

Table of Contents

ntroduction	4
Methods	6
Study Lakes	6
Zooplankton Sampling	7
Zooplankton Quantification	8
Statistical Analysis	9
Maine Lakes Analysis	
Light Absorption	
Melanin Quantification in <i>Daphnia</i>	11
Results	12
Zooplankton Community in ANP lakes	12
Invertebrate Predators	24
UV-B Analysis	27
Conclusions	
Acknowledgments	
Literature Cited	
Appendix A: Weather conditions during sampling period	35
Appendix B: Sampling dates and nets used	
Appendix C: Sampling net types	
Appendix D: Counting equipment pictures	
Appendix E: T-Test p-values	
Appendix F: Spearman rho values	40

	Арр	pendix G: July chlorophyll measurements40
	Арр	pendix H: Copepod and cladoceran images and ecology 41
	Арр	pendix I: Zooplankton community composition44
	Арр	pendix J: Copepod pigmentation patterns47
List	t of F	igures
	1.	Aquatic food chain
	2.	Abundance and body size 14-15
	3.	Monthly copepod and cladoceran abundance 16-17
	4.	Monthly community composition
	5.	Average cladoceran body size 20-21
	6.	Cladoceran abundance and size in alewife lakes23
	7.	Invertebrate predator abundance 25-26
	8.	Light absorbance27
	9.	Epilimnion / hypolimnion29
	10.	Pigmented Daphnia pictures
	11.	Percent pigmented Daphnia and absorbance
List	of Ta	ables
	1.	2007 Water chemistry data7
	2.	Lake morphometry data7
	3.	Fish stocking information24

Introduction

Zooplankton are an essential part of aquatic ecosystems. They have numerous roles, the two most prominent of which are serving as a food resource for higher trophic levels and providing grazing pressure on the algal community. These roles are a result of their intermediate position in the aquatic food chain (Figure 1). The zooplankton community is influenced by top-down or predation pressure and by bottom-up factors related to food availability (Gliwicz 2002). A top-down force on a focal organism is imposed by predators higher up in the food chain. Bottom-up forces are driven by nutrients that support production at lower trophic levels needed by the focal organism needs. As a result, changes in the fish (Brooks and Dodson 1965) or algal communities (Macedo and Pinto-Coelho 2001) affect zooplankton community structure. By observing zooplankton community changes, lake managers have an indication of possible changes happening in the lake ecosystem. For instance, if the zooplankton community of a lake change in the predation pressure by fish. Other lake conditions influence zooplankton community and size structure. For example, since cladocerans such as *Daphnia* and *Bosmina* require calcium for their carapace, changes in calcium availability in the water column can affect the cladoceran community (Tessier and Horwitz 1990).



Figure 1: A simple representation of freshwater lake food chains. Cladocerans (represented by the *Daphnia*) are both influenced by bottom-up (nutrients) and top-down (predation) factors, putting them in a good position indicate the influence of both factors at once.

Since zooplankton, cladocerans in particular, graze upon algae they are an important control on the algal community. According to the size efficiency hypothesis larger cladocerans can filter algae from the water column more efficiently than smaller zooplankton (Hulsmann et al. 2005). A change in the zooplankton community from large cladocerans to small cladocerans can result in an increase in the concentration of algae in the water column.

Another stressor that influences lake ecosystems and zooplankton is exposure to UV-B radiation in the water column. UV-B radiation can be lethal to zooplankton if they are subjected to prolonged exposure (Storz and Paul 1998). Leech and Williamson (2000) found that the response to UV-B radiation differed among species. Defenses against UV-B radiation include migrating down the water column where UV exposure is less (Leech et al. 2005) and producing protective pigments (Rautio and Korhola 2002). The production of the pigment melanin in *Daphnia* carapaces puts them at risk to predation and is only for the purpose of shielding the animal from UV radiation (Boeing et al. 2004). Huebner and others (2006) found that *Daphnia* mortality increased and reproduction decreased as UV-B exposure increased. They also found that juvenile *Daphnia* were more susceptible to UV-B related mortality than adults. If the amount of UV-B radiation in clear-water lakes is enough to cause pigmentation in *Daphnia* it could be influencing the zooplankton community composition.

This research has three objectives: (1) characterize seasonal patterns and relationships in the zooplankton communities (e.g., cladoceran and copepods) in the eight Acadia National Park (ANP) eutrophication study lakes; (2) compare the size structure of zooplankton communities in ANP to a broader study of 75 Maine lakes; and (3) determine if UV-B radiation is affecting *Daphnia* in three clearwater ANP lakes. To accomplish the first objective we counted and measured the body size of copepods and cladocerans in monthly samples taken from the ANP study lakes each month between June and August. For the second objective we will use a statistical model being developed as part of another research project to compare the ANP lakes to a broader set of lakes in Maine. Results from this objective

will be provided in an addendum to this report. For the last objective we used visual inspection of melanin stripes to quantify the percentage of *Daphnia* responding to UV-B radiation in three clear lakes. We then compared these results to the amount of UV B radiation penetrating into the water column of the lakes for each month between June and August. Since producing melanin is a response to UV-B radiation, if *Daphnia* in clear-water lakes are producing melanin then they are responding to UV-B radiation.

Methods

Study Lakes

Eight Acadia National Park (ANP) lakes located in Mount Desert Island (Seal Cove Pond, Echo Lake, Great Long Pond, Upper Hadlock Pond, Witch Hole Pond, Bubble Pond, Jordan Pond, and Eagle Lake) were sampled for zooplankton during the months of June, July, and August. These eight lakes were chosen because they are ANP eutrophication study lakes and we could coordinate our zooplankton sampling with routine water quality sampling by Park Staff for nutrients, TP, DOC, and alkalinity (Table 1). The study lakes vary in both depth (9.5-45.7m) and surface area (28-897 acres) so the sampling is not biased towards one kind of lake (Table 2). Of the eight lakes Bubble Pond, Jordan Pond, and Eagle Lake were chosen for the melanin analysis, because they are oligotrophic lakes with the lowest dissolved organic carbon (DOC) and color (Table 1 and 2). Table 1: The average of the 2007 data for dissolved organic carbon (DOC), Secchi disk depth, True color, total phosphorus (TP), chlorophyll, and calcium for the study lakes. Lakes with * were used for melanin analysis and lakes with ⁺ were used for the epilimnion / hypolimnion comparison. The data were collected by ANP Biologists William Gawley and Beth Arsenault.

