Zooplankton community diversity of upstream and downstream lakes in Mission Mountain watersheds

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Abstract

Zooplankton, more specifically three taxonomic groups: Phylum Rotifera; Subclass Copepoda; and Suborder Cladocera, are an integral component of freshwater ecosystems. At the local level there are a variety of factors which have been indentified that influence zooplankton species richness and/or diversity: lake area (Dodson 1992; Dodson et al. 2000), lake depth (Keller & Conlon, 1994), turbidity (Scheffer, 1998), predation (Brooks & Dodson, 1965), and competition (Shurin, 2000). The primary goal of this investigation was to describe the diversity of the pelagic zooplankton communities within several mountain lakes and reservoirs located in the Mission Mountains, and assess if any of the recorded physical characteristics of study sites influence these assemblages. Sampling was conducted in between July 2 and July 7, 2007 within eleven lakes/reservoirs located within four watersheds located in the Mission Mountain Range on the Flathead Indian Reservation: Upper and Lower Jocko Lakes in the Jocko River Drainage; Lucifer Lake and Mission Reservoir in the Mission Creek Drainage; Twin Lakes and Saint Mary's Lake in the Dry Creek Drainage; and Frog Lakes, Summit and McDonald Lake in the Post Creek Drainage. A positive correlation existed only between calculated Shannon Index values and lake/reservoir area ($p = 0.009$; $df = 1$; $F = 11.366$). Though this investigation yields little in terms of statistical significance, several factors were eliminated and several were identified as potentially having an effect on zooplankton diversity with the study sites.

Introduction

Zooplankton, more specifically three taxonomic groups: Phylum Rotifera; Subclass Copepoda; and Suborder Cladocera, are an integral component of freshwater ecosystems. Not only does zooplankton provide sustenance for higher trophic levels, but they are also a critical element in the control of algal blooms via herbivory. The role of algal control becomes even more crucial in temperate lakes and ponds which experience vernal peaks in phytoplankton populations.

At the local level there are a variety of factors which have been indentified that influence zooplankton species richness and/or diversity: lake area (Dodson 1992; Dodson et al. 2000), lake depth (Keller & Conlon, 1994), turbidity (Scheffer, 1998), predation (Brooks & Dodson, 1965), and competition (Shurin, 2000). Many such studies have assumed lakes and ponds are closed units even though dispersal and colonization events have been identified as factors influencing species richness and diversity (Cottenie & De Meester, 2003). Cottenie & De Meester (2003) suggest that lakes with similar environmental conditions may contain different species compositions because of different chances of colonization and extinction for each species.

In western Montana on the Flathead Indian Reservation lies one of the world's largest freshwater, oligotrophic lakes, Flathead Lake. The zooplankton community within the lake has been well documented and remains the focus of researchers who are documenting the introduction of non-native species over the past 100 years. The Mission Mountains, also located within the reservation boundaries, contain numerous glacial filled alpine lakes, reservoirs, and streams. However unlike Flathead Lake, the

zooplankton communities within these lakes, reservoirs, and streams have received little attention.

The lakes within the Mission Mountains are relatively young having formed with the most recent glaciation meaning there has been limited time for colonization by local fauna. Unlike with many aquatic organisms, dispersal of zooplankton does not seem to be limited by waterways. Terrestrial vectors, such as aquatic birds or mammals may aid in the dispersal of zooplankton species and become increasingly important in locations which lack the potential for aquatic dispersal due to various migration barriers. Regardless of the mode of dispersal species must still successfully establish within a habitat, which is contingent on a variety of biotic and abiotic factors.

Natural means of dispersal and colonization could be considered to be random events that can happen over a great temporal scale and may have been historically restricted to native species. Recent introductions of native and non-native fishes into some of the mountain lakes/reservoirs located within the Missions may have also resulted in the unintentional introduction of various zooplankton species, native and non-native, which may have potentially established viable populations within a system. These introductions may potentially result in alterations in a system's diversity which may affect the overall stability of a system. The relationship between species diversity and system stability is a central issue in ecological research and has recently gained renewed attention in light of widespread, human-induced alterations of global biodiversity (Tilman, 1999; Loreau et al., 2002). Dispersal by natural means

The primary goal of this investigation was to describe the diversity of the pelagic zooplankton communities within several mountain lakes and reservoirs located in the

Mission Mountains. The use of species richness estimators will aid in gauging the success of my samples representing the potential zooplankton community within a lake/reservoir. Species richness is the simplest and most intuitive concept for describing community diversity (Chao 2000). Ecologists have often discovered that sampling entire ecosystems and/or complete species censuses require extraordinary effort, and have thus often resorted to subsampling techniques and statistical methods to infer total species richness (Chao 2000, Muirhead et al. 2006). Species richness and diversity have often been used interchangeably in text (Honnay et al. 2000, Brown 2001 Lobo et al. 2001), though Spellerberg and Fedor (2003) suggest that "species richness" should be used to refer to the number species within a given area or sample while "species diversity" should refer to an expression or function of the number of species and individuals observed in a sample or area (Spellberg 1991).

