A Functional Approach Reveals Zooplankton Responses to Environmental Change in Mountain Lakes

by

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A thesis submitted in partial fulfillment of the requirements for the degree of

Master of Science

in

Ecology

Department of Biological Sciences

University of Alberta

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Abstract

Concern is increasing over the future cumulative impacts of multiple stressors on freshwater biodiversity and ecosystem function, especially in alpine environments where climatic warming increases with elevation. Here, consideration of individual species traits enables translation of changes in biodiversity into functional responses by communities to environmental change. I integrated data for 170 mountain lakes and ponds spanning large latitudinal (2276 km) and elevational (1959 m) gradients across the mountains of Western Canada to assess how climatic and other environmental factors affect the taxonomic composition and functional structure of zooplankton communities. Unconstrained ordination and RLQ analyses revealed that certain functional groups consisting of relatively small-bodied, shoreline-associated (littoral) species were significantly associated with several climatically dependent environmental changes, namely higher water temperatures, shallower water depths, and lower ionic concentrations. My findings highlight how species turnover (beta-diversity) in shrinking alpine lakes will depend on limited dispersal from nearby ponds or lower montane elevations as environmental conditions become more variable in a warmer and drier climate.

Table of Contents

List of Tables and Figures

Tables

Figures

Acknowledgements

First and foremost, I would like to thank my supervisor, Dr. Rolf Vinebrooke, for supporting this process, and allowing me to explore my diverse interests within alpine limnology. His guidance and encouragement allowed me to pursue many new ideas and skills throughout my degree. I would also like to thank Dr. David Hik for his expertise in mountain ecology, and for willingly joining my committee late in the game. It was a pleasure and a privilege to work with you both. Thanks also to Dr. Erin Bayne and Dr. Richard Palmer for being on my defense committee.

I would like to thank my fearless field team- Mitchell Johnsen, Katie Keenan, Sarah Heemskerk and Lindsay Boucher- for their endless excitement in the mountains, and moral and technical support in the lab. Parks Canada staff, including Mark Taylor (Banff) and Barb Johnston (Waterton), helped with permits and facilitating access to helicopter and backcountry sites. I would also like to thank Charlie Loewen for being an integral part of my chapter 2 development, for compiling the immense data set used in analysis and for his sound statistical advice. Thanks to Dr. Shelley Arnott for her daphnia culturing advice, and for acting as a key mentor in my development as a limnologist.

Funding for this research was provided by a discovery grant from the Natural Sciences and Engineering Research Council of Canada (NSERC) awarded to R. D. Vinebrooke. This work was also supported by a Canadian Graduate Scholarship from NSERC, an Alberta Lake Management Society Scholarship, a Queen Elizabeth II Scholarship, and Graduate Teaching Assistant Scholarships awarded by the University of Alberta.

On a personal note, I would like to thank my family and friends back in Ontario for their boundless love and encouragement. Special thanks to my adventure accomplice, Milly, for her support through the dark Edmonton winters. Thanks to my 603 cohort for their friendship and for completing my graduate student experience. Finally, I would like to thank my partner, Blaine, for his unwavering love and support, even through many daphnia mass dying events.

Chapter 1: Alpine Limnology in the Context of Environmental Change

Introduction

Alpine lakes and ponds are considered to be prime sentinels of climate change as they integrate the effects of precipitation and temperature across their entire catchments in the relative absence of confounding local human disturbances (Hauer *et al.,* 1997, Battarbee *et al.,* 2009, Parker *et al.,* 2008, Williamson *et al*., 2009). These chemically dilute ecosystems are relatively species-poor and characterized by cold stenothermic organisms, making them highly sensitive to warming (Vinebrooke & Leavitt, 2005). Further, the expected impacts of climate change on the timing of biological events (i.e., phenology) are especially pronounced in alpine lakes given that the rate of warming generally increases with elevation (Pepin *et al.,* 2015). In addition, the retreat of glaciers has altered the state of glacially fed lake systems from highly turbid to clear systems dominated by non-glacial organic matter (Wolfe *et al.* 2013). However, the net impacts of climate change on these lakes are complicated by potential cross-scale interactions involving local factors (i.e., exotic sportfish, treeline position) mediating the more regional effects of meteorological events. As a result, forecasting the cumulative impacts of climate change on mountain lakes must include modelling of direct and indirect effects of multiple environmental factors (Figure 1).

The purpose of this chapter is to highlight the current and future effects of climate change on alpine lakes and ponds. The alpine region can be defined using factors including treeline, elevation, and regional climate. In this review, I define “alpine” as the region above treeline (approximately 2000 m asl as in the Canadian Rocky Mountains), where water bodies are chemically dilute and temperatures are cold. This review will assess environmental change in a multiple stressor context of a warmer and drier future climate, where we expect to see more variable pond-like environments (Parker *et al.,* 2008).

Endemic alpine lake and pond species

Species endemic to high elevations play an important role in alpine diversity, and are, as evidenced below, at risk to environmental change. For example, daphniids ("water fleas") are key species affecting both water quality through their key roles as the primary herbivores, and vectors of energy transfer to higher trophic levels (Miner *et al.,* 2012). *Daphnia middendorffiana* Fischer 1851*,* has a narrow temperature tolerance range of 13-15 °C in laboratory conditions, with peak activity, growth and reproduction at colder temperatures than temperate species (Yurista, 1999). *D. middendoriffiana* inhabits arctic ponds (O'Brien *et al.*, 1979) and fishless alpine lakes (Wilhelm *et al.,* 1998). *Daphnia pulex* O.F. Müller 1785 is a widespread temperate species, and inhabits alpine ponds as a key grazer (Anderson, 1974, Strecker *et al.,* 2014). These two daphniids species do not often coexist, potentially due to different energy budgets mediated by temperature variation dependent on depth (MacIsaac, 1985).

Alpine calanoid copeods such as *Hesperodiaptomus arcticus* Marsh 1920 and *Hesperodiaptomus shoshone* S.A. Forbes 1893 are also endemic to arctic and alpine regions. They also occur in subarctic regions due to north-south migratory patterns of birds (i.e., Northern Quebec, Canada; Swadling *et al.,* 2001). *H. arcticus,* or *H. shoshone* in more southern sites of the Canadian Rockies (Fischer *et al.,* 2011), are generally the top invertebrate predator in alpine systems with a few exceptions when amphipods are observed (Fischer *et al.,* 2015).

Amphipod species *Gammarus lacustris* G.O. Sars 1864 play a key role in structuring plankton communities in alpine lakes by predating on daphniids grazers. Thus, *G. lacustris* can release pressure on phytoplankton and recycle nutrients such as phosphorus (Wilhelm & Schindler, 1999). However, deep-water lakes can provide refugia to prey, diminishing the effects of amphipod predation on community structure. *H. arcticus* also acts as a key predator in regulating planktonic rotifer populations (Andrew & Schindler, 1994, Wilhelm & Schindler, 1999).

In many alpine plankton species, a "cocktail of defenses" are combined as adaptive measures to protect against ultraviolet radiation (UV), including pigmentation and daytime vertical migration that are exacerbated with greater UV transparency (Hansson *et al*., 2010, Fischer *et al*., 2015). There is an apparent trade-off however, between pigmentation production and growth rates in zooplankton, making pigmented individuals competitively inferior, particularly when there are predatory fish present (Hessen, 1996). Alpine daphniids and calanoid copepods are acutely adapted to alpine conditions, making these species sensitive to warming conditions, especially at higher elevations and latitudes.

Higher and more variable temperatures

Warming

Model projections of climate change indicate significant increases in temperature along the American Cordillera region, with predictions of greater than 3 degrees Celsius for the high mountains of the North American Rockies (Bradley *et al*., 2004). Warming of high elevation regions in the winter and spring has been amplified due to decreased perennial snow pack and therefore increased snow-albedo feedback (Giorgi *et al.*, 1997, Fyfe & Flato, 1999). According to the Intergovernmental Panel on Climate Change (IPCC), inland water reservoirs are some of the most sensitive ecosystems to the predicted climate change conditions (IPCC, 2007). As global warming decreases snow and ice cover extent, coldwater species become threatened as mountain catchments warm more rapidly (Battarbee *et al.*, 2009).

Temperature decreases ~10 degrees Celsius per 1000 m rise in elevation (Lyons & Vinebrooke, 2016). Therefore, alpine sites are often used as indicators for investigations of climate change because warming can damage their cold-adapted communities. The effects of warming on alpine community composition have been investigated (i.e. Strecker *et al.*, 2004, Holzapfel & Vinebrooke, 2005, Thompson *et al.,* 2008, Fischer *et al.*, 2011). Moderate warming stimulates large daphniid species, but suppresses (Weidman *et al.*, 2014, Fischer *et al.*, 2011) or reduces reproduction (Thompson *et al.,* 2008) in larger *Hesperodiaptomus*. Alternatively, in growth chamber experiments warmed to 20 °C, total biomass decreases due to a decline in both large alpine species (i.e., *D. middendorffiana* and *H. arcticus*; Holzapfel & Vinebrooke, 2005). Natural warming of 3.6 °C also suppresses total zooplankton biomass due to a taxonomic shift away from larger daphniids, such as *D. pulex*, and towards smaller rotifer species (Strecker *et al.,* 2004). Varying expectations for warming in the alpine predict different community composition changes for the future, with shifts being more prominent at higher temperatures.

Temperature Variation

With investigations of climate change predicting not just warmer, but also drier conditions, shifts by lake ecosystems towards greater instability are increasingly probable (Parker *et al.*, 2008, Bradley *et al*., 2004). In addition, an increase in the frequency of extreme weather events, such as drought, is predicted (Easterling *et al.* 2000). As water levels decline, numerous buffered lakes could shift towards shallower ponds and have consequences for biodiversity and ecosystem function (Parker *et al*., 2008). Shallow alpine ponds are considered especially sensitive to environmental change with their small volume and high solar energy absorption (Vinebrooke *et al.,* 2014). Pond ecosystems are polymictic so they lack the stratification needed to buffer against rapid changes in temperature (Lewis, 1983). They experience marked diurnal cycles in water temperature making them one of the most physically variable aquatic environments in the world (Neldner & Pennak, 1955), with daily temperature ranges as large as 23.6°C (McMaster, 2003). Such thermal variability can significantly decrease the somatic growth and fitness of planktonic species that are unable to adjust metabolic rates accordingly (Reichwaldt *et al*., 2005, Chen & Stillman, 2012). In contrast, deeper alpine lakes are less variable and have higher thermal stability (Vallentyne, 1957) making these non-adapted populations potentially sensitive to larger scale temperature variation predicted by climate models (Weyhenmeyer *et al*., 2011, Fischer *et al.,* 2015).

Littoral habitats within smaller basins have greater short-term temperature variation than deeper, larger lake basins (Finlay *et al*., 2001). *D. pulex* has been found to acclimate and reduce their metabolic rates under more variable thermal conditions, resulting in slower growth rates (Chen & Stillman, 2012). Lower available energy for growth compared to temperate daphniids may be a hindrance for *D. middendorffiana*, as their competitive abilities are also dependent on temperatures (Yurista, 1999). As the climate begins to favour species acclimated to warmer and shallower conditions (i.e., *D. pulex*) it is possible that they could replace cold-adapted *D. middendorffiana* (Fischer *et al*., 2015). In addition to lower growth rates of large taxa and taxonomic shifts with higher thermal variation, the effects of warming that will shift the community to smaller, more metabolically plastic zooplankton taxa may also be compounded.

Given the overall consensus that warming will cause a shift in community composition towards smaller taxa, and decrease grazing pressure, it is interesting to examine the combined effects of warming with further stressors. By reviewing the individual and additive or non-additive effects of stressors related to environmental change, we can get a more holistic view of future ecosystem changes.

Biological Invasion

Thermophilization, or the increase in competition among cold-adapted species in response to warming and immigration of warm-adapted species, has been assessed in plant communities, and is an imminent threat to alpine systems (Gottfried *et al.,* 2012). Upwards shifts in ranges of alpine species to cooler regions in response to warming may cause range contraction rather than expansion due to the nature of the mountain topography, making them thermally and physically isolated to areas that do not support survival or reproduction (Forero-Medina *et al*., 2010). Although vegetative communities experience more direct range expansion and competition, this too is a concern in aquatic mountain communities.

Montane invaders may persist under colder lab conditions, bringing to question dispersal limitation, predation exclusion or competition in natural environments (Holzapfel & Vinebrooke, 2005). These limitations may be reduced under changing environmental conditions at higher elevations, making upward shifts of montane species more likely. Regional dispersal of montane species can also mediate the response of an introduced stressor (i.e., trout stocking) by evoking different responses to environmental change (Loewen & Vinebrooke, 2016). Despite the potential adaptive capacity of montane species to alpine environments, topographic barriers may limit transverse dispersal (Jenkins & Underwood, 1998, Donald *et al*., 2001).

Many alpine systems are naturally fishless, creating dynamic pelagic communities that are particularly sensitive to external changes (Kong *et al*., 2016). In the Canadian Rockies, 20% of naturally fishless lakes were stocked with cutthroat, rainbow and/or brook trout from the early 1900s to 1980s for recreational angling (Donald, 1987). Non-native fish stocking has been shown to drastically alter food web dynamics in pelagic alpine communities (Schindler & Parker, 2002, Knapp *et al*., 2005, Eby *et al*., 2006, Loewen & Vinebrooke, 2016). Given the small size and isolation of alpine lakes, it is relatively easy to examine the cascading effects across trophic levels (Eby *et al*., 2006). Trout stocking in the Canadian Rockies typically eliminated larger alpine zooplankton such as *D. middendorffiana* and *H. arcticus*, leaving smaller zooplankton to compensate, resulting in larger phytoplankton taxa flourishing and decreasing water clarity (McNaught *et al*., 1999, Schindler & Parker, 2002). The initial low species richness in alpine ecosystems compound the trophic cascade effects because these smaller species play a minor role and are unable to functionally compensate for the loss of larger alpine-adapted species (Parker & Schindler, 2006).

In Yosemite National Park (California, USA), fish stocked sites had 16% fewer taxa than expected in the absence of stocking, with these effects strengthening with increasing elevation (Knapp *et al*., 2005). Resilience of rare species is much lower in lakes with longer fish residence times because resting egg stores in the sediment become depleted, and the Allee effect may limit mating potential in copepods (McNaught *et al*., 1999, Sarnelle & Knapp, 2004). In the alpine, mountains may act as topographic barriers to overland dispersal, again limiting the recovery of native zooplankton species (Jenkins & Underwood, 1998, Donald *et al*., 2001). There is also evidence that introduced trout persists better under alpine conditions given the low accessibility of sites to anglers and favourable living conditions (i.e., low temperature, high dissolved oxygen concentration; Messner *et al*., 2013).

Fish may also increase the thermal sensitivity of zooplankton communities, exacerbating the size selection for smaller body size by releasing smaller taxa from predation by larger invertebrate zooplankton and competition for algal food sources (MacLennan *et al*., 2015). In warmer lakes, this can allow total zooplankton biomass and species richness to increase (Messner *et al.,* 2013). In contrast, introduction of non-native trout appears to override any effect of warming on fishless alpine planktonic communities, which require the greater regional species pool to rescue them from the negative functional impact of introduced fish (Loewen & Vinebrooke 2016). Management practice therefore must include the combined effects of both the legacy of fish introductions and the future forecasts for warming.

Nutrient Deposition

Increases in anthropogenic emissions have increased the deposition of reactive nitrogen (N) into environmental reservoirs globally (Galloway & Cowling, 2002). N-deposition in mountainous regions adjacent to urban areas has increased since the intensification of agricultural and industrial processes in the 1950s, with algal communities indicative of nutrient enrichment (i.e., Colorado Rockies; Baron *et al.,* 2000, Wolfe *et al.,* 2001). Nitrogen plays a role as a limiting or co-limiting nutrient in many oligotrophic alpine lakes with small watersheds, making these systems sensitive to external deposition (Elser *et al*., 2007, Paerl & Scott, 2010). The sensitivity of mountain lake ecosystems to increased N deposition (i.e., greater nitrogen limitation) is negatively correlated with lake size (Murphy *et al*., 2010). Although most water bodies in the Canadian Rockies are P-limited, shallow alpine ponds are more likely to be N-limited, and could therefore be used as early indicators of N-deposition (Murphy *et al.,* 2010). However, top-down effects by predators (i.e., crustacean zooplankton) and competition between algal species may limit responses by phytoplankton (Van Geest *et al*., 2007, Vinebrooke *et al*., 2014).

Forest fire also has a profound impact on the dry deposition of phosphorus over boreal and mountain watersheds in North America (McEachern *et al.,* 2000, Emelko *et al*., 2016). Phosphorus and to a lesser degree N can be liberated by such fires, depending on the magnitude of the disturbance and the catchment's ability to retain nutrients (McEachern *et al*., 2000).

The synergistic effects of warming and N-deposition optimize growing conditions for phytoplankton due to longer growing seasons and increased vertical stratification (Thompson *et al*., 2008, Paerl & Scott, 2010). Taxonomic shifts of zooplankton communities related to the synergistic effects of warming and N-deposition are habitat-dependent, and grazers are more likely to be suppressed in shallow pond environments (Thompson *et al*., 2008). In turn, there are dramatic implications for water clarity and productivity given the top-down mechanisms of grazing, however this is dependent on the strength of grazing pressure (Thompson *et al*., 2008). With N-deposition abolishing important large grazers, the effects of warming on community assemblages will also tend towards smaller taxa.

UV Radiation

UV-B radiation increases by ~20% per 1000 m of elevation (Blumthaler *et al.,* 1992), therefore, high elevation alpine ponds are particularly sensitive to its damaging effects. Dissolved organic carbon (DOC) can mediate the effects of UV exposure by attenuation mechanisms (Scully *et al*., 1994). However, high altitude ponds are often located in treeless catchments, nearly free of vegetation, so there are few sources of DOC compounds to reduce UV irradiance (Mcknight *et al*., 1997). Total DOC concentrations of alpine systems can be < 20 μM, and predicted warming and drought conditions will decrease DOC inputs from the catchment, further reducing the beneficial buffer of DOC attenuation (Williamson *et al*., 1996, Schindler & Curtis, 1997). Vinebrooke & Leavitt (2005) suggested that under such changing conditions, UV impacts would be greatest near treeline where small changes can affect the influx of DOC and therefore UV exposure. Changes in water clarity would alter depth refugia and diurnal vertical migration processes of UV-sensitive zooplankton (Weidman *et al.,* 2014, Fischer *et al*., 2015). However, warming may mitigate the effects of increased UV exposure in some taxa by increasing enzymatic photo-repair processes (Williamson *et al*., 2002). The adaptive capacity of alpine species to develop pigmentation (i.e., melanin, carotenoid) may also decrease the impact of long-term increased exposure, however these mechanisms come with both metabolic and ecological costs (Hessen, 1996) and increased sensitivity to predation by visual predators (Hansson *et al*., 2000). In the case of decreased DOC inputs, warming and the adaptive capacity of alpine species may be able to compensate for increased UV penetration.

Alternatively, in some lakes, climate change may increase DOC in response to increased glacial ablation and treeline advancement, which could in turn increase productivity and diversity (Vinebrooke & Leavitt, 1999, Messner *et al.,* 2013). An increase in DOC in alpine systems could give smaller, UV-sensitive taxa a competitive advantage over endemic alpine species (Weidman *et al.,* 2014). Under these circumstances, the effects of warming may also add to these shifts, further solidifying a community dominated by taxa of smaller size classes. Therefore, the fate of DOC under changing climate conditions relies on the physical water catchment, and whether or not it is fed by glacial sources, as well as its proximity to the imminently advancing treeline. By tracking DOC levels, we can get a better understanding of the changes occurring on a broader climate scale (i.e., drought, warming), and assess sensitivities to UV exposure.

Organic Pollutants

Alpine lakes are relatively pristine systems, with minimal or absent direct anthropogenic inputs. However, they are not immune to the atmospheric deposition of contaminants (Blais *et al*., 1998, Kallenborn, 2006). Cold condensation is a mechanism involving the transport of relatively volatile compounds from warmer regions to ultimately condense and contaminate areas of cooler temperature (i.e., higher latitudes; Simonich & Hitest, 1995). Even though many of these contaminants have been restricted in developed countries since the early 1970s, long distance transport of these volatile compounds allows for later deposition in colder regions (Donald *et al*., 1999). Studies on the accumulation of persistent organochlorine compounds (POPs) in the mountains of Western Canada showed a significant positive correlation between volatile compound concentrations and elevation, exhibiting this phenomenon at an altitudinal level (Blais *et al*., 1998). Fish from mountainous and arctic environments have been equally contaminated with POPs, indicating that bioaccumulation is affecting these systems detrimentally and raising substantial concern about overall ecosystem function (Kallenborn, 2006). With many alpine communities being structured around a large zooplankter as its apex predator, the effects of POP accumulation is less clear. Amphipods (*Gammarus lacustris*) in alpine environments have higher accumulation of POPs than at lower elevations, which is compounded by their slower growth rates (Blais *et al.,* 2003). These trends could also be assigned to other large alpine zooplankton species, creating repercussions at higher trophic levels.

Due to warmer summer temperatures, glaciers are becoming an increasingly important source of POPs (Blais *et al*., 2001). Melting glaciers supplied between 50 to 97% of organochloride compounds to a subalpine lake, between 2 and 50 times higher than from valley stream inputs (Blais *et al*., 2001). Given that melting layers contain POP contaminants deposited from the 1950s to the early 1990s, peak deposition in alpine lakes could be later than at mid-latitudes (Donald *et al*., 1999). As climate warming increases glacial melt, discharge of these compounds into many freshwater sources around the world could increase. As well, increased warming could increase evaporation of lower volatile organochloride compounds, further increasing deposition of new contaminants at higher altitudes (Blais *et al*., 2003).

Conclusion

It is imperative that environmental changes be examined through interactions with other stressors to assess future impacts (Figure 1). With multiple environmental stressors acting on alpine lakes to increase the thermal sensitivity of these fragile communities, some stressors may add to the shifts towards smaller body size at the expense of endemic alpine species (i.e., nutrient deposition, alien fish introductions). Conversely, certain stressors (i.e., increased UV) may act to enhance the adaptive capacities of such large alpine species. There is a need to test these stressors in combination with not just warming, but with each other in order to assess the overall effect on community composition, and the long-term consequences for ecosystem function.

