Benthic Macroinvertebrates in the Alpine Lakes of Green Lakes Valley

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Abstract

The community composition of different benthic macroinvertebrates can be used as an indicator of environmental conditions. Benthic macroinvertebrates are small organisms that lack a backbone (invertebrate), live on the bottom (benthic), and are visible with the naked eye (macro). To better understand the community structure of benthic macroinvertebrates communities and how they respond to changing environmental conditions, 53 benthic macroinvertebrate samples were collected over the summer of 2018 from three alpine lakes in the Green Lakes Valley, CO. Benthic macroinvertebrates were sampled from the shoreline, inlet (inflow of water), and outlet (outflow of water) of each sampled lake, within lake variables (surrounding vegetation, temperature (C°), rock size (cm^3), nitrate (mg/L,) pH, and percent dissolved oxygen were also measured every time benthic macroinvertebrates were sampled. The results of this study suggest that lake identity and duration of ice-off period are major driving forces of benthic macroinvertebrate taxonomic richness and density when compared to within lake level variables. Considering that aquatic alpine environments are experiencing average sooner ice off times, benthic macroinvertebrates communities are expected to change in future years, establishing a need for future studies of benthic macroinvertebrates in changing alpine environments.

Introduction

Lakes found in the upper to middle elevational portions of a mountain ranges, referred to as alpine lakes, often share some defining characteristics. Alpine lakes occupy a relatively small area compared to the surrounding terrestrial environment. They are located at or near geographical low spots throughout alpine areas and therefore, control the flux of nutrients and water to downstream areas (Caine 1995; Wurtsbaugh et al. 2005). Thus, high elevation lakes are excellent indicators of change in the surrounding watershed (Williams et al. 1996; Miller and McKnight 2015). Furthermore, alpine lakes are considered to be oligotrophic, meaning they are characterized by low levels of dissolved nutrients and relatively low primary productivity levels (low rates of photosynthetic organisms converting atmospheric carbon dioxide into organic compounds) (Carlson 1977; Dodds et al. 1998; Catalan et el. 2009). This oligotrophic nature of aquatic alpine systems is in part due to the lack of biological buffering (soils natural ability to absorb various nutrients and minerals). High elevation lakes are typically surrounded by unvegetated slopes and therefore a lack of biological buffering capacity (Caine 1995; Williams et al. 1995).

The harsh climate of alpine lakes has the ability to influences and shapes the structure of aquatic communities. Species diversity in alpine environments are considered to be species limited compared to montane environments (areas below alpine and subalpine areas) (Rahbek 1995; Loewen et al. 2016). With low diversity in alpine environments there are relatively few organisms occupying each trophic level (e.g. position in food web). The three lowest trophic levels in alpine aquatic food webs are the primary producers, primary consumers, and secondary consumers (Hernández et al. 2015). Phytoplankton are microscopic photosynthesizing organisms that are suspended in the water column. Phytoplankton represent the base level of the web and are the primary producers. Zooplankton are primary consumer macroscopic organisms which are suspended or swim within the water column and represent the next connection in the food web as they consume phytoplankton. Benthic macroinvertebrates, small organisms that lack a backbone (invertebrate), live on the bottom (benthic), and are visible with the naked eye (macro), represent the next connection as they feed on zooplankton as secondary consumers. Animals that feed on secondary consumers are called tertiary consumers, a juvenile fish feeding on benthic macroinvertebrates is an example of a tertiary consumer. All trophic levels or communities of aquatic organisms in alpine lakes are affected by intense flushing rates driven by snowmelt, as well as limited growing seasons during short ice-free periods relative to ice-on periods (Miller and McKnight 2015; Pepin et al. 2015). However, each trophic level can be affected in various ways. For instance, because of their high elevational position, alpine communities have less atmosphere between them and the sun and therefore face high levels of ultra violet light (UV) which has been shown to decrease growth in phytoplankton (Davidson and Heijden 2000; Doyle and Saros 2005). Contrastingly, certain species of *Daphnia*, a member of the alpine zooplankton community, have been shown to adapt to increased UV light exposure with elevation by increasing levels of UV blocking pigment as elevation increases (Rhode et al. 2001; Hessen et al. 2002; Hansson et al. 2007). By understanding how alpine aquatic communities at each trophic levels respond to different conditions, the community composition of different trophic levels can be used as an indicator of environmental conditions: phytoplankton (Flanangan et al. 2009; Miller and McKnight 2015), zooplankton (Rhode et al. 2001; Hessen et al. 2002), and benthic macroinvertebrates (Goodnight 1973; Krieger 1984; Lenat 1988). Further research is needed on how different alpine aquatic communities respond to change, considering the aquatic alpine environment is changing as a result of climate warming and excess nutrient deposition.

There is strong evidence to suggest that over the past 50 years alpine aquatic ecosystems and the surrounding alpine environments have been significantly affected by global warming trends (Thompson et al. 2005; Benson et al. 2012; O'Reilly et al. 2015; Pepin et al. 2015; Preston et al. 2016). Recent anthropogenic actions like the burning of fossil fuels and deforestation have rapidly sped up the process of climate change by releasing unprecedented amounts of carbon dioxide into the atmosphere in a short amount of time, relative to the history of the earth (Barnola et al.1987; Petit 1999; Thompson et al. 2003). This result is known as the greenhouse effect (Ekholm 1901). Specifically in aquatic alpine ecosystems, recent climate warming trends have been correlated to higher average water temperatures and sooner ice of times (Livingstone 1997; Gerten & Adrian 2002; Benson et al. 2012; Preston et al. 2016; Hewitt et al. 2018). For instance, ice-off dates, the date a given lake is completely ice free, have been trending towards an earlier date each year in the Green Lakes Valley of Colorado, ice-off dates have shifted seven days earlier over the past 33 years (Preston et al. 2016). However, the consequences of earlier ice-off times can vary among lakes due to unique geographical position and shape (morphology), resulting in different chemical and physical characteristics. Nevertheless, general trends include warmer average water temperatures and earlier ice-off dates that reduce summer stream flow and therefore increase lake residence time (an increase in the amount time it takes a given lake to cycle through all its water), while increased melting of permafrost and glacial ice alter nutrient regimes, leading to an increase in lake inputs, such as dissolved nitrogen or phosphorus (Schindler et al.1996; Preston et al. 2016). Global warming has also increased lake residence times and higher average water temperatures which influence the stratification of lakes (the layering of lakes water columns by different temperature-density gradients). Altering the stratification changes nutrient regimes which have strong impacts on the community composition of lakes, like earlier spring phytoplankton blooms (Gerten & Adrian 2002; Miller and McKnight 2015). These alterations of phytoplankton communities can have cascading effects on the entire ecosystem. For instance, an increase in the amount of phytoplankton (phytoplankton bloom) will results in large phytoplankton communities consuming more dissolved organic carbon, which absorbs harmful UV light, resulting in increased UV levels and a decrease in phytoplankton growth (Davidson and Heijden 2000; Doyle and Saros 2005). Also, with less dissolved organic carbon, more UV light will penetrate the water column, causing higher rates of UV exposure for other communities (Schindler et al.1996; Schindler 1997; Miller and McKnight 2015). The effect of climate change on high elevation aquatic ecosystems is complex, it alters the abiotic (non-living) and biotic (living) interconnected processes of these systems, ultimately creating long lasting and far-reaching effects on the entire ecosystem. In particular, the effect climate change has on phytoplankton community structure may be further influenced by the process of excess nutrient deposition.