A species diversity index will then be used to access the potential effects that system type, area, depth, and elevation have on the effects of species richness and abundance within our study sites. I expect observe a difference in the effect of system type on species diversity, as it may be that several of the reservoirs included in this study were established within the past hundred years. I also expect to observe a positive correlation between species diversity and increased area and depth, and a negative correlation between diversity and increased elevation. We will then use a similarity index, function of species richness, to potentially identify factors that may or may not create similarities of zooplankton assemblages between systems. It is also likely that lakes and reservoirs located within the same drainages will be more similar than those located in separate drainages. Decreased differences in geographic distance, area, depth,

and elevation should also yield zooplankton communities that appear to be more similar that those that are further apart in terms geographic distance, area, depth, and elevation.

Methods

Sampling

Sampling was conducted in between July 2 and July 7, 2007 within eleven lakes/reservoirs located within four watersheds located in the Mission Mountain Range on the Flathead Indian Reservation: Upper and Lower Jocko Lakes in the Jocko River Drainage; Lucifer Lake and Mission Reservoir in the Mission Creek Drainage; Twin Lakes and Saint Mary's Lake in the Dry Creek Drainage; and Frog Lakes, Summit and McDonald Lake in the Post Creek Drainage. Lake/reservoir area, elevation and geographic distance from other systems were calculated using Google Earth. Area was calculated by using the most relevant geometric shape for each lake/reservoir and taking the appropriate measurements in order to calculate area for that shape. Elevation and geographic distance were established by pacing a waypoint at the center of each sample site. Elevation of each waypoint was then recorded followed by its distance from other waypoints.

Zooplankton communities in each lake/reservoir were sampled by taking, three plankton tow samples in the deep portions of each lake/reservoir we could identify without topographic maps of the basins. We first recorded the depth at each tow site within the sampled lake/reservoir and submerged our plankton net roughly one meter from the lake/reservoir bottom, and then recorded the tow length. The 30cm diameter net is then brought back up though the water column.

The plankton net's mesh, 80 µm, allowed water and most phytoplankton pass while trapping zooplankton within the net. Once the net was removed from the water the contents of the net were carefully placed in a labeled (date/time/depth/tow length) jar. The plankton net was then rinsed thoroughly to avoid any potential cross contamination of samples and lake systems. Once on shore, the three plankton samples were further filtered using the plankton net to remove excess water. Plankton was rinsed back into the sample jar and 95% ethanol was added creating a concentration of 70% preservative. Samples were then brought back to the lab for identification and quantification.

The volume of the samples in the jars, containing zooplankton and preservative, were recorded. Samples then were well mixed and 12.0 mL sampled from the center of the jar using a pipette. This sub-sample was then placed in a 20 mL vial and an additional 3 mL of ethanol added to increase preservation of the samples. Using the second subsample, we removed 5ml for identification of zooplankton down to species or genus. Samples were identified using both a 40x stereomicroscope and a 100x compound microscope and the University of New Hampshire Center for Freshwater Biology's Image-Based Key to the Zooplankton of the Northeast (http://cfb.unh.edu/CFBkey/html/index.html). The number of each taxonomic group was also counted and then used to calculate zooplankton abundance using the following equation:

A / B = # of taxon X per liter lake water
A = # of taxon X counted * Volume of Fixative
B = Area of the net (cm^2) * Tow Length (cm) / 1000

Corrections were made to account for the 12 mL subsample removed by dividing the volume of fixative by four. This is due to the fact that our abundance equation is setup for 1 mL subsamples used for identification.

Species Richness

The statistical approach we chose to pursue to calculate species richness utilizes non-parametric estimators of species abundance or occurrence patterns in samples (Muirhead et al. 2006). These types of estimators are based on the observed frequency of rare species in the community and add the number of species that occur in only a few of one's total samples to the observed total richness, or add the proportion of samples that have a particular species present for bootstrap estimation to correct the bias (Smith & van Belle 1984; Chao 1987). Such approaches have thus been termed, sample-based rarefaction curves, and of these the bias-corrected Chao2 and Jackknife2 have been determined to be two of the most reliable predictors of species richness (Maiphae et al. 2005; Hortal et al. 2006). However like all statistical approaches used in ecology, one must use such approaches with caution. In a study of cladocerans in Canadian Shield lakes, Arnott et al. (1998) noted that single samples only represented only 33% of the total estimated species and that richness increased with the number of seasonal, inter-annual, and spatial samples collected. Chao2 and Jackknife values for our samples will be generated using the species richness estimator, ws2mb (Turner et al. 2003), and will only be used for observational purposes.

Species Diversity and Similarity Index

Once I accessed the accuracy of our samples using the species richness estimators, I then used the Shannon-Wiener Index (SI), a diversity index based on the number of species or genera in a sample and their relative abundance (Shannon and Weaver, 1949). Shannon-Wiener Index is a frequently used index of diversity that is dimensionless, independent of sample size, and expresses the relative importance of each

species (Wilhm 1968). The most appealing aspect of this index is that it is independent of sample size which makes it extremely useful in this investigation, as time limitations prevent further temporal sampling efforts. For the purpose of this study, SI values were calculated using total number taxa observed within a site and the mean abundance of each taxa from the three plankton tows.