Research Objectives

My thesis research addressed four main objectives:

1. Identify the environmental factors that significantly capture taxonomic variation in zooplankton communities across an elevational gradient *(Chapter 2).*

   To achieve this objective, I used forward model selection to determine the significant environmental variables explaining the distribution of species across 170 sites across the mountains of Canada. I then used an unconstrained principal component analysis (PCA) to visualize the distribution of species across different elevational regions. From there, I constrained this ordination using redundancy analysis (RDA) to show the general species trends against each environmental vector.

2. Identify the environmental factors that best explain shifts in the functional structure of mountain zooplankton communities *(Chapter 2)*.

   To achieve this objective, I used fourth corner and RLQ analysis to determine the bivariate associations between trait clusters and significant environmental variables. I also visualized these trends using an RLQ plot.

3. Predict how functional niche groups will react to changes along an environmental gradient *(Chapter 2)*.

To achieve this objective, I identified trait clusters sensitive to climate change predictions, and used species distribution analysis to predict the risk factor of individual species.

4. Describe the shortcomings of using *Daphnia* species as “model organisms” for culturing and adaptation experiments *(Chapter 3)*.

To achieve this objective, I conducted a literature review and discussed deficiencies in using single clone lines grown under lab conditions to generalize for natural populations. I also drew on personal experiences culturing *D. pulex* and *D. middendorffiana* from natural alpine environments for temperature variation experiments.

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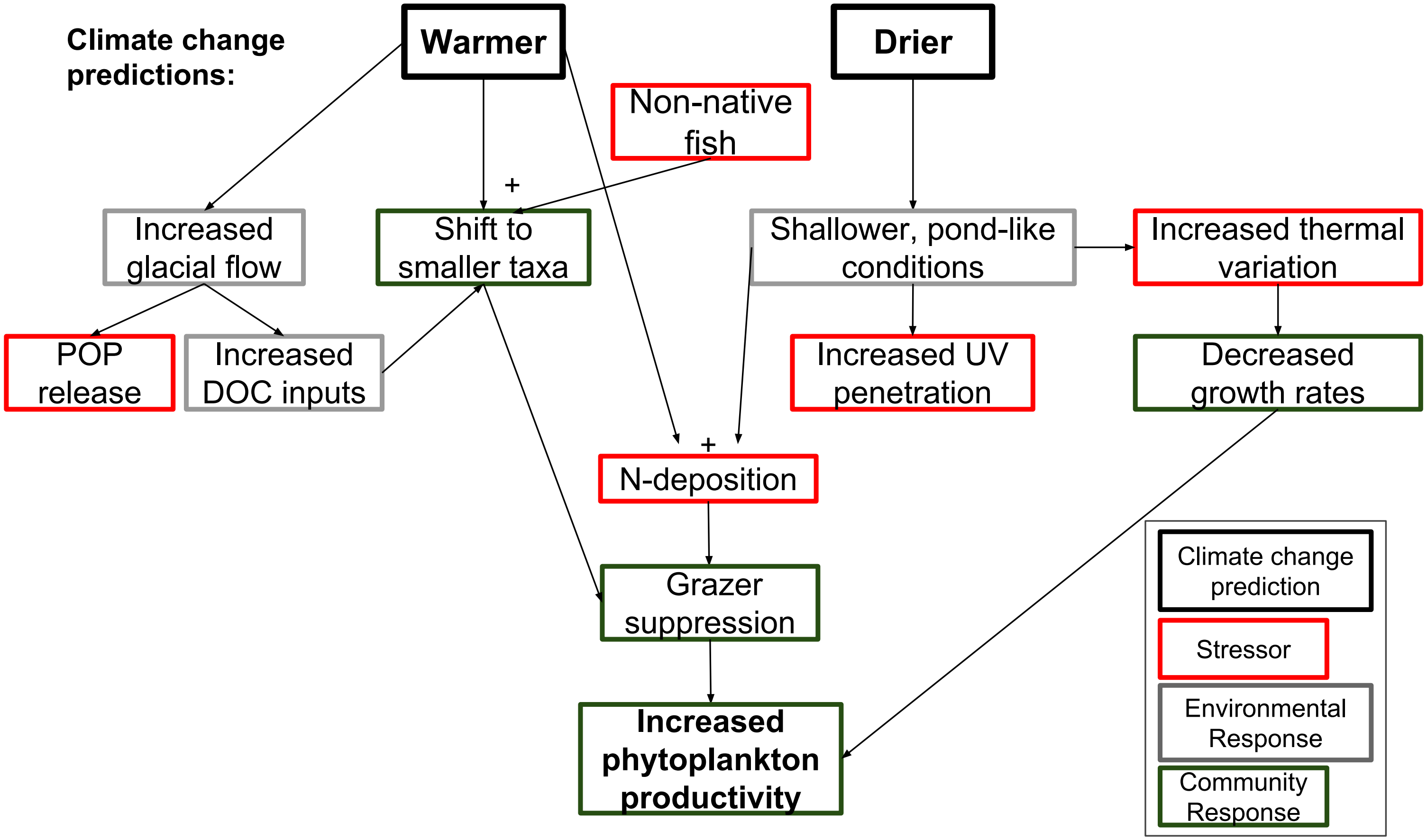

Figure 1: Flow diagram identifying the cumulative effects of multiple stressors (red) and climate change (black) on the community composition and function of alpine lakes (green). Addition signs (+) represent synergistic interactions of stressors.

Chapter 2: A Functional Approach Reveals Zooplankton Responses to Environmental Change

Introduction

Species turnover (β-diversity) across gradients

Regional (i.e., connectivity, species dispersal) and local (i.e., environmental filtering and biotic interactions) processes drive community composition (Ricklefs, 1987, Leibold *et al*., 2004). Regional factors allow the spatial migration of species in response to environmental changes, and regional diversity can act as a rescue effect by maintaining total species richness (Loewen *et al.,* 2016, Thompson & Shurin, 2012). However topographic barriers and low propogule pressure may limit dispersal in mountainous environments (McNaught *et al.,* 1999, Donald *et al*., 2001, Sarnelle & Knapp, 2004).

Even across highly interconnected systems, local environmental factors play an important role in structuring zooplankton communities (Cottenie *et al*., 2003). Under the species-sorting paradigm, heterogeneous habitats elicit the sorting of species based on their respective environmental tolerances, thereby minimizing the relative importance of dispersal ability that is more important under mass effects paradigms (Leibold *et al*., 2004). At higher elevation, species-sorting is expected to outweigh mass effects due to limited dispersal. Community composition is also influenced by interspecific interactions (i.e., competition, predation) that may further structure local communities and diversity (Ricklefs, 1987).

Beta-diversity is commonly defined as the variance of species across sites (Anderson *et al.,* 2011). Taxonomic variation along an environmental gradient is often explored to determine how abiotic or other biotic changes affect shifts in species composition across communities (i.e., β-diversity; Anderson *et al*., 2011, s *et al*., 2011). Geographic positions (including latitude and elevation) drive environmental gradients that can be used as proxies for changes in climate and landscape features that influence lake environments (Dunne *et al*., 2004). For example, changes that occur along an elevational gradient, such as temperature and position of key landscape features (i.e., treeline), can be used to infer expected temporal changes (i.e., warmer temperatures in lower elevation montane regions can be proxies for warming at higher elevations as a result of climate change). Given that climatic predictors of community structure are expected to change in the future, with warming generally occurring more rapidly at higher elevation (Pepin *et al*., 2015), it is of interest to test taxonomic variation along elevational gradients to assess the sensitivity of communities to anthropogenic environmental changes (i.e., stressors).

Functional diversity

Although the traditional taxonomic analyses of communities along environmental gradients has provided insights into how species abundances change in response to various environmental changes, they do not directly address the related potential impacts on ecosystem function (Tilman *et al.,* 1997, Walker *et al.,* 1999, Norberg, 2004). The functional trait diversity of a community can better assess resilience to change based on the ecological roles of species (Barnett *et al*., 2007, Mouillot *et al.,* 2013). A functional trait is defined as a measureable feature that impacts the performance or fitness of the individual (Violle *et al.,* 2007). For the purpose of this analysis, functional response traits will be used. Response traits are those that allow species to persist under certain environmental conditions, and do not refer to the effects that species have on the total ecosystem functioning (Nock *et al.*, 2016, Hooper *et al.,* 2005). Functional traits act on species fitness by influencing their feeding, growth, survival and reproductive rates (i.e., morphological, physiological, behavioural traits; Litchman *et al.,* 2013). In this chapter, functional redundancy refers to the ability of multiple species with the same functional responses in a community to compensate if a single species is eliminated. Having multiple species with similar response traits creates a buffer for change, and maintains biodiversity. Functional diversity refers to the range of functional responses within a community. In addition to assessing the impacts of disturbance and taxonomic structures, species traits can be used to understand the functional structure of communities in relation to environmental change (Mouillot *et al.,* 2013, Norberg*,* 2004). Individuals allocate energy to various functions, and in a changing environment, ecological tradeoffs may influence the functional complexity of communities. Therefore, relating environmental conditions to species functional groups can provide critical insight to the non-neutral niche effects of global change (Mouillot *et al.,* 2013).

Functional resistance to climate change

With environmental changes being an imminent threat to the functional diversity of communities, and driving shifts towards taxa with traits adapted for new conditions (Moulliot *et al.,* 2013), it is interesting from a conservation perspective to assess the resilience capacity of sensitive cold-adapted alpine lakes. Biodiversity is critical to ecosystem function because redundancies help maintains function under changing environmental conditions, increasing resistance (Yachi & Loreau, 1999). Having a range of species with different functional responses to environmental perturbations can maintain ecosystem function, even under environmental flux (Hooper *et al.,* 2005). The classic species distribution indicates that a few species make up the bulk of all biomass and that multiple minor species are functionally similar to dominant species, providing functional resistance to changing environmental conditions (Walker *et al.,* 1999). This is known as the species insurance hypothesis (Yachi & Loreau, 1999); however, alpine systems may offer limited resistance to change due to low species richness and heightened functional specialization (Lyons & Vinebrooke, 2016).

Research Objectives

Here, I address the following objectives to develop predictions for how crustacean zooplankton communities may respond both taxonomically and functionally to future environmental changes in mountainous regions:

1. Identify significant, independent environmental predictors of shifts in species composition along elevational and latitudinal climate gradients.
2. Identify environmental variables that best explain shifts in functional structure along elevational and latitudinal climate gradients.
3. Assess the potential functional consequences of future environmental changes for zooplankton communities in mountainous regions.

Methods

Geographic Extent

An environmental-zooplankton species database was assembled from limnological reports published between 1975 and 1997 for mountain lakes and ponds across Western Canada (Table 1), spanning large latitudinal (2276 km) and elevational (1959 m) gradients. Most data are from within the national mountain parks along the Continental Divide between Alberta and British Columbia, including Waterton Lakes, Banff, Yoho, Kootenay, Mt. Revelstoke, Glacier and Jasper National Parks. Mountain lakes within Mt. Revelstoke and Glacier National Parks are in granite formations, and lakes in Jasper National Park are in feldspar-quartz formations, while the remainder of mountain lakes are in Paleozoic limestone formations (Anderson, 1974). Northern sites are situated in northern B.C., and across the Yukon Territory (Figure 2). All sites lie within the rugged Western Cordillera geological region and the Cordillera climatic zone, characterized by cool, wet winters and warm, dry summers (Average temperatures: 0.5-7.5 °C, Average precipitation: 1200-1500mm at higher elevation; Ecological Stratification Working Group, 1996**)**. The sites all lie within harsh plant hardiness zones, and are therefore areas of low terrestrial vegetation diversity (McKenney *et al.,* 2000).

Limnological surveys

Sampling methods were fairly consistent across the dataset, with specifics of each stated in individual reports (Table 1). Zooplankton samples were collected with vertical hauls from ~1 m above a lake bottom using #20 Wisconsin-style conical nets consisting of mesh sizes of 63-70-µm and diameters of 25 cm. Water samples were taken a few centimetres below the surface with a Van Dorn sampler or by hand. Temperature was measured with electronic thermometers and often verified with a mercury thermometer. Zooplankton samples were preserved in either 37% formaldehyde or 5% formalin in the field, and enumerated using a dissecting microscope. Identification was done under a compound microscope using the dichotomous-based taxonomic keys of Edmondson (1959) and Brooks (1957).

Statistical Methods

To address the following objectives, a variety of statistical tests were done to visualize and test the sensitivity of species and functional clusters to climatic change.

1. Identify significant, independent environmental predictors of shifts in species composition along elevational and latitudinal climate gradients:
    - 1.1. Principal Component Analysis (PCA) to visualize the distribution of sites and species in ordination space.
    - 1.2. Redundancy analysis (RDA) constrained by environmental factors determined by forward selection.
    - 1.3. Overlay of PCA and RDA with elevational regions data.
2. Identify environmental variables that best explain shifts in functional structure along elevational and latitudinal climate gradients:
    - 2.1. Cluster analysis of species based on functional traits.
    - 2.2. Overlay of PCA and RDA with cluster groups.

    2.3. R-mode linked to Q-mode (RLQ) analysis to assess the distribution of species in functional space; overlay of elevational regions.

3. Assess the potential functional consequences of future environmental changes for zooplankton communities in mountainous regions:
    3.1. RLQ analysis to visualize relationships between cluster groups and environmental variables.
    3.2. Fourth corner analysis to test relationship between cluster groups and environmental variables to test trait cluster sensitivity to climate change.
    3.3. Compare species abundance curves of different elevations to assess sensitivity of regions based on functional composition.
    3.4 After distinguishing sensitive functional groups, assess each species within that group for climatic indicator potential.

1.1 PCA

Ordination is a vital tool in the visualization of variance between sites and species. It consists of plotting observational points along one or more orthogonal axes that represent large fractions of variation in the data. For example, principal component analysis (PCA) involves the creation of synthetic axes (principal components) that are rotated around the original coordinates to correspond to the directions of maximum variance (Legendre & Legendre, 1998). These unconstrained techniques are used to visualize the relationships between sites and species across ordination space, with the eigenvalue representing the strength along each gradient. PCA assumes linear relationships between variables, and therefore transformations are often necessary to fit a Euclidean distance matrix (Legendre *et al.* 2001).

All ordinations were performed using the ‘vegan’ package (Oksanen *et al.*, 2014) for the R software environment (R Development Core Team, 2014). Matrix editing removed sites that had no species present, and rare species, defined as being present at < 5 sites, because those species could be below detection rates not consistent across sites. Species data were in the form of presence-absence (1,0) rather than abundance data due to variation in sampling methods and unstandardized units of abundance over the course of data collection. Outlier sites were removed if their robust score distance (SD), measured as the robust statistical distance from a PC score to the center of the scores, was above the critical cut-off value (97.5%; Varmuza & Filzmoser, 2009). A nonmetric multi-dimentional scaling (NMDS) analysis was tested given its ability to reduce spatial distortion, but the stress function was > 3, and therefore the fit was deemed not representative of the community. A Principal Component Analysis (PCA) was used to best visualize the distribution of taxa and sites in ordination space.

A detrended correspondence analysis (DCA; Hill & Gauch, 1980) of the species-by-site data was used initially to test gradient length and determine the appropriate distribution model for ordination analyses (Legendre & Legendre, 1998). DCA acts to reduce the polynomial relationship between the first and second axes (an artifact of the method), by constraining against such correlations (Legendre & Legendre, 1998). Therefore, axes are scaled to units of standard deviation of turnover, with a complete species turnover occurring across 4 standard deviation (SD) units (Gauch, 1982). If the gradient length of the first axis is < 2 SD units, a linear model can be used with raw data (Legendre & Legendre, 1998). Otherwise, using a linear model with raw data on a long gradient increases the risk of an arc effect that appears as a curve in ordination space because species replace one another along the gradient thus generating many zeros (Legendre &

Legendre, 1998).

Given the long gradient lengths (6.26 SD units for first DCA axis) species data was Hellinger transformed to reduce the effect of zeros along a longer gradient showing complete species turnover (Legendre & Gallagher, 2001). Therefore, as sites get further apart in the distance matrix and asymptote at zero species in common, the effect of these zeros is reduced for a clean visualization of ordination distance. Hellinger transformation involves a square-root transformation of proportional site abundances, thus reducing the effect of zeros and offering the best compromise between linearity and resolution (Legendre & Gallagher, 2001). After Hellinger transformation, rank-index analysis increased the fit of a Euclidean matrix to the data, confirming linear relationships between variables.

1.2 RDA

Constrained ordination with forward selection was performed to identify significant ($p < 0.05$) and independent environmental predictors of variation in zooplankton community composition across sites. Environmental variable distributions were checked for normality using the Shapiro-Wilk Normality Test, although given the range of the values, normality was not achieved. Therefore, non-parametric tests of resampling for statistical significance were used throughout analysis. Due to the large spread of environmental gradients, variables were scaled and centered for comparison. Multicollinearity among environmental variables was assessed using a Variance Inflation Factor (VIF) equaling 10, and collinear variables were removed to reduce the risk of overestimating the significance of correlated variables.

The measurement and reporting of the chemical and environmental data were incomplete because chemical and physical variables were not always consistently recorded at each site. Therefore, constrained analysis was conducted on a smaller subset of sites (174- before outlier removal) for which measures of elevation, depth, surface area, total dissolved solids (TDS), temperature, pH, Calcium (Ca), Magnesium (Mg), Sodium (Na), Sulphate (SO_4^{2-}) and Potassium (K) were available.

Forward selection with the function 'add1' (Chambers, 1992) identified and added environmental variables that explained successive significant variance ($\alpha = 0.05$, n=500) to increase the fit of the model to be used in constrained analysis. RDA is a form of constrained ordination that displays the variance of the response variable (species data) that is explained by the constraining explanatory variables (environmental data). This process combines a multivariate linear regression followed by a PCA of those fitted values. Following forward selection using permutation tests, a redundancy analysis (RDA) was used to constrain the PCA by significant environmental variables.

1.3 Ordination overlay with elevational regions

A linear regression analysis compared species richness across elevation. Next, each taxon was put into a regional group based on elevation. Species were labeled as alpine (> 2000 m asl), subalpine (1500-2000 m asl) or montane (< 1500 m asl) based on the highest proportion of sites in which they were found (Donald *et al.,* 2001). This is meant to be an estimation of trends across elevation, given that treeline declines with increasing latitude across the wide site extent. These regions were plotted as categorical hulls overlaying unconstrained and constrained ordinations to display the changes in species richness and diversity across elevation.

2.1 Cluster analysis

Species were also assigned functional traits based on a review of the literature pertaining to mountain zooplankton ecology. Literature was searched for studies that had either categorical or quantitative values for species functional traits for each taxon present within our study, and transformed into categorical traits for statistical analysis. Traits were chosen based on fitness as a function of survival, reproduction, feeding and growth (Litchman *et al.,* 2013). All traits relate to species abilities to thrive and persist in a particular habitat (i.e., response traits; Nock *et al.,* 2016). Traits included pigmentation potential (yes or no), trophic status (herbivore, omnivore, carnivore), habitat type (pelagic, littoral, benthic), feeding behaviour (filter, raptorial, Barnett *et al.,* 2007), reproduction mode (asexual, sexual, Barnett *et al.,* 2007), and body-size class (small: 0-1 mm, medium: 1-2 mm, large: >2 mm; Table 5). Traits were assigned to species based on literature values, but in some cases were categorized based on similarities in taxa (i.e., *Simocephalus vetulus* O. F. Müller, 1776 was categorized as herbivore given its filtration morphology).

Cluster analysis was then performed to assemble species into groups based on functional traits, weighted by species presence/absence data. Ward's minimum variance method of hierarchical agglomeration was used to cluster groups based on minimizing the growth of within-group variance (Husson *et al.,* 2010), as Benedetti *et al.* (2015) used to identify marine copepod functional groups. The cluster-wise cluster stability assessment by bootstrap resampling with replacement was used to assess the stability of each cluster to see how well they were reproduced using the 'fpc' package (Hennig, 2015).

2.2 Ordination overlay with cluster groups

The resulting clusters were overlaid on constrained and unconstrained ordinations using ordination hulls to visualize the effect of cluster group on ordination distribution.

2.3 RLQ analysis of species and functional trait variances across elevation

Using the RLQ methods described below, species were ordinated in functional space according to the trait identity of each species to assess the distribution of species from a functional perspective. These methods are described further in section 3.1, and involve a multiple correspondence analysis (MCA) of categorical trait identities that is weighted by the species presence/absence data. Species points were overlain by elevational regions, described above, to visualize the functional space occupied at different elevations, and the functional shifts that occur along an elevational gradient. The area occupied by each elevational functional niche was then calculated.

3.1 RLQ analysis to compare cluster groups across environmental gradients

After visualizing the relationships between functional cluster groups along environmental gradients in an RDA, the significance of these relationships were tested with R-mode linked to Q-mode (RLQ) analysis. RLQ and fourth corner analyses visualized the covariance between environmental variables and trait groups, mediated by species presence/absence data (Dray *et al.,* 2014). This analysis aims to uncover the relationship between functional traits and environmental predictors of community composition shifts. The "Fourth Corner Problem" involves the inability to compare species traits with environmental gradients (Figure 3). Fourth corner analysis tests the associations between individual traits and environmental variables by permutating sites and species together to detect the significance of each bivariate environment-trait association. RLQ analysis integrates three matrices to create a matrix that represents the fourth corner that compares the relationship between functional traits and environmental gradients by maximizing covariance based on species abundances across sites. The R matrix is a site x environment table, the L matrix is a site x species table, and the Q matrix is a species x trait table. RLQ creates a new matrix that compares environment x traits in a table, and then plots their relationship in ordination space using eigen decomposition.

In RLQ analysis, table L was computed by conducting a correspondence analysis (CA) on untransformed species presence/absence data. Next, row (site) and column (species) weights derived from correspondence analysis were used for PCA of environmental variables (R) and a MCA of categorical trait groups (Q), respectively. RLQ analysis was combined with Fourth corner analysis to test associations between trait groups and environmental gradients. To address objective 2.3, matrices L and Q were isolated to assess the distribution of species in functional space.

3.2 Fourth corner analysis

A multivariate Monte-Carlo test (n = 49,999) was applied to evaluate the global significance of the trait-environment associations computed by the RLQ by randomly selecting for the specified number of permutations, without replacement. Fourth corner analysis was used to detect the significance of each bivariate environment-trait group association (Legendre *et al*., 1997). RLQ and fourth corner analysis were completed using the ‘ade4’ package (Dray & Dufour, 2007) for R. P-values were adjusted to correct for multiple univariate tests using the false discover rate (fdr) method.