Finally, alpine aquatic ecosystems can be exposed to excess nutrient deposition, which can cause cascading effects across trophic levels. Excess nutrient deposition is defined here as above average levels of input or deposition of nutrients into ecosystems from external sources, predominantly human (anthropogenic) sources. The main excess nutrients in alpine aquatic ecosystems are nitrogen (N) and phosphorus (P) which are sourced from a variety of anthropogenic sources such as, agricultural fertilizer runoff, livestock waste, and combustion of fossil fuels (Smith et al. 1999; Parmesan 2006; Carpenter et al. 2011). Previous research from a high elevation environment has demonstrated that lakes within the Colorado Front Range and the Green Lakes Valley are displaying trends of increased inorganic nitrogen deposition from anthropogenic origin (Caine 1995; Baron et al. 2000; Williams and Tonnessen 2000; Williams et al. 2001; Wolfe et al. 2001; Wolfe et al. 2003). External inputs of N and P enter aquatic ecosystems from underground water sources, surface runoff (e.g. melting snow flowing on top of the soil), and atmospheric inputs (e.g. attachment of nutrients to water molecules in snow or rain) (Smith et al. 1999; Parmesan 2006; Carpenter et al. 2011). The reason external inputs of N and P induce change in aquatic ecosystems is that N and P are both considered limiting nutrients (Redfield 1958; Hecky and Kilman 1988; Smith et al. 1999). Liebig's Law of the minimum shows that plant growth is limited by the nutrient that is present in the lowest quantity relative to the plant growth demands (Liebig 1855) and the Redfield ratio shows the nitrogen-phosphorus ratio (N:P) found in ocean phytoplankton, represents the ratio of limiting nutrients within the water and is characteristic of the phytoplankton's nutrient growth requirements (Redfield 1958). Therefore, once excess N and P are deposited in aquatic ecosystems the nutrient limiting ratio for phytoplankton growth becomes unbalanced and phytoplankton respond according to the new nutrient ratios, ultimately changing the phytoplankton community structure (Anneville et al. 2005; Flanangan et al. 2009; Miller and McKnight 2015). For example, previous phytoplankton research from Green Lake 4, a lake within the Green Lakes Valley, demonstrated that increased nitrogen deposition over several years lead phytoplankton communities to switch from N limited to P limited, ultimately, increasing the number of nitrophilic species (Williams et al. 1996; Bowman et al. 2014; Miller and McKnight 2015). Changes in the structure of an aquatic community, such as the phytoplankton community, will likely affect the community composition of surrounding aquatic communities as they are interconnected through trophic interactions (Smith et al. 1999; Parmesan 2006; Catalan et el. 2009; Carpenter et al. 2011).

To better understand how alpine aquatic communities are responding and interacting in a changing climate, benthic macroinvertebrates were studied in the Green Lakes Valley, CO. The benthic macroinvertebrate community was chosen to be studied because within the Green Lakes Valley they are the most understudied community in relation to the phytoplankton and zooplankton community. The Green Lakes Valley and the surrounding ecosystem (Niwot Ridge, CO) has long been studied under the Niwot Ridge Long-Term Ecological Research program, (Niwot LTER). Green Lake 4 is the most studied lake in the valley and has been researched since the early 1980's. However, Green Lake 4's long term ecological record consists of a primary producer phytoplankton record from 2000 to present and a primary consumer zooplankton record from 2009 to present. By studying benthic macroinvertebrates in the Green Lakes Valley and adding a missing piece to the Niwot LTER ecological recorded, future Niwot LTER research can be conducted using the three base trophic levels to understand how aquatic alpine communities are responding and interacting in a changing climate.

Background

I. Benthic macroinvertebrates

In alpine lakes and streams the term benthic macroinvertebrate usually refers to aquatic insect larvae that inhabit the bottom of lakes and streams. However, the term benthic macroinvertebrate encompasses other macroscopic invertebrates that live in the benthic zone like oligochaetes (freshwater worms), Amphipoda (malacostraca crustaceans), and Tricladida (free living flatworms). Therefore, the term benthic macroinvertebrate describes a community of macroscopic invertebrates that inhabit the benthic zone. Most benthic macroinvertebrates have two main life stages, an immature larval stage and a mature adult form (Ward et al. 2002). The larval stage lives in the benthic zone of freshwater lakes, streams, and wetlands (Ward et al.

2002). Benthic macroinvertebrates spend the majority of their life in the larval stage and may undergo several sub transformational events (metamorphosis) until they reach their final metamorphosis stage and pupate into mature adult form, nearly all adult forms have wings (Ward et al. 2002). The adult stage is a short-lived stage with the main purpose of mating. Adults mate and the females drop their eggs back into the water to restart the process over (Ward et al. 2002). However, since most adult benthic macroinvertebrates are able to fly, they are able to preferentially select for their preferred environments, therefore the presence or absence of certain benthic macroinvertebrates is a strong indicator of certain environmental conditions (Goodnight 1973; Krieger 1984; Lenat 1988). The presence and abundance of certain benthic macroinvertebrate taxa depends on several controlling factors: temperature, speed of current, chemical conditions, dissolved gas and nutrient levels, type of substrate, and type of aquatic vegetation (Vannote et al. 1980; Benke 1984; Huryn and Wallace 1987; Wallace 1996; Ward et al. 2002). These controlling factors create several diverse microhabitats within a particular body of water (Frissell et al. 1986; Huryn and Wallace 1987; Wallace 1996). The main characteristic of most benthic macroinvertebrate microhabitat is running water (lotic water). Therefore, as a group, benthic macroinvertebrates have developed many physical adaptations to live in flowing waters: body flattening and streamlining, suckers, friction pads, claws, hooks, and production of silk, which all allow the macroinvertebrates to better attach themselves to various substrates (Statzner and Holm 1989; Ward et al. 2002).

II. Benthic macroinvertebrates as bioindicators

The presence or absence of certain benthic macroinvertebrate taxonomic group has long been used as tool by humans to indicate a particular state of water quality. Cairns and Pratt

(1993) summarize the European work of Kolkwitz and Marsson in 1908 and 1909 and describe how they developed the idea of ‘saprobity’, which measures the degree of pollution (primarily organic sewage) in rivers by evaluating dissolved oxygen levels. Kolkwitz and Marsson made observations of certain macroinvertebrate taxa in relation to the environmental conditions present, leading to a list of indicator species that represented a particular state of water quality (Cairns and Pratt 1993). Using their foundational idea others have expanded their use, linking benthic macroinvertebrates as bioindicators for certain aspects of water quality (Illies and Botosaneanu 1963; Vannote et al. 1980; Goodnight 1973; Krieger 1984; Lenat 1988). While Strahler’s work in 1957 didn’t involve benthic macroinvertebrates it also contributed to how alpine lakes and streams are viewed in the context of studying benthic macroinvertebrates. Strahler developed a methodology to measure the relative size of streams based on the number of tributaries that flow into a particular stream, low order streams have few to none small tributaries, while high order streams are fed by an extensive number of large tributaries (Strahler 1957). Building upon the idea of benthic macroinvertebrates as bioindicators and the stream order concept, Illies and Botosaneanu (1963) developed a zonation system, classifying streams based on changes in benthic macroinvertebrate faunal composition over a longitudinal profile. Further expanding upon zonation systems Vannote and colleagues (1980) developed the river continuum concept. The river continuum concept is a model used for describing and classifying flowing water based on the dynamic equilibrium of abiotic and biotic factors that allow certain feeder groups of benthic macroinvertebrates to be present; the model classifies four main types of feeding groups: shredders, grazers, collectors, and predators (Vannote et al. 1980). Shredders feed on coarse particulate matter by breaking the organic matter into smaller pieces and are characteristic of high order streams, grazers feed by scarping algae of attached surfaces (periphyton) and are characteristic of mid order streams, collectors filter and trap particulate matter and are characteristic of low order streams, lastly predators feed by preying on other animals and are fairly evenly distributed throughout all stream orders (Vannote et al. 1980). Using the foundational ideology of previous research, many studies have been conducted using macro benthic invertebrates as tools to bio-monitor water quality (Goodnight 1973; Krieger 1984; Lenat 1988). Using benthic macroinvertebrates as a biomonitoring tool to evaluate ecosystem health can be superior to using chemical and physical aspects to evaluate ecosystem health because the presence or absence of certain benthic macroinvertebrate taxa represents not only present environmental conditions but past and extreme environmental conditions (Wilhm and Dorris 1968; Goodnight 1973; Ward et al. 2002).