The Jacquard's similarity index (JI) was chosen to identify potential similarities between communities. The Jacquard index is a proportion of the total number of species that two communities have in common divided by the sum of the total number species in both communities less the species they have in common. JI values equal to one mean the two communities are identical while a values of zero indicated complete community differentiation. Though the Jacquard index is not frequently found throughout the literature, it allows for a quick and relatively easy method for accessing community similarities.

Statistical Analyses

Statistical analyses were conducted using SYSTAT 10.0 (SYSTAT Software, Inc.; Point Richmond, CA). The calculated SI values were assessed first using a one-way ANOVA, to determine the potential effects of system type. A backward, stepwise multiple regression was conducted with the SI values to test for significant correlations with area, depth, elevation or any combination of these three abiotic factors. The least significant α-values ≥ 0.15 were progressively removed, leaving only factors yielding α-value ≤ 0.15. Calculated JI values were also assessed using the same tests conducted as our SI values. A one-way ANOVA was used to determine if JI values differ significantly between lakes/reservoirs found within the same drainage and lakes/reservoirs.

Significance for both ANOVAs was determined at an $\alpha = .05$ level. Another backward, stepwise multiple regression using our calculated JI to determine if any correlations exist with differences in area, depth, elevation, and/or geographic distance between sites. Again, the least significant α-values ≥ 0.15 were progressively removed, leaving only factors yielding α-value ≤ 0.15.

A hierarchal cluster analysis was computed using the Arcsine transformed proportion of the mean abundance of each individual species/genera found at each site to the mean of the total number of organisms found within the same site. A principal component analysis (PCA) was accessed using this same the same proportion data. Using the first four factors produced by our PCA, we conducted another hierarchal cluster analysis. The intent of these both cluster analyses was to identify potential similarities in diversity across sites. The difference between the two cluster analyses however, is that the use of a PCA reduces the amount of variables retaining those that explain an increased proportion of the data sets variation. The distance metric for both cluster analyses was one Pearson correlation coefficient. SPSS determines significance of clusters based on our distance metric, and clusters determined to be significant have their branches on the cladogram highlighted.

Results

In my survey of the eleven lakes and reservoirs, I was able to indentify three sixteen species of zooplankton and species of three genera which could not be indentified to the species level. The number of species identified in each lake varied for 3 to 12 with the greatest number of species observed in Twin Lake A and Summit Lakes and only 3

species being observed in North Frog Lake (Figure 1). Total mean abundance varied from 115 organisms per liter in Twin Lake A to 0.003 organisms per liter in North Frog (Figure 2). Mean abundance of individual species also differed across sites suggesting that biotic and abiotic factors are not uniform throughout our sites, which may potentially explain variations in mean total and individual taxa abundance across sites.

Species richness estimate values were similar to the actual observed number of taxa per site (Figure 3). These estimates suggest that our sampling design, though only a single sampling event consisting of three collections, was accurate indicator of the potential zooplankton assemblages within our study sites. This is important as the likelihood of observing rare species within a system decreases with decreased sampling.

Shannon-Wiener Index (SI) values varied from North Frog with an SI value of 0.69 to an SI value of 2.03 for Mission Reservoir (Table 1). The results from the one – way ANOVA suggest that system type, reservoir vs. lake, has very little effect on species diversity, or SI values ($p = 0.321$; $df = 1$; $F = 1.104$). Neither mean depth ($p = 0.858$; $df = 1$; $F = 0.004$) nor elevation ($p = 0.341$; $df = 1$; $F = 1.023$) were correlated with our SI values in the multiple regression analysis. However, the multiple regression analysis did suggest that a positive correlation exists between SI values and lake/reservoir area ($p = 0.009$; $df = 1$; $F = 11.366$) (Figure 4).

Jacquard's similarity index values calculated using species richness data for each of this study's sample locations, along with the number of species each location had in common with each other are displayed in a matrix in Table 2. Results from our one-way ANOVA suggest that JI values produced from lakes and reservoirs found within the same drainage did not significantly differ in JI values calculated for lakes and reservoirs

located in different drainages ($p = .989$; $df = 53$). Results from the multiple regression values did not yield any significant relationships between JI values and differences in geographic distance ($p = 0.378$; $df = 1$; $F = 1.317$), area ($p = 0.430$ $df = 1$; $F = 1.543$), mean depth ($p = 0.550$; $df = 1$; $F = 1.294$), or elevation ($p = 0.738$; $df = 1$; $F = 0.947$).

According to cluster analysis using Arcsine transformed proportion of the mean abundance of each individual species/genera found at each site and the mean of the total number of organisms found within the same site, lakes and reservoirs located within the Jocko River and Mission Creek drainages cluster very close to one another. The lakes within the Dry Creek drainage also cluster together suggesting potential similarities between sites located within the same drainage. This contradicts the results of comparisons of JI values and may be due to the inclusion of abundance values which are not factored into the Jacquard similarity index.

