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# Zooplankton of the Belgrade Lakes: The Influence of Top-Down and Bottom-Up Forces in Family Abundance

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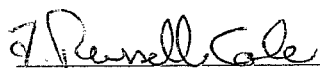
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# Zooplankton of the Belgrade Lakes: The Influence of Top-Down and Bottom-Up Forces in Family Abundance


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May 6, 2011

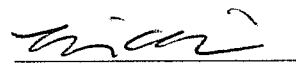
A thesis submitted to the faculty of the Environmental Studies program in partial fulfillment of the graduation requirements for the Degree of Bachelor of Arts with honors in Environmental Studies



F. Russell Cole, Advisor



Philip Nyhus, Reader



Liam O'Brien, Reader

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## **EXECUTIVE SUMMARY**

The purpose of this study was to assess the abundance and family diversity of zooplankton communities in the Belgrade Lakes, and to identify the broad scale and local variables that structure zooplankton communities in this region. The local effects of shoreline development and the presence of macrophyte patches were compared to larger scale variables, such as watershed wide residential development. Zooplankton are an intermediate link in the freshwater food web, and communities respond both to predation pressures as well as nutrient inputs. Shoreline development was expected to influence zooplankton densities by the increased nutrient inputs via erosion off developed sites with no buffer. The presence of macrophytes was expected to increase the densities of zooplankton since macrophytes serve as a refuge for zooplankton from zooplanktivorous fish.

Zooplankton were sampled in all seven of the Belgrade Lakes from macrophyte patches, in the water adjacent to undeveloped shorelines, and in the water adjacent to developed shorelines with no vegetated buffer. 142 sites were sampled in total, and at least five replicates of each of the three primary habitat types were assessed from all of the Belgrade Lakes. Each zooplankton specimen was identified to the Family level, with the exception of copepods, which were identified to the Order. Abundance of each family was established for each site as number of individuals per liter. Aggregate density and the density of each Family were tested for significant differences between sites with no buffer and undeveloped sites, as well as between sites with macrophytes and sites without macrophytes. The relative influence of all variables on density were analyzed using a two step model. The first step was a zero inflated negative binomial regression which predicts the log odds of observing a density of zero individuals. This accounted for the high number of zero densities observed in each family. The second step was a logarithmic regression to predict the non-zero densities of each family.

No significant differences were observed between sites with no buffer and undeveloped sites for the density of any Family. Aggregate density and the Cladoceran Families Chydoridae, Daphniidae and Sididae all had significantly higher densities within macrophyte patches compared to outside of macrophyte patches.

Flushing rate was linked to decreases in several families of zooplankton, including Chydoridae, Daphniidae, and the Order Cyclopoida. Increased flushing rate is generally associated with better water quality and lower nutrient levels in lakes, which is consistent with our prediction that nutrient levels will be important determinants of zooplankton abundance. Residential development was linked with decreases in several families of zooplankton, including Bosminidae, Chydoridae, Sididae and the Order Cyclopoida. While this is counter-intuitive to the logic that increased nutrient loading will increase zooplankton densities, nutrient and chlorophyll levels were held constant in several models. Another mechanism, such as changes in pH may be responsible for the declines predicted. The decline in densities in the presence of Northern Pike was not expected, since the presence of an aggressive piscivore was predicted to lower the grazing pressure of zooplanktivores on zooplankton, and an increase in zooplankton abundance. The opposite effect was observed, suggesting that predation controls on zooplankton abundance are not as important as other environmental variables in the Belgrade Lakes. Management of lake water quality should focus on controlling nutrient inputs into the lakes to improve water quality, because top-down drivers are unlikely to be structuring the zooplankton communities of the Belgrade Lakes.

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## INTRODUCTION

The Belgrade Lakes are a system of seven lakes near Waterville, Maine, which are directly connected by streams and wetlands. All of the lakes are connected, so each lake influences the water quality of the other lakes, or is affected by changes in the water quality of another lake. There are differences of water quality across the lakes, making them a unique and fascinating system to study.

Current research in the Belgrade Lakes is being spearheaded by the National Science Foundation (NSF) EPSCoR (Experimental Program to Stimulate Competitive Research) grant awarded to the University of Maine. This grant funds the Belgrade Lakes Sustainability Project, which brings investigators from Colby College, University of Maine at Farmington, Maine Congress of Lakes, the Belgrade Lakes Regional Conservation Alliance, and local stakeholders together. The project aims to model resilience and adaptation in the Belgrade Lakes watershed, and to use the Belgrades as a model of a dynamic system of lakes. The tasks of this group include conducting shoreline investigations to determine the impact of development and different quality of buffer strips, biodiversity assessments, and creating data sets of spatial resources (Colby 2011). My project investigated the impacts of shoreline development on zooplankton, and contributes to the initiatives of the EPSCoR grant.

The focus of this study is on the abundance and family diversity of zooplankton communities of littoral zones of lakes and ponds in the Belgrade Lakes region. The goal of this study was to identify both the broad and local variables that structure zooplankton communities in this system. I quantified the role of localized shoreline development and investigated the importance of macrophyte presence in structuring these communities in comparison to broader scale factors. Specifically, the difference between undeveloped shorelines and shorelines that have been developed with no buffer were compared. The broader scale factors these local effects will be compared to include lake-wide water quality and chemical parameters such as phosphorus levels, chlorophyll levels, and pH. Biological factors will also be considered, such as the presence of warm and cold water

fish assemblages, and the presence of the aggressive piscivore, Northern Pike (*Esox lucius*).

I expected that zooplankton density will be higher in macrophyte patches due to the refuge effect (Jeppesen et al. 1997, Perrow et al. 1999, Benndorf et al. 2002). Additionally, I expected that zooplankton densities will be higher at highly developed sites compared to undeveloped sites as a result of increased local nutrient loading. I did not anticipate broad-scale chemical factors such as conductivity to be important drivers of zooplankton density within the Belgrade Lakes region, although we do expect that lake wide water quality parameters will be important measures of bottom up influences since measures of chlorophyll and total phosphorus are direct measures of nutrient status (Finlay et al. 2007, Gelinias and Pinel-Alloul 2008a). I expected the presence of warm or cold water fish assemblages to correlate to the trophic status of lakes, and for these to be indicators of bottom-up forces (Stemberger and Lazorchak 1994, Finlay et al. 2007). If top-down forces are important drivers of zooplankton communities, we expect to find higher densities of zooplankton in lakes where Northern Pike are present as a result of increased piscivory (Haliwel and Evers 2008).

## **Lakes**

Lakes are an important resource to the state of Maine, providing ecosystem functions, recreational opportunities, and drawing tourist revenue. The economic value of Maine's lakes has been established by several studies that have linked poor water quality to economic losses. Michael et al. (1996) found that a one meter increase in water clarity, measured by secchi depth readings, raises property values between \$11 per foot shoreline for Echo Lake in Augusta and \$200 per foot shoreline for Sabattus Lake in Auburn in an evaluation that encompassed lakes across Maine. Boyle et al. (1999) also found a variable link between water clarity and real estate prices, ranging from \$2,337 per meter in Bangor to \$12,938 in Camden when real estate values were aggregated to the entire lake.

In addition to the real estate value of shoreline homes, fishermen and boaters also contribute to local economies. Lakes provide critical habitat for Maine's iconic wildlife, including the common loon, land locked salmon, and trout and bass fisheries (IFW 2011).

Maintaining good water quality is critical for protecting these important resources and economic values.

Maintaining the water quality of Maine lakes is an important objective for lake managers (Michael et al. 1996, MDEP 2010). Up to \$250,000 has been committed each year to restoring and managing Maine lake water quality by the state from 1986 to 1996 (Michael et al. 1996). Programs such as Lake Smart (MDEP 2010) have been started to help mitigate the effects of development (discussed in the next section) and groups such as the Belgrade Lakes Regional Conservation Alliance (BRCA) have organized to help maintain the health of the dynamic Belgrade Lakes system. Lakes are an important resource that both the state government and local groups are committed to protecting.

## **Development**

Despite the importance of Maine's pristine lakes, they are threatened by the impacts of development. Development in Maine has increased steadily since the 1970s (Davis et al. 1978, UMO 2011). Within the Belgrade Lakes watershed, development is projected to increase mostly within the towns of Oakland, Belgrade and Sidney as a result of growing populations and residential development (McCullough 2010). The impacts of development include loss of habitat complexity from the removal of coarse woody debris (Christensen et al. 1996, Jennings et al. 2003), the loss of macrophytes (Jennings et al. 2003, Cheruveli and Sorrano 2007), increased erosion, and the subsequent nutrient loading (Nelson and Booth 2002). The roots of vegetation stabilize the soil where the plants are growing, especially along shorelines. When this vegetation is removed during development, the roots can no longer hold the soil in place, and it can wash into water bodies more easily. When natural processes are interrupted, nutrients are likely exported from terrestrial systems into aquatic systems in a process called "Nutrient Loading". Additional nutrient loading associated with development occurs from erosion of camp roads and outdated septic systems, which is especially important near the shoreline (MDEP 2007, McCullough 2010)

In most freshwater lakes, phosphorus is the nutrient which limits growth of plants (Marsden 1989). Phosphorus is therefore the main nutrient of concern for nutrient loading



resulting from the impacts of development. Phytoplankton are small plants which live in the water columns of bodies of water, including lakes. Phytoplankton are one of many types of algae. When higher levels of phosphorus are available in a lake, phytoplankton populations are the first organisms to respond by reproducing rapidly to take advantage of the new resources available. This phenomenon is known as an “algal bloom”, and can have widespread effects both for people and the ecosystem (Sharpley et al. 1994). Algal blooms are one symptom of eutrophication, the process of increasing productivity in a lake over time. When that increase in productivity is driven by human activities, the process is referred to as cultural eutrophication (Barnes and Mann 1991).

Lakes exist on a continuum of eutrophication, as measured by their “trophic state”, from oligotrophic to eutrophic. Oligotrophic lakes are characterized by low nutrient inputs, low productivity, and tend to be deeper lakes (Barnes and Mann 1991). Eutrophic lakes tend to be more shallow, have higher nutrient inputs, higher productivity, lower secchi depths and lower dissolved oxygen levels (Barnes and Mann 1991). Between these extremes are mesotrophic lakes. Most of the Belgrade Lakes are classified by the DEP as mesotrophic lakes (PEARL 2010).

### **Algal Blooms**

Algal blooms are often triggered by high phosphorus levels (Gove et al. 2001). In Maine, phosphorus levels above 12 to 15 parts per billion (ppb) are sufficient to trigger algal blooms (MDEP 2002). Elevated nutrient levels occur during specific times of the year and during mixing events. Mixing events can occur due to high winds as well as seasonal changes in the density of water due to temperature changes. Water is most dense at 4 degrees Celsius (Manahan 2010), although the year round temperatures fluctuate between 0 degrees Celsius and 21 degrees Celsius (NOAA 2010). In the fall, as the water temperature on the surface falls to 4 degrees Celsius, the lake becomes isothermic and is easily mixed by wind. In the spring the warming of water to 4 degrees Celsius again results in constant temperatures throughout the water column, allowing wind-agitated mixing (Manahan 2010).

During most of the year, deep lakes are thermally stratified with a denser layer that is closer to 4 degrees Celsius at the bottom, and the upper layer that is warmer during the biologically active summer months near the surface. While biologically available phosphorus in the upper layer is rapidly sequestered by actively growing biological organisms such as phytoplankton, phosphorus in the lower layer increases. Phytoplankton in the surface water form conglomerates then fall into the lower layer. When the dead phytoplankton is decomposed, the phosphorus held in the biomass of the phytoplankton is released. This “biological pump” of phosphorus down into the lower layers, as well as internal loading mechanisms account for higher levels of phosphorus observed in the deeper areas of stratified lakes (Lake et al. 2007). When the lake becomes mixed, the phosphorus in the lower layer is re-distributed to the upper layer, where algal blooms can occur since there is not enough light in the lower layer to support phytoplankton growth (Manahan 2010).

While the lake is stratified, no nutrients or oxygen can be exchanged between the upper and lower layer because the differences in density of the water in each layer prevents mixing. However, coagulations of algae can sink from the upper layer into the lower layer. Once in the lower layer, aerobic decomposition in the substrate releases biologically active phosphorus and consumes oxygen. The oxygen consumed cannot be replenished since there is no gas exchange between the upper and lower layers of water in the lake while it is stratified, and only the upper layer can replenish oxygen from gas exchange with the atmosphere. Oxygen levels in the lower layer may fall to hypoxic levels (less than 2 ppb) or anoxic levels when oxygen is completely absent. When anoxic levels are reached near the substrate, additional phosphorus is released from the sediments. This positive feedback mechanism of internal nutrient loading is one of the reasons why restoring lakes that have been damaged by nutrient loading is a difficult, long, and expensive process (Amirbahman et al. 2003, Lake et al. 2007, Manahan 2010, King pers. comm.).

Additionally the hypoxic and anoxic oxygen levels have effects on organisms living in the lake which need oxygen to survive. These species include fish, which have recreational value to sportsmen. Fish that are dependent on cooler temperatures, such as

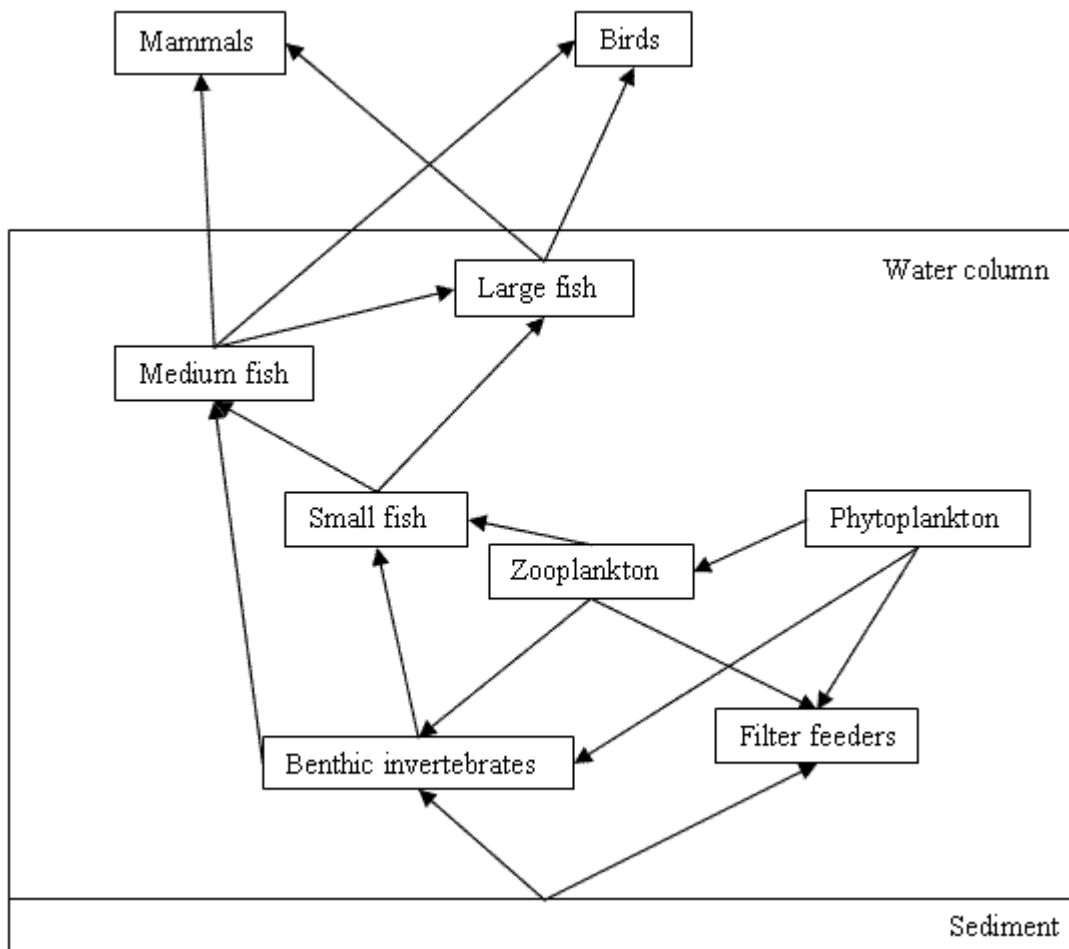
the salmonids, are especially sensitive (IFW 2010). These coldwater fishes typically live in the lower, cooler waters of deep lakes. However, when anoxia occurs in the cooler waters, these fish can no longer survive in the lake. In the Belgrade lakes, Land Locked Salmon (*Salmo salar sebago*) have been lost from Salmon Lake due to increasing anoxia in lower, cooler waters, which was a result of nutrient loading (IFW 2010).

