Sublethal effects of ultraviolet radiation on the cladocerans, *Daphnia catawba* and *Daphnia pulex*

Peter Pryzbylkowski

Department of Biology, Franklin and Marshall College

Advisor: Janet Fischer

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Abstract- The effects of UV radiation on zooplankton are well documented. Most research has analyzed the effects of relatively high doses of UV radiation on the survival of these organisms. The levels of radiation used in these experiments tend to be unnatural in order to elicit a response from the organism. This study analyzes the effects of sublethal levels of UV radiation in order to determine the responses of zooplankton to these lower levels of radiation. In order to assess sublethal effects, I monitored respiration rates as the response variable. My results indicate that sublethal levels of UV radiation increase respiration rates of *Daphnia* suggesting that there is an energetic cost of DNA repair within the organisms. Furthermore, additional experiments point to nucleotide excision repair (NER) as the likely mechanism causing increased respiration rates following UV exposure. Results of experiments exploring effects of temperature on the metabolic cost of UV exposure were inconclusive due to weak responses at the range of UV levels tested. The sublethal effects of UV radiation have important consequences for *Daphnia* since energy reserves are used to repair damaged DNA, rather than being allocated to processes such as growth and reproduction.
Introduction- In recent decades, the amount of UVB radiation reaching the earth’s surface has been increasing at temperate, as well as polar latitudes, due to stratospheric ozone depletion (Kerr and McElroy 1993, Madronich 1994). It is important to study the effects that UV radiation is having on freshwater ecosystems where, in some cases, UV radiation penetrates to great depths (Williamson et al. 2002). In freshwater ecosystems, chromophoric dissolved organic matter (CDOM) strongly absorbs UV radiation, providing organisms with a certain level of protection from UV damage (Scully and Lean 1994, Morris et al. 1995). While UV is attenuated in the top one meter of water in lakes with high CDOM concentrations, UV can penetrate to much deeper depths (e.g., ≥10m) in lakes with lower CDOM concentrations (Morris et al. 1995). In highly UV transparent lakes, organisms rely on behavioral, physiological, and molecular mechanisms to avoid, reduce, and repair UV damage (Williamson and Zagarese 1994, Roy 2000).

UV radiation is damaging to organisms because it alters DNA structure (Sinha and Hader 2002). Following exposure to UVB radiation, a disruption in the DNA sequence occurs creating cyclobutane pyrimidine dimers (CPDs) and/or pyrimidine (6-4) pyrimidone photoproducts (64PPs) that can affect transcription and translation.

Concerning the effects of UV radiation on aquatic organisms, the shortest wavelengths of UVB radiation in sunlight are potentially the most damaging per photon. However, due to the greater photon flux density of longer wavelength UV in sunlight, the net potential damage to these organisms in nature is greatest for the longer wavelength UVB and shorter wavelength UVA radiation in the 305-322nm range. Following exposure to damaging UVB radiation, aquatic organisms exhibit a dramatic increase in survival in the presence of longer wavelength UVA radiation due to the stimulation of DNA repair
mechanisms. These results suggest that UVA radiation plays an important role in allowing aquatic organisms to survive harmful shorter wavelength radiation (Williamson et al. 2001).

Organisms can use two mechanisms to repair damaged DNA: photoenzymatic repair (PER) and nucleotide excision repair (NER), also known as dark repair (Williamson et al. 2002). Not all aquatic organisms are capable of PER, however NER is present in all aquatic taxa. PER uses a single enzyme, photolyase, which captures a photon of light to break the DNA dimer. Because this process transfers electrons without a net redox reaction, it is not thought to be energetically costly to the organism. On the other hand, nucleotide excision repair is a complex mechanism that synthesizes new DNA using ATP. Since energy is required for the nucleotide excision repair process, it is expected to be metabolically costly. Like many other enzyme-catalyzed processes, DNA repair is temperature-dependent (Keeton et al. 1973). Nucleotide excision repair rates have been demonstrated to increase with temperatures between 5°C and 18°C in yeast (Giese et al. 1957), while PER repair rates increase with increasing temperatures in cell-free extracts (Langenbacher et al. 1997) and mold spores (Coohill and Deering 1969). Within many fish species, PER rates of repair increase from 6°C to 25°C, while nucleotide excision repair rates level off at 12°C (Malloy et al. 1997). In aquatic bryophytes, the influence of temperature on the effects of UVB radiation varies among species. Specifically, temperature-dependence of UV radiation is lower for species with higher overall UV tolerance (Nunez-Olivera et al. 2004).

The effects of UV radiation on a wide range of freshwater species are well documented and can operate by changing behavior, survival rates, and respiration rates.
Several different types of aquatic organisms have the ability to detect and avoid harmful UV radiation. For example, juvenile coho salmon react to UV exposure by migrating to areas of the river that are more shaded (Kelly et al. 2002). Similarly, coregonid fish larvae actively swim away from harmful UV radiation in the lab and the field (Ylonen et al. 2004). Finally, the freshwater cladoceran, *Daphnia*, also exhibits a strong behavioral response to UV radiation. An immediate downward migration at broadband UV illumination levels (with a major part in the UVB) has been demonstrated in *Daphnia pulex* (Hessen 1994). Also, experiments with the cladoceran *Daphnia magna* have shown that the organism reacts to UV light (290-380nm) through negative phototaxis whereas visible light (420-600nm) causes positive phototaxis (Storz et al. 1998). These UV-dependent movements often negatively affect *Daphnia* because the organisms are forced to move towards deeper, cooler waters where food is more scare and physiological functions occur at slower rates.