The results of the cluster analysis of the PCA factors also lend support for increased similarity of sites located within the same drainage. The results of this analysis suggest that this relationship may even more significant than even the prior cluster analysis of the Arcsine transformed data suggest. The use of the PCA helps to provide information into the importance of a given taxa within sample sites. We then attempted to determine whether any of recorded physical properties or system type influenced any of the top three variables of each the four factors weighted by the PCA. Unfortunately however, none of the recorded physical properties or system type had an effect on mean abundance values of these taxa.

Discussion

The use of the species richness estimators, Chao 2 and Jackknife 2, helped use determine how successful our sampling attempts were at representing the potential community composition of the lakes and reservoirs sampled in this study. Though these values suggest that our sampling may have been adequate, increased temporal sampling efforts would still be preferred. Zooplankton communities in temporal lakes are subject to vernal peaks due to changes in lake productivity over time. We were not able to assess this effect due to sampling design.

Along with increased temporal sampling, it would be advantageous to obtain more physical characteristics of sample sites. The physical characteristics collected in this study had little effect on both richness and diversity. Area was the only recorded physical attribute that provided any significant results, as it was positively correlated with calculated Shannon Index diversity estimates. Physical attributes such as temperature or dissolved oxygen may provide much more useful information, but would need to be collected. A majority of these attributes would be fairly easy to obtain in the field so long as one had properly functioning equipment.

Examining the total and individual taxa mean abundance value led me to believe that individual lake productivity and fish predation may be extremely important in shaping zooplankton assemblages within individual lakes and streams. In terms of productivity, we observed macrophyte beds on the bottom of only two of our sample sites, Twin Lake A and Mission Reservoir. The increase number of marcophytes within these lakes suggests that they are likely to be much more productive than other sample

sites. The increased number of marcophytes would help explain why these two lakes support much more diverse community of zooplankton, both in abundance and overall richness. Twin Lake A would have to be fairly productive due to the fact that not only is at the upper reaches of its drainage but it was one of the smallest in terms of depth and area. Measuring nitrate and phosphate levels in future sampling attempts may provide a feasible means of assessing productivity of sample sites to help determine if individual productivity plays a role in shaping zooplankton communities.

Fish were observed in every lake and reservoir sampled in this study, in fact minnows were trapped in our plankton net on at least one occasion at both Mission Reservoir and Twin Lake A. Fish predation has been suggested to have dramatic impact on zooplankton diversity within a system, and severity will vary between fish species depending on the proportion zooplankton comprise of their diet. *Daphnia* species have been suggested to be preferred prey of planktivorous fish because of their large body size and slow swimming speeds. In systems where *Daphnia* species are heavily preyed upon, they are replaced with smaller *Bosmina* species. It would be interesting to examine the fish communities within each lake/reservoir and to help determine the effect of fish predation on zooplankton assemblages. Examining Figures 7 and 8 lead me to believe that this would be worth the effort as it appears that *Daphnia* species are less abundant than the combination of *Bosmina longirostris* and *Diaphanasoma brachyurum* in the lakes where small minnows were trapped in the plankton net.

Though this investigation yielded little in terms of statistical significance, I was able to eliminate a couple potenial factors influencing zooplankton diversity while discovering future avenues to pursue. There is little doubt that one sample session,

though it may provide some useful information, is inadequate to address a majority of the questions raised. Increased temporal sampling and futher detailed sampling of aboitic and biotic factors are required, but my priliminary data suggest that this may yield successful results. One may also be be able to address what may bethe primary mode of dispersal of zooplankton within these systems.

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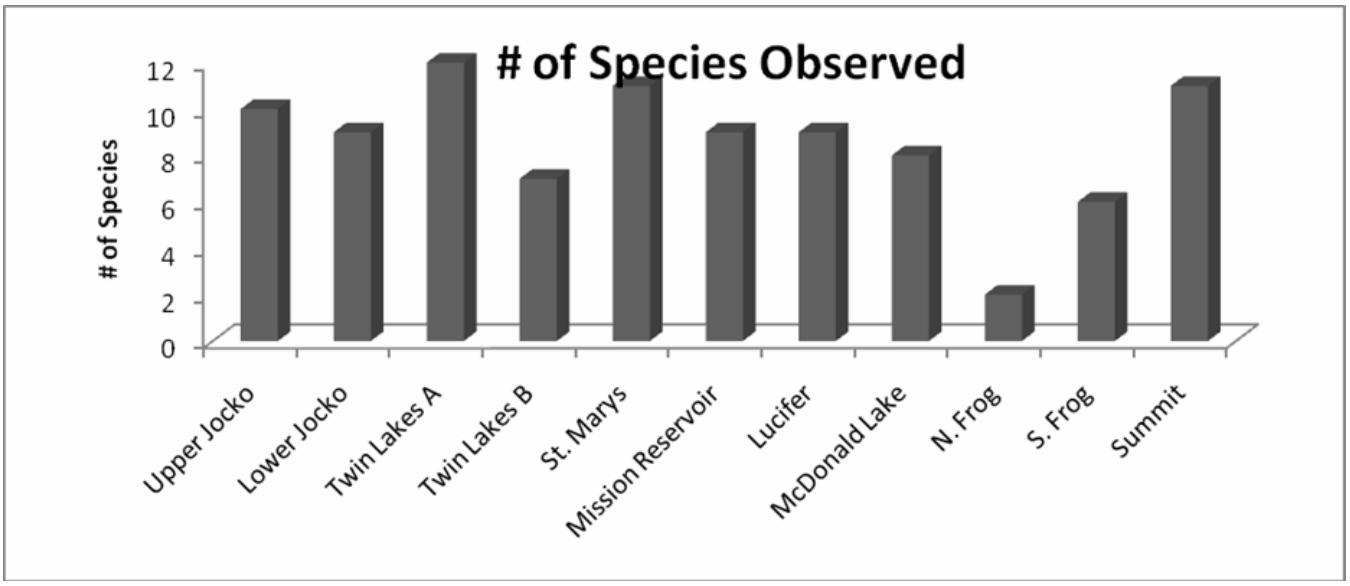