	DOC (mg/L)	Secchi (m)	Color (PCU)	Total Phosphorus (₪g/L)	Chlorophyll (团g/L)	Calcium (mg/L)
* ⁺ Eagle Lake	2.0	10.7	3.4	1.7	1.4	69.9
* ⁺ Jordan Pond	2.0	11.4	3.4	1.9	1.1	83.6
Great Long Pond	2.8	8.4	9.3	3.1	2.8	79.1
*Bubble Pond	2.3	10	4.7	3.6	1.3	85.1
Upper Hadlock	3.4	6.9	9.3	4.7	1.8	93.1
Pond						
Seal Cove Pond	4.0	6.9	12.3	4.7	2.4	70.4
Echo Lake	2.8	7.9	7.2	5.2	1.9	115
Witch Hole Pond	4.6	4.0	16.0	11.7	3.8	84.3

Table 2: Lake morphometry data for the eight study lakes (Seger et al. 2006)

	Max Depth (m)	Area (hectares)	Flush Rate yr ⁻¹	Trophic state
Eagle Lake	33.6	177	0.3	Oligotrophic
Jordan Pond	45.7	75.7	0.2	Oligotrophic
Great Long Pond	34.5	363.3	0.3	Oligotrophic
Bubble Pond	11.9	13	1.9	Oligotrophic
Upper Hadlock Pond	10.7	14.2	4.1	Mesotrophic
Seal Cove Pond	13.4	114.6	1.9	Mesotrophic
Echo Lake	20.1	96	0.6	Oligotrophic
Witch Hole Pond	9.5	11.3	1.2	Mesotrophic

Zooplankton Sampling

The sampling location for each lake was sample station 1, the deep point for the lake. Each sampling location was reached by canoe. On some days the sample station could not be reached due to unsafe weather conditions (Appendix A). In those cases the samples were taken from the deepest area possible. For Eagle Lake the deep spot was difficult to find because it was small and hard to locate by GPS. When the deep spot could not be located, zooplankton samples were taken as close to the deep spot as possible. Each zooplankton sample consisted of 3 or 5 pooled vertical tows taken from about 2m above the sediment to the surface. Except for samples collected for melanin analysis with a 243 μ , 0.3 m diameter net, most zooplankton were collected with an 80 μ Wisconsin or closing net. The three types of nets used during the course of this study are described in Appendices B and C.

Zooplankton samples were collected for three types of analyses. The first sample type was for determining density and body length of major zooplankton taxa including cladoceran genera and copepod major group (calanoid or cyclopoids) monthly from June to August from the eight study lakes. The second type was designed to collect *Daphnia* for melanin analysis from the three UV lakes. Zooplankton samples for melanin analysis were collected each month on a cloudy day, sunny day, and at night. We collected melanin samples at different times because Herbert and Emery (1990) found that for different amounts of light there were different concentrations of pigmented and non-pigmented *Daphnia*. We organized our sampling time by the moon cycle so that our night sample would be taken when there was the least amount of moonlight. This way our samples would not be biased towards pigmented or non-pigmented *Daphnia*. The third sample type consisted of paired epilimnion / hypolimnion zooplankton samples collected with the closing net in August. These paired samples were collected at midday and after sunset two days later on two lakes, Jordan Pond and Eagle Lake. All zooplankton samples were preserved in 70% ethanol. The count and epilimnion / hypolimnion samples were kept at room temperature while the melanin samples were refrigerated to prevent the breakdown of melanin.

Zooplankton Quantification

The zooplankton taxa were counted and measured using a Wards counting wheel and a Nikon SMZ800 dissecting microscope with a Nikon CoolPix 995 digital camera attached (Appendix D), and the computer software Image J(http://rsb.info.nih.gov/ij/). Zooplankton were identified to genus (for cladocerans) or major group (for copepods) and counted in subsamples obtained using a Henson

Stemple pipette. Every mature zooplankton was counted to a total of 200 individuals. An image of every cladoceran and of 20 or more copepods was taken. Body length was measured using Image J (Appendix D). Because invertebrate predators were lower in abundance than other cladoceran and copepod taxa, we counted the entire sample instead of taking a subsample to improve our density estimates. For the three lakes chosen for melanin analysis we used the melanin samples instead of the count samples to calculate invertebrate predator abundance. The densities of invertebrates captured with the plankton tow net were generally higher than those determined from samples collected with the Wisconsin and closing net, most likely because the wider-diameter plankton net was more efficient at sampling these rarer taxa.

Statistical Analysis

In order to determine relationships between density and body size metrics of the zooplankton communities and lake chemistry, Spearman rho correlation tests were run using the statistics program R (http://www.rproject.org). We chose to run this test because our data were not normally distributed. The zooplankton community metrics we tested were zooplankton abundance and body size, cladoceran abundance and body size, and *Daphnia* abundance and body size with separate analyses by month. The lake features used included both bottom-up measures such as TP, DOC, and chlorophyll, and top-down measures such as invertebrate predator abundance. Spearman rank correlation coefficients (r_s) with absolute values greater than 0.833 (98% confidence interval) were considered significant; this CI was chosen to account for the multiple correlations.

To test if there was a difference among months when it came to abundance and body size, paired T-tests were run. These tests were performed using Excel, comparing the months in pairs. We chose a confidence of 95%. Our body size data were normally distributed but our abundance data were not, so we log (base 10) transformed the abundance data. The variables we compared among months were: total zooplankton abundance, total zooplankton body size, total cladoceran abundance, total cladoceran body size, *Daphnia* abundance, and *Daphnia* body size.

Maine Lakes Analysis

To complete the second objective we will compare the results from the ANP lakes to a statistical model we will create using 75 Maine lakes. In that study we will identify what abiotic and biotic lake features influence cladoceran size structure, and then we will determine how cladoceran size structure is related to lake features. We will then be able to predict the cladoceran size structure of a lake based on how it is classified by lake features (such as depth, area, location, and water chemistry). After this research is completed we will be able to better assess how the zooplankton from the ANP lakes compare to other similar lakes in the state.