In cases where behavioral changes fail to completely prevent UV exposure, UV radiation can also affect survival of many aquatic organisms. Both field and laboratory experiments with teleost fish have demonstrated that exposure to UVB radiation causes sunburn, cataracts, immune depression, and increased larval mortality (Alemanni 2003). Other field studies with Northern pike larvae and newly hatched zebrafish have shown decreased survival at higher levels of UVB exposure (Hakkinen 2004, Charron et al. 2000). Laboratory studies with different frog species have shown that ambient levels of solar radiation cause 100% mortality in *Rana pipiens, Rana clamitans* and *Rana septentrionalis*. In subsequent experiments where PER is eliminated, survival is low
within the three species of frogs suggesting that UVB radiation alone is responsible for causing the increased rates of mortality (Tietge et al. 2001).

UV radiation also negatively effects the survival rates of many types of freshwater zooplankton. *Daphnia catawba* experience high mortality rates (71%) when exposed to full spectrum solar radiation for seven hours (Williamson et al. 2001). *Daphnia* are very sensitive to UVB intensity with significant levels of mortality (15%) seen at even the lowest doses of radiation (10kJ/m²) within a laboratory setting. An inverse correlation between the dissolved organic carbon (DOC) concentration and individual mortality rates has also been documented in *Neobosmina chilensis*, a small cladoceran, exposed to high levels of UV radiation further supporting the hypothesis that DOC effectively screens out UV radiation in aquatic ecosystems (De Los Rios, 2004). Both *Daphnia menucoensis* and the copepod *Metacyclops mendocinus* show high efficiency in repairing UVB induced damage to the DNA molecule, with a significant decrease in mortality when the species are exposed to visible radiation (PAR), in addition to UVB (Goncalves et al. 2002). PER contributes significantly towards the degree of total tolerance to UVB light in almost all groups of cladocerans (Ramos-Jiliberto et al. 2004). The beneficial effects of PER in allowing organisms to survive harmful UV radiation is well documented across many aquatic species (Tietge et al. 2001, Williamson et al. 2001).

Several recent studies have suggested that organisms may also experience a metabolic cost at levels of UV exposure that are nonlethal. In these cases, the sublethal effects of UV radiation are manifested in the respiration rates of aquatic organisms. UV radiation damages DNA, which forces DNA repair mechanisms to turn on. As these repair mechanisms are turned on they increase the respiration rates of aquatic organisms
because some of the repair mechanisms require energy in the form of ATP. The sublethal effects of UV radiation on respiration rate are species-specific. For example, juvenile rainbow trout react to UVB exposure by increasing their rate of oxygen consumption and altering their behavior (Alemanni 2003), while UVB exposure decreases the maximum oxygen consumption rates of both vendace and whitefish larvae (Ylonen et al. 2004). In amphibian larvae, exposure to environmental levels of UVA radiation causes increased rates of oxygen consumption within *Bufo bufo* tadpoles whereas exposure to UVB radiation causes significant decreases in oxygen consumption (Formicki et al. 2003). The effects of UV radiation on the respiration rates of zooplankton, including both *Daphnia catawba* and *Daphnia pulex*, have yet to be examined.

The purpose of this independent study is to investigate the sublethal effects of UV radiation on *Daphnia catawba* and *Daphnia pulex*. *Daphnia* are a critical link in lake food webs and tend to be less tolerant than either their food or their predators, thus making their response pivotal in how UV impacts pelagic food webs in lakes (Williamson et al. 2002). Most research has focused on the survival rates of *Daphnia* and, consequently, the UV exposure levels tended to be very high (55KJ/m^2^ UVB, 34KJ/m^2^ UVB, 26KJ/m^2^ UVB: Williamson et al. 2001) in order to elicit a response in the organism. Due to high UV exposure levels, zooplankton research may be missing a more subtle, yet important, effect of sublethal UV radiation. Zooplankton responses to natural levels of UV radiation could have a significant effect on other physiological processes such as growth and reproduction. Therefore, a better approach to zooplankton research would be to use more natural levels of UV radiation and to monitor a more sensitive response variable, such as respiration rates. This is exactly the purpose of this
independent study project. The goals of this study were to determine: 1) how respiration rate changes across a broad range of UV doses, 2) what mechanism is responsible for increasing the respiration rates of *Daphnia* after UV exposure, 3) the temporal pattern of the respiration response during exposure, and 4) the influence of temperature (22°C and 12°C) on the respiration rates of *Daphnia* during and after UV exposure.

**Material and Methods** – Both *Daphnia catarbba* and *Daphnia pulex* are two types of zooplankton that are present in many of the freshwater lakes, streams, and ponds found throughout the United States (Williamson et al. 2002). Female adult *Daphnia catarbba*, originating from Lake Giles, and *Daphnia pulex*, obtained from Carolina Biological Supply Company, were studied during these UV experiments. All *Daphnia catarbba* were collected from Lake Giles, a highly UV transparent lake, located in the Pocono Mountains of Northeastern PA. They were collected using a 60µm, 0.5m diameter vertical plankton net towed from 18m to the surface. Once back in the lab, *Daphnia catarbba* were isolated into Lake Giles water and fed *Cryptomonas* overnight. Typically, after collection and feeding the *Daphnia catarbba* underwent artificial UV exposure the very next day.