Figure 1. Graphical representation of the number of species recorded at each of this study's sample site.

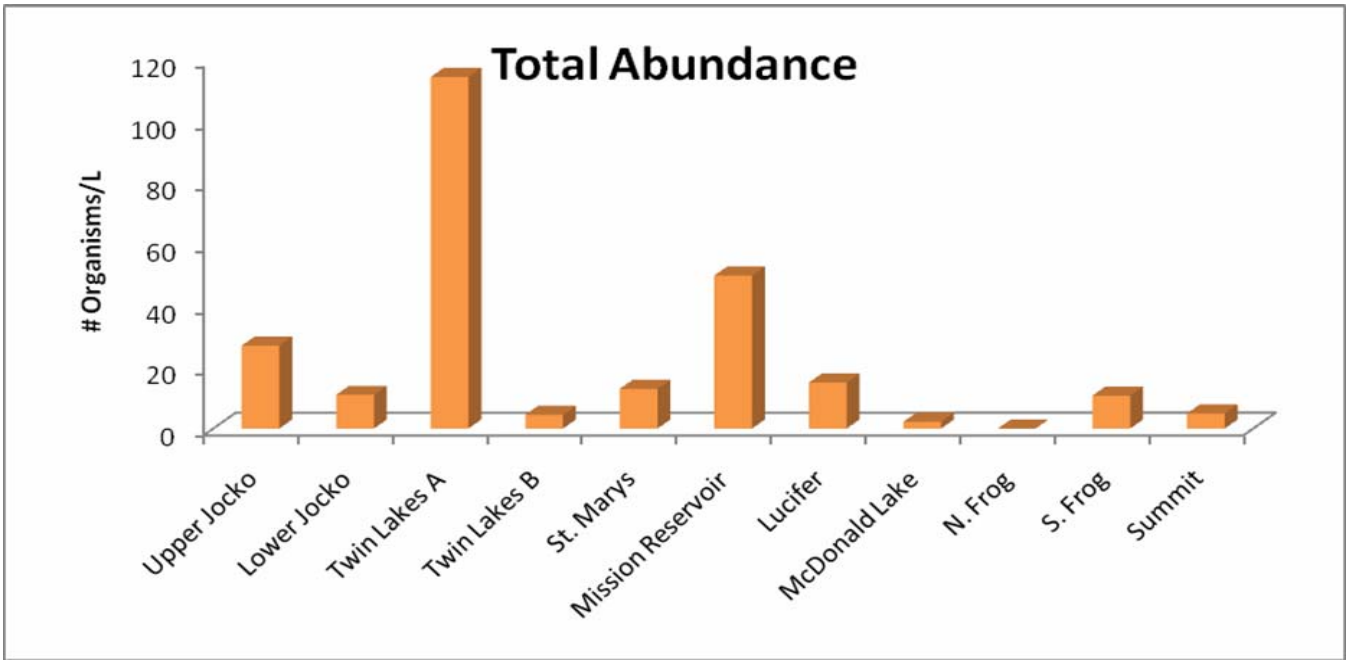

Figure 2. Graphical representation of the total mean abundance of organisms per liter of lake water at each of this study's sample.

