigments (Loewen et al. 2021). This variability between assemblages with identical function but different generic composition may account for the non-significance of the genus-level ordination. Pigments may be a better approximation of functional composition as the broad taxonomic groups distinguished by pigments often respond very differently to environmental change (Vinebrooke and Leavitt 1999).

## *Conclusions*

Precipitation, lake size, and temperature explained among-lake variance in mountain phytoplankton assemblage structure as assessed by pigments. These environmental variables are related to risk of climate change induced warming and drought stress, therefore warming and drought are likely to cause significant changes in phytoplankton biomass and assemblage in mountain lakes. An insight from this study is that for our goals, taxonomically diagnostic pigments are more strongly correlated with the environmental variables than were algal genera. Future directions for this area of research would be to experimentally manipulate warming and drought stress to provide evidence for the predicted changes in biomass and phytoplankton assemblage.



**Table 2.1** Number key for surveyed lakes.

1	Agnes	25	Temple	49	Hamilton
2	Baker	26	Waterfowl	50	Hungabee
3	Boom	27	Annette	51	Oesa
4	Copper	28	Beauvert	52	Opabin
5	Eiffel	29	Cabin	53	Mary
6	Grizzly	30	Caledonia	54	Schaffer
7	Herbert	31	Celestine	55	Sherbrooke
8	Island	32	Christine	56	Wapta
9	Kingfisher	33	Cutt	57	Alderson
10	Laryx	34	Dorothy	58	Allison
11	Lost (B)	35	Edith	59	BC Pond
12	Louise	36	Honeymoon	60	Cameron
13	Moraine	37	Horseshoe	61	Crandell
14	Mud	38	Iris	62	IS W47
15	Mummy	39	Leach	63	Linnet
16	O'Brien	40	Little Honeymoon	64	Lonesome
17	Pharaoh	41	Marjorie	65	Lower Rowe
18	Pilot	42	Mile 16 1/2	66	Maskinongne
19	Ptarmigan	43	Mina	67	TP W60
20	Rock Isle	44	Osprey	68	Upper Rowe
21	Rockbound	45	Patricia	69	Cobb
22	Smith	46	Pyramid	70	Floe
23	Sentinel	47	Virl	71	Kootenay Pond
24	Taylor	48	Celeste	72	Olive

**Table 2.2** Numbered list of phytoplankton taxa as shown in the ordination plots

Number	Genus	26	<i>Cyanodictyon</i>	52	<i>Nannochloris</i>
1	<i>Achnanthes</i>	27	<i>Cyclotella</i>	53	<i>Navicula</i>
2	<i>Anabaena</i>	28	<i>Cymbella</i>	54	<i>Neidium</i>
3	<i>Ankistrodesmus</i>	29	<i>Diatoma</i>	55	<i>Nitzschia</i>
4	<i>Aphanocapsa</i>	30	<i>Dictyosphaerium</i>	56	<i>Ochromonas</i>
5	<i>Aphanothece</i>	31	<i>Didymocystis</i>	57	<i>Oocystis</i>
6	<i>Asterionella</i>	32	<i>Dinobryon</i>	58	<i>Oscillatoria</i>
7	<i>Bitrichia</i>	33	<i>Discostella</i>	59	<i>Pediastrum</i>
8	<i>Carteria</i>	34	<i>Euastrum</i>	60	<i>Peridinium</i>
9	<i>Ceratium</i>	35	<i>Euglena</i>	61	<i>Phacus</i>
10	<i>Chilomonas</i>	36	<i>Eunotia</i>	62	<i>Phormidium</i>
11	<i>Chlamydomonas</i>	37	<i>Fragilaria</i>	63	<i>Pinnularia</i>
12	<i>Chlorella</i>	38	<i>Frustulia</i>	64	<i>Planktolyngbya</i>
13	<i>Chromulina</i>	39	<i>Glenodinium</i>	65	<i>Pseudanabaena</i>
14	<i>Chroococcus</i>	40	<i>Gloeocapsa</i>	66	<i>Pseudokephyrion</i>
15	<i>Chroomonas</i>	41	<i>Gloeocystis</i>	67	<i>Pterosperma</i>
16	<i>Chrysochromulina</i>	42	<i>Gomphosphaeria</i>	68	<i>Rhabdoderma</i>
17	<i>Chrysococcus</i>	43	<i>Gymnodinium</i>	69	<i>Rhodomonas</i>
18	<i>Chrysophaerella</i>	44	<i>Katablepharis</i>	70	<i>Scenedesmus</i>
19	<i>Closterium</i>	45	<i>Kephyrion</i>	71	<i>Selenastrum</i>
20	<i>Coelastrum</i>	46	<i>Lyngbya</i>	72	<i>Sennia</i>
21	<i>Coelosphaerium</i>	47	<i>Mallomonas</i>	73	<i>Snowella</i>
22	<i>Cosmarium</i>	48	<i>Melosira</i>	74	<i>Synechococcus</i>
23	<i>Crucigenia</i>	49	<i>Merismopedia</i>	75	<i>Synedra</i>
24	<i>Crucigeniella</i>	50	<i>Microcystis</i>	76	<i>Tabellaria</i>
25	<i>Cryptomonas</i>	51	<i>Monoraphidium</i>	77	<i>Tetraedron</i>
				78	<i>Tetraspora</i>

**Table 2.3** Taxonomically diagnostic pigments and their algal groups. Modified from Vinebrooke and Leavitt (1999).

Pigment	Algal Group
Chlorophyll a	All algae
Chlorophyll b	Chlorophytes, euglenophytes
Alloxanthin	Cryptophyte
Canthaxanthin	Filamentous cyanobacteria
Diadinoxanthin	Chromophytes, Euglenophytes
Diatoxanthin	Diatoms, few chromophytes
Fucoxanthin	Chromophytes
Lutein	Chlorophytes
Zeaxanthin	Cyanobacteria
Myxoxanthophyll	Colonial cyanobacteria
Violaxanthin	Chlorophytes

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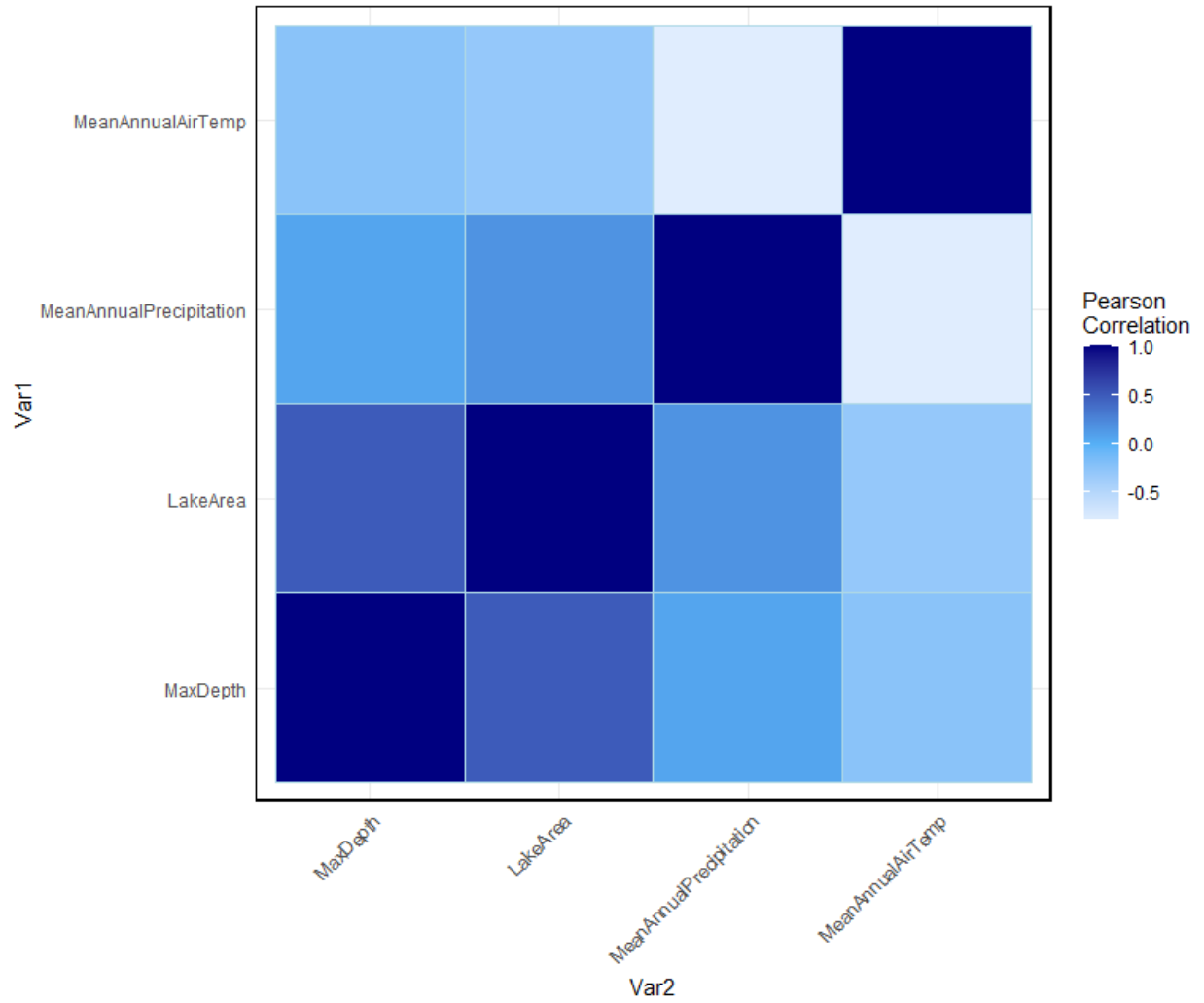
**Figure 2.3.** A) Principal component analysis (PCA) of environmental variables linked to increased risk of warming and drought stress in mountain lakes. Environmental data was collected in the summer of 2017 and 2018 across 72 lakes in the Canadian Rockies. Number key for the lakes is in Table 2.1. B) PCA with centroids calculated and drawn as polygons representing elevational grouping.

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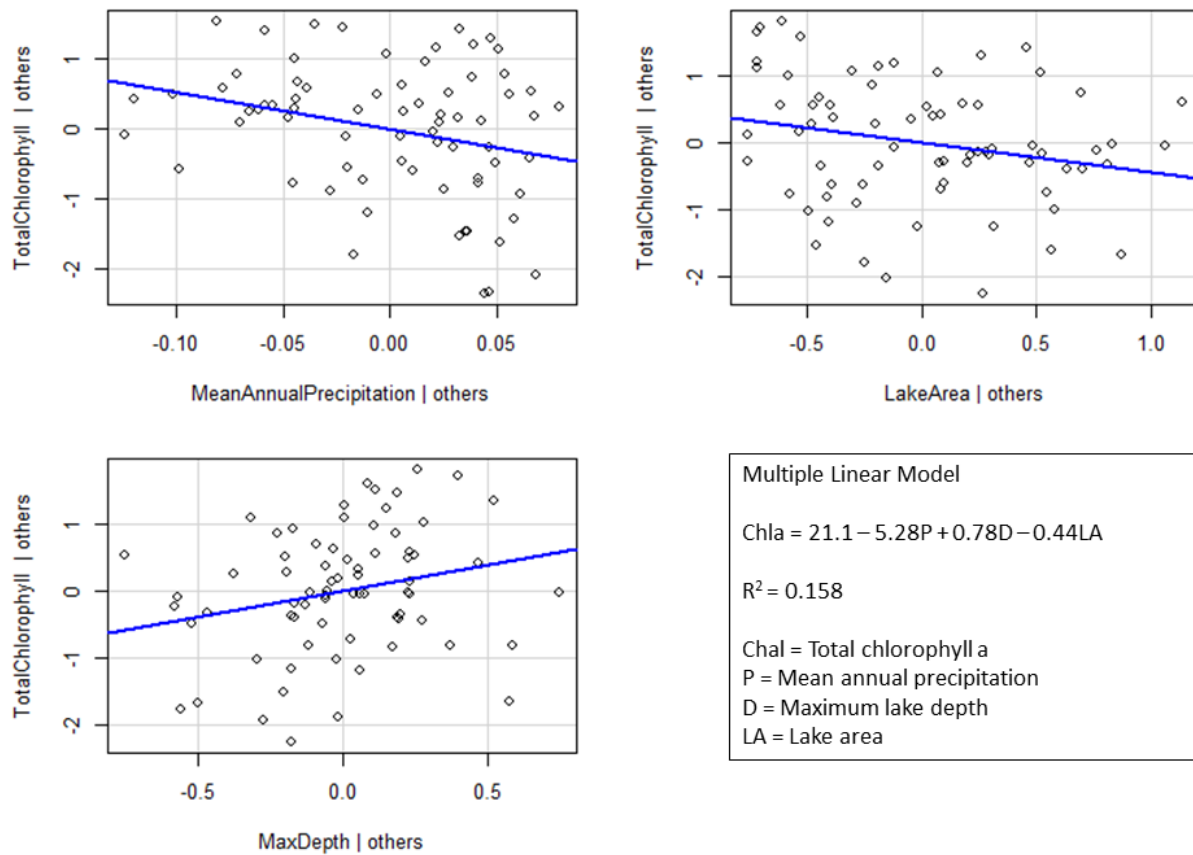
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**Figure 2.6.** Redundancy analyses (RDA) of taxonomically diagnostic phytoplankton pigments and forward selected environmental variables ( $P < 0.05$ ) linked to increased risk of warming and drought stress in mountain lakes measured in the summer of 2017 and 2018 across 72 lakes in the Canadian Rockies. Centroids were calculated and drawn as polygons representing A) elevational grouping and B) park of origin.

