# Effects of Solar Ultraviolet Radiation on Littoral Benthic Macroinvertebrate Feeding

BIOS 35502: Practicum in Field Biology

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## *Abstract*

Human-induced changes in the environment are resulting in increased exposure to ultraviolet radiation for forest floors, streams, and lakes. Aquatic ecosystems are particularly sensitive to ultraviolet radiation, which can reduce algal growth rates and overall ecosystem productivity. Increased ultraviolet radiation also affects the invertebrate inhabitants of aquatic ecosystems by altering distributions or even damaging and eventually killing them if levels are high enough. These combined effects have the potential to create a scarcity of food at lower trophic levels which could cascade up the trophic chain to affect larger aquatic organisms. I investigated the effects of ultraviolet radiation on littoral benthic macroinvertebrates by measuring colonization preference and algal growth rate in shaded and unshaded locations of varying depths. I hypothesized that sites with higher solar radiation penetrance would have lower macroinvertebrate colonization, and that primary production would be highest in sites with intermediate levels of solar radiation penetrance. My results showed that macroinvertebrate diversity was positively correlated to solar radiation penetrance, and that shallow sites were preferred over deep sites.

## *Introduction*

Currently, depletion of the ozone layer is resulting in higher ultraviolet radiation levels for the earth's surface (Kerr and McElroy 1993, Madronich et al. 1998). Compounding this problem is the fact that anthropogenic habitat modification is reducing vegetative cover over enormous areas globally, thus exposing previously shaded forest floor, streams, and lakeshores to greater overall solar radiation.(Clare 2000). Considering these two points raises questions about what effect this increase in solar radiation will have on the biosphere.

Aquatic ecosystems are particularly sensitive to ultraviolet radiation and many of these systems, especially those in the polar and temperate latitudes, have seen marked increases in UV radiation levels in recent decades (Williamson 1995). Previous studies have shown that increased UV radiation can reduce algal growth rates (Banaszak and Trench 1995) and overall aquatic ecosystem productivity (Häder et al. 2007). However, these primary producers also

require solar radiation to grow, so their ideal growth conditions could be dictated by a tradeoff between obtaining solar radiation for photosynthesis and avoiding areas of dangerously high ultraviolet radiation.

In addition to primary producers, increased UV radiation is also known to alter littoral benthic macroinvertebrate distributions (Boeing et al. 2004), as well as damage and eventually kill macroinvertebrates if exposure is great enough (Hurtubise et al. 1998, Cywinska et al. 1998). These detrimental effects on both primary producers and macroinvertebrates could combine to have a large impact on larger organisms by creating a scarcity of food at the lowest trophic levels. Despite extensive study in recent years, much remains to be investigated, especially because these effects vary significantly by habitat and species (Vinebrooke and Leavitt 1999, Kelly et al. 2003).

In this study, I investigated the effects of UV radiation on littoral benthic macroinvertebrate colonization and food availability. The study was conducted in 5 lakes on the University of Notre Dame Environmental Research Center (UNDERC) property, and measured invertebrate colonization preference at shallow and deep locations, each with shaded and unshaded sites, in order to gauge avoidance of high UV areas. The study also measured algal growth rate at each of these sites in order to quantify the effects of UV radiation on food production for littoral benthic macroinvertebrates, as algae is typically the base of their trophic chain (Hamilton et al. 1992).

I hypothesize that sites with higher solar radiation penetrance will have lower macroinvertebrate colonization, and that primary production will be highest in sites with

intermediate levels of solar radiation penetrance. Specifically, I predict that invertebrate diversity will be greater at shaded sites than unshaded sites.

## *Methods*

The experiment took place in Bergener, Brown, Crampton, Long, and Plum lakes on the UNDERC property (Figure 1). These lakes were chosen based on pH, turbidity, lake area and total lake depth in order to incorporate a representative spectrum of different lake attributes for the region (Figure 2; Table 1).

Each lake contained two experimental setups; one at a shallow site and the other at a deep site along the lakeshore slope. The depths of these sites varied by lake, and were selected in order to have the shallow site receiving nearly 100% solar radiation and the deep site receiving approximately 50% solar radiation.

At each site, the experimental setup was marked by a buoy anchored by a weighted bucket. The setup consisted of 6 shallow plastic containers set on the lake bottom immediately adjacent to the anchor and tied to holes in the side of the bucket. The bottom of each container was lined with pebble gravel, a 7.5 x 7.5 cm tile was placed in, and holes were cut in the sides to allow benthic invertebrates to enter and feed on algal growth in the container (Appendix A). Three of the six containers were covered with a shade cloth over the top which filtered out 77.1 ± 0.9% (mean ± std. error) of light to represent sites in the shade under tree cover or other vegetation.

We revisited three times:(1) 4 days, (2) 21 days and (3) 49 days after the initial installment to pick up a pair of tiles (shaded and unshaded), measure light intensity in the

water, and other water chemistry. We also measured light intensity with a LI-COR light meter, from which I calculated solar radiation penetrance. Dissolved oxygen concentration and water temperature were measured using a YSI 55 Dissolved Oxygen Meter, and a water sample was collected from just below the surface with a 250 mL bottle. Once the water samples were returned to the lab, we measured conductivity and pH from them. We also filtered100 mL of the sample water and weigh the product to get a measure of dissolved solute concentrations.