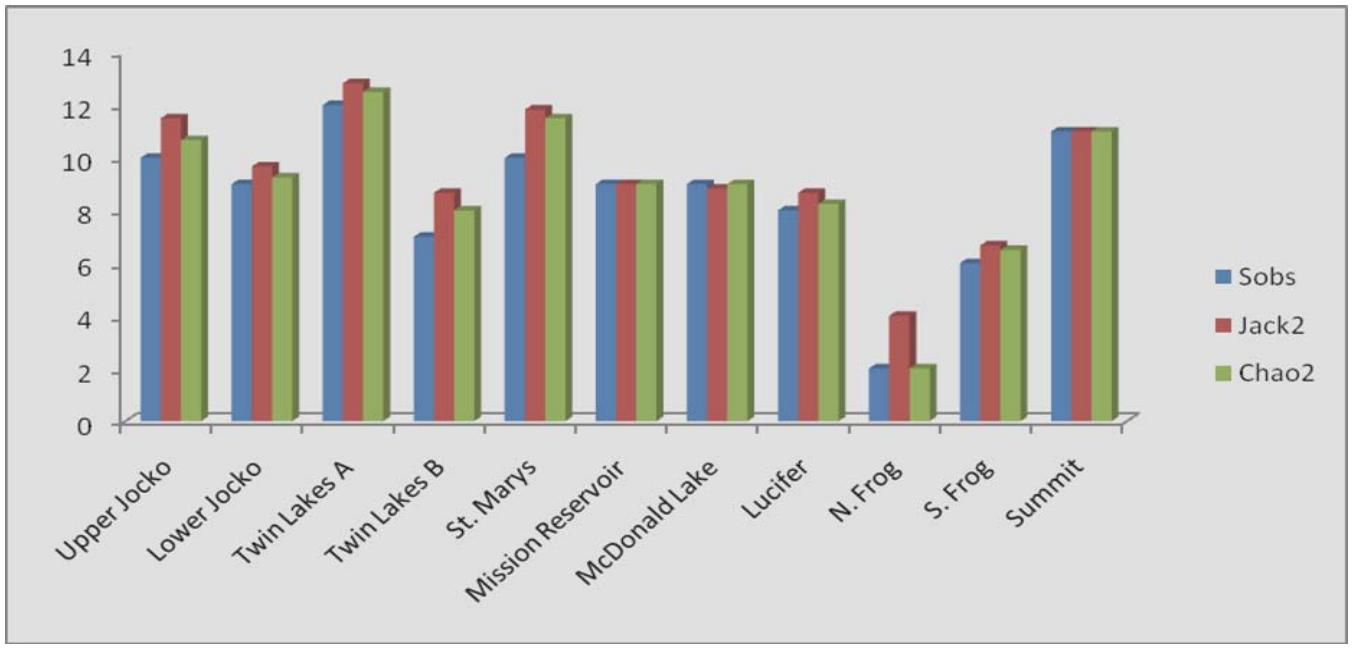

Figure 3. Graph of the results of our species richness estimators, Chao2 and Jackknife2 (Jack2), along with the actual number of total species observed (Sobs).

Site	Elevation (m)	Depth (m)	Area (m^2)	SI
Upper Jocko	1475.232	9	84300	1.09
Lower Jocko	1453.896	19.16666667	316415	2.02
Twin Lakes A	1261.872	6.416666667	25434	1.63
Twin Lakes B	1261.872	14.58333333	34948.985	0.77
St. Marys	1223.772	29	567840	2.02
Mission Reservoir	1040.892	13.58333333	749430	2.03
McDonald Lake	1097.28	25.83333333	118177.04	0.86
Lucifer	1911.096	22.66666667	125600	1.43
N. Frog	1885.188	1.5	12265.625	0.69
S. Frog	1888.236	2.833333333	18486.72771	1.33
Summit	1926.336	12.16666667	211147.0054	1.81

Table 1. Elevation, mean depth, area, and Shannon-Wiener Index (SI) values for each study site.

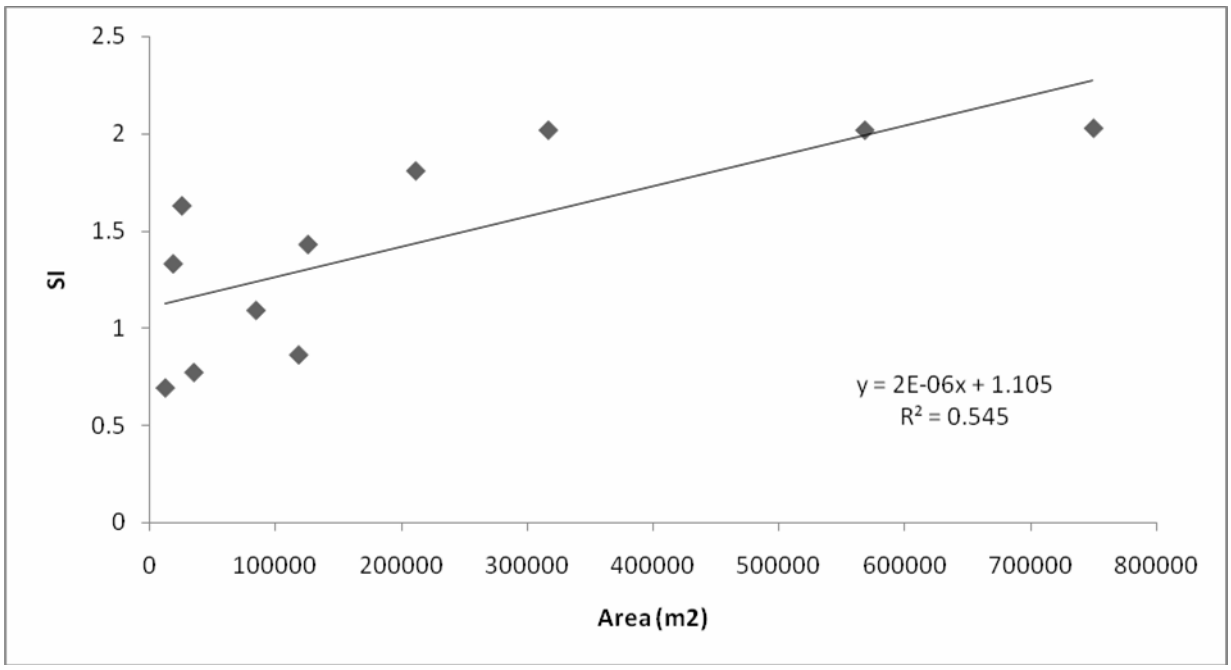

Figure 4. Graphical representation of the positive correlation between lake/reservoir area and species diversity, or SI ($R^2 = 0.545$; $p = 0.009$).