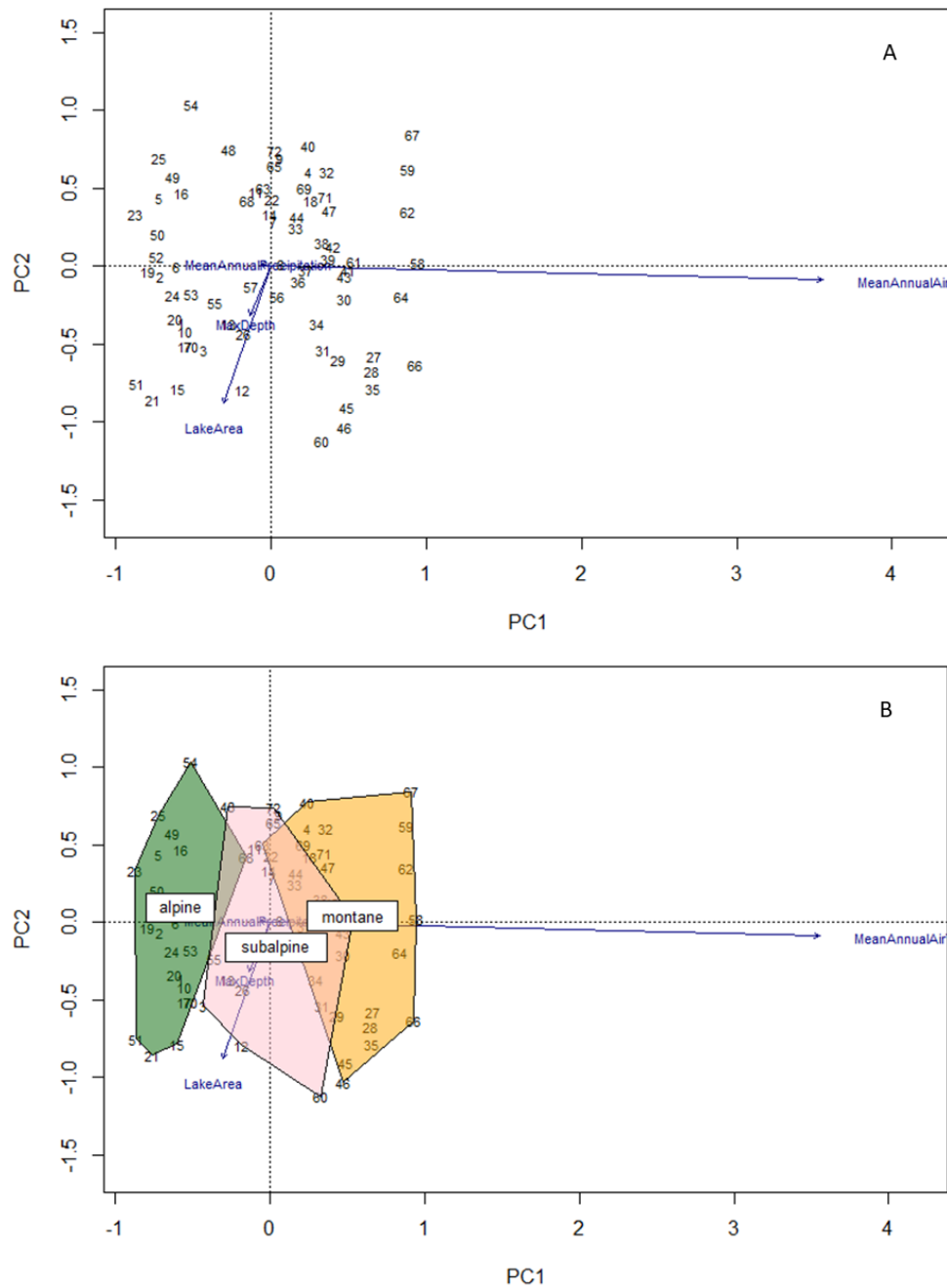
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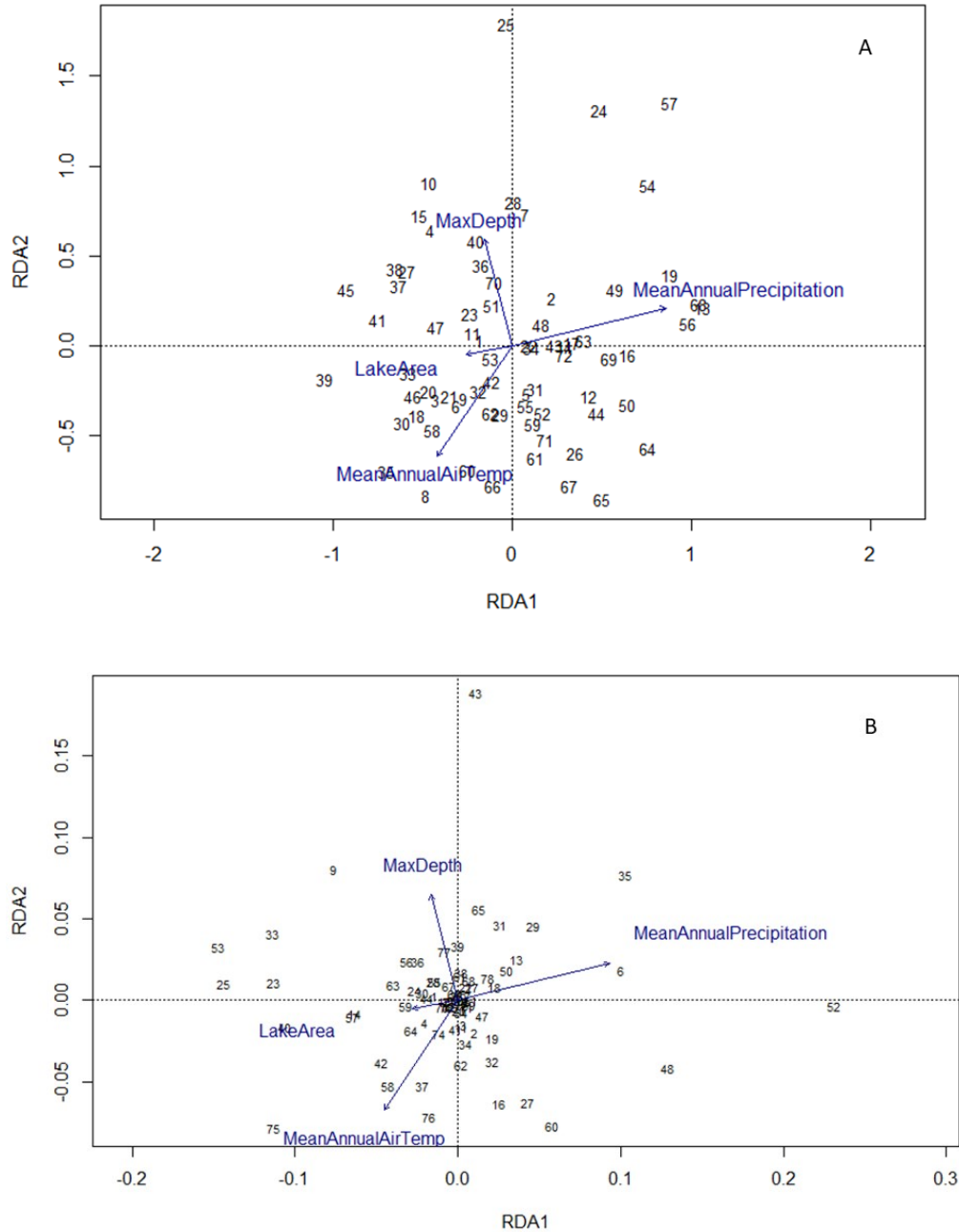
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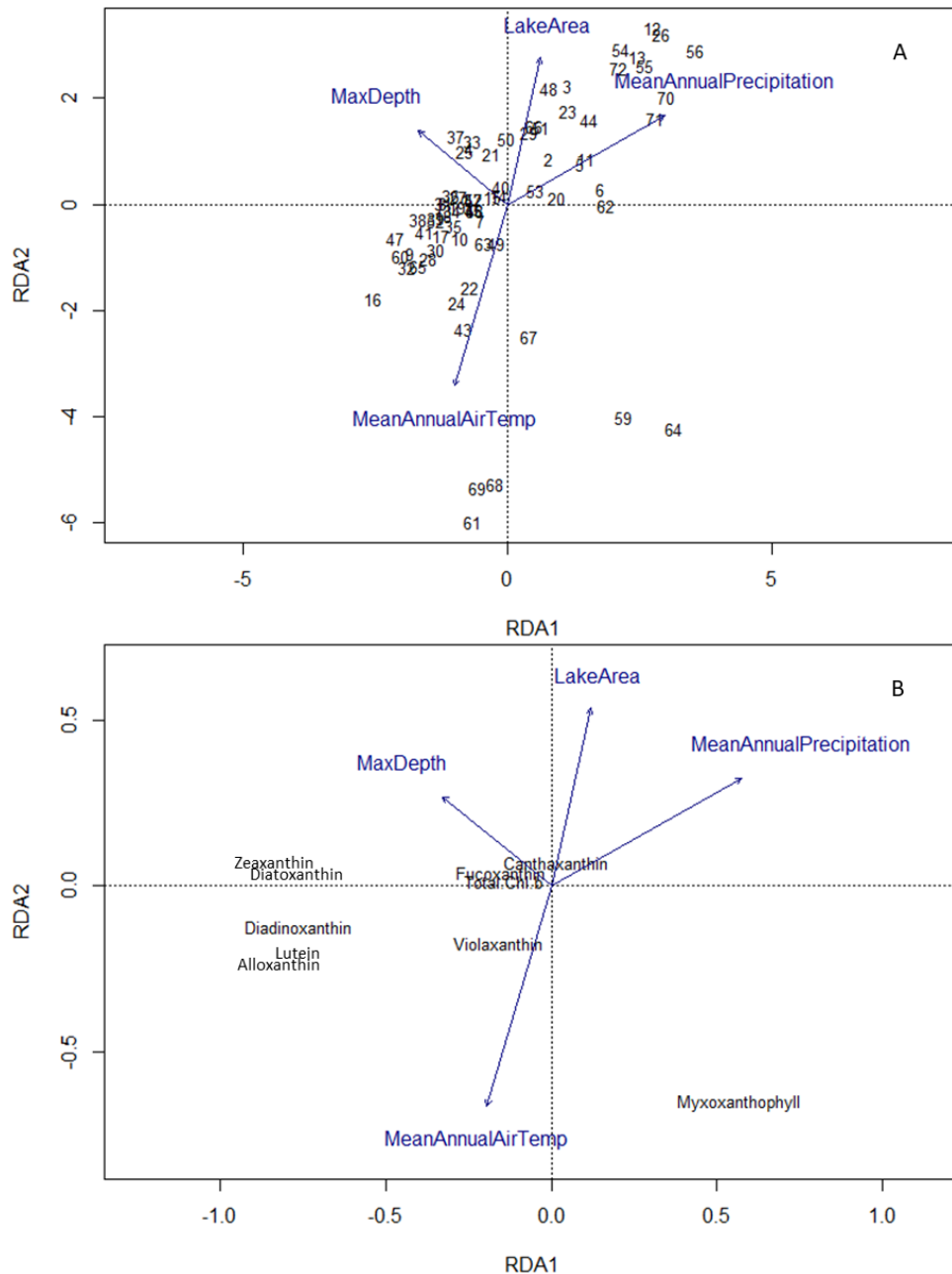