3.3 Species abundances across elevation

Species abundance curves compared the functional diversity of species at montane and alpine elevations to assess resistance and resilience to environmental change. By comparing the number of functional groups present at different elevations, we can assess resistance to change and functional redundancy at different altitudes.

3.4 Indicator species of sensitive group and climatic gradients

After sensitive functional groups were established, individual species within such functional groups were scored across environmental gradients to assess sensitivity to climate change. Each species was tested using a non-parametric Wilcoxon Rank-Sum test for significant differences in environmental predictors (temperature and depth) across sites in which they were present versus absent to test for climatic indicator species. Species that displayed strong differences and associations with colder and deeper waters were classified as highly sensitive to warmer and drier conditions.

Spatial Analysis

Given the large geographic extent of this dataset, a multi-scale ordination (MSO) was conducted to test how the variance structure changed as a function of geographic distance, to ensure that residual variance in community structure was not driven spatially (Wagner, 2004). A RDA constrained by geographic coordinates gave a value for the variance explained by the spatial extent of the data. As well, additional reanalysis was conducted to compare the results of the fourth corner and RLQ analysis of the entire dataset, and a subset of exclusively the southeastern Canadian Rockies sites to ensure functional clusters and relations to environmental gradients were consistent and not spatially focused.

Results

Species Richness

Species richness decreased with increasing elevation (Richness ~ Elevation, $r^2 = 0.10$, $p = 1.7 \times 10^{-5}$). There was a greater average number of montane species than alpine species (Table 4, Figure 4). After elimination of outliers, 170 sites (Table 1) and 49 crustacean zooplankton species (Table 5) were used in analysis.

Unconstrained Ordinations

PCA constructed two primary axes that together captured 16.54 % of cumulative variance (Figure 5, 6). The remainder of axes did not fulfill broken stick criterion given that the percentage of variance they explained were less than the broken stick cumulative variance. Although the variance captured by these figured was minor given the number of sites and species, it still acts as a visual representation of some of the turnover that occurs across elevation. Species *H. arcticus* (34), *D. middendorffiana* (25) and *H.shoshone* (35) were strongly negatively associated with axis two (Figure 5). *Cyclops scutifer* G. O. Sars, 1863 (19), *Leptodiaptomus angustilobus* Sars G.O., 1898 (39), *Daphnia longiremis* G. O. Sars, 1861 (23) and *Bosmina coregoni* Baird, 1857 (11) were positively related to axis one (Figure 5). *Aglaodiaptomus leptopus* S. A. Forbes, 1882 (5) was associated with axis 2. Sites were separated in ordination space having two main groupings (Figure 6). PCA visualizations of elevational regions showed a visibly larger distribution of montane species along axis one, taking up a greater proportion of ordination space compared to alpine species that were clustered along axis two (Figure 7).

Forward Selection and Constrained Ordinations

Forward selection identified significant, independent explanatory environmental variables as being elevation, temperature, pH, maximum depth, surface area and potassium (K). Elevation was removed as an environmental variable to assess the significant interactions of strictly climate driven variables (Table 1). The five remaining significant variables chosen after forward selection showed large ranges, therefore justifying their scaling for analysis (Table 2). K was significantly correlated with measures of productivity in the dataset such as total phosphorus (TP; $r = 0.82$, $p = 2.2 \times 10^{-16}$, $df = 123$), chlorophyll *a* ($r = 0.81$, $p = 2.2 \times 10^{-16}$, $df = 78$) and total nitrogen (TN; $r = 0.99$, $p = 2.2 \times 10^{-16}$, $df = 85$). K was also correlated with total dissolved solids (TDS; $r = 0.72$, $p = 2.2 \times 10^{-16}$, $df = 304$).

RDA using the reduced model terms revealed 14.54% of total variance explained by the constraining environmental variables, with 11.09% of explained variance displayed on the first two axes (Figure 8). *D. middendorffiana* (25) and *H. arcticus* (34) were both associated with decreasing temperature. *D. middendorffiana* (25), *H. arcitcus* (34), *Holopedium gibberum* Zaddach, 1855 (37) and *Leptodiaptomus sicilis* S. A. Forbes, 1882 (41) were all positively associated with increasing depth. *C. scutifer* (19), *L. angustilobus* (39), *D. longiremis* (23) and *B. coregoni* (11) were all associated with increasing surface area and K. *A. leptopus* (5) was strongly associated with increasing temperature and pH (Figure 8). After applying elevational region filters, alpine species were strongly associated with RDA axis 1 (positively associated with maximum depth, and negatively associated with temperature; Figure 9). Montane species showed a larger breadth of responses along environmental gradients, but had higher associations along vectors of increasing temperature, K and pH (Figure 9).

Cluster Analysis

Cluster analysis using the results from the literature review (Table 4) revealed six trait clusters based on categorical trait values (Table 5), of which only two had lower stability values after bootstrapping analysis (Group C and E; 0.60, 0.71 on a scale from 0 to 1). Cluster A was defined as larger pigmented species (i.e., *D. middendorffiana* & *H. arcticus*). Cluster B are herbivores of varying sizes (i.e., bosmidiids & daphniids). Clusters D and F are shallow water littoral species, with cluster D being entirely parthenogenic (i.e., *Alona* & *Chydorus* spp.). Cluster C and E are mixed feeders of differing pigment levels.

Trait clusters were then overlain on PCA ordination (Figure 10). Trait clusters spanned from generalist to specialist groups with varying degrees of ordination space occupied after PCA analysis. Trait cluster A occupied the greatest proportion of ordination space. Trait cluster D and F occupied the smallest proportion of ordination space, having a narrower site dependence. RDA plots overlain by trait clusters revealed a corroboration with PCA analysis, with species within trait cluster A occupying the largest proportion of ordination space and having diverse responses along environmental predictors surface area and K (Figure 11). Additionally, trait cluster A species primarily followed the vector in the direction of decreasing temperature. Species in trait cluster D, E and F were driven by increasing temperature and pH, and decreasing depth.

RLQ Analysis

RLQ analysis revealed a significant global model ($p = 2.0 \times 10^{-5}$) after 49, 999 Monte-Carlo permutations, with the first two axes accounting for 97.9% of the association between trait clusters and environmental factors (total inertia; Figure 12). Bivariate analysis between individual trait clusters and explanatory environmental variables revealed significant interactions ($p < 0.05$) between the following: trait cluster A and maximum depth (pos, $p = 0.05$), temperature (neg, $p = 0.014$), and pH (neg, $p = 0.006$). Trait cluster C had a positive interaction with K ($p = 0.038$), and trait cluster F had a negative interaction with depth ($p = 0.025$; Figure 13). Across elevational regions, the alpine region had negative relationships with both temperature and K ($p = 0.005$, $p = 0.038$); and the montane region had positive relationships with temperature, pH and K ($p = 0.005$, $p = 0.013$, $p = 0.005$).

RLQ analysis involving species distribution across functional space indicated that species from the alpine, subalpine and montane regions occupy 13.86%, 7.51% and 78.63% of functional space, respectively (Figure 14). Alpine and subalpine regions consist of a subset of the total larger functional area that is occupied by lower elevation montane species, with the exception of one point representing three alpine species (*H. arcticus*, *H. shoshone* and *Branchinecta paludosa* O. F. Müller, 1788) outside of the montane functional space. Axis one was driven primarily by habitat and size, while axis two was driven by trophic level and pigmentation (Figure 14).

Species Abundance Curves

Species abundance curves compared the functional species composition of alpine to montane elevational regions (Figure 15). A species abundance curve of alpine species showed that the four major species (*D. middendorffiana*, *H. arcticus*, *H. shoshone* and *B. paludosa*) are from trait cluster A (Figure 15A). Minor species (*Eucyclops speratus* Lilljeborg, 1901, *Acroperus harpae* Baird, 1834 and *Alona rectangular* Sars, 1861) are from trait clusters D and F, which are functionally distinctive from trait cluster A. The species abundance curve of montane species showed no particular pattern in trait cluster occurrences, and species present in montane environments were from all six trait clusters (Figure 15B).

Indicator Species Tests

Significant differences in climate predictors (depth and temperature) between sites in which members of trait cluster A were present/absent were determined. *H. arcticus* had the highest ranked change for temperature between sites in which they were present versus absent, and were present in cooler temperatures ($W = 4356.5$, $p = 2.82 \times 10^{-9}$). *D. middendorffiana (*W=3299, p= 0.00016) was also present in significantly cooler temperatures. *L. angustilobus* (W=937, p= 0.015), *Orthocyclops modestus* Herrick, 1883 (W= 1608.5, p = 0.022) and *S. vetulus* (W= 97.5, p= 0.0019) were present in significantly warmer temperatures.

*H. arcticus (*W= 1827.5, p= 0.00061), *H. shoshone* (W= 278, p= 0.036), *L. angustilobus* (W=963.5, p= 0.020), and *L. sicilis* (W= 521.5, $p=9.12 \times 10^{-6}$) were present in significantly deeper waters.

Spatial Analysis

A multiscale ordination (MSO) analysis revealed that there was no significant autocorrelation between the spatial coordinates and variance structure ($p > 0.05$), and explained plus residual variance fell within the confidence intervals for total variance. An

RDA ordination constrained species by latitude and longitude coordinates, and 10.54% of variance was explained with a visible separation between northwestern and southeastern sites (Figure 17).

Discussion

My study highlights the potential for shifts in the taxonomic composition of zooplankton communities along elevational and latitudinal gradients to also affect their functional response to change. Analysis revealed species richness decreased with increasing elevation. Constrained ordinations revealed that environmental variables related to climate best explained taxonomic and functional shifts by these communities along such environmental gradients. Together, my findings reveal the relative lack of functional redundancy that exists within and among species-poor alpine zooplankton communities, thereby making them sensitive to certain environmental changes, especially those related to climatic warming.

Given the lack of species richness and functional redundancy in the alpine, which environmental predictors best explain taxonomic distribution of species across elevation? The five environmental variables that were deemed significant in model selection include climatically influenced aquatic variables, including those that potentially affect lake productivity. For instance, lake surface area and depth as significant predictors of zooplankton communities could easily be seen to decline under a warmer and drier future climate. Similarly, the dilute chemical environments of many alpine lakes (Vinebrooke & Leavitt, 2005) could become more concentrated, thereby highlighting the potential importance of K as a significant explanatory variable of zooplankton community composition and functional structure. For example, changes in lake chemistry are anticipated as warming increases weathering rates due to decreased perennial snow coverage, therefore increasing the ionic composition of remote alpine lakes (i.e., K; Sommaruga-Wograth *et al.,* 1997). In this dataset, although other measures of productivity were not used in analysis due to inconsistent data, K was significantly correlated with total phosphorus (TP), chlorophyll-*a* and total nitrogen (TN). It may therefore have been selected for in model creation due to its important role in comparing different production levels between sites and across elevation. Importantly, K can contribute to nutrient limitation in lake ecosystems (Elser *et al.,* 2007), thereby helping explain why it might have been discovered to be a predictor of zooplankton species turnover.

Alpine species were positively associated with depth and negatively associated with temperature, suggesting that they are likely less tolerant of the more thermally variable conditions that exist near shore or in shallower lakes (Finlay *et al.,* 2001, McMaster, 2003). Two alpine species, *H. arcticus* and *D. middendorffiana*, and one subalpine species, *H. gibberum* had the highest associations with these responses. Montane species (i.e., *A. leptopus*, *D. pulex* and *Polyphemus pediculus* Linnaeus, 1761) had higher associations with increasing temperature, K and pH, and decreasing depth. These species are littoral species, and would therefore have higher tolerance of increasing thermal variability as water depth declines. Therefore, under changes in climate conditions, these species located in the lower right-hand quadrant of Figure 8 may be better suited. Given that these species are primarily montane species (with the exception of subalpine species *Daphnia schodleri* Sars, 1862), dispersal limitations of obligate sexual taxa (i.e., copepods) across long elevational distances may inhibit the transport of thriving species to alpine sites (Jenkins & Underwood, 1998).

Unlike the distinct niche separation of alpine from lower montane species, analysis of the distribution of their traits revealed that there existed a fair amount of functional overlap. However, given the small amount of functional space occupied by alpine and subalpine species (21.37% combined; Figure 14), there is less functional diversity at higher elevations. Although alpine species are within a subset of the greater functional space occupied by montane species, there are still species with functional identities unique to the alpine. For example, the point that lies outside of the montane functional space (Figure 14) represents three alpine species, *H. arcticus, H. shoshone* and *B. paludosa.* These three species all occupy alpine regions, and are members of trait group A, given that they have identical functional identities. Since these functionally similar species lie outside of the range of montane functional space, they are at higher risk to complete functional eradication given they are all cold-adapted alpine species. Additionally, *H. arcticus* and *H. shoshone* do not coexist in nature (Fischer *et al*., 2011), so for these species to compensate for one another functionally, long distance latitudinal dispersal must be possible. However, as obligate sexually reproducing species, overland dispersal as a rescue device is unreasonable (Jenkins & Underwood, 1998). Therefore, this group could be particularly sensitive to environmental changes that could alter community composition, because different functional components of the community could be completely eliminated.

My functional approach also revealed that trait cluster A was sensitive to changes in climate-related increases in water temperature and lake chemistry. Trait cluster A distribution was driven by decreasing temperature, depth and ionic composition, whereas trait clusters D and F were driven by increasing temperature and pH. Climate predictions anticipate increasing temperature and ionic concentrations (Sommaruga-Wograth *et al.*, 1997, Parker *et al.*, 2008), which could favour species of trait cluster F. These species are littoral mixed feeders, and therefore would be fit for shallow waters as climate warms and dries lakes into more temporally variable pond environments (Finlay *et al.,* 2001). Species from trait cluster D are all asexual reproducers, and therefore create resting eggs capable of dispersal. However, given the functional differences between trait cluster A and D/F, littoral mixed feeders would be unable to functionally compensate for losses in alpine species of trait cluster A, if dispersal were available.

Dispersal of regional species could reduce the magnitude of ecosystem changes under certain stress conditions (Thompson & Shurin, 2012). In times of environmental change, the montane species within cluster A could compensate for cold-adapted alpine species if they have a higher tolerance as climate changes; however, these taxa may be limited due to the topography of mountain environments and distance in elevation between montane and alpine sites (Donald *et al.,* 2001). In addition, there are only four cluster A species that are present in montane sites (*S. vetulus*, *L. angustilobus*, *O. modestus*, *L. sicilis*), and therefore there exists only a weak buffering potential of montane species under warmer conditions. Also, only one montane species within trait cluster A (*S. vetulus*) is parthenogenic and produces resting eggs capable of wind-dispersal, making a regional rescue effect unlikely. The buffering effect of regional dispersal on ecosystem function is also less able to withstand the effect of multiple stressors (Thompson & Shurin, 2012), making the future bleak in the face of predicted climate conditions (Parker *et al.,* 2008). Furthermore, the subalpine transition zone between montane and alpine regions occupies a trivial functional niche (7.51% of total functional area), with minimal overlap of the alpine region therefore lacking any added redundancy if dispersal limitations were not an issue.

Species in the alpine region lacked both taxonomic and functional diversity and redundancy needed to compensate under dynamic climate conditions. Generally, species abundance curves show a few major species that are then compensated for by functionally similar minor species if they are extirpated (Walker *et al.,* 1999). In the alpine, however, lower species richness reduces the probability of functional compensation because there is a lack of redundancy (Figure 15; Lyons & Vinebrooke, 2016). The most frequently occurring species in the alpine are all of the same functional group (trait cluster A), compared to the vast functional diversity and redundancy in species of the montane region (Figure 15B). The minor species that occupy the alpine less frequently are functionally dissimilar (trait clusters D and F) to the major species, therefore eliminating the opportunity for compensation after environmental perturbation (Figure 15). Alpine lakes and ponds lack functional diversity, which could have consequences if climatic changes begin to eliminate the most sensitive cold-adapted species (i.e., *D. middendorffiana*, *H. arcitcus*).

Functional overlap among alpine species themselves was also minimal compared to their wide distribution (Figure 10). Trait cluster A has a combination of both montane and alpine species, so the differences in occupancy across elevation could be driving some of this apparent separation. However, 57% of alpine sites have zero coexistence between trait cluster A members, and only 0.1% of sites contain greater than two trait cluster A species, therefore, reducing the functional redundancy within alpine lakes. Whether the lack of co-occurrence of these species is spatially (Fischer *et al.,* 2011) or biotically driven by interspecific interactions is unknown and demands greater examination.

Endemic alpine species (i.e., *H. arcticus* and *D. middendorffiana*) associated with climate predictors may be at pronounced risk as climate instability becomes more apparent. *D. middendorffiana* and *H. arcticus* were present in significantly colder sites, making these species prone to decline in warmer conditions. This corroborates with both laboratory and field experiments that showed a decline in large alpine species under warmer conditions (i.e., Strecker *et al.,* 2004, Holzapfel & Vinebrooke, 2005). In addition, *H. arcitcus* was also present in significantly deeper sites, indicating that they may also be sensitive to predicted shallow conditions. Since both of these environmental parameters are likely to change as climate becomes warmer and drier (Parker *et al.,* 2008), it is of value to use these species as indicators for early climate transitions in alpine communities (i.e., Khamis *et al.,* 2014). The significant association between these two cold-adapted species and temperature make them more sensitive to warming, whereas other species may be less prone to extinction under such conditions (i.e., *S. vetulus*, Table 7), maybe allowing them to replace them functionally within the ecosystem. Given that *H. arcticus* has the same functional identity as both *H. shoshone* and *B. paludosa*, there is some redundancy in function (although not often within sites). The functional diversity of alpine systems is small, and by eliminating key endemic species, it jeopardizes overall ecosystem function.

Given that there was no significant autocorrelation between spatial coordinates and variance structure, it can be assumed that the variance explained by environmental variables in RDA analysis is true across sites. There is a separation of sites between the Yukon Territory sites in the Northwest, and the sites of the Rocky Mountains towards the southeast (Figure 17). This explains why there is a considerable amount of variance explained by latitude and longitude coordinates. Additional analysis was done to compare the spatial extent of the dataset by running RLQ and fourth corner analysis on 147 sites from only the southeast sites in the Canadian Rockies, and similar clusters were revealed. One cluster was comparable to cluster A from the original analysis, including both *H. arcitcus* and *D. middendorffiana* and was positively associated with depth, and negatively associated with temperature and K. Therefore, spatially driven regional interactions of community composition are not statistically affecting the locally driven interactions in this study, however biological interactions warrant greater investigation.

My findings highlight certain knowledge gaps in mountain limnology and zooplankton ecology that could serve as a basis for future research directions. For example, the low amount of taxonomic variance explained (~15%) when constrained by the set of environmental variables points to the need for more comprehensive, repeated sampling of additional variables over a greater time scale. As well, inconsistencies in the data meant that I was not able to test limiting nutrients as measures of productivity (i.e., total phosphorus (TP), total nitrogen (TN)). Furthermore, future research should look at a broader number of environmental and chemical factors to address the synchronous effects of multiple environmental changes. Limitations of this study include the shortage of functional trait descriptors at the species level (Benedetti *et al.,* 2015). Future studies should aim to compile a large database of zooplankton functional traits at both large scale and local levels that are imperative to improve the precision of functional group analysis. Locally specific functional traits, such as pigmentation, should also be developed to address site-specific stressors and selection for fitness predictor traits around the globe.

Conclusions

My findings enabled me to offer predictions of how and why crustacean zooplankton communities will respond to future climate conditions. Species richness, taxonomic diversity and functional diversity decreased with increasing elevation, creating a sensitive alpine realm. The environmental predictors of community composition across elevation were primarily climate-related, highlighting how these communities show pronounced changes under increasingly more variable climatic conditions. Additionally, these predictors drive the functional structure of mountain lake and pond communities, revealing a lack of functional redundancy at high elevations. Most alpine species are classified into trait cluster A, the larger, pigmented group, which have low site redundancy and dispersal potential. Trait cluster A species are associated with decreasing temperature and K, as well as increasing depth, therefore making them sensitive to climate change. *H. arcitcus* and *D. middendorffiana* could act as indicator species for early signs of climate change, as they have the strongest associations with these climatic predictors. There is little functional redundancy in alpine communities, making these fragile communities sensitive to environmental perturbation. With mountain climate shifting towards warmer and drier conditions, I anticipate a shift in zooplankton community composition towards smaller, littoral species, at the expense of cold-adapted pigmented endemic species, therefore altering the functional responses and effect traits of alpine ecosystems.

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Table 1: Site names, regional distribution and source of historical data for each site in analysis. Note: BANF= Banff National Park, BC NORTH= Northern British Columbia, GLAC= Glacier National Park, JASP= Jasper National Park, KOOT= Kootenay National Park, REVE= Revelstoke National Park, WATE= Waterton Lakes National Park, YOHO= Yoho National Park, YUK NORTH= Northern Yukon Territory, YUK SOUTH= Southern Yukon Territory.