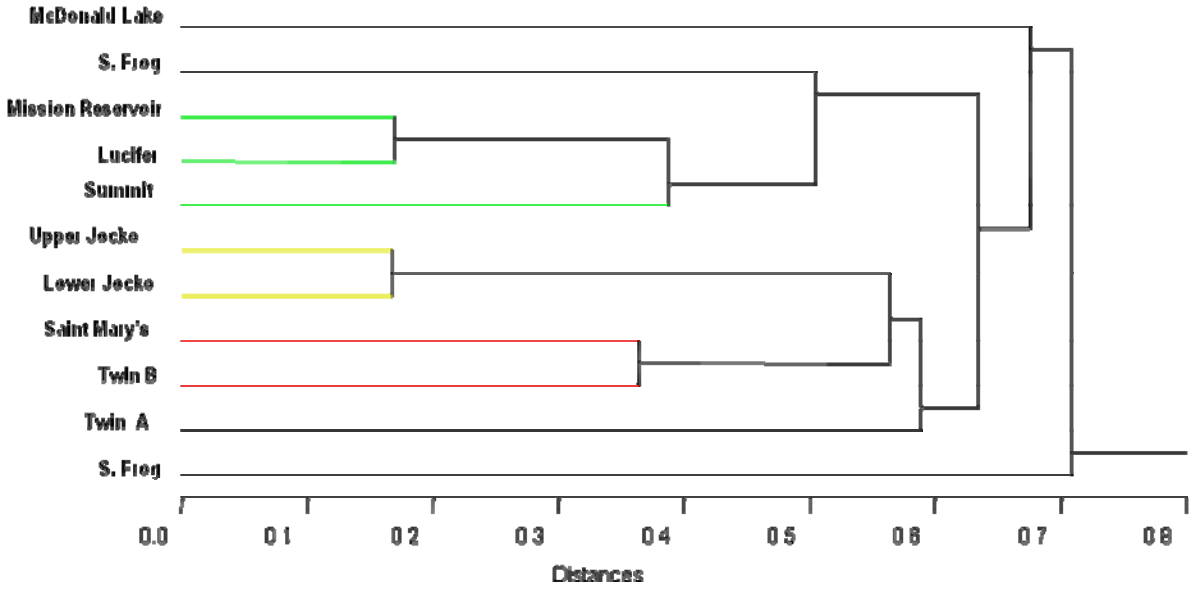

Figure 5. Result of the cluster analysis using Arcsine transformed proportion of the mean abundance of each individual species/genera found at each site and the mean of the total number of organisms found within the same site.

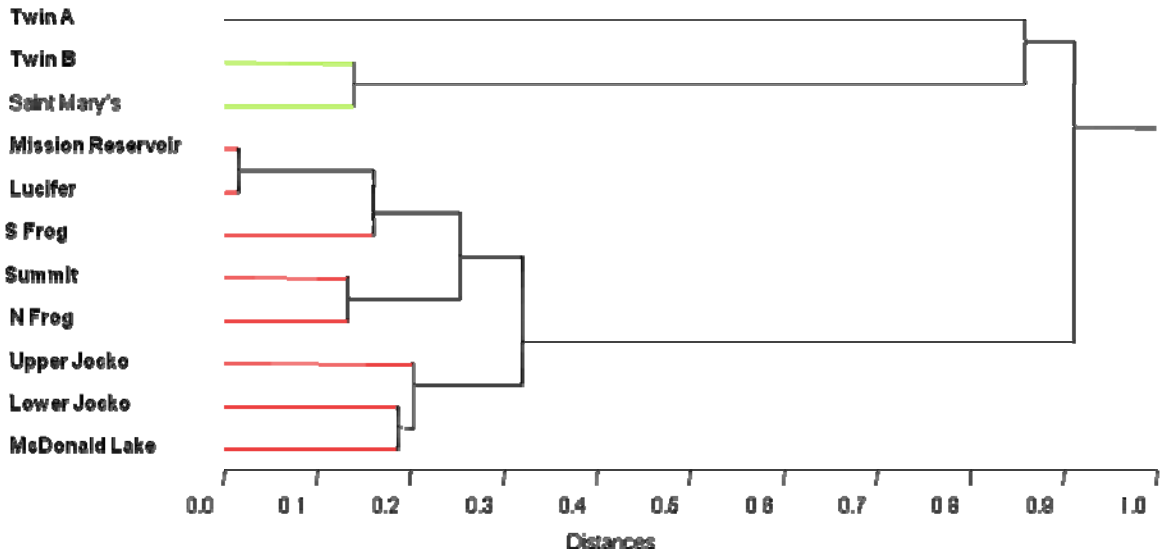

Figure 6. Result of the cluster analysis using the first four factors of our PCA.