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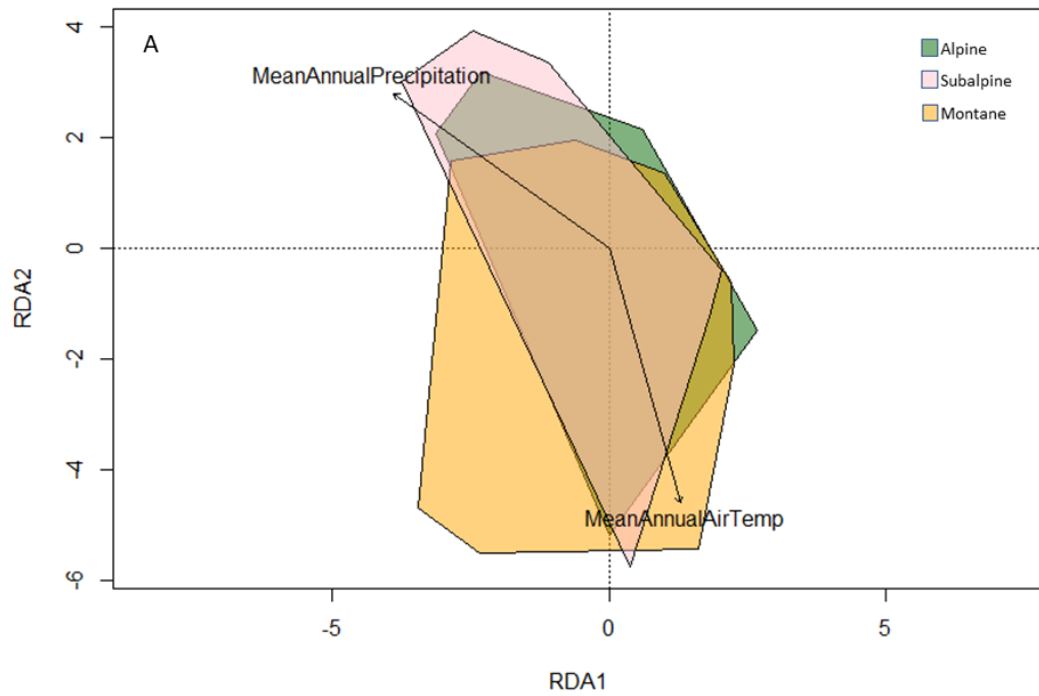
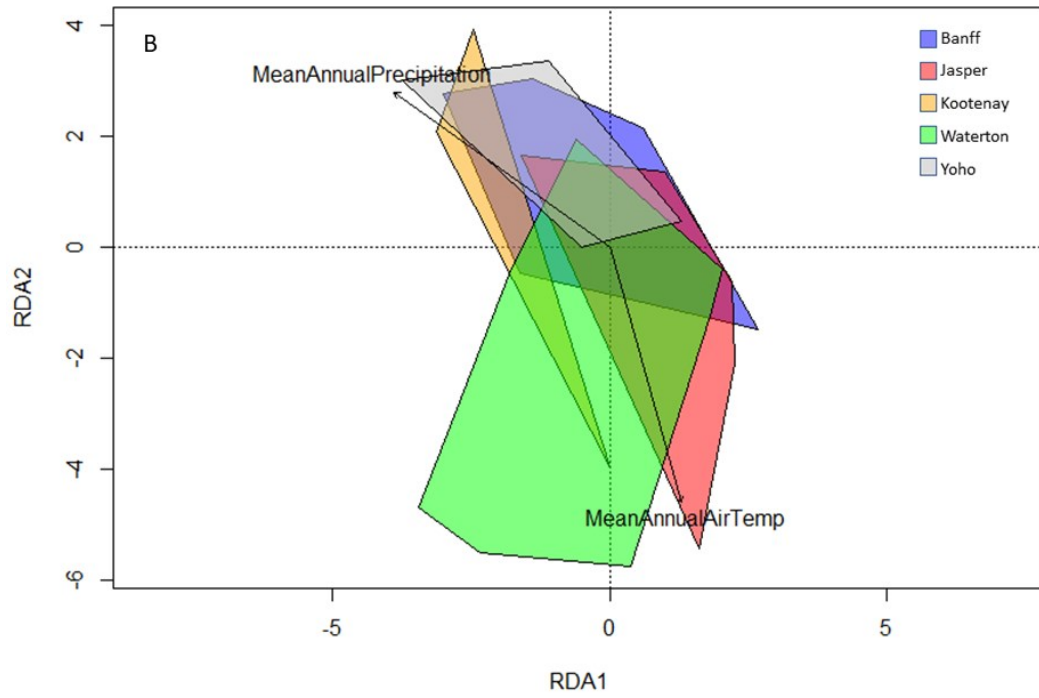
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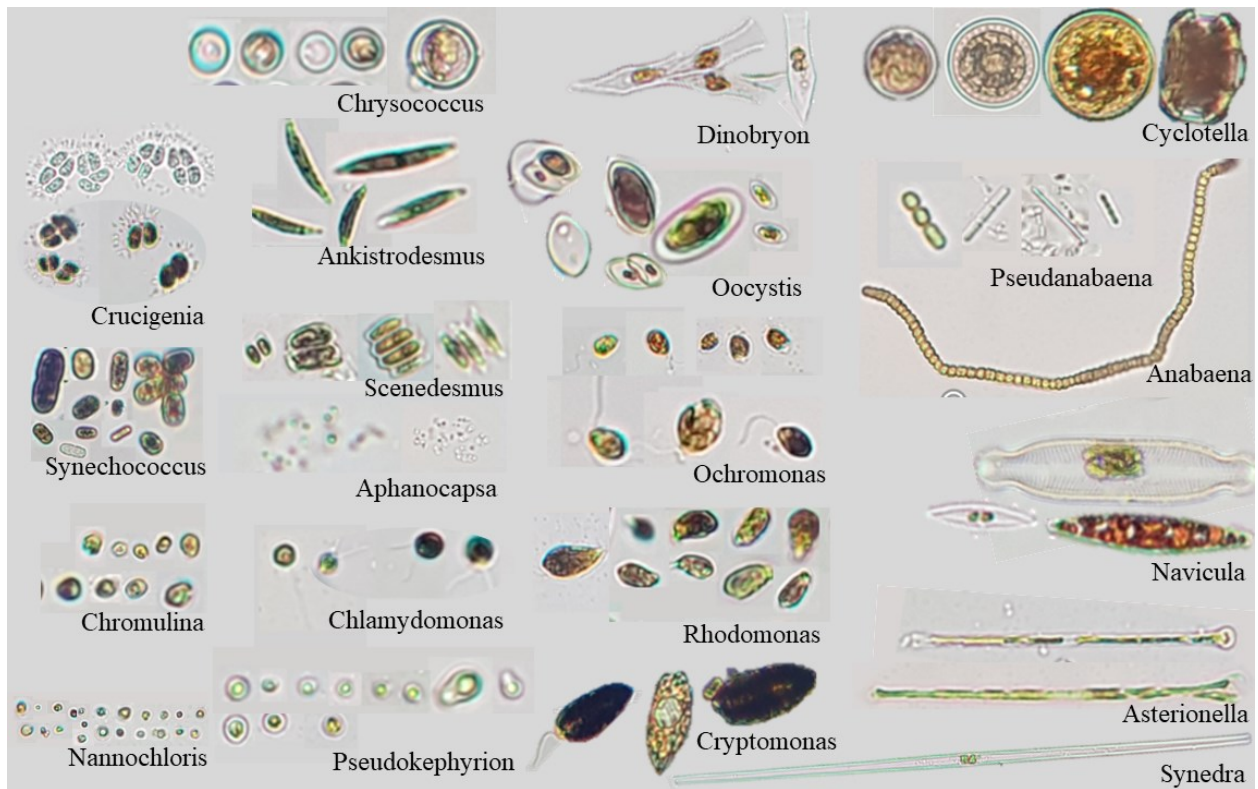


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using forward selected ( $P < 0.05$ ). Number key for the lakes in Table 2.1, phytoplankton pigment taxonomic association in table 2.3.



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**Figure 2.7** Major genera identified in the survey as examples of phytoplankton diversity across lakes in the Canadian Rocky Mountains. Images not to scale.

# **Chapter 3 Interactive and exposure order effects of simulated drought and heatwave events on alpine lake communities**

## **Introduction**

A mounting challenge in ecology is developing a better understanding of how interactions between novel or extreme environmental perturbations influence cumulative ecological impacts (Jackson et al. 2016; Orr et al. 2020; Jackson et al., 2021). Interactions between such stressors are at the core of frequently unexpected net impacts termed “ecological surprises” (sensu Paine et al. 1998). Further, order of exposure to consecutive rather than simultaneous stressors has long been expected to confound predictions of their combined ecological effects (Breitburg et al. 1998). There is some empirical evidence of exposure order effects, where reversal of sequence of stressors alters their cumulative impact (Brooks and Crowe 2019; MacLennan and Vinebrooke 2021), but such studies are rare. In particular, a knowledge gap exists concerning the potential for order of exposure to climatic stressors (e.g., drought events, summer heatwaves) to affect their interactive effects on ecological communities.

With climate change, droughts and heatwaves are increasingly likely to be stressors of freshwater ecosystems (Easterling et al. 2000; O’Reilly et al. 2015), especially at higher elevations (Calanca 2007). Alpine environments are experiencing faster warming than lower elevations (Wang et al. 2014; Bradley et al. 2004). Small lakes and ponds are shrinking under warmer and drier conditions (Magnuson 1997; Riordan et al. 2015; Smol and Douglas 2007) due to the compounding effects of unprecedented heat waves, loss of snowpack and glaciers, and inconsistent precipitation (ICPP 2022). Recent research has demonstrated how different parts of

mountain lake communities respond to various environmental and climate components, but there remains a lack of evidence of thermal and desiccation tolerance or vulnerability in these cold-adapted taxa. Composition and abundance of benthic and planktonic algae in Canadian Mountain lakes and ponds can be predicted by environmental and chemical variables such as elevation, conductivity, and DOC (Vinebrooke and Leavitt 1999). Catchment features, temperature, and precipitation are predictors for assemblage structure of zooplankton in Canadian mountain lakes (Loewen et al. 2019). Warming of communities from alpine sites in the Canadian Rockies can result in significant changes in zooplankton biomass throughout the summer (Johnsen et al. 2019). The focus of my study was to build on previous research and assess effects of interactions and exposure order of warming and drought on several community components in fishless alpine ponds.

Drought can impact aquatic communities by altering nutrient composition, shortening the growing season, and changing water clarity (Schindler et al. 1996; Oleksy et al. 2020; Parker et al. 2008). Drought events can decrease inflow and increase evaporation causing nutrients to become concentrated in the water column; conversely, reduced run-off can decrease nutrients and dissolved organic carbon (DOC) entering waterbodies (Schindler et al. 1996; Oleksy et al. 2020; Parker et al. 2008). Reduced allochthonous DOC in North American lakes can cause a significant increase in penetration of UV radiation, especially in oligotrophic alpine ponds (Schindler et al. 1996). Severe drought can eliminate shallow ponds entirely, even ones with no recent history of drying up (Magnuson 1997; Riordan et al. 2015; Smol and Douglas 2007). While many planktonic and benthic species can form resting structures and survive drought, a shortened growing season, or several consecutive years of unfavourable condition, could deplete the egg and seed banks beyond their recovery potential (Brock et al. 2003), or significantly alter

community composition (Arnott et al. 2002; Findlay et al. 2001). Ponds that do not dry completely during a drought may become shallower and would more closely track changes in air temperature, additionally stressing the ecosystem (Kelly et al. 2018; Kraemer 2016).

Warming can result in a shift towards smaller faster reproducing species (Daufresne 2009), and cause changes to the physical and chemical properties in aquatic systems (Oleksy et al. 2020; Parker 2008). Warmer water and warming induced mixing may result in higher chlorophyll concentrations, especially in the epilimnion (Michelutti et al. 2016; Kraemer et al. 2016 : Kelly et al. 2018 ) due to heat increasing the metabolic and reproductive rates of algae (Kraemer 2016). This can cause a shift towards species that are adapted for warmer water, demonstrated in alpine pond mesocosms in Banff National Park (Strecker, et al 2003). Shallow aquatic systems often lack a temperature refuge in a hypolimnion, so species in the entire water column and sediment may be affected by air temperature (Schindler, et al. 1996). In fishless alpine ponds, water temperature increases of just a few degrees can decrease zooplankton biomass by causing a shift from large cladocerans to rotifers, and shift phytoplankton assemblages (Strecker et al. 2004). In a multiple stressor pond experiment, Shurin et al. (2012) observed that warming increased the total rate of whole-system production but led to top-heavy food webs. Warming affected the entire food web through indirect top-down and bottom-up effects. A decrease in benthic and pelagic producer biomass was observed. Warming additionally increased the effect of fish presence on phytoplankton but reduced the effect of eutrophication on periphyton. Shifts in phytoplankton assemblages following warming have included decreases in cryptophytes and dinophytes and some chlorophytes in shallow systems in the UK (Moss et al. 2003) and shifts towards phytoflagellates away from filamentous chlorophytes in North America (Strecker et al. 2004). Both Moss et al. (2003) and Shurin et al. (2012) found that warming had

weaker direct effects than predation or nutrient addition but did interact with these stressors significantly. While there have been some observations of individual drought and warming effects, the combined effects of drought and warming on freshwater communities have not been previously documented. Also, most experimental evidence of interactions between two stressors is generated using simultaneous stressors, leaving the interaction of sequential and alternate exposure order stressors another area of interest (Gunderson et al. 2016).

We conducted an in-vitro experiment using alpine pond mesocosms to test the following hypotheses:

- 1) Drought suppresses community biomass by eliminating aquatic habitat and desiccation-sensitive resting stages of species in sediments while warming stimulates tolerant primary producers and rotifers (Strecker et al. 2004; Brock et al. 2003; Daufresne et al. 2009; Kratina et al. 2012).
- 2) As the worst of two stressors, drought overrides the weaker effect of warming to result in a net antagonistic impact on community biomass (Folt et al. 1999; Jackson et al. 2016; Morris et al., 2022).
- 3) Reversal of exposure to drought and warming events alters their combined effect on the community as they differ in how they impact organisms (i.e. desiccation versus thermal stress; MacLennan & Vinebrooke 2020).

## **Methods**

### *Experimental design and setup*

In July 2021, we collected a total of 30 litres of sediments from Opabin, Hungabee, and Sentinel lakes within Banff and Yoho National Parks in Alberta and British Columbia. Ten litres

of surface sediments were collected along a ~1m-depth transect, parallel to the shoreline in each lake. All 30 litres of sediments were homogenized to create a composite biological inoculum consisting of live and resting stages of algae, zooplankton and benthic invertebrates. Depth-integrated algae and zooplankton samples were taken using conical nets attached to nylon towlines, apart from Hungabee lake, where an unfiltered surface grab of the phytoplankton was used due to shallow water. Zooplankton samples were preserved in 70% ethanol, and phytoplankton samples were preserved in Lugol's solution. We assessed light penetration, depth, and chemical components, using a Secchi disk, a depth sounder and surface water samples at the deepest point in the lake according to bathymetric maps. These water samples were analysed for eight water quality variables in the BASL lab at the University of Alberta (<http://www.biology.ualberta.ca/basl>) (Table 3.1). Sediments were kept in loosely tied bags in coolers at 4°C for 4 days prior to the experiment.