As cold water species are lost from lakes, they are replaced by the dominance of warmwater species, such as the centrarchids, or sunfish. Warmwater species found in the Belgrade Lakes include Smallmouth Bass (*Micropterus dolomieu*), Largemouth Bass (*Micropterus salmoides*), Chain Pickerel (*Esox niger*), Northern Pike (*Esox lucius*) and White Perch (*Morone americana*) (IFW 2010, PEARL 2011). Some studies have attempted to use the ratio of warmwater fish biomass compared to coldwater fish biomass as a measure of the effects of eutrophication in lakes (Stemberger and Lazorchak 1994).

### **Role of Zooplankton**

Zooplankton are tiny, free swimming organisms that feed on phytoplankton and sometimes on other zooplankton (Figure 1). Zooplankton are consumed by piscivorous fish and macroinvertebrates such as *Chaborous* larvae. Zooplankton include animals in the Phyla Arthropoda and Rotifera. Within Arthropoda, the Suborder of Cladocera includes families such as Daphniidae, and the Subclass of Copepoda includes the Orders Calanoida and Cyclopoida (Figure 2).

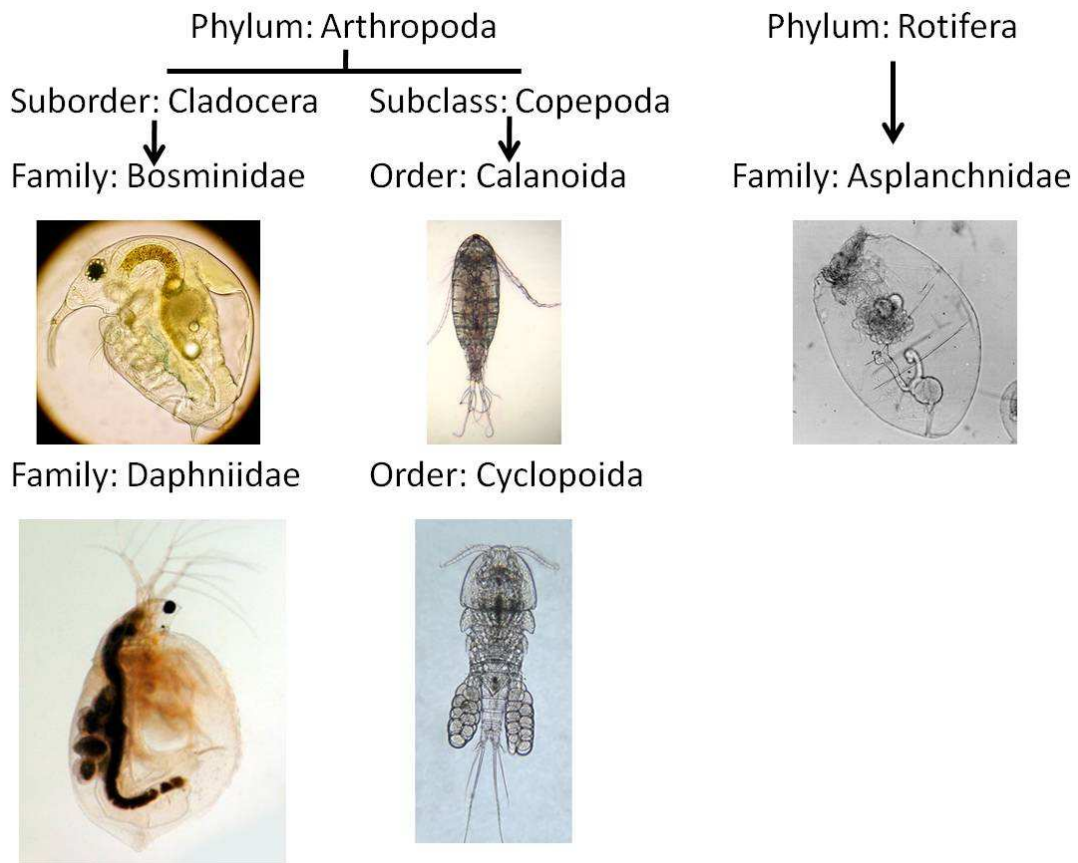
Zooplankton in the Phylum Rotifera tend to have short development times and a rapid intrinsic rate of increase. Rotifers reproduce asexually throughout the growing season, and reproduce sexually during unfavorable conditions to form a resting egg that can survive overwintering. Rotifers are suspension feeders that feed on both phytoplankton and sometimes upon other rotifers, although they are limited to feeding on particles less than 20  $\mu\text{m}$  (Allan 1976).



**Figure 1: Freshwater food web tracking the transfer of energy between trophic levels (EPA 2011).**

Zooplankton in the Order Cladocera (Phylum Arthropoda) include the families Daphniidae, Bodminidae, Chydoridae, Sididae and Polyphemidae. Cladocerans have a rapid intrinsic rate of increase, although this rapid reproduction is not as rapid as that of Rotifera. Cladocerans also reproduce asexually, reproducing sexually under stressful conditions to form resting eggs that can survive overwintering. Cladocerans are filtration feeders, which use appendages to create a current that they use to help filter particles out of the water column. Cladocerans are generally limited to consuming particles less than 50  $\mu\text{m}$  (Allan 1976).

Copepods, on the other hand reproduce only sexually, and must mature through several larval stages to reach sexual maturity. Body size is directly related to egg output, although the maximal rate of increase is less than that of either Cladocerans or Rotifers, and the time to maturation is also significantly longer. Copepods are capable of feeding upon larger particles than Cladocerans or Rotifers, and are capable of altering feeding behavior although they tend to be feeding specialists. (Allan 1976).



**Figure 2: Taxonomy of common zooplankton found in the Belgrade Lakes (UNH 2011)**

Some studies suggest that zooplankton have a critical role in controlling and preventing algal blooms (Lampert et al. 1987, Arnott and Vanni 1993, Xu et al. 2001, Buskey et al. 2007). This is known as “top-down” control, since a predator, the zooplankton, is keeping the prey, phytoplankton, under control. When an additional

trophic level is added, such as zooplanktivorous fish, this can reduce zooplankton abundance. When zooplankton biomass is decreased by zooplanktivorous fish, their control on phytoplankton is reduced, allowing phytoplankton biomass to increase. This phenomenon is known as a “trophic cascade”, and occurs in systems where top-down controls are important. In a system controlled by top-down effects, we expect to observe similar changes in trophic levels that are separated by another trophic level, such as the zooplanktivore-phytoplankton coupling described (Brett and Goldman 1996).

Other studies have suggested that “Bottom up” controls are more important than top-down. Bottom up processes occur when nutrient levels determine phytoplankton levels, which determine the levels of zooplankton and upper trophic levels. In a system where bottom up controls are more important, we would expect to see an increase in phytoplankton and zooplankton when nutrient levels are increased.

The dichotomy between top-down and bottom-up effects is the subject of many studies (Gliwicz 1980, Jeppesen et al. 1997, Agrawal 1998, Perrow et al. 1999, Bennedorf et al. 2002, Jones et al. 2002, Finlay et al. 2007), which have found that several aspects of zooplankton ecology can modify these effects.

For instance, several studies have suggested that larger zooplankton are more efficient grazers on algae in the “size-efficiency hypothesis”(Gliwicz 1980). This is because larger zooplankton can grow and reproduce more successfully at low food concentrations relative to small zooplankton. Therefore, large zooplankton can maintain a high grazing pressure on phytoplankton, even when phytoplankton levels are low. The size-efficiency hypothesis is reviewed by Gliwicz (1980).

Some researchers suggest that the break down of top-down controls is due to the increase in biomass of phytoplankton that are resistant to grazing over time. Mechanisms of phytoplankton resistance to grazing include developing spines, large size, and toxins. In the “defense theory” there is a life-history trade-off between growth rates resistance to grazing, which holds for phytoplankton as well (Agrawal 1998). Phytoplankton resist grazing with structures such as spines or achieving large sizes that few zooplankton can consume. This theory is complicated by variation in how functional resistance is defined,

although there is an inverse relationship between resistance and growth rates in phytoplankton. Defense theory is reviewed by Agrawal (1998).

Another important aspect of zooplankton ecology are interactions with macrophytes, or aquatic vegetation. Macrophytes provide important refuges for zooplankton to hide from zooplanktivorous fish. Zooplanktivorous fish are known to preferentially select larger zooplankton, resulting in a shift both to smaller body sizes and smaller species when zooplanktivory is increased in the system. (Gliwicz 1980, DeMott et al. 2001, Kircheis et al. 2002, Finlay et al. 2007, Gelinas and Pinel-Alloul 2008a, Gelinas and Pinel-Alloul 2008b). Macrophyte patches are important refuges for zooplankton to hide from zooplanktivorous fish (Jeppesen et al. 1997, Perrow et al. 1999, Benndorf et al. 2002, Jones et al. 2002) despite chemical repulsion from macrophytes (Benndorf et al. 2002).

### **Macrophytes**

Macrophytes are rooted aquatic plants that typically grow in the shallow areas of a lake, and tend to grow in areas that have finer substrates and less extreme slopes (Duarte and Kalff 1986, Ness 2006), including near wetland areas.

Macrophytes grow in several forms, including emergent, floating, and submergent. Emergent macrophytes tend to grow in the shallower areas of the littoral zone, and extend above the surface of the water. Floating macrophytes typically have leaf structures that grow on the surface of the water, although the plant itself is rooted in the substrate. Submergent macrophytes grow entirely within the water column, although they remain rooted in the substrate.

As plants, macrophytes use many of the same nutrients as phytoplankton. Macrophytes and phytoplankton compete for limited nutrients, including phosphorus, which limits all plant growth in Maine lakes. While macrophytes may be able to limit the growth of phytoplankton using chemical cues (Benndorf et al. 2002), phytoplankton can outcompete macrophytes for light since algal blooms result in turbidity. Increased turbidity decreases the depth at which submergent macrophytes can grow. This antagonistic relationship between macrophytes and phytoplankton fuels the hypothesis of two stable states for shallow eutrophic lakes: a macrophyte-dominated state, and a

phytoplankton dominated state. In lakes with high nutrient loading, either macrophytes will dominant and outcompete phytoplankton for nutrients, or phytoplankton will dominant and outcompete macrophytes for light (Benndorf et al. 2002).

### **Previous Research Completed in Region**

The Maine Volunteer Lake Monitoring Program was founded in 1971 and now boasts 1,000 active volunteers on 500 lakes including the Belgrade region lakes. This program is the primary source of lake data in Maine, producing the “Maine Lakes Report” each year and providing data to the Maine Department of Environmental Protection (MDEP) PEARL database. There are many lakes on which extensive data have been collected, but the Belgrade Lakes system has been of particular focus for many studies due to their dynamic, highly interconnected, and varied nature. Students at Colby College and at the University of Maine have studied many of these lakes intensively.

For example, Colby students evaluate the water quality of a lake near the Waterville area each year for the senior Environmental Science capstone project. This assessment includes establishing land use patterns within the watershed of each lake, creating a land-use based model of phosphorus loading to the lake, and tracking intensive water quality parameters throughout the summer. Many of the Belgrade Lakes have been intensively studied by Colby students (Colby Environmental Assessment Team, CEAT), including Great Pond (CEAT 1999, 2010), Long Pond (CEAT 2007, 2008), Salmon Lake and McGrath Ponds (CEAT 2009). Other researchers at Colby have characterized the geology and sediments of East Pond (Nesbeda 2004), and *Gleotricha echinulata* blooms on Great and Long Ponds (King and Laliberte 2005).

Ian McCullough (2010) studied the patterns of residential development in the watershed of the entire Belgrade Lakes region at Colby College, and made predictions of increases in phosphorus loading to each lake as a result of development. McCullough (2010) predicted that Salmon/McGrath Ponds and Messalonskee Lake would have the greatest increase in phosphorus loading as a result of more rapid residential growth. This highlights individual lakes and watersheds that are likely to experience changes in the near future. Broad scale monitoring projects such as this study are important in providing baseline data to track these changes.



Zooplankton in the Belgrade Lakes were monitored in East and North Ponds by Ditzler (2010) during a bioremediation project. White perch (*Morone americana*) were removed from East Pond, in an attempt to lower the biomass of zooplanktivores, and lower the predation pressure on zooplankton (Haliwell and Evers 2008). The project investigated whether or not the removal of zooplanktivores would result in higher zooplankton biomass, and whether the higher biomass of zooplankton would keep noxious blooms of cyanobacteria under control in East Pond (Haliwell and Evers 2008). Ditzler (2010) monitored the size structure, taxonomic composition and abundance of zooplankton communities in East and North Ponds before and after the bioremediation project. Ditzler did not find significant differences that were expected from the bioremediation, such as an increase in zooplankton biomass. Instead, Ditzler concluded that bottom-up forces were dominant in both lakes, and that external factors such as weather drove the changes observed in zooplankton compositions before and after the bioremediation, which were similar between East and North Ponds. Although zooplankton communities have been intensively studied in North and East Ponds by Ditzler (2010) as discussed, no studies have investigated the zooplankton communities across the entire Belgrade Lakes system.