Light Absorbance

In June and August we took water samples using a closing net from the epilimnion for the three melanin focus lakes to measure light absorbance at both visible and UV wavelengths. No samples were analyzed for the month of June because we did not have access to the needed equipment. We collected integrated epilimnetic samples using a plastic tube lowered into the water column to the top of the thermocline. The tube was then closed off at the top, pulled back up, and then the water was released into a churn sample splitter. While mixing, the sample was poured into acid washed 90ml plastic bottles. The water was filtered through 0.7µ Millipore GFF filters. The filtrate was then transferred to another acid washed 90ml bottle. The absorbance of the water was measured in a 1cm quartz cuvette with a Varian 50 Bio UV-Visible Spectrophotometer at wavelengths of 320,380, and 440nm. These wavelengths represent UV-B radiation, UV-A radiation, and visible light, respectively. Samples from the other five lakes were collected and analyzed in the same manner in August only.

Melanin Quantification in Daphnia

The melanin was extracted from Daphnia collected from Jordan Pond and Eagle Lake and analyzed using methods in Rautio and Korhola (2002). Each sample consisted of a minimum of 30 pooled Daphnia individuals; when possible up to two replicates were also prepared. Bubble Pond could not be used for the melanin analysis because Daphnia were rare throughout the sampling period. Prior to melanin extraction, each Daphnia body length was measured using Image J. Pooled individuals were stored in 95% ethanol and refrigerated until analysis. The presence of visible melanin production was observed when the Daphnia were separated for melanin extraction. The numbers of pigmented and non-pigmented Daphnia were counted before removing the Daphnia for melanin extraction. The melanin was extracted in 5ml of 5M NaOH. Following extraction, the sample was homogenized using an ultrasonic rod, and then heated in a warm water bath at 65[°]C. Each sample was then cooled to room temperature. This heating and cooling sequence was repeated on a daily basis for five days. To account for the melanin naturally occurring in the Daphnia eyespots, the spherical volume of each eyespot was determined using the eyespot diameter determined using the program Image J. The spherical volume was calculated using the formula $(4/3)\pi r^3$. The amount of extracted melanin was quantified in a 1cm glass cuvette with a Varian 50 Bio UV-Visible Spectrophotometer at a wavelength of 350 nm and compared to standards made from synthetic melanin (Sigma # M-8631) at concentrations of 1, 2, and 5μ g/ml. Once the total amount of melanin per μ g dry weight was calculated, the amount of melanin in the eyespot was subtracted so we would only have the melanin produced in the carapace.

Results and Discussion

Zooplankton Communities in the ANP lakes

In the ANP lakes the zooplankton community (i.e., cladocerans plus copepods) was variable in abundance and body size from month to month with no consistent pattern across lakes arrayed by TP concentration (Figure 2 A and B). Cladoceran and cyclopoid abundance and body size did not change consistently across lakes (Figure 2 C and D; Figure 3). To determine if the abundance of zooplankton, all cladocerans, and *Daphnia* differed from June to August we used a paired t-test. At *p*<0.05 we found that for all lakes combined, the abundances for each month for total zooplankton, total cladoceran, and *Daphnia* were similar (Appendix E). When we look at the lakes individually we see that for some lakes the abundances increased while in other lakes they decreased (Figures 2 and 3). The zooplankton community in the ANP lakes was dynamic in that the abundances and body size changed from month to month but patterns were unpredictable.

A few patterns did appear when individual taxa were examined. Calanoid copepod abundance decreased and the cyclopoid copepod community increased throughout the summer (Figure 4). In June the two most prominent taxa were calanoid copepods and *Bosmina;* by August cyclopoid copepods, *Diaphanasoma* and *Daphnia* had increased (Figure 4). This change in the cladoceran community dominance followed typical seasonal patterns described in Balcer et al. (1984) including higher abundance of *Holopedium* in early summer than late summer and more abundant *Diaphanasoma* in late summer.

Out of 72 correlations relating abundance or body size to lake features, only three were significant: total cladoceran abundance and chlorophyll were negatively related in June; *Daphnia* abundance and TP were positively related in June; and *Daphnia* abundance and invertebrate predator abundance were positively related in July (Appendix F). We found no significant correlations for August or for body size or total zooplankton. We hypothesize that the zooplankton community is being influenced by a combination of lake features working together rather than one single predictor. Further, the ANP lake set consisted of only 8 lakes across a narrow and relatively low range of TP concentrations making detection of strong patterns related to eutrophication problematic. The next step is to test hypotheses about top-down vs. bottom-up controls with a larger set of lakes, as we are currently doing with the 75-Maine lake dataset.

Based on the t-test results, we found that across the eight lakes total zooplankton and total cladoceran body size differed across all three months. For both total zooplankton and total cladocerans body size was smaller in July than August. For *Daphnia*, body size was larger in June than July but not August, and was smaller in July compared to August.





Figure 2: Abundance (A) and average body size (B) for the total zooplankton community each month. Abundance (C) and average body size (D) for the cladocerans each month.



July





Figure 3: The abundance of the two copepod taxa (calanoid and cyclopoid) and cladoceran genera in (A) June, (B) July, and (C) August.







Figure 4: The relative abundance of zooplankton taxa for each of the eight study lakes in (A) June, (B) July and (C) August.









We were unable to explore the relationship between fish as a top-down factor and zooplankton because we could not accurately estimate fish predation pressure. Also, we could not compare stocked and non-stocked lakes because at some point in their history all have been stocked, and within the last five years the only lake that was not stocked was Seal Cove Pond (Table 3). We were able to look at the three lakes that reportedly have alewife (*Alosa pseudoharengus*) and compare the zooplankton and cladoceran abundance and body size to past research findings. According to the fish community data from both ANP and PEARL (http://pearl.maine.edu) Great Long Pond and Seal Cove Pond have sea run alewife and Echo Lake has landlocked alewife.