We established six treatment levels, each replicated five times for a total of 30 units (Figure 3.1). The experimental units were 8-litre aquaria, filled with 1 litre of sediment as biotic inoculum and six litres of commercial 'spring water' (<https://www.compliments.ca/en/products/spring-water-15-l/>) (Table 3.1). The experiment was divided into three phases: the establishment phase, the stress phase, and the sampling phase. The aquaria were randomly assigned to one of six groups. These groups were control (C), warming (W), drought (D), simultaneous warming-and-drought (SIM), drought-then-warming (D→W) and warming-then-drought (W→D) treatments. The W and D treatments were used to determine the individual effect of each stress on the final community compositions, and abundance or biomass. The SIM treatment was used to assess evidence of interaction of drought and warming treatments the community. The other two treatments, D→W and W→D, were used to assess evidence of exposure order effects.



All aquaria were allowed to settle and establish for one month at 14°C and 12h L/D cycles in an experimental growth chamber at the University of Alberta. Throughout the establishment and treatment phases (Figure 3.1), we topped-up aquaria with deionized water from a Milli-Q EQ7000 to account for evaporation as needed. To control for any placement effects in the growth chamber, aquaria were randomly rearranged weekly. After one month, each aquarium was randomly assigned to one of the six different treatments. The W treatment was increased to 22°C for 2 months using 50-Watt aquarium thermostatic heaters (Petland, Canada). Warming of 8°C above the control was based on current moderate prediction of short-term warming events caused by climate change (Magnuson et al. 1997; ICPP 2022). Drought for the D treatment was simulated by drawing the water down using a J-shaped siphon and filtering the removed water through a 63- $\mu$ m mesh. The solids were put back into the aquaria immediately. Drought aquaria were not topped up until after the stress phase. For the W→D treatment, we first increased the temperature to 22°C for 1 month, then removed the heaters and applied drought stress for the second month. For the D→W treatment we removed the water for one month, after which we refilled the aquaria with the filtered water and increased the temperature to 22°C for the second month. For the SIM treatment, we removed the water for two months and simultaneously warmed the dry aquaria to 22°C using 40 Watt CHEs (Ceramic Heat Emitters) and a thermostat temperature controller (Inkbird ITC-306T Pre-wired Electronic Heating Temperature Controller and Digital Timer Controller). CHEs were placed at a 45-degree angle 5 cm above the top of the aquaria, as per the manufacturer's instructions. After the 2-month stress-treatment phase (Figure 3.1), we removed all heaters and refilled all empty tanks. The removed and filtered water was added back in a slow stream along the wall of the aquaria to avoid

suspension of the sediment. When initiating the drought portion of the W→D treatment, we used a 180- $\mu\text{m}$  net to filter the siphoned water because mucilage in the tanks clogged the 63- $\mu\text{m}$  net.

### *Sampling protocol*

Phytoplankton and rotifers were sampled weekly over a period of three weeks (Figure 3.1). On each sampling date, we filtered 750 mL of water from each aquarium through a 63- $\mu\text{m}$  mesh to capture zooplankton samples, which were preserved in Lugol's solution for taxonomic enumeration. Water was filtered through a GF/F Whatman filter paper and frozen for phytoplankton pigment analysis. On the final sampling day phytoplankton, rotifers, benthic macroinvertebrates and periphyton were sampled. Phytoplankton and rotifers were samples in the same manner as the previous sample days. Sediment from each aquarium was collected day, homogenized, and 1g was freeze-dried for periphytic algal pigment analysis. The remaining sediment was stored at 4°C for taxonomic enumeration of benthic macroinvertebrates. An additional 125 mL of water was collected from each aquarium and mixed to create a composite of replicates for each treatment. Water chemistry analysis was provided by the University of Alberta BASL lab (<http://www.biology.ualberta.ca/basl>)

### *Taxonomic and statistical analyses*

To identify taxonomically diagnostic algal pigments, High Pressure Liquid Chromatography (HPLC) was performed using the protocol of Vinebrooke and Leavitt (1999). Filter papers containing the concentrated phytoplankton from each of the sampled aquaria were frozen and the pigment was extracted using a standard 80:20 solution of acetone and methanol over 24 hours. The samples were filtered again through a 0.7- $\mu\text{m}$  Whatman GF/F filter and dried completely using  $\text{N}_2$  gas (Jeffrey et al. 2005; Vinebrooke and Leavitt 1999). HPLC was

performed in an Agilent 1100 Series HPLC. Taxonomically diagnostic pigments were identified from each sample using Agilent ChemStation software. Taxonomic enumeration of invertebrates was performed using microscopy. Zooplankton community composition consisting of rotifers (Figure 3.2) was determined using a compound light microscope. Identifications were made down to the species level referencing Haney et al. (2013). Benthic macroinvertebrates were counted by sequential filtering of the sediments and identified using a stereo dissecting scope as either chironomids or oligochaetes, referencing Clifford (1991).

Chlorophyll *a* concentrations were used to quantify treatment effects on algal assemblages while total abundance was used to quantify responses by rotifer and benthic invertebrate assemblages. All pigment and abundance data were log-transformed prior to statistical analysis. The effect of each treatment was tested for significance using a nonparametric Kruskal-Wallis ANOVA. If the ANOVA showed a significant difference between the treatment groups at a 95% confidence interval ( $P < 0.05$ ), we then used a pairwise Wilcoxon rank sum test to determine which groups were significantly different from each other. Once we determined which treatments had a significant effect on abundance or biomass, we calculated the magnitude and direction of this effect as a relative effect size. Rotifers were identified and counted as a single composite per treatment resulting in a simple response ratio instead of an effect size.

The relative effect size was calculated as:

$$S_t = (\mu_t - \mu_c) / \mu_c$$

Where  $\mu_t$  is the mean biomass or abundance of a treatment group, and  $\mu_c$  is the mean biomass or abundance of the control group.

Based on the additive null model, the sum of the individual effects of drought and warming were compared to the effects of the combined simultaneous (SIM) treatment. Hypotheses of interactions between drought and warming were supported if the sum of the effect sizes of D and W were not equal to the effect size of the SIM treatment on average across trophic groups. If there was an interaction, we then calculated the predicted effect of the SIM treatment using additive, dominance, and multiplicative null models to see which best predicted the actual effect of the SIM treatment. The null model equations used were modified from Morris et al. (2022).

Additive Null Model equation:

$$S_W + S_D$$

Dominance Null Model equation:

$$S_W \text{ if } S_W > S_D$$

$$S_D \text{ if } S_D > S_W$$

Multiplicative Null Model equation:

$$S_W S_D$$

$S_W$  was the relative effect size of the warming treatment and  $S_D$  was the relative effect size of the drought treatment. The null model equation that best approximated the actual

combined effect size  $S_{SIM}$  determined the type of interaction between warming and drought stress. The additive null model was calculated with the absolute values first, if the null model that best fit the actual effect was the additive model, we then used direction to determine if the effect was antagonistic or synergistic.

Similarly, to test for evidence of order-of-exposure effects, we used a Kruskal-Wallis ANOVA, with pairwise Wilcoxon rank sum tests in R studio using base R (RStudio Team 2020), to determine the significance of any order of exposure effects. If there was a significant difference found between the  $W \rightarrow D$  and  $D \rightarrow W$  treatments, then altering the order of exposure did have an effect as predicted. Magnitude and direction of the effect sizes of the  $W \rightarrow D$  and  $D \rightarrow W$  treatments on each trophic group were compared to determine the direction of this effect.

To visualize treatment effects on community composition, ordination analyses were performed on the algal pigment and taxonomic data. Ordinations were done in R studio (RStudio Team 2020). Using the package *ggplot2* (Wickham 2016) centroids were calculated and drawn as assemblage polygons for each treatment within each taxonomic group using the replicates from the final sample day. The rotifer assemblage polygons were drawn using three different sample dates as pseudo-replicates to visualize ecological distance between treatments.

## **Results**

### *Responses of primary producers*

Drought treatment significantly suppressed chlorophyll-inferred phytoplankton biomass, which was not affected by warming (Fig. 3.3; Table 3.2). The net effect of simultaneous exposure to both stressors on phytoplankton biomass did not differ significantly from the control, denoting community response that best matched the prediction of the multiplicative null model (Table

3.3). Differences in order of exposure to drought and warming also did not significantly alter phytoplankton biomass (Fig. 3.3; Table 3.4). However, drought and warming did affect phytoplankton assemblage as assessed by pigments, as there was minimal overlap with the control group in ordination space (Fig. 3.4). Treatment groups were separated from the control based on a general shift away from fucoxanthin to higher concentrations of canthaxanthin, chlorophyll *b* and neoxanthin.

Drought also had a significant negative effect on periphyton biomass while it was unaffected by warming (Fig. 3.5; Table 3.2). The negative impact of simultaneous exposure to drought and warming did not differ significantly from that of drought only, indicating an antagonistic response as predicted by the dominance null model (Fig. 3.5; Table 3.3). An exposure-order effect by drought and warming was detected as final biomass was significantly lower in the D→W than in the W→D treatment groups (Fig. 3.5; Table 3.4). The periphyton assemblages also differed between the two treatments with the W→D and D→W polygons being distinct in ordination space (Figure 3.6). The aquaria were also visually distinct, as the sediments appeared more scoured and less greenish in the D→W than in the W→D treatment (Figure 3.7). Treatment groups involving drought were taxonomically separated from controls, which contained higher concentrations of diatoxanthin and fucoxanthin (Fig. 3.6).

### *Responses of consumers*

Drought exerted its strongest negative effect on the abundance of benthic macroinvertebrates, which was not significantly affected by warming (Figure 3.8). Effects of drought and drought combined with warming did not differ significantly, indicating an antagonistic interaction in which drought was dominant (Figure 3.8; Table 3.2). Effect sizes of

the two sequences of drought and warming also did not differ significantly (i.e. no exposure order effect; Table 3.5). Drought altered the taxonomic composition of the benthic macroinvertebrate assemblages by suppressing chironomids, which otherwise defined the control and warmed assemblages (Table 3.6).

Rotifers were less abundant in most treatment groups relative to that in the control, except for in the D→W mesocosms (Figure 3.9; Table 3.2). Similarly, taxonomic composition of the rotifer assemblages consistently differed between the controls and the manipulated mesocosms (Figure 3.10). However, statistical analysis of the rotifer data was not possible (Tables 3.2 and 3.4) given the need to assemble composite samples by combining replicates to achieve adequate rotifer counts. Rotifer assemblages appeared to group by treatment. The rotifer genera *Monostyla*, *Trichocerca*, and *Kellicottia*, were associated with the control and warmed treatments, while the genera *Keratella*, *Euchlanis*, and *Lepadella*, were more indicative of treatments involving drought (e.g., D, DW, WD, and SIM; figure 3.11).

## **Discussion**

In this novel in-vitro study of simulated climate-related effects in fishless mountain pond communities, the results revealed the overriding impact of drought relative to that of higher temperatures. Drought directly suppressed all taxonomic groups and altered their taxonomic composition. The dominance null model in most cases best captured the net effect of drought and warming when they were applied simultaneously, further highlighting the key role of the worst single stressor, namely drought. The one exception to these results involved the phytoplankton. Reversal of the drought and warming treatments altered their net effect only for periphyton,

further demonstrating that stressor asymmetry rather than timing determines their cumulative impact on the pond community. Collectively, these findings suggest that drought events will be the key drivers of the future community structures of shallow alpine lake and pond ecosystems.

#### *Direct drought and warming effects*

The drought treatment exerted the largest negative effects on the benthic assemblages in the mesocosms. Benthic invertebrates were the most impacted as they were completely extirpated following the drought event. Here, lack of drought resistance likely reflected the inability of chironomids to tolerate desiccation and. Similarly, reproductive output could not have compensated for high mortality following the drought event because these invertebrates would have had to mature into sexually reproductive aerial adults. Drought also suppressed periphyton and consistently favoured cyanobacteria over diatoms. Cyanobacteria are considered more drought-tolerant than other freshwater algae because of their protective mucilage and ability to form thick filamentous mats on the sediment can prevent desiccation (Lin and Wu 2014). Barthès et al. (2015) observed benthic algae and bacterial communities on biofilms after various levels of drought stress and found that the communities would recover to the original state only from the weakest drought stress. In all other cases the community's function was significantly altered and the algae had significant assemblage shifts. We also observed assemblage shifts after the drought stress. While certain algae can form resting structures, others cannot, nor do they necessarily remain viable over an extended period (Brock et al. 2003).

Ordination analyses of three sample dates showed that there was effect of drought on the rotifer assemblage, although the lack of replicates precluding statistical testing. Drought appeared to suppress *Monostyla* spp. and *Cephalodella* spp. while favouring *Keratella* spp.,



*Euchlanis* and *Lepadella*. Although crustacean zooplankton are well known to emerge from sedimentary resting egg banks and populate lake communities (Sarnelle and Knapp 2004), information regarding the rotifers is lacking. In general, armoured rotifers (e.g., *Keratella* spp.) appeared to be more tolerant of drought than are soft-bodied species (e.g., *Cephalodella* spp.) during the experiment possibly due to a higher potential for retaining water and avoiding desiccation.