For experiments exploring the temperature dependence of UV effects, female individuals of *Daphnia pulex* were obtained from Carolina Biological Supply and cultured for a period of four weeks. These organisms were obtained from Carolina Biological Supply because it was difficult to collect *Daphnia* in the field due to the fact that Lake Giles is covered with ice during the winter months. These organisms were cultured in temperature appropriate (22°C or 12°C) spring water and fed *Cryptomonas* as
needed. The incubators the *Daphnia pulex* were cultured in were on a 12-hour day/night cycle to mimic conditions that are seen in their natural habitat.

I exposed both *Daphnia catawba* and *Daphnia pulex* to damaging UV radiation on a phototron in the laboratory (Fig. 1). The phototron simulates UV exposure on the surface of the water at 40° N latitude on a sunny day around summer solstice when weighted for the spectral sensitivity of *Daphnia catawba* (Williamson et al. 2001). The phototron (Fig. 1) consisted of a rotating exposure wheel with one UVB lamp providing radiation from above the wheel. Acetate was placed under the UVB lamp to screen out any UVC radiation the lamp may have emitted. Two lamps containing UVA and cool white bulbs, photorepair radiation (PRR) lamps, were present underneath the phototron. The PRR lamps underneath the wheel provide light for PER. Mylar was placed on the wheel, where appropriate, to screen out any UVB radiation emitted from the PRR lamps. The wheel contains 40 exposure holes and rotates at 2 revolutions per minute to provide uniform UV exposure. Flat bottom quartz dishes fit into the exposure holes. Each dish was covered with a piece of circular quartz. UVB exposure was controlled by using mesh screens that yielded desired transmittances. Black discs are used to shield some dishes from PRR from below and/or UVB from above as necessary. *Daphnia* were placed on the phototron with the appropriate mesh screens and exposed to UV radiation for 12 hours, without food.

After the 12-hour exposure period, two *Daphnia* were transferred into 10 ml gas-tight syringes containing filtered Lake Giles water or temperature appropriate spring water. Oxygen concentration was measured for each replicate at 2 hr intervals. I used the slope of the oxygen concentration vs. time data to estimate respiration rate. Syringes that
contained *Daphnia* that either reproduced or died were dropped from analyses. After UV exposure and subsequent respiration measurements, both *Daphnia catawba* and *Daphnia pulex* were kept in Petri dishes containing either filtered Lake Giles or spring water to assess survival rates in the organisms. The dishes were housed in temperature appropriate incubators and the individuals were fed *Cryptomonas* as needed. These individuals were all covered during the incubation period after exposure. An overview of the protocol is seen in Figure 2.

**Range finding experiment**- To determine how respiration rate changes across a broad range of UV doses, I varied the percent transmittance of UVB on the phototron. Treatments included 1.04 KJ/m$^2$, 2.08 KJ/m$^2$, 4.16 KJ/m$^2$, 11.96 KJ/m$^2$, and 29.64 KJ/m$^2$ UVB levels, and a dark control. All treatments, except the dark control, received repair radiation from the PRR lamps below the wheel. *Daphnia catawba* were collected on October 17, 2004 and exposed on October 18, 2004. Each treatment was replicated five times and twelve *Daphnia* were used in each replicate.

**Respiration mechanism experiment**- To determine what mechanism was responsible for increasing the respiration rates of *Daphnia catawba* after UV exposure, I varied the percent transmittance of UVB and varied exposure to PRR. Treatments included PRR only, 2.08 KJ/m$^2$ UVB without PRR, 2.08 KJ/m$^2$ UVB with PRR, 4.16 KJ/m$^2$ UVB without PRR, 4.16 KJ/m$^2$ UVB with PRR, and a dark control. *Daphnia catawba* were collected on November 15, 2004 and exposed on November 16, 2004. Each treatment contained five replicates and twelve *Daphnia* were used in each replicate. I measured respiration rates and survival following exposure.
**Temporal Pattern of Response** - To determine the temporal pattern of the respiration response during exposure, I varied the lengths of exposure for a 4.16 KJ/m² UVB with PRR treatment. Treatments included 4 hr, 8 hr, and 12 hr exposures at 4.16 KJ/m² UVB with PRR along with dark controls for each temporal period. *Daphnia catawba* were collected on November 19, 2004 and exposed on November 20, 2004. Each treatment was replicated five times and five *Daphnia* were used for each replicate.

Because my results indicated that the protocol was missing a respiration response that was occurring during the actual exposure (*See Results*), I developed a new protocol to quantify respiration rates during UV exposure either in the field or the laboratory. First, UV-transparent quartz containers (diameter= 1.4 cm, height= 3.6 cm), that were small enough to fit on the phototron’s holes, were tested using silicone and rubber stoppers to determine gas permeability of the container/stopper system. Gas permeabilities of these container/stopper systems were compared to the permeability of 10 ml gas-tight syringes used in previous experiments. I bubbled nitrogen gas through 500 ml of distilled tap water for a period of forty minutes and then placed this water into the different container/stopper systems along with the 10 ml gas-tight syringes. There were three replicates per treatment. The oxygen concentration of the containers was then monitored for a period of six hours at two-hour intervals.