Lake name	**Region**	**Source**
Agnes Lake	BANF	Mayhood & Anderson 1976 (LAKE LOUISE, Part 2)
Akamina Lake	WATE	Anderson & Donald 1976 (WATERTON-SMALL LAKES)
Amethyst Lake	JASP	Anderson & Donald 1978 (JASP Part 3. 'amethyst & moat')
Amiskwi Lake	YOHO	Mudry & Anderson. 1975 (Yoho National Park ARI)
Annette B	BANF	Mayhood & Anderson 1976 (LAKE LOUISE, Part 2)
Annette J	JASP	Anderson & Donald 1978 (JASP Part 4. townsite)
Atlin	BC NORTH	Patalas, Patalas & Salki. 1994. Can. Tech. Rep. Fish. Aquat. Sci.
Baker Lake	BANF	Mayhood & Anderson 1976 (LAKE LOUISE, Part 2)
Baker Lake (little)	BANF	Mayhood & Anderson 1976 (LAKE LOUISE, Part 2)
Beauvert Lake	JASP	Anderson & Donald 1978 (JASP Part 4. townsite)
Beaver Pond A (W33)	WATE	Anderson & Donald 1976 (WATERTON-THE PONDS)
Bench Lake	JASP	Anderson & Donald 1980 (JASP 'westblock')
Bertha Lake	WATE	Anderson & Donald 1976 (WATERTON-SMALL LAKES)
Bighorn Lake	BANF	Anderson & Donald 1978 (Cascade Trail)
Boom Lake	BANF	Mayhood & Anderson 1976 (LAKE LOUISE, Part 2)
Bow Lake	BANF	Anderson.1974. JFRBD
Brachiopod Lake	BANF	Mayhood & Anderson 1976 (LAKE LOUISE, Part 2)
Braeburn	YUK SOUTH	Patalas, Patalas & Salki. 1994. Can. Tech. Rep. Fish. Aquat. Sci.
Buck Lake	JASP	Donald & De Henau 1981 (JASP Part 8. 'icefields')
Buffalo Lake	WATE	Anderson & Donald 1976 (WATERTON-SMALL LAKES)
Cabin Lake	JASP	Anderson & Donald 1980 (JASP 'westblock')
Caledonia Lake	JASP	Anderson & Donald 1980 (JASP 'westblock')
Caribou Lake (lower)	JASP	Donald, Alger & Antoniuk 1985 (JASP Part 10. 'north boundary')
Caribou Lake (upper)	JASP	Donald, Alger & Antoniuk 1985 (JASP Part 10. 'north boundary')
Carthew Lake (lower)	WATE	Anderson & Donald 1976 (WATERTON-SMALL LAKES)
Celestine Lake	JASP	Donald, Alger & Antoniuk 1985 (JASP Part 10. 'north boundary')
Chalet Pond	JASP	Donald & De Henau 1981 (JASP Part 8. 'icefields')
Christine Lake	JASP	Anderson & Donald 1980 (JASP 'westblock')
Cobb Lake	KOOT	Donald & Alger 1984 a (KOOTENAY)
Colfair Lake (lower)	JASP	Donald & De Henau 1981 (JASP Part 8. 'icefields')
Colfair Lake (upper)	JASP	Donald & De Henau 1981 (JASP Part 8. 'icefields')
Consolation Lake (lower)	BANF	Mayhood & Anderson 1976 (LAKE LOUISE, Part 2)
Consolation Lake (upper)	BANF	Mayhood & Anderson 1976 (LAKE LOUISE, Part 2)
Crandell Lake	WATE	Anderson & Donald 1976 (WATERTON-SMALL LAKES)

Crypt Lake	WATE	Anderson & Donald 1976 (WATERTON-SMALL LAKES)
Crystal Lake	YOHO	Mudry & Anderson. 1975 (Yoho National Park ARI)
Cuthead Lake	BANF	Anderson & Donald 1978 (Cascade Trail)
Cutt Lake	JASP	Anderson & Donald 1980 (JASP 'westblock')
Daer Lake	KOOT	Donald & Alger 1984 a (KOOTENAY)
Dalayee	YUK SOUTH	Patalas, Patalas & Salki. 1994. Can. Tech. Rep. Fish. Aquat. Sci.
Dezadeash	YUK SOUTH	Patalas, Patalas & Salki. 1994. Can. Tech. Rep. Fish. Aquat. Sci.
Diamain	YUK NORTH	Patalas, Patalas & Salki. 1994. Can. Tech. Rep. Fish. Aquat. Sci.
Dog Lake K	KOOT	Donald & Alger 1984 a (KOOTENAY)
Dolly Lake	JASP	Donald, Alger & Antoniuk 1985 (JASP Part 10. 'north boundary')
Dorothy Lake	JASP	Anderson & Donald 1980 (JASP 'westblock')
Dragon Lake	JASP	Donald & De Henau 1981 (JASP Part 8. 'icefields')
Dragon YS	YUK SOUTH	Patalas, Patalas & Salki. 1994. Can. Tech. Rep. Fish. Aquat. Sci.
Duchesnay Lake	YOHO	Mudry & Anderson. 1975 (Yoho National Park ARI)
Edith Lake	JASP	Anderson & Donald 1978 (JASP Part 4. townsite)
Edwards Lake	JASP	Donald & De Henau 1981 (JASP Part 8. 'icefields')
Eiffel Lake	BANF	Mayhood & Anderson 1976 (LAKE LOUISE, Part 2)
Elliot	YUK NORTH	Patalas, Patalas & Salki. 1994. Can. Tech. Rep. Fish. Aquat. Sci.
Emerald Lake	YOHO	Mudry & Anderson. 1975 (Yoho National Park ARI)
Eva Lake	REVE	Donald & Alger 1984 b (GLAC/REVE)
Fairy Lake	YOHO	Mudry & Anderson. 1975 (Yoho National Park ARI)
Finlayson	YUK SOUTH	Patalas, Patalas & Salki. 1994. Can. Tech. Rep. Fish. Aquat. Sci.
Floe Lake	KOOT	Donald & Alger 1984 a (KOOTENAY)
Fox	YUK SOUTH	Patalas, Patalas & Salki. 1994. Can. Tech. Rep. Fish. Aquat. Sci.
Frances	YUK SOUTH	Patalas, Patalas & Salki. 1994. Can. Tech. Rep. Fish. Aquat. Sci.
G7 Pond	GLAC	Donald & Alger 1984 b (GLAC/REVE)
Geraldine Lake (lower)	JASP	Donald & De Henau 1981 (JASP Part 8. 'icefields')
Geraldine Lake #3	JASP	Donald & De Henau 1981 (JASP Part 8. 'icefields')
Giant's Mirror	WATE	Anderson & Donald 1976 (WATERTON-SMALL LAKES)
Golden Lake	JASP	Anderson & Donald 1980 (JASP 'westblock')
Grouse Lake	BANF	Anderson & Donald 1978 (Cascade Trail)
Hamilton Lake	YOHO	Mudry & Anderson. 1975 (Yoho National Park ARI)
Harrison Lake	BANF	Anderson & Donald 1978 (Cascade Trail)
Harvey Lake	JASP	Donald, Alger & Antoniuk 1985 (JASP Part 10. 'north boundary')
Herbert Lake	BANF	Mayhood & Anderson 1976 (LAKE LOUISE, Part 2)
Herbert Lake (little)	BANF	Mayhood & Anderson 1976 (LAKE LOUISE, Part 2)
Herbert Pond	BANF	Mayhood & Anderson 1976 (LAKE LOUISE, Part 2)
Hibernia Lake	JASP	Anderson & Donald 1980 (JASP 'westblock')
Hidden Lake B	BANF	Mayhood & Anderson 1976 (LAKE LOUISE, Part 2)
Hidden Lake J	JASP	Anderson & Donald 1980 (JASP 'westblock')
High Lake Five	JASP	Anderson & Donald 1980 (JASP 'westblock')

High Lake Four	JASP	Anderson & Donald 1980 (JASP 'westblock')
High Lake One	JASP	Anderson & Donald 1980 (JASP 'westblock')
High Lake Seven	JASP	Anderson & Donald 1980 (JASP 'westblock')
High Lake Three	JASP	Anderson & Donald 1980 (JASP 'westblock')
Honeymoon Lake	JASP	Donald & De Henau 1981 (JASP Part 8. 'icefields')
Honeymoon Lake (little)	JASP	Donald & De Henau 1981 (JASP Part 8. 'icefields')
Horseshoe Lake	JASP	Anderson & Donald 1978 (JASP Part 4. townsite)
Hostel Pond (Mile 54)	JASP	Donald & De Henau 1981 (JASP Part 8. 'icefields')
Hungabee Lake (mid Opabin)	YOHO	Mudry & Anderson. 1975 (Yoho National Park ARI)
Indian Springs Lake (W46)	WATE	Anderson & Donald 1976 (WATERTON-THE PONDS)
Indigo Lake (aka Frigid)	YOHO	Mudry & Anderson. 1975 (Yoho National Park ARI)
Iris Lake	JASP	Anderson & Donald 1980 (JASP 'westblock')
Island Lake	BANF	Mayhood & Anderson 1976 (LAKE LOUISE, Part 2)
Jade Lake (Lower)	REVE	Donald & Alger 1984 b (GLAC/REVE)
Jade Lake (Upper)	REVE	Donald & Alger 1984 b (GLAC/REVE)
K13 Pond	KOOT	Donald & Alger 1984 a (KOOTENAY)
K16 Pond	KOOT	Donald & Alger 1984 a (KOOTENAY)
K20 Pond	KOOT	Donald & Alger 1984 a (KOOTENAY)
K24 Pond	KOOT	Donald & Alger 1984 a (KOOTENAY)
K4 Pond	KOOT	Donald & Alger 1984 a (KOOTENAY)
Kerkeslin Lake	JASP	Donald, Alger & Antoniuk 1985 (JASP Part 10. 'north boundary')
Kesler Lake	WATE	Donald, Alger & Antoniuk 1985 (JASP Part 10. 'north boundary')
Kingfisher Lake	BANF	Donald, Alger & Antoniuk 1985 (JASP Part 10. 'north boundary')
Kingfisher Pond	BANF	Donald, Alger & Antoniuk 1985 (JASP Part 10. 'north boundary')
Kiwetinok Lake	YOHO	Donald, Alger & Antoniuk 1985 (JASP Part 10. 'north boundary')
Kluane	YUK SOUTH	Donald, Alger & Antoniuk 1985 (JASP Part 10. 'north boundary')
Kootenay Pond	KOOT	Donald, Alger & Antoniuk 1985 (JASP Part 10. 'north boundary')
Leach Lake	JASP	Donald, Alger & Antoniuk 1985 (JASP Part 10. 'north boundary')
Lectern Lake	JASP	Donald, Alger & Antoniuk 1985 (JASP Part 10. 'north boundary')
Lineham Lake (north)	WATE	Donald, Alger & Antoniuk 1985 (JASP Part 10. 'north boundary')
Little Gem Lake	YOHO	Mayhood & Anderson 1976 (LAKE LOUISE, Part 2)
Lone Lake	WATE	Mayhood & Anderson 1976 (LAKE LOUISE, Part 2)
Long Lake	JASP	Mayhood & Anderson 1976 (LAKE LOUISE, Part 2)
Lost Lake B	BANF	Mayhood & Anderson 1976 (LAKE LOUISE, Part 2)
Lost Lake W	WATE	Mayhood & Anderson 1976 (LAKE LOUISE, Part 2)
Louise Lake	BANF	Mayhood & Anderson 1976 (LAKE LOUISE, Part 2)
Maligne Lake	JASP	Mayhood & Anderson 1976 (LAKE LOUISE, Part 2)
Marjorie Lake	JASP	Mayhood & Anderson 1976 (LAKE LOUISE, Part 2)
Mary Lake	YOHO	Mayhood & Anderson 1976 (LAKE LOUISE, Part 2)
McClintock	YUK SOUTH	Mayhood & Anderson 1976 (LAKE LOUISE, Part 2)
McNair Pond	BANF	Mayhood & Anderson 1976 (LAKE LOUISE, Part 2)

Meadow Creek Beaver Pond	KOOT	Mayhood & Anderson 1976 (LAKE LOUISE, Part 2)
Miette Lake	JASP	Mayhood & Anderson 1976 (LAKE LOUISE, Part 2)
Mile 16 1/2 Lake	JASP	Mayhood & Anderson 1976 (LAKE LOUISE, Part 2)
Millar Lake	REVE	Mayhood & Anderson 1976 (LAKE LOUISE, Part 2)
Mina Lake	JASP	Mayhood & Anderson 1976 (LAKE LOUISE, Part 2)
Minnow Lake	JASP	Mayhood & Anderson 1976 (LAKE LOUISE, Part 2)
Minto	YUK NORTH	Mayhood & Anderson 1976 (LAKE LOUISE, Part 2)
Moab Lake	JASP	Mayhood & Anderson 1976 (LAKE LOUISE, Part 2)
Mona Lake	JASP	Mayhood & Anderson 1976 (LAKE LOUISE, Part 2)
Moraine Lake	BANF	Mayhood & Anderson 1976 (LAKE LOUISE, Part 2)
Mud Lake	BANF	Mayhood & Anderson 1976 (LAKE LOUISE, Part 2)
Nares	YUK SOUTH	Mayhood & Anderson 1976 (LAKE LOUISE, Part 2)
Nixon Pond	KOOT	Mayhood & Anderson 1976 (LAKE LOUISE, Part 2)
O'Beirne Lake #1	JASP	Mayhood & Anderson 1976 (LAKE LOUISE, Part 2)
O'Beirne Lake #2	JASP	Mayhood & Anderson 1976 (LAKE LOUISE, Part 2)
O'Beirne Lake #4	JASP	Mudry & Anderson. 1975 (Yoho National Park ARI)
O'Brien Lake	BANF	Mudry & Anderson. 1975 (Yoho National Park ARI)
O'Hara Lake	YOHO	Mudry & Anderson. 1975 (Yoho National Park ARI)
Oesa Lake	YOHO	Mudry & Anderson. 1975 (Yoho National Park ARI)
Olive Lake	KOOT	Mudry & Anderson. 1975 (Yoho National Park ARI)
Opabin Lake (upper)	YOHO	Mudry & Anderson. 1975 (Yoho National Park ARI)
Osprey Lake	JASP	Mudry & Anderson. 1975 (Yoho National Park ARI)
Palmer	BC NORTH	Mudry & Anderson. 1975 (Yoho National Park ARI)
Patricia Lake	JASP	Mudry & Anderson. 1975 (Yoho National Park ARI)
Pipit Lake	BANF	Mudry & Anderson. 1975 (Yoho National Park ARI)
Ptarmigan Lake	BANF	Mudry & Anderson. 1975 (Yoho National Park ARI)
Pyramid Lake	JASP	Mudry & Anderson. 1975 (Yoho National Park ARI)
Quiet	YUK SOUTH	Mudry & Anderson. 1975 (Yoho National Park ARI)
R11 Pond	REVE	Mudry & Anderson. 1975 (Yoho National Park ARI)
R14 Pond	REVE	Mudry & Anderson. 1975 (Yoho National Park ARI)
Rathlin Lake	JASP	Mudry & Anderson. 1975 (Yoho National Park ARI)
Redoubt Lake	BANF	Mudry & Anderson. 1975 (Yoho National Park ARI)
Reid	YUK NORTH	Mudry & Anderson. 1975 (Yoho National Park ARI)
Riley Lake	JASP	Patalas, Patalas & Salki. 1994. Can. Tech. Rep. Fish. Aquat. Sci.
Rowe Lake (lower)	WATE	Patalas, Patalas & Salki. 1994. Can. Tech. Rep. Fish. Aquat. Sci.
Rowe Lake (upper)	WATE	Patalas, Patalas & Salki. 1994. Can. Tech. Rep. Fish. Aquat. Sci.
Ruby Lake	WATE	Patalas, Patalas & Salki. 1994. Can. Tech. Rep. Fish. Aquat. Sci.
Saturday Night Lake	JASP	Patalas, Patalas & Salki. 1994. Can. Tech. Rep. Fish. Aquat. Sci.
Sentinel Lake	BANF	Patalas, Patalas & Salki. 1994. Can. Tech. Rep. Fish. Aquat. Sci.
Sherbrooke Lake	YOHO	Patalas, Patalas & Salki. 1994. Can. Tech. Rep. Fish. Aquat. Sci.
Sink Lake	YOHO	Patalas, Patalas & Salki. 1994. Can. Tech. Rep. Fish. Aquat. Sci.

Snowflake Lake	BANF	Patalas, Patalas & Salki. 1994. Can. Tech. Rep. Fish. Aquat. Sci.
Squanga	YUK SOUTH	Patalas, Patalas & Salki. 1994. Can. Tech. Rep. Fish. Aquat. Sci.
Storm Lake (Upper)	KOOT	Patalas, Patalas & Salki. 1994. Can. Tech. Rep. Fish. Aquat. Sci.
Summit Lake Y	YOHO	Patalas, Patalas & Salki. 1994. Can. Tech. Rep. Fish. Aquat. Sci.
Tatchum	YUK SOUTH	Patalas, Patalas & Salki. 1994. Can. Tech. Rep. Fish. Aquat. Sci.
Taylor Lake	BANF	Patalas, Patalas & Salki. 1994. Can. Tech. Rep. Fish. Aquat. Sci.
Teenah	YUK SOUTH	Patalas, Patalas & Salki. 1994. Can. Tech. Rep. Fish. Aquat. Sci.
Tilted Lake	BANF	Patalas, Patalas & Salki. 1994. Can. Tech. Rep. Fish. Aquat. Sci.
Topaz Lake	JASP	Patalas, Patalas & Salki. 1994. Can. Tech. Rep. Fish. Aquat. Sci.
Twin Lake (upper)	WATE	Patalas, Patalas & Salki. 1994. Can. Tech. Rep. Fish. Aquat. Sci.
Twintree Lake	JASP	Patalas, Patalas & Salki. 1994. Can. Tech. Rep. Fish. Aquat. Sci.
Virl Lake	JASP	Patalas, Patalas & Salki. 1994. Can. Tech. Rep. Fish. Aquat. Sci.
Wapta Lake	YOHO	Patalas, Patalas & Salki. 1994. Can. Tech. Rep. Fish. Aquat. Sci.
Watson	YUK SOUTH	Patalas, Patalas & Salki. 1994. Can. Tech. Rep. Fish. Aquat. Sci.
YUK16 (Barlow)	YUK NORTH	Pienitz, R., Smol, J.P., and D.R.S. Lean. 1997. Can. J. Fish. Aquat. Sci.
YUK21 (Chapman)	YUK NORTH	Pienitz, R., Smol, J.P., and D.R.S. Lean. 1997. Can. J. Fish. Aquat. Sci.
YUK7 (Twin)	YUK SOUTH	Pienitz, R., Smol, J.P., and D.R.S. Lean. 1997. Can. J. Fish. Aquat. Sci.

Table 2: Environmental variables and associated variance explained after manual forward selection that identified significant ($p < 0.05$) and independent environmental predictors of variance in zooplankton community composition across sites. ** = significant $p < 0.01$

Variable	Variance
Temperature	0.03884**
Maximum Depth	0.02152**
Potassium (K)	0.01884**
Surface Area	0.01840**
pH	0.01800**

Table 3: Mean, median and range of values for each significant, independent environmental variable used to explain variance in zooplankton community composition (before scaling) across sites.

Variable	Mean	Median	Range
Temperature (°C)	12.6	11.9	4.7- 22.3
Maximum Depth (m)	15.5	11.0	0.5- 100
Potassium (mg/L)	0.5	0.4	0.1- 2.8
Surface Area (ha)	255.3	8.2	0.4- 10610
pH (unit)	8.0	8.1	6.7- 9.5

Table 4: Species richness as number of zooplankton species from *n=174* sites in three altitudinal regions Montane = < 1500 m, Subalpine = 1500-2000 m, Alpine= > 2000 m

Region	**Mean**	**Median**	**Range**
Montane ***(n= 77)***	6.8	7	2-14
Subalpine ***(n=58)***	4.5	4	1-15
Alpine ***(n=39)***	4.4	4	1-9

Table 5: Literature review database of six functional traits for each of the 49 species and associated references that were used in cluster analysis of trait cluster groups. MAA= mycosporine-like amino acids, N.D.= not determined, S= small: 0-1 mm, M= medium: 1-2 mm, L= large: >2 mm.

Species name	**Pigmentation**	**Trophic**	**Habitat**	**Feeding**[4]	**Reproduction**[4]	**Size Class**
Acanthocyclops capillatus G.O. Sars, 1863	N.D.	Omnivore[6]	Pelagic[6]	Rap	Sexual	M[8]
Acanthocyclops vernalis Fischer, 1853	MAA[14]	Omnivore[4]	Pelagic[4]	Rap	Sexual	M[13]
Acanthodiaptomus denticornis Wierzejski, 1887	Carotenoid[15]	Omnivore[33]	Pelagic	Filter	Sexual	M[8]
Acroperus harpae Baird, 1834	N.D.	Herbivore[1]	Littoral[1]	Filter	Asexual	S[8]
Aglaodiaptomus leptopus S. A. Forbes, 1882	N.D.	Omnivore[7]	Littoral[7]	Filter	Sexual	M[7]
Alona affinis Leydig, 1860	Melanin[16]	Herbivore[1]	Littoral[1]	Filter	Asexual	S[8]
Alona guttata G. O. Sars, 1862	N.D.	Herbivore[1]	Littoral[1]	Filter	Asexual	S[8]
Alona rectangular Sars, 1861	N.D.	Herbivore[1]	Littoral[1]	Filter	Asexual	S[8]
Alonella nana Baird, 1850	N.D.	Herbivore[1]	Littoral[1]	Filter	Asexual	S[8]
Arctodiaptomus arapahoensis Dodds, 1915	Carotenoid[17]	Omnivore	Pelagic	Filter	Sexual	M[13]
Bosmina coregoni Baird, 1857	N.D.	Herbivore[1]	Pelagic[1]	Filter	Asexual	S[8]
Bosmina longirostris O. F. Müller, 1776	N.D.[18]	Herbivore[1]	Pelagic[1]	Filter	Asexual	S[7]
Branchinecta paludosa O. F. Müller, 1788	Carotenoid[19]	Omnivore	Pelagic[2]	Filter	Sexual	L[9]
Ceriodaphnia affinis Lilljeborg, 1900	N.D.	Herbivore[1]	Pelagic[1]	Filter	Asexual	S[11]
Ceriodaphnia lacustris Birge, 1893	N.D.	Herbivore[1]	Pelagic[1]	Filter	Asexual	S[8]

Ceriodaphnia quadrangular O. F. Müller, 1785	N.D.[18]	Herbivore[1]	Pelagic[1]	Filter	Asexual	S[8]
Ceriodaphnia reticulate Jurine, 1820	Melanin[20]	Herbivore[1]	Pelagic[1]	Filter	Asexual	S[8]
Chydorus sphaericus O. F. Mueller, 1785	Melanin[21]	Herbivore[1]	Littoral[1]	Filter	Asexual	S[8]
Cyclops scutifer G. O. Sars, 1863	N.D.[22]	Omnivore[4]	Pelagic[4]	Rap	Sexual	M[8]
Daphnia catawba Coker, 1926	N.D.[23]	Herbivore[1]	Pelagic[1]	Filter	Asexual	M[8]
Daphnia dentifera Forbes, 1893	Melanin[24]	Herbivore[1]	Pelagic[1]	Filter	Asexual	M[8]
Daphnia galeata mendotae Birge, 1918	N.D.[25]	Herbivore[1]	Pelagic[1]	Filter	Asexual	L[8]
Daphnia longiremis G. O. Sars, 1861	N.D.	Herbivore[1]	Pelagic[1]	Filter	Asexual	S[8]
Daphnia longispina O. F. Mueller, 1785	Melanin[26]	Herbivore[1]	Pelagic[1]	Filter	Asexual	M
Daphnia middendorffiana Fischer, 1851	Melanin[27]	Herbivore[1]	Pelagic[1]	Filter	Asexual	L[8]
Daphnia pulex Leydig, 1860	Melanin[25]	Herbivore[1]	Pelagic[1]	Filter	Asexual	M[8]
Daphnia schodleri Sars, 1862	N.D.	Herbivore[1]	Pelagic[1]	Filter	Asexual	M[8]
Diacyclops thomasi S. A. Forbes, 1882	MAA[14]	Omnivore[1]	Pelagic[1]	Rap	Sexual	S[8]
Diaphanosoma birgei Korinek, 1981	N.D.	Herbivore[1]	Littoral[1]	Filter	Asexual	M[12]
Eucyclops agilis Koch, 1838	N.D.	Omnivore[7]	Littoral[7]	Rap	Sexual	M[8]
Eucyclops speratus Lilljeborg, 1901	N.D.	Omnivore	Littoral	Rap	Sexual	M[8]
Eurycercus lamellatus O. F. Mueller, 1776	N.D.	Herbivore[1]	Littoral[1]	Filter	Asexual	L[8]
Gammarus lacustris G. O. Sars, 1863	N.D.	Omnivore	Benthic	Filter	Sexual	L[10]
Hesperodiaptomus arcticus Marsh, 1920	Carotenoid[21]	Omnivore[2]	Pelagic[2]	Filter	Sexual	L[8]