The entire container was removed and used to quantify food production and invertebrate colonization for each site. We scraped the algae from the tiles with a toothbrush and used this to measure algal biomass through chlorophyll-α abundance. This extraction was done as described in Kiffney et al. 2007, with the exception being that we used acetone as the solvent as opposed to methanol. I also identified and counted all invertebrates greater than 6 mm in length in the substrate of my samples, then took three 2 mL subsamples from each using a micropipette with a tip diameter of 6 mm. I identified and counted all invertebrates present in these subsamples, then extrapolated to determine abundances for the full sample volumes. This provided me with a measure the invertebrate communities present in the substrate of the container to determine potential organisms feeding upon the algae.

The data from this experiment was analyzed using a two-way ANOVA on macroinvertebrate diversity versus site (shallow or deep) and type (shaded or unshaded). Pearson's R correlations, and t-tests for those correlations, were calculated for macroinvertebrate diversity against both solar radiation penetrance and chlorophyll-α concentration separately, and for chlorophyll concentration versus solar radiation penetrance.

A multiple regression was then calculated for macroinvertebrate diversity against both solar radiation intensity and chlorophyll-α concentrations.

## *Results*

This study demonstrated that littoral benthic macroinvertebrate diversity did not differ significantly (ANOVA $F_{1,56}$=0.2459, p=0.6219) between shaded and unshaded sites, but did differ significantly (ANOVA $F_{1,56}$=13.6157, p=0.0005093) between shallow and deep sites (Figure 3). No significant interaction was found between these two factors (ANOVA $F_{1,56}$=1.9780, p=0.1651).

Solar radiation penetrance showed a significant positive correlation with macroinvertebrate diversity ($t_{52}$=2.3931, p=0.02035), with a Pearson's r value of 0.315 (Figure 4). Chlorophyll concentration also showed a marginally significant correlation with macroinvertebrate diversity ($t_{52}$=1.9912, p=0.05172), with a Pearson's r value of 0.315 (Figure 5). Solar radiation penetrance and chlorophyll concentration, however, were not found to covary (Pearson's r=0.1354, $t_{52}$=0.9854, p=0.329).

When the effects of solar radiation penetrance and chlorophyll concentration were considered in conjunction, solar radiation penetrance was shown to significantly influence macroinvertebrate diversity ($t_{51}$=2.181, p=0.0338) and the effect of chlorophyll concentration was marginally significant ($t_{51}$=1.748, p=0.0865). Together, they explained 15% (multiple $R^2$=0.1501) of variance in macroinvertebrate diversity, and their influence was significant ($F_{2,51}$=4.504, p=0.0.01580).

## *Discussion*

The results from my experiment did not support my hypothesis about the negative effects of ultraviolet radiation on littoral benthic macroinvertebrates. In fact, the significant positive correlation between solar radiation penetrance and macroinvertebrate diversity directly contradicts my prediction that macroinvertebrate diversity would be higher in shaded locations than in unshaded ones. The fact that diversity was significantly higher in shallow sites than in deep sites is also somewhat contradictory to this hypothesis. The results also failed to support my secondary hypothesis, that algal growth would be highest at sites of intermediate solar radiation penetrance. Instead, there was no significant correlation between solar radiation penetrance and algal growth as measured by chlorophyll concentration.

That solar radiation penetrance was positively correlated to macroinvertebrate diversity would seem to suggest that invertebrates might prefer higher radiation levels over lower ones, directly in contradiction to my hypothesis as well as previous literature. The fact that shallow sites had significantly higher diversity than deep sites could also be taken in this way, however I believe that both of these points do not serve as evidence against the deleterious effects of increased ultraviolet radiation, but rather are the result of unexpected complications, highlighting shortcomings in the design and scope of this study. The higher macroinvertebrate diversity at shallower sites may also reflect a factor (i.e. refuge from predation)that we did not test but one that plays a significant role in the distribution of macroinvertebrates.

I believe that the lack of difference between shaded and unshaded sites is largely due to the difference in ease of colonization between shaded and unshaded containers. Unshaded

containers were open on top as well as through the four approximately 1” x 0.5” holes cut in the sides, while the tops of shaded containers were closed off to colonization by macroinvertebrates by the shade cloth, leaving only the four holes in the sides as conduits for colonization, a combined area only a fraction of the size of the top of the container. This difference could have made it much more difficult for invertebrates to colonize the shaded containers in the first place. Thus, the preference due to shading from ultraviolet radiation was likely cancelled out by the difference in ease of colonization, resulting in no apparent preference for shaded or unshaded containers by littoral benthic macroinvertebrates.

Another problem which could have led to the lack of preference between shaded and unshaded containers is seasonal growth of vegetation, which effectively shaded both containers in some replicates, resulting in the unshaded container being protected from ultraviolet rays, and the shaded container possibly being blocked out of all light. This effect was especially evident in Bergner and Brown Lakes where submergent and floating vegetation, which was low when we selected sites, grew up to the surface of the water and almost completely obscured the experimental setups.