Because the silicone and rubber stoppers were not gas-tight, I performed a follow up experiment testing the gas permeability of a quartz 10 mm path length spectrophotometer cell with a Teflon stopper (*Fig. 3*). This spectrophotometer cell was borrowed from the Chemistry department. The gas permeability of the system was again compared to the 10ml gas-tight syringes used in previous experiments following the same
methods as outlined above. However, a twelve-hour monitoring period was used for this experiment with only an initial and final measurement taken for the quartz spectrophotometer cell/Teflon stopper system. There were two replicates per treatment.

**UV/Temperature experiments** - To determine the influence of temperature (22°C and 12°C) on the respiration rates of *Daphnia pulex* during and after UV exposure, I varied the percent transmittance of UVB and varied exposure to PRR. Treatments included PRR only, 1.04 KJ/m² UVB without PRR, 1.04 KJ/m² UVB with PRR, 2.08 KJ/m² UVB without PRR, 2.08 KJ/m² UVB with PRR, 4.16 KJ/m² UVB without PRR, 4.16 KJ/m² UVB with PRR, and a dark control. For the 22°C experiment, each treatment contained four replicates and seven *Daphnia pulex* were used in each replicate. The experiment was performed on April 4, 2005 with respiration rates and survival being measured following exposure. The sizes of *Daphnia pulex* used in the experiment varied greatly between individuals; therefore *Daphnia* were saved for individual length measurements. These measurements were used to estimate biomass using the equation $\mu g$ dry weight $= 6.99 \times (\text{length in mm})^{22}$ (McCauley 1984), so that respiration rate per unit biomass could be calculated.

For the 12°C UV/temperature experiment *Daphnia pulex* were used. Each treatment contained five replicates and twelve *Daphnia pulex* were used in each replicate. The experiment was performed on April 11, 2005 with respiration rates, survival, and biomasses being measured following exposure as described for the 22°C experiment above.
Results-

*Range finding experiment with Daphnia catawba*- My range finding experiment suggested that UV exposure may result in increased respiration rates of *Daphnia catawba*. Although an ANOVA testing for differences in respiration rate among all treatments was not statistically significant ($F_{5,26}=1.78$, $p=0.16$), I used planned contrasts to compare respiration rates in each UV exposed treatment to the dark control for exploratory purposes. Mean respiration rate was significantly higher in the 2.08 KJ/m² UVB with PRR treatment than in the dark control (Fig. 4, $F_{1,6}=6.34$, $p=0.012$). However, mean respiration rate for all other UV exposed treatments was not significantly different from the dark control (Fig. 4, dark vs 1.04 KJ/m² UVB with PRR: $F_{1,6}=0.53$, $p=0.47$; dark vs 4.16 KJ/m² UVB with PRR: $F_{1,6}=0.0$, $p=1.0$; dark vs 11.96 KJ/m² UVB with PRR: $F_{1,6}=0.24$, $p=0.63$; dark vs 29.64 KJ/m² UVB with PRR: $F_{1,7}=1.59$, $p=0.22$).

*Respiration mechanism experiment with Daphnia catawba*- My mechanism experiment suggested that nucleotide excision repair (dark repair) is the DNA repair mechanism causing increased respiration rates within *Daphnia catawba*. ANOVA indicated significant differences in respiration rates among treatments (Fig. 5, $F_{5,26}=14.20$, $p=0.0001$). Specifically, mean respiration rate was significantly higher in the 2.08 KJ/m² UVB with PRR treatment than in the dark control ($F_{1,9}=11.80$, $p=.0023$) and the PRR only treatment ($F_{1,9}=6.02$, $p=0.022$). Mean respiration rate was significantly lower in the 4.16 KJ/m² UVB treatment than in the dark control ($F_{1,8}=16.39$, $p=0.0005$). The mean respiration rate in the 2.08 KJ/m² UVB treatment was also significantly higher than in the dark control ($F_{1,9}=11.8$, $p=0.0023$) and the PRR only treatment ($F_{1,9}=6.02$, $p=0.022$). Mean respiration rate in all other UV exposed treatments was not significantly different
from the dark control (dark vs PRR: $F_{1,5}=0.96$, $p=0.34$; dark vs 4.16 KJ/m$^2$ UVB with PRR: $F_{1,5}=2.17$, $p=0.15$). Repeated measures ANOVA indicated no significant difference in average survival or the trajectory of survival through time (i.e. the UV x time interaction) between the 2.08 KJ/m$^2$ UVB with PRR treatment and the dark control (Fig. 6, Table 1). However, statistically significant UV x time interactions indicated that survival trajectories in the 4.16 KJ/m$^2$ UVB with PRR, 4.16 KJ/m$^2$ UVB, and 2.08 KJ/m$^2$ UVB with PRR treatments differed from the dark control (Table 1). At these levels of UV exposure, survival was low (Fig. 6).