We know from the classic Brooks and Dodson (1965) paper that alewife radically changes both the zooplankton community and size structure. When comparing the zooplankton community before and after alewife, they found that after alewife was introduced the invertebrate predator *Leptodora* kindtii disappeared along with large cladoceran species like Daphnia Catawba. Based on the results from studies by Post and others (in press) in Connecticut lakes, we would expect that ANP lakes with landlocked alewife would have low cladoceran abundance and small body size throughout the summer. However, in Echo Lake which has land locked alewife, cladoceran size and abundance increased from June to August (Figure 6). Mean cladoceran body size in Echo Lake increased from 0.56mm in June to 0.96mm in August. This does not correspond to the Connecticut study where the body size in eight landlocked alewife lakes remained between 0.2 and 0.4mm and the biomass changed very little from June to August. In the Connecticut study there were no large cladocerans and almost no small cladocerans in three lakes with sea run alewife. For the two sea run alewife lakes in ANP (Great Long and Seal Cove) we found that cladoceran abundance increased from June to July then declined in August, but still remained above the June values. Cladoceran body size increased in Great Long Pond (0.54-0.63mm), but decreased in Seal Cove (0.86-0.55mm). In the sea-run alewife lakes in Connecticut, cladoceran abundance would increase in the winter and spring (while the alewife were at sea) and crash in June when the alewife returned. The abundance for cladocerans was close to 0 μ g/L in the summer. The average cladoceran body size in the Connecticut lakes was around 0.2mm from June to August, which is less than half the smallest average cladoceran body size for either ANP lake. Based on this information we conclude that the alewife population has not been having as strong an effect on the three ANP lakes, but it is unknown if this is due to differences in other fish species present in the lakes, the zooplankton community composition, or some other reason.



Figure 6: Cladoceran abundance (A) and body size (B) for the three study lakes that contained alewife. Note that alewife are land-locked in Echo and sea-run in the other two lakes.

Table 3: Fish Community structure and Fish Stocking information for the ANP study lakes, Fishcommunity information came from the PEARL website and fish stocking information came from BruceConnery (most recent stocking date 2005)

	Fish	Stocked Species
Eagle Lake	Brown Bullhead, American Eel, White Sucker, Banded Killifish, Threespine Stickleback, Pumpkinseed, Common Shiner, Golden Shiner, Rainbow Smelt, Northern Redbelly Dace, Landlocked Salmon, Brook Trout, Lake Trout, Fallfish	Brook Trout, Landlocked Salmon
Jordan Pond	American Eel, , Banded Killifish, Golden Shiner, Rainbow Smelt, Landlocked Salmon, Brook Trout, Lake Trout	Landlocked Salmon
Great Long Pond	Alewife (Sea run), American Eel, White Sucker, Chain Pickerel, Banded Killifish, Threespine Stickleback, Pumpkinseed, Smallmouth Bass, Golden Shiner, Rainbow Smelt, Northern Redbelly Dace, Landlocked Salmon, Brook Trout	Landlocked Salmon
Bubble Pond	American Eel, White Sucker, Banded Killifish, Three-spine Stickleback, Pumpkinseed, Common Shiner, Golden Shiner, Rainbow Smelt, Northern Redbelly Dace, Brook Trout	Brook Trout
Upper Hadlock Pond	American Eel, White Sucker, Banded Killifish, Redbreast Sunfish, Pumpkinseed, Golden Shiner, Rainbow Smelt, Brook Trout,Brown Trout	Splake
Seal Cove Pond	Alewife (Sea run), American Eel, White Sucker, Chain Pickerel, Banded Killifish, Sunfish, Smallmouth Bass, White Perch, Golden Shiner, Rainbow Smelt, Yellow Perch, Brown Trout.	No Stocked Species
Echo Lake	Alewife (Landlocked), Brown Bullhead, American Eel, Banded Killifish, Pumpkinseed, Common Shiner, Golden Shiner, Rainbow Smelt, Ninespine Stickleback, Landlocked Salmon, Brook Trout, Fallfish	Brook Trout, Landlocked Salmon
Witch Hole Pond	American Eel, Banded Killifish, Pumpkinseed, Golden Shiner, Northern Redbelly Dace, Ninespine Stickleback, Brook Trout	Brook Trout

Invertebrate Predators

We found three invertebrate predators in the water column of the ANP lakes. *Chaoborus* or the phantom midge is an insect in the order Diptera while *Polyphemus* and *Leptodora* are cladocerans.

Unlike the cladoceran predators Chaoborus spends its larval stage in the lake, emerging from the lake as

a pupa and reproducing. The invertebrate predators Chaoborus and Leptodora are much larger than

their prey (up to 10 times larger) which also make them targets for fish predation. *Leptodora* prey on small zooplankton species, such as small cladocerans and rotifers, more often than the larger cladoceran species like *Daphnia* (McNaught et al. 2004). *Polyphemus* tend to feed on the same prey items that *Leptodora* do but are much smaller (0.7-2mm,Balcer et al. 1984). We quantified densities of the invertebrate predators *Polyphemus pediculus* and *Leptodora kindtii*, (both cladocerans) and *Chaoborus spp.* (dipteran larvae) (Figure 7). In July the *Chaoborus* abundance in Witch Hole Pond was much larger than any other of the lakes. It was not surprising that *Polyphemus* was absent from all lakes in June because it tends to occur in highest abundance in July and August (Balcer et al. 1984). The number of invertebrate predator genera in for most lakes increased throughout the study period and in July and August all lakes had at least one of the three invertebrate predators.





Figure 7: Invertebrate predator abundance for all study lakes throughout the sampling time (A=June, B=July, and C=August). Note that for Witch Hole in July, the abundance of *Chaoborus* was 419.99 Individuals m⁻³.



Light Absorbance

Figure 8: Epilimnion light absorbance in August. These water samples were taken at the same time the zooplankton samples were taken.

UV-B Analysis

In August we took water samples from each lakes' epilimnion to measure light absorbance for three wavelengths; 320 nm (UV-B range), 380 nm (UV-A range), and 440 nm (visible light). We did this to compare the three lakes selected for melanin analysis to the other study lakes (Figure 8). The three melanin focus lakes had lower light absorbance values for all three wavelengths compared to the other study lakes. The lower light absorbance, the deeper that particular wavelength of light can penetrate into the water column. Particulates (such as chlorophyll) and colored dissolved organic carbon in the water column absorb light passing through the water increasing the absorbance value.