III. Current study and objectives

The four central objectives of this study are: (1) understand how benthic macroinvertebrate communities differ across lakes using taxonomic richness (number of different taxa present) and density (number of individual macroinvertebrates per given area); (2) evaluate how within lake variables (surrounding vegetation, temperature (C°), rock size (cm^3), nitrate (mg/L,) pH, and percent dissolved oxygen) are related to benthic macroinvertebrate taxonomic richness and density; (3) evaluate how benthic macroinvertebrate taxonomic richness and density changes through the sampling season; and finally (4) compare the benthic macroinvertebrate community composition to historic benthic macroinvertebrate community composition from the Green Lakes Valley reported by Bushnell and colleagues in 1987 (Bushnell et al. 1987). The long-term objective of this study is to establish a foundational methodology and community composition list for the future long-term study of benthic macroinvertebrates in the Green Lakes Valley. To achieve the objectives of this study, in the summer of 2018 during a two-month period I sampled benthic macroinvertebrates, from three lakes in the Green Lakes Valley approximately every other week for eight weeks. Before sampling three main questions were derived: (1) what is the relative influence of within lake variables and lake identity on benthic macroinvertebrate taxonomic richness and density? (2) is benthic macroinvertebrate taxonomic richness and density positively correlated to dissolved oxygen? as most macroinvertebrates in oligotrophic environments require high levels of dissolved oxygen (Ward et al. 2002; Connolly et al. 2004) and (3) will benthic macroinvertebrate density increase throughout the sampling season? as it is the limited summer growing season, characterized by a short ice-free period relative to ice-on period (Pepin et al. 2015; Preston et al. 2016) therefore benthic macroinvertebrates will grow and multiply as much as they can, at the time of year when nutrients needed for growth and reproduction are readily available.

Methods

I. Sample locations and descriptions

During the summer of 2018, I sampled three alpine lakes for benthic macroinvertebrates in Green Lakes Valley, Colorado: Green Lake 1, Green Lake 4, and Lake Albion (Figure 1.) Each lake was sampled approximately every two weeks for eight weeks (53 samples total) to evaluate benthic macroinvertebrate taxonomic richness and density over time. Within each lake there were six sample sites: inlet, outlet, two shoreline sites on the east side of the inlet-outlet orientation, and two shoreline sites on the west side of the inlet-outlet orientation. The exact amount of times each location was sampled was based on physical accessibility and time constraints. The sample locations are as follows and are listed following the elevational gradient:

Green Lake 4 (3550 m), Green Lake 1 (3425 m), and Lake Albion (3345 m). Each lake had three sampling events except Green Lake 4 which was visited four times.

II. Collecting protocol

At each sampling location I randomly selected five fully submerged rocks that were similarly sized (2000-4000 cubic centimeters each) and within one meter of the shore. Each rock was removed from the water and placed into a four-gallon bucket with a known quantity of water to estimate rock size via measurement of displaced water. Once in the bucket the rock was thoroughly scrubbed to remove all benthic macroinvertebrates (Huryn and Wallace 1987; Hannaford and Resh 1995; Barbour et al. 1999). While the rock was in the bucket the sediment where the rock had previously been was agitated for 20 seconds and followed by three dip-net sweeps to catch any macroinvertebrates living in the sediment under the rock (Lenat 1988; Barbour et al. 1999). The rock was then placed back in the place it was removed from and the water in the bucket was poured through a 500 µm Mesh D-frame dip-net to accumulate all macroinvertebrates (Huryn and Wallace 1987; Hannaford and Resh 1995; Barbour et al. 1999). The dip-net was then flipped inside out, and the contents of the net were placed back into the bucket in a small amount of corresponding water. The contents of the bucket were then poured into a 500 mL Nalgene bottle using a funnel and preserved with an 80% ethanol solution. The bottle was then labeled with: date collected, time collected, sample location, site location, and stored until microscope analysis.

Due to the natural chemical variation between lakes at different elevations and variations in lake morphology, certain environmental parameters were quantified at each site. Using visual observation, I observed dominant surrounding vegetation within three meters of each site and estimated the area to be vegetated or unvegetated, an estimation of more than 50% vegetation was considered vegetated. A Garmin eTrex 10 Global Positioning System (GPS) was used to measure the site coordinates at each site and a YSI 556 multi-probe meter (YSI of the Americas) was used to measure temperature (C°), percent dissolved oxygen, pH, and nitrate (mg/L). YSI probe measurements were made 0.1 m under the surface and 0.5 m off shore. All YSI probe measurements were recorded at every site before benthic macroinvertebrates were sampled.

III. Taxonomic identification and quantification

All preserved samples of benthic macroinvertebrates were taxonomically identified using an Olympus SZ51 Stereomicroscope (Olympus Corporation of the Americas) using a magnification range of 8-40x. The full contents of each sample (Nalgene bottle) were analyzed to the lowest taxonomic resolution using taxonomic guides (Ward et al. 2002; Thorp and Covich 2009; Iowa State University Department of Entomology 2019). The taxonomic identifications of all benthic macroinvertebrates were cross referenced with previous taxonomic information from the Green Lakes Valley (invertebrate surveys in 1960 and early 1980's) (Bushnell et al. 1987). All benthic macroinvertebrates were taxonomically identified to at least family. In most cases Trichoptera (caddisflies), Ephemeroptera (mayflies), and Plecoptera (stoneflies) were taxonomically identified to genus level and in few cases species level. Since the full contents of each sample were analyzed taxonomically and each sample represents one site on a given date at a given lake, benthic macroinvertebrate taxonomic richness was estimated at a site by date by lake level. At each sample site every individual macroinvertebrate of the same taxonomic family was quantified, ultimately creating a category for total number of individuals within each taxonomic family to be used to calculate taxonomic richness and density in statistical analysis.

IV. Statistical analysis

To evaluate how benthic macroinvertebrate taxonomic richness and density changed over time and among sites, I used a generalized linear mixed modeling framework. Taxonomic richness was calculated by summing the total number of different taxonomic families present per sample site and was modeled as a Poisson distribution with a log-link function. Sample density was calculated by dividing the total count of individuals pooled among all taxa per sample site by total rock volume (summed across the five sampled rocks per site, volume in cm^3). An offset for total rock volume was added to the model of benthic macroinvertebrate density to standardize for the various size of rocks collected. The generalized linear mixed model of benthic macroinvertebrates density with poisson distribution (log-link function) and offset for total rock volume was determined to be over-dispersed (Variance > Mean, 29.06 > 4.17). To correct for over-dispersion an observational category was created and run as a random effect (Harrison 2014). The use of observational category random effect with poisson distribution is similar to using a negative binomial model, it allows the variance to be independently adjusted from the mean. Total rock volume per site was verified to have a non-significant effect on taxonomic richness, suggesting that that there was no relation to effort (total rock volume collected) and taxonomic richness, indicating that the sampling effort was adequate.

Each model included fixed effects for water temperature (C°), percent dissolved oxygen, pH, nitrate (mg/L), surrounding vegetation, visit number, running water, and lake identity. Vegetation of each site was recorded as a dichotomous variable: vegetated or unvegetated. Visit was a numeric value of 1-4 accomplished by dividing the sampling season into four parts denoted by visit one through four. Running water was recorded as a presence-absence variable, generated by using a one when running water was present and a zero when running water was absent. Lake was a three-factor variable: Green Lake 1, Green Lake 2, and Lake Albion; lake identity was run as a fixed effect (GL1, GL4, or Albion). Model selection was accomplished by starting with a full model (containing all fixed effects) and then eliminating variables in backward selection after the model was computed, the fixed effect with the highest p-value was dropped each time. After model selection Akaike information Criterion (AIC) was used to ensure that the selected model best represented the data (had lowest AIC score when compared to other models) (AIC package, R Development Core Team 2008). Lakes were determined to be different from each other by using the re-level function and comparing the p-values in a univariate general linear model with Poisson distribution as a log-link function. All analysis was performed within the lme4 package in the statistical framework R (R Development Core Team 2017).