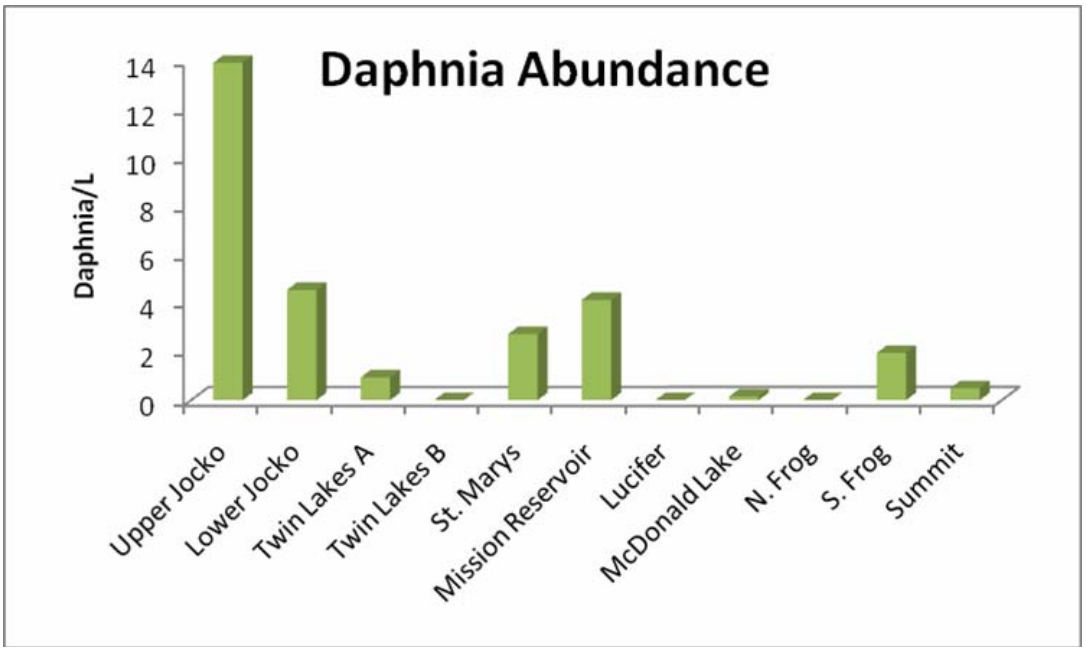

Figure 7. Graphical representation of the total mean abundance of Daphnia speices per liter of lake water at each of this study's sample.

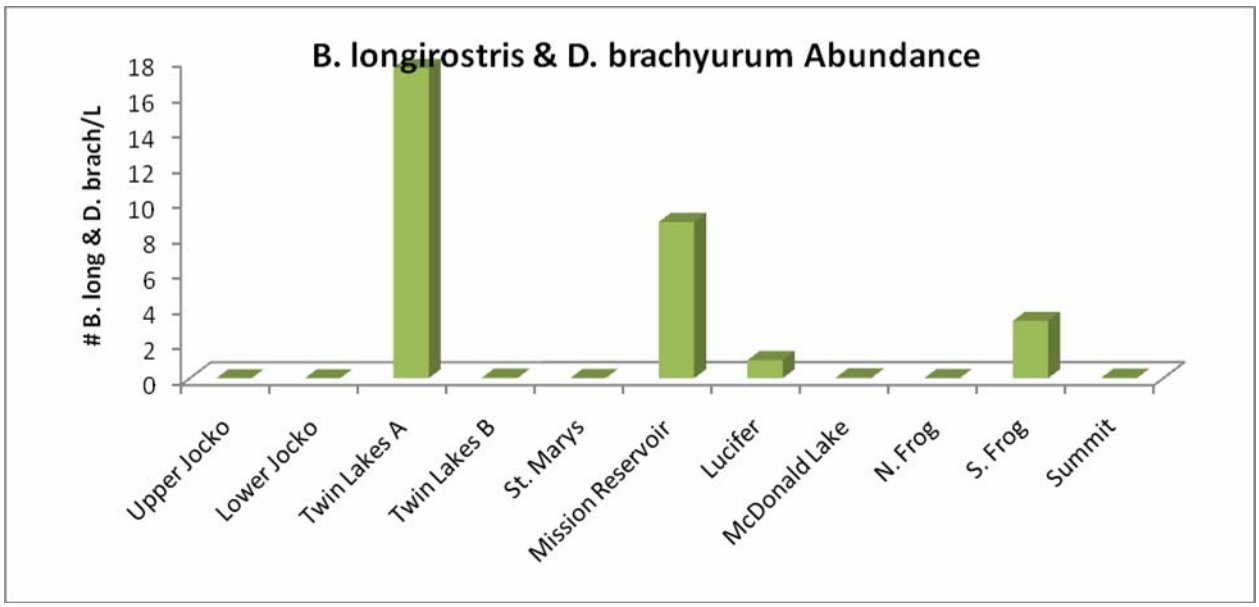

Figure 8. Graphical representation of the total mean abundance of *Bosmina longirostris* and *Diaphanasoma brachyurum* per liter of lake water at each of this study's sample.