In August the epilimnion and hypolimnion of two of the melanin focus lakes were studied to determine if vertical migration was occurring. Bubble Pond did not stratify on the days we took epilimnion and hypolimnion samples so we focused on the stratified Jordan Pond and Eagle Lake for this part of the study. In Eagle Lake there was a higher abundance (both night and day) of cladocerans in the hypolimnion than in the epilimnion (Figure 9A) whereas in Jordan Pond the opposite pattern was

observed (Figure 9B). In both lakes, Leptodora was found in the epilimnion and hypolimnion at night but was only found in the epilimnion during the day (Figure 9C and D). *Polyphemus* and *Chaoborus* were in higher abundance in Eagle Lake, with *Chaoborus* reaching highest densities in the hypolimnion at night. This suggests that during the day *Chaoborus* are near the sediments, and migrate up at night, a typical behavioral response to fish predation. The invertebrate predator data in Figure 9 are likely underestimates of actual density because the net used was smaller than that used to collect the samples shown in Figure 7. Although we don't have the data to support this, the difference in vertical distribution between the two lakes could be because Eagle Lake has a higher concentration of invertebrate predators and thus higher invertebrate predation pressure (Figure 7). We did not see the vertical migration in cladocerans that we would expect if predation pressures were strong. If the primary predation pressure is fish predation we would expect there to be a higher abundance of larger cladocerans in the hypolimnion during the day, shifting to a higher abundance of cladocerans in the epilimnion at night. For lakes with high enough concentrations of the invertebrate predator Chaoborus we would expect higher cladoceran abundance in the epilimnion during the day and higher abundances in the hypolimnion at night (Lampert and Sommer 2007). Instead in both lakes they are either staying in the hypolimnion (Eagle) or they are staying in the epilimnion (Jordan).



Figure 9: A and B- Zooplankton species abundance in the epilimnion and hypolimnion. The Eagle Lake and Jordan Pond day samples were taken on 8/12/07, while the night samples for both were taken on 8/14/07. C and D- Invertebrate predator abundances in the eagle and Jordan elilimnia and hypolimnia.

We had visual confirmation that *Daphnia* were producing melanin (Figure 10). However, we were not able to detect melanin in the samples as all measurements were below standard values. There are several possible reasons for this. One could be that instead of selecting only the more heavily pigmented Daphnia like previous researchers have done we selected individual Daphnia for melanin measurement at random. Since we wanted to look at the entire Daphnia community our subsamples were a mixture of both pigmented and non-pigmented individuals. Therefore, if our subsamples were larger or we focused on only pigmented *Daphnia* our ability to quantify melanin would have been improved. At most 40% of the *Daphnia* in a sample produced enough melanin in their carapace that it could be seen by the human eye. Second, when we made our standards we let the Daphnia samples soak in KOH for 12 days instead of 5 because it took longer than expected to get the synthetic melanin to dissolve into KOH. This may have lead to a breakdown of the melanin, even though we kept the samples in a dark hood and at room temperature. Finally, we based the concentration of our melanin standards on previous research by Hansson and others (2007). They found Daphnia in Siberia with melanin ranging from 29µg/ml to 0.15µg/ml. Since the amount of melanin observed in the Daphnia in ANP was less than 1µg/ml, far less than the heavily pigmented Daphnia found in Siberia, our standards were too high to accurately estimate the amount of melanin in the ANP samples.

Another issue with the melanin research was that we could only get data from two of the three focus lakes. We took melanin measurement samples from three of the study lakes; Jordan Pond, Eagle Lake, and Bubble Pond. All three lakes had clear-water that would allow sub-surface UV-B radiation penetration. The *Daphnia* population in Bubble Pond throughout the study period was too sparse to provide the necessary minimum 30 *Daphnia* for melanin measurement (Appendix G and Figure 4).

Although we were not able to quantify melanin concentrations, we were able to make visual counts of *Daphnia* with visible melanin stripes in their helmets. In Jordan Pond melanin was being produced throughout the study period, while in Eagle Lake melanin production was not seen until July

(Figure 11). In Jordan Pond the highest percentage of pigmented *Daphnia* was found in June (40%); but we cannot compare that to the June UV-B absorbance because we were unable to measure UV-B absorbance for June. While In Jordan Pond the percentage of pigmented *Daphnia* decreased over the summer, it increased in Eagle Lake. It appears that UV-B radiation is enough to cause some *Daphnia* to respond by producing melanin. One explanation for the lower percentages of pigmented Daphnia in Eagle Lake than in Jordan Pond could be because the *Daphnia* species that produce pigment were not present until July (Herbert 1990).



Figure 10: (A) Pigmented *Daphnia* from Jordan Pond, (B) non-pigmented *Daphnia* from Upper Hadlock, and (C) two *Daphnia* samples from a study done by Hansson et al. (2007) study. In C the top *Daphnia* is unpigmented and the bottom *Daphnia* is heavily pigmented.



Figure 11: The absorbance and % pigmented *Daphnia* (A) Jordan Pond and (B) Eagle Lake. Note that the % pigmented individuals are shown in different scales on the two panels.

Conclusions

In conclusion, we detected only a few significant correlations between zooplankton abundance and body size and either bottom-up and top-down factors, but they were not consistent and indicative of strong effects. It is likely that multiple factors interact to influence the zooplankton community composition and size structure of these eight ANP lakes. By putting the ANP lakes in a broader context of a larger set of Maine lakes we will be able to determine how other factors related to lake morphometry and water chemistry may be important. By accounting for these variables we can determine if the zooplankton communities of the ANP lakes are similar to other lakes in the state. Our results do provide baseline data on the zooplankton community composition data for future comparisons. Even though we did not have extensive fish community and abundance information to make conclusions about the effects of fish populations on the zooplankton community, we were able to compare the cladoceran community and size structure data of ANP lakes that contain alewife to Connecticut alewife lakes. We found that the seasonal patterns of cladoceran density and size structure in the ANP lakes were different from the alewife lakes in Connecticut. The results of the UV study showed that UV-B penetration into the clearest ANP lakes is sufficient to induce the production of visible melanin in the *Daphnia* suggesting that future work elucidating the role of UVB stress might be warranted.