Results

I. Community composition

In 53 samples collected over 57 days, I identified a total of 10 different taxonomic families of benthic macroinvertebrates from the three sampled lakes (Table 1). Community composition was composed of seven different orders of benthic macroinvertebrate: Trichoptera (caddisflies), Ephemeroptera (mayflies), Plecoptera (stoneflies), Diptera (flies), Amphipoda (malacostraca crustaceans), Coleoptera (beetles), and Tricladida (free living flatworms). Across all sampled lakes two different families of Trichoptera (caddisflies) were identified: Rhyacophilidae and Leptoceridae. Interestingly, the majority of the two Trichoptera families were found in relation to the elevation of each sampled lake, the Rhyacophilidae family appears to prefer higher elevation lakes while the Leptoceridae family appears to prefer lower elevation lakes (Table 1). Two different families of Ephemeroptera (mayflies) were identified: Baetidae and Ameletidae of which both families were absent from Green Lake 1 (Table 1), potentially an indication of a of habitat preference for flowing water as Green Lake 1 is a seepage lake and does not contain running water (does not have an inlet or outlet). However, Green Lake 4 and Lake Albion both contain running water. Similar ideology applies to the one family of Plecoptera (stoneflies) identified: Perlodidae, as the Perlodidae family was also found in Green Lake 4 and Lake Albion but not in Green Lake 1 (Table 1). The same ideology can also be applied in reverse to the one family of Amphipoda (malacostraca crustaceans) sampled: Gammaridae, as all sampling encounters occurred in Green Lake 1 (Table 1), indicating again a possible habitat preference of non-running water. Across all sampled lakes two different families of Diptera (flies) were identified: Chironomidae and Simuliidae. The Chironomidae family was found in all sampled lakes, while the Simuliidae family was found only in Green Lake 4 (Table 1). The presence of the Simuliidae family only in Green Lake 4 appears to indicate a possible elevational preference in habitat, but the Simuliidae family was only encountered in sampling one time (7/17/18), therefore more sampling is needed to infer any possible relationship to elevation; this same ideology applies the one family of Coleoptera (beetles) found in Green Lake 4, as it was encountered only in Green Lake 4 on one sampling visit (7/17/18). Across all lakes only one family of Tricladida (free living flatworms) was identified: Planariidae, the Planariidae family was only encountered in the two lakes with higher elevations (Table 1). The three most frequently encountered benthic macroinvertebrate families in this study were: (1) the Chironomidae family (non-biting flies) found in 68% of samples, (2) the Rhyacophilidae family (caddisflies) found in 53% of samples, and (3) the Ameletidae family (mayflies) found in 32% of samples.

II. Taxonomic richness

Across all sampled lakes, benthic macroinvertebrate taxonomic richness increased over time as the sampling season progressed (Figure 2). For instance, from the first visit to the fourth visit the average benthic macroinvertebrate taxonomic richness increased by 0.76-fold. Overall, the variables measured within each lake had no significant influence on benthic macroinvertebrate taxonomic richness. Stronger effects on taxonomic richness were associated with lake identity. Benthic macroinvertebrate taxonomic richness in Lake Albion was determined to be significantly different from Green Lake 1 and Green Lake 4, taxonomic richness was significantly lower in Lake Albion (Mean±=SD, 1.3 ± 0.28) than Green Lake 1 (Mean±=SD, 2.4 ± 0.28) or Green lake 4 (Mean±=SD, 2.6 ± .027) (Figure 3).

III. Density

Across all sampled lakes, benthic macroinvertebrate density increased over time as the sampling season progressed (Figure 4), from the first visit to the fourth visit the average benthic macroinvertebrate density increased by 1.14-fold. Benthic macroinvertebrate density was found to be influenced by one variable measured within lake. pH was negatively correlated to benthic macroinvertebrate density ($\boldsymbol{\beta}_{\mathrm{pH}}$: - 0.57 ± 0.17, p=0.002) (Figure 5), while the within lake variable of pH did influence benthic macroinvertebrate density. Lake identity was still a major driving force of benthic macroinvertebrate density such that Green Lake 1 was significantly different from Lake Albion and Green Lake 4. Benthic macroinvertebrate density was significantly higher in Green Lake 1 (Mean±=SD, 5.4 ± 0.43) than Green Lake 4 (Mean±=SD, 3.8 ± 0.41) or Lake Albion (Mean±=SD, 3.4 ± 0.44) (Figure 6).

Discussion

To better understand the structure of aquatic alpine communities in the Green Lakes Valley and how these communities respond to changing environmental conditions, 53 benthic macroinvertebrate samples were collected over the summer of 2018 from three alpine lakes in the Green Lakes Valley. I taxonomically identified a total of ten different benthic macroinvertebrate families. Across all sampled lakes, benthic macroinvertebrate taxonomic richness and density increased over the summer, however, benthic macroinvertebrate taxonomic richness and density was not significantly correlated to any within lake level variable except pH. Thus, lake identity appears to be a stronger driving force of benthic macroinvertebrate taxonomic richness and density than within lake variables.

The results of my study were compared to previous research on benthic macroinvertebrates in the alpine lakes of the Swiss Alps (Füreder et al. 2006) and Tatra Mountains (Krno et al. 2006; Hamerlík et al. 2014;) as well historic research from the Green Lakes Valley in 1960 and the early 1980's (Bushnell et al. 1987). Measures of density are absent from the examined studies, as benthic macroinvertebrates were sampled using mesh nets (Füreder et al. 2006; Krno et al. 2006; Hamerlík et al. 2014) or rock picked with forceps (Bushnell et al. 1987), therefore a volume metric to be used to calculate density was not quantified in any sampling method. However, the frequency at which the Chironomidae family (non-biting flies) was found in my study is comparable to previous research, in this study the Chironomidae family was found in 68% of samples, previous research from alpine lakes in the Swiss Alps showed that depending on the species, the Chironomidae family constituted 72-96% of samples (Füreder et al. 2006). While previous research from alpine lakes in the Tatra Mountains showed that the Chironomidae family constituted over 50% of the diversity of each sample (Hamerlík et al. 2014) and was found in 100% of samples

(Krno et al. 2006). The seconded most frequently encountered benthic macroinvertebrate family in this study was the Rhyacophilidae family (caddisflies) found in 53% of samples, the Rhyacophilidae family belongs to the trichoptera order (caddisflies). Research from alpine lakes in the Tatra Mountains showed that the trichoptera order was the seconded most frequently encountered benthic macroinvertebrate group (Hamerlík et al. 2014), found in 69% of samples (Krno et al. 2006). Research from the Green Lakes Valley in 1981 showed that at any particular lake site the Chironomidae family constituted 22-67% of samples (Bushnell et al. 1987). Since the frequency at which the Chironomidae family was found in this study is similar to reported frequencies in other alpine lakes across the world, this study confirms that the Chironomidae family of non-biting flies constitute a large percent of the benthic macroinvertebrate densities in alpine lakes.

To accurately evaluate the taxonomic richness and community composition of benthic macroinvertebrates found in my study (Table 1**)**, findings were compared to historic studies of benthic macroinvertebrates from Green Lakes Valley (Bushnell et al. 1987). To accurately cross compare the studies it is necessary to understand the differences between the two studies. My study is focused exclusively on sampling benthic macroinvertebrates from the alpine lakes of the Green Lakes Valley, while the historic studies presented by Bushnell and colleagues (1987) samples benthic macroinvertebrates in alpine lakes as well as alpine streams found throughout Green Lakes Valley. The historic studies also sampled lakes within the valley that are not sampled in this study (Green Lake 2, Green Lake 3, and Green Lake 5) (Bushnell et al. 1987). To further complicate matters Bushnell and others (1987) taxonomically identified benthic macroinvertebrates to genus level, while my study taxonomically identified to family level. To account for the differences between studies, the benthic macroinvertebrate taxonomic identification presented by Bushnell et al. (1987) was adjusted to family level and restricted to identifications made from lakes sampled in this study (Lake Albion, Green Lake 1, and Green Lake 4). The adjusted taxonomic identifications of Bushnell and colleagues (1987) also excludes identifications made of of adult benthic macroinvertebrates, on the account that only benthic macroinvertebrate larvae were sampled in this study. A total of ten different benthic macroinvertebrate families were identified in my study, the adjusted number of benthic macroinvertebrate families identified by Bushnell et al. (1987) is 18 families. The average benthic macroinvertebrate taxonomic richness of each lake in this study was not compared to the adjusted benthic macroinvertebrate taxonomic richness of Bushnell et al. (1987) because this study estimated taxonomic richness on a site level (average richness of a given site at a given lake on a given date) while the presented data of Bushnell et al. (1987) estimated taxonomic richness on a lake level. However, the total number of different benthic macroinvertebrate families identified in this study can be compared to the adjusted number of benthic macroinvertebrate families identified by Bushnell and colleagues (1987) because the values are both estimated on a collective lake level (taxonomic families present or absent in one of the three lakes sampled in this study), 1.8x more benthic macroinvertebrate taxonomic families were identified in the Green Lakes Valley historically then identified in my study. The observed decrease in benthic macroinvertebrate taxonomic richness in the Green Lakes Valley from the early 1980's (Bushnell et al. 1987) to 2018 (current study) could be attributed to the changing climate in the alpine environment via climate warming (Thompson et al. 2005; Benson et al. 2012; O'Reilly et al. 2015; Pepin et al. 2015; Preston et al. 2016) or excess nutrient deposition (Caine 1995; Baron et al. 2000; Williams and Tonnessen 2000; Williams et al. 2001; Wolfe et al. 2001; Wolfe et al. 2003). However, it is more likely that then sampling methods of my study where insufficient in the catching of particular benthic macroinvertebrate groups, leading to a low reported richness value, this study failed to catch and identify any aquatic worms (Oligochaeta), aquatic spiders (Acarina), or filter feeders (Bryozoa and Hydridae), these groups were all identified by Bushnell and colleagues (1987). While the benthic macroinvertebrate taxonomic richness of my study did not compare well to the findings presented by Bushnell and colleagues (1987), the community composition of both studies is very similar.