The significant difference in diversity between shallow and deep sites is likely the result of habitat preference, which is known to be determined by the trade-off between solar radiation and both quantity and quality of algal food available (Donahue et al. 2003). However, in addition to not seeing an inverse correlation between solar radiation intensity and diversity as expected, the correlation between algal growth and diversity was only marginally significant. Furthermore, there was no significant correlation between solar radiation intensity and algal growth, which is directly contradictory to previous studies (Donahue et al. 2003) as well as basic

principles of photosynthesis. This suggests that the measure of algal growth were inaccurate, which could be due to either problems with the spectrophotometer, or foreign matter included with the chlorophyll extractions. During the June 14th session of data collection for the chlorophyll extractions, it was realized that the spectrophotometer was not reading correctly, and all of the values from the first week had to be discarded. It appeared to function correctly for the rest of that session, but was showing erroneous values and had to be corrected again during the July 12th data collection, so it is possible that some of the values in between were incorrect.

Aside from technical problems with the spectrophotometer, we have the possible problem of foreign organic matter getting included in the chlorophyll extractions. While scrubbing the tiles for algal growth, I noticed that some of the tiles appeared to have organic detritus which settled on them rather than having grown as algal production. Having no way to distinguish between the two, all organic matter on the tiles was included in the measurements. If there was in fact foreign organic detritus included, this could artificial inflate some of the chlorophyll values, thus leading to our lack of a significant correlation between solar radiation penetrance and chlorophyll production.

In order to more accurately investigate the affects of solar ultraviolet radiation on littoral benthic invertebrates, I would correct some of the experimental design from this study before conducting another experiment. For one, I would standardize the ease of colonization between both shaded and unshaded containers so as to not mask the predicted preference of shaded containers over unshaded. This could be done by either covering the unshaded containers in an ultraviolet and light transparent membrane so that invertebrates could only

enter through the sides of all containers, or by suspending the shade clothes above the shaded containers, so that invertebrates could colonize both shaded and unshaded containers through the top as well as the sides. Also, I would gently rinse all tiles in de-ionized water before scrubbing the algae off for chlorophyll extractions. This would help to ensure that all chlorophyll measured in the extractions came from algae grown on the tile, thus providing an accurate measurement of algal growth at different levels of the experiment. Finally, I would make sure to select sites with no submergent vegetation which could grow up to shade the experimental setup over the course of the season. Although this study yielded little in the form of biologically significant results, increased ultraviolet radiation is nonetheless a pressing issue which should be investigated further. Given the significance of aquatic invertebrates as food for higher trophic levels, including economically important fish, a modified reattempt of this study would be a worthwhile effort for aquatic ecology and conservation research.

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# Tables and Figures

Table 1. Lake attributes for study lakes. Sites were chosen based off of these attributes as well as lake area and bathymetry. (Credit: S. Carpenter)

| Lake | Maximum Depth (m) | Secchi Depth (m) | Chlorophyll$_a$ (mg/L) | Alkalinity (mg/L $CaCO_3$) | pH | Conductivity (µS) |
|---|---|---|---|---|---|---|
| Bergner | 12.00 | 2.40 | 6.19 | 4.64 | 5.20 | 15.00 |
| Brown | 5.49 | 0.70 | 114.96 | 64.00 | 8.39 | 133.50 |
| Crampton | 15.25 | 4.25 | 4.13 | 0.09 | 6.00 | 18.00 |
| Long | 14.00 | 3.60 | 6.28 | 0.71 | 6.00 | 15.00 |
| Plum | 12.00 | 25.00 | 6.87 | 8.30 | 7.50 | 50.00 |