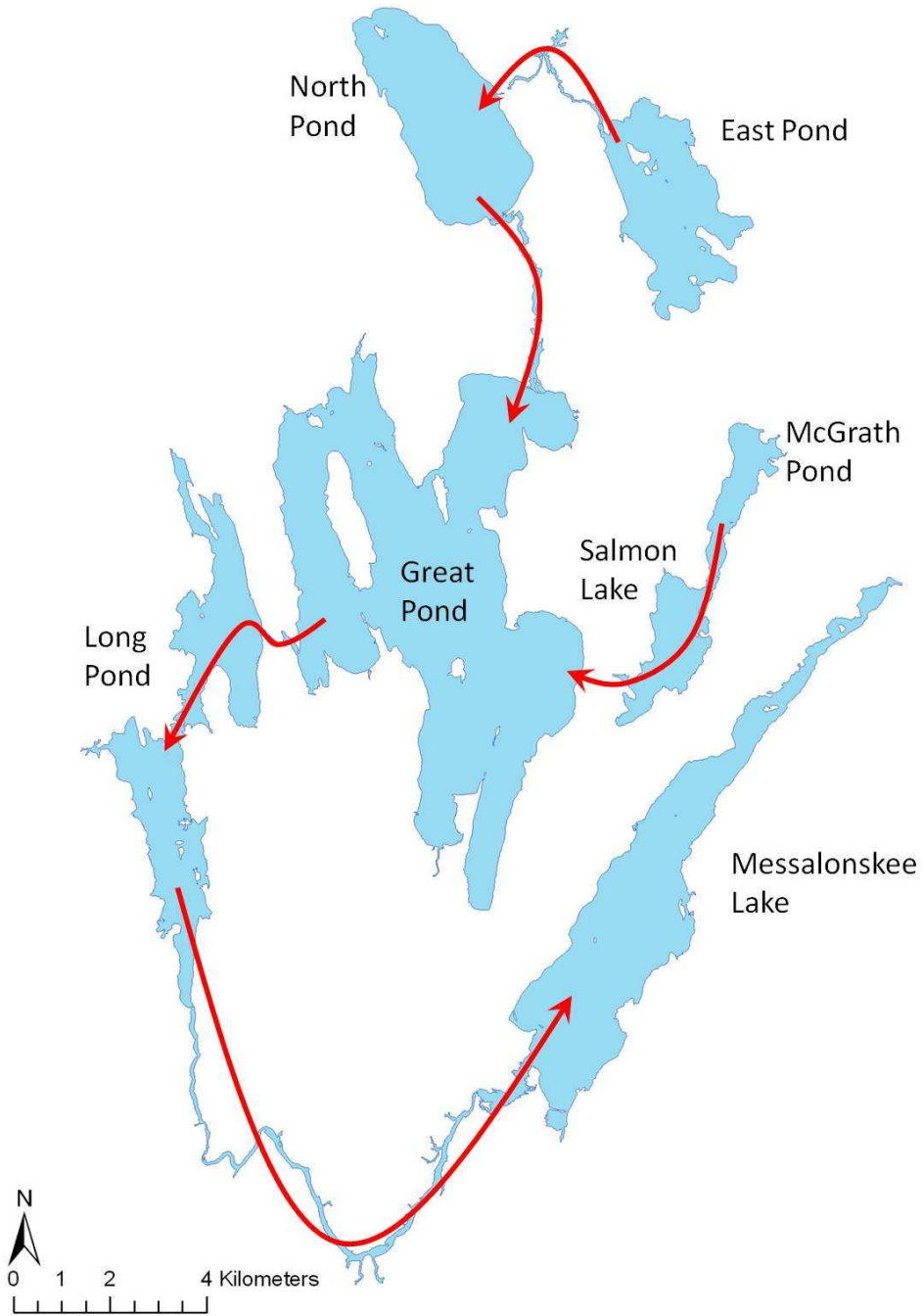
## **METHODS**

### **Study Area**

#### *Location*

The focus of this study is on the Belgrade Lake system of central Maine. All seven lakes are connected, with East Pond flowing into North Pond then Great Pond, and McGrath Pond flowing into Salmon Lake then Great Pond. Great Pond drains into Long Pond, which flows into Messalonskee Lake through the Belgrade Stream (Figure 3). Messalonskee Lake drains into Messalonskee Stream, which is part of the lower Kennebec Watershed. Each of the Belgrade Lakes was sampled for this study, with McGrath Pond and Salmon Lake treated as a single water body, and Long Pond North

basin and Long Pond South basin treated as a single water body as well (due to immediate connectivity).



**Figure 3: Water bodies of the Belgrade Lakes region and direction of water movements (Maine Office of GIS 2010).**

### *Temporal*

All sites were sampled in the month of October, 2010. East Pond was sampled on October 6, 2010, while the remainder of the sites were sampled from October 17 to October 25, 2010. All sampling was done between noon and 5 pm, except for North Pond, which was sampled from 8 am to noon. Samples from Great Pond were collected by the Colby Environmental Assessment Team (CEAT) from September through October, 2010.

### **Field Methods**

#### *Zooplankton Sampling Site Selection*

This study sampled zooplankton abundance in three distinct habitats: macrophyte patches, littoral areas adjacent to undeveloped shoreline, and littoral areas adjacent to developed shoreline with no buffer. This study sampled these habitats from all seven of the Belgrade Lakes (Figure 3).

In each lake, all macrophyte patches that were easily accessible by boat were sampled for zooplankton. At least five macrophyte samples were collected from each lake where macrophytes were found, with the exception of the North basin of Long Pond where no macrophytes were found on the date of sampling. In lakes where there were fewer than five distinct macrophyte patches, a second zooplankton sample was taken from a different area of one of the larger patches.

At least five shoreline sites with no buffer, as we defined in the land use classifications section, were sampled from each lake. Zooplankton tows were taken as close to shore as possible, although all tows were within five meters of the shoreline and were taken parallel to the shoreline. At least five sites with undeveloped shorelines were sampled for each lake in the same method as the no buffer sites. Samples from Great Pond were collected by CEAT with a compatible methodology described by CEAT (2010). These samples were collected 2-3 m from the shoreline as two 10 m tows combined into one sample. Counts from these samples were normalized by volume to be equivalent to counts taken from samples I collected myself (CEAT 2010).

The minimum sample size of five sites of each habitat type from each lake was chosen to represent a distribution of sites across each lake (that could be sampled within time constraints). Additionally, when all sites are aggregated within the Belgrade Lakes system, a sample size of sufficient statistical power was collected (n=142) to perform statistical tests.

#### *Land Use Classifications Used When Describing Sites*

The shoreline land uses were classified into one of five categories: developed sites with no buffer, developed sites with a buffer between 1 to 5 m, developed sites with a buffer greater than 5 m, undeveloped sites, and undeveloped wetland sites. A buffer was defined as woody vegetation greater than 1 m in height. These land use classifications were selected for easy identification from the water, and to represent the relative levels of shoreline disturbance.

#### *Site Selection*

Shoreline uses of the lakes were investigated using online aerial imagery such as MSN Bird's Eye View search engine (Bing 2011). Once in the field, large areas of the land use of interest were identified. These large stretches were selected to be well distributed throughout the lake. The specific point sampled on each large stretch was randomly selected by visually dividing the area into sections, and selecting the section to be sampled using a random number generator.

#### *Collection of Zooplankton Tows*

Zooplankton was collected in 12 cm diameter, 83  $\mu$ m cone plankton net towed over 10 meters. Once the tow was completed, ambient lake water was poured down from outside the net to wash any specimens that may have been caught on the net into the sample collection following standard sampling methodologies. The sample was poured into a collection bottle pre-labeled with a unique number, and stored on ice during sampling until preserved in the laboratory. Only one tow was taken on most sites.

Macrophyte and midlake samples taken on East Pond were not collected over a 10 m tow. Instead, 10 L of water collected using a wide-mouth jar was drained through the net.

However, the densities of these samples were much lower than the densities of samples collected over the 10 m tow, so this method was abandoned and all other macrophyte and mid lake samples were collected as 10 m tows.

#### *Additional Local Data Collected at Sites*

In sites sampled as a macrophyte patch, the land use of the nearest shoreline point was recorded, according to the designations outlined in the land use classification section. The size of the patch and distance from shore were visually estimated. The types of aquatic vegetation were noted and any macrophytes that could be easily identified were noted as well.

Canopy cover was measured at every site using a densitometer. At macrophyte sites, this was taken facing the shoreline from the middle of the patch where the zooplankton tow was taken. At no buffer and undeveloped shoreline sites, this measurement was taken from the same location as the zooplankton tow, facing the shoreline. Substrate was quantified at every site based on the presence of sand, silt or rocky materials using an AquaScope. The presence of submerged macrophytes was noted as well. A Lowerance GPS unit was used to record the location of each zooplankton site with the UTM coordinates.

### **Laboratory Methods**

#### *Preservation and Storage of Zooplankton Samples*

Zooplankton samples were preserved in 70% ethanol and transferred to storage vials labeled with the date and collection bottle number on the day of sampling immediately upon return to the laboratory. Samples were drained through a 90  $\mu\text{m}$  sieve and rinsed into storage vials with 50 mL of 70% ethanol solution.

#### *Analysis of Family Composition and Abundance of Zooplankton Samples*

One milliliter of each preserved sample of zooplankton was observed under 10x magnification on Sedgewick-Rafter slides. Each zooplankton specimen was identified to Family using the UNH zooplankton key (UNH 2011). Copepods were identified to the Order. At least two slides were examined from each sample. If at least 10 individuals

were not observed in the first two slides, up to three more slides were examined; either until 10 individual specimens in total were observed or until five slides had been examined. This sampling procedure was held constant across all samples. The zooplankton counts were normalized to account for differences in subsampling. While the number of zooplankton observed from each sample is low, this only reduces the power of the analysis and does not introduce sampling bias (O'Brien, pers. comm.). Because 145 samples were collected, the use of time favors collecting more independent samples rather than increasing subsampling. The abundance of each family was converted to represent density per liter. Density by family was used to calculate overall density and diversity using the Shannon-Weiner Index.

### **Statistical Methods**

Several biotic and abiotic environmental variables were selected to explain the variability of zooplankton communities in the Belgrade Lakes based on the findings of previous research, easily measured field conditions, and limitations of existing data. Substrate character, land use, macrophyte presence, and type of macrophyte patches were observed while sampling. Whole-lake morphometric, water quality, chemistry, and fish data were obtained from the Maine DEP from the PEARL dataset (PEARL 2010).

Several studies have suggested that top-down effects characteristic of systems experiencing trophic cascades are strong regulators of zooplankton community structure. Top-down effects include high levels of zooplanktivory depressing the abundance of zooplankton; or the presence of high piscivore biomass, which suppresses the abundance of zooplanktivorous fish and relieve grazing pressure on zooplankton. Since fish biomass data are not available for all lakes, we incorporated the presence or absence of the aggressive piscivore, Northern Pike to test for top-down effects. Stemberger and Lazorchak (1994) used coldwater and warmwater fish assemblages as supplementary indicators of lake health. We included the number of species present from the “warmwater” families cyprinidae and centrarchidae as proxies for the same trends Stemberger and Lazorchak (1994) investigated.

Lake chemistry and morphometric variables were included since Allen et al. (1999) found pH and lake depth to be the dominant variables structuring zooplankton

communities. The water quality variables are included to directly test our hypotheses of bottom-up controls that act on the lake-wide scale. Watershed development variables were taken from McCullough (2010). These metrics were included to investigate a larger-scale effect of development, as Gelinias and Pinel-Alloul (2008b) found these watershed wide development metrics to have a greater magnitude influence on zooplankton community structure than smaller scale riparian disturbance.

All explanatory variables were classified into groups, with specific variables listed in Table 1. These groups include substrate, vegetation, morphometry, chemistry, water quality and fish.

**Table 1: Candidate variables to predict densities of zooplankton families in the Belgrade region lakes. Variables are listed by group, and were later narrowed down using statistical tests (McCullough 2010, PEARL 2010).**

Morphometry	Chemistry	Water Quality	Development	On-Site Observations	Fish
Perimeter	pH	Minimum chlorophyll	Road	Vegetation	Salmonid species count
Mean depth	Alkalinity	Maximum chlorophyll	Forest	Canopy cover	Centrarchid species count
Maximum depth	Conductivity	Mean chlorophyll	Wetland		Cyprinind species count
Volume		Total Phosphorus (epicore)	Developed		
Direct drainage		Total Phosphorus (surface)	Agricultural		
Total drainage		Trophic State	Residential		
Flushing rate		Index			

Non parametric statistics were used since none of the data are normally distributed and transformations of the data were unsuccessful. Explanatory variables were chosen from the large initial dataset using the Spearman correlation for ordinal or continuous data (O'Brien, pers. comm.). Many of the significant variables were autocorrelated, so only the variable with the highest correlation with the outcome after checking for collinearity was used. Binomial variables were selected for modeling if the distribution of zooplankton densities (the outcome variable) between values of 0 and 1 were

significantly different ( $p < 0.05$ ) in a Mann-Whitney Rank Sum test. Categorical variables that are not ordinal (such as land use and lake number) were tested for significant differences in zooplankton density with the Kruskal-Wallis test. The variables that exhibited significant differences in zooplankton density, or were correlated with the Spearman Rank Correlations were then used in a zero inflated negative binomial model. Not all selected variables were significant predictors once used in the model, but it was necessary to narrow down the pool of variables for the model to run successfully (O'Brien, pers. comm.).

In addition to evaluating each potential variable for the aggregated data set for the entire Belgrade Lakes region, land use and macrophyte presence were tested for significance within each lake. By conducting intra-lake tests, we hoped to establish if the relationships between our main variables could be masked by strong differences in other lake components (such as trophic state).

The most significant predicting variables from our tests conducted on the aggregated data set were selected for each family. Binomial variables were selected if the p-value of the rank sum test was less than 0.05, as were categorical variables tested with the Kruskal-Wallis test. Ordinal data tested with the Spearman Rank correlations were selected if the spearman coefficient was greater than 0.35. Many statistical tests were conducted during the variable selection process, which increases the likelihood of finding false differences, which is a limitation of our variable selection process.

The variables that met the standards of significance were then used in a two step zero inflated negative binomial model. The first step is a logarithmic regression that predicts the occurrence of zero values in the outcome variable, zooplankton density. The second step is a negative binomial regression that predicts the count of the outcome variable, in this case, zooplankton density. This type of model is used in situations where the data have high variance. A zero-inflated model produces a coefficient which predicts the log odds of observing a value of zero. Log odds are a metric that is derived from the logarithm of the ratio of the probability that an event will occur to the probability that the event will not occur. In this case, the event which the probability is derived from is the observation of a density of zero. This type of model was used because there were high



observations of zero values for the densities of all families modeled, resulting in skewed data. Zero data is difficult for most models to predict; therefore, by adding in a step to specifically account for zero values, we can construct a model that is more representative of data observed (O'Brien, pers. comm.).

The first step used macrophyte presence to predict the log odds of finding no zooplankton of a family in a sample in a zero inflated negative binomial regression. If the macrophytes were not a significant predictor of zero values, then a constant was used instead. All families had significant inflation of zero values, which is why a two step model was used. The second step was a logarithmic regression of the densities of each family of zooplankton under conditions when values are not zero.

## **GIS Modeling Methods**

### *Zooplankton Density Interpolation*

Since zooplankton densities of individual samples were significantly different among the individual lakes, each lake was interpolated separately. For the baseline prediction, the Inverse Distance Weighted (IDW) interpolation method was used (Demers 2008). IDW predicts the spatial distribution of a metric, in this case zooplankton density, over continuous areas that were not actually sampled using a simple linear interpolation between sample points (Demers 2008). IDW was used over other interpolation methods for simplicity. Since the initial zooplankton interpolation is a baseline that will be linked to other within-lake factors such as macrophyte presence, simple distance methods of interpolation were the most appropriate (Gimond, pers. comm.).

The UTM NAD 1983 Zone 19 N coordinate system and datum were used for all GIS analyses. The cell size for raster output was set to 10 by 10 meters since each zooplankton tow was 10 meters long. A variable search radius was used since the distribution of the sampling points across the lake was widely distributed. The shoreline of the lake, as mapped from the Maine Office of GIS (2010) was used as a polyline barrier to prevent sites which were not directly connected by water from influencing each other. The same shoreline layers were used to set the extent of the analysis.

## **Procedure for Constructing the Macrophyte Presence Prediction**

Areas where macrophytes are likely to be present were mapped in GIS using bathymetry data from CEAT (2007, 2008, 2009, 2010), PEARL (2010), and Whitney King (unpublished data). Areas deeper than five meters were excluded from analysis since aquatic vegetation does not grow successfully at greater depths. The remaining bathymetry raster was used to calculate a new raster representing the slope of the lake bed. Slopes below 2.24% (Duarte and Kalff 1986) were retained, and other slopes were excluded. The remaining raster was converted to a uniform value that represents areas likely to have aquatic vegetation for this analysis.