**Temporal Pattern of Response in Daphnia catawba**— My temporal pattern experiment suggested that I might be missing a respiration response within *Daphnia catawba* that is occurring during the actual exposure period. Mean respiration rate was not significantly affected by UV exposure after 4 hours (Fig. 7, $t_{4.6}=1.4$, $p=0.22$). Similarly, there was no significant difference in respiration rate between UV exposed and dark control treatments after 12 hours ($t_{7}=0.24$, $p=0.82$). However, after 8 hours, mean respiration rate was significantly elevated in the UV exposed treatment compared to the dark control ($t_{7}=2.87$, $p=0.024$). Since my results indicated that I may be missing a respiration response that is occurring during UV exposure I created a new protocol to assess respiration rates during the exposure period.

My results for the new protocol experiments indicated that the quartz 10 mm path length spectrophotometer cell/Teflon stopper system should be used to monitor respiration rates during UV exposure periods. Compared to the 10 ml gas-tight syringes, both the rubber and silicone stoppers used in the glass quartz tubing were unable to prevent oxygen from entering the system (Fig. 8A). However, the follow up experiment
with the 10 mm quartz spectrophotometer cell and Teflon stopper system was promising. It was determined that compared to the 10 ml gas-tight syringe, the quartz spectrophotometer cell/Teflon stopper system was indeed gas tight (Fig. 8B). The relatively slight changes in oxygen concentration within both the 10 ml gas-tight syringes and the quartz spectrophotometer cell/Teflon stopper system may be due to changes in water temperature during the experiment. The new quartz spectrophotometer cell/Teflon stopper system will be used in upcoming years to monitor respiration rates as they occur in the field or on the phototron.

**UV/Temperature experiments with Daphnia pulex**- My results for the temperature experiments indicated that there was, in general, no major significant interaction between UV radiation and temperature. However, a marginally significant result was obtained at the 4.16 KJ/m² UVB with PRR treatment level for the 12°C temperature experiment. For the 22°C temperature experiment, ANOVA indicated no significant differences in respiration rates among treatments (Fig. 9, F_{7,27}=0.44, p=0.8670). Mean respiration rates in all UVB with PRR exposed treatments were not significantly different from the dark control (dark vs PRR: F_{1,6}=0.01, p=0.94; dark vs 1.04 KJ/m² UVB with PRR: F_{1,7}=0.97, p=0.34; dark vs 2.08 KJ/m² UVB with PRR: F_{1,7}=0.85, p=0.37; dark vs 4.16 KJ/m² UVB with PRR: F_{1,6}=0.40, p=0.53). Repeated measures ANOVA indicated no significant difference in average survival or the trajectory of survival through time (i.e. the UV x time interaction) between the 1.04 KJ/m² UVB with PRR, 2.08 KJ/m² UVB with PRR, 4.16 KJ/m² UVB with PRR, and PRR only treatments and the dark control (Table 2). However, statistically significant UV x time interactions indicated that survival trajectories in the 1.04 KJ/m² UVB, 2.08 KJ/m² UVB and 4.16 KJ/m² UVB treatments
differed from the dark control (Table 2). At these levels of UV exposure, survival was low (Fig. 10).

For the 12°C temperature experiment, ANOVA indicated no significant differences in respiration rates among treatments (Fig. 11, $F_{7,35}=0.75$, $p=0.64$). ANOVA also indicated that, except for the 4.16 KJ/m² UVB with PRR treatment, the mean respiration rates in all UVB with PRR exposed treatments were not significantly different from the dark control (dark vs PRR: $F_{1,9}=0.04$, $p=0.84$; dark vs 1.04 KJ/m² UVB with PRR: $F_{1,8}=0.22$, $p=0.65$; dark vs 2.08 KJ/m² UVB with PRR: $F_{1,9}=0.43$, $p=0.52$).

However, I was intrigued by the trend in increased respiration rates found within the 4.16 KJ/m² UVB with PRR treatment. Therefore, I felt that a one-tailed t-test may be appropriate to use because previous experiments have suggested a directional hypothesis that respiration rate increases with increased levels of UV exposure. Using this test where $p<0.1$ is significant, I concluded that there was a marginally significant effect of UV radiation on respiration rate in the 4.16 KJ/m² UVB with PRR treatment as compared to the dark control (dark vs 4.16 KJ/m² UVB with PRR: $F_{1,9}=2.59$, $p=0.11$). Repeated measures ANOVA indicated no significant difference in average survival or the trajectory of survival through time (i.e. the UV x time interaction) between the 1.04 KJ/m² UVB with PRR, 2.08 KJ/m² UVB with PRR, 4.16 KJ/m² UVB with PRR, and PRR only treatments and the dark control (Table 3). However, statistically significant UV x time interactions indicated that survival trajectories in the 1.04 KJ/m² UVB, 2.08 KJ/m² UVB and 4.16 KJ/m² UVB treatments differed from the dark control (Table 3). At these levels of UV exposure, survival was low (Fig. 12).
Since there was no significant effect of temperature during UV exposure and knowing that the sizes of *Daphnia pulex* differed between the two experiments, the biomasses of both sets of *Daphnia pulex* were analyzed. The biomasses were analyzed in order to verify that the experiment testing for the effects of temperature on UV radiation was not confounded by differences in *Daphnia pulex* size. A two-sample t-test assuming unequal variances indicated that there was no significant difference between the biomasses of the *Daphnia pulex* used in the experiments ( t<sub>37</sub>=1.6, p=0.33) indicating that differences in biomasses had no major effect on the experiments performed.