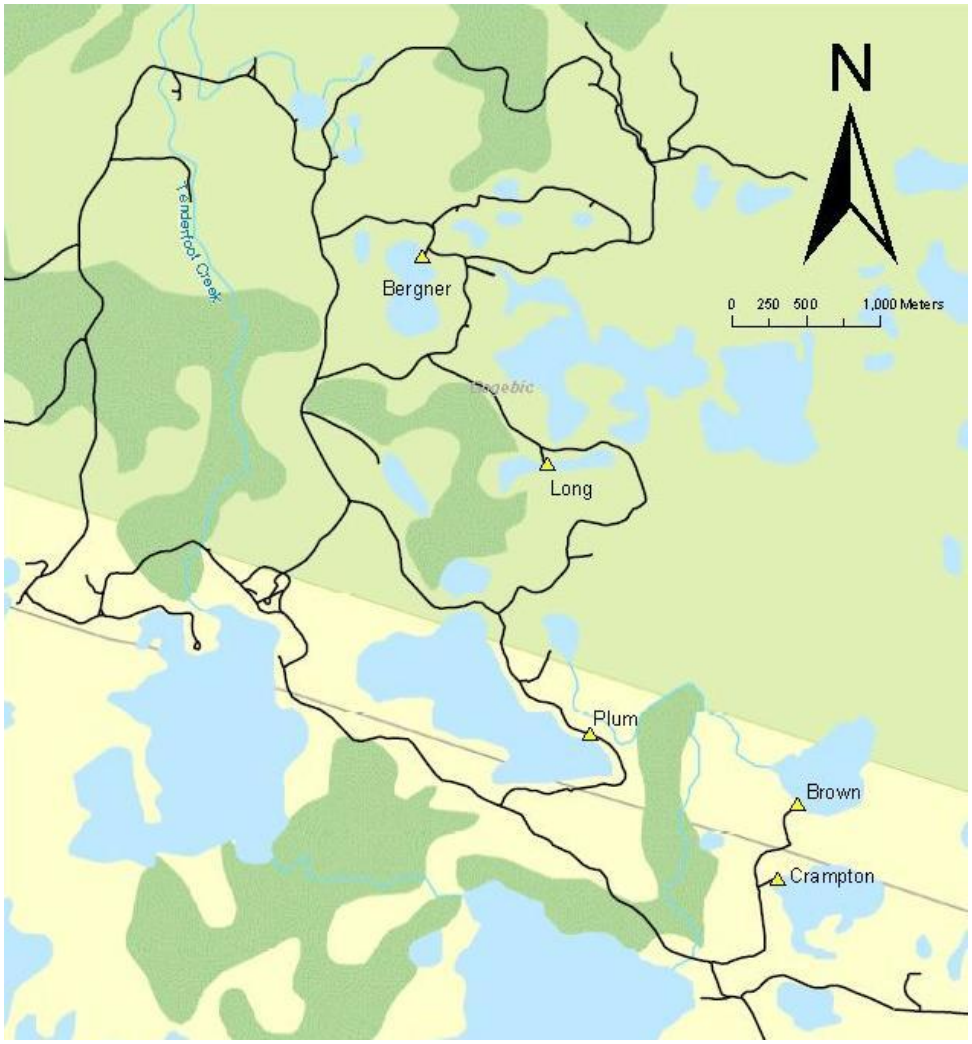


Figure 1. Map of UNDERC property showing locations of study sites taken from GPS coordinates.