Hesperodiaptomus shoshone S. A. Forbes, 1893	Carotenoid[14]	Omnivore[2]	Pelagic[2]	Filter	Sexual	L[7]
Heterocope septentrionalis Juday & Muttkowski, 1915	Carotenoid[19]	Predator[1]	Pelagic[1]	Filter	Sexual	L[8]
Holopedium gibberum Zaddach, 1855	N.D.[17]	Herbivore[4]	Pelagic[4]	Filter	Asexual	M[8]
Hyalella Azteca Saussure, 1858	Melanin[28]	Herbivore[5]	Benthic[5]	Filter	Sexual	L
Leptodiaptomus angustilobus Sars G.O., 1898	Carotenoid[29]	Omnivore	Pelagic	Filter	Sexual	M[8]
Leptodiaptomus nudus Marsh, 1904	N.D.	Omnivore	Pelagic	Filter	Sexual	M[7]
Leptodiaptomus sicilis S. A. Forbes, 1882	Carotenoid[30]	Omnivore[4]	Pelagic[4]	Filter	Sexual	M[7]
Leptodiaptomus tyrrelli Poppe, 1888	Carotenoid[17]	Herbivore	Pelagic	Filter	Sexual	M[13]
Leptodora kindtii Focke, 1844	N.D.	Predator[3]	Pelagic	Rap	Asexual	L[13]
Macrocyclops albidus Jurine, 1820	Carotenoid[31]	Omnivore[1]	Littoral[1]	Rap	Sexual	M[8]
Microcyclops varicans G. O. Sars, 1863	N.D.	Omnivore[6]	Littoral[6]	Rap	Sexual	S[6]
Orthocyclops modestus Herrick, 1883	Carotenoid[17]	Omnivore[2]	Pelagic[2]	Rap	Sexual	M[8]
Polyphemus pediculus Linnaeus, 1761	Carotenoid[19]	Predator[1]	Pelagic[1]	Filter	Asexual	M[8]
Scapholeberis kingi G. O. Sars, 1888	Melanin[20]	Herbivore[1]	Littoral[1]	Filter	Asexual	S[8]
Simocephalus vetulus O. F. Müller, 1776	Carotenoid[32]	Herbivore	Pelagic	Filter	Asexual	L[8]

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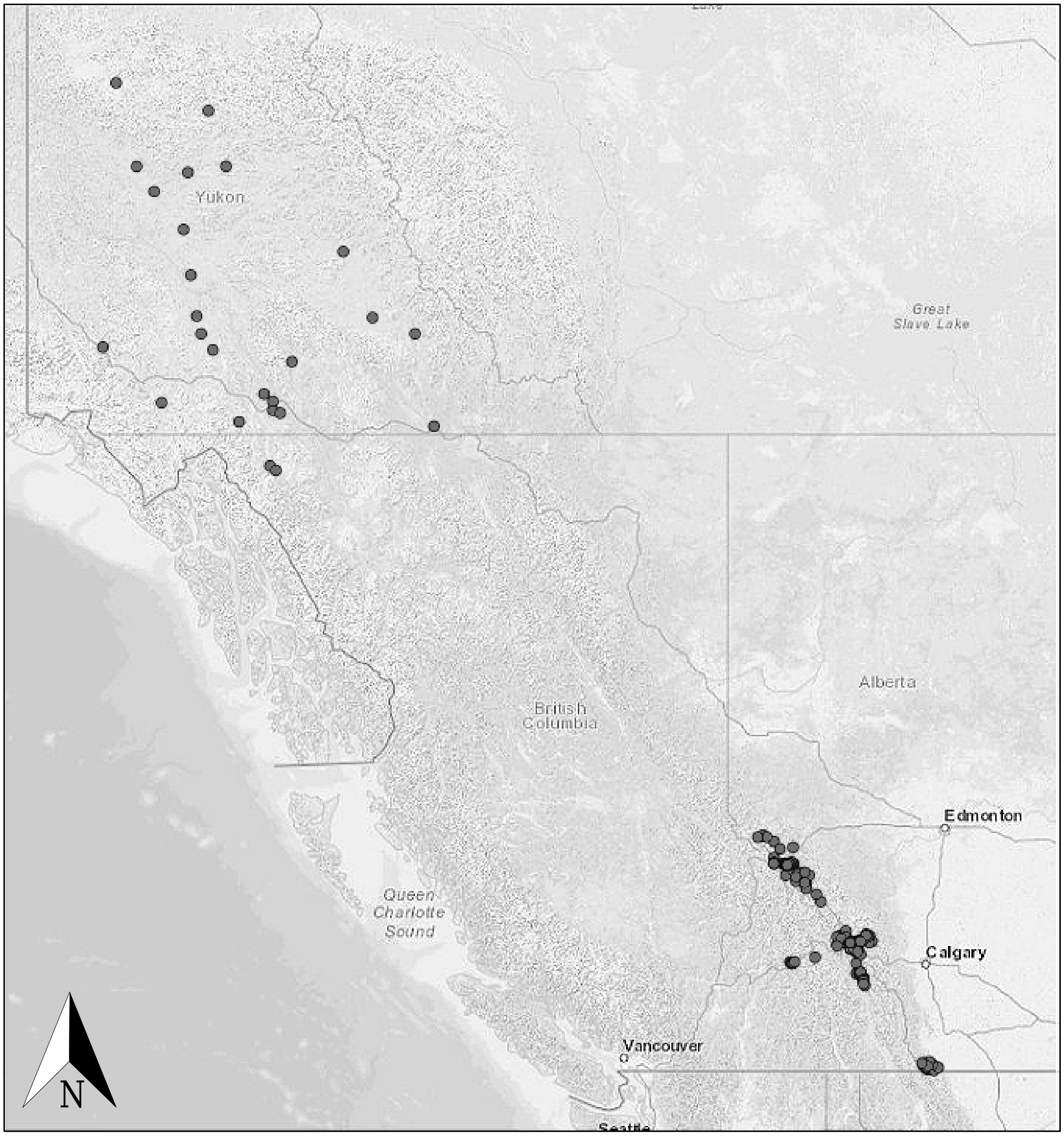
Note: Species without a citation were categorized based on taxonomic similarity to cited species.

Table 6: Six trait cluster groups based on literature trait review after cluster analysis using Ward's minimum variance method of hierarchical clustering.

Trait Cluster	**Species**	**Trait Cluster**	**Species**
A: large, pigmented	*Acanthocyclops vernalis* *Arctodiaptomus arapahoensis* *Branchinecta paludosa* *Daphnia middendorffiana* *Hesperodiaptomus arcticus* *Hesperodiaptomus shoshone* *Leptodiaptomus angustilobus* *Leptodiaptomus sicilis* *Orthocyclops modestus* *Simocephalus vetulus*	D: littoral, asexual herbivores	*Acroperus harpae* *Alona guttata* *Alona rectangular* *Alonella nana* *Diaphanosoma birgei*
B: herbivores	*Bosmina coregoni* *Bosmina longirostris* *Ceriodaphnia affinis* *Ceriodaphnia lacustris* *Ceriodaphnia quadrangula* *Daphnia catawba* *Daphnia longiremis* *Daphnia schodleri* *Holopedium gibberum*	E: high pigment, mixed-feeders	*Acanthodiaptomus denticornis* *Ceriodaphnia reticulata* *Daphnia dentifera* *Daphnia longispina* *Daphnia pulex* *Diacyclops thomasi* *Gammarus lacustris* *Hyalella azteca* *Leptodiaptomus tyrrelli*
C: low pigment, mixed-feeders	*Acanthocyclops capillatus* *Cyclops scutifer* *Daphnia galeata mendotae* *Heterocope septentrionalis* *Leptodiaptomus nudus* *Leptodora kindtii* *Polyphemus pediculus*	F: littoral mixed feeders	*Aglaodiaptomus leptopus* *Alona affinis* *Chydorus sphaericus* *Eucyclops agilis* *Eucyclops speratus* *Eurycercus lamellatus* *Macrocyclops albidus* *Microcyclops varicans* *Scapholeberis kingi*

Table 7: Wilcoxon Signed-Rank test compared parameters (temperature and depth) across sites which species of Trait cluster A are present versus absent. Note: '-' represents a non-significant relationship.

Species	**Parameter**	**W**	***p*-value**	**Interpretation**
H. arcticus	Temperature	4356.5	2.8×10^{-9}	Present in lower mean temperatures
	Depth	1827.5	0.00061	Present in higher mean depth
H. shoshone	Temperature	-	-	-
	Depth	278	0.036	Present in higher mean depths
D. middendorffiana	Temperature	3298.5	0.00016	Present in lower mean temperatures
	Depth	-	-	-
L. angustilobus	Temperature	937	0.015	Present in higher mean temperatures
	Depth	963.5	0.020	Present in higher mean depth
L. sicilis	Temperature	-	-	-
	Depth	521.5	9.1×10^{-6}	Present in higher mean depth
S. vetulus	Temperature	97.5	0.0019	Present in higher mean temperatures
	Depth	763	0.00062	Present in lower mean depth
O. modestus	Temperature	1608.5	0.022	Present in higher mean temperatures
	Depth	-	-	-



Sources: Esri, HERE, DeLorme, USGS, Intermap, INCREMENT P, NRCan, Esri Japan, METI, Esri China (Hong Kong), Esri Korea, Esri (Thailand), MapmyIndia, NGCC, © OpenStreetMap contributors, and the GIS User Community

Figure 2: Map of 174 study sites across Western Canada.

	Environment	**Species**
Sites	R	L
Traits	Missing corner	Q

Figure 3: A table R (sites x environment), a table L (sites by species) and a table Q (traits by species) used in fourth corner analysis. Fourth corner and RLQ analysis allows the associations between trait values and environmental gradients to be assessed, filling the Missing corner. Adapted from Jamil *et al.,* 2013.

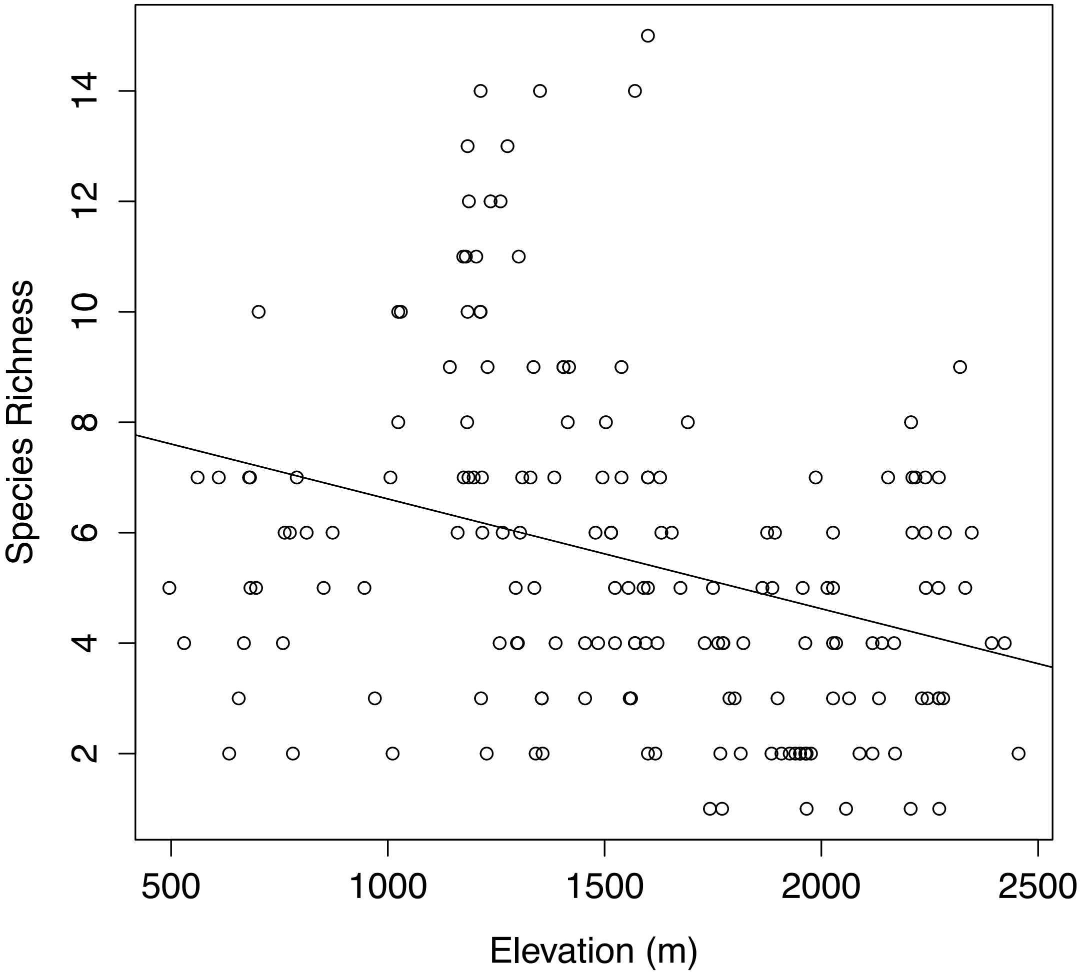


Figure 4: Linear regression of elevation against species richness with linear fit (Species richness ~ Elevation; $r^2 = 0.1$, $p = 1.65 \times 10^{-5}$).

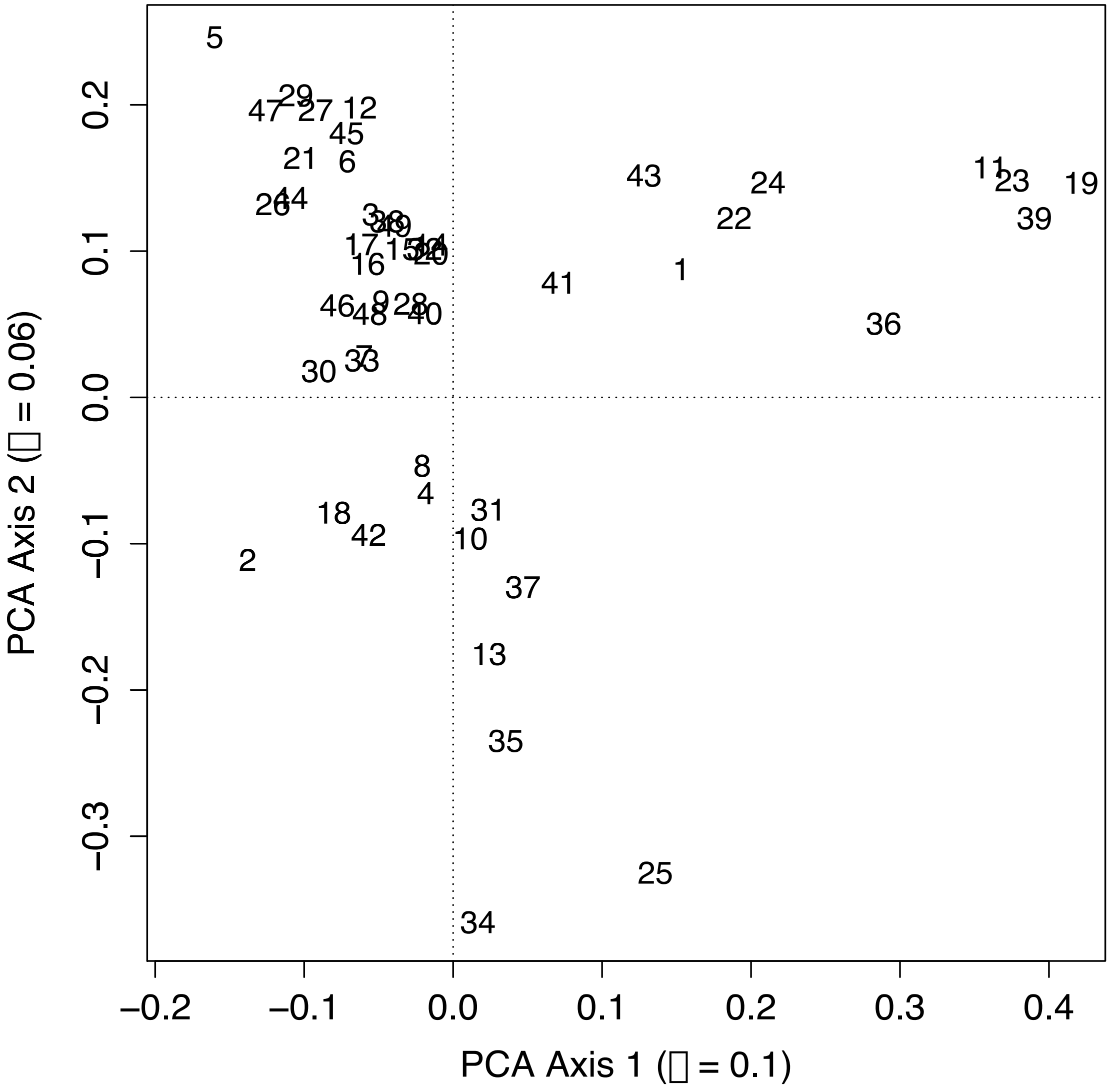


Figure 5: Principal component analysis (PCA) plot displaying 49 crustacean zooplankton species along the first two significant PCA axes based on variance of zooplankton community structure (16.54% total species variance explained).

1. *Acanthocyclops capillatus*
2. *Acanthocyclops vernalis*
3. *Acanthodiaptomus denticornis*
4. *Acroperus harpae*
5. *Aglaodiaptomus leptopus*
6. *Alona affinis*
7. *Alona guttata*
8. *Alona rectangular*
9. *Alonella nana*
10. *Arctodiaptomus arapahoensis*
11. *Bosmina coregoni*
12. *Bosmina longirostris*
13. *Branchinecta paludosa*
14. *Ceriodaphnia affinis*
15. *Ceriodaphnia lacustris*
16. *Ceriodaphnia quadrangula*
17. *Ceriodaphnia reticulata*
18. *Chydorus sphaericus*
19. *Cyclops scutifer*
20. *Daphnia catawba*
21. *Daphnia dentifera*
22. *Daphnia galeata mendotae*
23. *Daphnia longiremis*
24. *Daphnia longispina*
25. *Daphnia middendorffiana*
26. *Daphnia pulex*
27. *Daphnia schodleri*
28. *Diacyclops thomasi*
29. *Diaphanosoma birgei*
30. *Eucyclops agilis*
31. *Eucyclops speratus*
32. *Eurycercus lamellatus*
33. *Gammarus lacustris*
34. *Hesperodiaptomus arcticus*
35. *Hesperodiaptomus shoshone*
36. *Heterocope septentrionalis*
37. *Holopedium gibberum*
38. *Hyalella azteca*
39. *Leptodiaptomus angustilobus*
40. *Leptodiaptomus nudus*
41. *Leptodiaptomus sicilis*
42. *Leptodiaptomus tyrrelli*
43. *Leptodora kindtii*
44. *Macrocyclops albidus*
45. *Microcyclops varicans*
46. *Orthocyclops modestus*
47. *Polyphemus pediculus*
48. *Scapholeberis kingi*
49. *Simocephalus vetulus*

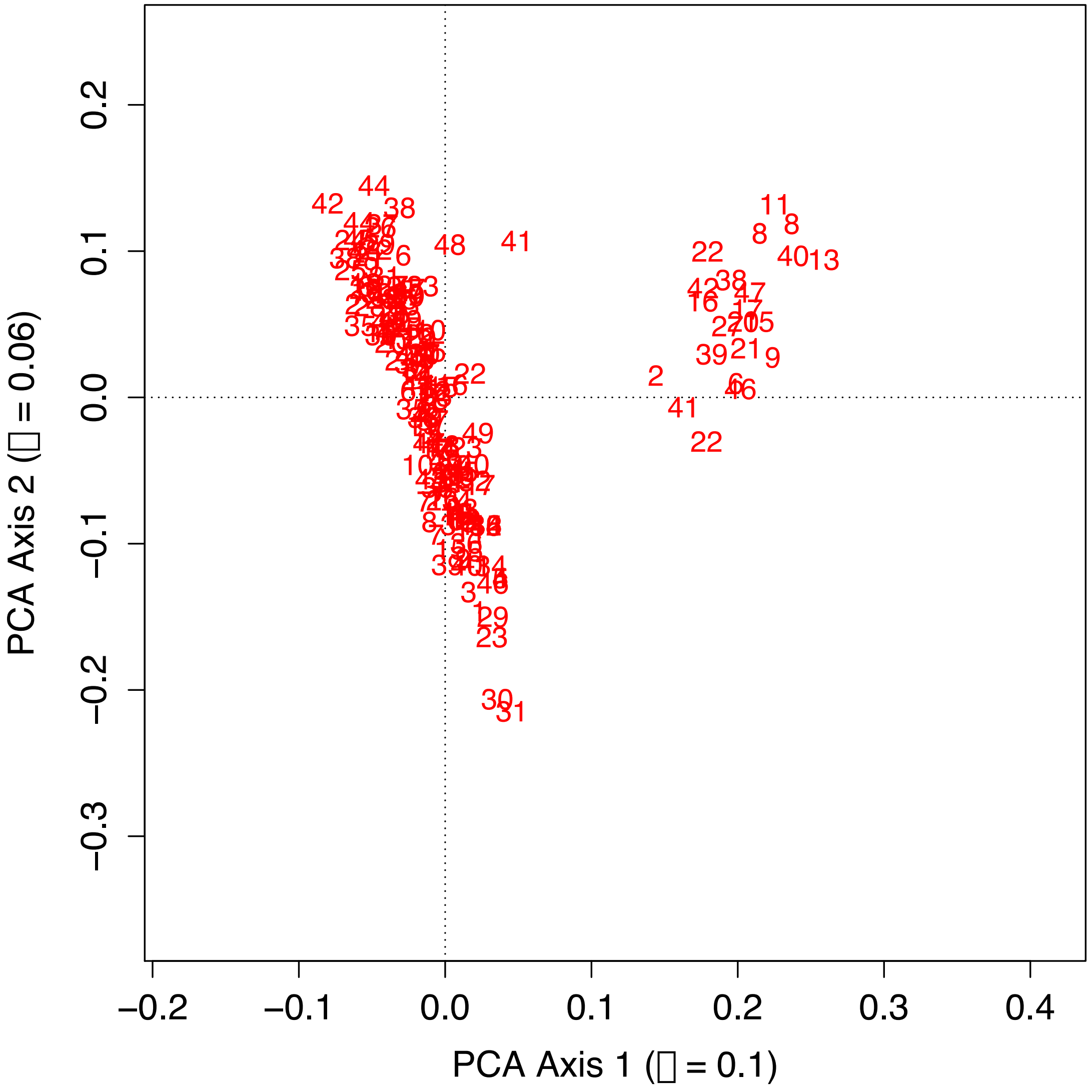


Figure 6: PCA plot displaying the distribution of sites along the first two axes based on variance of zooplankton community structure.