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Appendix A: Sampling dates and weather conditions

Date	Lake	Wind	Cloud Cover	Time	Comments
6/13/2007	Upper Hadlock Pond		Overcast		
6/14/2007	Witch Hole Pond		Patchy		
6/15/2007	Eagle Lake		None		
6/17/2007	Jordan Pond		Slight	10:27am	
6/18/2007	Seal Cove Pond	13	Slight	11:48am	
6/18/2007	Echo Lake	13	Slight		
6/19/2007	Bubble Pond				
6/20/2007	Jordan Pond		Dense	9:27am	No Rain
6/20/2007	Bubble Pond		Dense	10:20am	No Rain
6/20/2007	Eagle Lake		Dense	11:00am	Light Rain
6/20/2007	(Great) Long Pond		Dense	2:42pm	No Rain
6/20/2007	Eagle Lake		Dense	9:00pm	
6/21/2007	Bubble Pond		Overcast	9:10am	
6/21/2007	Jordan Pond		Overcast	10:51am	
7/10/2007	Eagle Lake			11:32am	
7/10/2007	Upper Hadlock Pond			3:03pm	
7/10/2007	Echo lake			4:30pm	
7/11/2007	Jordan Pond	17	Overcast		
7/11/2007	Witch Hole Pond	16	Overcast	3:00pm	
7/12/2007	Bubble Pond		Overcast	9:00am	
7/12/2007	Seal Cove Pond		Overcast	1:00pm	
7/13/2007	Jordan Pond				
7/16/2007	Bubble Pond			8:46am	
7/16/2007	(Great)Long Pond			10:46am	
7/16/2007	Echo Lake			1:00pm	
7/16/2007	Bubble Pond			10:50pm	
7/17/2007	Jordan Pond			8:30pm	
7/17/2007	Eagle lake			9:30pm	
7/18/2007	Eagle Lake		Dense	Day	Light Rain
8/12/2007	Jordan Pond		Slight	Day	
8/12/2007	Bubble Pond		Slight	Day	
8/12/2007	Eagle Lake		Slight	Day	
8/12/2007	Upper Hadlock Pond		Slight	Day	
8/13/2007	Seal Cove Pond			Day	
8/13/2007	Echo Lake			Day	
8/13/2007	(Great) Long Pond			Day	
8/13/2007	Witch Hole Pond			Day	
8/14/2007	Jordan Pond			Night	
8/14/2007	Bubble Pond			Night	
8/14/2007	Eagle Lake			Night	
8/15/2007	Jordan Pond		Cloudy	Day	
8/15/2007	Bubble Pond		Cloudy	Day	
8/17/2007	Eagle Lake		Cloudy	Day	

	June						
Lake	Date	Sample Type	Net	Tow Depth (m)	# of tows	# of replicates	
Seal Cove Pond	6/18	Count	Wisconsin	5	3	1	
Echo Lake	6/18	Count	Wisconsin	10	3	1	
(Great) Long Pond	6/20	Count	Wisconsin	15	3	1	
Upper Hadlock Pond	6/13	Count	Wisconsin	10	3	1	
Witch Hole Pond	6/14	Count	Wisconsin	5	3	1	
Bubble Pond	6/21	Count	Wisconsin	8	3	1	
Bubble Pond	6/19	Sunny Melanin	Wisconsin and Closing	10	5	2 (one per net type)	
Bubble Pond	6/20	Cloudy Melanin	Wisconsin and Closing	5	5	2 (one per net type)	
Bubble Pond	6/18	Night Melanin	Wisconsin and Closing	8	5	2 (one per net type)	
Jordan Pond	6/21	Count	Wisconsin	10	5	1	
Jordan Pond	6/17	Sunny Melanin	Wisconsin and Closing	20	5	2 (one per net type)	
Jordan Pond	6/20	Cloudy Melanin	Wisconsin and Closing	10	5 (C net) 4 (W net)	2 (one per net type)	
Jordan Pond	6/18	Night Melanin	Wisconsin and Closing	20	3	2 (one per net type)	
Eagle Lake	6/15	Count	Wisconsin	10	3	1	
Eagle Lake	6/15	Sunny Melanin	Wisconsin and Closing	10	5 (C net) 3 (W net)	2 (one per net type)	
Eagle Lake	6/20	Cloudy Melanin	Wisconsin and Closing	8	5	2 (one per net type)	
Eagle Lake	6/20	Night Melanin	Wisconsin and Closing	10	5	2 (one per net type)	
			July				
Lake	Date	Sample Type	Net	Tow Depth (m)	# of tows	# of replicates	
Seal Cove Pond	7/12	Count	Wisconsin	10	5	1	
Echo Lake	7/10	Count	Closing	15	3	1	
(Great) Long Pond	7/16	Count	Wisconsin	26	3	1	
Upper Hadlock Pond	7/10	Count	Closing	10	3	1	
Witch Hole Pond	7/11	Count	Closing	8	3	1	
Bubble Pond	7/12	Count	Wisconsin	10	3	1	

Appendix B: Sampling Types and Equipment Used to Collect Zooplankton Samples

Bubble Pond 7/16 Sunny Melanin 10 6 Tow 2 **Bubble Pond** 7/12 Cloudy Melanin Tow 10 6 2 7/16 Night Melanin Tow 8 6 2 **Bubble Pond**

Jordan Pond	7/11	Count	Closing	20	3	1
Jordan Pond	7/13	Sunny Melanin	Tow	45	6	2
Jordan Pond	7/11	Cloudy Melanin	Tow	45	6	2
Jordan Pond	7/17	Night Melanin	Tow and	45	6	2 (one per net
			Wisconsin			type)
Eagle Lake	7/10	Count	Closing	15	3	1
Eagle Lake	7/10	Sunny Melanin	Tow	15	6	2
Eagle Lake	7/18	Cloudy Melanin	Tow and	20	6	2 (one per net
			Wisconsin			type)
Eagle Lake	7/17	Night Melanin	Tow and	16	6	2 (one per net
			Wisconsin			type)