## **Integrated Model**

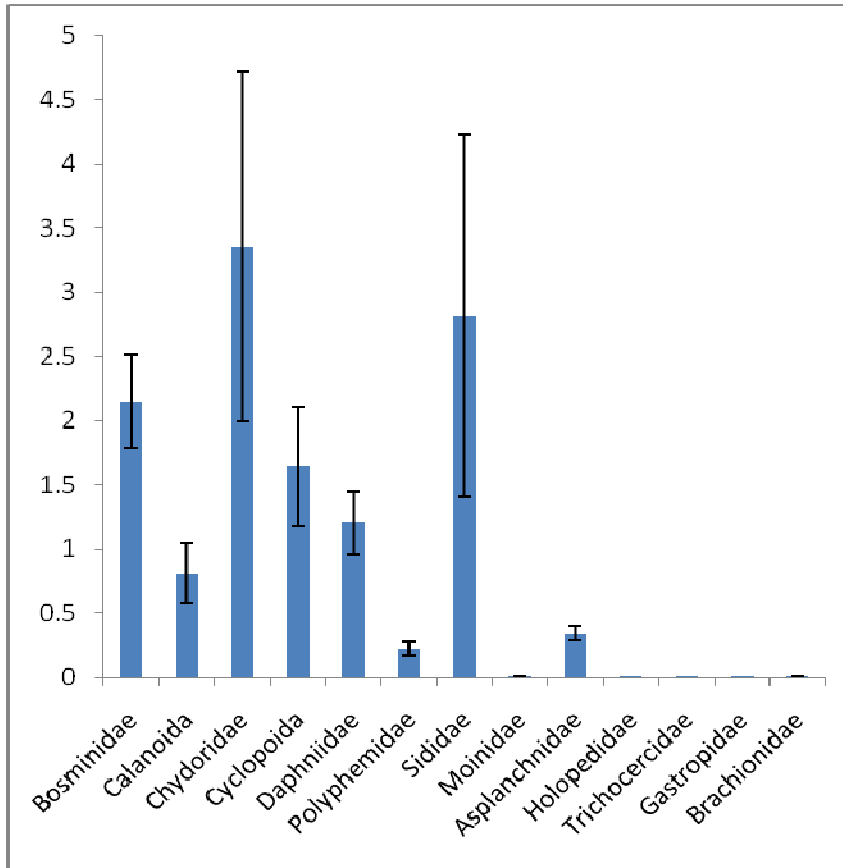
Since all littoral sites sampled were located in areas identified as likely to have macrophytes in the slope-based macrophyte model, a fully integrated raster model of densities could not be constructed. However, the validity of our models was tested by interpolating all zooplankton samples from the lake, which were taken at sites where macrophytes were absent. The predicted density of zooplankton from the macrophyte-absent raster layer were extracted to the sites where macrophytes were observed. This value was multiplied by the coefficient for macrophyte density to make a prediction for what zooplankton densities would be based on the non-macrophyte values and the numerical model. These predictions were compared to the actual densities observed at the macrophyte sites using the Mann-Whitney rank sum test (O'Brien, pers. comm.).

## **RESULTS**

### **Overall Summary of Data**

I found 13 distinct families of zooplankton in my samples. I found Cladocerans from the Families Bosminidae, Chydoridae, Daphniidae, Polyphemidae, Sididae, Moinidae, and Holopedidae. Copeopods were found from the Orders of Calanoida and Cyclopoida, and rotifers were found from the Order Ploima from the Families Asplanchnidae, Brachionidae, Gastropidae and Trichoceridae. (Figure 4). The densities of all families were highest in East Pond, with the exception of the Order Calanoida, which was highest

in Salmon Lake and McGrath Pond; Polyphemidae, which was highest in Great Pond; and Asplanchnidae, which was highest in Long Pond (Table 2).

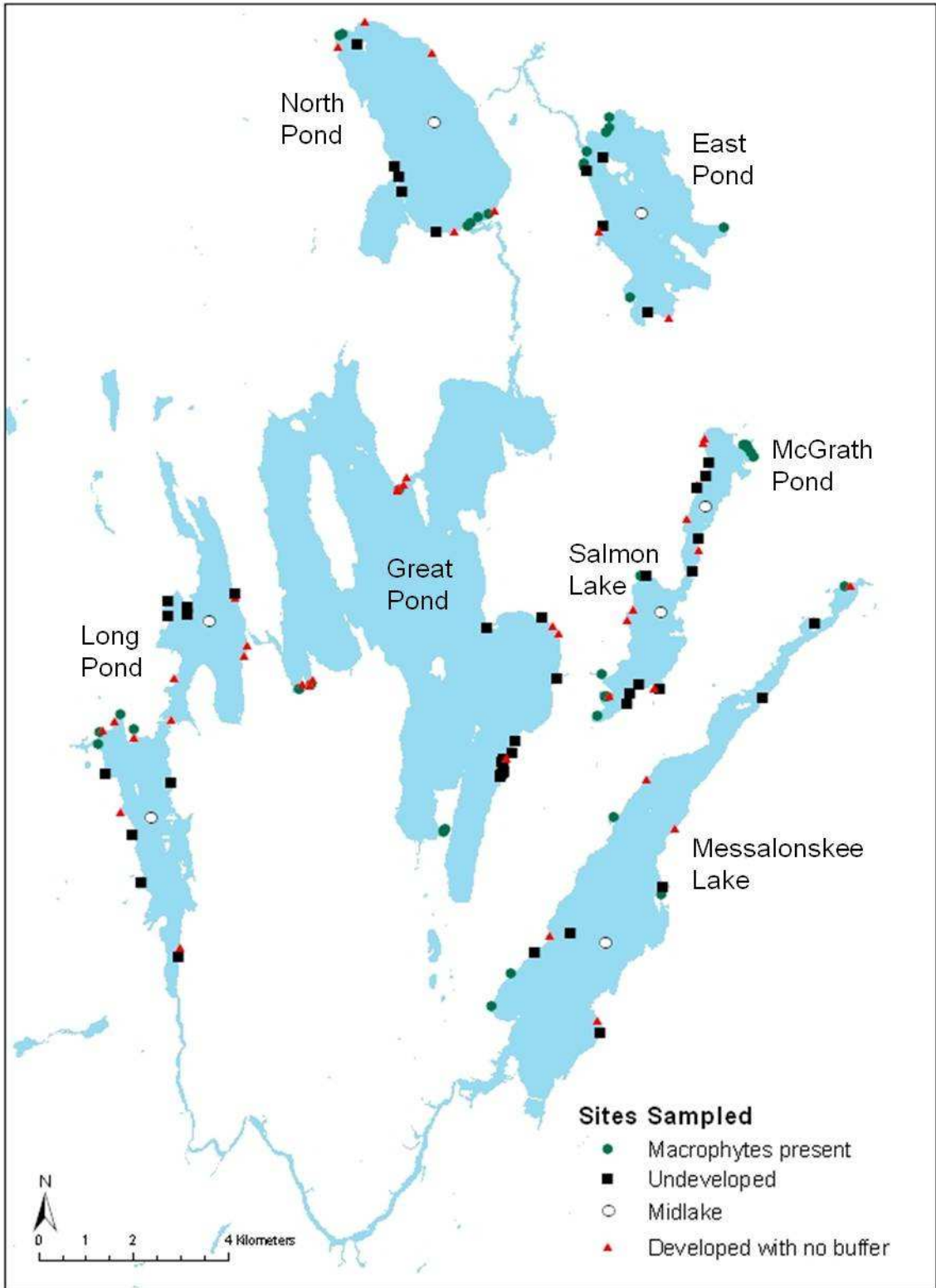


**Figure 4: Mean ( $\pm$  SE) zooplankton density observed per site by Family across all sites sampled in the Belgrade Lakes.**

No macrophyte patches were found in the North basin of Long Pond, and a mid lake sample was not collected from Great Pond. Only ten sites were sampled in the north basin of Long Pond since no macrophytes were found, and 16 to 33 sites were sampled on all other lakes (Figure 5).

**Table 2: Mean densities (per Liter) of individual zooplankton families in each of the Belgrade Lakes across all sites within each lake.**

	Bosminidae	Calanoida	Chydoridae	Cyclopoida	Daphniidae	Polyphemidae	Sididae	Asplanchnidae
East Pond	7.11	1.09	22.31	10.83	5.13	0.00	18.91	0.00
North Pond	0.25	0.04	0.29	0.27	0.00	0.01	0.12	0.33
Salmon/McGrath	1.78	2.97	1.44	0.75	2.40	0.08	1.27	0.53
Great Pond	1.62	0.07	0.74	0.27	0.09	0.71	0.26	0.09
Long Pond	2.27	0.01	0.04	0.05	0.02	0.09	0.44	0.85
Messalonskee	0.41	0.02	0.19	0.11	0.12	0.21	0.08	0.05



**Figure 5: Locations of all sites sampled in the Belgrade Lakes during study. Sites were sampled from October 6-25, 2010.**

## Results of Hypotheses Tests

### *The Presence or Absence of Macrophytes and Zooplankton Density*

Aggregate zooplankton density is significantly higher in sites sampled in macrophyte patches compared to sites where macrophytes were not observed (Mann Whitney U test,  $df=141$ ,  $p=0.0033$ ). Chydoridae (Mann Whitney U test,  $df=141$ ,  $p=0.0031$ ), Daphniidae (Mann Whitney U test,  $df=141$ ,  $p=0.0003$ ) and Sididae (Mann Whitney U test,  $df=141$ ,  $p=0.0000$ ) were found at significantly higher densities within macrophyte patches. The p-values for the remaining families and metrics are listed on Table 3.

**Table 3: P-values for Mann-Whitney U test between sites with macrophytes present and sites with no macrophytes across all Belgrade Lakes for density (per Liter) of individual families and aggregate density ( $df=141$ ). Significant differences are in bold.**

Family	p-value
Density	<b>0.0033</b>
Bosminidae	0.4718
Calanoida	0.3650
Chydoridae	<b>0.0031</b>
Cyclopidae	<b>0.0449</b>
Daphniidae	<b>0.0003</b>
Polyphemidae	0.1966
Sididae	<b>&gt;0.0001</b>
Moinidae	0.1079
Asplanchnidae	0.1100
Holopedidae	0.8935
Trichoceridae	0.1210
Gastropidae	0.5190
Branchionidae	0.6358

Since strong inter-lake differences were observed across many variables, we tested our hypothesis in each lake individually since stronger interlake signals may drown out the macrophyte signal. Aggregate zooplankton density is not significantly higher within macrophyte patches when compared within a single lake in any of the Belgrade region lakes. However, specific families did have significant differences in density among sites within macrophyte patches and sites with no macrophytes. These families varied between

lakes, although Sididae density is significantly higher in macrophyte patches in every lake except Great Pond.

**Table 4: P-values of Mann-Whitney U test comparing zooplankton densities by family of sites with and without macrophytes within individual lakes. Missing values in table are due to no variance observed within family by lake. Bolded p-values indicate significance ( $p < 0.05$ )**

Family	East Pond	North Pond	Sal/McG	Great Pond	Long Pond	Messalonskee
Bosminidae	0.1541	0.9615	0.1036	0.0867	0.8913	0.7505
Calanoida	<b>0.0115</b>	0.1224	0.4325	0.9556	<b>0.0165</b>	0.7509
Chydoridae	0.7556	0.9111	0.4318	<b>0.0262</b>	0.3169	0.5942
Cyclopoida	0.2123	0.4922	0.0808	<b>0.0345</b>	0.9646	0.1235
Daphniidae	<b>0.0327</b>	0.4795	0.7340	0.0654	<b>0.0005</b>	0.4589
Polyphemidae		0.5483	<b>0.0430</b>	0.4400	0.4531	0.0670
Sididae	<b>0.0140</b>	<b>0.0339</b>	<b>&lt;0.0001</b>	0.2013	<b>0.0102</b>	<b>0.0006</b>
Moinidae	0.1037		0.4902	0.4468	0.6767	
Asplanchnidae		0.3411	0.2217	0.5880	0.3904	0.5524
Holopedidae		0.1573	0.4902		0.6767	
Density	0.1424	0.133	0.1330	0.9455	0.1942	0.2249

In East Pond, Calanoida, Daphniidae and Sididae have significant differences in density within and outside of macrophyte patches. In North Pond, Messalonskee Lake and Salmon/McGrath Pond, only Sididae showed a significant difference. In Great Pond, Chydoridae and Cyclopoida have significant differences within and outside of macrophyte patches. In Long Pond, Calanoida, Daphniidae and Sididae were significantly different in density between macrophyte patches and sites without macrophytes (Table 4).

*Undeveloped Land Uses compared to Developed lands with no buffer*

There was no significant difference in zooplankton densities of any family between sites adjacent to undeveloped shoreline and sites adjacent to shoreline that was developed with no buffer. Within lake comparisons between no buffer and undeveloped sites also did not have significant differences in density of most zooplankton families, with the

exception of Sididae and Asplanchnidae in Salmon and McGrath Ponds, and Cyclopoida in Long Pond (Table 5).

**Table 5: p-values of Mann-Whitney U test comparing sites sampled adjacent to undeveloped shoreline and sites sampled adjacent to developed shoreline with no buffer for region aggregated and within individual lakes. No significant differences were found ( $p < 0.05$ )**

Family	Belgrade Region	East Pond	North Pond	Salmon/McGrath	Great Pond	Long Pond	Messalonskee
Bosminidae	0.9314	0.5192	0.5192	0.6664	0.8450	0.5543	0.2505
Calanoida	0.6019	0.3120	0.3120	0.3050	0.2547	0.3173	0.9093
Chydoridae	0.1001	0.1161	0.1161	0.2318	0.1918	0.1478	0.5318
Cyclopoida	0.3766	0.8301	0.8301	0.0834	0.5154	0.0350	0.8253
Daphniidae	0.4782	0.2518	0.2518	0.6656	0.6500	1.0000	0.3507
Polyphemidae	0.2115			0.1384	0.2631	0.4746	0.8155
Sididae	0.1350	0.5650	0.5650	0.0296	0.5051	0.6228	0.8609
Asplanchnidae	0.5552			0.0287	0.7258	0.5085	0.0790
Total density	0.7821	0.6682	0.6682	0.8949	0.9222	0.8179	0.7748

## Regression

### *Autocorrelation of Predicting Variables*

None of the field based observations experienced autocorrelations with any of the other explanatory variables. Variables were considered to be highly autocorrelated if a Spearman Rank correlation coefficient of over 0.5 was reported between variables (O'Brien, pers. comm.). Therefore these variables were excluded from Tables 6-9, which report the Spearman correlation values of all variables and the risk for autocorrelation.

Within the chemistry variable group, all variables were significantly autocorrelated. None of the fish species count variables were autocorrelated to each other. Most of the water quality variables were autocorrelated to each other, with the exception of mean chlorophyll, which was not correlated to any variable within the group. Most of the morphometry variables were autocorrelated to each other.



Autocorrelation was also assessed between groups of variables. The chemistry group was not correlated with any variables from any other group except for variables in the watershed land use group. All chemistry variables were correlated with the road/residential development and forest cover variables, while pH and conductivity were correlated with overall development.

Variables from the morphometry group were correlated with many variables from several other groups, especially variables from the water quality group. All morphometric variables were correlated with trophic state index. Most morphometric variables were correlated with maximum chlorophyll, total phosphorus from both the surface and the epilimnion, Centrarchid species count, and the proportion of the watershed composed of wetlands.

The water quality group was correlated with several variables from other groups, including the morphometry group previously discussed, fish group, and watershed land use group. The most consistent correlations were with the wetland land use variable, with only mean chlorophyll and trophic state index not correlated with wetlands.

Variables from the fish group were correlated with variables from other groups. Cyprinid species count was correlated to only minimum chlorophyll and mean chlorophyll. Salmonid species count was correlated to both measures of total phosphorus, trophic state index, and development. Centrarchid species count was correlated to many variables, including minimum and maximum chlorophyll, TSI, Road/Residential land use, Wetlands and Agriculture (Tables 6-10).