Warming did not exert the expected positive effects on algal growth. The warming treatment had the most variable effect sizes during the experiment, which may account of the lack of significance. Moderate warming typically stimulates algal growth while suppressing grazers (e.g., Petchey et al. 1999), resulting in a shift towards smaller algal species (Daufresne et al. 2009) and cyanobacteria (Kratina et al. 2012). Strecker et al. (2004) also reported that moderate warming stimulated phytoplankton growth in while reducing the abundance of grazing cladocerans and copepods in experimental alpine ponds in Canada. These crustacean zooplankton were scarce in our experiment, and therefore, release from grazing pressure in the warmed treatments, likely did not occur even though the abundance of micro-filter feeding rotifers appeared to be negatively affected. A potential alternate explanation for the lack of a positive effect of warming on phytoplankton growth is that our treatment was more extreme (8 versus ~3 degrees Celsius), possibly inducing more thermal stress than established in other similar studies (e.g., Strecker et al., 2004; Thompson et al. 2018). Elsewhere, Kratina et al. (2012) showed that warming increased grazing pressure and reduced the positive effect of nutrient additions, resulting in a cumulative negative effect on primary producer biomass in freshwater mesocosms in Vancouver, BC. This is attributed to increased metabolic demands of

predators. Here, shifts in consumer assemblages in the warming treatment, that may have similarly resulted in suppression of primary producers, masking a positive effect of warming.

### *Combined effects of drought and warming*

The dominance null model best predicted the nature of interactions between the warming and drought treatments on most communities (except phytoplankton) during the experiment. The dominance null model was likely the most appropriate to use in our experiment given the degree of asymmetry between the magnitudes of the effects sizes of the two stressors. In general, drought was the worst simulated individual stressor as its effect size greatly exceeded that of the warming treatment (Table 3.2). Here, drought can be anticipated to have a greater effect than warming because it can completely remove aquatic habitats while higher temperatures only modify them. For phytoplankton, it is unclear why drought did not have a stronger effect on chlorophyll *a* inferred biomass but this may be due to high variability among replicates. Nevertheless, our hypotheses of non-additive effects of drought and warming were partially supported by the responses by the various measured communities. In particular, our identification of the appropriateness of the dominance null model for our study parallels the meta-analytic finding of antagonistic interactions between stressors of other freshwater ecosystems (Jackson et al., 2016; Morris et al., 2022). Our findings also reflected those of earlier findings where experimental warming did not exert direct significant effects, but instead interacted with other stressors to generate indirect non-additive effects on freshwater communities (e.g., Moss et al., 2004; Shurin et al., 2012).

### *Order of exposure*

Altering the order of exposure to warming and drought only affected their net effect on the periphyton. Here, a possible explanation for this exposure-order effect involves how each stressor uniquely affected periphyton growth and its taxonomic composition. As the first stressor, warming appeared to enable sufficient time for biomass accrual by cyanobacteria and diatoms before periphyton was exposed to the stronger drought effect. In contrast, drought as the first stressor appeared to scour surface sediments (Figure 3.7) so as to suppress algal biomass accrual and favour chlorophytes and euglenoids once later exposed to warming. MacLennan and Vinebrooke (2021) proposed that such contrasting species responses to two stressors (i.e. negative co-tolerance) could increase the importance of order of exposure on their cumulative impact. In comparison, a potential explanation for the lack of exposure-order effects on the other taxonomic groups involves how stressors that do not affect species in distinctly different ways are redundant and therefore interchangeable (MacLennan & Vinebrooke 2021). However, this was likely not the case in our experiment. Instead, the overriding dominance of the worst stressor, namely drought, likely drove the cumulative impact of drought and warming regardless of when the subordinate stressor was applied.

An additional observation was made when comparing the SIM treatment to the D→W and W→D treatments across all taxonomic groups (Table 3.3, Table 3.4). An unexpected result was the difference between these treatment responses. When we compare the effect of either of the order of exposure treatments to the effect expected by the null models, treating each of the order of exposure treatments as if they were a combined effect treatment, we see that the actual effect is best predicted by the multiplicative model in most cases, not the dominance model. This could indicate that the interaction has a temporal component, as the D→W, and W→D treatment

only had one month of drought stress each, and the SIM treatment had 2 months of drought stress. It is possible that drought only becomes a dominant stressor after that time frame.

However, we would need further experiments to determine if this is the case.

### *Conclusions*

The key ecological finding of this research was that after a 2-month period, drought alone or in combination with warming has the potential to be catastrophic for some taxonomic groups. It appears that the 2-month simultaneous treatment effect is best predicted by the dominance null model, and sequential 1 month stress effects are best predicted by a multiplicative null model. From this study it appears that the order of exposure effects were only important for the periphytic assemblages, which could offer future insights to the mechanism of the stressor interactions. Innovative insights from this study are that the length of the drought and warming stress and whether they are simultaneous or sequential affect the interaction between these two stressors. This experiment opens many opportunities for future research. Observed differences in interaction between drought and warming in the simultaneous versus the sequential treatments, shows potential for a threshold where the interaction may change from multiplicative to dominant. A temporal experiment with different lengths of drought would help further understanding in this area. An in-vivo or survey-based experiment could help demonstrate how these results reflect a larger study system in a more realistic setting. This experiment is a good starting point for sites with longer food webs, which may include top predators like fish. Additional larger experiments may highlight more complex factors. DuBose et al. (2019), found that drought killed off mussel communities in a mesocosm experiment and the resulting pulse of nutrients caused by decomposition had cascading effects. Similarly, drought responses may vary

in more complex systems, so understanding the mechanism and interaction in simpler systems will help predict more complex outcomes.

The anticipated significance of our findings pertain to highlighting how increasingly frequent and severe drought events will drive the future structure of alpine pond ecosystems under a warming climate. Riordan et al. (2006) already documented changes to the sizes of ponds in Alaska due to several factors, including higher temperatures, variation in precipitation and duration of drought events, and increased drainage as permafrost melts. Elsewhere, warming and drought have also affected the habitat size of European alpine ponds (Calanca 2007). Since these small ecosystems at high elevations are regarded as sentinels of the ecological impacts of climate change (Wang et al. 2014; Williamson et al. 2008), my findings emphasize the need to better understand their resurrection ecology and the role of resting stages in sediments to predict the future resilience of these ecosystems following increasingly frequent and intense drought events.

**Table 3.1** Water chemistry from the three alpine ponds that acted as sedimentary inoculum for the in-vitro experiment. PP is particulate phosphorus ( $\mu\text{g}/\text{mg}$  as P), TP is total phosphorus ( $\mu\text{g}/\text{mg}$  as P), TN is total nitrogen ( $\mu\text{g}/\text{mg}$  as N), conductivity is measured in  $\mu\text{S}/\text{cm}$ , turbidity is measured in NTU, TOC is total organic carbon ( $\text{mg}/\text{L}$ ), and TIC is total inorganic carbon ( $\text{mg}/\text{L}$ ).

Sample	PP	TP	TN	pH	Conductivity	Turbidity	TOC	TIC
Opabin	2.46	29	55	8.14	71.6	0.30	0.4	8.3
Hungabee	3.61	23	45	7.91	16.9	0.19	1.8	0.6
Sentinel	2.80	26	102	7.28	140	0.27	0.9	15.8
Commercial Spring Water	1.48	27	1010	7.58	557	0.08	2.3	37.8
Reportable Detection Limit	1	1	11	N/A	N/A	N/A	0.1	0.1

**Table 3.2** Relative effect sizes of the drought (D), warming (W), and simultaneous exposure to both (SIM) on major taxonomic groups at the end of the experiment various treatment levels. Calculated as  $(\mu_t - \mu_c) / (\mu_c)$  on different levels of the community. Bolded effect sizes were considered significantly different from the control by a Wilcoxon rank sum test (\* significant at 0.05, \*\* significant at 0.02).

Treatment	Relative effect size $(\mu_t - \mu_c) / (\mu_c)$		
	W	D	SIM
Phytoplankton	-0.17	-0.91 *	-0.24
Periphyton	-0.27	<b>-0.40 **</b>	<b>-0.49 **</b>
Rotifer (as composites)	-0.74	-0.74	-0.91
Benthic invertebrates	0.51	<b>-1 **</b>	<b>-1 **</b>

**Table 3.3** Results as predicted by each null model. Comparing actual relative effect sizes to predicted relative effect sizes by each model; Additive, multiplicative, dominance. Formulae for each null model. Additive:  $|S_a + S_b|$ . Multiplicative:  $S_a S_b$ . Dominance:  $S_a$  if  $S_a > S_b$ ,  $S_b$  if  $S_a < S_b$ . Additive with direction:  $S_a + S_b$

Group	SIM	Add.	Multi.	Dom.	Add. with direction	Best Model
Phytoplankton	-0.24	1.08	0.1547	0.91	-1.08	Multi.
Periphyton	-0.49	0.67	0.108	0.4	-0.67	Dom.
Rotifers	-0.91	1.48	0.5476	0.74	-1.48	Dom.
Benthic invertebrates	-1	1.51	0.51	1	-0.49	Dom.

**Table 3.4** Relative effect sizes and pairwise Wilcoxon rank-sum test significance values comparing the mean biomass or abundance between order of exposure treatments across groups.

Group	Relative effect size ( $u_t - u_c$ )/( $u_c$ )		Pairwise Wilcoxon Rank-Sum test $P$
	D→W	W→D	
Phytoplankton	-0.33	-0.43	1
Periphyton	-0.42	-0.17	<b>0.02 **</b>
Rotifers (as composites)	4.66	-0.46	N/A
Benthic invertebrates	0.50	0.55	0.841

**Table 3.5** Water chemistry in each treatment from composites taken on the final sample day.

Sample	TP ( $\mu\text{g/L}$ )	NO <sub>2</sub> +NO <sub>3</sub> ( $\mu\text{g/L}$ )	NH <sub>4</sub> ( $\mu\text{g/L}$ )	pH	Conductivity ( $\mu\text{S/cm}$ )	DOC ( $\text{mg/L}$ )
Control	15	<2	<3	8.18	386	10.5
Warming	19	<2	12	8.66	302	10.4
Drought	46	2	6850	7.91	562	15.6
W→D	21	26	276	8.37	431	9.8
D→W	10	2	4	8.32	380	7.4
SIM	47	5	7850	7.87	601	21.8
Reportable Detection Limit	1	2	3	N/A	N/A	0.1

**Table 3.6** Mean abundances and standard deviations of benthic macroinvertebrate groups across treatments.

Treatment	Chironomidae	St. Dev.	Oligochaeta	St. Dev.
Control	13.8	3.97	78.6	21.4
Warming	14.8	3.49	125	45.2
Drought	0	0	0	0
W→D	3.2	2.92	72.6	35.3
D→W	0	0	72.2	20.4
SIM.	0	0	0	0

### List of Figures

**Figure 3.1.** Experimental set up showing the three phases of the experiment. The control temperature was 14°C, and the warming treatment temperature was 22°C. C is the control, W is warming treatment, D is drought treatment, DW is drought-then-warming, WD is warming-then-drought treatment, and SIM is simultaneous warming and drought.

**Figure 3.2.** Taxonomic diversity of rotifers in the mesocosm experiment.



**Figure 3.3.** Mean total phytoplankton chlorophyll *a* among treatments on the final day of the experiment (n=5). C is the control, W is warming treatment, D is drought treatment, DW is drought-then-warming, WD is warming-then-drought treatment, and SIM is simultaneous warming and drought. The control temperature was 14°C, and the warming treatment temperature was 22°C.

**Figure 3.4.** DCA plot showing the taxonomically diagnostic algal pigment of phytoplankton assemblages among treatments on the final day of the experiment. C is the control, W is warming treatment, D is drought treatment, DW is drought-then-warming, WD is warming-then-drought treatment, and SIM is simultaneous warming and drought. The control temperature was 14°C, and the warming treatment temperature was 22°C.

**Figure 3.5** Mean total periphyton chlorophyll *a* among treatments on the final day of the experiment (n=5). C is the control, W is warming treatment, D is drought treatment, DW is drought-then-warming, WD is warming-then-drought treatment, and SIM is simultaneous warming and drought. The control temperature was 14°C, and the warming treatment temperature was 22°C.

**Figure 3.6.** PCA plot showing the taxonomically diagnostic algal pigment composition of periphyton assemblages among treatments on the final day of the experiment. C is the control, W is warming treatment, D is drought treatment, DW is drought-then-warming, WD is warming-then-drought treatment, and SIM is simultaneous warming and drought. The control temperature was 14°C, and the warming treatment temperature was 22°C.