All benthic macroinvertebrate families taxonomically identified in my study where taxonomically identified in the historic studies presented by Bushnell and colleagues (1987), with the exception of the mayfly (Ephemeroptera) Ameletidae *Ameletus* identified in this study and not in the historic studies presented by Bushnell and colleagues (1987). A summary of the historic study's benthic macroinvertebrate community composition (Bushnell et al. 1987) compared to the current study's community composition can be seen in Table 2. It is unclear if the genus of mayfly *Ameletus* spp. found in the current study was found historically in the Green Lakes Valley (Bushnell et al. 1987), as the historic study presents the genus *Ameletus* under the mayfly family of Siplonuridae and not Ameletidae (Table 2); considering the age of the presented historic studies (Bushnell et al. 1987) it is possible that the taxonomic groupings have changed in recent years. The other notable difference was that this current study identified the caddisfly (Trichoptera) family of Leptoceridae in larvae forum while the historic studies presented by Bushnell et al. (1987) only identified the caddisfly family in adult forum (Table 2). Besides the two described discrepancies the benthic macroinvertebrate community composition identifications (to family level) of the Green Lakes Valley currently and the Green Lakes Valley historically (Bushnell et al. 1987) are identical (Table 2); this finding supports the historic findings of Bushnell et al. (1987). In the studies presented by Bushnell et al. (1987), there are two surveys of benthic macroinvertebrates, a 1960 sampling and a 1981-1982 sampling (additional samples in 1983, 1984, and 1985), the conclusion is drawn that between the 1960 survey and the early 1980's survey the distribution of major benthic macroinvertebrate taxonomic groups remained relatively stable (Bushnell et al. 1987). My study is in support of the historic data because benthic macroinvertebrate community composition identifications in 2018 where similar to historic identifications presented by Bushnell et al. (1987) (Table 2).

The results of my study indicate that variables measured within lake (temperature, percent dissolved oxygen, pH, and nitrate concentration) had very little influence on benthic macroinvertebrate taxonomic richness or density. Across all sampled lakes the only within lake level variable that showed a correlation to either benthic macroinvertebrate taxonomic richness or density was pH, it showed a negative correlation to density($\boldsymbol{\beta}_{\mathrm{pH}}$: -0.57 ± 0.17, p=0.002), meaning sample sites with low pH's had significantly higher benthic macroinvertebrate densities. A possible rationale behind this result is that the breaking down of organic matter (ex. leaves) is an acidic process (Donahue et al. 1998; Miller et al. 2009) and organic matter is a food source for benthic macroinvertebrates (Ward et al. 2002) Therefore, a large amount of organic matter breaking down at a particular sample site could account for a low pH level and an abundant food source to draw in higher densities of macroinvertebrates, although it is also possible that lake identity (the different abiotic and biotic characteristics present in a given lake) was influencing benthic macroinvertebrate density and not pH.

Lake identity was determined to be a major driving force on benthic macroinvertebrate taxonomic richness and density in my study. Benthic macroinvertebrate taxonomic richness was significantly lower in Lake Albion than Green Lake 1 or Green lake

4 and benthic macroinvertebrate density was significantly higher in Green Lake 1 than Green Lake 4 or Lake Albion. The low benthic macroinvertebrate taxonomic richness observed in Lake Albion compared to the other two sampled lakes, could be attributed to Lake Albion being considered more of an alpine reservoir than a lake, as the flow is highly regulated by the City of Boulder. Lake Albion has been dammed and partially drained historically to make repairs on the dam (Bushnell et al. 1987). The anthropogenic factors influencing Lake Albion could decrease overall water quality, limiting the benthic macroinvertebrate community to species that are more tolerate of poor water quality, reducing the overall taxonomic richness, this relationship has been observed in rivers affected by anthropogenic factors (Azrina at el. 2006; Maloney et al. 2011). The high benthic macroinvertebrate density observed in Green Lake 1 compared to the other two sampled lakes, could be attributed Green Lake 1's unique geographic position. Bushnell et al. (1987) found that "species diversities of Green Lake 1 and Stream 1 were both higher than mean diversities of all lakes and streams", the high taxonomic richness observed in Green 1 and subsequent stream was attributed to Green Lake 1 having longer ice-off seasons due shallow lake depth and full southern exposure to the sun (Bushnell et al. 1987). The Depths of the three lakes sampled in this study are: Green Lake 4, 13 meters deep, Green Lake 1, 7 meters deep, and Lake Albion, 15 meters deep (Niwot LTER SOP). Lake size and ice cover duration have been shown to influence the community composition of aquatic macroinvertebrate fauna of European alpine lakes (Catalan et el. 2009). Therefore, it is likely that the unique characteristics of Green Lake 1 (shallow depth and full southern exposure) create a unique lake identity that is a driving force of benthic macroinvertebrate taxonomic richness and density. Further evidence that lake identity is a driving force on benthic macroinvertebrate taxonomic richness and density can be seen in the fact that

certain benthic macroinvertebrate families encountered in my study were found only in certain lakes (Table 1). Different benthic macroinvertebrate families present in different lakes supports the idea that lake identities (unique chemical and physical characteristics) create diverse microhabitats that particular benthic macroinvertebrate families or groups are adapted too (Frissell et al. 1986; Huryn and Wallace 1987; Wallace 1996).

The results of my study also show that both benthic macroinvertebrate taxonomic richness and density increased over the sampling season, regardless of lake identity. From the first visit of the sampling season to the fourth visit (last visit) of the sampling season the average benthic macroinvertebrate taxonomic richness increased by 0.76-fold while the average benthic macroinvertebrate density increased by 1.14-fold. In examination of the scientific literature there appears to be an absence of research on how benthic macroinvertebrate taxonomic richness and density change throughout an ice-off period in an alpine lake. The historic studies of benthic macroinvertebrates in the Green Lakes Valley presented by Bushnell et al. (1987) state that there were two peaks in benthic macroinvertebrate abundance (highest total amount of benthic macroinvertebrates), the seconded peak occurred in early September in both the 1981-1982 survey and subsequent 1985 survey; however, the first peak in benthic macroinvertebrate abundance occurred in late July in the 1981-1982 survey, while the first peak abundance in the 1985 survey occurred in August. The results of my study demonstrate that benthic macroinvertebrate taxonomic richness and density increase throughout ice-off periods but historic studies (Bushnell et al. 1987) from the Green Lakes Valley suggest that benthic macroinvertebrate abundance varies from year to year; in comparison these results suggest that benthic macroinvertebrates increase in taxonomic richness and density during ice-off periods but these periods of increase fluctuation from year to year with the fluctuation of ice-off times in alpine lakes from year to year (Preston et al. 2016).