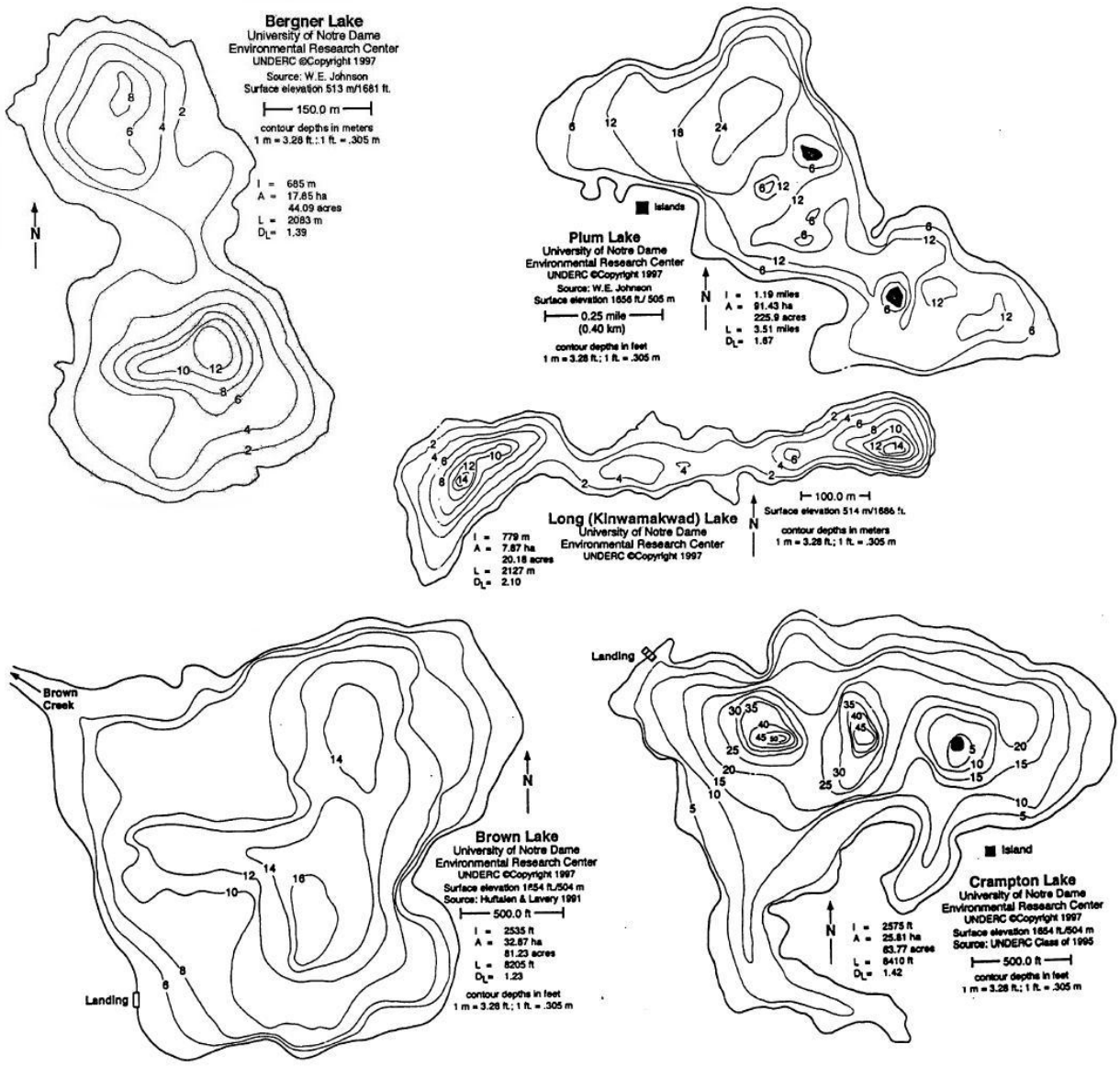


Figure 2. Bathymetric maps with area characteristics for each study lake(Credit: W. E. Johson, Hufton and Lavery 1991, UNDERC Class of 1995).

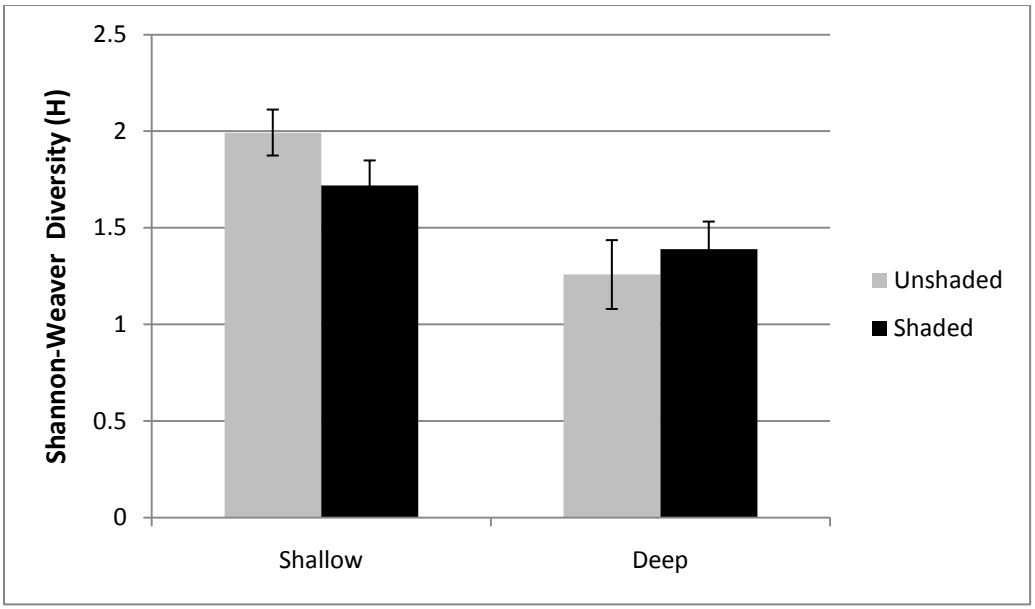


Figure 3. Mean Shannon-Weaver Diversity Index values (H) for macroinvertebrate diversity in shaded versus unshaded containers and shallow versus deep sites.

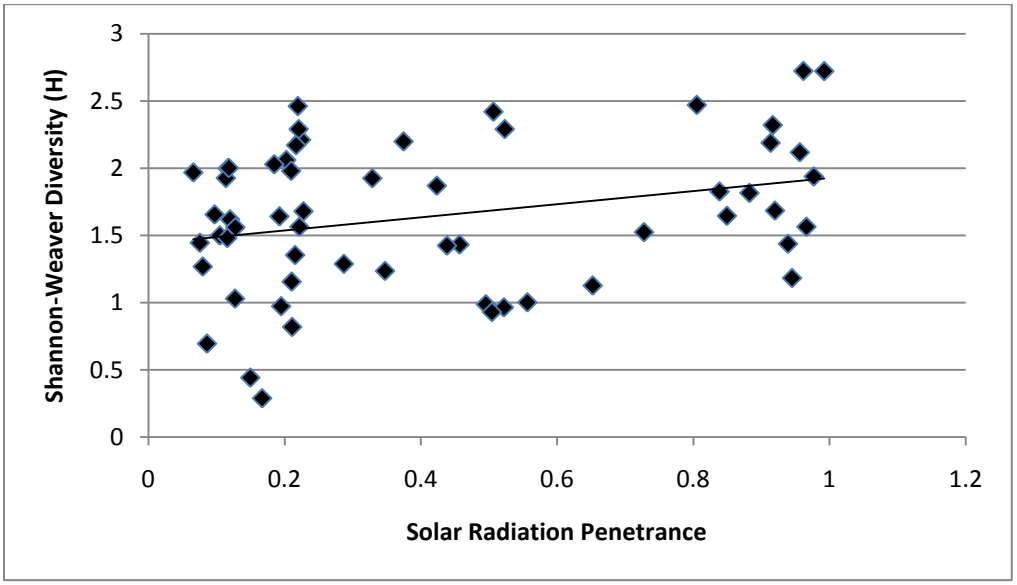


Figure 4. Plot of Shannon-Weaver Diversity Index values (H) for macroinvertebrate diversity against solar radiation penetrance.