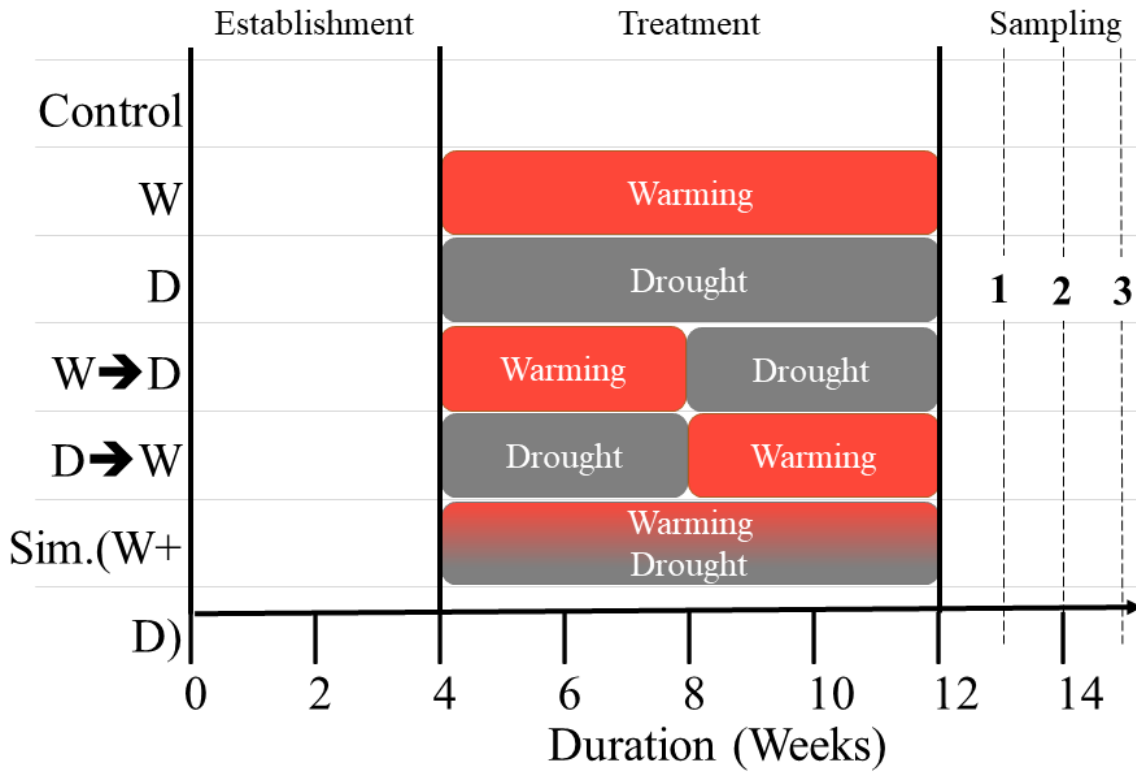
**Figure. 3.7.** Top-down photos of the surface sediments exposed to the various treatment levels for the duration of the experiment. The control temperature was 14°C, and the warming treatment temperature was 22°C.

**Figure 3.8.** Total benthic invertebrate abundance among treatments on the final day of the experiment (n=5). C is the control, W is warming treatment, D is drought treatment, DW is drought-then-warming, WD is warming-then-drought treatment, and SIM is simultaneous warming and drought. The control temperature was 14°C, and the warming treatment temperature was 22°C.

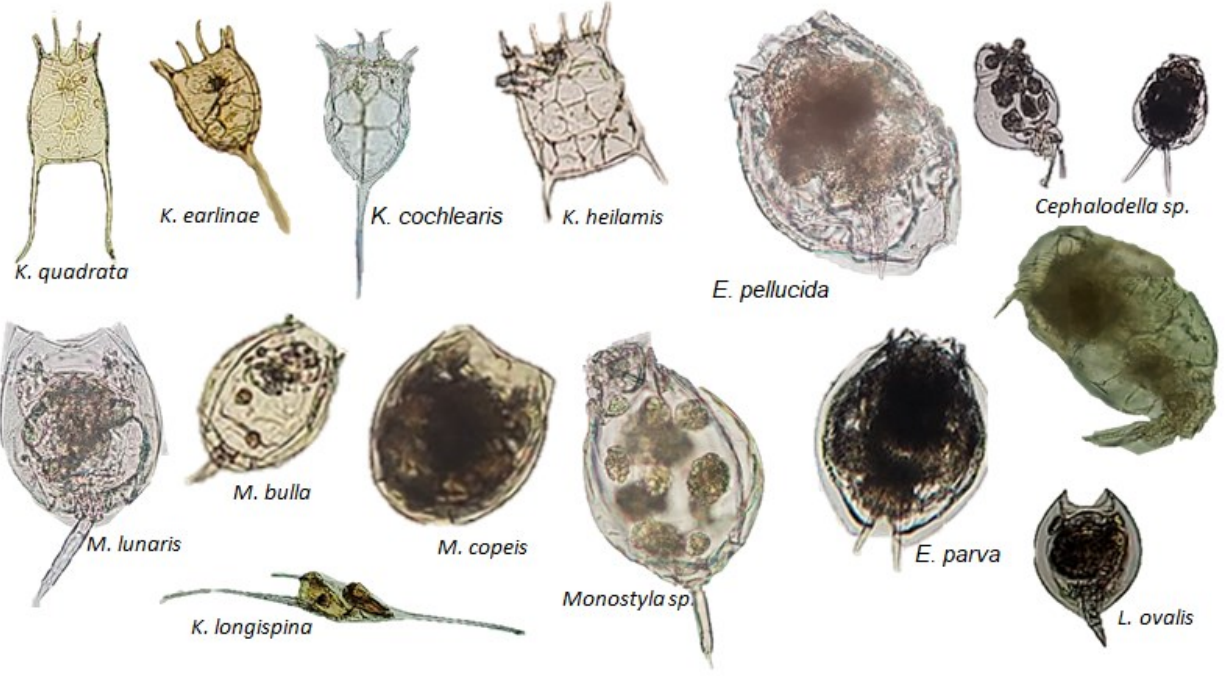
**Figure 3.9.** Total rotifer abundance among treatments on the final day of the experiment (n=1). C is the control, W is warming treatment, D is drought treatment, DW is drought-then-warming, WD is warming-then-drought treatment, and SIM is simultaneous warming and drought. The control temperature was 14°C, and the warming treatment temperature was 22°C.

**Figure 3.10.** DCA plot showing taxonomic composition of rotifer assemblages among treatments the three sample dates as pseudo-replicates in order to visualize an assemblage polygon. C is the control, W is warming treatment, D is drought treatment, DW is drought-then-warming, WD is warming-then-drought treatment, and SIM is simultaneous warming and drought. The control temperature was 14°C, and the warming treatment temperature was 22°C.

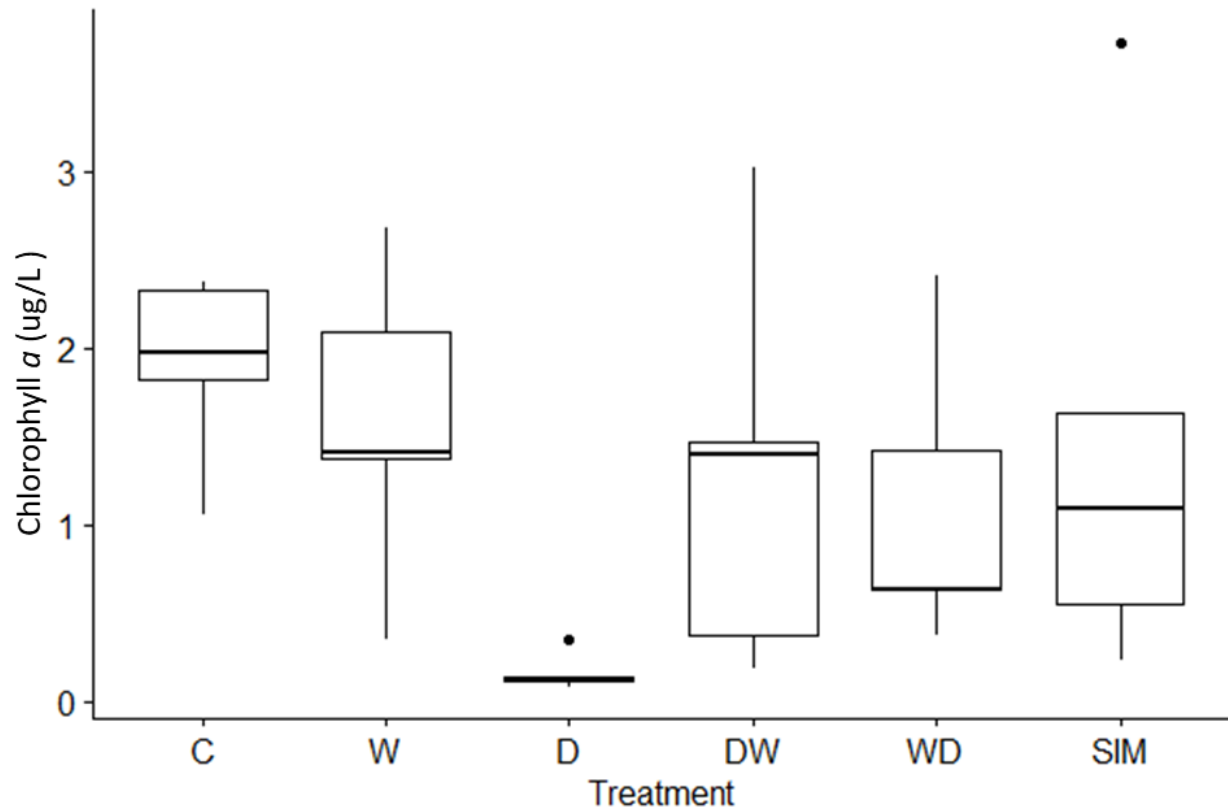
**Figure 3.11.** Drought and warming effects on alpine pond experimental setup. A) First day of the experiment, sediment remained suspended for approximately 48 hours. B) Mesocosms during the treatment phase. C) Simultaneous drought and warming (SIM) treatment using ceramic heat emitters (CHEs) with thermostatic control. D) Thick bacteria or algal mucilage in a Warming then Drought treatment mesocosm.



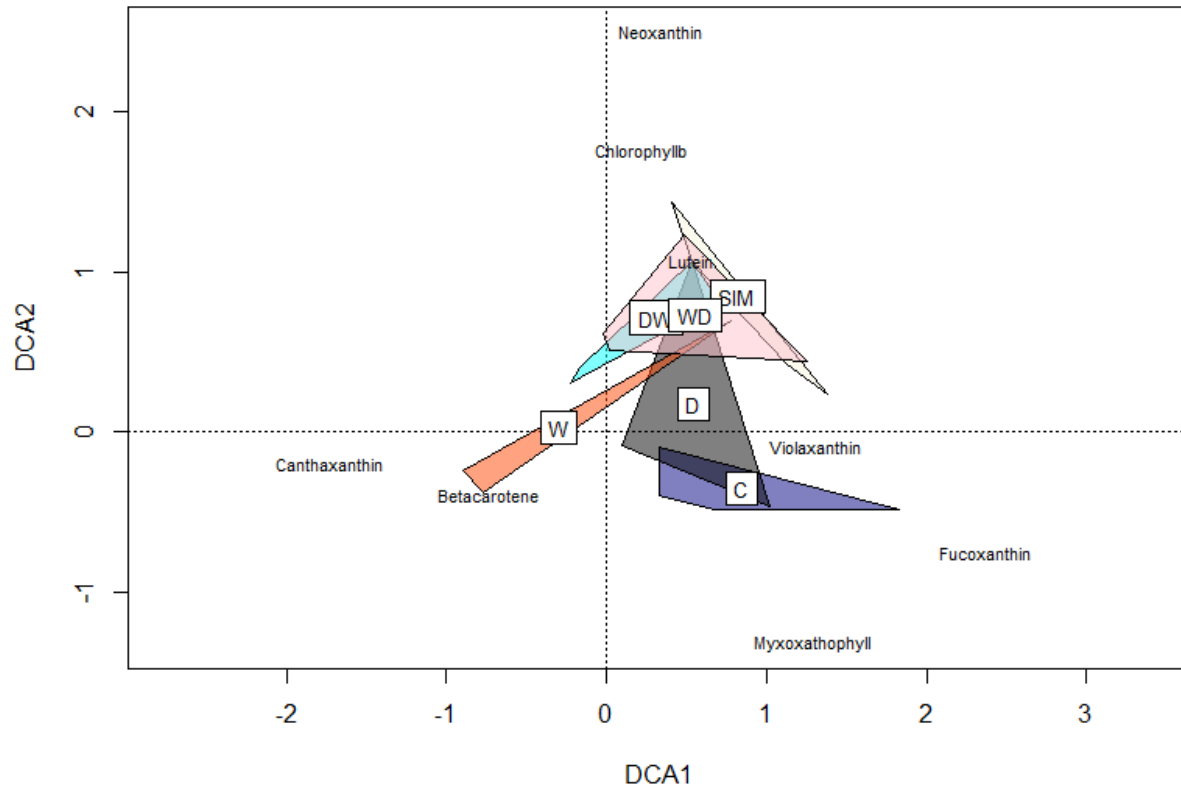
**Figure 3.1** Experimental set up showing the three phases of the experiment. The control temperature was 14°C, and the warming treatment temperature was 22°C. C is the control, W is warming treatment, D is drought treatment, DW is drought-then-warming, WD is warming-then-drought treatment, and SIM is simultaneous warming and drought.