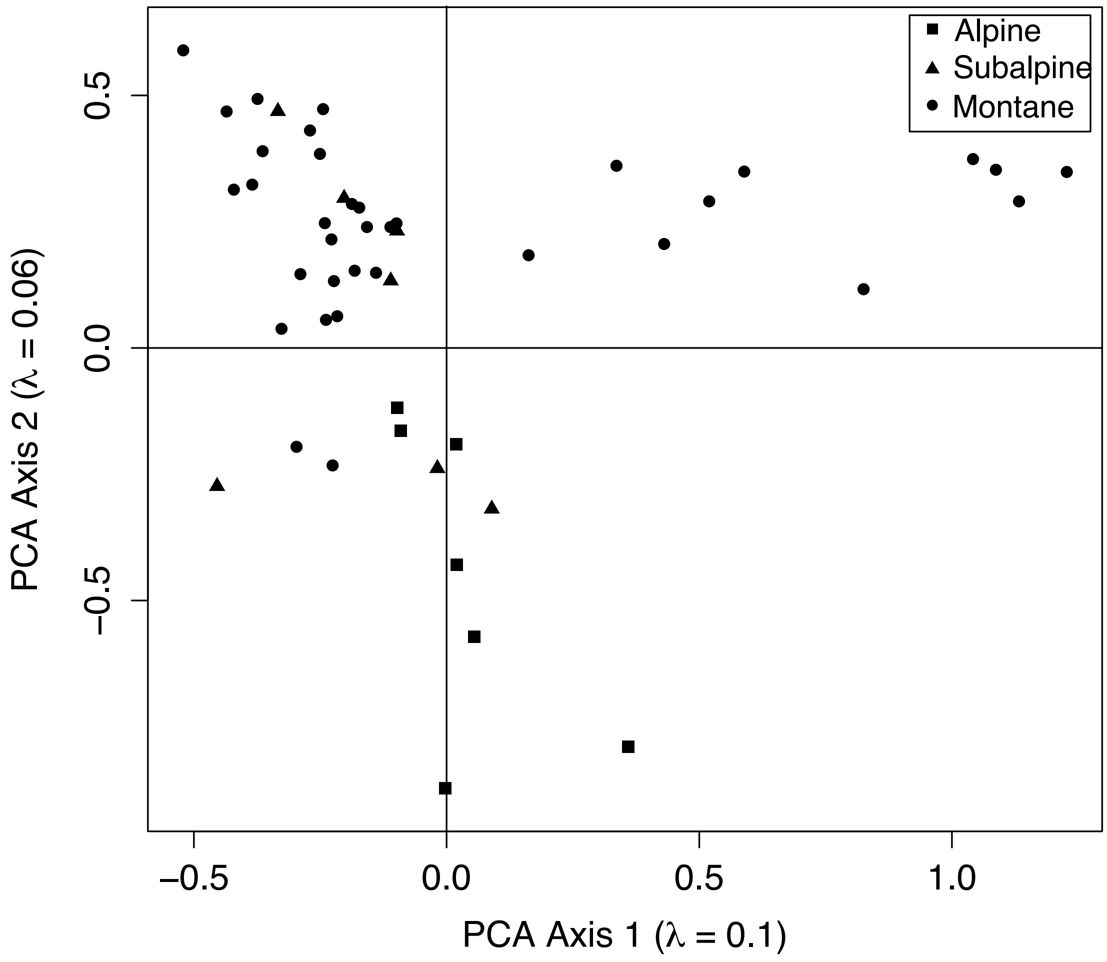


Figure 7: PCA plot displaying 49 crustacean zooplankton species from different elevational regions based on variance of zooplankton community structure (Alpine > 2000 m, Subalpine = 1500- 2000 m, Montane < 1500 m).

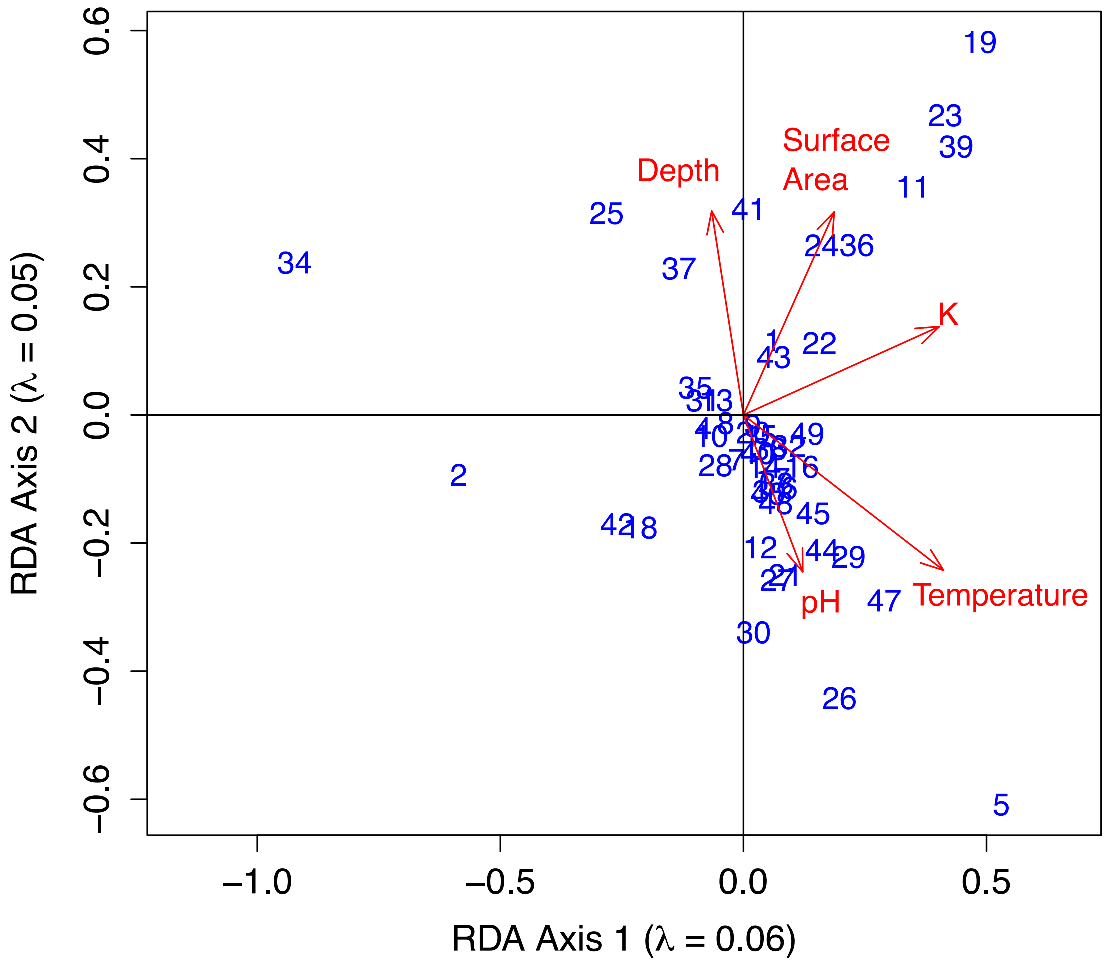


Figure 8: Redundancy analysis (RDA) of variance of zooplankton community structure with 49 crustacean zooplankton species as constrained by five significant ($p < 0.05$) explanatory variables (11.09% variance explained by two axes).

1. *Acanthocyclops capillatus*
2. *Acanthocyclops vernalis*
3. *Acanthodiaptomus denticornis*
4. *Acroperus harpae*
5. *Aglaodiaptomus leptopus*
6. *Alona affinis*
7. *Alona guttata*
8. *Alona rectangular*
9. *Alonella nana*
10. *Arctodiaptomus arapahoensis*
11. *Bosmina coregoni*
12. *Bosmina longirostris*
13. *Branchinecta paludosa*
14. *Ceriodaphnia affinis*
15. *Ceriodaphnia lacustris*
16. *Ceriodaphnia quadrangula*
17. *Ceriodaphnia reticulata*
18. *Chydorus sphaericus*
19. *Cyclops scutifer*
20. *Daphnia catawba*
21. *Daphnia dentifera*
22. *Daphnia galeata mendotae*
23. *Daphnia longiremis*
24. *Daphnia longispina*
25. *Daphnia middendorffiana*
26. *Daphnia pulex*
27. *Daphnia schodleri*
28. *Diacyclops thomasi*
29. *Diaphanosoma birgei*
30. *Eucyclops agilis*
31. *Eucyclops speratus*
32. *Eurycercus lamellatus*
33. *Gammarus lacustris*
34. *Hesperodiaptomus arcticus*
35. *Hesperodiaptomus shoshone*
36. *Heterocope septentrionalis*
37. *Holopedium gibberum*
38. *Hyalella azteca*
39. *Leptodiaptomus angustilobus*
40. *Leptodiaptomus nudus*
41. *Leptodiaptomus sicilis*
42. *Leptodiaptomus tyrrelli*
43. *Leptodora kindtii*
44. *Macrocyclops albidus*
45. *Microcyclops varicans*
46. *Orthocyclops modestus*
47. *Polyphemus pediculus*
48. *Scapholeberis kingi*
49. *Simocephalus vetulus*

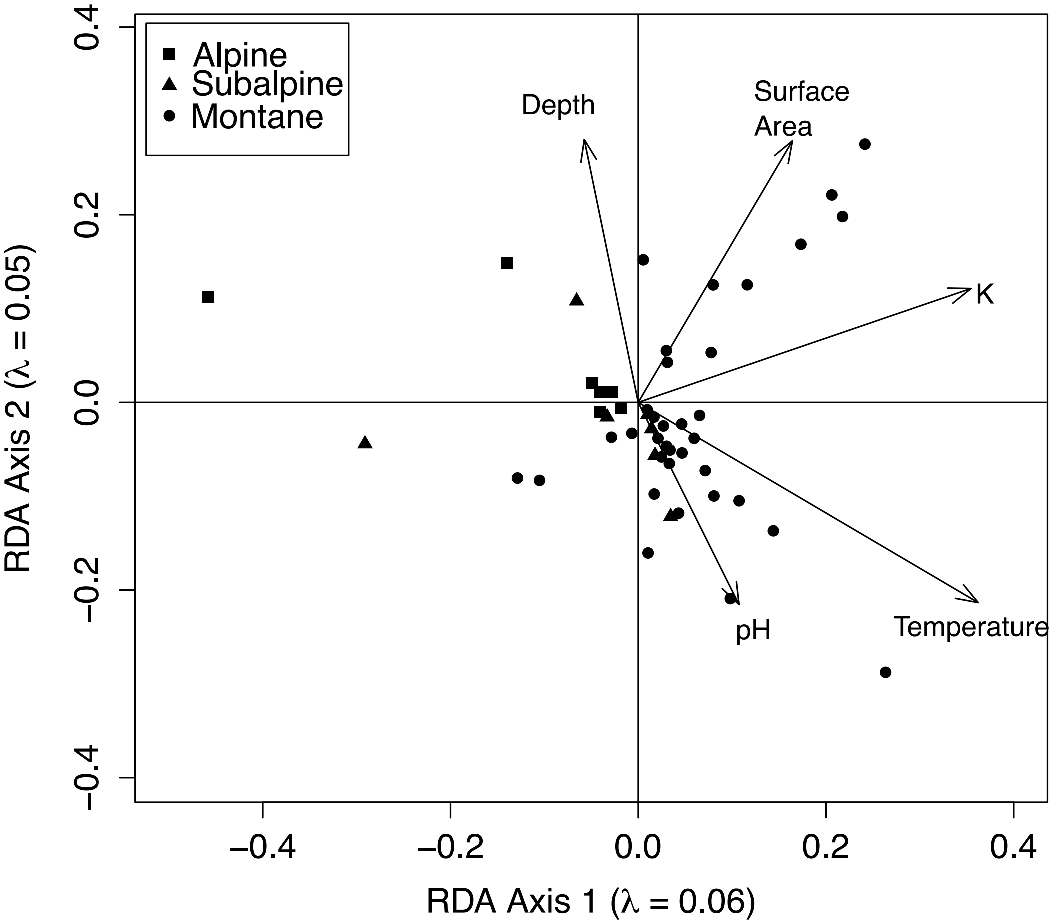


Figure 9: RDA of the variance of community composition of 49 crustacean zooplankton species from different elevational regions (Alpine > 2000 m, Subalpine = 1500- 2000 m, Montane < 1500 m) as constrained by five significant explanatory variables**.**

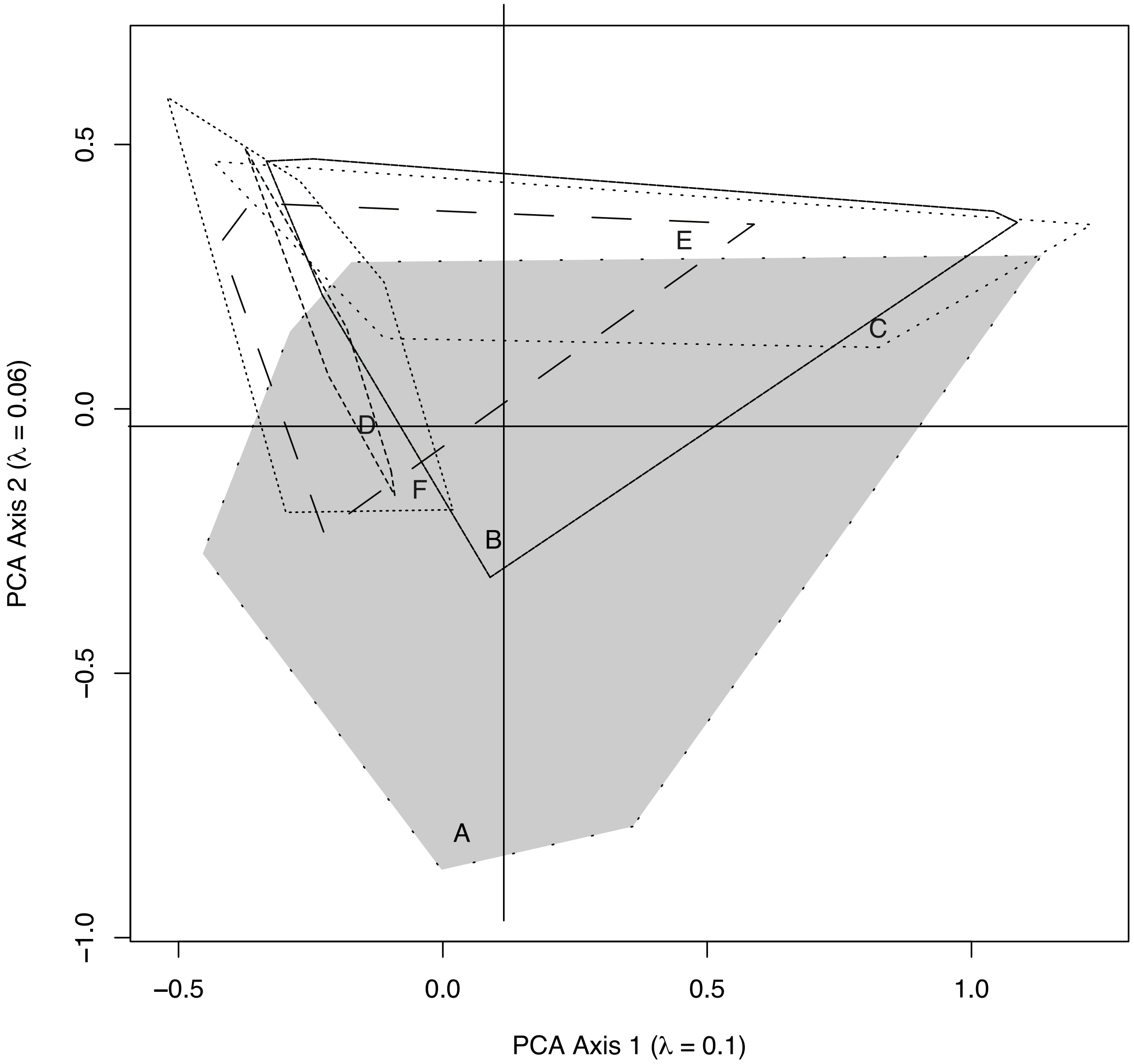


Figure 10: PCA plot displaying variance of community composition for 49 crustacean zooplankton species within six trait clusters (A-F; Table 6). Trait cluster **A**: Large, pigmented **B**: Herbivores **C**: Low pigment mixed-feeders **D**: Littoral, asexual herbivores **E**: High pigment mixed-feeders **F**: Littoral mixed-feeders.

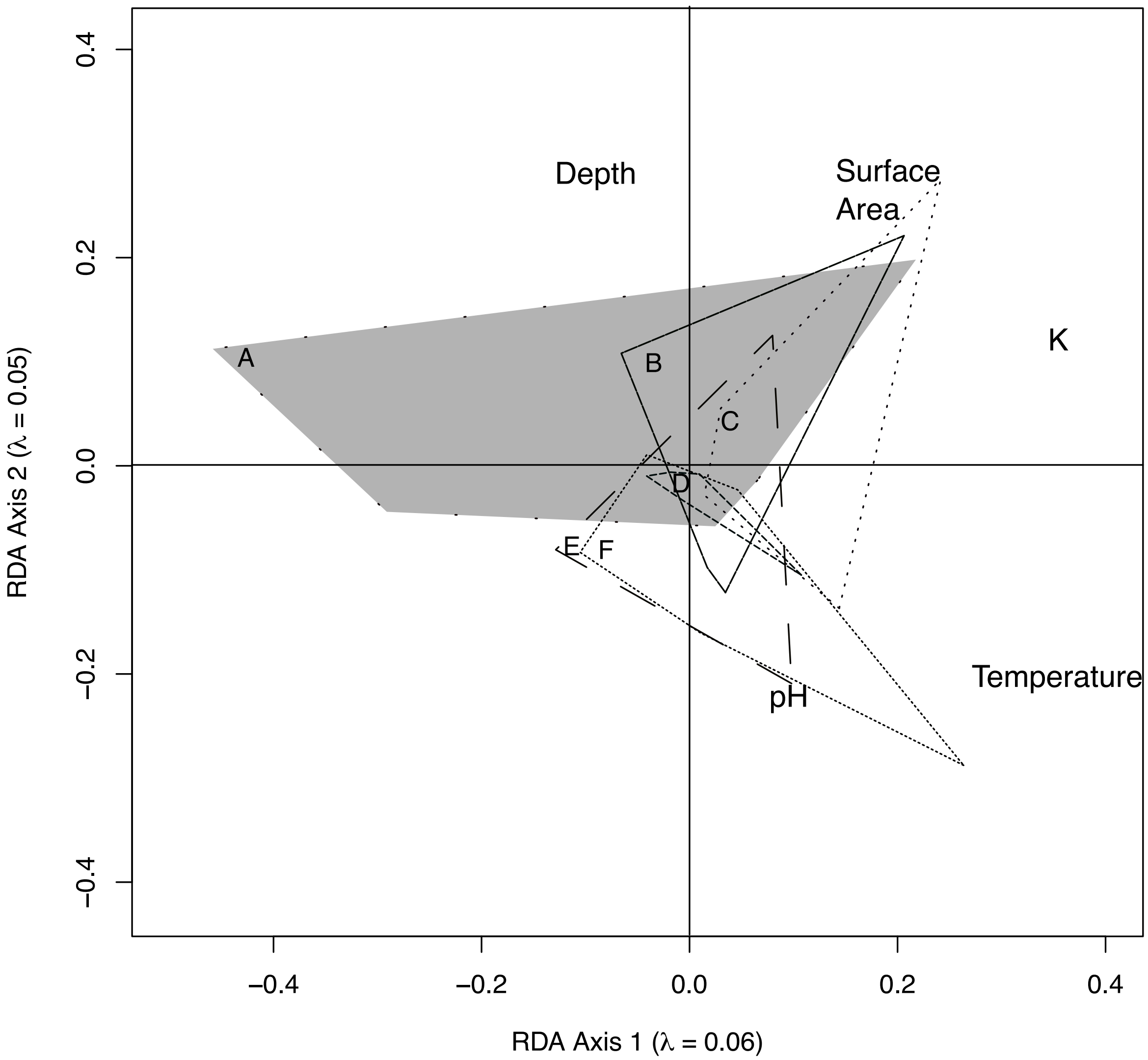


Figure 11: RDA of of the variance of community composition of 49 crustacean zooplankton within six trait clusters (Table 6), constrained by five significant environmental variables (Temperature, pH, Depth, Surface Area, K). Trait cluster **A**: Large, pigmented **B**: Herbivores **C**: Low pigment mixed-feeders **D**: Littoral, asexual herbivores **E**: High pigment mixed-feeders **F**: Littoral mixed-feeders