August

Lake	Date	Sample Type	Net	Tow Depth	# of tows	# of replicates
				(m)		
Seal Cove Pond	8/13	Count	Wisconsin	11	3	1
Echo Lake	8/13	Count	Wisconsin	17	4	1
(Great) Long	8/13	Count	Wisconsin	25	3	1
Pond						
Upper Hadlock	8/12	Count	Wisconsin	9	3	1
Pond						
Witch Hole Pond	8/13	Count	Wisconsin	8	3	1
Bubble Pond	8/12	Count	Wisconsin	8	3	1
Bubble Pond	8/12	Sunny	Tow	8	6	2
Bubble Pond	8/15	Cloudy	Tow	8	6	2
Bubble Pond	8/14	Night	Tow and	8	6	2 (one per net
			Wisconsin			type)
Jordan Pond	8/12	Count	Wisconsin	45	3	1
Jordan Pond	8/12	Sunny	Tow	45	6	2
Jordan Pond	8/15	Cloudy	Tow	45	6	2
Jordan Pond	8/14	Night	Tow and	45	6	2 (one per net
			Wisconsin			type)
Jordan Pond	8/12	Hypolimnion	Closing	45-10	3	1
		Sunny Day				
Jordan Pond	8/12	Epilimnion	Closing	10-0	3	1
		Sunny Day				
Jordan Pond	8/14	Hypolimnion	Closing	45-10	3	1
		Night				
Jordan Pond	8/14	Epilimnion	Closing	10-0	3	1
		Night				
Eagle Lake	8/12	Count	Wisconsin	18	3	1
Eagle Lake	8/12	Sunny	Tow	17	6	2
Eagle Lake	8/17	Cloudy	Tow	17	6	2
Eagle Lake	8/14	Night	Tow and	17	6	2 (one per net
			Wisconsin			type)
Eagle Lake	8/12	Hypolimnion	Closing	17-11	3	1
		Sunny Day				

Eagle Lake	8/12	Epilimnion	Closing	11-0	3	1
		Sunny Day				
Eagle Lake	8/14	Hypolimnion	Closing	17-11	3	1
		Night				
Eagle Lake	8/14	Epilimnion	Closing	11-0	3	1
		Night				

Appendix C: Net types

Plankton Tow Net: Diameter – 0.3m Mesh – 243µ Length – 1.07m

Notes: The original bucket for this net was lost and the bucket for the closing net was used for this experiment. This net was unavailable for use during the June sampling.

Birge Closing Net: Diameter – 0.12m $Mesh-200\mu \label{eq:mesh}$ Length – 0.9m

Notes: The length of the line for this net was about 22m, which was too short for Jordan Pond. After the July sampling more rope was added increasing the length to over 50m.

Wisconsin Net: Diameter – 0.12m

 $\begin{array}{l} \text{Mesh}-200\mu\\ \text{Length}-0.4m \end{array}$



Appendix D: Microscope set-up and counting equipment



Nikon SMZ800 dissecting microscope set up with a Nikon Coolpix 995 digital camera.



Wards Counting Wheel with counting tools



Diaphanasoma and cyclopoid copepods being measured in Image J

Appendix E: Paired T-Test for abundance and body size. Shown are the probability levels for the t-test.

	June:July	June:August	July:August
Total zooplankton abundance	0.0982	0.1563	0.3652
Total zooplankton body size	0.3518	0.0788	0.0380 (JY <a)< th=""></a)<>
Total cladoceran abundance	0.4447	0.1443	0.1799
Total Cladoceran body size	0.1183	0.3227	0.0438 (JY <a)< th=""></a)<>
Daphnia abundance	0.0675	0.0844	0.3957
Daphnia body size	0.2042	0.0043 (J <a)< th=""><th>0.0349 (JY<a)< th=""></a)<></th></a)<>	0.0349 (JY <a)< th=""></a)<>

correlation coefficients r	narked with 0 were not	significant; - were negativ	le and + were positive.
Total Zooplankton	June	July	August
Size:TP	0.310 (NS)	-0.072 (NS)	0.238 (NS)
Size:DOC	0.395 (NS)	0.405 (NS)	-0.095 (NS)
Size:CHL	0.381(NS)	0.395 (NS)	0.602 (NS)
Size: InvPred	-0.292 (NS)	0.216 (NS)	-0.095 (NS)
Abund:TP	-0.452 (NS)	0.611 (NS)	0.262 (NS)
Abund:DOC	-0.695 (NS)	0.119 (NS	0.143 (NS)
Abund:CHL	-0.452 (NS)	0.132 (NS)	0.265 (NS)
Abund:InvPred	0.571 (NS)	0.539 (NS)	0.476 (NS)
Total Cladocerans	June	July	August
Size:TP	0.238 (NS)	-0.168 (NS)	0.095 (NS)
Size:DOC	0.395 (NS)	-0.071 (NS)	-0.286 (NS)
Size:CHL	0.500 (NS)	0.419 (NS)	0.325 (NS)
Size: InvPred	-0.152 (NS)	0.455 (NS)	-0.262 (NS)
Abund:TP	-0.095 (NS)	0.635 (NS)	0.262 (NS)
Abund:DOC	-0.419 (NS)	0.048 (NS)	0.143 (NS)
Abund:CHL	-0.833*	0.036 (NS)	0.265 (NS)
Abund:InvPred	0.304 (NS)	0.419 (NS)	0.476 (NS)
Daphnia	June	July	August
Size:TP	0.563 (NS)	-0.491 (NS)	0.071 (NS)
Size:DOC	0.542 (NS)	-0.238 (NS)	-0.238 (NS)
Size:CHL	-0.072 (NS)	0.252 (NS)	0.53 (NS)
Size: InvPred	-0.281 (NS)	0.108 (NS)	-0.19 (NS)
Abund:TP	0.850*	0.79 (NS)	0.214 (NS)
Abund:DOC	0.590 (NS)	0.69 (NS)	0.381 (NS)
Abund:CHL	0.311 (NS)	0.731 (NS)	0.024 (NS)
Abund:InvPred	-0.281 (NS)	0.862*	0.69 (NS)

Appendix F: Spearman Rho Correlation coefficient values for relationships between body size or abundance and lake features. The critical value was 0.833 for n=8 and a 98% confidence interval; correlation coefficients marked with 0 were not significant; '-' were negative and '+' were positive.