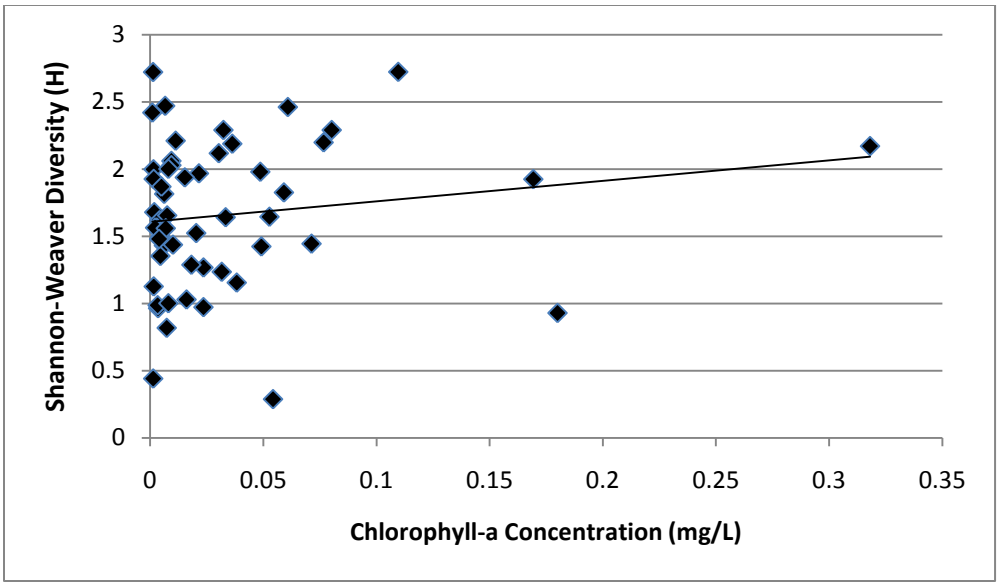


Figure 5. Plot of Shannon-Weaver Diversity Index values (H) for macroinvertebrate diversity against solar radiation penetrance.

# Appendix A

Appendix A-1. All field data collected and values calculated for lake properties, chlorophyll concentrations, and macroinvertebrate Shannon-Weaver Diversity.