**Table 6: Spearman Rank correlation values between all ordinal predicting variables to test for autocorrelates. Highly significant correlations are in bold (coefficient > 0.5). (Part 1 of 5)**

	Date	n	Area	Perimeter	Mean Depth	Maximum Depth	Volume	Direct Drainage	Total Drainage	Flushing Rate
Observations	-0.3909									
Area	0.2706	-0.246								
Perimeter	0.1409	-0.0926	<b>0.9329</b>							
Mean Depth	<b>0.5571</b>	-0.0295	0.3794	0.4683						
Max Depth	<b>0.6163</b>	-0.151	<b>0.615</b>	<b>0.6079</b>	<b>0.9043</b>					
Volume	0.1188	-0.0011	<b>0.8755</b>	<b>0.8998</b>	<b>0.5403</b>	<b>0.7402</b>				
Direct Drainage	0.3474	-0.2054	<b>0.8429</b>	<b>0.7849</b>	0.4707	<b>0.7762</b>	<b>0.8912</b>			
Total Drainage	<b>0.787</b>	-0.3981	<b>0.7169</b>	<b>0.6075</b>	<b>0.7284</b>	<b>0.8697</b>	<b>0.5924</b>	<b>0.7136</b>		
Flushing Rate	<b>0.839</b>	-0.3809	0.0251	-0.0429	<b>0.5184</b>	0.4696	-0.1431	0.0781	<b>0.6742</b>	
pH	-0.171	0.2875	-0.2255	-0.3033	0.015	0.1222	0.1146	0.0607	-0.1694	-0.2602
Alkalinity	0.1136	0.1352	-0.1179	-0.247	0.1458	0.246	0.0938	0.0604	0.1095	0.0972
Conductivity	-0.0523	0.1212	-0.1379	-0.3101	-0.1484	-0.0268	-0.0249	-0.0846	-0.0621	-0.0689
Minimum Chlorophyll	0.2898	-0.3253	<b>0.8806</b>	<b>0.6995</b>	0.1009	0.4051	<b>0.6558</b>	<b>0.683</b>	<b>0.6472</b>	0.0928
Maximum Chlorophyll	<b>-0.5203</b>	0.2905	<b>-0.8099</b>	<b>-0.6604</b>	-0.4588	<b>-0.7685</b>	<b>-0.7667</b>	<b>-0.8755</b>	<b>-0.8381</b>	-0.3215
Mean Chlorophyll	<b>-0.5703</b>	0.3794	0.07	0.273	0.0797	-0.1223	0.2403	-0.1157	-0.3684	<b>-0.666</b>
Total phosphorus epicore	-0.4378	0.0497	<b>-0.5634</b>	<b>-0.6592</b>	<b>-0.6852</b>	<b>-0.7189</b>	<b>-0.607</b>	<b>-0.5377</b>	<b>-0.7073</b>	-0.4888
Total phosphorus surface	-0.2072	0.0963	<b>-0.7497</b>	<b>-0.7031</b>	-0.2573	<b>-0.5175</b>	<b>-0.7326</b>	-0.701	<b>-0.5771</b>	-0.1067
Trophic state index	-0.4201	0.0988	<b>-0.5459</b>	<b>-0.6362</b>	<b>-0.5413</b>	<b>-0.6004</b>	<b>-0.5233</b>	<b>-0.5003</b>	<b>-0.6605</b>	<b>-0.5037</b>
Salmonids	0.1507	-0.2254	-0.2093	-0.4152	-0.1153	-0.0917	-0.2932	-0.1192	-0.0492	-0.0174
Centrarchids	<b>0.7974</b>	-0.452	<b>0.6628</b>	<b>0.592</b>	<b>0.6573</b>	<b>0.7661</b>	0.4701	<b>0.6419</b>	<b>0.9549</b>	<b>0.7381</b>
Cyprinids	0.0793	-0.3657	0.2518	-0.0329	<b>-0.599</b>	-0.2916	-0.0847	0.1119	0.0875	-0.0028

**Table 7: Spearman Rank Correlations continued (part 2 of 5)**

	Date	n	Area	Perimeter	Mean Depth	Maximum Depth	Volume	Direct Drainage	Total Drainage	Flushing Rate
Road	-0.4034	0.4224	<b>-0.5114</b>	-0.4874	-0.0961	-0.1119	-0.1028	-0.1818	<b>-0.505</b>	-0.4575
Forest	-0.0317	0.0103	0.0461	0.3057	0.135	-0.0818	-0.0626	-0.1258	-0.0181	0.1456
Wetland	0.4698	-0.3614	<b>0.9397</b>	<b>0.8102</b>	0.4385	<b>0.7241</b>	<b>0.8147</b>	<b>0.9196</b>	<b>0.8304</b>	0.2023
Developed	-0.0572	0.0071	-0.4509	<b>-0.6612</b>	-0.2931	-0.1812	-0.3259	-0.1639	-0.2903	-0.1811
Agricultural	<b>0.8183</b>	-0.5101	0.0296	-0.1961	0.2632	0.313	-0.24	0.0501	<b>0.6154</b>	<b>0.8721</b>
Residential	-0.4034	0.4224	<b>-0.5114</b>	-0.4874	-0.0961	-0.1119	-0.1028	-0.1818	-0.505	-0.4575

**Table 8: Spearman Rank Correlations continued (Part 3 of 5)**

	pH	Alkalinity	Conductivity	Minimum Chlorophyll
Alkalinity	<b>0.8925</b>			
Conductivity	<b>0.8301</b>	<b>0.9391</b>		
Minimum Chlorophyll	-0.083	0.1303	0.2074	
Maximum Chlorophyll	-0.1721	-0.3636	-0.2604	<b>-0.8444</b>
Mean Chlorophyll	-0.1285	-0.3965	-0.3681	-0.2585
Total phosphorus (epicore)	0.0527	-0.2199	-0.0471	-0.4877
Total phosphorus (surface)	-0.1641	-0.3632	-0.3400	<b>-0.8350</b>
Trophic state index	0.1601	-0.1317	0.0011	-0.5178
Salmonids	0.0343	-0.1073	-0.0890	-0.2216
Centrarchids	-0.4216	-0.1438	-0.3054	<b>0.5620</b>
Cyprinids	-0.0205	0.1332	0.3691	<b>0.6330</b>
Road	<b>0.8711</b>	<b>0.5952</b>	<b>0.5282</b>	-0.4998
Forest	<b>-0.8346</b>	<b>-0.8130</b>	<b>-0.8444</b>	-0.2130
Wetland	-0.1621	-0.0541	-0.1304	<b>0.8356</b>
Developed	<b>0.6648</b>	0.4911	<b>0.5050</b>	-0.2810
Agricultural	-0.1755	0.1483	0.0801	0.2092
Residential	<b>0.8711</b>	<b>0.5952</b>	<b>0.5282</b>	-0.4998

**Table 9: Spearman Rank Correlations continued (part 4 of 5)**

	Maximum Chlorophyll	Mean Chlorophyll	Total phosphorus (epicore)	Total phosphorus (surface)	Trophic state index
Mean Chlorophyll	0.4130				
Total phosphorus (epicore)	<b>0.6731</b>	0.2082			
Total phosphorus (surface)	<b>0.8575</b>	0.2854	<b>0.7775</b>		
Trophic state index	<b>0.6454</b>	0.3026	<b>0.9764</b>	<b>0.7933</b>	
Salmonids	0.2031	0.0157	<b>0.7174</b>	<b>0.6322</b>	<b>0.775</b>
Centrarchids	<b>-0.7127</b>	-0.3795	<b>-0.6654</b>	-0.4656	<b>-0.6633</b>
Cyprinids	-0.3478	<b>-0.5644</b>	0.1585	-0.3559	0.0295
Road	0.2528	0.1658	0.3641	0.2583	0.4735
Forest	0.3263	0.3272	-0.2261	0.1614	-0.3034
Wetland	<b>-0.8865</b>	-0.1452	<b>-0.5028</b>	<b>-0.6723</b>	-0.4805
Developed	0.1307	-0.2303	<b>0.6524</b>	0.3972	<b>0.7117</b>
Agricultural	-0.3315	<b>-0.7370</b>	-0.1458	0.0050	-0.1643
Residential	0.2528	0.1658	0.3641	0.2583	0.4735

**Table 10: Spearman Rank Correlations continued (Part 5 of 5)**

	Salmonids	Centrarchids	Cyprinids	Road	Forest	wetland	developed	agricultural
Centrarchids	-0.0642							
Cyprinids	0.0963	0.073						
road	0.1897	<b>-0.6804</b>	-0.3076					
forest	-0.3744	0.2281	-0.404	<b>-0.6047</b>				
wetland	-0.0003	<b>0.7787</b>	0.2871	-0.4568	-0.082			
developed	<b>0.7113</b>	-0.4255	0.1942	<b>0.7096</b>	<b>-0.8089</b>	-0.2413		
agricultural	0.3665	<b>0.6399</b>	0.3258	-0.4005	-0.1733	0.2619	0.1666	
residential	0.1897	<b>-0.6804</b>	-0.3076	<b>1.0000</b>	<b>-0.6047</b>	-0.4568	<b>0.7096</b>	-0.4005

### *Predicting Variables Selected for Use in Numerical Model*

The final predicting variables for the density of each family of zooplankton and the metrics used were chosen as outlined in statistical methods. The Families Moinidae, Brachionidae, Gastropidae, Trichoceridae and Holopidae were excluded due to low number of observations.

The density of the Family Bosminidae was best correlated to the conductivity of the lake, cyprinid species count, and the mean chlorophyll (Table 13). Chydoridae was best correlated to centrarchid species count, flushing rate of the whole lake, and proportion of the watershed composed of roads/residential development. Daphniidae was best correlated to the percent cover of vegetation at sites, the number of centrarchid species found in the lake, flushing rate of the lake, and the proportion of the watershed developed as roads/residential development. Polyphemidae was best correlated to the offshore minimum chlorophyll levels of a lake. Sididae was best correlated to the total drainage area of the whole lake. The one rotifer family observed in quantities high enough to show significant relationships was Asplanchnidae, which was best correlated to flushing rate.

The copepod Order Calanoida was best correlated to the number of centrarchid species present in the lake, and the watershed cover of roads/residential development. The other copepod Order identified (Cyclopoida) was best correlated to the number of centrarchid species in a lake, the flushing rate, and the proportion of the watershed developed as roads or residences (Table 13).

### *Binomial Predicting Variables Selected for Use in Numerical Model*

The binomial variables fell into three categories: substrate (presence of organic substrates, silt, sand or rock), macrophyte characterization (presence of macrophytes, emergent macrophytes, submergent macrophytes, floating macrophytes), and fish (presence of Northern Pike, Land Locked Salmon).

Density of the Family Bosminidae was significantly different for Northern Pike presence or absence. Calanoida density was significant for organic substrate presence, and northern pike. Chydoridae was significant for silt presence, floating macrophytes, emergent macrophytes, macrophyte presence, and northern pike. Cyclopidae was significant for floating macrophyte, macrophyte presence, and northern pike. Daphniidae

was significant for organic substrate, silt, rock, floating macrophytes, emergent macrophytes, macrophyte presence, and northern pike. Polyphemidae was significant for rock presence and northern pike. Sididae was significant for silt, rock, all macrophyte variables, and northern pike. No binomial variables were significant for density of Families Moinidae, Asplanchnidae, Holopedidae, Trichocercidae, Gastropidae, or Branchionidae (Tables 11-13, 15)

*Categorical Variables Selected for Use in Numerical Model*

The two categorical variables tested were lake and land use. Strong significant differences were observed in most of the zooplankton family densities and all metrics between different lakes (Table 11). Only the families with low number of observations, Moinidae, Holopedidae, Trichoceridae, Gastropidae, and Brachionidae did not have significant differences between lakes. These strong intralake correlations are accounted for by clustering the data by lake within the numerical model. The relationship between zooplankton family densities or metrics and land use was not as strong or consistent. Only Bosminidae (Kruskal-Wallis test,  $df=141$ ,  $p=0.0001$ ) and Sididae (Kruskal-Wallis test,  $df=141$ ,  $p=.0087$ ) densities were significantly different between different shoreline land uses when the Belgrade region is analyzed as a whole.

**Table 11: P-values for Kruskal-Wallis test for categorical variables, land use and lake. Significant values are in bold ( $p<0.05$ )**

	By lake	By land use
Bosminidae	<b>0.0001</b>	<b>0.0001</b>
Calanoida	<b>0.0001</b>	0.8731
Chydoridae	<b>0.0001</b>	0.2324
Cyclopidae	<b>0.0001</b>	0.2636
Daphniidae	<b>0.0001</b>	0.3781
Polyphemidae	<b>0.0008</b>	0.1074
Sididae	<b>0.0001</b>	0.0087
Moinidae	0.9987	0.999
Asplanchnidae	<b>0.0001</b>	0.5128
Density	<b>0.0001</b>	<b>0.0443</b>



**Table 12: p-values of Mann Whitney U rank sum test for all binomial variables, including substrate variables, vegetation types, and presence of key indicator species. Significant values (p<0.05) are in bold. (Part 1 of 2)**

	Aggregate Density	Bosminidae	Calanoida	Chydoridae	Cyclopoida	Daphniidae	Polyphemidae
Organic substrate	<b>0.0210</b>	0.4079	<b>0.0384</b>	0.5557	0.4116	<b>0.0171</b>	0.5466
Silt substrate	<b>0.0076</b>	0.0919	0.1892	<b>0.0253</b>	0.1829	<b>0.0015</b>	0.6394
Sand substrate	0.0399	0.0826	0.8309	0.5452	0.2109	0.3266	0.4583
Rocky substrate	0.2448	0.5013	0.6366	0.1077	0.2629	<b>0.0452</b>	<b>0.0030</b>
Floating vegetation	<b>0.0445</b>	0.5455	0.7657	<b>0.0293</b>	<b>0.0143</b>	<b>0.0143</b>	0.2411
Emergent vegetation	<b>0.0283</b>	0.2924	0.8796	<b>0.0434</b>	0.3495	<b>0.0118</b>	0.3108
Submergent vegetation	<b>0.0494</b>	0.9071	0.4937	0.1509	0.7992	0.2208	0.2320
Macrophyte presence	<b>0.0033</b>	0.4718	0.3650	<b>0.0031</b>	0.0449	<b>0.0003</b>	0.1966
Northern Pike	< <b>0.0001</b>	<b>0.0028</b>	< <b>0.0001</b>	< <b>0.0001</b>	< <b>0.0001</b>	< <b>0.0001</b>	<b>0.0082</b>
Land Locked Salmon	< <b>0.0001</b>	<b>0.8936</b>	< <b>0.0001</b>	< <b>0.0001</b>	< <b>0.0001</b>	< <b>0.0001</b>	<b>0.0001</b>

**Table 13: p-values of Mann Whitney U rank sum tests for significance of binomial variables. Significant values are in bold.  
(Part 2 of 2)**

	Sididae	Moinidae	Asplanchnidae	Holopedidae	Trichocercidae	Gastropidae	Branchionidae
Organic substrate	0.5628	0.4260	0.0638	0.6146	0.7728	0.7728	0.5126
Silt substrate	<b>&lt;0.0001</b>	0.5483	0.4383	0.8266	0.5403	0.5403	0.5261
Sand substrate	0.1672	0.6979	0.1009	0.2940	0.0965	0.5475	0.7303
Rocky substrate	0.0329	0.6188	0.4136	0.6942	0.3749	0.3749	0.0993
Floating vegetation	<b>&lt;0.0001</b>	0.2512	0.2614	0.4221	0.6453	0.6453	0.2965
Emergent vegetation	<b>&lt;0.0001</b>	0.2186	0.6808	0.5592	0.0427	0.6217	0.9872
Submergent vegetation	<b>0.0001</b>	0.4505	0.7064	0.5976	0.7621	0.7621	0.4924
Macrophyte presence	<b>&lt;0.0001</b>	0.1079	0.1100	0.8935	0.1210	0.5190	0.6358
Northern Pike	<b>&lt;0.0001</b>	0.3707	0.1952	0.9932	0.4726	0.4726	0.4977
Land Locked Salmon	<b>0.0002</b>	0.3707	0.7935	0.9932	0.4726	0.4726	0.4977