Appendix G: Chlorophyll Concentrations from the UM Environmental Chemistry Laboratory

Lake Name	MIDAS	Sampling Date	ChI a μg/L	
Eagle Lake	4606	7/10/07	1.0	
Upper Hadlock	4612	7/10/07	2.2	
Jordan Pond	4608	7/11/07	<1	
Witch Hole	4458	7/11/07	2.3	
Seal Cove Pond	4630	7/12/07	2.1	
Bubble Pond	4452	7/16/07	<1	
Echo Lake	4624	7/16/07	1.6	

(Great) Long Pond chlorophyll measurements were not taken for July. Appendix H: Images and characteristics of cladoceran genera and copepod groups found in the ANP lakes: Information on species was from Balcer et al. (1984).

Copepods





Calanoid Copepods- These copepods can be filter feeders or omnivores depending on the species. Their size can vary from 0.5mm to almost 2mm (size range observed for Maine lakes). They have antennae equal in length to their body length. They produce one large cluster of eggs over their caudal ramus. In response to UV-B radiation they will produce carotenoids in their carapace.

Cyclopoid Copepods - These copepods are omnivores (consuming small zooplankton and algae) and their size can vary from 0.5mm to almost 2mm (size range observed for Maine lakes by Elizabeth Whitmore). They have antennae that are about half their body length. They have a forked caudal ramus which can hold two clusters of eggs. In response to UV-B radiation they will produce carotenoids in their carapace.

Cladoceran Grazers



Chydorus sphaericus- This species can be found in both littoral and pelagic areas. Its size can range from 0.2-0.5mm. It is known for its spherical shape and having both a compound eye and ocellus. They will sometimes attach themselves to filamentous algae. It has not been determined if they will produce any kind of pigment in response to UV-B radiation. They are one of the few species that can overwinter and reproduce under the ice. *Chydorus* can tolerate a wide range of conditions and tends to reach highest abundance in eutrophic lakes. They are filter feeders and feed on algae, bacteria and protozoans.



Bosminids- Bosmina and *Eubosmina* can be found in both the littoral and pelagic zones of lakes . Their size can range from 0.2-0.6mm. They are known for their long rostrum (trunk-like appendage) and two small tail spines. So far it has not been determined if they will produce any kind of pigment in response to UV-B radiation. They prefer cool and well-oxygenated waters. They are one of the first groups to reproduce (early-mid June) and their numbers tend to decline in August and October. They are filter feeders and feed on algae, bacteria and protozoans.



Ceriodaphnia- This genus is pelagic. Their size ranges from 0.4-0.8mm. Their most distinguishing features are their pin shaped heads, they lack a tail spine, and they have a "cigar-like" protrusion near their mouth-parths. So far it has not been determined if they will produce any kind of pigment in response to UV-B radiation. They are usually found in the warmer waters and nearshore. They are filter feeders and feed on algae, bacteria and protozoans.



Diaphanasoma- This is a pelagic species. Their size can range from 0.4-0.9mm. So far it has not been determined if they will produce any kind of pigment in response to UV-B radiation. Their abundance tends to peak in the fall months. They are filter feeders and prefer to stay in the epilimnion.



Daphnia- This genus ranges in size from 0.5-2.3mm. The species differ in their preference for many lake conditions. Some respond to predation by going through cyclomorphosis which causes changes (elongation) in their tail spine and helmet (area above the eyespot), and the development of ridges on their back called "neck teeth". They are a cornerstone genus in pelagic lake ecosystems because they are an important prey for planktivorus fish and are efficient grazers on algae. *Daphnia* filter feeds by moving its appendages to create a current that draws algae towards its mouth



Holopedium gibberum- This species is large, 0.5-2mm and unique for two reasons; it has long featherlike feeding appendages and it produces a large gelatinous sheath that protects it from invertebrate predation. The gelatinous sheath does not protect them from fish predation however. So far it has not been determined if they will produce any kind of pigment in response to UV-B radiation. They tend to peak in abundance during the early summer months. They filter feed, and spend much of their time in the epilimnion. They tend to swarm, so are patchily distributed, and prefer water that is slightly acidic (6.0-6.8), less than 25^oC, and low in calcium (less than 20mg/L)

Cladoceran Predators



Leptodora kindti- This is a cladoceran, although very different in size and shape compared to other cladocerans. This species tends to swarm and are considered to be ideal as prey species for fish. They feed mostly on small cladocerans and rotifers. Feeding behavior has been described as "vampire-like". They will grab their prey with modified legs and consume the interior of their prey. They are nearly transparent except for a large eyespot, so they are difficult to observe even though they range in size from 5-13 mm and larger.



Other Invertebrate Predators

Polyphemus pediculus- This cladoceran species is known for its large eyespot. This large eyespot and darker body pigmentation makes them easy to see and therefore they are frequently targeted by fish. They have modified thoracic appendages for grabbing prey, rotifers, protozoans, and small cladocerans. *Polyphemus* abundance tends to be highest in July and August. They tend to be found in high abundance where prey such as *Bosmina* are in high abundance.



Chaoborus - This zooplankton is the only representative of the insects to be found in the open water of lakes. It is a dipteran called the phantom midge. They spend the larval stage of their life cycle in lakes. They are common in lakes and frequently undergo vertical migration, spending the daylight hours near the bottom sediments to avoid fish predation. and their size is



Appendix I: Zooplankton community composition in each study lake during June, July, and August.-

Panels on the left represent the whole community and panels on the right show the cladoceran community structure.





Appendex J: Copepod Pigmentation Patterns in Jordan Pond and Eagle Lake.

Carotenoid Pigmentation: The pigment is Carotene is suspended in a lipid and can range in color from yellow-orange to bright red. There was a wide variety of pigmentation patterns observed.





Hemoglobin- This is produced in reaction to a low oxygen environment, and despite the fact that it is dark red, should not be confused with carotenoids. These specimens were collected in Jordan Pond and Eagle Lake