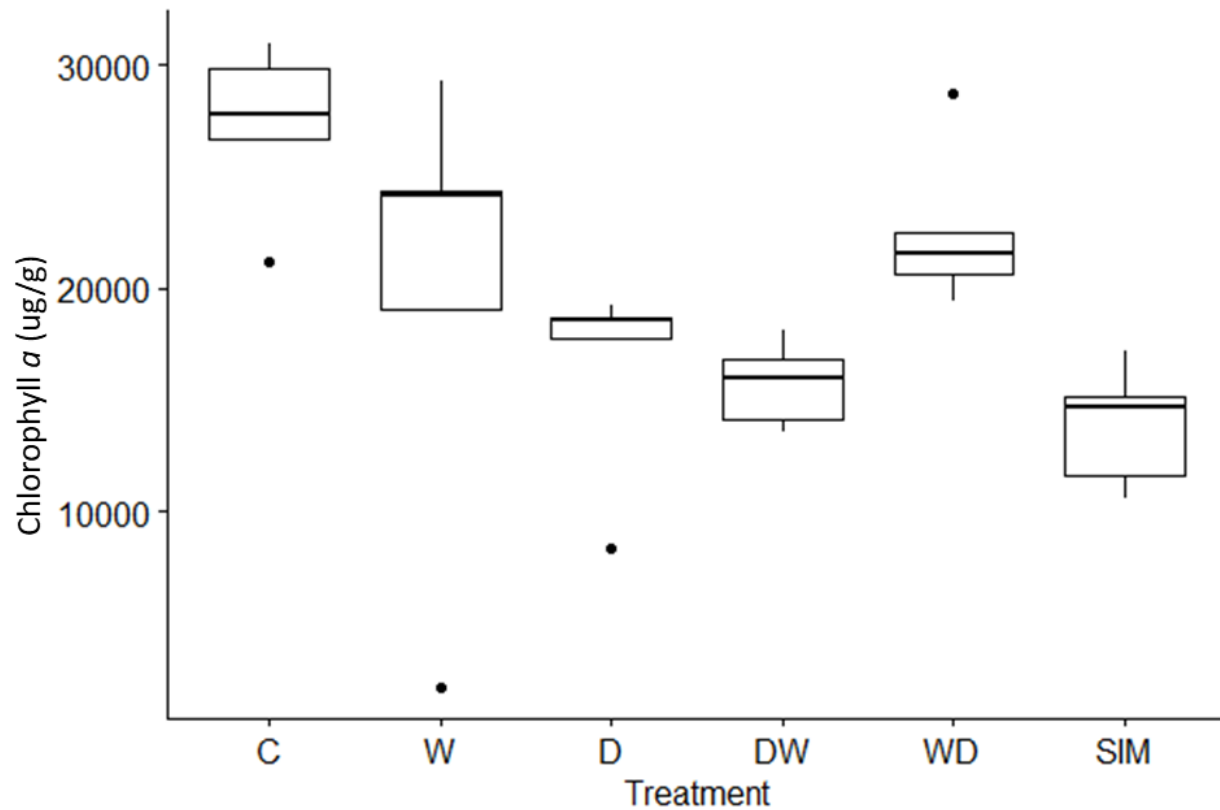
**Figure 3.2** Taxonomic diversity of rotifers in the mesocosm experiment.



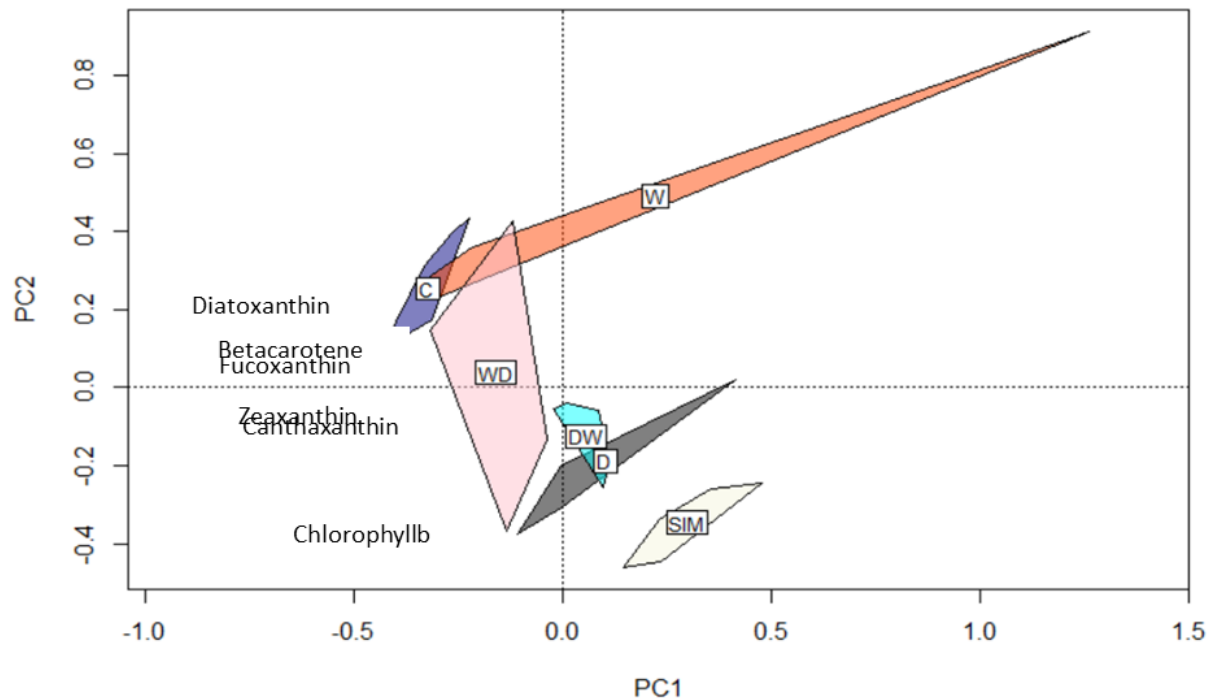
**Figure 3.3** Mean total phytoplankton chlorophyll *a* among treatments on the final day of the experiment (n=5). C is the control, W is warming treatment, D is drought treatment, DW is drought-then-warming, WD is warming-then-drought treatment, and SIM is simultaneous warming and drought. The control temperature was 14°C, and the warming treatment temperature was 22°C.



**Figure 3.4** DCA plot showing the taxonomically diagnostic algal pigment of phytoplankton assemblages among treatments on the final day of the experiment. C is the control, W is warming treatment, D is drought treatment, DW is drought-then-warming, WD is warming-then-drought treatment, and SIM is simultaneous warming and drought. The control temperature was 14°C, and the warming treatment temperature was 22°C.



**Figure 3.5** Mean total periphyton chlorophyll *a* among treatments on the final day of the experiment (n=5). C is the control, W is warming treatment, D is drought treatment, DW is drought-then-warming, WD is warming-then-drought treatment, and SIM is simultaneous warming and drought. The control temperature was 14°C, and the warming treatment temperature was 22°C.



**Figure 3.6** PCA plot showing the taxonomically diagnostic algal pigment composition of periphyton assemblages among treatments on the final day of the experiment. C is the control, W is warming treatment, D is drought treatment, DW is drought-then-warming, WD is warming-then-drought treatment, and SIM is simultaneous warming and drought. The control temperature was 14°C, and the warming treatment temperature was 22°C.



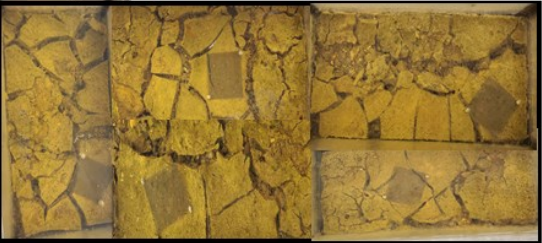
Control (C)



Drought then Warming (D→W)



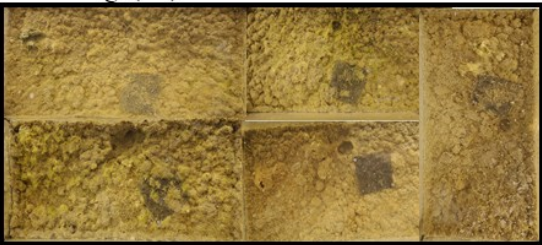
Drought (D)



Warming then Drought (W→D)



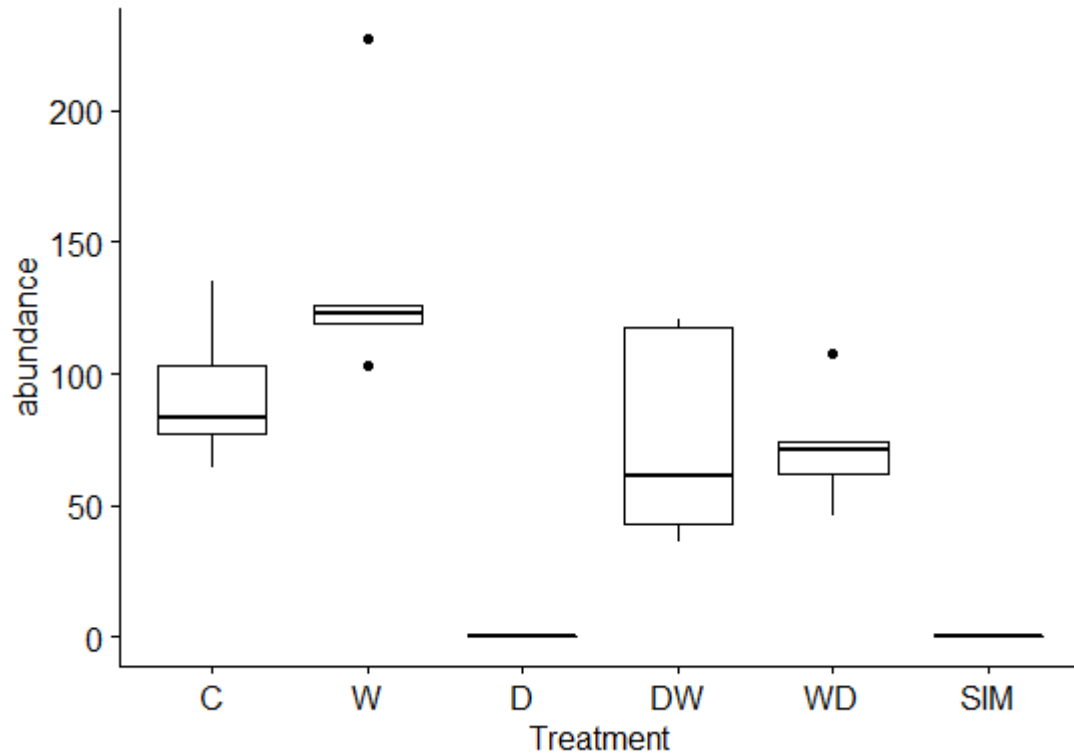
Warming (W)



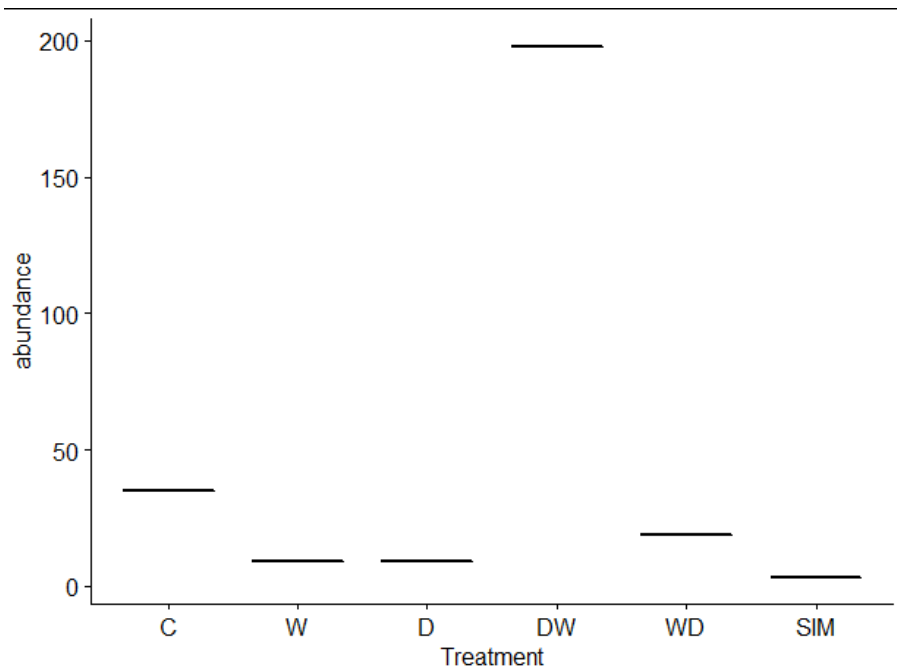
Simultaneous (SIM)