Conclusion and future work

While the results of my study suggest that lake identity is a major driving force of benthic macroinvertebrate taxonomic richness and density, my results also suggest that benthic macroinvertebrate taxonomic richness and density is influenced by other environmental variables such duration of ice-off season and date of ice-off/ice-on. Considering that recent climate warming trends in alpine lakes have been correlated to higher average water temperatures and sooner ice of times (Livingstone 1997; Gerten & Adrian 2002; Benson et al. 2012; Preston et al. 2016; Hewitt et al. 2018), the results of my work suggest that communities of benthic macroinvertebrates in alpine lakes are going to experience future changes regardless of lake identity. To evaluate exactly how benthic macroinvertebrate communities, respond to future change, further studies on benthic macroinvertebrates need to be conducted on a long term time scale, with the quantification of environmental variables like duration of ice-off season and date of ice-off/ice-on. This long-term future work on benthic macroinvertebrate communities in a changing climate should also seek to improve upon the established sampling and taxonomic identification methods of my study, in order to capture and taxonomically identify benthic macroinvertebrates groups encountered in the historic studies of the Green Lakes Valley (Bushnell et al. 1987) but not encountered in my study.

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Figures

Figure 1. An elevational map (meters) of the alpine lakes within the Green Lakes Valley, Colorado. The yellow stars signify the three lakes sampled for benthic macroinvertebrates in 2018: Green Lake 4, Green Lake 1, and Lake Albion. Figure modified from the Niwot Long Term Ecological Research program standard operating procedure.

Benthic Macroinvertebrate Taxonomic Family	**Lake Albion (3345 m)**	**Green Lake 1 (3425 m)**	**Green Lake 4 (3550 m)**
Trichoptera Rhyacophilidae *Rhyacophila*	0 %	12 %	88 %
Trichoptera Leptoceridae	15 %	80 %	5 %
Ephemeroptera Baetidae *Baetis*	16 %	0 %	84 %
Ephemeroptera Ameletidae *Ameletus*	2 %	0 %	98 %
Plecoptera Perlodidae	67 %	0 %	33 %
Diptera Chironomidae	58 %	28 %	14 %
Diptera Simuliidae	0 %	0 %	100 %
Amphipoda Gammaridae	0 %	100 %	0 %
Coleoptera Dytiscidae	0 %	0 %	100 %
Tricladida Planariidae Polycelis *coronata*	0 %	22 %	78 %

Table 1. Represents the percentage of benthic macroinvertebrate taxonomic families found in each sampled lake (Green Lakes Valley, 2018). The percentage constituted of each benthic macroinvertebrate taxonomic family is based on the total number of samples collected from each lake throughout the entire sampling season.

Benthic Macroinvertebrate Taxonomic Family Identified in Current Study	**Benthic Macroinvertebrate Taxonomic Family Identified in Historic Study**
Trichoptera Rhyacophilidae *Rhyacophila*	Yes (*Rhyacophila* genus found in 1960 and 1981-1982)
Trichoptera Leptoceridae	Yes (Leptoceridae adult forum identified in 1981-1982)
Ephemeroptera Baetidae *Baetis*	Yes (*Baetis* genus identified in 1960 and 1981-1982)
Ephemeroptera Ameletidae *Ameletus*	No (Ameletidae family not identified but *Ameletus* genus found in 1981-1982, listed under Siplonuridae family)
Plecoptera Perlodidae	Yes (Perlodidae family identified in 1960 and 1981-1982)
Diptera Chironomidae	Yes (Chironomidae family identified in 1960 and 1981-1982)
Diptera Simuliidae	Yes (Simuliidae family identified in 1960 and 1981-1982)
Amphipoda Gammaridae	Yes (Gammaridae family identified in 1960 and 1981-1982)
Coleoptera Dytiscidae	Yes (Dytiscidae family identified in 1981-1982)
Tricladida Planariidae Polycelis *coronata*	Yes (Polycelis *coronata* found in 1960 and 1981-1982)

Table 2. Compares whether the benthic macroinvertebrate taxonomic families identified in this current study (Green Lakes Valley 2018) where identified in historic studies from the Green Lakes Valley (1960 survey and 1981-1982 survey) presented by Bushnell et al. (1987).

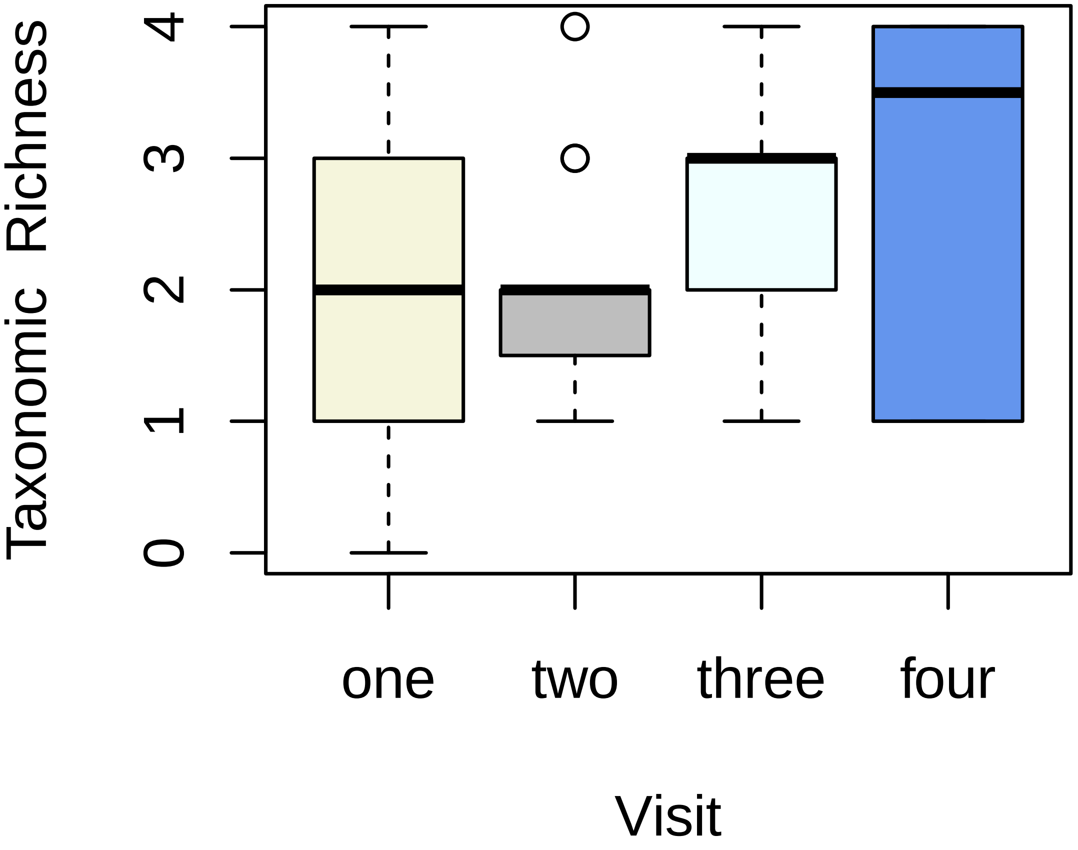

Figure 2. The relationship between benthic macroinvertebrate taxonomic richness and visit (number of different taxonomic families) over the sampling season (Green Lakes Valley, 2018). The Sample season is divided into four equal sections of time denoted by visit one, two, three, four. Means are depicted by black horizontal lines, the horizontal edges of the boxes represents the 25th and 75th percentiles, the dashed lines extending vertically from the boxes represent 10th and 90th percentiles, and the horizontal lines on the at the end of the dashed lines represent the outliers (5th and 95th percentiles).