**Table 14: List of variables selected for each zooplankton family for use in models of density in the Belgrade Lakes from Spearman Rank Correlations (continuous and ordinal data). These were chosen based on the magnitude of correlation (Spearman Rank Coefficient < 0.35)**

Bosminidae	Calanoida	Chydoridae	Cyclopoida	Daphniidae	Sididae	Polyphemidae	Asplanchnidae
Conductivity		Centrarchid species count	Centrarchid species count	Vegetation	Total drainage	Minimum chlorophyll	Flushing rate
Cyprinid species count				Centrarchid species count	Residential	Residential	
Mean Chlorophyll		Flushing rate	Flushing rate				
Residential		Residential	Residential	Flushing rate			
				Residential			

**Table 15: List of binomial variables selected for each zooplankton family for use in models in the Belgrade Lakes of zooplankton study. Variables listed were determined to be significant by the Mann Whitney U tests ( $p < 0.05$ ).**

Bosminidae	Calanoida	Chydoridae	Cyclopoida	Daphniidae	Sididae	Polyphemidae	Asplanchnidae
Northern Pike	Northern Pike	Northern Pike	Northern Pike	Northern Pike	Northern Pike	Northern Pike	
Land Locked Salmon	Land Locked Salmon	Land Locked Salmon	Land Locked Salmon	Land Locked Salmon	Land Locked Salmon	Land Locked Salmon	
	Organic substrate	Floating vegetation	Floating vegetation	Organic substrate	Floating vegetation	Rocky substrate	
		Emergent vegetation		Rocky substrate	Emergent vegetation		
		Macrophyte presence		Floating vegetation	Submergent vegetation		
		Silt substrate		Emergent vegetation	Macrophyte presence		
				Macrophyte presence	Silt substrate		
				Silt substrate			

*Results of the Numerical Model for Individual Zooplankton Families*

The significant variables described were used in a non-parametric two-step model. In the first step, the presence of macrophytes was used to predict the log odds of a value of zero for most families. Macrophyte presence was not a significant predictor of zero values for Bosminidae or Daphniidae. For these families, a constant was a significant predictor of zero values, indicating that zero-inflation was still significant despite the fact that macrophyte presence or absence did not predict the probability (zero inflation negative binomial model,  $df=141$ ,  $p<0.0001$ ).

For the Family Bosminidae, the number of cyprinid species present in a lake was the most significant variable predicting the non-zero densities. For an increase of one cyprinid species in a lake, a decrease of Bosminidae density by a factor of 0.42 is expected, holding all other factors constant. For an increase of one microgram per liter of mean chlorophyll, an increase of 2.12 is expected, and for an increase in watershed residential development by one percent, a decrease in density by a factor of 0.92 is predicted, holding all other variables constant. Conductivity was another significant predictor of Bosminidae density in our model (Table 16).

**Table 16: Logistic regression model results for non-zero densities of Bosminidae in the Belgrade Lakes. Significant predicting variables in bold ( $p<0.05$ ).**

Predicting Variable	Logistic Coefficient	Factor of influence	Z-statistic	p-value
<b>Conductivity (mS)</b>	<b>0.0950</b>	<b>1.0998</b>	<b>2.63</b>	<b>0.0090</b>
<b>Cyprinids (species count)</b>	<b>-0.8576</b>	<b>0.4241</b>	<b>-4.35</b>	<b>&lt;0.0001</b>
<b>Mean chlorophyll (<math>\mu\text{g/L}</math>)</b>	<b>0.7515</b>	<b>2.1203</b>	<b>3.76</b>	<b>&lt;0.0001</b>
<b>Residential (% of watershed)</b>	<b>-0.0879</b>	<b>0.9158</b>	<b>-2.45</b>	<b>0.0140</b>
Northern pike	1.4385	4.2148	1.38	0.1660
Constant	-7.2657	0.0007	-2.04	0.0410

Macrophyte presence was a significant predictor of the absence of the Order Calanoida, although this predicted a higher instance of zero densities in the presence of macrophytes (zero inflated negative binomial, df=141,  $p < 0.0001$ ). The only significant predictor of non-zero densities was the presence of an organic substrate, predicting Calanoid densities to decrease by a factor of 0.06 at sites where organic substrates are present if all other factors are held constant (Table 17).

**Table 17: Logistic regression model results for non-zero densities of Calanoid Copepods in the Belgrade Lakes. Significant predicting variables ( $p < 0.05$ ) in bold.**

Predicting Variable	Logistic Coefficient	Factor of influence	Z-statistic	p-value
<b>Organic substrate</b>	<b>-2.8726</b>	<b>0.0566</b>	<b>-25.81</b>	<b>&lt;0.0001</b>
Northern Pike	6.1345	461.4940	0.72	0.4690
Land locked salmon	0.0301	1.0305	0.04	0.9700
Centrarchids (species count)	-4.1783	0.0153	-1.36	0.1750
Residential (% of watershed)	0.6295	1.8766	1.07	0.2870
Constant	9.8615	19177.5	1.82	0.0690

The presence of macrophytes at a site significantly predicted the absence or presence of Chydoridae (zero inflated negative binomial,  $df=141$ ,  $p=0.001$ ), as well as the non-zero densities of Chydoridae. An increase in Chydoridae densities by a factor of 14.87 is expected at sites where macrophytes are present compared to sites where macrophytes are absent, holding all other variables constant. The most significant variable determining the non zero densities of Chydoridae is the presence of Northern Pike, predicting a decrease in Chydoridae densities by a factor of 0.0001 when Northern Pike are present, holding all other variables constant. The presence of Land Locked Salmon was also a significant predictor of Chydoridae density, predicting an increase by a factor of 4.73 when Land Locked Salmon are present compared to lakes where they are absent. Significant decreases in Chydoridae were predicted for an increase in flushing rate and residential development as well (Table 18).

**Table 18: Logistic regression model results for non-zero densities of Chydoridae in the Belgrade Lakes. Significant predicting variables ( $p<0.05$ ) in bold.**

Predicting Variable	Logistic Coefficient	Factor of influence	Z-statistic	p-value
Centrarchids (species count)	2.9127	18.4067	1.69	0.0910
<b>Flushing rate (times/yr)</b>	<b>-2.2062</b>	<b>0.1101</b>	<b>-4.05</b>	<b>&lt;0.0001</b>
<b>Residential (% of watershed)</b>	<b>-0.5205</b>	<b>0.5942</b>	<b>-2.93</b>	<b>0.0030</b>
Silt substrate	0.2839	1.3282	0.47	0.6380
<b>Floating vegetation</b>	<b>-2.3225</b>	<b>0.0980</b>	<b>-2.93</b>	<b>0.0030</b>
Emergent vegetation	-0.6186	0.5387	-1.21	0.2270
<b>Macrophyte presence</b>	<b>2.6993</b>	<b>14.8692</b>	<b>7.19</b>	<b>&lt;0.0001</b>
<b>Land locked salmon</b>	<b>1.5536</b>	<b>4.7285</b>	<b>4.00</b>	<b>&lt;0.0001</b>
<b>Northern Pike</b>	<b>-9.8678</b>	<b>0.0001</b>	<b>-3.51</b>	<b>&lt;0.0001</b>
Constant	-3.5364	0.0291	-0.75	0.4550

Zero densities of the Order Cyclopoida were significantly predicted by the presence of macrophytes, although the presence of macrophytes predicted increased observations of zero densities (zero inflated negative binomial,  $df=141$ ,  $p<0.0001$ ). Densities of the Order Cyclopoida were most significantly modeled to changes in the flushing rate. For an increase in flushing rate by replacing all the water in the lake and additional time per year, a decrease in Cyclopoid densities by a factor of 0.01 is expected. For an increase in residential development within the watershed by one percent, a decrease in Cyclopoid densities by a factor of 0.30 is expected. Significant increases in Cyclopoid densities are expected with increases in the number of Centrarchid species present in a lake, as well as in the presence of macrophytes. Significant decreases in Cyclopoids are expected in the presence of Northern Pike and Land Locked Salmon (Table 19).

**Table 19: Logistic regression model results for non-zero densities of Cyclopoid Copepods in the Belgrade Lakes. Significant variables ( $p<0.05$ ) in bold.**

Predicting Variable	Logistic Coefficient	Factor of influence	Z-statistic	p-value
<b>Centrarchids (species count)</b>	<b>10.0725</b>	<b>23681.5</b>	<b>16.01</b>	<b>&lt;0.0001</b>
<b>Flushing rate (times/yr)</b>	<b>-4.2765</b>	<b>0.0139</b>	<b>-19.33</b>	<b>&lt;0.0001</b>
<b>Residential (% of watershed)</b>	<b>-1.2195</b>	<b>0.2954</b>	<b>-16.23</b>	<b>&lt;0.0001</b>
Floating vegetation	-0.7719	0.4621	-1.61	0.1070
<b>Macrophyte presence</b>	<b>0.6146</b>	<b>1.8490</b>	<b>3.94</b>	<b>&lt;0.0001</b>
<b>Land locked salmon</b>	<b>-0.5793</b>	<b>0.5603</b>	<b>-6.09</b>	<b>&lt;0.0001</b>
<b>Northern pike</b>	<b>-21.5058</b>	<b>&lt;0.0001</b>	<b>-19.25</b>	<b>&lt;0.0001</b>
Constant	-22.6896	<0.0001	-13.34	<0.0001



Zero densities of Daphniidae were not significantly predicted by the presence of macrophytes, although they were significantly predicted using a constant (zero inflated negative binomial, df=141,  $p < 0.0001$ ). Daphniidae densities were most significantly predicted by the presence of organic substrates, with a decrease in densities by a factor of 0.15 expected in the presence of an organic substrate when all other variables are held constant. Increases in Daphniidae densities are expected in the presence of Land Locked Salmon by a factor of 5.90, holding all other variables constant. Decreases in density by a factor of 0.0003 are predicted in the presence of Northern Pike. The model also predicted significant decreases in non-zero densities of Daphniidae in the presence of silty substrates and with increases in the flushing rate (Table 20).

**Table 20: Logistic regression model results for non-zero densities of Daphniidae in the Belgrade Lakes. Significant variables ( $p < 0.05$ ) in bold.**

Predicting Variable	Logistic Coefficient	Factor of influence	Z-statistic	p-value
Vegetation (%)	0.0066	1.0066	1.62	0.1060
Centrarchids (species count)	1.7633	5.8317	1.40	0.1600
<b>Flushing rate (times/yr)</b>	<b>-1.3496</b>	<b>0.2593</b>	<b>-2.40</b>	<b>0.0160</b>
Residential (% of watershed)	-0.0667	0.9355	-0.46	0.6450
<b>Organic substrate</b>	<b>-1.9197</b>	<b>0.1467</b>	<b>-8.47</b>	<b>&lt;0.0001</b>
<b>Silt substrate</b>	<b>-0.7829</b>	<b>0.4571</b>	<b>-2.03</b>	<b>0.0420</b>
Rock substrate	-0.2133	0.8079	-0.68	0.4950
Floating vegetation	0.0496	1.0508	0.09	0.9250
Emergent vegetation	0.5324	1.7029	1.84	0.0660
<b>Land locked salmon</b>	<b>2.4549</b>	<b>11.6455</b>	<b>5.90</b>	<b>&lt;0.0001</b>
<b>Northern pike</b>	<b>-8.0165</b>	<b>0.0003</b>	<b>-3.47</b>	<b>0.0010</b>
Constant	-4.5102	0.0110	-1.44	0.1490

Zero densities of Sididae were significantly predicted by the absence of macrophytes (zero inflated negative binomial, df=141,  $p < 0.0001$ ). The presence of floating vegetation was the most significant predictor of non zero densities of Sididae. An increase in densities by a factor of 14.45 was predicted at sites with floating vegetation compared to sites without floating vegetation, holding all other variables constant. This significant increase in densities was also noted in the presence of emergent vegetation and rocky substrates. Significant decreases in density were found in the presence of Northern Pike and with increases in the residential development of the watershed (Table 21).

**Table 21: Logistic regression model results for non-zero densities of Sididae in the Belgrade Lakes. Significant variables ( $p < 0.05$ ) in bold.**

Predicting Variable	Logistic Coefficient	Factor of influence	Z-statistic	p-value
Total drainage (square miles)	0.0231	1.0234	1.20	0.2310
<b>Residential (% of watershed)</b>	<b>-0.8930</b>	<b>0.4094</b>	<b>-2.59</b>	<b>0.0100</b>
Silt substrate	0.1951	1.2155	0.49	0.6260
<b>Rock substrate</b>	<b>0.9404</b>	<b>2.5609</b>	<b>2.62</b>	<b>0.0090</b>
<b>Floating vegetation</b>	<b>2.6711</b>	<b>14.4562</b>	<b>10.55</b>	<b>&lt;0.0001</b>
<b>Emergent vegetation</b>	<b>2.0605</b>	<b>7.8497</b>	<b>6.65</b>	<b>&lt;0.0001</b>
Submergent vegetation	-0.6676	0.5129	-0.57	0.5670
Land locked salmon	-0.0962	0.9083	-0.08	0.9360
<b>Northern pike</b>	<b>-11.7239</b>	<b>&lt;0.0001</b>	<b>-3.48</b>	<b>&lt;0.0001</b>
Constant	9.6671	15789.1	2.25	0.0250

Zero densities of Polyphemidae were significantly predicted by the presence of macrophytes (zero inflated negative binomial,  $df=141$ ,  $p<0.0001$ ). Densities of Polyphemidae were best predicted by the presence of land locked salmon in our model. In the presence of land locked salmon, an increase in Polyphemidae densities by a factor of 17.4 is expected. In the presence of Northern Pike, a decrease by a factor of 0.006 is expected. For an increase in the minimum chlorophyll levels in the lake of one micro gram per liter, we expect Polyphemidae densities to increase by a factor of 25.4, and for an increase in residential development by one percent within the watershed, we expect a decrease in Polyphemidae densities by a factor of 0.88 (Table 22).

**Table 22: Logistic regression model results for non-zero densities of Polyphemidae in the Belgrade Lakes. Significant variables ( $p<0.05$ ) in bold.**

Predicting Variable	Logistic Coefficient	Factor of influence	Z-statistic	p-value
<b>Minimum chlorophyll (<math>\mu\text{g/L}</math>)</b>	<b>3.2358</b>	<b>25.4271</b>	<b>5.45</b>	<b>&lt;0.0001</b>
Rock substrate	0.1820	1.1996	0.50	0.6190
<b>Residential (% of watershed)</b>	<b>-0.1286</b>	<b>0.8794</b>	<b>-4.85</b>	<b>&lt;0.0001</b>
<b>Land locked salmon</b>	<b>2.8544</b>	<b>17.3636</b>	<b>10.47</b>	<b>&lt;0.0001</b>
<b>Northern pike</b>	<b>-5.1339</b>	<b>0.0059</b>	<b>-7.90</b>	<b>&lt;0.0001</b>
Constant	-6.2602	0.0019	-4.76	<0.0001

The one rotifer family that was observed often enough to be modeled was Asplanchnidae, whose non-zero densities were significantly predicted only by the flushing rate. For an increase in flushing rate by one time per year, an increase in Asplanchnidae densities by a factor of 1.58 is expected, holding all other variables constant (Table 23).