**Discussion –**

*Sublethal Effects on Daphnia catawba* - Overall, my experiments involving *Daphnia catawba* were successful in identifying sublethal levels of UV exposure and suggest that there is an energetic cost of DNA repair. Similar to previous studies (Williamson et al. 2001), higher doses of UV (>4.16 KJ/m²) were lethal to *Daphnia catawba* and PRR was critical to survival (Fig. 6). Furthermore, respiration rate was elevated following exposure to 2.08 KJ/m² UVB with PRR compared to dark controls in two experiments (Fig. 4,5). Enhanced respiration has been found in studies of rainbow trout (Alemanni 2003); however, the mechanism causing increased respiration was not determined. My results suggest that the energetic cost of DNA repair was the mechanism driving enhanced respiration (Fig. 5). In particular, nucleotide excision repair (dark repair) seems to be responsible for the increase in respiration rates seen in the experiments. If photo-oxidative damage or behavior was the mechanism, then respiration rates would have been enhanced in the PRR only treatment because both of these responses are caused by exposure to UVA radiation alone (Williamson et al. 2002). The involvement of
nucleotide excision repair (dark repair) in repairing damaged DNA after UV exposure makes sense since the organisms are housed in a non-lighted incubator after they have been exposed to radiation on the phototron. This result has applications to situations in the field where organisms are exposed to natural levels of UV radiation during the day and repair damaged DNA during the night.

**Temporal Response of Daphnia catawba** - The temporal response experiment suggests that my standard protocol may be missing a respiration response that is occurring during the exposure to UV radiation. After eight hours of exposure, respiration was higher in the UV exposed treatment than in the control (Fig. 7). Because I measured respiration rates after the 12-hour exposure period in my earlier experiments, I failed to detect this response. Consequently, I may have underestimated the energetic cost of UV exposure because the respiration response could have been occurring long before I measured it. After this finding, a new protocol was developed in the second semester of this project. The new protocol involves using gas-tight, UV transparent, 10 mm path length quartz spectrophotometer cells that are able to house *Daphnia* both in the field and in the laboratory during UV exposure. This new protocol will allow for the measurement of respiration rates during the UV exposure period because the quartz cells are gas-tight, UV transparent, and easily manipulated in both the field and laboratory. Follow up experiments should be performed where respiration rates are measured during the actual UV exposure period. Performing an experiment similar to my respiration rate/mechanism experiment, but monitoring respiration rates during UV exposure, may serve to ultimately determine what mechanism/mechanisms play a role in DNA damage repair in *Daphnia catawba*. 
Temperature-Dependent Effects in Daphnia pulex - Enzyme catalyzed processes, such as photoenzymatic repair (PER) and nucleotide excision repair (NER), are temperature-dependent with increased temperatures causing these processes to work faster and more efficiently. If the effects of UV radiation are temperature-dependent than one would expect to see increased respiration rates overnight in organisms exposed to lower temperatures as compared to organisms exposed to higher temperatures. This is due to the hypothesis that more DNA damage is present at the end of the day within organisms exposed to lower temperatures because DNA repair rates are expected to be slower in cold conditions. However, increased respiration rates will still depend on the level of UV radiation used during exposure. For example, organisms exposed to low levels of UV radiation should be able to efficiently repair damaged DNA at both low and high temperatures because low levels of radiation are unable to cause large amounts of DNA damage. More moderate levels of UV radiation cause increases in net DNA damage that are quickly repaired at high temperatures. At moderate levels of radiation, lower temperatures cause increased respiration rates because the DNA repair mechanisms are unable to keep up with the total amount of DNA damage. The highest levels of UV radiation cause tremendous amounts of DNA damage, which are not able to be repaired efficiently at both high and low temperatures resulting in increased rates of respiration. These temperature-dependent processes have applications to the field where sensitivity to UV radiation is expected to be highest during the springtime when lake water temperatures are cold (Williamson et al. 2001).

The two UV/temperature experiments showed a weak effect of temperature during sublethal UV exposure. A significant difference in respiration rates was expected
to be seen between the dark control and UV exposed treatments in the 12°C experiment. However, only a marginally significant result was seen in the 4.16 KJ/m² UVB with PRR treatment (dark vs 4.16 KJ/m² UVB with PRR: $F_{1,9}=2.59$, $p=0.11$). It is possible that larger UV treatment levels may elicit the response that is hypothesized to be seen at low and high temperatures.

It is important to keep in mind that for the UV/temperature experiments *Daphnia pulex* were used, while for all other previous experiments *Daphnia catawba* were manipulated. Previous research has shown that zooplankton tolerance to UV radiation is not based on body size, rather tolerance has been found to be species-specific. Specifically, *Daphnia catawba* has been found to be significantly less tolerant of UV radiation than *Daphnia pulearia*, a species that is closely related to *Daphnia pulex* (Leech et al. 2000). My results support previous research, which has shown that the effects of UV radiation on *Daphnia* are species-specific. This statement is based on the observation that, at relatively similar temperatures (19°C for *catawba* and 22°C for *pulex*), there was a significant difference in respiration rate between the 2.08 KJ/m² UVB with PRR treatment and the dark control in two experiments for *Daphnia catawba* (Fig. 3, $F_{1,8}=6.34$, $p=0.012$; $F_{1,9}=11.80$, $p=0.0023$), whereas for *Daphnia pulex* there was no significant difference within the same 2.08 KJ/m² UVB with PRR treatment group ($F_{1,7}=0.85$, $p=0.37$). The survival data of both *Daphnia catawba* and *pulex* shows that PRR, which induces PER, is key in preventing increased levels of mortality (Figs. 6, 10, 12) This finding further supports the hypothesis that PER is critical to the survival of any *Daphnia* species (Williamson et al. 2001). Unfortunately, the results of my study do not allow for any significant conclusions to be made regarding the temperature
dependence of UV radiation on *Daphnia pulex*. More experiments, at temperatures similar to those used in my studies, should be performed using a wider range of UV exposure levels in order to determine the effect of temperature on UV radiation within *Daphnia pulex*. Also, experiments using individuals obtained from the wild might yield the true importance of the UV/temperature interaction since these organisms have to deal with varying UV/temperature interactions on a seasonal basis.