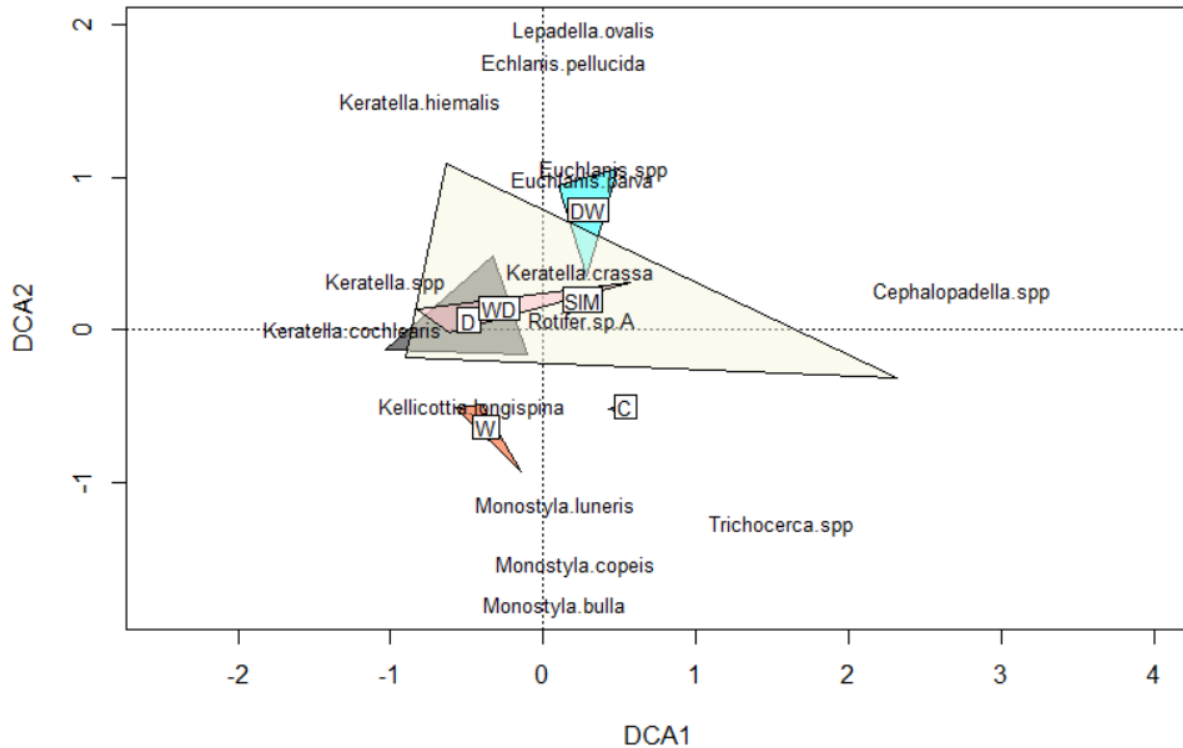
**Figure 3.7** Top-down photos of the surface sediments exposed to the various treatment levels for the duration of the experiment. The control temperature was 14°C, and the warming treatment temperature was 22°C.



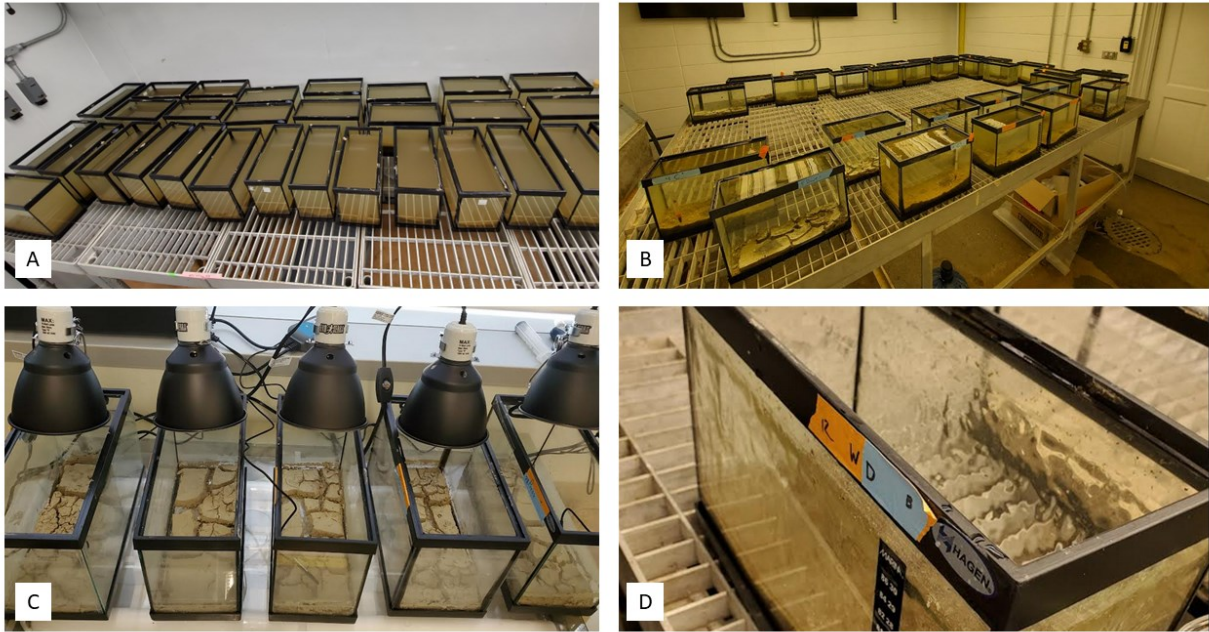
**Figure 3.8** Total benthic invertebrate abundance among treatments on the final day of the experiment (n=5). C is the control, W is warming treatment, D is drought treatment, DW is drought-then-warming, WD is warming-then-drought treatment, and SIM is simultaneous warming and drought. The control temperature was 14°C, and the warming treatment temperature was 22°C.



**Figure 3.9** Total rotifer abundance among treatments on the final day of the experiment (n=1). C is the control, W is warming treatment, D is drought treatment, DW is drought-then-warming, WD is warming-then-drought treatment, and SIM is simultaneous warming and drought. The control temperature was 14°C, and the warming treatment temperature was 22°C.



**Figure 3.10** DCA plot showing taxonomic composition of rotifer communities among treatments the three sample dates as pseudo-replicates in order to visualize an assemblage polygon. C is the control, W is warming treatment, D is drought treatment, DW is drought-then-warming, WD is warming-then-drought treatment, and SIM is simultaneous warming and drought. The control temperature was 14°C, and the warming treatment temperature was 22°C.



**Figure 3.11.** Drought and warming effects on alpine pond experimental setup. A) First day of the experiment, sediment remained suspended for approximately 48 hours. B) Mesocosms during the treatment phase. C) Simultaneous drought and warming (SIM) treatment using ceramic heat emitters (CHEs) with thermostatic control. D) Thick bacteria or algal mucilage in a Warming then Drought treatment mesocosm.

## Chapter 4 Conclusions

### Synthesis

Using a comparative experimental approach, I aimed to generate a fuller understanding of the current and future climate stressors affecting lentic systems in the Canadian Rocky Mountains, and how the communities in these systems may respond to this stress. Using environmental variables and phytoplankton data from 72 mountain lakes across five national parks, I determined that current phytoplankton biomass and species composition are related to environmental variables that predict high risk of warming and drought. By experimentally manipulating warming and drought stress in mesocosms based on mountain-pond sediments, I provided evidence for the negative and neutral effects of drought and warming respectively, a dominant and multiplicative interaction between drought and warming, and order of exposure effects in periphyton biomass and assemblage.

Phytoplankton biomass was negatively related to mean annual precipitation and lake surface area and positively related to maximum lake depth across my surveyed sites (Figure 2.2). Assemblages in these lakes was best predicted by mean annual precipitation and mean annual air temperature, but only when measured as taxonomically diagnostic pigment concentrations and not genus-level biovolumes (Figure 2.5). These environmental variables related to increased risk of drought and warming stress predicted current phytoplankton assemblages, which indicate that these lakes may have significant biomass and assemblage changes when faced with climate stress. I used previously collected data on mean annual precipitation, mean annual air temperature, lake surface area and maximum depth in 72 lakes across national mountain parks in Canada. Mean annual precipitation, lake depth, and lake surface area, were all identified as

significant predictors of phytoplankton biomass. Mean annual precipitation and air temperature significantly explained variance in taxonomically diagnostic algal pigment concentration but not the relative genus-level biovolumes. These findings highlight how phytoplankton in alpine ponds rather than those in larger lakes may be more sensitive early indicators of the ecological impacts of accelerating rates of climate change at higher elevations.

My in-vitro experiment showed no significant effect of an 8°C increase in temperature but a significant negative effect of drought on alpine pond mesocosms. Periphyton and benthic macroinvertebrates abundance and biomass were especially affected. Drought altered the community composition across most groups, as did simultaneous drought and warming. The interaction between drought and warming was best predicted by a dominance null model when the stressors were simultaneously applied for 2 months, and by a multiplicative null model when sequentially applied for 1 month each. Altering the order of exposure to warming and drought had a significant effect on periphyton biomass and assemblage. This offers insights into the vulnerability of these ecosystems to climate stress, as drought may be the determining stressor for the recovery potential of these ecosystems.

My findings in this thesis indicated that predicted climate warming and precipitation changes will affect mountain lake and pond communities. Phytoplankton and periphyton in alpine ponds may be sensitive early indicators of the ecological impacts of accelerating rates of climate change at higher elevations. Phytoplankton assemblages are strongly associated with lake size and mean annual air temperature and precipitation. Periphyton assemblages showed a significant response to alternate of exposure orders and all benthic assemblages were the most significantly affected by extended independent drought and simultaneous drought and warming. If the egg and seed bank are repeatedly depleted by unfavourable environmental conditions,

without the means to replenish, it could have drastic effects on the whole community function in the long term (Arnott et al. 2002). A shift in the phytoplankton assemblage or biomass would affect the entire community through bottom-up effects (Kratina et al. 2012). My finding in the importance of lake size, drought, and temperature on entire lake communities is supported by previous surveys and experiments (Oleksy et al. 2020; Parker et al. 2008).

### **Shortcomings**

My analysis of the lake survey included 72 lentic sites from montane to alpine in the Canadian Rocky Mountains. While this is a large data set, it does not mean that the relationships I found were universal. Watershed specific factors do influence phytoplankton assemblages and can affect the impact of other climate variables (Oleksy et al. 2020; Parker et al. 2008). The climate variables I chose to include as indicators of increased risk of warming and drought may not tell the whole story. Other variables that influence the relationship between climate and phytoplankton include glacier cover and latitude (Oleksy et al. 2020; Schneider 2010). This survey only included the phytoplankton assemblage and did not include zooplankton or fish which may have exerted top-down effects that masked or strengthened relationships between phytoplankton and climate variables. For example, Parker et al. (2008) observed that the reintroduction of native zooplankton in some lakes strengthened the negative relationship between climate stress and phytoplankton biomass. Additionally, chlorophyll *a* is not a perfect measure of phytoplankton biomass as chlorophyll per cell can vary significantly in response to environmental change (MacIntyre et al. 2002; Westberry et al. 2008; Krause 1988).

Mesocosm experiments are efficient in manipulating experimental variables across a variety of ecosystems, however all mesocosm experiments have fundamental shortcomings



(Schindler 2012). My experiment ran for four months, however the ice-free growing season in some mountain lakes is longer than that. It is possible that a longer recovery period before sampling would have identified different community responses to the stressors. The aquaria I used held 8 litres of water, and were not stratified, this represented warming in mountain ponds, but not deeper lakes, that may offer temperature refugia in a hypolimnion. The drought stress I used was very extreme, as I depleted the entire water column. While this is accurate for many ponds (Riordan 2006), it limits our understanding of what partial drought might do to the community. It was not feasible to use water from our source ponds in the mesocosms, store bought mineral water was a suitable replacement but did not exactly replicate the nutrients and turbidity in the source ponds. An additional shortcoming of my mesocosm experiment was having to rely on composites for the rotifer samples, thus lacking true replication and statistical power.

### **Future Research**

The mountain phytoplankton survey and mesocosm experiment both indicate a need for further investigation of community responses to climate variables. Precipitation regimes and mean air temperatures are predicted to continue to change in these mountain parks (Easterling 2000) and the community responses to these changes in the 2020's remains a knowledge gap. Another survey of these lakes could include the zooplankton and periphyton assemblages to get a broader picture of community dynamics in a changing climate. The 2017-2018 survey did not include small ponds that may be the most affected by drought. Including these previously unsampled ponds may change which environmental and lake variables have the strongest impact on biomass and community composition.

My mesocosm experiment showed a possible temporal threshold for the types of interaction between drought and warming and possible variation in the interaction when sequential one-month versus simultaneous two-month stressors were applied. A follow-up mesocosm experiment in which duration and timing of stressor exposure are manipulated would provide evidence of this finding. The warming treatment in the mesocosm experiment was 8 °C above the control. This warming was not enough to cause significant changes in composition or biomass. Increasing water temperature to the extreme end of predicted changes would be a natural follow-up experiment. To simulate drought stress, I drew down the entire water column and filtered it for particulates. An experiment in which there is only a partial drought may not decimate the benthic assemblages as we saw, but rather allow insight into the mechanism of drought response if an assemblage change was observed instead. Additionally, allowing natural evaporation of the water instead of filtering, would include the concentration of nutrients as a factor.

An in-vivo or survey-based experiment that looks specifically at lakes after warm, dry summers, may reflect the study system more realistically than a mesocosm experiment. For example, DuBose et al. (2019), found that the increase in nutrients from decomposing larger bodied species killed by drought had cascading effects on the community. Scaling up the mesocosm experiments or using whole lakes with top predators like fish may highlight more complex factors. Larger deeper lakes, or larger mesocosm may allow larger copepods and cladocerans to survive and reproduce, creating a more complete food web. My study opens up many opportunities for further exploration of drought and warming effects on lentic mountain systems and the communities they house. Understanding the mechanisms, interactions, and

responses involved in lake communities under climate stress will be key to predicting the future of these imperilled and important ecosystems.

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