**Table 23: Logistic regression model results for non-zero densities of Asplanchnidae in the Belgrade Lake. Significant variables (p<0.05) in bold.**

Predicting Variable	Logistic Coefficient	Factor of influence	Z-statistic	p-value
<b>Flushing rate (times/yr)</b>	<b>0.4557</b>	<b>1.5774</b>	<b>2.64</b>	<b>0.0080</b>
Constant	-1.7956	0.1660	-3.56	<0.0001

## DISCUSSION

### The Significance of Macrophyte Presence on Zooplankton Density

Our hypothesis that zooplankton densities would be higher within macrophyte patches compared to outside of macrophyte patches was supported. This hypothesis was supported for the aggregate density of zooplankton, as well as for the Families Chydoridae, Daphniidae, and Sididae. This increase in density within macrophyte patches during the daytime, when all our samples were collected is consistent with the work of Jeppesen et al. (1997), Perrow et al. (1999), and Benndorf et al. (2002). The family Sididae is especially associated with macrophytes (Fairchild 1981), as are Daphniidae, which as larger zooplankton experience higher grazing pressure from planktivorous fish (Perrow et al. 1999, DeMott et al. 2001, Kircheis et al. 2002).

Macrophytes provide a visual refuge for zooplankton from highly visual fish predators, which we could easily observe with our surface sampling scheme that captured the horizontal distributions of zooplankton. This diurnal horizontal migration has been demonstrated by Perrow et al. (1999) in Daphniidids, which experience a trade-off between reduced grazing opportunities within macrophyte patches and reduced predation

risk. However, many large zooplankton also participate in vertical diurnal migrations, migrating to down in the water column into the darker areas of the lake to escape the predation of planktivorous fish as well (Jeppesen et al. 1997, Gelinas and Pinel-Alloul 2008a). Since we only collected surface samples, we were unable to observe the contingent of the zooplankton population that participates in vertical migration. However, we were still able to provide additional evidence that several families seem to favor macrophyte patches during the day within the horizontal distribution of surface waters. These families were Chydoridae, Daphniidae and Sididae.

### **The Significance of Shoreline Development on Zooplankton Density**

No significant difference was found between developed sites with no buffer and undeveloped sites. This suggests that shoreline land use is not a significant factor structuring zooplankton communities at the 10 meter scale we investigated. This may be due to physical mixing of the water at a larger scale, or the rapid diffusion of substances throughout the water column. The substances that can freely diffuse throughout the water column include the excess nutrients from shoreline development, which we expected to influence local zooplankton communities via bottom up mechanisms.

Another explanation for our rejection of this hypothesis is derived from the fact that zooplankton are free to move about the lake. Many studies have demonstrated that zooplankton will migrate into macrophyte patches to escape predation during the day despite less grazing potential within these macrophyte patches due to antagonistic relationships between macrophytes and phytoplankton (Jeppesen et al. 1997, Perrow et al. 1999, Benndorf et al. 2002). We found higher densities of zooplankton within macrophyte patches as well. The findings of high density in macrophytes and no difference between different land uses suggests that during the day, when this study was conducted, refuges from predators may be a more important driver for the horizontal distribution of zooplankton compared to grazing options. Grazing options may be determined by bottom up drivers such as nutrient inputs from shoreline development and affect zooplankton compositions at a larger scale, while the refuge seeking behavior suggests a top-down influence at a finer scale.

## **Interpretations of Numerical Models of Zooplankton Density for Each Family**

### *Bosminidae*

Bosminidae densities were predicted to slightly increase by a factor of 1.100 for an increase in one milli Siemens per meter (mS) of conductivity within the Belgrade Lakes. Conductivity is a measure of the total ions dissolved in a solution, and ranges from 76 mS in McGrath Pond to 40 mS in East Pond. Conductivity was found to be autocorrelated with the watershed residential development and watershed road area (Spearman rank correlation=0.5282). This link may be a result of salting roads during the winter, because salt disassociates readily into ions in solution, raising conductivity.

Despite the high correlation with conductivity, residential development was still significantly correlated with Bosminidae densities. The model accounts for autocorrelation, and will reduce the significance of predictor variables that are highly autocorrelated. Bosminidae densities were observed to fall slightly by a factor of 0.9158 for a one percent increase in residential development. This effect is in the opposite direction of the effect of conductivity, suggesting that increases in conductivity as a result of residential development may not be the mechanism by which watershed-wide residential development influences Bosminidae densities. The mechanisms by which watershed residential development influence zooplankton densities will be discussed in detail in the General Trends section.

The most significant variable correlated with Bosminidae density is the number of cyprinid species within the lake. Bosminidae levels are predicted to fall by a factor of 0.4241 for an increase of one species of cyprinids in the lake. Cyprinids are commonly referred to as minnows. Members of this family are usually better adapted to “warmwater conditions”, including warmer temperatures, lower dissolved oxygen, shallower and more productive systems (Stemberger and Lazorchak 1994). Cyprinids are planktivorous (Stemberger and Lazorchak 1994), and the decrease in Bosminid density may be an effect of increased zooplanktivory. A major limitation of this predictor variable is that it gives no indication of the biomass of cyprinids present in the lake, so the true grazing pressure exerted on zooplankton cannot be modeled with these data.

The final predictor of Bosminid density is the mean chlorophyll, as calculated over the whole year. Chlorophyll is a proxy for the biomass of phytoplankton in a lake, and a measure of lake productivity. For a one  $\mu\text{g/L}$  increase in chlorophyll levels, an increase in Bosminidae densities by a factor of 2.12 is predicted by our model. The positive correlation of density with chlorophyll levels is a signature of bottom-up effects and is consistent with the work of Finlay et al. (2007), who found that smaller zooplankton such as Bosminids respond disproportionately to nutrient loading.

### *Calanoida*

Calanoida densities were significantly predicted only by the presence of macrophytes and the presence of organic substrates (Table 16). In contrast to Cladoceran families that were linked to macrophytes, the presence of macrophytes increased the log odds of finding a zero density of Calanoids, which means that the probability of observing a density of zero of Calanoida in a macrophyte patch is increased. This suggests that Calanoid Copepods do not favor macrophytes as habitat, and may prefer the open-water or deeper areas of lakes. This is consistent with finding of lower densities at sites with organic substrates. Since sites sampled in mid-lake areas did not have substrate observations due to increased depths, the finding of decreased densities at sites with organic substrates may be coincidental with sites that are more likely to be in the littoral zone, which is not the deeper water habitat favored by most Calanoids (Visconti and Manca 2010).

### *Chydoridae*

Chydoridae densities are modeled to fall with increases in flushing rate, residential development and with the presence of Northern Pike. Densities were modeled to increase in the presence of Land Locked Salmon. These were trends that held across several families, and are discussed more in depth in the General Trends section.

Chydoridae densities were best predicted using macrophyte presence as both a predictor of zero values and as a predictor of non-zero densities. For non-zero densities, Chydoridae density was predicted to increase by a factor of 14.87. However, among the

Chydoridae observed within macrophyte patches, floating vegetation was correlated with a decrease in Chydoridae densities by a factor of 0.0980. By multiplying these coefficients together, we have an estimate of the factor of floating vegetation alone without relying on the presence of macrophytes. The presence of floating vegetation still results in an increase in Chydoridae densities by a factor of 1.45, although this is of a much smaller magnitude than macrophyte presence considered together.

Macrophytes serve as an important visual refuge for zooplankton, as discussed in our hypotheses. The complexity of the macrophyte patches may be an important determinant of how well they serve as visual refugia, and densities of zooplankton observed. Since floating vegetation tends to have more surface area on the surface of the water, this blocks light attenuation to lower reaches of the water, where submerged vegetation could grow to offer more complex refugia. Floating vegetation, while offering refugia, may not offer as complex a refugia, resulting in lower Chydoridae densities. The differences observed between the different types of macrophytes are significant for this family, and may be significant for other families and even based on finer designations of macrophytes, such as by taxonomy.

### *Cyclopidae*

Cyclopidae densities fell with increases in flushing rate, residential development and in the presence of Land Locked Salmon and Northern Pike. These trends were noted across several families, and are discussed in more detail in the General Trends section.

The presence of macrophytes was a significant predictor of zero densities of Cyclopidae, increasing the log odds of observing a zero density. The presence of macrophytes was a significant predictor of non-zero densities, predicting an increase in cyclopids by a factor of 1.849. The observed increase in non-zero densities is consistent with our hypothesis that densities will be higher within macrophyte patches due to the refugia effect. However, the significant role in predicting zero inflation is counter-intuitive. These data suggest that Cyclopoid Copeopods favor limnetic areas over littoral areas, accounting for the zero-inflation. However, when found in littoral areas,



Cyclopoids are likely to engage in refuge-seeking behaviors accounting for the increased densities found in macrophyte patches. More study is necessary to validate such a link.

The number of Centrarchid species present in a lake was associated with a significant increase in Cyclopoid densities 23,000 times with an increase in one Centrarchid species. Centrarchids are the family of the sunfishes, and were classified as a “warm water” species by Stemberger and Lazorchak (1994) in a similar study that investigated factors that structure zooplankton communities. This study found that the presence of cold or warmwater fish stocks, along with chlorophyll measurements and land use explained the major differences in body size and taxonomic groups observed across a larger sample size of lakes across New England.

A limitation of my metric for Centrarchids is that it is only a count of species, and has no bearing on the actual biomass of Centrarchids in lakes. Also, Centrarchid species richness only varied from 4 to 6 throughout the lakes. This means that any differences observed may also be coincidental. Few biomass studies have been conducted on the Belgrade Lakes, with the exception of East and North Ponds (Ditzler 2010), but wider-scale estimates of biomass would offer stronger data to draw conclusions of trophic interactions from.

### *Daphniidae*

Daphniidae experienced decreases in density with increases in flushing rate and the presence of Northern Pike, and increases in density in the presence of Land Locked Salmon. These trends held across several families, and are discussed in depth in the section on General Trends.

The strongest correlations observed in Daphniidae densities are decreases in the presence of organic substrates. Significant decreases in the presence of silt substrates were also observed, although the magnitude of this correlation is neither as extreme nor significant. Relationships with substrate were found for the Calanoid Copepods as well, and may be more strongly related to shallow depths or distances from shore than to the specific characteristics of the substrates themselves. Further investigations explicitly

testing the effects of site depth and distance from shore are necessary to tease out the true relationships.

An alternative explanation of our data is that the smaller particle sizes of silt and organic substrates are more easily agitated into the water column by physical disturbance such as wave action. This could lead to increased turbidity at sites with organic and silt substrates, which may block light availability for macrophytes and phytoplankton, both of which can impact zooplankton densities. This mechanism is only speculation, although further studies can investigate the interactions of turbidity, phytoplankton biomass, macrophyte biomass, and zooplankton density.

### *Sididae*

Sididae densities experienced declines with increases in residential development and Northern Pike, as discussed in the General Trends section. Zero inflation within this family was also significantly predicted by the presence of macrophytes.

In addition to predicting zero densities of Sididae, specific types of aquatic vegetation were associated with strong increases in Sididae density. The presence of floating vegetation is associated with an increase in Sididae densities by a factor of 14.46. The presence of emergent vegetation is associated with an increase in Sididae densities by a factor of 7.85. The strong association of Sididae with vegetation was established by Fairchild (1981), and is consistent with our observations and hypotheses.

Additionally, Sididae were observed to increase by a factor of 2.56 in the presence of rocky substrates when all other factors are held constant. Since rocky substrates are composed of larger sized particles, they are not easily agitated nor suspended in the water quality. Rocky substrates may contribute to less turbidity than smaller sized substrates such as silt, organic materials and sand. Further study is necessary to validate such a link between Sididae densities and turbidity, which was not measured in this study; as well as links between substrate type and turbidity.

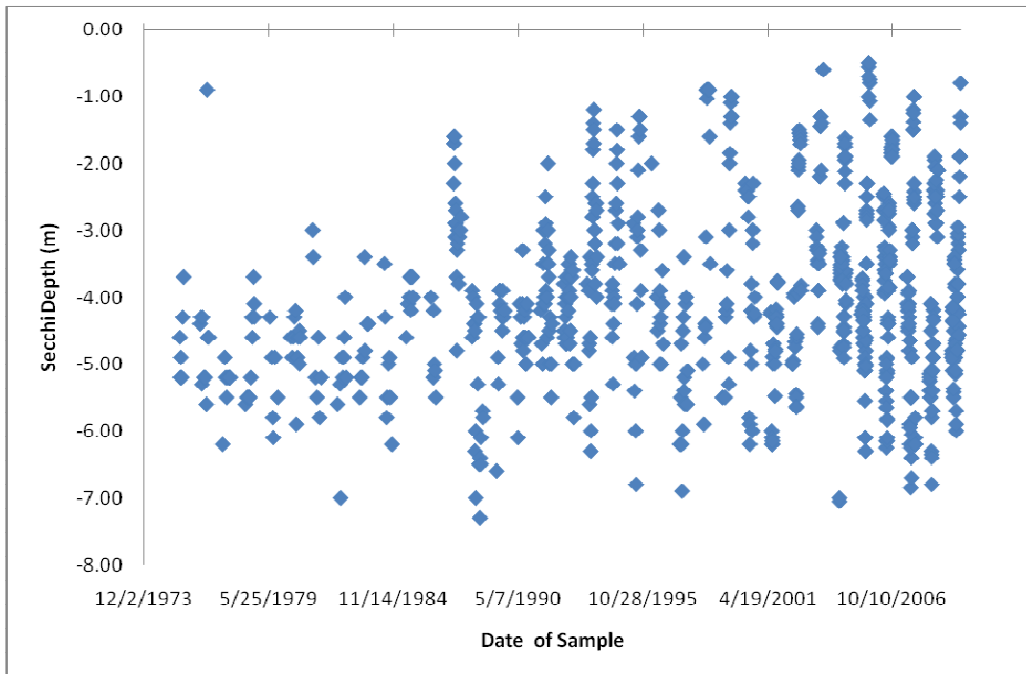
## *Polyphemidae*

Polyphemidae densities were significantly associated with minimum chlorophyll levels. The magnitude of this correlation was impressive, with an increase in density by a factor of 25.43 predicted for an increase in minimum chlorophyll levels by one  $\mu\text{g/L}$ . While this information may seem to suggest bottom up controls at first pass, the lakes with the lowest minimum chlorophyll levels are also the lakes with the highest maximum chlorophyll levels, mean chlorophyll levels and nutrient levels as measured by total phosphorus (Table 24). For instance, East Pond has the highest mean and maximum chlorophyll levels, while also having the lowest minimum chlorophyll levels.

**Table 24: Minimum, Maximum and Mean Chlorophyll Annual Distribution, as well as total phosphorus levels in 2009 in each of the Belgrade Lakes (PEARL 2010).**

Lake	Minimum Chlorophyll ( $\mu\text{g/L}$ )	Maximum Chlorophyll ( $\mu\text{g/L}$ )	Mean Chlorophyll ( $\mu\text{g/L}$ )	Total Phosphorus (ppb)
East Pond	1.1	66	9.6	18
Great Pond	2.7	8.5	4.9	9.5
Long Pond	2.0	9.6	4.9	8
McGrath	1.7	10.3	3.2	11
Messalonskee	2.5	5.3	3.9	11
North Pond	2.3	18.3	4.5	17.3
Salmon Lake	1.4	25.9	6.1	14

Long term analysis of secchi depth readings in the Belgrade Lakes have demonstrated a similar pattern of increased divergence over time. For instance, in the heavily degraded East Pond (Figure 6), current secchi depth readings are either much deeper or much shallower than readings observed earlier in the lake's development (King, unpublished data). Secchi Depth integrates turbidity over depth, and is a measure of water clarity and light attenuation. Chlorophyll is loosely correlated to secchi depth since the pigments in phytoplankton dissipate light, contributing to an increase in turbidity and a decrease in secchi depth (Benndorf et al. 2002).