If the tolerance to UV radiation within *Daphnia* is species-specific then differing results might be expected to be seen within the survival rates of the two types of *Daphnia* used in these studies. Indeed, it seems that *Daphnia pulex* are better able to withstand UV radiation as compared to *Daphnia catawba*. This statement is based on looking at the similar treatments both the *Daphnia pulex* and *Daphnia catawba* experienced. It seems that especially in the 2.08 KJ/m² UVB with PRR, and 4.16 KJ/m² UVB with PRR treatments *Daphnia pulex* survived better than *Daphnia catawba* (Fig. 6,10). These results further support the hypothesis that *Daphnia catawba* are less tolerant of UV radiation as compared to other zooplankton species (Leech et al. 2000). However, it should be noted that both of these experiments were run at different temperatures (19°C for *catawba* and 22°C for *pulex*).

**Conclusions and Significance**- This independent study demonstrates that there is a sublethal effect of UV radiation on *Daphnia catawba*. This sublethal effect is seen in the increased respiration rates of *Daphnia catawba* following exposure to sublethal levels of UV radiation. The increase in respiration rate is caused by the damage to and subsequent repair of the DNA molecule by *Daphnia*. This result could have important implications on growth and development of *Daphnia*. If *Daphnia* increase their respiration rates in
order to provide energy for DNA repair mechanisms, then less energy may be available for processes such as growth, reproduction, and vertical migration within the water column. These changes could have important implications for aquatic food webs because zooplankton are a main food source for many aquatic organisms and act as important grazers on algae.

Subsequent experiments with *Daphnia catawba* indicated that nucleotide excision repair (NER) and not photoenzymatic repair (PER) is the mechanism causing increased respiration rates in the organism. Never before has research analyzed the sublethal effects of UV radiation on zooplankton. All previous studies tend to analyze the survival of the organisms after they receive a large and unnatural dose of UV radiation. Since the experiment mimics a daytime exposure/night time repair cycle, the results seen with *catawba* may have important implications in the field where the organisms are exposed to the same day/night cycle. Follow up experiments in which respiration rates are measured during exposure should be performed in order to obtain a better understanding of the DNA repair processes that occur during the daytime within *Daphnia*.

My final temperature experiments showed only a weak interaction of UV and temperature. An interesting trend was seen with the marginally significant difference in respiration rate at the 4.16 KJ/m² UVB with PRR treatment as compared to the dark control in the 12°C experiment, however no other significant differences were found. Larger levels of UV radiation could possibly elicit the expected temperature/UV results and follow up experiments should be performed using these larger levels of radiation. These types of experiments will provide insights into how UV radiation effects vary seasonally (i.e. spring vs. summer) within aquatic ecosystems.
Table 1. Survival statistics of *D. catarina* exposed to UV radiation on November 17, 2004. P values reported for Time and Time x UV treatment interaction have been corrected for departure from sphericity using the Huynh-Feldt adjustment.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>UV Treatment</th>
<th>Time</th>
<th>Time x UV Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>df</td>
<td>F</td>
<td>p</td>
</tr>
<tr>
<td>All treatments</td>
<td>5.24</td>
<td>54.48</td>
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</tr>
<tr>
<td>PRR vs Dark</td>
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<tr>
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<td>0.01</td>
<td>0.93</td>
</tr>
<tr>
<td>2.08 KJ/m² UVB vs Dark</td>
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</tr>
<tr>
<td>4.16 KJ/m² UVB with PRR vs Dark</td>
<td>1.8</td>
<td>3.96</td>
<td>0.08</td>
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<tr>
<td>4.16 KJ/m² UVB vs Dark</td>
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<td>205.29</td>
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Table 2. Survival statistics of *D. pulex* exposed to UV radiation at 22°C on April 4, 2005. P values reported for Time and Time x UV treatment interaction have been corrected for departure from sphericity using the Huynh-Feldt adjustment.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>UV Treatment</th>
<th>Time</th>
<th>Time x UV Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>df</td>
<td>F</td>
<td>p</td>
</tr>
<tr>
<td>All treatments</td>
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<tr>
<td>PRR vs Dark</td>
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<td>4.16 KJ/m² UVB vs Dark</td>
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<td>102.69</td>
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Table 3. Survival statistics of *D. pulex* exposed to UV radiation at 12°C on April 11, 2005. P values reported for Time and Time x UV treatment interaction have been corrected for departure from sphericity using the Huynh-Feldt adjustment.