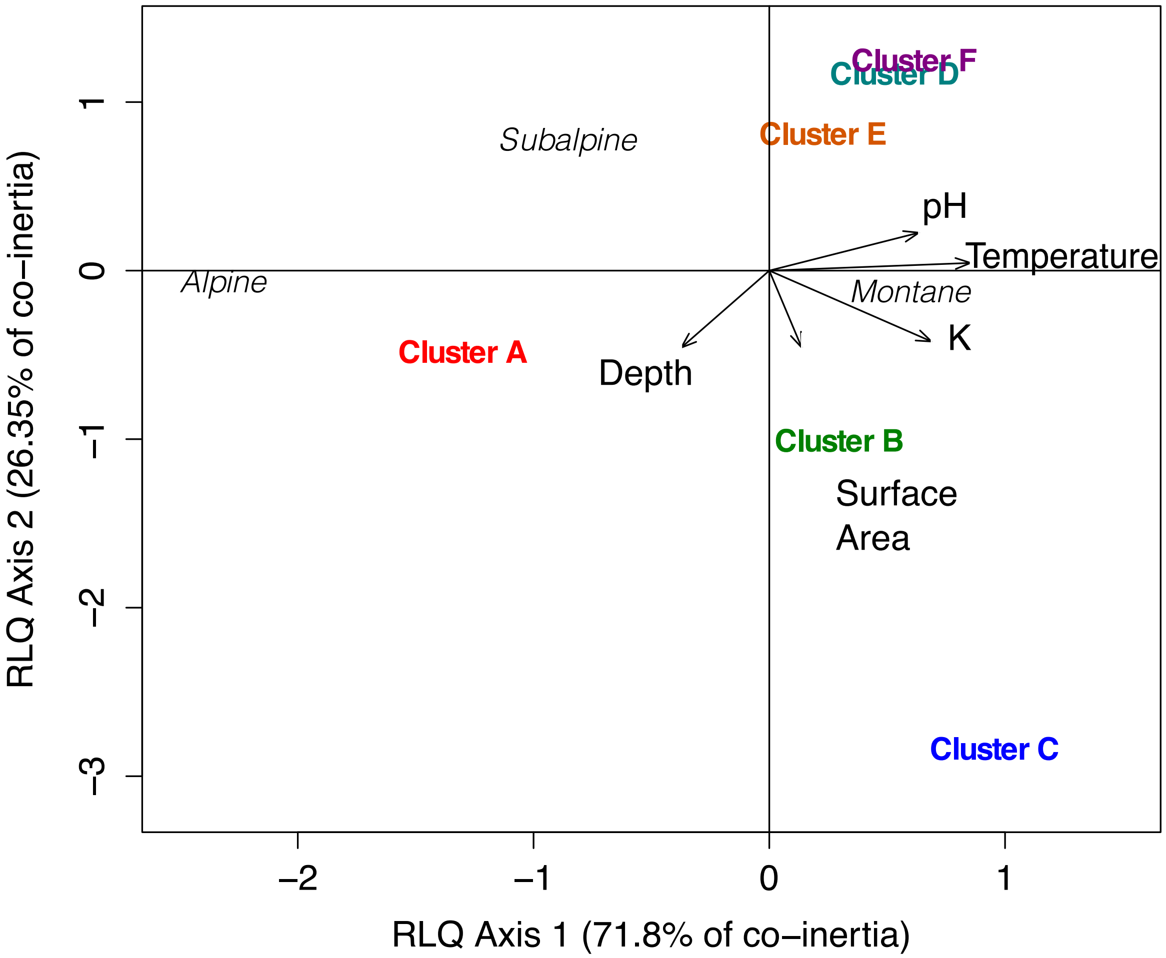
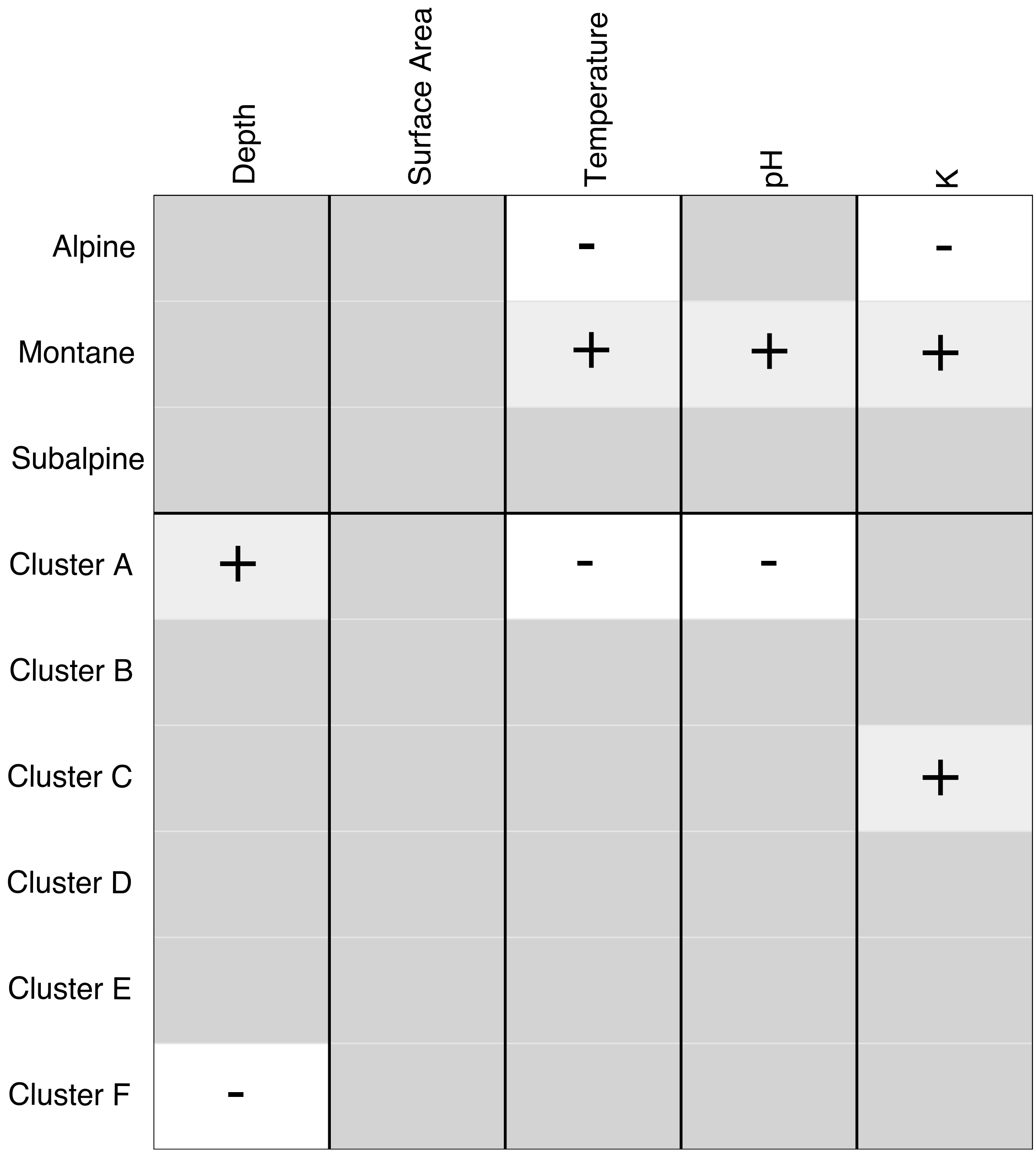


Figure 12: RLQ ordination displaying the relationships between six trait clusters (Trait cluster **A**: Large, pigmented **B**: Herbivores **C**: Low pigment mixed-feeders **D**: Littoral, asexual herbivores **E**: High pigment mixed-feeders **F**: Littoral mixed-feeders), elevational regions (black italic) and environmental variables (black).



	Depth	Surface Area	Temperature	pH	K
Alpine			-		-
Montane			+	+	+
Subalpine					
Cluster A	+		-	-	
Cluster B					
Cluster C					+
Cluster D					
Cluster E					
Cluster F	-				

Figure 13: Fourth corner bivariate analysis revealing significant ($p < 0.05$) positive (+) and negative (-) relationships between elevational regions (Alpine, Subalpine, Montane), Trait Clusters (A-F; Trait cluster A: Large, pigmented B: Herbivores C: Low pigment mixed-feeders D: Littoral, asexual herbivores E: High pigment mixed-feeders F: Littoral mixed-feeders) and environmental variables.

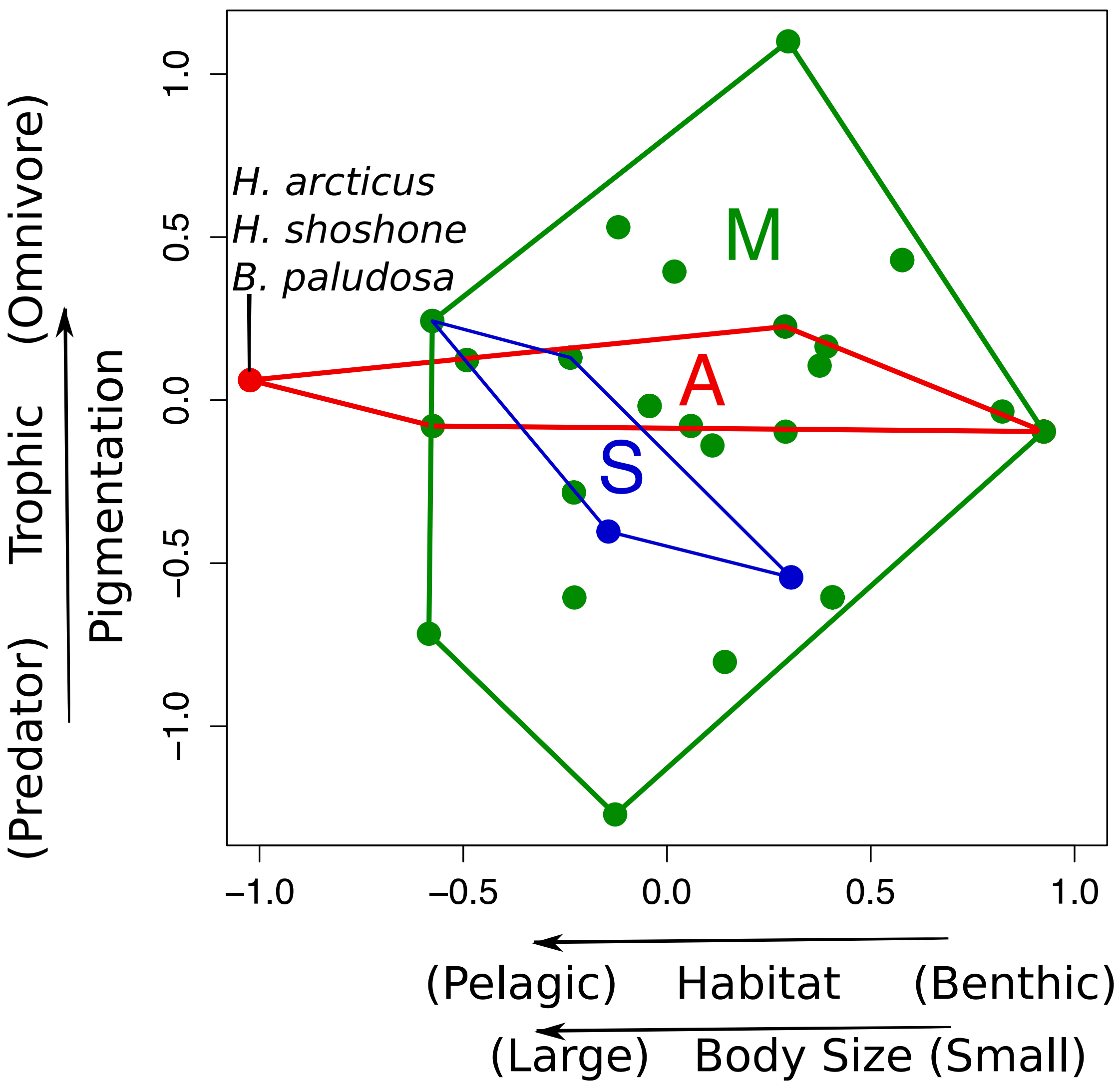


Figure 14: Unconstrained multiple correspondence analysis (MCA) ordination of 49 species in functional space, mediated by species presence/absence data. A=Alpine region, S=Subalpine region, M=Montane region. Arrows below axes indicate the functional traits that are driving the distribution of species on each of the two axes.

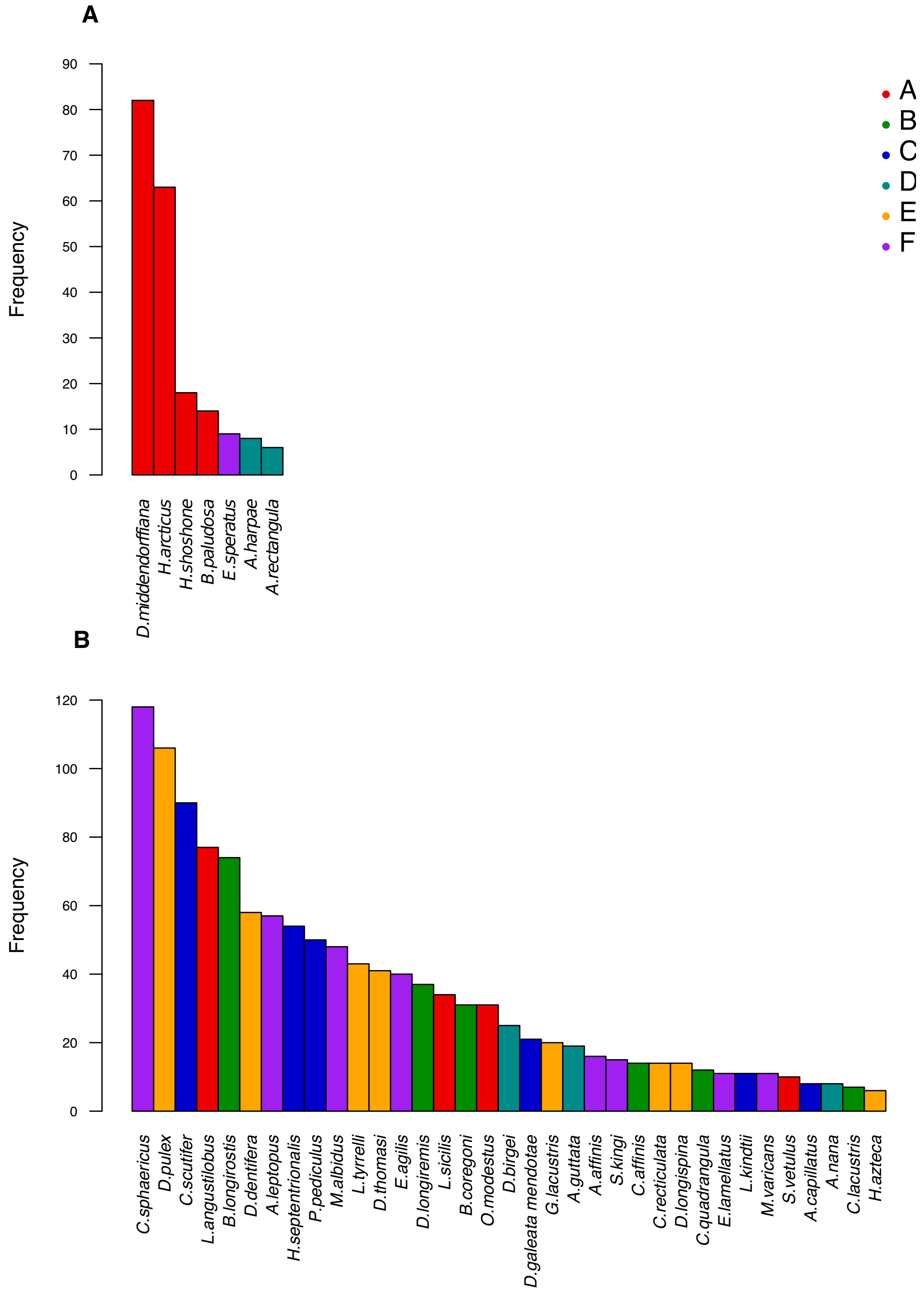


Figure 15: Species abundance curves for **A**: alpine species and **B**: montane species whereby frequency refers to the number of sites occupied by each species across the study extent. Colour indicates membership within one of the six trait clusters (A-F).

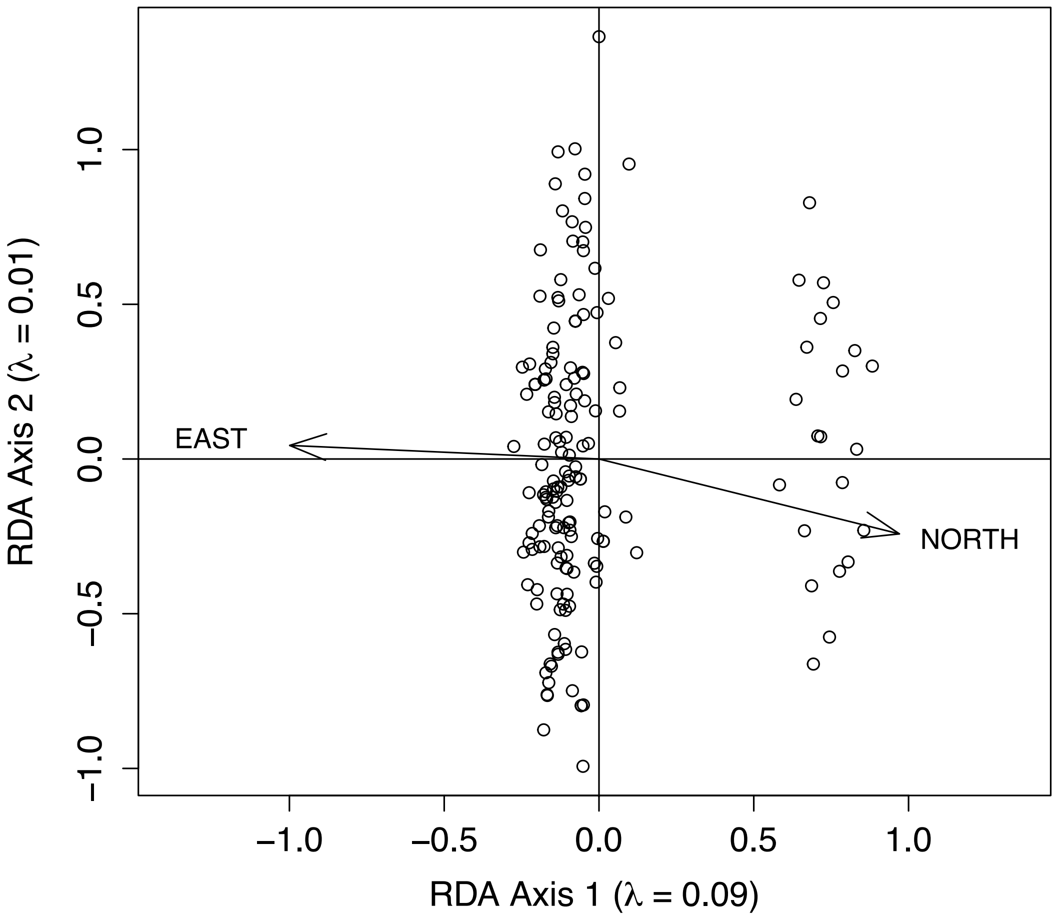


Figure 16: RDA ordination displaying the variance of 170 sites constrained by latitude and longitude coordinates showing the spatial extent of the study area.

Chapter 3: Trials and Tribulations of Culturing Alpine Daphnia

Introduction

The adaptability of a species plays a key role in its ability to cope with more frequent extreme events (i.e., stressors). Adaptation is the development of a feature that increases Darwinian fitness relative to alternative features (Williams, 1966). The direct non-lethal effects of moderate warming and the role of local adaptation have been investigated (Van Doorslaer *et al.,* 2009a, De Meester *et al.,* 2011), and daphniid species from different environments react to warming in different ways. Local populations able to exhibit adaptive responses to climate change can reduce susceptibility to invasion by immigrant genotypes (Van Doorslaer *et al.,* 2009b). This becomes increasingly important as elevation shifts occur because lower elevation temperate species are pre-adapted to predicted warm conditions, threatening endemic alpine diversity. In particular, the potential for ecological tradeoffs is key to the examination of mechanisms of microevolution in response to climate change (Hessen, 1996).

I planned to conduct an evolutionary impact investigation using two sister species of water flea: *Daphnia pulex* O.F. Müller 1785 (widespread temperate to alpine pond species) and *Daphnia middendorffiana* Fischer 1851 (cold-adapted alpine lake specialists) as models for the effects of thermal variation on phenotypic plasticity. Across the Canadian Rockies, *D. pulex* are found primarily in ponds, and *D. middendorffiana* in lakes. Given the differences in temperature variation between lakes and ponds, it is suggested that they may have diverged due to differences in energy budgets (MacIsaac *et al.,* 1985). The experimental manipulation of temperature variation in a controlled environment would allow for the comparison of plasticity between the two species (Hendry, 2016). For complete field and statistical methods and culturing protocol, see Appendix A.

Research Objectives

The study planned to address the following questions:

1) Do alpine daphniids show local adaptation to thermal variation of their native lake or pond environments? I hypothesized that populations will exhibit greater survival and reproductive fitness under native than foreign conditions (i.e., local adaptation).

2) If so, then are they also adaptive or maladaptive to less/more variable environments? Here, I expected populations from more climatically stressful sites (i.e., ponds) to show low fitness under less stressful foreign conditions (i.e., maladaptation).

3) Can the adaptability of the species be attributed to genetic variation? I predict that across sites, pond and lake populations will have adapted similarly to temperature variation. I also expect that within a population, different genetic clones will have varying levels of adaptability, depending on their habitat of origin. I predict pond clones will react similarly to temperature variation, given the homogeneity of their native habitats. I predict lake clones will have some adapted clones, and other maladapted clones, given the heterogeneity of their native habitats. However, lake populations could also be dominated by a single generalist genotype given the permanence of their habitat, and therefore survival will be similar between clones.

Results

Given the cold-adapted nature of alpine species, transplanting daphniid species from alpine environments was a chancy endeavour. Although the transport from the alpine back to the growth chambers at the University of Alberta had its own tribulations, the daphniids were transferred with relative ease. From each site, 30 individuals were isolated into 100 mL jars, and transferred into 100% COMBO solution over the course of two weeks.

D. middendorffiana were larger and hardy at time of collection, with an average survival rate of 16.42 days. *D. pulex* had an average survival rate of 12.07 days. However, only 56.25% of all clones from both species reproduced in culture. Although some clone lines were reproducing successfully, mass declines ensued, and entire populations died off. As a result, the experiments could not be conducted given the lack of replication power after 2-8 weeks of culturing.