**Figure 6: Annual divergence of secchi depths in East Pond (Belgrade Lakes) from 1973 through 2008 (MDEP 2010).**

When considered with the underlying trends in chlorophyll levels, there are two potential explanations for Polyphemidae response to minimum chlorophyll levels specifically. The first is that Polyphemidae may be unable to effectively compete for resources under eutrophic conditions that decreased minimum chlorophyll levels are associated with. This means that Polyphemidae may be a more sensitive family that could be a useful indicator of water quality if investigated in further studies with a more explicit link to nutrient loading.

The second explanation for the trend between Polyphemidae and minimum chlorophyll is that a minimum level of chlorophyll must be maintained as a food source for Polyphemidae, below which Polyphemidae is subject to starvation. The higher starvation threshold is a key component of the size-efficiency hypothesis of zooplankton grazing, where larger bodied zooplankton are more capable of surviving periods of starvation. Larger zooplankton are more important in keeping phytoplankton populations low via grazing (Gliwicz 1980). Since we observed Polyphemidae levels to decline with declining minimum chlorophyll levels regardless of nutrient loading to the system,

Polyphemidae are unlikely to be an important grazer if the size-efficiency hypothesis holds since they cannot maintain a high grazing pressure throughout the cycle.

Polyphemidae densities also decreased significantly with increases in residential development, and in the presence of Northern Pike, which is discussed in depth in the General Trends section.

### *Asplanchnidae*

Asplanchnidae densities significantly increase with an increase in flushing rate. This trend is opposite to the correlations of flushing rate to the other families. This may be due to suboptimum conditions for members of other families, allowing Asplanchnidae densities to rise in the absence of competition. As soft bodied zooplankton, there is evidence that rotifers are mechanically repressed by the activities of larger, shelled, cladocerans. Gilbert (1988) reviewed the interactions of *Daphnia* and rotifers, demonstrating that high levels of *Daphnia* reduced rotifer populations not only through resource competition, but mechanical interference as well. Mechanical interference occurs when rotifers become caught in the feeding groove of large cladocerans and are damaged. This inverse relationship between Daphniidae and Asplanchnidae was also found in our study, as indicated by a negative Spearman Rank correlation coefficient of -0.1863 (Spearman Rank Correlation, df=141, p=0.0259).

### *General Trends Held Across Models of Several Families*

#### Flushing Rate

The densities of three families were noted to decrease significantly with a one unit increase in flushing rate. Flushing rate is measured in times per year that the entire volume of the lake is replaced. A one unit increase in flushing rate represents a substantial difference between lakes, and is also related to morphological characteristic of a lake that is not subject to change. It is important to consider the scale of the one unit increase in flushing rate when considering the decreases in Chydoridae density by a factor of 0.1101, Cyclopidae by a factor of 0.0139, and Daphniidae by a factor of 0.2593.

Higher flushing rates in a lake mean that the water within the lake is replaced more rapidly. Lakes with higher flushing rates tend to be more resilient to water quality changes as a result of nutrient loading events, since the water will be quickly replaced. This is not to say that lakes with high flushing rates cannot become eutrophic, but generally lakes with higher flushing rates will have better water quality. Generally, lakes with better water quality will have lower nutrient levels. The lower levels of zooplankton observed in lakes with higher flushing rates may be a function of less nutrient availability in less eutrophic lakes. This trend between higher flushing rates and lower productivity, measured as chlorophyll levels, was significant in our analysis, with Spearman Rank correlation coefficient of -0.6660 (Tables 6-9).

### Residential Development

Five of the eight families modeled predicted significant decreases in density for a one percent increase in residential development, when all other variables including nutrient levels are held constant. Bosminidae densities decrease modestly by a factor of 0.9158, Chydoridae densities by a factor of 0.5942, Sididae densities by a factor of 0.4094, and Cyclopidae densities by a factor by 0.2954.

Increases in residential development are typically associated with increased nutrient loading via increased erosion, leaky septic systems, and fertilizers used on lawns (McCullough 2010). Under bottom-up mechanisms, increases in nutrient levels are expected to increase the density of zooplankton. However, we did not see an increase in zooplankton with residential development as expected under a nutrient loading hypothesis, instead we saw the opposite. While zooplankton densities are generally associated with increasing nutrient and chlorophyll levels, these more direct effects of nutrient loading are held constant in our model. The decrease in densities, holding any effects of nutrient loading constant, may involve another mechanism.

To find potential mechanisms of the link between watershed wide residential development and decreases in zooplankton density independent of changes in productivity, we investigated autocorrelations between predictor variables (Tables 5-7) Of all the predictor variables utilized in this study (Table 1), pH was the most strongly correlated to residential development. pH within the lake is determined by many

environmental factors, including the levels of tannins, coniferous forests, and hydrogeologic regimes in the watershed ( Kortelainen and Saukkonen 1995, Buffam et al. 2007, Irfanullah 2009, King, pers. comm.). Lower pH tend to be associated with greater coniferous forests in a watershed, the amount of time water spends within these more acidic areas, and the erosion of bicarbonate from the soil to buffer these changes in pH (Manahan 2010) It is possible that changes in the flow regimes and forest composition in watersheds, potentially an effect of development, may drive minor pH changes. Allen et al. (2001) linked pH as a broad-scale determinant of zooplankton compositions, validating pH as a potential link between residential development and decreased zooplankton density.

Residential development was also highly correlated with conductivity in the lake. Conductivity is a measure of the ions in solution. Residential development is highly correlated to roads in the watershed. Salt spread on the roads in winter can increase the conductivity of a lake, so increased road density within a watershed can affect conductivity of the lake. Conductivity may have an effect on zooplankton densities in a lake independent of nutrient loading, although experimental studies are necessary to validate and quantify any link.

### Land Locked Salmon

Land Locked Salmon are stocked in some of the Belgrade Lakes as a cold water fishery. However, some stocked populations of Land Locked Salmon have crashed in the Belgrade Lakes due to declines in water quality, including the population in Salmon Lake (IFW 2010). Brown trout, another coldwater fish is now stocked in Salmon Lake since it is less sensitive to declining water quality than Land Locked Salmon. Even though Land Locked Salmon are stocked in the Belgrade Lakes, their sensitivity to changing conditions within the Belgrade system validates their use as an indicator for this study. A limitation to using the presence and absence of an indicator fish species is that the distribution of a binomial variable across a small number of lakes may be coincidental.

Cladocerans responded in a similar manner with increases in density in the presence of Land Locked Salmon, when all other factors, including the presence of Northern Pike, were held constant. The only lake where Northern Pike were present but Land Locked

Salmon were absent in North Pond, giving both sets of data very similar distributions that have the potential to confound results.

The only taxa noted to decrease with the presence of Land Locked Salmon were the Cyclopoid copepods, when all other factors, including water quality parameters, were held constant. There may be a variable link between cyclopoid copepods and eutrophication in the Belgrade Lakes. Stemberger and Miller (1998) described a conceptual model linking Cyclopoid dominance to increasing phosphorus loading and the loss of coldwater refuges, which is consistent with our findings since the presence of the coldwater refuge, as measured with the presence of Land Locked Salmon, is correlated with a decrease in Cyclopoid densities.

### Northern Pike

Northern Pike were illegally introduced into the Belgrade lakes in the 1980's (IFW 2010), and act as aggressive piscivores in the food web (Haliwell and Evers 2008). If top-down influences are important in structuring zooplankton communities, then zooplankton densities were predicted to increase in the absence of Northern Pike due to the effects of the trophic cascade. However, the opposite was observed to occur across five distinct taxa, including the copepod Order Cyclopoida. Since Northern Pike presence or absence is a binomial variable distributed across a small sample size of lakes in the Belgrade region, the distinction may be coincidental. However, the significant decreases in zooplankton densities counter to the expectations of top-down control suggest that top-down controls are not important in structuring zooplankton communities in the Belgrade Lakes.

### *Bottom-Up versus Top-Down Roles in Structuring Zooplankton Communities*

Overall, the highest aggregate densities of zooplankton were observed in the lakes with the highest levels of nutrient loading and eutrophication (Table 25). Our finding of decreased zooplankton densities with increased flushing rate also supported our initial hypothesis that bottom-up forces are the dominant structuring force of zooplankton communities.



**Table 25: Aggregate zooplankton density per Liter compared to the trophic status of the Belgrade Lakes, as indicated by mean annual chlorophyll for 2009 and total phosphorus measured in the epilimnion (PEARL 2010)**

	Aggregate Density (per L)	Mean Chlorophyll ( $\mu\text{g/L}$ )	Total Phosphorus(ppb)
East Pond	65.41	9.6	18.0
North Pond	1.36	4.5	17.3
Salmon/McGrath	11.25	6.1	14.0
Great Pond	3.86	4.9	9.5
Long Pond	3.77	4.9	8.0
Messalonskee	1.23	3.9	11.0

The presence of Northern Pike, an aggressively piscivorous and invasive fish was included to determine if top-down forces were important in the Belgrades. (Haliwell and Evers 2008). We hypothesized that if top-down forces are important in structuring zooplankton communities, zooplankton densities would be higher in lakes where Northern Pike are present due to the trophic cascade. Instead, the opposite was found across several Families, suggesting that Northern Pike presence in lakes of better water quality may be coincidental and that top-down forces are not as important in structuring zooplankton communities.

A caveat to our evidence supporting bottom-up mechanisms of control over zooplankton communities is our finding of decreased densities with increased residential development. This finding is counter intuitive to our findings that bottom-up forces are the more important drivers, since increased residential development tends to be associated with increased nutrient loading. However, the metrics of our model are for a change in residential development alone, holding all other variables, including nutrient levels and chlorophyll, constant. This effect may be acting through another mechanism than nutrient loading, as discussed previously.

### *GIS based results*

The failure of our GIS-based model to accurately predict the density of zooplankton families suggests that variables other than macrophyte patches are important. For instance, proximity to macrophyte patches may be just as important as physical sampling within a macrophyte patch. Some studies have suggested that zooplankton tend to aggregate around the edges of macrophyte patches due to chemical repellence and lack of grazing potential within the patch (Benndorf et al. 2002). Additionally, some families demonstrated significant relationships with the composition of the substrate, which may be an important variable excluded from our initial analysis.

This model can be further improved by incorporating a more accurate depiction of macrophyte locations in the lake, including types of macrophytes. Although I attempted to create a slope-based model to predict the locations of macrophytes in the Belgrade Lakes, this model did not have fine enough resolution to be meaningful for my study. A finer resolution than 10 by 10 m may yield a more useful distribution of macrophyte locations in the Belgrade Lakes. Additionally, the types of macrophytes were significant predictors of density for some families, such as Sididae. Substrate composition was important for some families, and could also be mapped for the littoral areas of the lakes more accurately in future studies.

Wind direction is an additional variable that may be important, but was not measured in this study. Future studies should record meteorological data to control for these factors in analysis. While wind is an important driver for phytoplankton families (Jones et al. 1995), patchiness of zooplankton communities has been reported as well (Folt et al. 1993). Our GIS-based models capture the patchiness of zooplankton communities, especially in the littoral zones, which are likely to be less influenced by wind than mid-lake areas due to wind breaking effects of vegetation. Additionally, our models explain some of the factors structuring the patchiness of zooplankton communities within lakes as a factor of macrophyte presence, type, and for some families, substrate composition.

### **Limitations of Study**

While several broad-scale trends were observed in this study, caution should be taken when observing the results. There was a high degree of collinearity between many of the

predicting variables, which may be due overlapping ecological processes. It is possible that different variables could have been selected for use, and have been significant, within our models that were created if a different methodology was utilized in our variable selection process. Therefore, while strong associations were observed, these do not imply causation. Additionally, confounding factors such as time of sampling, wind direction, and weather were not controlled for by this study. Despite the limitations of our analysis, strong patterns emerged, and this study provided evidence that there are strong differences between lakes and made preliminary suggestions as to what may be driving those differences.

### **Recommendations for future research**

Future research should focus on refining the broad relationships found within this study. By collecting supporting data on a smaller scale than whole-lake data in real time, tighter correlations may be found. For instance, dissolved oxygen, pH, chlorophyll and turbidity data can be collected at the same scale as the sampling sites using a data sonde.

This study should also be conducted during other seasons, such as in the spring and throughout the summer. With this type of data, changes in composition and the controlling variables can be determined throughout the year, and dynamic models can be created. If macrophytes are mapped out with greater accuracy than the slope model attempted, the densities of zooplankton could be extrapolated to other areas of the lake. When these macrophyte patches are tested independently, and in the same season, they can form a subset of data that tests the predictions of our models. Additionally, data on the densities of zooplankton families can be linked to the grazing rates of the families, and help lake managers determine which areas of the lake are most important in controlling algal blooms.

### **CONCLUSIONS**

In the Belgrade Lakes, bottom up forces appear to be more important than top-down forces in structuring zooplankton communities. This is consistent with other work conducted on zooplankton in the region (Ditzler 2010). In order to effectively manage

water quality, nutrient loading should be controlled. Top-down controls on water quality tend to be temporary and weak in most mesotrophic lakes (Gliwicz 1980, Jeppesen et al. 1997, Benndorf et al. 2002, Finlay et al. 2007). Additionally, the cyanobacteria blooms which remediation projects in East Pond were attempting to control (Haliwel and Evers 2008) tend to be resistant to zooplankton grazing (Gliwicz 1980), further dampening effects of top-down control.

Zooplankton densities are higher within macrophyte patches, which may be due to a refuge effect as zooplankton hide from planktivorous fish. Macrophyte patches should be protected to ensure their role as a refuge for zooplankton is maintained, and zooplankton densities can be protected from predation and serve their trophic role as grazers on phytoplankton. While no statistically sound relationship was found between undeveloped and developed sites with no buffer, this does not mean shoreline land uses are insignificant drivers of zooplankton composition, but rather aggregate shoreline uses may have significant influence. When all other factors were held constant, including nutrient loading, increases in watershed-wide residential development were linked to decreases in zooplankton density. While the mechanisms of such a relationship are not established, decreases in zooplankton density independent of nutrient loading may have negative implications for the control of phytoplankton populations within the Belgrade Lakes.



## **PERSONAL COMMUNICATIONS**

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## APPENDIX 1. REFERENCE OF ORGANISMS IDENTIFIED OR REFERRED TO IN STUDY.

### Zooplankton of the Belgrade Lakes (UNH 2011)

- Kingdom: Animalia
  - Phylum: Artropoda
    - Subphylum: Crustacea
      - Class: Brachiopoda
        - Suborder: Cladocera
          - Family: Bosminidae, Chydoridae, Daphniidae, Holopedidae, Moinidae, Polyphemidae, Sididae
      - Subclass: Copepoda
        - Order: Calanoida, Cyclopoida
  - Phylum: Rotifera
    - Class: Monogonota
      - Order: Ploima
        - Family: Asplanchnidae, Brachionidae, Gastropidae, Trichocercidae

### Fishes of the Belgrade Lakes (PEARL 2010)

- Kingdom: Anamalia
  - Class: Actinopterygii
    - Order: Perciformes
      - Family: Centrarchidae (sunfishes),
        - Species: Northern Pike (*Esox lucius*), Yellow Perch (*Perca flavescens*), White Perch (*Morone americana*), Smallmouth Bass (*Micropterus dolomieu*), Chain Pickerel (*Esox niger*), Pumpkinseed Sunfish (*Lepomis gibbosus*)
      - Family: Cyprinidae (minnows)
        - Species: Fallfish (*Semotilus corporalis*), Golden Shiner (*Notemigonus crysoleucas*).
      - Family: Salmonidae (salmon, trout)
        - Species: Land Locked Salmon (*Salmo salar Sebago*), Brook Trout (*Salvelinus fontinalis*), Brown Trout (*Salmo trutta*).