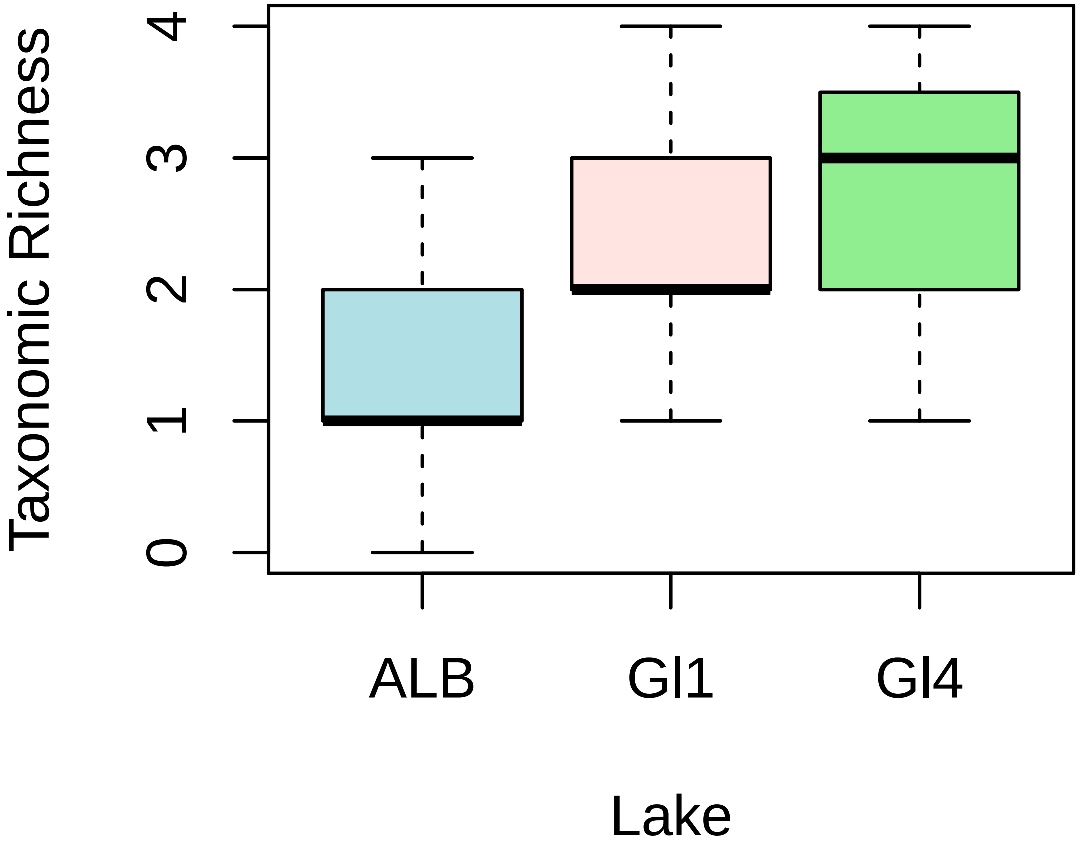

Figure 3. Represents the benthic macroinvertebrate taxonomic richness (number of different taxonomic families) from the three alpine lakes sampled: Lake Albion (ALB), Green Lake 1 (Gl1), and Green Lake 4 (Gl4) in the Green Lakes Valley, 2018. Means are depicted by black horizontal lines, the horizontal edges of the boxes represents the 25th and 75th percentiles, the dashed lines extending vertically from the boxes represent 10th and 90th percentiles, and the horizontal lines on the at the end of the dashed lines represent the outliers (5th and 95th percentiles).

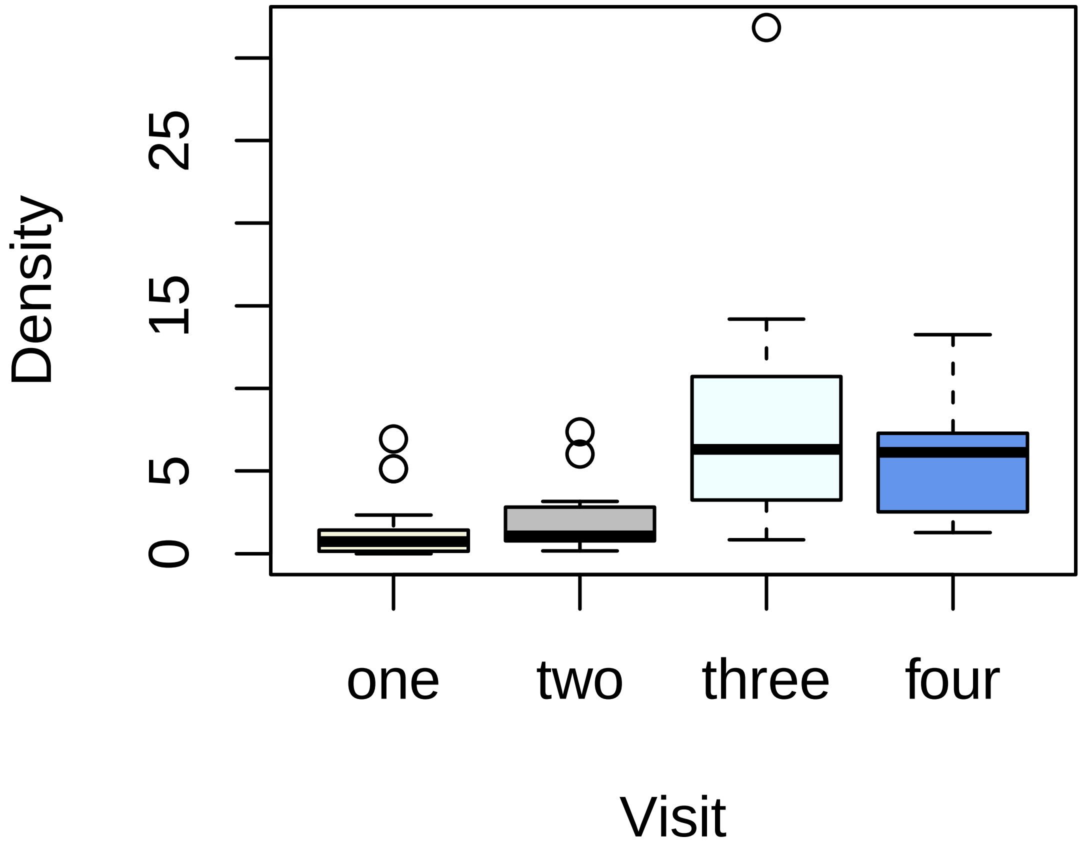

Figure 4. The relationship between benthic macroinvertebrate density (number of benthic macroinvertebrates collected at a given site divide by the total rock volume collected at each site, count/centimeters cubed) over the sampling season (Green Lakes Valley, 2018). The Sample season is divided into four equal sections of time denoted by visit one, two, three, four. Means are depicted by black horizontal lines, the horizontal edges of the boxes represents the 25th and 75th percentiles, the dashed lines extending vertically from the boxes represent 10th and 90th percentiles, and the horizontal lines on the at the end of the dashed lines represent the outliers (5th and 95th percentiles).

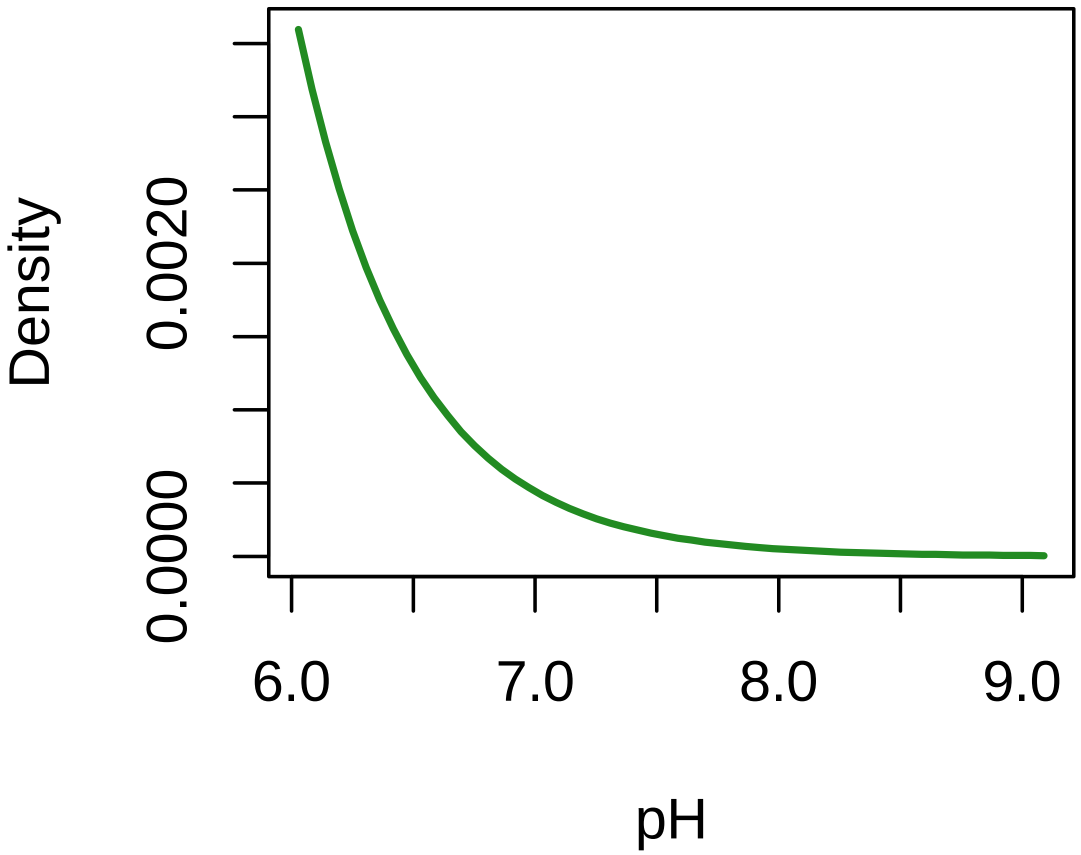

Figure 5. The predicated relationship between benthic macroinvertebrate density (number of benthic macroinvertebrates collected at a given site divide by the total rock volume collected at each site, count/centimeters cubed) and pH of each sampling site, across all sampled lakes in the Green Lakes Valley, 2018.