<table>
<thead>
<tr>
<th>Treatment</th>
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<th>df</th>
<th>F</th>
<th>p</th>
<th>Time</th>
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<td>4.16 KJ/m² UVB with PRR vs Dark</td>
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<td>3,24</td>
<td>140.3</td>
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Figure 1. A diagram of the phototron used for UV exposures. Simulates solar radiation during the summer solstice at 40° N latitude (Diagram from Williamson 2001). The phototron rotates at a rate of two revolutions per minute providing uniform exposure to all experimental replicates.
Collection of *Daphnia* from Lake Giles or obtain from Carolina Biological Supply

Culture *Daphnia* in incubators at appropriate temperature, feed as needed.

Place *Daphnia* into quartz exposure chambers containing either Lake Giles or spring water. (3 hours)

After placing mesh screens above quartz containers, to yield desired transmittances, expose *Daphnia* on phototron. (12 hours)

Take *Daphnia* off phototron and place two individuals per replicate into a 10 ml gas-tight syringe. (3 hours)

All other unused individuals are placed into Petri dishes containing appropriate water. Survival is monitored for the next five days.

Monitor the change in oxygen consumption within the gas-tight syringes. (12 hours)

*Figure 2.* Overview of experimental protocol used in these studies.
Figure 3. 10 mm path length spectrophotometer cell with Teflon stopper. Outside dimensions 12.5 mm sq x 49.0 mm high.
**Figure 4.** Respiration rates of *D. catawba*, following a 12-hour UV exposure, calculated by the change in oxygen concentration in gas-tight syringes. The treatments included 1.04 KJ/m² UVB with PRR, 2.08 KJ/m² UVB with PRR, 4.16 KJ/m² UVB with PRR, 11.96 KJ/m² UVB with PRR, 29.64 KJ/m² UVB with PRR, and a dark control. Each treatment was replicated five times.
Figure 5. Respiration rates of *D. catawba*, following a 12-hour UV exposure, calculated by the change in oxygen concentration in gas-tight syringes. The treatments included PRR, 2.08 KJ/m² UVB with PRR, 4.16 KJ/m² UVB with PRR, 2.08 KJ/m² UVB, 4.16 KJ/m² UVB and a dark control. Each treatment was replicated five times.
Figure 6. Survival rates of *D. catawba*, following a 12-hour UV exposure on November 17, 2004. The treatments included PRR, 2.08 KJ/m² UVB with PRR, 4.16 KJ/m² UVB with PRR, 2.08 KJ/m² UVB, 4.16 KJ/m² UVB and a dark control. Each treatment was replicated five times.
Figure 7. Respiration rates of *D. catawba*, after different time intervals of UV exposure, calculated by the change in oxygen concentration in gas-tight syringes over a 8 hour period. The treatments were divided into three exposure periods: 4 hours, 8 hours, and 12 hours. Each treatment contained its own individual dark control, and all exposure levels were at 4.16 KJ/m² UVB with PRR. Each treatment was replicated five times.
Figure 8. A) Oxygen concentration (ml/L) versus time (mins) for different quartz glass/stopper systems and 10 ml gas-tight syringes. The different stoppers used in the quartz glass tubing were rubber and silicone. Each line represents one replicate. B) Oxygen concentration (ml/L) versus time (mins) for quartz cell/Teflon stopper systems and 10 ml gas-tight syringes. Each line represents one replicate.
Figure 9. Respiration rates per unit biomass of *D. pulex* at 22°C, following a 12-hour UV exposure, calculated by the change in oxygen concentration in gas-tight syringes. The treatments included PRR, 1.04 KJ/m² UVB with PRR, 2.08 KJ/m² UVB with PRR, 4.16 KJ/m² UVB with PRR, 1.04 KJ/m² UVB, 2.08 KJ/m² UVB, 4.16 KJ/m² UVB and a dark control. Each treatment was replicated four times.
Figure 10. Survival rates of *D. pulex*, following a 12-hour UV exposure at 22°C on April 4, 2005. The treatments included PRR, 1.04 KJ/m² UVB with PRR, 2.08 KJ/m² UVB with PRR, 4.16 KJ/m² UVB with PRR, 1.04 KJ/m² UVB, 2.08 KJ/m² UVB, 4.16 KJ/m² UVB and a dark control. Each treatment was replicated four times.
Figure 11. Respiration rates per unit biomass of *D. pulic* at 12°C, following a 12-hour UV exposure, calculated by the change in oxygen concentration in gas-tight syringes. The treatments included PRR, 1.04 KJ/m² UVB with PRR, 2.08 KJ/m² UVB with PRR, 4.16 KJ/m² UVB with PRR, 1.04 KJ/m² UVB, 2.08 KJ/m² UVB, 4.16 KJ/m² UVB and a dark control. Each treatment was replicated five times.
Figure 12. Survival rates of *D. pulex*, following a 12-hour UV exposure at 12°C on April 11, 2005. The treatments included PRR, 1.04 KJ/m² UVB with PRR, 2.08 KJ/m² UVB with PRR, 4.16 KJ/m² UVB with PRR, 1.04 KJ/m² UVB, 2.08 KJ/m² UVB, 4.16 KJ/m² UVB and a dark control. Each treatment was replicated five times.
Literature Cited


**Acknowledgments**- I would like to thank Prof. Janet Fischer for the support and guidance she provided me with throughout the independent study process.