| Date | Lake | Site | Type | Depth (cm) | Dissolved Oxygen (mg/L) | Temp (°C) | Light ($W/m^2$) | | Solar Radiation Penetrance | pH | Conduc-tivity (µS/cm) | Solutes (mg/L) | Chlorophyll-a (mg/L) | Shan-non H |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | Air | At Depth | | | | | | |
| 5/28/2010 | Plum | S | Un. | 50.6 | 11.02 | 20.8 | 70.28 | 69.74 | 0.99 | 7.6 | 0.33 | 2.64 | 0.00126* | 2.720 |
| | | | Sh. | 50.6 | 11.02 | 20.8 | 70.28 | 69.74 | 0.23 | | | | 0.00178* | 1.677 |
| | | D | Un. | 100 | 11.05 | 20.8 | 100.27 | 50.79 | 0.51 | | | | 0.00094* | 2.418 |
| | | | Sh. | 100 | 11.05 | 20.8 | 100.27 | 50.79 | 0.12 | | | | 0.00143* | 1.997 |
| 5/28/2010 | Crampton | S | Un. | 39.5 | 10.85 | 21.3 | 1136.00 | 1045.00 | 0.92 | 7.9 | 0.01 | 4.49 | - | 1.682 |
| | | | Sh. | 39.5 | 10.85 | 21.3 | 1136.00 | 1045.00 | 0.21 | | | | 0.00728* | 0.817 |
| | | D | Un. | 87.5 | 10.63 | 21.2 | 296.80 | 193.60 | 0.65 | | | | 0.00153* | 1.125 |
| | | | Sh. | 87.5 | 10.63 | 21.2 | 296.80 | 193.60 | 0.15 | | | | 0.00131* | 0.439 |
| 5/28/2010 | Brown | S | Un. | 93.8 | 11.85 | 22.1 | 638.50 | 563.40 | 0.88 | 7.6 | 0.00 | 7.72 | 0.00616* | 1.814 |
| | | | Sh. | 93.8 | 11.85 | 22.1 | 638.50 | 563.40 | 0.20 | | | | 0.00936* | 2.059 |
| | | D | Un. | 112.9 | 11.91 | 22.0 | 1201.70 | 548.90 | 0.46 | | | | 0.00602* | 1.430 |
| | | | Sh. | 112.9 | 11.91 | 22.0 | 1201.70 | 548.90 | 0.10 | | | | 0.00448* | 1.497 |
| 5/28/2010 | Bergner | S | Un. | 31.0 | 10.39 | 22.4 | 167.40 | 157.20 | 0.94 | 6.9 | 0.05 | 0.93 | 0.01003* | 1.435 |
| | | | Sh. | 31.0 | 10.39 | 22.4 | 167.40 | 157.20 | 0.22 | | | | 0.00448* | 1.352 |
| | | D | Un. | 74.2 | 10.32 | 22.8 | 1498.00 | 781.70 | 0.52 | | | | 0.00347* | 0.963 |
| | | | Sh. | 74.2 | 10.32 | 22.8 | 1498.00 | 781.70 | 0.12 | | | | 0.00410* | 1.617 |
| 5/28/2010 | Long | S | Un. | 28.0 | 10.13 | 22.5 | 1565.00 | 1512.00 | 0.97 | 7.1 | 0.18 | 4.73 | 0.00291* | 1.562 |
| | | | Sh. | 28.0 | 10.13 | 22.5 | 1565.00 | 1512.00 | 0.22 | | | | 0.00214* | 1.562 |
| | | D | Un. | 127.5 | 10.19 | 22.5 | 1024.00 | 507.20 | 0.50 | | | | 0.00326* | 0.985 |
| | | | Sh. | 127.5 | 10.19 | 22.5 | 1024.00 | 507.20 | 0.11 | | | | 0.00152* | 1.925 |
| 6/14/2010 | Plum | S | Un. | 50.5 | 7.11 | 17.9 | 168.30 | 135.50 | 0.81 | 6.8 | 0.14 | 0.11 | 0.00659 | 2.469 |
| | | | Sh. | 50.5 | 7.11 | 17.9 | 168.30 | 135.50 | 0.18 | | | | 0.00936 | 2.027 |
| | | D | Un. | 116.5 | 7.81 | 17.9 | 84.10 | 35.60 | 0.42 | | | | 0.00495 | 1.868 |
| | | | Sh. | 116.5 | 7.81 | 17.9 | 84.10 | 35.60 | 0.10 | | | | 0.00748 | 1.654 |
| 6/14/2010 | Crampton | S | Un. | 41.0 | 7.40 | 17.8 | 103.20 | 94.60 | 0.92 | 7.2 | 0.01 | 4.09 | - | 2.319 |
| | | | Sh. | 41.0 | 7.40 | 17.8 | 103.20 | 94.60 | 0.21 | | | | 0.03822 | 1.153 |
| | | D | Un. | 93.5 | 7.70 | 17.9 | 90.60 | 50.40 | 0.56 | | | | 0.00802 | 1.000 |
| | | | Sh. | 93.5 | 7.70 | 17.9 | 90.60 | 50.40 | 0.13 | | | | 0.00686 | 1.558 |
| 6/14/2010 | Brown | S | Un. | 92.5 | 8.35 | 17.8 | 64.50 | 123.30 | 1.91 | 6.5 | 0.13 | 9.14 | 0.03234 | 2.289 |
| | | | Sh. | 92.5 | 8.35 | 17.8 | 64.50 | 123.30 | 0.44 | | | | 0.04912 | 1.423 |
| | | D | Un. | 118.5 | 7.40 | 17.8 | 145.70 | 50.60 | 0.35 | | | | 0.03161 | 1.234 |
| | | | Sh. | 118.5 | 7.40 | 17.8 | 145.70 | 50.60 | 0.08 | | | | 0.02352 | 1.266 |

| | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 6/14/2010 | Bergner | S | Un. | 32.0 | 7.67 | 17.9 | 264.90 | 225.00 | 0.85 | 7.1 | 0.01 | 0.05 | 0.05268 | 1.643 |
| | | | Sh. | 32.0 | 7.67 | 17.9 | 264.90 | 225.00 | 0.19 | | | | 0.02352 | 0.971 |
| | | D | Un. | 114.0 | 7.82 | 17.9 | 358.70 | 102.90 | 0.29 | | | | 0.01820 | 1.286 |
| | | | Sh. | 114.0 | 7.82 | 17.9 | 358.70 | 102.90 | 0.07 | | | | 0.02152 | 1.966 |
| 6/14/2010 | Long | S | Un. | 35.0 | 7.83 | 17.7 | 60.60 | 59.20 | 0.98 | 7.2 | 0.03 | 0.30 | 0.01528 | 1.936 |
| | | | Sh. | 35.0 | 7.83 | 17.7 | 60.60 | 59.20 | 0.22 | | | | 0.01123 | 2.209 |
| | | D | Un. | 79.5 | 7.54 | 17.6 | 119.70 | 61.60 | 0.51 | | | | 0.01711 | 0.000 |
| | | | Sh. | 79.5 | 7.54 | 17.6 | 119.70 | 61.60 | 0.12 | | | | 0.00802 | 2.000 |
| 7/12/2010 | Plum | S | Un. | 64.0 | 11.55 | 24.3 | 735.00 | 703.00 | 0.96 | 7.3 | 0.06 | 0.02 | 0.03029 | 2.116 |
| | | | Sh. | 64.0 | 11.55 | 24.3 | 735.00 | 703.00 | 0.22 | | | | 0.06081 | 2.460 |
| | | D | Un. | 121 | 10.95 | 24.2 | 834.00 | 462.00 | 0.55 | | | | 0.04277 | 0.000 |
| | | | Sh. | 121 | 10.95 | 24.2 | 834.00 | 462.00 | 0.13 | | | | 0.01604 | 1.028 |
| 7/12/2010 | Crampton | S | Un. | 54.5 | 11.35 | 25.4 | 183.00 | 176.00 | 0.96 | 7.3 | 0.01 | 1.11 | 0.10959 | 2.721 |
| | | | Sh. | 54.5 | 11.35 | 25.4 | 183.00 | 176.00 | 0.22 | | | | 0.08019 | 2.289 |
| | | D | Un. | 100 | 11.19 | 25.0 | 283.00 | 93.00 | 0.33 | | | | 0.16929 | 1.923 |
| | | | Sh. | 100 | 11.19 | 25.0 | 283.00 | 93.00 | 0.08 | | | | 0.07128 | 1.443 |
| 7/12/2010 | Brown | S | Un. | 100.0 | 13.43 | 24.7 | 67.30 | 63.60 | 0.95 | 7.0 | 0.14 | 17.27 | - | 1.181 |
| | | | Sh. | 100.0 | 13.43 | 24.7 | 67.30 | 63.60 | 0.22 | | | | 0.31809 | 2.169 |
| | | D | Un. | 126.5 | 14.01 | 24.6 | 291.00 | 109.00 | 0.37 | | | | 0.07663 | 2.197 |
| | | | Sh. | 126.5 | 14.01 | 24.6 | 291.00 | 109.00 | 0.09 | | | | - | 0.693 |
| 7/12/2010 | Bergner | S | Un. | 42.0 | 11.28 | 25.4 | 117.00 | 98.10 | 0.84 | 7.1 | 0.01 | 2.38 | 0.05907 | 1.824 |
| | | | Sh. | 42.0 | 11.28 | 25.4 | 117.00 | 98.10 | 0.19 | | | | 0.03332 | 1.640 |
| | | D | Un. | 90.5 | 10.85 | 24.8 | 257.00 | 187.00 | 0.73 | | | | 0.02031 | 1.522 |
| | | | Sh. | 90.5 | 10.85 | 24.8 | 257.00 | 187.00 | 0.17 | | | | 0.05426 | 0.286 |
| 7/12/2010 | Long | S | Un. | 20.0 | 9.95 | 25.4 | 937.00 | 856.00 | 0.91 | 6.6 | 0.02 | 1.34 | 0.03626 | 2.187 |
| | | | Sh. | 20.0 | 9.95 | 25.4 | 937.00 | 856.00 | 0.21 | | | | 0.04865 | 1.978 |
| | | D | Un. | 142.0 | 8.50 | 25.0 | 696.00 | 351.00 | 0.50 | | | | 0.17998 | 0.928 |
| | | | Sh. | 142.0 | 8.50 | 25.0 | 696.00 | 351.00 | 0.12 | | | | 0.00392 | 1.478 |

S=Shallow D=Deep Un.=Unshaded Sh.=Shaded *back-calculated from values from 6/14

# Appendix B

The original proposed experiment was to include experiments investigating the effects of ultraviolet radiation on macroinvertebrate reproduction and development, in addition to the feeding experiment described above. The original proposed procedure for this portion of the study is as follows:

> *This study will focus on two studies species, the aquatic gastropod Helisoma trivolvis, and the aquatic amphipod Hyalella azteca. This experiment will take place in four large tubs of water located in an unshaded area outdoors, with two tubs dedicated to each species. Each tub will contain 12 120 mL plastic containers with holes cut in the sides and 250 µm mesh glued around them to create an enclosure. Six of these will have shade cloth secured across the top, and the other three will have the same 250 µm mesh secured across the top. Pebble gravel and natural substrate from Tenderfoot Lake will be in the bottom, and two individuals (one male and one female) will be placed in each container. Each individual will be measured at the start of the experiment, after three weeks, and after seven weeks. The lengths of each individual will be used to monitor growth and development progress.*
>
> *Presence of any new individuals, egg clusters from snails, or brood pouches on amphipods will be recorded in order to get a measure of reproductive rates. Egg patches from snails and any new individuals will be removed upon discovery. Also, any dead individuals will be replaced with fresh ones. For the snails, these replacements will be freshly caught individuals from Tenderfoot Lake and for the amphipods they will be taken from group maintained in a holding tank throughout the duration of the study. In between these scheduled checks, the containers will be monitored to ensure food and water levels remain adequate.*
>
> *The data on reproduction rates will be analyzed using an ANOVA, testing the association between reproduction and UV intensity. Containers will be grouped based on whether or not they were shaded and whether or not reproduction had taken place at each given check, as evidenced by new offspring individuals, brood pouches in amphipods, or egg clusters for snails. From the length measurements, I will calculate regressions of growth versus time separately for shaded and unshaded containers, which will be compared in order to gauge the affects of UV intensity on growth rate.*

The experiment described above failed because all organisms, except for one snail, had died prior to the scheduled check after 21 days, likely due to eutrophication of the water but also possibly due to food not reaching their containers. The experiment was modified twice to include better aerated water, more food, and shorter durations, but both of these attempts failed as well, with all organisms either dying or escaping. Due to time constraints, the development and reproduction portions of the study were abandoned.