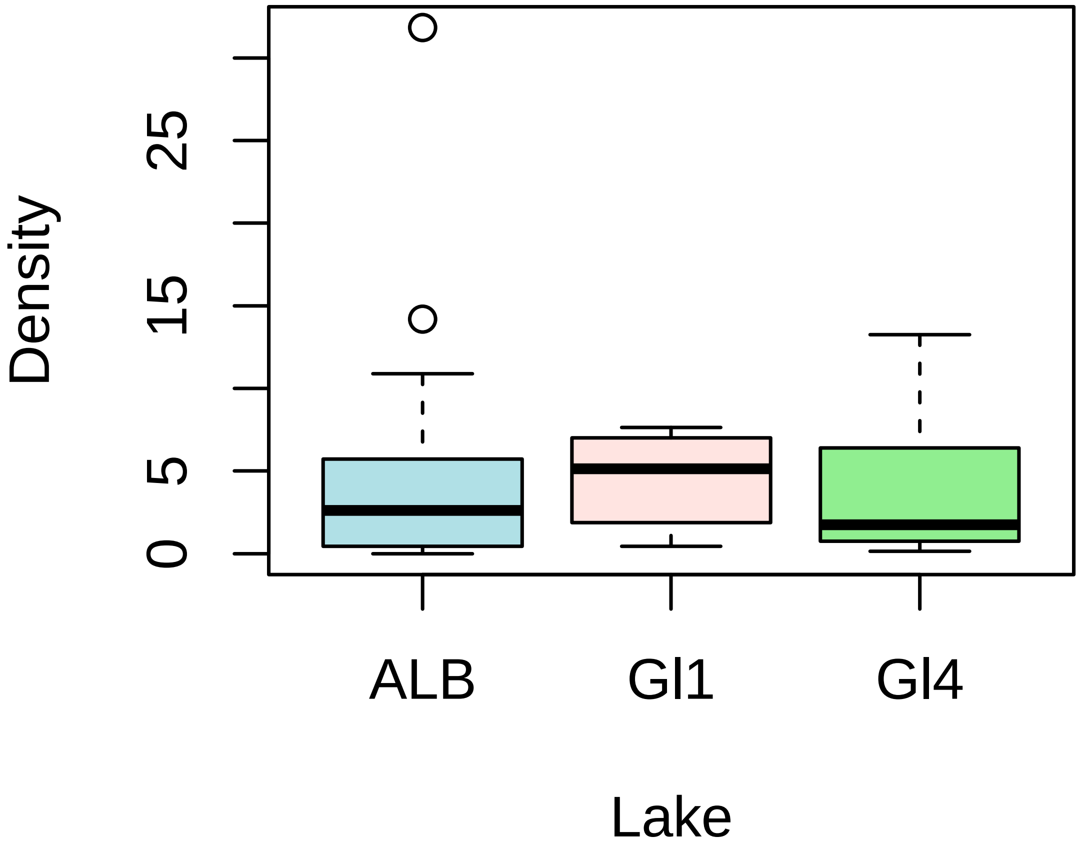

Figure 6. Represents the benthic macroinvertebrate density (number of benthic macroinvertebrates collected at a given site divide by the total rock volume collected at each site, count/centimeters cubed) from the three alpine lakes sampled: Lake Albion (ALB), Green Lake 1 (Gl1), and Green Lake 4 (Gl4) in the Green Lakes Valley, 2018. Means are depicted by black horizontal lines, the horizontal edges of the boxes represents the 25th and 75th percentiles, the dashed lines extending vertically from the boxes represent 10th and 90th percentiles, and the horizontal lines on the at the end of the dashed lines represent the outliers (5th and 95th percentiles).

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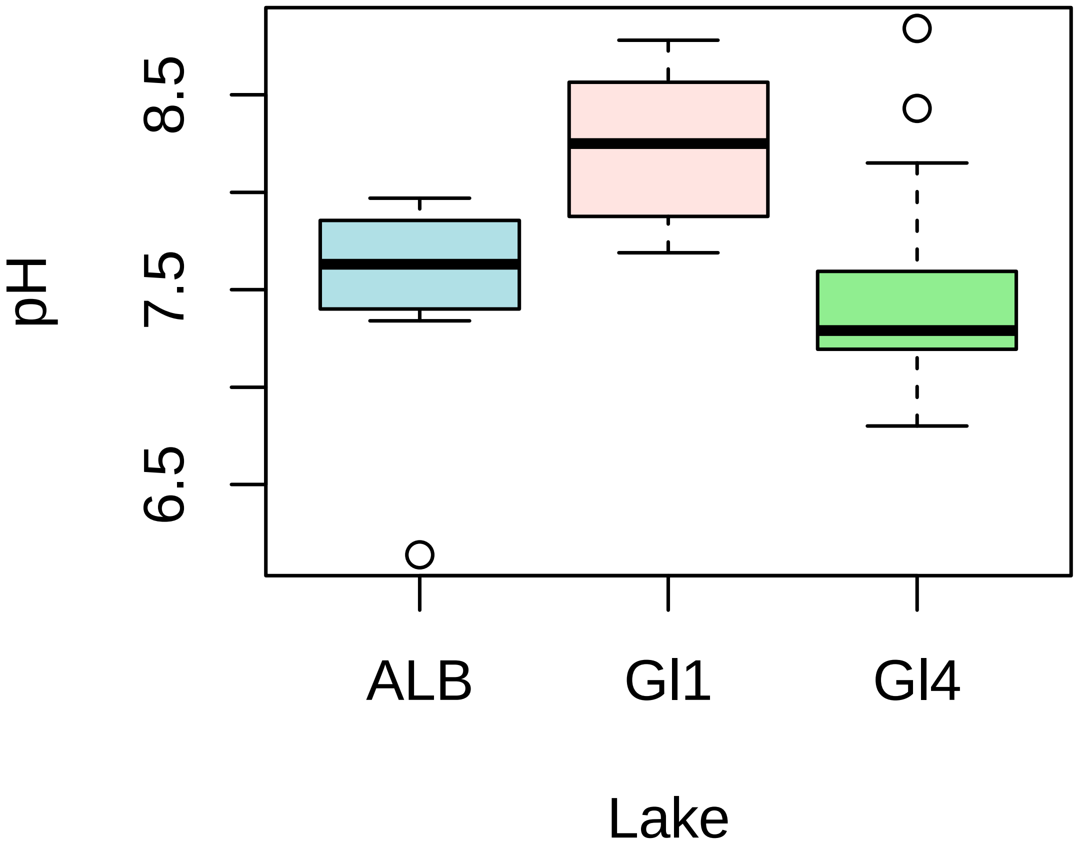

Figure 7. Represents the pH levels of within lake sites across the whole sampling season from the three alpine lakes sampled: Lake Albion (ALB), Green Lake 1 (Gl1), and Green Lake 4 (Gl4) in the Green Lakes Valley, 2018. Means are depicted by black horizontal lines, the horizontal edges of the boxes represents the 25th and 75th percentiles, the dashed lines extending vertically from the boxes represent 10th and 90th percentiles, and the horizontal lines on the at the end of the dashed lines represent the outliers (5th and 95th percentiles).