Insights and Discussion

Daphniids are key species affecting both water quality through its role as a consumer of phytoplankton, and through the productive capacity for harvestable fish in lakes by transferring energy and nutrients to forage and sportfish (Miner *et al.,* 2012). The genome of *Daphnia pulex* has been described as having only 200 megabases and at least 30,907 genes (Colbourne *et al.,* 2011). Their short generation time and potential for high clonal population growth via parthenogenesis makes them typically considered a ‘model organism’ for eco-evo research (De Meester, 1996).

However, daphnia clonal lines may differentiate within a single habitat (i.e., lake), and therefore distinct lines may have different reactions to stressors. The number of clone lines (unique genotypes) increases in systems with greater niche specialization (i.e., lakes; Laberge & Hann, 1990). However, a few generalist clonal lines may dominate in permanent lake systems (Lynch, 1983, Mort & Wolf, 1986, Holmes *et al.,* 2016). Permanent ponds have also been found to have low clonal variation that is highly structured and rearranged in times of environmental change (Hebert, 1974). This may be due to the permanence and long-term establishment and selection for a few dominant clone lines that have saturated the environment (De Meester *et al.,* 2002). In cyclical parthenogenesis of many daphniids, sporadic bouts of sexual reproduction allow for selection, and then adaptive genotypes are able to replicate efficiently via asexual clonal reproduction (Hebert, 1974). This may allow for dynamic clonal diversity within and between populations. Neighbouring populations are often highly genetically differentiated (Lynch & Spitze, 1994, Vanoverbeke & De Meester, 1997) as a response to rapid population growth and local adaptation upon colonization (De Meester *et al.,* 2002). Genetic differentiation between populations increases as clonal diversity decreases because it is inflated by high clonal selection for a few generalist clones (Vanoverbeke & De Meester, 1997). So it is therefore expected that two sites that have lower genetic diversity will be more differentiated than two sites that have high genetic diversity.

Previous work in the Holarctic used allozymic variation to establish clonal structure and distribution in arctic ponds (Weider *et al.,* 1999). These findings suggest that only a few clones were widespread across the range (1000s of km's), which could be attributed to parallel evolution. Most clones were restricted to a single region, or even a single site, indicating that the local adaptation of clone lines is prevalent. Therefore, low diversity populations (i.e., arctic or alpine) may have a single, or few clone lines that dominate in a specific population or region.

Clone by environment interactions have been established in response to temperature as well as food availability (i.e., Steiner *et al.,* 2016), exhibiting the idea that distinct clones will be more or less favoured under changing conditions. Therefore, given that transport from a natural environment to laboratory conditions involves multiple changes and stressors, certain clone lines or individuals may be favoured under such conditions. Local adaptation by resident species of extreme environments can provide them with a 45% advantage over species from elsewhere (Hereford, 2009). Therefore, although alpine species are considered to reside in *extreme* natural environments, they are adapted to such conditions. Maladaptation refers to a reduction in relative fitness under less stressful conditions (Kawecki & Ebert, 2004). The introduction of alpine species from the stressful conditions that they are adapted to into less stressful laboratory conditions may in fact have resulted in maladaptation.

Conclusions

Water chemistry of common medium

Although completely disentangling the reasons behind the failed culturing of alpine daphnia is challenging, there are some speculations that can be made. Firstly, given the geological diversity of the Canadian Rocky Mountains, the chemical composition of alpine water bodies may be site dependent. Culturing mediums are based on the chemical composition of a model lake, and so cannot encompass the variation between sites. For example, FLAMES medium (Celis-Salgado *et al.,* 2008) is based off the chemical composition of Red Chalk Lake in Lake of Bays, Ontario, and as a result, daphniids from that lake typically have a very high fitness (Personal communication with S. Arnott, Queen's University 2016). Therefore, the COMBO medium used in the culturing of alpine daphniids may not have been suitable for all sites. As such, the development of a common medium based on the dilute chemistry of alpine lakes would better suit the culturing of alpine species from the field.

Clonal specialization

Lake and pond systems may vary in the genetic diversity of clone lines from site to site. Although I was not able to test for the number of clone lines at a given site, the literature concludes that permanent systems are typically genetically different between sites, and through selection are very specifically locally adapted (Lynch & Spitze, 1994, Vanoverbeke & De Meester, 1997). Although I expect a single generalist clone line to dominate, there still exists different clone lines from the same site that may react differently to stressors, and in pond systems, that may even have temporal consequence throughout the growing season (De Meester, 1996). Therefore, given the number of environmental changes that an individual experiences after transfer to lab conditions, clone lines may react differently, with some being favoured over others. It is therefore sensible to assume that some clone lines may do better than others under laboratory conditions, and therefore only a small proportion of individuals collected were able to survive and reproduce. However, the proportion of individuals that were reproducing was lower than clone line selection would expect, implying that other factors may be at play.

Maladaptation to laboratory conditions

Alpine lakes reside in extreme environments, with a number of stressors affecting lakes in this region, which has forced the selection for cold-adapted, resilient taxa. Remote mountain water bodies have cold temperatures, dilute chemistry and low biocomplexity, limiting the types of species that can survive (Vinebrooke & Leavitt, 2005). Low connectivity between lakes in the alpine makes selection pressures especially high, making clone lines from different sites very specialized (De Meester *et al.,* 2002). Therefore, it is possible that when alpine species were transferred to laboratory conditions where food was abundant, and conditions were consistent, they were unable to cope, and were maladapted to these new conditions. Given that there was no substantial difference between pond and lake species, we can speculate that species-specific characteristics were not a factor in the failure to culture.

Future Directions

Daphnia are considered a model organism for ecological evolution experiments across many disciplines including ecotoxicology and ecogenomics (Lampert, 2011, Miner *et al.,* 2012). However, differences in clone lines across regions, populations and sites may affect the "culturability" of daphnia species. Many studies make generalizations about results from laboratory experiments that extend to natural environments. However, the clone lines used in such experiments are often highly "culturable", and are well suited to lab conditions over multiple generations. So, what can be said for the clone lines that have high fitness in natural environments, but are unable to reproduce and thrive under laboratory conditions? Can we confidently draw conclusions by comparing lower fitness lab daphnia to their extreme environment-adapted sisters? Comparisons of this nature can be informative in a general sense, but must be conducted with caution. With the increasing prevalence of genetic analysis of populations, we can get a better idea of the genetic composition of the populations of which we are drawing conclusions, and corroborate with lab experiment results.

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Chapter 4 Conclusions

Synthesis

Alpine lakes and ponds as sentinels of climate change are coldwater, chemically dilute ecosystems containing relatively few species, showing evidence of thermal niche specialization based on their limited geographic range (Vinebrooke & Leavitt, 2005). Several lines of evidence show that environmental stressors may increase the thermal sensitivity of these fragile alpine communities given that the effects of warming increase with elevation (Pepin *et al.,* 2015). Here, my investigation showed that the best environmental predictors of mountain zooplankton community composition were in fact related to climate-influenced variables. Regional alpine communities were dominated by a functional group that was associated with decreasing temperature and increasing depth, indicating that certain functional components could be completely eliminated under predicted warmer and drier conditions. In particular, the endemic alpine species *H. arcitcus* and *D. middendorffiana* could act as indicators of the early stages of the biological impacts of climate changes as they have strong associations with temperature and depth. The use of endemic species in laboratory experiments may be useful in the analysis of acute tolerances to changes in temperature and depth; however, caution should be taken when generalizing laboratory results to natural environments. Maladaptation of alpine species transferred to lab environments may also occur, limiting the accessibility of such experiments. As climate change shifts towards warmer temperatures and shallower pond-like conditions, we anticipate a shift in zooplankton community composition towards smaller, littoral species, at the expense of endemic alpine species, therefore altering the functional responses of alpine ecosystems.

Future Research

Environmental monitoring should be integrated and standardized across Canada in order to reconcile differences across collection methods and monitoring units (Table 8). In the North American Rockies, there is a lack of meteorological stations at high elevations (Bradley *et al.,* 2004). Given that warming is expected to be greater at higher elevations (Pepin *et al.*, 2015), it is vital to have baseline and change data for climatic shifts in temperature and precipitation. In addition to meteorological data, monthly surveys of chemical, physical and biological data should be completed over the course of the ice-off and ice-on season (Hampton *et al.,* 2017) to identify community and ecosystem function shifts that accompany climate change in alpine environments. Since this study used historical data (Chapter 2), it would be valuable to resample these sites to identify any shifts in zooplankton communities or indicator species that have already occurred as a result of warming.

However, the effects of warming can be exacerbated by additional multiple environmental and anthropogenic perturbations. It is therefore important to test these stressors in combination, not just individually or combined with the effects of warming. Mesocosm experiments in alpine environments could be used to manipulate communities with multiple stressors including warming, nutrient deposition, atmospheric deposition of pollutants and non-native fish introduction in order to disentangle the additive and synergistic effects of perturbations on zooplankton community composition and ecosystem function. The threat of decreased pelagic zones due to decreased summer precipitation may also affect temperature variation in shallow systems (i.e., ponds), which has not yet been tested in a community context.

To better assess the functional diversity of sites across elevation, future studies should aim to compile a zooplankton functional trait database with regional and local traits for given species (Benedetti *et al.,* 2015). By better defining species functional identities, greater precision can be gathered in the process of understanding the effects of climate change on the functional diversity of communities. The functional structure of alpine communities is limited, and therefore these extreme environments may be more susceptible to warmer and drier environments, but the extent of these shifts is unknown. Model simulations of predicted climate conditions could be used to understand the magnitude of taxonomic shifts at higher elevations.

In addition, the rescue effect of regional diversity has been tested in experiments where dispersal is not limited (Loewen *et al.,* 2016, Thompson & Shurin, 2012), however dispersal capacities of species across elevation have not been tested in natural environments. Future studies should quantify the dispersal limitations that are anticipated in remote mountain environments that have only been evidenced by an absence of reestablishment (Donald *et al.,* 2001). Also, transplant experiments could determine if functionally similar montane species are in fact capable of establishing alpine sites under warmer conditions and therefore able to compensate functionally for a loss in alpine diversity under expected climate conditions.

Table 8: Recommended parameters to be measured in mountainous lakes and ponds to assess changes over time that may be important for climate change.

Parameter	**Method**	**Importance**
Temperature variation	Temperature profile	Increased climatic variation may affect community composition and species adaptability
Turbidity	Secchi disk depth	Decreased glacial inputs may decrease turbidity (Wolfe *et al.* 2013)
Dissolved oxygen	Probe	Important for the survival of introduced fish species
pH	Probe	Enhanced weathering from warming may cause the acidification of alpine lakes (Sommaruga-Wograth *et al.* 1997)
Multiple zooplankton hauls	Zooplankton haul nets	Multiple tows allows for greater precision of zooplankton community composition, as well as temporal changes during both the growing season and ice-on season
Algal biomass	Chlorophyll-*a* inferred algal biomass via filtration	Algal biomass is anticipated to increase given warming conditions and atmospheric nutrient deposition that will compound a trophic cascade that starts with the loss of key grazer species (Chapter 1, Figure 1)
Key limiting nutrients (total phosphorus, total dissolved phosphorus, total kjeldahl nitrogen, inorganic/organic nitrogen)	Laboratory analysis	Atmospheric deposition of key nutrients driving algal biomass may increase in alpine environments (Wolfe *et al.* 2001)
Dissolved organic carbon & colour	Laboratory analysis and observation	Increasing treeline may affect DOC concentrations (Vinebrooke & Leavitt, 2005)
Total suspended sediment (TSS)	Filtration	Contaminant transport (heavy metals, POPs, nutrients)
Anion and cation concentrations	Laboratory analysis	Increased weathering may increase the anion and cation concentrations of typically dilute alpine systems (Sommaruga-Wograth *et al.* 1997)

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Appendix A

Chapter 3 Methods and Culturing Protocol

Field Methods

To test my hypotheses, I planned to run a laboratory common garden experiment and genetic variation analysis to assess the role of local adaptation to temperature variation. By isolating for temperature variation as the independent environmental factor, I would directly test its role as a selection agent for local adaptation. Zooplanktons were collected from two environments: Five high-elevation ponds (<5 m maximum depth) and five lakes (>5 m maximum depth), each located above treeline (alpine) across the Canadian Rockies (Alberta and British Columbia, Canada; Table 1). By using a parallel local adaptation design (Kawecki & Ebert, 2004), the two habitat types were defined based on temperature variation (lake versus pond), and replicates were paired by type across 5 sites (1. Waterton Lakes NP, 2. Kananaskis Country, 3. East Banff NP, 4. West Banff NP/Yoho NP, 5. Jasper NP). Therefore, at each site, there was one pond and one lake selected for comparison. Collection took place from south to north to compensate for latitudinal differences in ice-off timing between sites.

Experimental Methods

Individual populations of *D. pulex* and *D. middendorffiana* from each pond and lake site, respectively, were brought back to the University of Alberta in Edmonton, AB and cultivated in growth chambers under standard light conditions (16:8 hrs L/D cycles) at ~10 °C until experiments are conducted. Populations were separated into individual clonal lines within 48 hours of collection to avoid erosion of genetic variation by a dominant genotype. Clones were grown in 100-mL jars containing COMBO, a standardized freshwater medium that supports growth of zooplankton and their green algal food supply (Kilham *et al.,* 1998). Water was changed twice weekly, and individuals were fed 20 x 10^6 cells/ individual of green microalgae paste (*Nannochloropsis;* Innovative Aquaculture Products Ltd.) every other day to not limit food quantity across treatments.

Multiple clone lines (50) from each of the five lake and pond populations were preserved in 90% EthOH to be sequenced for genetic variation using the restriction site-associated DNA (RAD) approach (Baird *et al.,* 2008) to obtain genetic markers and allow for the disentanglement of genetic variation within pond and lakes as a measure of adaptive potential. Five genetically distinct clonal lines were to be randomly chosen as a subsample from each site, and used in acclimation experiments.

For the acclimation experiments, growth chambers were to be set at two temperature regimes (a pronounced diurnal “pond” cycle and a less variable diurnal “lake” cycle). Each treatment was to be replicated across five pond and five lake populations to account for site effects (Hargreaves *et al.,* 2014) and allow for the comparison of adaptation across sites (Figure 17). From each site, the five distinct clonal lines would have eight individuals randomly chosen and assigned to one of the two treatments, allowing each experimental unit to have 4 replicates (n=400; Figure 18). Heating treatments were to be conducted by setting the temperatures of the growth chamber rooms with respective diurnal fluctuations in 12 hour intervals synchronized with light/dark cycle (2-18 °C and 9-11 °C) and allowing culture medium to adjust accordingly. To isolate for temperature variation as the independent stressor, daily average temperature was standardized at 10 °C, around the average temperature of alpine water bodies.

Life history parameters were to be used to quantify individual performance over 21 day trials (Van Doorslaer *et al.,* 2007). Reproductive fitness was to be tracked as age at first and second clutch and number of offspring in first clutch and second clutch. Acclimation was to take place for 2 generations before parameters were counted to allow for potential phenotypic acclimation and account for maternal effects.

Statistical Methods

I had then planned to perform a multivariate analysis to determine the degree at which local (mal)adaptation occurs for each habitat type across the two warming regimes. I planned to perform a nested two-factor ANOVA to compare the reproductive fitness of pond versus lake genotypes against two temperature variation treatments. The main effects were to be used to disentangle the effects of habitat type and treatment type. The interaction effect would allow for the disentanglement of (mal)adaptation and test for a genotype (habitat type) x environment (treatment) interaction. Quantitative measures of genetic variation between habitat types (i.e., lake versus pond populations) were to be used as a proxy for adaptive potential between environments, and used to corroborate experimental results. I also wanted to run a two-way ANOVA to test for a genotype x environment interaction across the 5 pond sites and across the 5 lake sites, to check for genotypic variation between sites. This would have allowed us to explore the following question: Will different sites adapt to the same selection agent (temperature variation) in the same way? The interaction of genotypes within each population (clones) was to be tested as well, to see if the clonal lines within a population respond differently to temperature variation, therefore implying a genetic polymorphism. By analyzing genotype x environment interactions at different levels of population structure, a hierarchy of polymorphisms for temperature variation would be developed.

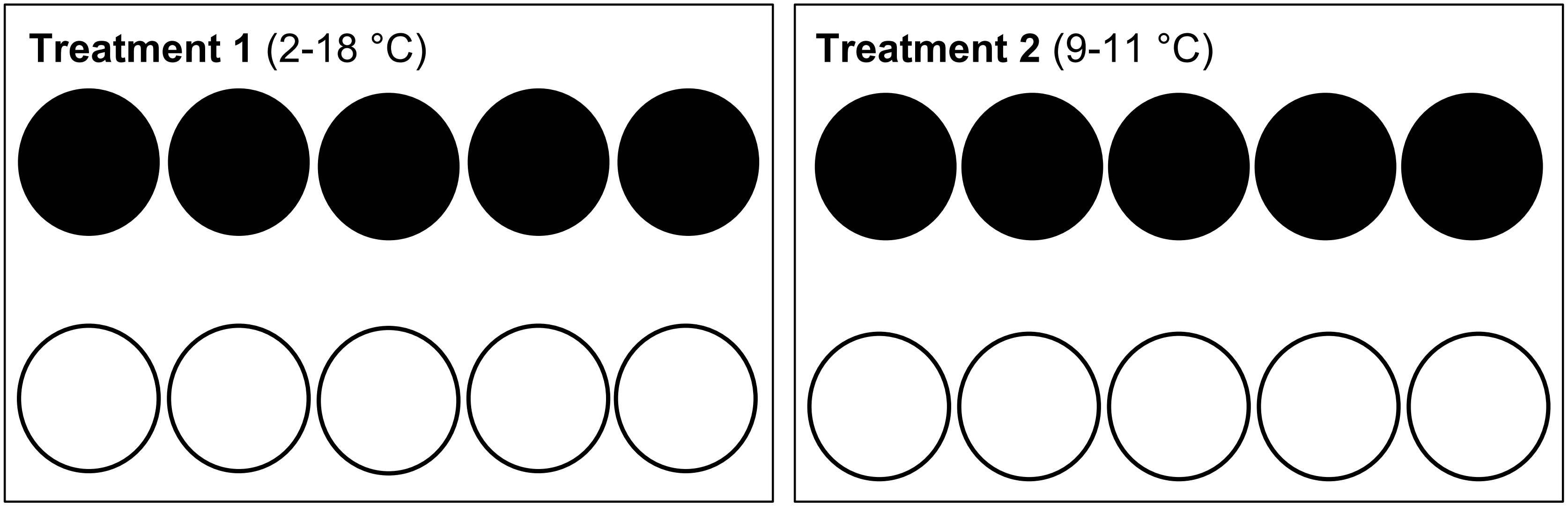

Figure 17: Design for reciprocal transplant experiment involving two source populations (pond versus lake) and two thermal regimes (high versus low variability). Treatment 1 growth chamber set with diurnal fluctuations of 2-18 °C and Treatment 2 growth chamber set at 9-11°C. Five sites of both pond populations (filled circles) and lake populations (open circles) under each treatment combination to be replicated 5 times. Acclimation time of 2 generations.

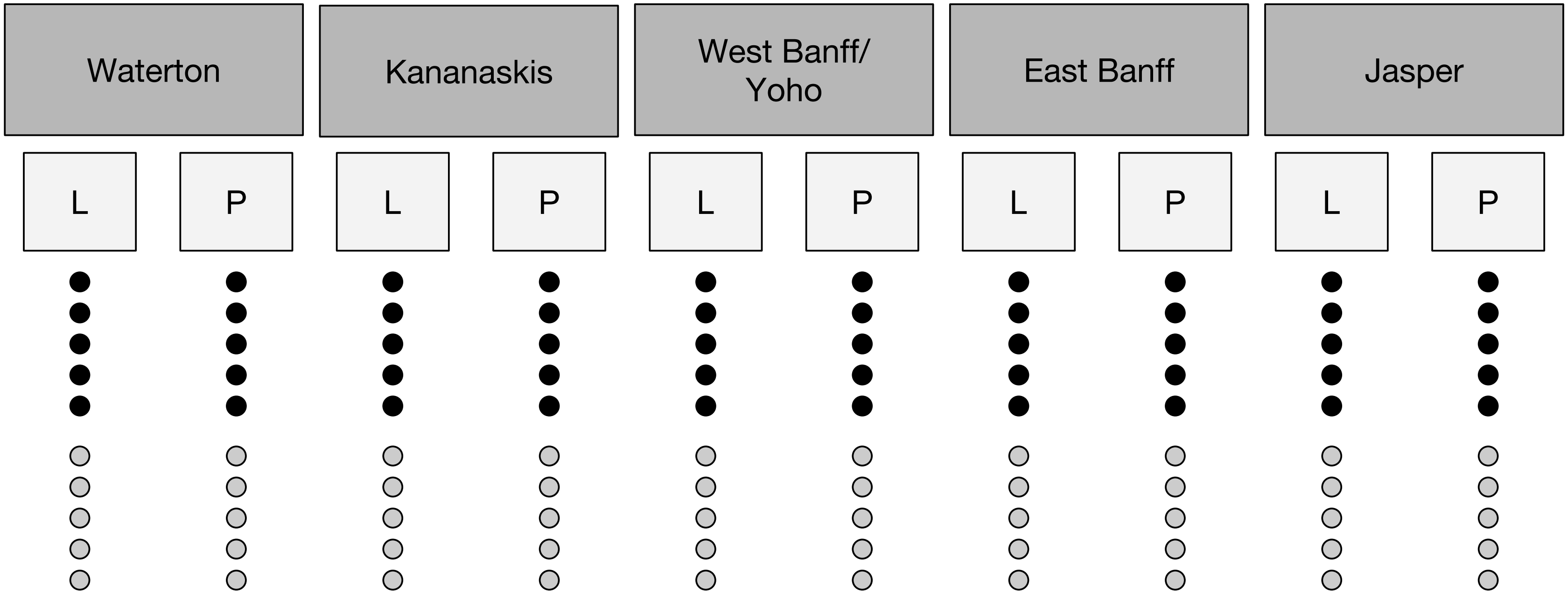

Figure 18: Nested design of multivariate statistics. Each of 5 sites have 2 habitat types (Lake and Pond) which will be placed in 2 temperature treatments (black and grey) with 5 clone lines for each. Each clone line will have 4 replicates.